Scientific-Based Explanation of Thai Traditional Medicine Theory for Thai Traditional Herbal Remedy (A Case Study of Mo-Ha-Rak Formula)

Chinnaphat Chaloemram

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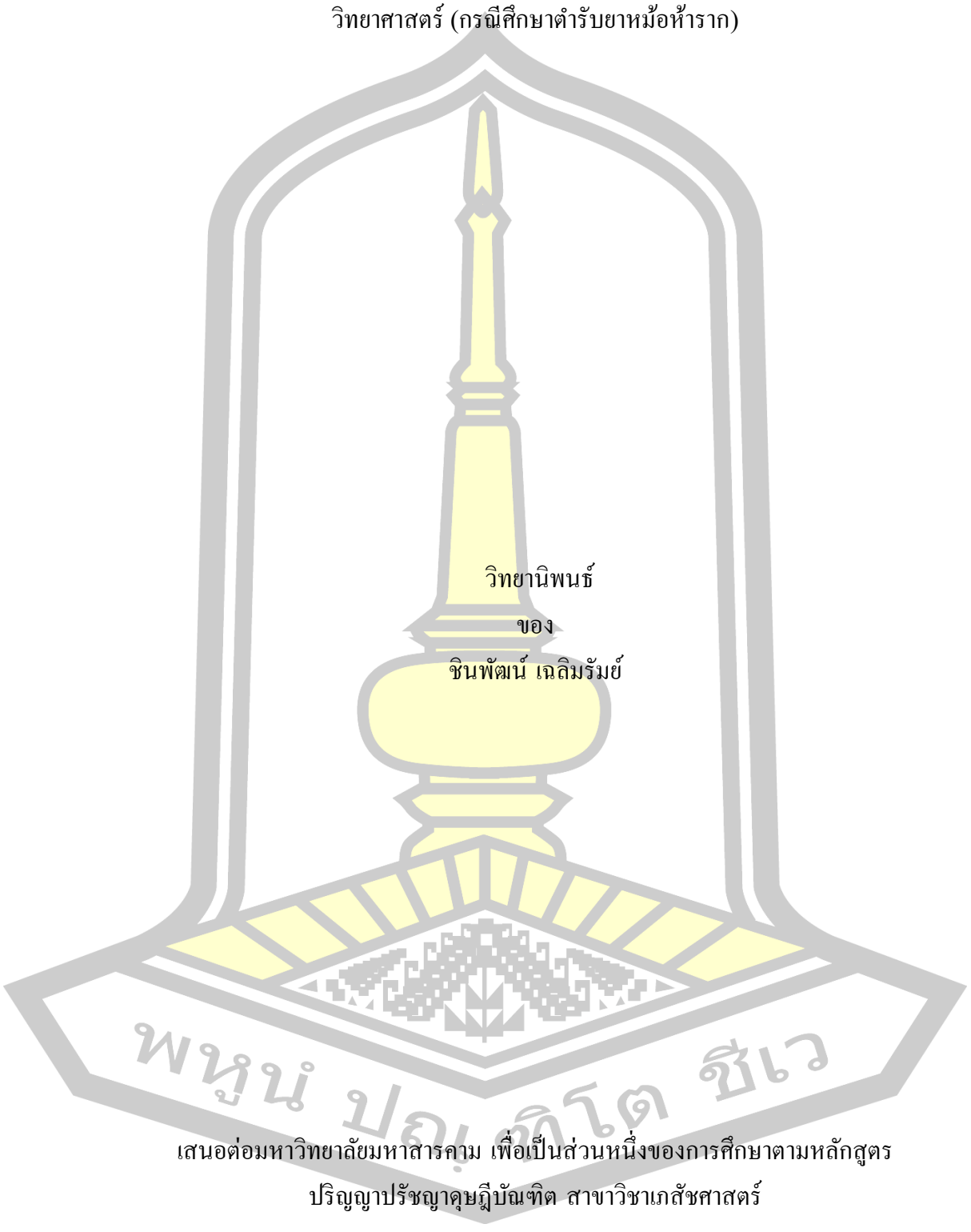

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ABSTRACT

Thai traditional preparation (polyherbal medicine) typically contains more than two herbs, with up to 30 or more herbs. There is limited information available regarding the formulation theory of Thai traditional preparations. This study aims to provide further insights into the formulation theory underlying Thai traditional polyherbal medicine. The structure of Thai traditional preparation comprises four distinct groups of herbs, each serving different functions: primary herbs (PH), adjunct herbs (AH), supportive herbs (SH), and flavoring herbs (FH). Additionally, each group might be further divided into subgroups.

Mo-Ha-Rak (MHR), a polyherbal medicine comprising 21 medicinal plants was selected for this study. It is used by Thai traditional practitioners for the treatment of high fever. In this study, modifications of herbs in various factors of the 92 modified and original MHR of 70% ethanol extract were investigated in terms of relation to their chemicals, pharmacological activities (anti-inflammatory and antioxidant), and toxicity.

The anti-inflammatory activity of each herbal group and the combination of PH with other groups were listed as follows: PH (72.26%), PH+SH (53.47%), PH+AH (52.80%), MHR (31.32%), AH (21.39%) and SH (20.08%). The studies indicated that PH exhibits a direct anti-inflammatory effect. The combination of PH with other herbal groups displayed a lower anti-inflammatory activity.

The PH can be classified into 4 subgroups, reduced-toxic fever herbs (PH1), anti-kamdao and lohit fever herbs (PH2), anti-di fever herbs (PH3) and anti-semha and lom fever herbs (PH4). The anti-inflammatory activity of PH1 (75.92%) was not significantly different from PH (72.26%) and PH1+PH3 (72.05%) but significantly higher than PH1+PH4 (58.61%) and PH1+PH2 (39.87%). The combination of PH1 with other PH subgroups displayed different anti-inflammatory activity, PH1+PH3+PH4 (62.28%), PH1+PH2+PH3 (62.16%) and PH1+PH2+PH4

(41.13%). This study showed that herbs within the PH subgroup exhibit both synergistic and antagonistic effects.

The adjunct herbs (AH) consist of 2 subgroups: stimulant laxative (AH1) and sour-astringent laxative (AH2). The anti-inflammatory activity of PH was significantly higher than PH+AH (52.80%) and PH plus AH subgroups, PH+AH1 (46.08%) and PH+PH2 (41.53%) including AH (23.39%). The anti-inflammatory effect of AH was relatively low compared to PH and MHR. The anti-inflammatory effect of PH decreases when PH is combined with AH.

The antioxidant activity (% inhibition of NO radical scavenging) of herbal groups and their mixture were listed in the following order: AH (62.54%), MHR (61.93%), PH+ AH (60.40%), PH (56.23%), PH+SH (33.00%) and SH (28. 00%). Antioxidant activity is not directly related to PH but is directly related to AH. It may support other effects of the remedy.

The toxicity (% cell viability) of herbal groups and their mixture was listed in the following order: PH+SH (118.42%), MHR (104.64%), SH (101.67%), AH (100.31%), PH+AH (93.64%) and PH (89.47%). PH is relatively toxic to cells, especially in the PH subgroup; PH1 showed a significantly lowest % cell viability of 75.90%. SH has the potential to reduce the toxicity of PH.

Correlation analysis, most major compounds of PH were positively correlated with anti-inflammatory effects. PH revealed nine major compounds, including bergenin, chlorogenic acid, lourierin A, *O*-methyllaloptaeroxyrin, pectolinarigenin, perforatic acid, peucenin-7-methyl ether, resveratrol, and TT01. The TT01, resveratrol, lourierin A are major compounds in PH and demonstrated high anti-inflammatory activity, but TT01 and resveratrol showed a negative correlation to cell viability, while only lourierin A showed a positive correlation to cell viability.

This study indicates that only primary herbs exhibit a direct anti-inflammatory activity, while adjunct herbs and supportive herbs may reduce the effects of primary herbs. Increasing the number or ratio of adjunct and supportive herbs might reduce the activity of the primary herbs. In contrast, adjunct herbs may support other effects of the remedy, as well as, supportive herbs tend to reduce the toxicity of primary herbs.

The data obtained from this study aligns with the formulation theory of Thai traditional polyherbal medicine. However, the quantity and ratio of herbs within each group should be carefully adjusted to ensure the primary herbs's efficacy is maintained.

Keyword : Thai traditional drug, Polyherbal medicine, Mo-Ha-Rak, Anti-inflammatory, Cytotoxicity

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TABLE OF CONTENTS

Page

ABSTRACT ..... D

ACKNOWLEDGEMENTS ..... F

TABLE OF CONTENTS ..... G

LIST OF TABLES ..... O

LIST OF FIGURES ..... Q

CHAPTER I 1

INTRODUCTION 1

1.1 Background 1

1.2 Objectives 3

1.3 Hypothesis 4

1.4 Outcomes 4

1.5 Scopes of the study 4

1.6 Definition of specific terms and abbreviations 6

CHAPTER II 8

LITERATURE REVIEWS 8

2.1 Formulation Theory of Thai traditional preparation (polyherbal medicine) 8

2.1.1 Structure of Thai traditional preparation 8

2.1.1.1 The primary herbs 8

2.1.1.2 The adjunct herbs 9

2.1.1.3 The supportive herbs 9

2.1.1.4 The flavoring herbs 9

2.1.2 Formulation of Thai traditional preparation 9

2.1.2.1 Etiology in Thai traditional medicine 9

2.1.2.2 Herbal tastes of single herbs 20

2.1.2.3 Herbal tastes of remedy 22

2.1.2.4 Herbal taste for treating elemental imbalance 22
2.2 Fever 23
2.2.1 Fever from the perspective of Thai traditional medicine 23
2.2.1.1 Etiology of fever (Samutthān) 23
2.2.1.2 Pathology of fever 26
2.2.1.3 Therapy 31
2.2.2 Fever from the perspective of modern medicine 31
2.2.2.1 Definition and pathogenesis of fever 31
2.2.2.2 Inflammation and fever 32
2.2.2.3 Nitric oxide and fever 33
2.2.2.4 Therapy 34
2.3 MHR remedy 36
2.3.1 Definition 36
2.3.2 Herbal component of MHR remedy 38
2.3.2.1 Azadirachta indica A.Juss. 38
2.3.2.2 Bridelia ovata Decne. 40
2.3.2.3 Capparis micracantha DC. 41
2.3.2.4 Cassia fistula L. 43
2.3.2.5 Chan Khao 45
2.3.2.6 Clerodendrum indicum (L.) Kuntze 49
2.3.2.7 Dracaena cochinchinensis (Lour.) S.C.Chen 51
2.3.2.8 Ficus racemosa L. 53
2.3.2.9 Gymnopetalum chinense (Lour.) Merr. 55
2.3.2.10 Harrisonia erforate (Blanco) Merr. 56
2.3.2.11 Ligusticum sinense Oliv. cv. Chuanxiong 58
2.3.2.12 Mesua ferrea L. 60
2.3.2.13 Nelumbo nucifera Gaertn. 62
2.3.2.14 Phyllanthus emblica L. 64
2.3.2.15 Pinus kesiya Royle ex Gordon 67

2.3.2.16 Samo Thet 69
2.3.2.17 Terminalia bellirica (Gaertn.) Roxb. 71
2.3.2.18 Terminalia chebula Retz. 73
2.3.2.19 Tiliacora triandra (Colebr.) Diels 75
2.3.2.20 Tinospora crispa (L.) Hook. f. & Thomson 77
2.3.2.21 Vetiveria zizanioides (L.) Nash 80
2.4 Biological activities of plant components in MHR remedy 81
2.5 Related research 85
CHAPTER III 88
MATERIALS AND METHODS 88
3.1 Materials 88
3.1.1 Plant materials 88
3.1.1.1 Source of plant materials 88
3.1.1.2 Plant identification 89
3.1.2 Chemicals and reagents 92
3.1.3 Instruments and equipment 94
3.2 Methods 97
3.2.1 Experimental design 97
3.2.1.1 Formula analysis of MHR remedy 97
3.2.1.2 Experimental design 102
3.2.2 Quality control of plant materials 115
3.2.2.1 Physical determination 115
3.2.2.2 Extractive value 115
1) Ethanol-soluble extractive 115
2) Water-soluble extractive 116
3.2.4 Preparation of crude extracts 116
3.2.4.1 Maceration 116
3.2.4.2 Decoction 116
3.2.5 Anti-inflammatory and cytotoxicity activities 117

3.2.5.1 Animal cell lines 117
3.2.5.2 Preparation of sample solution 117
3.2.5.3 Assay for nitric oxide (NO) inhibitory effects in RAW 264.7 cells (Tewtrakul and Itharat, 2007) 117
3.2.5.4 Cytotoxicity by MTT assay (Tewtrakul and Itharat, 2007) 118
3.2.6 Antioxidant activities 119
3.2.6.1 Preparation of sample solution 119
3.2.6.2 Assay for nitric oxide free radical scavenging activity (Marcocci *et al*., 1994; Aktas *et al*., 2013) 119
3.2.6.3 Assay for super oxide free radical scavenging activity (Jaikong, 2021) 120
3.2.7 Characterization and identification of chemical marker by HPLC 120
3.2.7.1 Identification of original formula of MHR and modified MHR formulas 120
3.2.7.2 Identification of the component of MHR 121
3.2.7.3 Identification of the chromatographic peak 121
3.2.8 Characterization and identification of chemical marker by TLC 122
3.2.9 Phytochemical studies and isolation of chemical constituents of MHR 122
3.2.9.1 Isolation of perforatic acid 122
3.2.9.2 Isolation of O-methyllaloptaeroxyrin 123
3.2.9.3 Isolation of peucenin-7-methyl ether 123
3.2.10 Statistical analysis 124
CHAPTER IV 125
RESULTS 125
4.1 Standardization of MHR remedy 125
4.1.1 Quality control of herbal components of MHR 125
4.1.2 Characterization and identification of chemical marker by High-Performance Liquid Chromatograph (HPLC) 130
4.1.2.1 Identification of original MHR and its components 130
4.1.2.2 Peak identification in HPLC chromatogram of MHR 136

4.1.3 Characterization and identification of chemical marker by Thin-Layer Chromatography (TLC) 149
4.1.3.1 Identification of original MHR and its components 149
4.1.3.2 Identification of original MHR and modified MHR of primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs 151
4.1.2.3 Chemical marker identification in TLC chromatogram 153
4.2 Phytochemical studies of some components of MHR 162
4.2.1 Perforatic acid 162
4.2.2 *O*-methyllaloptaeroxyrin 164
4.2.3 Peucenin-7-methyl ether 166
4.3 Extractive value 168
4.4 Establishment of HPLC fingerprints of MHR 172
4.4.1 The ethanolic extract 172
4.4.1.1 The qualitative analysis 172
4.4.1.2 The quantitative analysis of major components in MHR 180
4.4.2 The aqueous extract 190
4.4.2.1 The qualitative analysis 190
4.4.2.2 The quantitative analysis of major components in MHR 198
4.5 Anti-inflammatory activity and toxicity of original MHR and modified MHR 205
4.5.1 Anti-inflammatory activity of original MHR and modified MHR of primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs 205
4.5.1.1 Anti-inflammatory activity and toxicity of original MHR and modified MHR ethanolic extract 205
4.5.1.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract 209
4.5.2 Anti-inflammatory activity and toxicity of primary herbs 211
4.5.2.1 Anti-inflammatory activity and toxicity of primary herbs ethanolic extract 211

4.5.2.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract by modification of herbs in primary herbs 219
4.5.3 Anti-inflammatory activity and toxicity of adjunct herbs 221
4.5.3.1 Anti-inflammatory activity and toxicity of adjunct herbs ethanolic extract 221
4.5.3.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract by modification of adjunct herbs 226
4.5.4 Anti-inflammatory activity and toxicity of original MHR and modified MHR by modification of supportive herbs.....228
4.5.4.1 Anti-inflammatory activity and toxicity of original MHR and modified MHR ethanolic extract by modification of supportive herbs.....228
4.5.4.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract by modification of supportive herbs.....231
4.5.5 Anti-inflammatory activity and toxicity of major compounds of MHR 232
4.6 Antioxidant activity of original MHR and modified MHR 235
4.6.1 Antioxidant activity of original MHR and modified MHR (primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs).....235
4.6.1.1 Antioxidant activity of original MHR and modified MHR ethanolic extract.....235
4.6.1.2 Antioxidant activity of original MHR and modified MHR aqueous extract 238
4.6.2 Antioxidant activity of primary herbs 241
4.6.2.1 Antioxidant activity of primary herbs ethanolic extract 241
4.6.2.2 Antioxidant activity of primary herbs aqueous extract.....247
4.6.3 Antioxidant activity of adjunct herbs 251
4.6.3.1 Antioxidant activity of adjunct herbs ethanolic extract.....251
4.6.3.2 Antioxidant activity of original MHR and modified MHR aqueous extract by modification of adjunct herbs 254

4.6.4 Antioxidant activity of original MHR and modified MHR by modification of supportive herbs 257
4.6.4.1 Antioxidant activity of original MHR and modified MHR ethanolic extract by modification of supportive herbs 257
4.6.4.2 Antioxidant activity of original MHR and modified MHR aqueous extract by modification of supportive herbs 259
4.6.5 Antioxidant activity of major compounds of MHR.....261
4.7 Investigation of the relationship between experimental parameters.....265
4.7.1 Relationship between pharmacological effects 265
4.7.2 Relationship between chemical markers 268
4.7.3 Relationship between pharmacological effects and chemical markers ..272
4.7.3.1 Relationship between pharmacological effects and chemical markers in the primary herbs 272
4.7.3.2 Relationship between pharmacological effects and chemical markers in the adjunct herbs 279
4.7.3.3 Relationship between pharmacological effects and chemical markers in the supportive herbs 285
CHAPTER V 289
CONCLUSIONS, DISCUSSIONS AND SUGGESTIONS 289
5.1 Conclusions.....290
5.2 Discussions 293
5.2.1 General discussions 293
5.2.1.1 Quality control of herbal components of MHR.....293
5.2.1.2 Characterization and identification of chemical marker 293
5.2.1.3 O-methyllaloptaeroxyrin and peucenin-7-methyl ether were identified in the HPLC chromatogram of aqueous extract 294
5.2.1.4 Models for pharmacological study 295
5.2.1.5 Selection of solvent extraction 296
5.2.2 Validation of the formulation theory for Thai traditional polyherbal medicine 297
5.2.2.1 The anti-inflammatory effect of each herbal group and their mixture of original and modified MHR.....297

5.2.2.2 The antioxidant activity of each herbal group and their mixture of original and modified MHR 301
5.2.2.3 The toxicity of each herbal group and their mixture of original and modified MHR 302
5.3 Suggestions 303
REFERENCES 304
APPENDIX 322
Appendix A 323
Biological activities of plant components in Mo-Ha-Rak remedy 323
Appendix B 373
HPLC chromatograms of the Mo-Ha-Rak component ethanolic extract 373
Appendix C 385
HPLC chromatograms of the Mo-Ha-Rak component aqueous extract 385
Appendix D 397
TLC data of Mo-Ha-Rak, its plant components and modified Mo-Ha-Rak 397
Appendix E 410
NMR spectra data 410
BIOGRAPHY 418

LIST OF TABLES

Page

Table 1 Comparison of some types of Khai-pit with modern medicine. 27

Table 2 Comparison of some types of Khai-kan with modern medicine.....29

Table 3 Herbal component of MHR remedy. 37

Table 4 Summary of biological activities of plant components in MHR remedy.....83

Table 5 Source of plant materials and reference voucher specimens. 90

Table 6 Sources of *Azadirachta indica* were purchased from traditional pharmacies in Thailand. 91

Table 7 Formula analysis of MHR remedy. 100

Table 8 Experimental design to decrease the number of herbs in various factors. ...109

Table 9 The extractive value of plant materials of MHR compared to the specification in THP or other references. 126

Table 10 The extractive value of Azadirachta indica petiole from fifteen traditional pharmacies. 129

Table 11 Selected sources of plant materials for the preparation of original MHR and modified MHR. 130

Table 12 The mobile phase for HPLC analysis of MHR ethanolic extract and its components. 131

Table 13 The mobile phase for HPLC analysis of MHR aqueous extract and its components. 134

Table 14 The major compounds in the HPLC chromatogram of MHR ethanolic extract. 139

Table 15 The major compounds in the HPLC chromatogram of MHR aqueous extract. 145

Table 16 The NMR data of perforatic acid. 163

Table 17 The NMR data of O-methyllaloptaeroxyrin. 165

Table 18 The NMR data of peucenin-7-methyl ether. 167

Table 19 Anti-inflammatory activity of the major compounds in MHR 233

Table 20 Nitric oxide radical scavenging activity of the major compounds in MHR. 263

Table 21 Superoxide radical scavenging activity of the major compounds in MHR.264

Table 22 Rf value (System I) of MHR compared with herbal components and standard chemicals, detected under UV 254 nm. 398

Table 23 Rf value (System I) of MHR compared with herbal components, modified Mo-Ha-Rak and standard chemicals, detected under UV 254 nm. 399

Table 24 Rf value (System I) of MHR compared with herbal components and standard chemicals, detected under UV 366 nm. 400

Table 25 Rf value (System I) of MHR compared with herbal components, modified MHR and standard chemicals, detected under UV 366 nm. 401

Table 26 Rf value (System I) of MHR compared with herbal components and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm. .402

Table 27 Rf value (System I) of MHR compared with herbal components, modified Mo-Ha-Rak and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm. 403

Table 28 Rf value (System II) of MHR compared with herbal components and standard chemicals, detected under UV 254 nm. 404

*T*able 29 Rf value (System II) of MHR compared with herbal components, modified MHR and standard chemicals, detected under UV 254 nm. 405

Table 30 Rf value (System II) of MHR compared with herbal components and standard chemicals, detected under UV 366 nm. 406

Table 31 Rf value (System II) of MHR compared with herbal components, modified MHR and standard chemicals, detected under UV 366 nm. 407

Table 32 Rf value (System II) of MHR compared with herbal components and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm. .408

Table 33 Rf value (System II) of MHR compared with herbal components, modified MHR and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm 409

LIST OF FIGURES

Page

Figure 1 Conceptual framework of a thesis. 5

Figure 2 Petiole of *Azadirachta indica* A.Juss 38

Figure 3 Leaf of *Bridelia ovata* Decne. 40

Figure 4 Root of *Capparis micracantha* DC. 42

Figure 5 Pulp of *Cassia fistula* L. 43

Figure 6 Wood of Chan Khao from different sources (a) Bangkok and (b) Nakhon Pathom. 45

Figure 7 Root of *Clerodendrum indicum* (L.) Kuntze 49

Figure 8 Wood of *Dracaena cochinchinensis* (Lour.) S.C.Chen 51

Figure 9 Root of *Ficus racemosa* L. 53

Figure 10 Fruit of *Gymnopetalum chinense* (Lour.) Merr. 55

Figure 11 Root of *Harrisonia erforate* (Blanco) Merr. 56

Figure 12 Rhizome of *Ligusticum sinense* Oliv. cv. *Chuanxiong* 58

Figure 13 Flower of *Mesua ferrea* L. 60

Figure 14 Stamen of *Nelumbo nucifera* Gaertn. 62

Figure 15 Fruit of *Phyllanthus emblica* L. 64

Figure 16 Wood of *Pinus kesiya* Royle ex Gordon 67

Figure 17 Fruit of Samo Thet 69

Figure 18 Fruit of *Terminalia bellirica* (Gaertn.) Roxb. 71

Figure 19 Fruit of *Terminalia chebula* Retz. 73

Figure 20 Root of *Tiliacora triandra* (Colebr.) Diels 75

Figure 21 Stem of *Tinospora crispa* (L.) Hook. f. & Thomson 77

Figure 22 Root of *Vetiveria zizanioides* (L.) Nash 80

Figure 23 HPLC chromatograms of MHR ethanolic extract (FG01E) detected at wavelengths 254 nm (a) and 280 nm (b). 133

Figure 24 HPLC chromatograms of MHR aqueous extract (FG01A) detected at wavelengths 254 nm (a) and 280 nm (b). 136

Figure 25 Identification of chromatographic peaks for ethanolic extract of MHR (FG01E) detected at wavelengths 254 nm (a) and 280 nm (b). 138

Figure 26 The chemical assignments in HPLC chromatogram for ethanolic extract of MHR (FG01E) detected at wavelengths 254 nm (a) and 280 nm (b). 139

Figure 27 The UV spectrum of major compounds in MHR ethanolic extract. 141

Figure 28 The chemical structure of major compounds in MHR ethanolic extract. . 142

Figure 29 HPLC chromatograms of MHR aqueous extract (FG01A) detected at wavelengths 254 nm (a) and 280 nm (b). 144

Figure 30 The chemical assignments in HPLC chromatogram of MHR aqueous extract (FG01A) detected at wavelengths 254 nm (a) and 280 nm (b). 145

Figure 31 The UV spectrum of major compounds in MHR aqueous extract. 147

Figure 32 The chemical structure of MHR aqueous extract. 148

Figure 33 TLC chromatograms of MHR and its plant components compared to chemical markers. 154

Figure 34 TLC chromatograms of MHR, its plant components and modified MHR compared to chemical markers. 156

Figure 35 TLC chromatograms of MHR and its plant components compared to chemical markers. 158

Figure 36 TLC chromatograms of MHR, its plant components and modified MHR compared to chemical markers. 160

Figure 37 The chemical structure of perforatic acid. 162

Figure 38 The chemical structure of O-methyllaloptaeroxyrin. 164

Figure 39 HMBC (a) and ^{1}H-^{1}H COSY (b) correlations of O-methyllaloptaeroxyrin. 165

Figure 40 The chemical structure of peucenin-7-methyl ether. 166

Figure 41 HMBC (a) and COSY (b) correlations of peucenin-7-methyl ether. 167

Figure 42 The percentage yields of the ethanolic and aqueous extracts of the original MHR and each modified MHR. 169

Figure 43 HPLC chromatogram of original MHR (01E) and modified MHR remedies, without adjunct and supportive herbs (02E), without supportive herbs (03E), only adjunct herbs (91E), without adjunct herbs (10E), and only supportive herbs (92E). 173

Figure 44 HPLC chromatogram of original MHR (01E) and modified MHR remedies by fixing reduced toxic fever herbs with one of the other components (45E-60E)...174

Figure 45 HPLC chromatogram of modified MHR showing only the modifications of primary herbs (31E-51E) compared to primary herbs (02E). 175

Figure 46 HPLC chromatogram of modified MHR showing reduced toxic herbs (33E) and their modified reduced toxic herbs by removing 4 herbs (61E-65E) and 3 herbs (66E-75E).....176

Figure 47 HPLC chromatogram of modified MHR showing reduced toxic herbs (33E) and their modified reduced toxic herbs by removing 2 herbs (76E-90E).....177

Figure 48 HPLC chromatogram of modified MHR by modification of adjunct herbs. 178

Figure 49 HPLC chromatogram of modified MHR by modification of adjunct herbs, focus on removing the number of sour-astringent laxative herbs. 179

Figure 50 HPLC chromatogram of modified MHR by modification of supportive herbs. 180

Figure 51 Peak area of gallic acid in modified MHR remedies. 183

Figure 52 Peak area of protocatechuic acid in modified MHR remedies. 183

Figure 53 Peak area of chebulanin in modified MHR remedies. 184

Figure 54 Peak area of corilagin in modified MHR remedies. 184

Figure 55 Peak area of chebulagic acid in modified MHR remedies. 185

Figure 56 Peak area of ellagic acid in modified MHR remedies. 185

Figure 57 Peak area of perforatic acid in modified MHR remedies. 186

Figure 58 Peak area of O-methyllaloptaeroxyrin in modified MHR remedies. 186

Figure 59 Peak area of rhein in modified MHR remedies. 187

Figure 60 Peak area of loureirin A in modified MHR remedies. 187

Figure 61 Peak area of peucenin-7-methyl ether in modified MHR remedies. 188

Figure 62 Peak area of PK02 in modified MHR remedies. 188

Figure 63 Peak area of GC01 in modified MHR remedies. 189

Figure 64 Peak area of MF02 in modified MHR remedies. 189

Figure 65 Peak area of GC04 in modified MHR remedies. 190

Figure 66 HPLC chromatogram of original MHR (01A) and modified MHR remedies, without adjunct and supportive herbs (02A), without supportive herbs (03A), only

adjunct herbs (91A), without adjunct herbs (10A), and only supportive herbs (92A). 191

Figure 67 HPLC chromatogram of original MHR (01A) and modified MHR remedies by fixing reduced toxic fever herbs with one of the other components (45A-60A). .192

Figure 68 HPLC chromatogram of modified MHR showing only the modifications of primary herbs (31E-51A) compared to primary herbs (02A). 193

Figure 69 HPLC chromatogram of modified MHR showing reduced toxic herbs (33A) and their modified reduced toxic herbs by removing 4 herbs (61A-65A) and 3 herbs (66A-75A). 194

Figure 70 HPLC chromatogram of modified MHR showing reduced toxic herbs (33A) and their modified reduced toxic herbs by removing 2 herbs (76A-90A). 194

Figure 71 HPLC chromatogram of modified MHR by modification of adjunct herbs. 196

Figure 72 HPLC chromatogram of modified MHR by modification of adjunct herbs, focus on removing the number of sour-astringent laxative herbs. 196

Figure 73 HPLC chromatogram of modified MHR by modification of supportive herbs. 197

Figure 74 Peak area of chebulic acid in modified MHR remedies. 199

Figure 75 Peak area of gallic acid in modified MHR remedies. 200

Figure 76 Peak area of protocatechuic acid in modified MHR remedies. 200

Figure 77 Peak area of FR02 in modified MHR remedies. 201

Figure 78 Peak area of chebulanin in modified MHR remedies. 201

Figure 79 Peak area of corilagin in modified MHR remedies. 202

Figure 80 Peak area of chebulagic acid in modified MHR remedies. 202

Figure 81 Peak area of ellagic acid in modified MHR remedies. 203

Figure 82 Peak area of perforatic acid in modified MHR remedies. 203

Figure 83 Peak area of O-methyllaloptaeroxyrin in modified MHR remedies. 204

Figure 84 Peak area of rhein in modified MHR remedies. 204

Figure 85 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01E) and modified MHR (Primary herbs (02E), without supportive herbs (03E), adjunct herbs (91E), without adjunct herbs (10E) and supportive herbs (92E)). 206

Figure 86 Comparison of nitric oxide inhibition (A) and cell viability (B) of original MHR (01E) and modified MHR (Primary herbs (02E), without supportive herbs (03E), adjunct herbs (91E), without adjunct herbs (10E) and supportive herbs (92E)). 207

Figure 87 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01E) and modified MHR by fixing reduced-toxic fever herbs plus one of the other components (45E-60E) compared to only one of the reduced-toxic fever herbs (61E-65E) and only reduced-toxic fever herbs (33E).....208

Figure 88 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01A) and modified MHR (Primary herbs (02A), without supportive herbs (03A), adjunct herbs (91A), without adjunct herbs (10A) and supportive herbs (92A)).....210

Figure 89 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01A) compared to only one of the reduced-toxic fever herbs (61A-65A) and only reduced-toxic fever herbs (33A). 211

Figure 90 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01E) and modified MHR (decrease one herb of group 1.4, decrease one herb of group 1.3 and decrease one and two herb of group 1.2). 214

Figure 91 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in different modifying primary herbs (formulas 01E-37E) at various concentrations. 215

Figure 92 Anti-inflammatory activity (A) and toxicity (B) of modified MHR by fixing reduced-toxic fever herbs and plus one of the primary components. 216

Figure 93 Anti-inflammatory activity (A) and toxicity (B) of modified MHR by decreasing the number of herbs in reduced-toxic fever herbs (subgroup 1.1).....218

Figure 94 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR aqueous extract by modification of herbs in primary herbs. 220

Figure 95 Anti-inflammatory activity (A) and toxicity (B) of original MHR compared to subgroup 1.1 (reduced-toxic fever herbs) and their single herbs.....221

Figure 96 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR by modification of adjunct herbs.....225

Figure 97 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in the different modifying adjunct herbs at various concentrations. 226

Figure 98 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR by modification of adjunct herbs.....227

Figure 99 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR by modification of supportive herbs.....230

Figure 100 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in the supportive herbs at various concentrations. 231

Figure 101 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR aqueous extract by modification of supportive herbs.....232

Figure 102 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in the major compounds at various concentrations. 234

Figure 103 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01E) and modified MHR (Primary herbs (02E), without supportive herbs (03E), adjunct herbs (91E), without adjunct herbs (10E) and supportive herbs (92E))....236

Figure 104 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01E) and modified MHR by fixing reduced-toxic fever herbs plus one of the other components (45E-60E) compared to only one of the reduced-toxic fever herbs (61E-65E) and only reduced-toxic fever herbs (33E). 238

Figure 105 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01A) and modified MHR (Primary herbs (02A), without supportive herbs (03A), adjunct herbs (91A), without adjunct herbs (10A) and supportive herbs (92A)). 239

Figure 106 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01A) and modified MHR by fixing reduced-toxic fever herbs plus one of the other components (45A-60A) compared to only one of the reduced-toxic fever herbs (61A-65A) and only reduced-toxic fever herbs (33A). 241

Figure 107 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of original MHR (01E) and modified MHR (decrease one herb of group 1.4, decrease one herb of group 1.3 and decrease one and two herb of group 1.2). 243

Figure 108 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of modified MHR by fixing reduced-toxic fever herbs and plus one of the primary components (45E-51E).....244

Figure 109 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of modified MHR by decreasing the number of herbs in reduced-toxic fever herbs (subgroup 1.1, 61-90E). 246

Figure 110 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of aqueous extract of original MHR (01A) and modified MHR (decrease one herb of group 1.4, decrease one herb of group 1.3 and decrease one and two herbs of group 1.2). 248

Figure 111 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of modified MHR aqueous extract by fixing reduced-toxic fever herbs and plus one of the primary components (45E-51E). 249

Figure 112 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of modified MHR aqueous extract by decreasing the number of herbs in reduced-toxic fever herbs (subgroup 1.1, 61-90A). 251

Figure 113 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of original MHR and modified MHR by modification of adjunct herbs. 254

Figure 114 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of aqueous extract of original MHR and modified MHR by modification of adjunct herbs. 256

Figure 115 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of original MHR and modified MHR by modification of supportive herbs. 258

Figure 116 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of aqueous extract of original MHR and modified MHR by modification of supportive herbs. 260

Figure 117 Comparison of nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities in the major compounds at various concentrations. 262

Figure 118 Correlation analysis between pharmacological effects. (A) Pearson's rank correlation plot, (B) PCA loading plot and (C) PCA score plot (n=92). 266

Figure 119 Heatmap cluster analysis for exploring the role of 92 modified MHR formulas in relation to their pharmacological effects. 267

Figure 120 Pearson's rank correlation analysis between chemical markers of the primary herbs (PH) and the adjunct herbs (AH). 269

Figure 121 Pearson's rank correlation analysis between chemical markers of the primary herbs (PH) and the supportive herbs (SH). 270

Figure 122 Pearson's rank correlation analysis between chemical markers of the adjunct herbs (AH) and the supportive herbs (SH). 271

Figure 123 Pearson's rank correlation analysis between pharmacological effects and chemical markers of the primary herbs (PH). 274

Figure 124 Pearson's Rho analysis between anti-inflammatory activity and chemical marker of the primary herbs (PH). 275

Figure 125 Pearson's Rho analysis between cell viability effects and chemical marker of the primary herbs (PH). 276

Figure 126 Pearson's Rho analysis between nitric oxide scavenging activity and chemical marker of the primary herbs (PH). 277

Figure 127 Pearson's Rho analysis between superoxide scavenging activity and chemical marker of the primary herbs (PH). 278

Figure 128 Pearson's rank correlation analysis between pharmacological effects and chemical markers of the adjunct herbs (AH). 280

Figure 129 Pearson's Rho analysis between anti-inflammatory activity and chemical markers of the adjunct herbs (AH). 281

Figure 130 Pearson's Rho analysis between cell viability effects and metabolites of the chemical markers of the adjunct herbs (AH). 282

Figure 131 Pearson's Rho analysis between nitric oxide scavenging activity and chemical markers of the adjunct herbs (AH). 283

Figure 132 Pearson's Rho analysis between superoxide scavenging activity and chemical markers of the adjunct herbs (AH). 284

Figure 133 Pearson's rank correlation analysis between pharmacological effects and chemical markers of the supportive herbs (SH). 286

Figure 134 Pearson's Rho analysis between anti-inflammatory activity and chemical markers of the supportive herbs (SH). 287

Figure 135 Pearson's Rho analysis between cell viability effects and the chemical markers of the supportive herbs (SH). 287

Figure 136 Pearson's Rho analysis between nitric oxide scavenging activity and chemical markers of the supportive herbs (SH). 288

Figure 137 Pearson's Rho analysis between superoxide scavenging activity and chemical markers of the supportive herbs (SH). 288

CHAPTER I
INTRODUCTION

1.1 Background

Globally, the popularity and interest in traditional medicine have been steadily growing. In many regions, a significant portion of the population still relies on their own traditional medicine for their primary healthcare needs (Che *et al*., 2017). Additionally, in numerous countries, traditional medicine is often used alongside modern medical treatments. Efforts are also underway worldwide to enhance the efficacy of traditional medicine, aiming to offer alternatives to chemical drugs in addressing widespread diseases.

Thai traditional medicine (TTM) has a long history in Thailand and was reintroduced into the country's healthcare system in 1977, in alignment with the WHO's Alma-Ata Declaration and global trends toward complementary and alternative medicine. Since then, policies promoting TTM practices in hospitals have been integrated into multiple national health development plans. However, the adoption of TTM in community hospitals progressed slowly for over two decades before experiencing a significant rise in the past decade (Thongruang, 2014).

Nowadays, TTM is recognized as an integral part of the health service system (Kongchanmitkul and Kasanit, 2019). Extensive research on herbal medicine products has since led to the broader use of single-herb remedies in public health settings. In 1999, the inclusion of herbal medicine products in the National List of Essential Medicines (NLEM) was announced, and these were later covered by the public health insurance system. Additionally, positions for Thai traditional practitioners as government officers were established, facilitating the increased use of both polyherbal and single-herbal remedies from the NLEM within the health service system (Department for Development of Thai Traditional and Alternative Medicine, 2014; Thongruang, 2014).

In Thailand, herbal medicines are classified into two categories: single-herb and polyherbal products. However, most Thai traditional practitioners favor polyherbal remedies for treating diseases. Their polyherbal remedies often contain more than 2 herbs, up to 30 or more herbs in one formula. According to the

formulation theory of Thai traditional medicine, each remedy includes four groups of herbs, each serving different functions: primary herbs (PH), adjunct herbs (AH), supportive herbs (SH), and flavoring herbs (FH). These groups are designed to address the primary symptoms and related complications (Nithetsukkit, Khun, 1973; Khrupanyamat, 2016). However, there is no clear explanation regarding the number or proportion of herbs in each group. This raises several questions, including the necessity of using such a large number of herbs, potential herb interactions, reduced efficacy of the primary herb ingredient, optimal herb ratios, preparation difficulties, and cost concerns. Additionally, some remedies include minerals and animal materials that are now difficult to source. Certain formulations also rely on rare or endangered plants, increasing production costs or making it impossible to follow the original recipe. Furthermore, some medicinal plants used in these remedies are known to be toxic. Mo-Ha-Rak (MHR) was selected as a case study for this study. The study aims to investigate the impact of removing the number of herbs in each group of MHR compared to the original MHR. The chemical composition, pharmacological activities, and toxicity of herbs in each group of modified MHR and original MHR were evaluated.

Mo-Ha-Rak (MHR) is a polyherbal medicine commonly used by Thai traditional practitioners to treat symptoms associated with severe fever (including high fever or fever with rash), drowsiness, internal heat, excessive thirst, restlessness, delirium, unconsciousness, and internal poisoning (Nualkaew, 2020). This remedy consists of 21 medicinal plants; *Azadirachta indica* A. Juss., *Bridelia ovata* Decne., *Capparis micracantha* DC., *Cassia fistula* L., *Clerodendrum indicum* (L.) Kuntze, *Dracaena cochinchinensis* (Lour.) S.C.Chen, *Ficus racemosa* L., *Gymnopetalum chinense* (Lour.) Merr., *Harrisonia perforata* (Blanco) Merr., *Ligusticum sinense* Oliv., *Mesua ferrea* L., *Nelumbo nucifera* Gaertn., *Phyllanthus emblica* L., *Pinus kesiya* Royle ex Gordon, *Tarenna hoaensis* Pit., *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* Retz., *Terminalia* sp. “Samo Thet”, *Tiliacora triandra* (Colebr.) Diels, *Tinospora crispa* (L.) Hook. f. & Thomson and *Vetiveria zizanioides* (L.) Nash.

The medicinal plants in MHR remedy are classified into three groups based on their herbal tastes and therapeutic activities. **1) Primary herbs:** These treat severe fever, hyperthermia, internal heat, and thirst. This group includes *A. indica, C.*

micracantha, *C. indicum*, *D. cochinchinensis*, *F. racemosa*, *G. chinense*, *H. perforata*, *L. sinense*, *P. kesiya*, *T. hoaensis*, *T. triandra*, and *T. crispa*. **2) Adjunct herbs**: These act as laxatives and assist in fever detoxification. This group includes *B. ovata*, *C. fistula*, *P. emblica*, *T. bellirica*, *T. chebula* and *Terminalia* sp. "Samo Thet" **3) Supportive herbs:** These function as heart tonics. This group includes *M. ferrea*, *N. nucifera*, and *V. zizanioides*.

This study aims to scientifically validate the formulation theory of polyherbal medicine by investigating the chemical profile and pharmacological activities of both modified and original formulas of MHR. Formulas with a reduced number of herbs and adjusted herb ratios within and across groups will be designed. High-performance liquid chromatography (HPLC) fingerprints will be used to determine the chemical profiles of the modified formulas, with major peaks identified through comparison with standard references. The anti-inflammatory, antioxidant, and cytotoxic activities of both the modified and original MHR formulas were evaluated. The relationship between the number and ratio of herbs, their chemical composition, pharmacological activities, and toxicity were also analyzed.

1.2 Objectives

1.2.1 Overall objectives

The overall objective of this research is to scientifically validate the formulation theory of Thai traditional polyherbal medicine by examining the chemical profile, pharmacological activities, and toxicity of both the original MHR formula and various modified formulations.

1.2.2 Specific objectives

1.2.2.1 To study the chemical profile, pharmacological activities, and toxicity of the original MHR formula and MHR without adjunct herbs, supportive herbs and without both adjunct herbs and supportive herbs.

1.2.2.2 To study the chemical profile, pharmacological activities, and toxicity of different modified MHR formulas by decreasing the number and ratio of herbs in the primary herbs.

1.2.2.3 To study the chemical profile, pharmacological activities, and toxicity of different modified MHR formulas by decreasing the number and ratio of herbs in the adjunct herbs.

1.2.2.4 To study the chemical profile, pharmacological activities, and toxicity of different modified MHR formulas by decreasing the number and ratio of herbs in the supportive herbs.

1.3 Hypothesis

1.3.1 The pharmacological activities of different modified MHR formulas and the original formula are different.

1.3.2 The toxicity of different modified MHR formulas and the original formula is different.

1.4 Outcomes

1.4.1 Understand the chemical profiles, pharmacological activities and toxicity of the original MHR formula and different modified MHR formulas.

1.4.2 Understand the relationship between the number and ratio of herbs, chemicals, pharmacological activity and toxicity in PH, AH and SH of MHR.

1.4.3 Provide deeper insights into the formulation theory of Thai traditional (polyherbal medicine).

1.4.4 Enable Thai traditional medicine students and Thai traditional practitioners to more easily comprehend the formulation theory behind Thai traditional (polyherbal medicine).

1.5 Scopes of the study

This experimental study aims to scientifically validate the formulation theory of Thai traditional polyherbal medicine, with MHR selected as the representative remedy. The chemical profiles, pharmacological activities, and toxicity of both the original MHR formula and various modified formulas were analyzed. Formulas with a reduced number of herbs and adjusted ratios within the PH, AH and SH were designed. HPLC fingerprints were used to determine the chemical profiles. The relationship between the number and ratio of herbs, pharmacological activities, and toxicity were also explored. The research was conducted at the Faculty of Pharmacy,

Mahasarakham University, Maha Sarakham Province, Thailand. The conceptual framework for this study is illustrated in **Figure 1**.

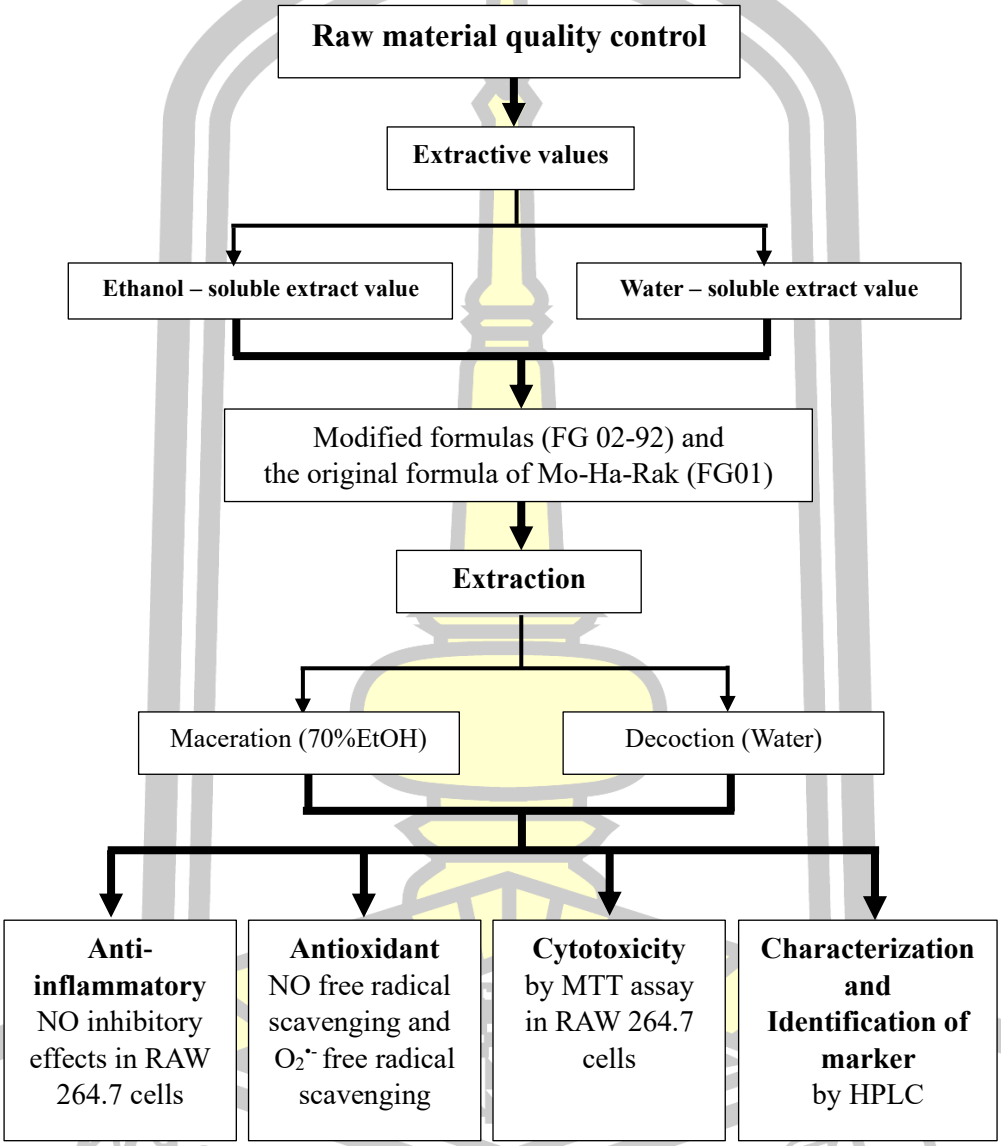

Figure 1 Conceptual framework of a thesis.

1.6 Definition of specific terms and abbreviations

Abbreviation	Term
%	Percent
>	More than
≥	More than or equal
<	Less than
≤	Less than or equal
=	Equal
+	Plus
^{o}C	Degree celsius
α	Alpha
β	Beta
µg	Microgram
µg/mL	Microgram per milliliter
µm	Micrometer
µM	Micromolar
µL	Microliter
AH	Adjunct herbs
AH1	Stimulant laxative herbs
AH2	Sour-astringent laxative herbs
AI	*Azadirachta indica* A. Juss.
BO	*Bridelia ovata* Decne.
CF	*Cassia fistula* L.
CI	*Clerodendrum indicum* (L.) Kuntze.
CM	*Capparis micracantha* DC.
CO_2	Carbon dioxide
DC	*Dracaena cochinchinensis* (Lour.) S.C.Chen.
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethylsulfoxide
FBS	Fetal bovine serum
FR	*Ficus racemosa* L.
GC	*Gymnopetalum chinense* (Lour.) Merr.
h	Hour
HP	*Harrisonia perforata* (Blanco) Merr.
HPLC	High-performance liquid chromatography
IC_{50}	Concentration causing 50% inhibition effect
LPS	Lipopolysaccharide
LS	*Ligusticum sinense* Oliv.
M	Molar (concentration)
mAU	Milli-absorbance unit

Abbreviation	Term
min	Minute
MF	*Mesua ferrea* L.
MHR	Original of Mo-Ha-Rak remedy
MTT	Thiazolyl blue tetrazolium bromide or 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
NBT	Nitro tetrazolium blue chloride
nm	Nanometer
NN	*Nelumbo nucifera* Gaertn.
NO	Nitric oxide
$O_2^{\bullet-}$	Superoxide anion
PBS	Phosphate buffer saline
PE	*Phyllanthus emblica* L.
pH	Potential of hydrogen ion
PH	Primary herbs
PH1	Reduced-toxic fever herbs
PH2	Anti-kamdao and lohit fever herbs
PH3	Anti-di fever herbs
PH4	Anti-semha and lom fever herbs
PK	*Pinus kesiya* Royle ex Gordon.
RAW 264.7	A macrophage cell line that was established from a tumor in a male mouse induced with the Abelson murine leukemia virus
ROS	Reactive oxygen species
RT	Retention time
SD	Standard deviation
SEM	Standard error of mean
SH	Supportive herbs
TB	*Terminalia bellirica* (Gaertn.) Roxb.
TC	*Tinospora crispa* (L.) Hook. f. & Thomson.
TCb	*Terminalia chebula* Retz.
TH	*Tarenna hoaensis* Pit.
THP	Thai Herbal Pharmacopoeia
TLC	Thin-layer chromatography
Ts	*Terminalia* sp. “Samo Thet”
TT	*Tiliacora triandra* (Colebr.) Diels.
VZ	*Vetiveria zizanioides* (L.) Nash.

CHAPTER II
LITERATURE REVIEWS

2.1 Formulation Theory of Thai traditional preparation (polyherbal medicine)

Thai traditional preparation is classified into two categories: single-herb and polyherbal medicine. However, most Thai traditional practitioners predominantly use polyherbal remedies for treating diseases. These formulas often contain more than two herbs, with some having up to 30 or more. Despite their widespread use, there is little detailed information about the formulation theory behind these remedies. The *Ayurvedhsuksa* textbook provides only a brief explanation of their structure. According to traditional practices, practitioners must first understand the etiology and symptoms of the disease before diagnosing and prescribing treatment. The structure of Thai traditional remedies is typically composed of four groups of herbs, each serving different functions: primary herbs (PH), adjunct herbs (AH), supportive herbs (SH), and flavoring herbs (FH). These groups are designed to address the primary symptoms as well as complications related to the disease (Nithetsukkit, Khun, 1973; Khrupanyamat, 2016). However, there is no detailed guidance on the specific number or proportion of herbs in each group.

2.1.1 Structure of Thai traditional preparation

Thai traditional remedies typically consist of four groups of herbs: primary herbs, adjunct herbs, supportive herbs and flavoring herbs. However, not every remedy includes all four groups; the composition depends on the complexity of the disease and the patient's condition. For less complicated ailments, a remedy may contain only the primary herbs.

2.1.1.1 The primary herbs

The herbs in this group are primarily used to address the main symptoms or root cause of the disease, or the primary health issue affecting the patient (Nithetsukkit, Khun, 1973; Khrupanyamat, 2016). Some textbooks suggest that the total weight of the primary herbs should be approximately double that of the adjunct herbs (Wutithamawech, 2011).

2.1.1.2 The adjunct herbs

The herbs in this group are used to treat secondary symptoms or to enhance the effectiveness of the primary herbs (Nithetsukkit, Khun, 1973). The activity of the adjunct herbs must not diminish the efficacy of the primary herbs (Wutithamawech, 2011; Khrupanyamat, 2016). Some textbooks recommend that the total weight of the adjunct herbs should be about half that of the primary herbs (Wutithamawech, 2011).

2.1.1.3 The supportive herbs

The herbs in this group are used to support the primary herbs, reduce potential side effects, prevent disease complications, or serve as a tonic (Nithetsukkit, Khun, 1973). Some textbooks suggest that the total weight of the supportive herbs should be half or one-quarter of the primary herbs, depending on the severity of the disease (Wutithamawech, 2011).

2.1.1.4 The flavoring herbs

The herbs in this group are used to enhance the flavor, aroma, and color of the remedy (Nithetsukkit, Khun, 1973). These herbs may be omitted if the remedy's taste is already satisfactory. Some textbooks recommend using flavoring herbs in an amount equal to half the total weight of the primary herbs (Wutithamawech, 2011).

2.1.2 Formulation of Thai traditional preparation

Formulation of Thai traditional drugs requires basic knowledge of Thai traditional medicine, including Thai traditional etiology (elements, seasons, age, time), herbal taste, remedy taste and the relationship between element imbalance and herbal taste. The specifics of each topic are outlined below.

2.1.2.1 Etiology in Thai traditional medicine

Thai Traditional medicine posits that diseases arise from imbalances in the four elements: earth, water, wind, and fire. When these elements are out of balance, the body falls into a state of disharmony. Therefore, treatments focus on restoring balance to the body. The etiology in Thai traditional medicine includes *thāt* (ธาตุ: the elements), *utu* (อุตุ: the seasons), *āyu* (อายุ: age), and *kāla* (กาล: time) are

considered the primary *samutthān* (สมุฏฐาน: causal factors or etiology) of disease. Other significant factors include *prathēt* (ประเทศ: place), and *mūnhēt* (มูลเหตุ: causes) arising from human actions and behavior (Traditional Thai Medicine Rehabilitation Foundation, 2015).

1) The elements

The human body is believed to be composed of four elements: *patthawi samutthān* (ปฐวีสมุฏฐาน) or *thāt din* (ธาตุดิน: the earth element), *āpo samutthān* (อาโปสมุฏฐาน) or *thāt nam* (ธาตุน้ำ: the water element), *wāyo samutthān* (วาโยสมุฏฐาน) or *thāt lom* (ธาตุลม: the wind element), and *tēchō samutthān* (เตโชสมุฏฐาน) or *thāt fai* (ธาตุไฟ: the fire element).

1.1) The earth element

The earth element or thāt din (ธาตุดิน) is composed of twenty sub-elements:

- Kesā (เกศา: the hair): Examples of hair disorders include scalp pain, hair loss, and premature greying.

- Lomā (โลมา: the body hair): Examples of body hair disorders include skin sensitivity or pain and hair loss.

- Nakhā (นขา: the nails): Examples of nail disorders include pain at the nail base, sometimes accompanied by inflammation and pus, and in some cases, nail loss.

- Thantā (ทันตา: the teeth): Examples of dental disorders include tooth root pain, cavities, pyorrhea (gum disease), and abscesses.

- Tacho (ตะโจ: the skin): Examples of skin disorders include itching, roughness or irritation to the touch, and stinging pain.

- Mangsang (มังสัง: the muscle): Examples of muscle disorders include the development of bruised, red patches, burning or stinging pain, as well as the appearance of moles and warts.

- Nahāru (นหารู: the tendons): Examples of tendon disorders include a sensation of constriction in the chest, leading to restlessness, weakness, and increased hunger.

- Atthi (อัฐิ: the bones): Examples of bone disorders include pain in the bones.

- Atthiminchang (อัฐิมิญชัง: tissue in the bones): Examples of bone marrow disorders include bone pain that can lead to the solidification of oil into fat, resulting in symptoms of beriberi

- Wakkang (วักกัง: the spleen): Examples of spleen disorders include alternating sensations of heat and cold, which can lead to conditions such as *krasai lom* (กระษัยลม) and splenic pain.

- Hatthayang (หทยัง: the heart): Examples of heart disorders include irritability, sensitivity, a short temper, and increased hunger.

- Yakanang (ยักนัง: the liver): Examples of liver disorders include liver enlargement, downward displacement of the liver, and painful symptoms associated with liver abscesses.

- Kilomakang (กิโลมกัง: fascia or connective tissue): Examples of fascia or connective tissue disorders include sensations of dehydration and thirst, as well as conditions such as hemorrhoids.

- Pihakang (ปิหกัง: the kidneys): Examples of kidney disorders include obstruction and congestion in the chest, leading to stomach distention, feelings of exhaustion and weakness, and puffiness.

- Papphāsang (ปัปผาสัง: the lungs): Examples of lung disorders include thirst, heartburn, and difficulty breathing.

- Antang (อันตัง: the large intestine): There are two sections: the upper part, which includes the stomach, and the lower part, extending from the small intestine to the rectum. Malfunctioning of this system can lead to weakness, as well as a feeling of fullness and bowel contraction.

- Antakhunang (อันตคุนัง: the small intestine): Examples of small intestine disorders include belching, yawning, the presence of blood and pus in the feces, faintness, aching muscles in the waist region, gripping pain on both sides and a burning sensation in the stomach and throat.

- Utthariyang (อุทริยัง: undigested food): Examples of undigested food disorders include diarrhea, colicky pain, dry retching, and hiccups.

- Krisang (กรีสัง: digested food): Examples of digested food disorders include irregular bowel movements, which can lead to hemorrhoids.

- Matthakematthalungkhang (มัตถเกมัตถลุงคัง: the brain): This substance is found in the skull and spinal cord. Disorders of the brain can lead to symptoms such as headaches, deafness, and stiffness in the tongue and jaw.

1.2) The water element

The water element or thāt nam (ธาตุน้ำ) consists of twelve sub-elements:

- Pittang (ปิตตัง: bile): This can be divided into two types: *phatthapitta* (พัทธะปิตตะ), referring to bile in the gallbladder, and *aphatthapitta* (อพัทธะปิตตะ), which refers to bile outside the gallbladder that flows into the intestines, secreted by the liver. Abnormal bile in the gallbladder can lead to symptoms of delirium, while bile outside the gallbladder may cause headaches, elevated body temperature, alternating sensations of heat and cold, jaundiced eyes and urine, as well as fever.

- Semhang (เสมหัง: mucus): This can be divided into three types: *sosemha* (ศอเสมหะ), referring to mucus in the throat; *urasemha* (อุระเสมหะ), the mucus in the windpipe; and *khutthasemha* (คูถเสมหะ), the mucus originating from the rectum. Disorders related to mucus in the throat may present as a sore throat, dry throat, or asthma. Mucus disorders in the windpipe can lead to wasting, jaundice, piercing chest pain, and dryness in the chest. Lastly, disorders related to rectal mucus can result in the presence of mucus and blood in the feces, as seen in dysentery.

- Puppho (ปุพโพ: pus): Examples of pus disorders include coughing, loss of appetite, and weight loss.

- Lohitang (โลหิตัง: blood): Examples of blood disorders include fever, which can lead to delirium, red urine, skin spots, and black and red patches, as well as diseases such as bubonic plague.

- Setho (เสโท: perspiration): Examples of perspiration disorders include restlessness, chills, weakness, exhaustion, and depression.

- Metho (เมโท: body fat): Examples of body fat disorders include the formation of patches on the skin that cause burning and stinging sensations, along with fluid exudation.

- Atsu (อัสสุ: tears): Examples of tear disorders include blurred vision, watery eyes, and conditions such as corneal opacity or corneal ulceration.

- Wasā (วสา: lymph): Examples of lymph disorders include jaundiced skin and eyes, as well as diarrhea.

- Khelo (เขโฬ: saliva): Examples of saliva disorders include a sore throat and the presence of pustules on the throat and at the base of the tongue.

- Singkhanikā (สิงฆานิกา: clear mucus): Examples of clear mucus disorders include changes in nasal mucus that can lead to pain within the skull, blurred vision, and nasal discharge.

- Lasikā (ลสิกา: joint fluid): Examples of joint fluid disorders include pain in the joints and within the bones.

- Muttang (มุตตัง: urine): Examples of urine disorders include changes in urine color, as well as a sharp pain in the urethra and bladder.

1.3) The air element

The air element or *thāt lom* (ธาตุลม) comprises six sub-elements:

- Utthangkhamāwātā (อุทธังคมาวาตา): The air which originates from the feet and rises to the head, or, as some suggest, begins in the stomach and ascends to the throat, as seen in yawning or belching. Disorders related

to this can result in symptoms such as restless hands and feet, abdominal discomfort, a sensation of heat in the stomach, frequent yawning and belching, and flatulence caused by mucus.

- Athokhamāwātā (อโธคมาวาตา): The air which originates from the head and descends to the feet, or, as some suggest, starts in the small intestine and moves down to the rectum, as seen in the act of passing gas. Disorders related to this can lead to an inability to lift the hands and feet, along with aching sensations in all the joints.

- Kutchisayāwātā (กุจฉิสยาวาตา): The air in the abdominal cavity. Disorders related to the air in the abdominal cavity can result in symptoms such as stomach rumbling (borborygmi), dizziness, and generalized joint pain.

- Kothāsayāwātā (โกฏฐาสยาวาตา): The air circulates in the intestines and stomach. Disorders related to this can lead to symptoms such as chest congestion, colicky pain, vomiting, and a strong aversion to food.

- Angkhamangkhānusāriwātā (อังคมังคานุสาริวาตา): The air circulates throughout the body. Disorders related to the air circulating throughout the body can result in symptoms such as blurred vision, dizziness, pain in the front of the thighs, spleen discomfort, dry retching, inability to eat, and alternating sensations of heat and cold.

- Atsāsapatsāsawātā (อัสสาสะปัสสาสะวาตา): The breath. Disorders affecting the breath can lead to difficulty in breathing, resulting in increasingly shallow breaths and, eventually, an inability to breathe.

1.4) The fire element

The fire element or thāt fai (ธาตุไฟ) comprises four sub-elements:

- Santappakkhi (สันตัปปัคคี): The heat in the body makes the body warm. Disorders can lead to a sensation of chilliness in the body.

- Parithaihakkhi (ปริทัยหัคคี): The heat in the body that makes the body feel hot and uncomfortable often necessitates bathing and fanning.

Disorders can create sensations of heat both internally and externally, accompanied by cold hands and feet, as well as excessive perspiration.

- Chiranakkhi (ชิรณัคคี): The heat causes the body to age. Disorders lead to the body's deterioration, causing it to wither, dry out, and lose overall vitality.

- Parināmakkhi (ปริณามัคคี): The heat for digestion plays a crucial role in breaking down food. Disorders can lead to stiffness in the wrists and ankles, phlegm buildup in the throat and air passages, and symptoms such as coldness accompanied by pain, resulting in coughing, painful palms and soles, abdominal rigidity, and nausea.

The primary causes of most diseases stem from an imbalance of the three fundamental elements: pitta (ปิตตะ: fire), vāta (วาตะ: wind or air), and semha (เสมหะ: water).

2) The seasons

The season is one of the etiology of diseases that causes a bodily imbalance in Thai traditional medicine. Thai traditional doctor believes that different seasons influence the balance of the elements within the body. For example, in the hot season, the high temperatures cause the body’s temperature to rise, potentially aggravating the fire element. Conversely, in the cold season, the cooler temperatures can make the body cold as well, disrupting its balance if not properly adjusted. Additionally, during the transitional periods between seasons, such as from winter to summer, summer to rainy season, or rainy season to winter, the body, which has adapted to one season, must adjust to a different climate. If the body cannot adapt in time or is not strong enough, it can lose its balance and lead to illness.

Thai traditional medicine divides the year into several seasonal systems: a three-season system, dividing the year into three seasons of four months each; a four-season system, dividing the year into four seasons of three months each; and a six-season system, dividing the year into six seasons of two months each. In this discussion, only the three-season system will be considered, as it aligns with the natural climate of Thailand.

- Khimhantareudū (คิมหันตฤดู: the hot season) traditionally begins on the first night of the waning moon in the fourth month and ends on the fifteenth night of the waxing moon in the eighth month. In this period of time, the fire element will easily flare up or become imbalanced.

- Wasantareudū (วสันตฤดู: the rainy season) is traditionally considered to begin on the first night of the waning moon in the eighth month and conclude on the fifteenth night of the waxing moon in the twelfth month. During this period, the wind element is susceptible to becoming aggravated or imbalanced.

- Hemantareudū (เหมันตฤดู: the cold season] is traditionally considered to begin on the first night of the waning moon in the twelfth month and end on the fifteenth night of the waxing moon in the fourth month. During this period, the water element is susceptible to becoming aggravated or imbalanced.

3) The age

Age is a fundamental factor that can lead to imbalances in the body. According to Thai Traditional Medicine, the lifespan is divided into three stages: childhood, middle age, and old age. Each stage is prone to different types of imbalances and diseases. For example, children tend to have an imbalance of the water element more easily than other elements. This is why children often get colds, which are caused by imbalances in the water element, especially during certain seasons.

Childhood, from birth to 16 years old, is a period when the water element or *semha* is easily imbalanced. Individuals within this age range, when falling ill, often experience diseases caused by imbalances or deficiencies in the water element.

Middle age, spanning from 16 to 32 years old, is a period where the fire element or *pitta* is easily imbalanced. Individuals within this age group, when falling ill, often experience diseases caused by imbalances or deficiencies in the fire element.

Old age, spanning from 16 to 32 years old, is a period where the wind element or *vata* is easily imbalanced. Individuals within this age

group, when falling ill, often experience diseases caused by imbalances or deficiencies in the wind element.

4) The time (Kalasamutthan)

Kalasamutthan refers to the time of day as a foundational factor for bodily imbalances or diseases influenced by different periods of time. A day is divided into 12 hours of daytime and 12 hours of nighttime. Each 12-hour period is further divided into 3 segments of 4 hours. Different times of day have different effects on the elements.

6:00 AM to 10:00 AM and 6:00 PM to 10:00 PM. At this time, the water element (semha) is likely to become imbalanced or aggravated.

10:00 AM to 2:00 PM and 10:00 PM to 2:00 AM. At this time, the fire element (pitta) is likely to become imbalanced or aggravated.

2:00 PM and 6:00 PM and 2:00 AM to 6:00 AM. At this time, the wind element (vata) is likely to become imbalanced or aggravated.

5) The place (prathētsamutthan)

Place or living environment is also a factor for bodily imbalances or diseases. For example, people who live in high mountainous areas are prone to illnesses caused by imbalances of the fire element. Additionally, people who suddenly change their living environment, such as travelling from one region of the world to another, may fall ill due to their bodies' inability to adapt to the new environment.

Hot country refers to places like high mountains or cliffs. People who live in hot countries tend to get sick due to an imbalance or impairment of the fire element.

Cold country refers to places that are muddy and rainy. People who live in cold countries tend to get sick due to an imbalance or impairment of the wind element.

Warm country refers to places that are sandy and have poor water retention. People who live in warm countries tend to get sick due to an imbalance or impairment of the water element.

Chilly country refers to places that are salty, muddy, and damp, such as the seaside. People who live in cold countries tend to get sick due to an imbalance or impairment of the water element.

6) The behavior

This is the basic knowledge in the Royal Medical Texts mentioned at the beginning. The mūnhet (มูลเหตุ: causes) of disease are mentioned elsewhere in the texts to facilitate the study of the origins of diseases. Those causes of disease arise from human actions and behavior. People must act, without exception, according to their thāt. They should not force their bodies beyond normal capacity. This occurs in the following ways:

As mentioned above, there are various factors that can disrupt bodily balance. These factors often originate from external environmental influences that are beyond our control, such as seasonal changes, time, and location. However, it is possible to prevent or restore bodily balance through various means, including physical conditioning, diet, and medication. For instance, during winter, one can wear warmer clothing, engage in physical activity to generate body heat or consume warm foods. If these adjustments prove insufficient and the body remains imbalanced or falls ill, medical intervention may be necessary. In addition to external factors, internal factors such as personal behaviors can also disrupt bodily balance.

6.1) Food

Food is indispensable for sustaining the human body and should be consumed regularly. Inappropriate dietary practices, such as overeating, under-eating, consuming contaminated food, eating raw or undercooked food, excessive consumption of unfamiliar foods, or irregular meal times, can have detrimental effects on health.

6.2) Body posture

Body posture encompasses standing, walking, sitting, and lying down. Varying these postures throughout the day is crucial for maintaining musculoskeletal health. Prolonged static postures can lead to musculoskeletal disorders, including tendonitis, muscle strain, and discomfort.

6.3) Heat and cold

Exposure to extreme temperatures, such as sudden changes from hot to cold or vice versa, as well as exposure to drafts, rain, dew, or prolonged immersion in water can predispose individuals to illness.

6.4) Lack of sleep, food and water

Food and water are essential for bodily sustenance, while sleep is crucial for energy replenishment and cellular repair. Inadequate consumption of nutrients, hydration, or rest can compromise overall health and increase susceptibility to disease.

6.5) Suppressing defecation and urinary retention

Habitual stool retention can lead to altered bowel motility and constipation. The prolonged retention of fecal matter can result in the absorption of toxins, potentially harming the body. Frequent urinary retention increases the risk of urinary tract infections, dysuria, and urinary incontinence.

6.6) Overworking

Physical strain from heavy lifting, overtraining, mental overexertion, and chronic stress can compromise one's health and increase susceptibility to illness.

6.7) Sadness

Emotional states, particularly sadness and grief, have a profound impact on physiological processes. The body's response to prolonged emotional distress can include alterations in appetite, sleep patterns, and metabolic rate, increasing susceptibility to various medical ailments.

6.8) Anger

A person who is always angry and cannot control their temper is likely to hold grudges, harbor resentment, and even contemplate harming others or themselves. This emotional turmoil can lead to both mental and physical suffering and may contribute to various illnesses.

2.1.2.2 Herbal tastes of single herbs

In Thai traditional medicine, the taste of a herb is believed to reflect its therapeutic actions and affinities with the body. The medicinal tastes of single herbs can be categorized into 10 tastes: astringent taste, sweet taste, oily taste, salty taste, sour taste, bitter taste, intoxicating taste, aromatic-cool taste, pungent-hot taste, and tasteless. Meanwhile, polyherbal medicines are classified into three tastes: hot taste, cold taste, and balanced taste.

1) Astringent taste

Herbs with an astringent taste have medicinal properties that help heal wounds, treat dysentery and control diarrhea. They can also regulate bowel movements. However, they are contraindicated for conditions such as cough, constipation, flatulence and imbalances of the fire element.

2) Sweet taste

Herbs with a sweet taste have the properties of nourishing the body, promoting moisture in the tissues, invigorating the body, and relieving fatigue. However, they are contraindicated for conditions such as tooth decay, excessive phlegm, vomiting, diabetes, lymph disorder and wounds.

3) Oily taste

Herbs with an oily taste are believed to have properties that penetrate deep into the tendons, relieving tendon disorders, nourishing tendons, alleviating aches and pains, strengthening joints and bone membranes, and providing warmth to the body. However, they are contraindicated for conditions related to excessive water elements, such as cough, asthma, various fevers, internal heat, and thirst.

4) Salty taste

Herbs with a salty taste have properties that penetrate the skin, treating skin diseases and constipation. They can also help purify the lymph system, cleanse the intestines of mucus and oil, purify the blood, and relieve thick phlegm. However, they are contraindicated for conditions such as abnormal bowel movements, bloody dysentery, and peptic ulcers.

5) Sour taste

Herbs with a sour taste have the properties of treating phlegm disorders, relieving thick phlegm, relieving cough, relieving constipation, promoting bowel movements, purifying the blood, and quenching thirst. However, they are contraindicated for conditions such as lymph disorder, diarrhea, and fever.

6) Bitter taste

Bitter herbs have the properties to treat symptoms occurring from the cause of bile and blood disorders, reduce fever caused by bile problems, purify the blood, nourish the bile, and stimulate appetite. However, they are contraindicated for conditions such as heart disease, bloating, and distension.

7) Intoxicating taste

Herbs with an intoxicating taste have the properties to treat symptoms occurring from the cause of bile, blood and phlegm disorders, fever, and relieve symptoms from animal bites. However, they are contraindicated for heart conditions and cough.

8) Aromatic-cool taste

Aromatic herbs have the properties to refresh the mind, nourish the heart, liver, and lungs, support pregnancy, relieve fatigue, and invigorate the body but they are generally contraindicated for conditions such as bloating, gas, and indigestion.

9) Pungent-hot taste

Pungent-hot herbs have the properties to relieve gas, bloating, and indigestion, promote burping, nourish the fire element, induce sweating, and aid in digestion. However, they are contraindicated for conditions such as fever, high body temperature, and delirium.

10) Tasteless

Tasteless herbs have the properties to alleviate conditions related to imbalances of the fire element and phlegm and promote urination. Additionally, they are not contraindicated for any specific conditions.

2.1.2.3 Herbal tastes of remedy

Herbal remedies are created by combining two or more herbs. Each herbal remedy has a unique taste that depends on the herb used. The taste of herbal remedies can be categorized into three tastes: hot, cold, and neutral.

1) Hot-tasting medicine refers to medicine made from hot-tasting herbs such as Benjakul, Trikatuk, *Piper retrofractum* and galangal. It is used to treat diseases related to the air element, relieves gas in the digestive tract, expels gas and promotes blood circulation

2) Cool-tasting medicine refers to medicine composed of ingredients such as flowers, animal horns, animal fangs, substances that have been burned into charcoal, and non-heating substances. It is used to treat diseases related to the fire element, toxic fever, severe fever and to extinguish internal heat.

3) Neutral (Sukhum) -tasting medicine refers to medicine composed of mildly hot or aromatic herbs such as Angelica, Costus, Agarwood, Clove, and Cinnamon. It is used to treat blood-related diseases, blood tonics, treat disorders of water elements and alleviate wind in blood circulation.

2.1.2.4 Herbal taste for treating elemental imbalance

Diseases caused by imbalances in different elements require herbs with different herbal tastes. Thai traditional medical theory provides the following recommendations:

1) Earth element disorders should be treated with astringent, sweet, oily, or salty taste herbs.

2) Water element disorders should be treated with sour, bitter, or intoxicating taste herbs.

3) Wind element disorders should be treated with neutral or hot-tasting herbs.

4) Fire element disorders should be treated with tasteless or cool-tasting herbs.

In Thai traditional medical theory, in addition to consideration of the herbal taste of herbs to treat disease due to elemental imbalances directly, the herbal

taste of herbs is also considered for treating elemental imbalances caused by age, season, and time.

2.2 Fever

2.2.1 Fever from the perspective of Thai traditional medicine

Fever refers to a physical or mental illness, such as severe fevers (Khai-pit, Khai-kan and Khai-nuea) or cold fever (khai-wat). In Thai traditional medicine, there is also a concept of "cold fever," which is caused by an imbalance of the fire element in the body. This type of fever may involve symptoms like chills, alternating hot and cold sensations, and body aches. Generally, fever refers to a condition where the body's temperature rises above the normal level due to illness. According to the *Ayurvedhsuksa* textbook by Nithetsukkit, Khun (1973), fever is defined by two main symptoms: elevated body temperature and a sensation of internal heat or heat on the skin. Additionally, fever is often accompanied by discomfort in the mind or the five senses. Various Thai traditional medical scriptures discuss the concept of fever, such as the Khamphi Chanthasat, the Khamphi Takkasila, the Khamphi Thatwiwon, and the Khamphi sitthisarasongkhro (Traditional Thai Medicine Rehabilitation Foundation, 2015).

2.2.1.1 Etiology of fever (***Samutthān***)

1) Fever disorders

1.1) Khai Eka-tosa (ไข้เอกโทษ: Primary fever disorders)

1.1.1) Kamdao fever: Symptoms include mental agitation, headaches, delirium, anxiety, high fever, yellowing of the eyes, reddish urine, yellow vomit, thirst, bitter taste in the mouth, dry saliva, cracked and dry skin, flushed face, yellowish skin, and insomnia at night. When touched, the person may experience moments of euphoria or disorientation, accompanied by tearfulness.

1.1.2) Semha fever: Symptoms include severe chills, goosebumps all over the body, chest tightness, aversion to cold, loss of appetite, sweet taste in the mouth, pale palms and soles, pale stools and urine, vomiting, food aversion, and experiencing chills when touched.

1.1.3) Lohit fever: Symptoms include high fever, headaches, intense thirst, muscle and body aches, yellowish urine, reddish skin, dry teeth, a stiff tongue and jaw, dry mouth, and thick saliva.

1.2) Khai Tuwan-tosa (ไข้ทุวันโทษ: Secondary fever disorders)

1.2.1) Lom and kamdao fever: Symptoms include intense chills, high fever, extreme thirst, sweating, restlessness, dizziness, and severe headaches.

1.2.2) Kamdao and semha fever: Symptoms include chills, aversion to cold, chest tightness, difficulty breathing, sweating, headaches, and high fever.

1.2.3) Lom and semha fever: Symptoms begin with chills and are followed by alternating hot and cold sensations, dizziness, sweating, headaches, blurred vision, and a lack of appetite.

1.2.4) Kamdao and lohit fever: Symptoms include insomnia at night with delirium when falling asleep, severe headaches, restlessness, internal heat, intense thirst, and loss of appetite.

1.3) Khai Tri-tosa (ไข้ตรีโทษ: Tertiary fever disorders)

1.3.1) Semha, kamado and lom fever: Symptoms include joint pain throughout the body, internal heat, intense thirst, restlessness, excessive sweating, and severe drowsiness.

1.3.2) Kamdao, lohit and lom fever: Symptoms include body aches, severe headaches, intense dizziness, chills, faintness, aversion to food, sluggishness, and drowsiness.

1.3.3) Lohit, semha and lom fever: Symptoms include a burning sensation, intense thirst, disturbed sleep at night, restlessness, sweating, yellowish face, vomiting yellowish substances with traces of blood, and intensely red eyes.

2) Severity of fever according to the elements in the body

1.2.1) Earth Element: This type of fever presents with symptoms such as a dull, cloudy mental state, unconsciousness, constipation,

difficulty with bowel movements, refusal to eat, and excessive vomiting within 1-2 days of onset. If these symptoms persist for 10-11 days, it is considered a sign of the Earth element and is often fatal.

1.2.2) Wind Element: This fever type presents with symptoms such as startled sleep, unconsciousness, delirium, burping, excessive saliva, cold hands and feet, within 3-4 days of onset. The condition is fatal in two out of three cases and is related to the Wind element. If the coldness in the hands and feet cannot be alleviated, the symptoms may persist for 9-10 days, leading to certain death.

1.2.3) Water Element: This fever type appears around the fourth day after onset and includes symptoms like diarrhea, sometimes with phlegm or blood in the stool or urine, and occasionally vomiting blood. This condition is associated with the Water element. If the symptoms do not improve after treatment within 8-9 days, the prognosis is usually fatal.

1.2.4) Fire Element: This fever manifests within 3-4 days with symptoms of severe internal and external heat, restlessness, confusion, requiring frequent cooling with water, dryness of the tongue, throat, and chest, intense thirst, delirium, and a state of unconsciousness accompanied by pains throughout the body. The person may crave unusual foods as if possessed. These symptoms are related to the Fire element. If the intense heat cannot be reduced and symptoms persist for 7-8 days, death is highly likely.

3) Khai Sannibat

Khai Sannibat (ไข้สันนิบาต) is characterized by the types of fever known as "eka-tosa," "tuwan-tosa," and "tri-tosa," which have not yet dissipated and persist continuously, resulting in a combination of these three types of ailments. It can be divided into three characteristics:

Symptoms include high body temperature, thirst, fatigue, dry mouth, red eyes, and pain throughout the body.

Symptoms include cold body temperature, a preference for sleeping, loss of appetite, bright red eyes, body aches, and yellow vomiting.

Symptoms include alternating hot and cold sensations, headache, extreme thirst, fatigue, and inability to urinate or defecate.

4) Khai Samprachuan

Khai Samprachuan (ไข้สำประชวร: **Chronic fever)** refers to a long-standing fever that does not heal, leading to a gaunt body, weakness, and loss of appetite. It consists of five causes:

4.1) Kamdao fever (กำเดา: dry heat): The symptoms of this fever are headache, body heat, chills and shivering, no tears, vomiting, insomnia, dry mouth, thirst, pain in the mouth and throat, and blood-red eyes.

4.2) Lohit fever (โลหิต: blood): The symptoms of this fever are headache, body heat, red face, blood-red eyes, tears, sore feet and heat all over the body, and anxious mind.

4.3) Semha fever (เสมหะ: secretions): The symptoms of this fever are cold, hairlessness, goosebumps, turmeric yellow eyes, dreaminess, excessive saliva in the mouth, cold hands and feet, craving for sweet and savory food, weakness, shivering hot and cold.

4.4) Di fever (ดี: bile): The symptoms of this fever are body heat, delirium, sleepover, headache, thirst, bitterness in the mouth, body aches, green eye rims.

4.5) Lom fever (ลม: air or wind): The symptoms of this fever are dizziness, lightheadedness, not hot, cloudy and cloudy eyes. In addition, the other has very few red eyes (fading red), body pain, colic, hiccups, vomiting, coughing, thirst, mouth ulcers, chest pains, and shortness of breath.

2.2.1.2 Pathology of fever

Types of fever can be categorized into three main groups: severe or toxic fevers (Khai-pit, Khai-kan and Khai-nuea), cold fever (khai-wat) and hyperpyrexia (khai-kamdao). These fevers may occur from the cause of water (semha, blood), wind and fire (kamdao, pitta) element imbalance or a combination of them.

1) Severe fever (Toxic fever)

Severe or toxic fevers consists of three types:

1.1) Khai-pit (ไข้พิษ): Sign and symptoms of pit (พิษ: toxic) include high fever, red eyes, cold hands and feet, headaches, alternating chills and

heat, pain, a stiff tongue and jaw, labored breathing, dry mouth, drowsiness, unconsciousness, delirium and rashes appearing on the body. A comparison of some types of Khai-pit with modern medicine is shown in **Table 1**.

Table 1 Comparison of some types of Khai-pit with modern medicine.

Khai-pit	**Sign and symptoms**	**Comparable to modern medicine (Bunyapraphatsara, 2008)**
Khai E-dam E-daeng (ไข้อีดำอีแดง)	Patches measuring 1-2 inches appear all over the body. If they are black, it is called E-dam; if they are red, it is called E-daeng.	Scarlet fever
Khai Pandam Pandaeng (ไข้ปานดำปานแดง)	Patches measuring 1-2 inches in size, appearing in black on half of the body, can be fatal if they cover the entire body. If the patches are black, it is referred to as Pandam; if they are red, it is called Pandaeng.	Leptospirosis
Khai Mahamek (ไข้มหาเมฆ)	Dark patches appear beneath the skin without raised surfaces, casting a shadow within the flesh. Key symptoms include unconscious passing of stool and urine.	Septicemia or sepsis or septic shock
Khai Mahanin (ไข้มหานิล)	Dark patches appear all over the body beneath the skin without raised surfaces, casting a shadow within the flesh all over the body. Key symptoms include unconscious passing of stool and urine.	
Khai Dan Hin (ไข้ดานหิน)	Rashes appear on both thighs in dark green or indigo circles. The body becomes cold as stone, while there is intense internal heat and thirst.	
Khai Kradan Hin (ไข้กระดานหิน)	Rashes appear all over the body, resembling hives, with red spots similar to a rash that later turn black and sink into the skin, causing itching. Key symptoms include severe headache and bone pain. If the rash does not subside within three months, it can be fatal.	
Khai Saifa Fat (ไข้สายฟ้าฟาด)	Streaks appear along the body, both front and back, ranging from 1 to 2 inches wide, with colors such as red, indigo, purple, or brown.	Septicemia or sepsis or septic shock
Khai Pleo Faifa (ไข้เปลวไฟฟ้า)	Extreme high fever causes the chest, nose, and face to darken with a smoky hue. Additional symptoms include dry mouth, blackened tongue, peeling of the palate, and dizziness.	
Khai Khao Mai Bai Kriam (ไข้ข้าวไหม้ใบเกรียม)	Rashes appear across the body, similar to hives, in patches 2-3 inches wide, sometimes in small dots that turn dark, like ant bites. Key symptoms include pain deep in the muscles and bones. If the fever reduces but the rashes darken and harden into thick, rhino-like skin, the person may survive for six months. However, if it affects the liver or lungs, it can lead to death.	
Khai Dao-rueang (ไข้ดาวเรือง)	Rash patterns resembling half-moon shapes appear on the skin, leading to frequent vomiting.	

Table 1 Comparison of some types of Khai-pit with modern medicine (Continued).

Khai-pit	**Sign and symptoms**	**Comparable to modern medicine (Bunyapraphatsara, 2008)**
Khai Rabu Chat (ไข้ระบุชาด)	Clusters of small, seed-like rashes, varying in size from that of a basella seed to a sesame seed, emerge across the body in bright red patches, 1-2 inches in size. These rashes cause a sense of heaviness and confusion, intense internal heat, extreme thirst, along with labored breathing and frequent hiccups.	Exantrem subitem or roseora infantum or sixt disease
Khai Fai Duean Ha (ไข้ไฟเดือนห้า)	Rashes can appear on the chest, with colors ranging from black to red, and some resembling flames. These cause internal heat and intense thirst, leading to confusion and loss of consciousness. Symptoms include a stiff tongue, locked jaw, and sometimes fainting.	Septicemia or sepsis or septic shock or hyperpyrexia with sepsis infantum or sixt disease
Khai Khao Mai Noi (ไข้ข้าวไหม้น้อย)	Rashes appear all over the body resembling ant bites, with pointed white tips.	Meningitis
Khai Khao Mai Yai (ไข้ข้าวไหม้ใหญ่)	Rashes appear all over the body resembling ant bites, with pointed white tips. Key symptoms include red eyes.	
Khai Sangwan Phra In (ไข้สังวาลพระอินทร์)	Red rashes appear in rows along the body, emerging on the left side for females and the right side for males. These cause shortness of breath, hiccups, and alternating chills and heat.	Acute phase of Herpes zoster
Khai Hong Rathot (ไข้หงส์ระทด)	There is no rash, but the skin appears burnt all over the body, as if the body were stiff like wood.	Malaria
Khai Chanthara Sut (ไข้จันทรสูตร)	There is no rash, but fainting occurs when the moon rises, and symptoms ease when it does not. The body stiffens like wood, with a rigid tongue and jaw.	
Khai Suriya Sut (ไข้สุริยะสูตร)	There is no rash, but fainting occurs when the sun rises, and symptoms ease when it does not.	
Khai Mek Sut (ไข้เมฆสูตร)	There is no rash, but symptoms appear when thunderstorms arise, with clouds forming across the sky in all directions.	

1.2) Khai-kan (ไข้กาพ): Characterized by high fever, headaches, alternating chills and heat, pain, a stiff tongue and jaw, drowsiness, unconsciousness, delirium and rashes appearing on the body. When it occurs, it creates intense internal heat. With this heat, small red bumps appear (*kan* /กาพ: inflammation within the body) in the intestines, kidneys, lungs, and spleen, causing swelling. It often results in death within 7 to 11 days. Upon death, black spots emerge on the skin in patches or circles. A comparison of some types of Khai-kan with modern medicine as shown in **Table 2**.

Table 2 Comparison of some types of Khai-kan with modern medicine.

Khai-kan	**Sign and symptoms**	**Comparable to modern medicine (Bunyapraphatsara, 2008)**
Khai Hat (ไข้หัด)	Small, sand-like bumps with sharp tips appear all over the body.	Measles
Khai Hueat (ไข้เหือด)	Small, sand-like bumps appear all over the body, without sharp tips.	Rubella
Khai Roem Namkhang (ไข้เริมน้ำค้าง)	Clusters of clear, watery blisters emerge in patches, each measuring about 1-4 inches in size.	Herpes simplex
Khai Roem Namkhao (ไข้เริมน้ำข้าว)	Clusters of opaque white blisters appear in patches, each about 1-4 inches in size.	
Khai Ngusawat (ไข้งูสวัด)	Small, sand-like bumps appear in rows, forming a shape resembling a snake. The bumps are swollen and have a glossy, pustular appearance. In women, they appear on the left side of the body, while in men, they appear on the right. If these bumps cross the spine, the condition becomes untreatable.	Herpes zoster
Khai Lam Lap Phloeng (ไข้ลำลาบเพลิง)	Rashes appear in patches on the skin, causing intense burning pain.	Erysipelas
Khai Failamthung (ไข้ไฟลามทุ่ง)	The skin has rashes that emerge rapidly in patches, causing intense burning pain.	
Khai Kamphaeng Thalai (ไข้กำแพงทะลาย)	It emerges as a single head and is highly toxic.	Abscess with cellulitis
Khai Lalok Kaeo (ไข้ละลอกแก้ว)	It occurs amidst khai-pit, taking the shape and size of a bean and appearing as a shiny blister.	Exanthematous fever
Khai Mareng Ta Moi (ไข้มะเร็งตะมอย)	It appears in sizes comparable to a thumb; if the base is white and the head is black, it indicates severe toxicity. Sometimes, it may also manifest on the body, arms, or legs.	Deep abscesses
Kan Fong Samut (กาฬฟองสมุทร)	It appears as small as a sesame seed or a bean, occurring in the mouth, on the palate, or on the tongue.	Typhoid fever
Kan Thum (กาฬทูม)	It presents with swelling on both sides of the jaw, although it may occasionally swell on just one side.	Mump

1.3) Khai-nuea (ไข้เหนือ): Also known as jungle fever or " Chattudong fever " (referring to the four regions of the forest). Sometimes referred to as "chills fever," it involves symptoms of fever characterized by chills that cause shaking, headaches, constipation, and cold hands and feet. It can be compared to the symptoms and signs of malaria.

2) Cold fever (khai-wat)

In the Khamphi Takkasila scriptures, it is stated that both types of colds are caused by weather conditions during the summer, rain, and winter. These colds occur when the body comes into contact with heat, dew, or raindrops, leading to the onset of a cold.

2.1) Khai-wat Noi (ไข้หวัดน้อย: common cold): Symptoms include alternating chills and fever, severe headache, cough, sneezing, and nasal discharge.

2.2) Khai-wat Yai (ไข้หวัดใหญ่: influenza): Symptoms include shivering, severe headache, cough, frequent sneezing, abundant nasal discharge, fever, vomiting, dry mouth, sour taste in the mouth, bitter taste, and loss of appetite. It causes significant coughing, dry throat, and dry mouth.

3) Hyperpyrexia (khai-kamdao)

3.1) Khai-kamdao Noi (ไข้กำเดาน้อย): Symptoms include headache, red eyes, high fever, cough, alternating chills and heat, bitter taste in the mouth, sour taste in the mouth, loss of appetite, vomiting, and insomnia. It can be compared to the symptoms and signs of dengue fever or chikungunya fever.

3.1) Khai-kamdao Yai (ไข้กำเดาใหญ่): Symptoms include severe headache, red eyes, high fever, cough, alternating chills and heat, dry mouth, confusion, general body aches, and occasionally presenting with red spots similar to mosquito bites, although these spots do not have a raised center. Sometimes, there may be coughing up blood through the nose or mouth, and in some cases, it may lead to convulsions with clenched fists. It can be compared to the symptoms and signs of dengue hemorrhagic fever or dengue shock syndrome.

2.2.1.3 Therapy

The treatment of fever by using Thai traditional drug in Taksila scriptures (Traditional Thai Medicine Rehabilitation Foundation, 2015) is the use of bitter and very cool taste to mild cool taste. Consider the severity of the fever to correct the recurrent elemental ethos to bring it back to equilibrium. Can be divided into 3 steps:

Step 1 : Use herbs to decrease the toxicity of fever with bitter and very cool taste to detoxify the internal organs to the outside organs.

Step 2 : Use herbs for fever-reducing with cold and tasteless to reduce the uncomfortable side effects.

Step 3: Use herbs with a mild cool taste to prevent the disease from recurring and treat the minor symptoms of the disease.

2.2.2 Fever from the perspective of modern medicine

2.2.2.1 Definition and pathogenesis of fever

Fever (pyrexia and febrile response) is defined as having a body temperature rise above the normal range occurring as a result of IL-1-mediated elevation of the hypothalamic set-point. There is not a single agreed-upon upper limit for normal temperature with sources using values between 37.5 and 38.3 °C. The increase in set-point triggers increased muscle contraction and causes a feeling of cold. This results in greater heat production and efforts to conserve heat. When the set-point temperature returns to normal a person feels hot, becomes flushed, and may begin to sweat. Rarely a fever may trigger a febrile seizure. This is more common in young children. Fevers do not typically go higher than 41 to 42 °C (Lowth, 2014).

Fever can be caused by many medical conditions ranging from not serious to potentially serious. This includes viral, bacterial, and parasitic infections such as the common cold, urinary tract infections, meningitis, malaria and appendicitis among others. Non-infectious causes include vasculitis, deep vein thrombosis, side effects of medication, and cancer among others. Other causes of fever are the destruction of tissues, such as trauma, tumors, and inflammatory disorders (Aronoff and Neilson, 2001). It differs from hyperthermia, in that hyperthermia is an increase in body temperature over the temperature set-point, due to

either too much heat production or not enough heat loss (Garmel and Mahadevan, 2009).

Many of the mediators underlying pyrexia have been described in recent years. The critical “endogenous pyrogens” involved in producing a highly regulated inflammatory response to tissue injury and infection are polypeptide cytokines. Pyrogenic cytokines, such as interleukin-1b (IL-1β), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to affect a fever response. Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacterial outer membrane lipopolysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1β.

2.2.2.2 Inflammation and fever

A febrile response is just one component of a complex array of host defense responses, collectively termed acute phase response (APR), which occurs during the time-course of systemic inflammation. The APR is a pronounced systemic reaction to disturbances of homeostasis, which is caused by infections, tissue injury, neoplastic growth, or immunological disorders; fever is one of the most prominent components of the APR, a hallmark of disease (Kluger, 1991; Roth and Blatteis, 2014). During systemic inflammation, phases of fever and phases of hypothermia can alternate, depending on the severity of a given inflammatory insult or on environmental conditions (Romanovsky and Székely, 1998; Romanovsky *et al*., 2015). It is a matter of debate, whether the strength of the fever or the degree of hypothermia is of prognostic value for the final outcome (i.e. survival) in critically ill patients (Harden *et al*., 2015).

According to the classical view, fever develops in several steps starting with the appearance of a given pathogenic agent, the “exogenous pyrogen”, in the afflicted host. This exogenous pyrogen, in turn, causes the release of fever-producing substances by the host’s polymorphonuclear leukocytes and by other cells. These substances are, therefore, called “endogenous pyrogens”. Traditionally, a number of cytokines, namely, interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α), interferons (IFNs), and others were identified and characterized as the endogenous

pyrogens (Kluger, 1991; Roth and Blatteis, 2014). Fever and other characteristic symptoms of sickness occur as rather stereotyped physiological and behavioral responses that are controlled by specific neuronal circuitries within the brain (Saper *et al*., 2012). Induction of these symptoms, therefore, depends on a propagation of the inflammatory response from the periphery into the brain. The febrile alterations of body temperature require the formation of prostaglandins, namely, prostaglandin E_2 (PGE_2). In the brain, pronounced formation of PGE_2 depends on the coupled induction of the inducible enzymes cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase 1 (mPGES1). In this context, cells forming the blood-brain barrier are not an obstacle for the inflammatory signal to be transferred into the brain.

Instead, the blood-brain barrier acts as a relay station, which is equipped with receptors for several pathogen associated molecular patterns such as lipopolysaccharide (LPS) as well as a number of critical cytokines. Indeed, brain endothelial cells and perivascular macrophages directly respond to inflammatory signals with an increased expression of both COX-2 and mPGES-1. The production of PGE_2 by these enzymes and its subsequent release into the brain on the abluminal side of the blood brain barrier is dependent on a concerted activation of inflammatory transcription factors (Rummel *et al*., 2016). PGE_2, at critical central sites of the thermoregulatory system, finally drives the fever and other alterations of body temperature during systemic inflammation.

2.2.2.3 Nitric oxide and fever

Fever is a phenomenon characterized by a raised thermoregulatory set point that leads to an elevation in body temperature. It is well known that fever can be initiated by a number of agents including lipopolysaccharide (LPS), viruses, yeast and gram-positive bacteria. Considerable efforts have been made to identify the mechanisms of fever and it is generally believed that fever results from the induction of cytokines, such as interleukin (IL)-1β, IL-6, interferons, and tumor necrosis factor (TNF), and subsequent generation of prostaglandins (PGs) in the CNS, particularly prostaglandin E_2 (PGE_2), thought to act as a proximal mediator of fever (Blatteis *et al*., 1998; Kluger, 1991).

Nitric oxide (NO) participates in several systems that are involved in body temperature regulation under euthermic conditions. However, when a pyrogen is

administered to an animal, a number of pathways in which NO might participate are thought to be activated. NO has been shown to participate in the febrile response by acting at both peripheral and central sites (Steiner and Branco *et. al*., 2001).

NO and its derivatives, including reactive nitrogen intermediates (nitrite and nitrate), produced by nitric oxide synthases (NOS) have been identified as important effector molecules that restrict pathogen growth in infected hosts, and monocytes and macrophages, endothelial cells, hepatocytes, and neutrophils can synthesize reactive nitrogen intermediates by constitutive pathways, inducible pathways, or both. Different members of the NOS family are encoded by separate genes. There are three known isoforms in mammals, two are constitutive (cNOS) and the third is inducible (iNOS). Cloning of NOS enzymes indicates that cNOS includes both brain constitutive (nNOS or NOS1) and endothelial constitutive (eNOS or NOS3); the third is the inducible (iNOS or NOS2) gene (Nathan and Xie, 1994).

In addition, NO is one of the inflammatory mediators causing inflammation in many organs and it is an inorganic free radical that has been implicated in physiological and pathological processes, such as vasodilation, non-specific host defense and acute or chronic inflammation (Kou and Schroder, 1995; Lantz *et al*., 2005). In inflammatory reactions, pro-inflammatory cytokines lead to the expression of the inducible NO synthase (iNOS) in monocyte/macrophages, neutrophil granulocytes and many other cells; in the case of bacterial infection, endotoxin is another strong inducer of expression. In consequence, large amounts of NO are synthesized, exceeding the physiological NO production by up to 1000-fold (Forstermann *et al*., 1994; Knowles and Moncada, 1994; Weinberg *et al*., 1995; Cook and Cattell,1996). Finally, the undesired effects of NO are due to its impaired production, including in short: vasoconstriction, inflammation and tissue damage.

2.2.2.4 Therapy

Fever drugs or antipyretics serve centrally by lowering the thermoregulatory set point of the hypothalamic center. This is achieved through the inhibition of cyclooxygenase (COX), the enzyme responsible for the conversion of arachidonic acid to prostaglandins (PG) and leukotrienes. Although several prostaglandins can induce fever, PGE_2 is the most important mediator. The lowering

of the hypothalamic set point leads to a series of physiological responses, including decreased heat production, increased blood flow to the skin, and increased heat loss through the skin by radiation, convection, and evaporation, resulting in a reduction in body temperature (Aronoff and Neilson, 2001). The major mechanism of action of aspirin and other antipyretics involves lowering PGE_2 by directly inhibiting COX enzyme activity (Flower and Vane, 1972).

Interestingly, clinically useful actions of antipyretics may also be COX independent (Cronstein *et al*., 1999), and relevant anti-inflammatory effects of aspirin, sodium salicylate, and other NSAIDs are seen only with doses much higher than those required to suppress COX activity. Thus, a variety of noncyclooxygenase-dependent functions have been proposed to explain the full effects of salicylates on the pyrogenic cascade. For example, salicylates and other antipyretics also suppress tissue inflammation through diminished leukocyte-endothelial cell interactions, reduced pyrogenic cytokine production, or enhanced expression of anti-inflammatory molecules. Other mechanisms, such as boosting the activity of endogenous antipyretic messengers, may further contribute (Wilkinson and Kasting, 1990).

Fever may be mitigated by antipyretic agents. The primary action of an antipyretic is inhibiting the enzyme cyclooxygenase and reducing the levels of PGE_2 within the hypothalamus. Other suggested mechanisms of antipyretic drugs include their ability to reduce inflammatory mediators, such as nitric oxide (NO) and prostaglandin E_2 (PGE_2) (Aronoff and Neilson, 2001).

Traditional healers acquire their heritage and experiences from their ancestors in formulating remedies with the purpose of maximizing the therapeutic efficacy and minimizing the adverse effects or toxicity. Thai traditional medicine (TTM) has been using Thai traditional remedies to treat fever and symptoms related to fever. TTM's medicinal plant therapy focuses on the structure of the formula, the taste of the drug and the main medicinal taste to cover the cause of disease and its complications.

2.3 MHR remedy

2.3.1 Definition

MHR (Mo-Ha-Rak) is a Thai traditional polyherbal preparation. The word "Mo-Ha-Rak" is derived from "Mo" and "Ha-Rak". Mo means a decoction or a mixed of many component herbs. Ha-Rak means the roots of five herbs: *Capparis micracantha* DC., *Clerodendrum indicum* (L.) Kuntze, *Ficus racemosa* L., *Harrisonia perforata* (Blanco) Merr. and *Tiliacora triandra* (Colebr.) Diels. It is used by Thai traditional practitioners as a treat for severe fever with drowsiness, internal heat with thirst, restlessness, delirium and unconsciousness (Nualkaew, 2020). This formula is composed of 21 medicinal plants: *Azadirachta indica* A.Juss. petioles, *Bridelia ovata* Decne. leaves, *Capparis micracantha* DC. roots, *Cassia fistula* L pulps, *Clerodendrum indicum* (L.) Kuntze roots, *Dracaena cochinchinensis* (Lour.) S.C.Chen woods, *Ficus racemosa* L. roots, *Gymnopetalum chinense* (Lour.) Merr. fruits, *Harrisonia perforata* (Blanco) Merr. roots, *Ligusticum sinense* Oliv. rhizomes, *Mesua ferrea* L. flowers, *Nelumbo nucifera* Gaertn. stamens, *Phyllanthus emblica* L. fruits, *Pinus kesiya* Royle ex Gordon woods, *Terminalia bellirica* (Gaertn.) Roxb. fruits, *Terminalia chebula* Retz. fruits, *Terminalia* sp. "Samo Thet" fruits, *Tarenna hoaensis* Pit. woods, *Tiliacora triandra* (Colebr.) Diels roots, *Tinospora crispa* (L.) Hook. f. & Thomson stems and *Vetiveria zizanioides* (L.) Nash roots are shown in **Table 3**.

Table 3 Herbal component of MHR remedy.

No.	Material	Part used	Taste	Amount (% w/w)	Function
1	*Azadirachta indica*	Petiole	Bitter	4.0	Primary herbs
2	*Bridelia ovata*	Leaf	Bitter-intoxicating	4.0	Adjunct herbs
3	*Capparis micracantha*	Root	Bitter	4.0	Primary herbs
4	*Cassia fistula*	Pulp	Sweet	12.0	Adjunct herbs
5	*Clerodendrum indicum*	Root	Tasteless-intoxicating	4.0	Primary herbs
6	*Dracaena cochinchinensis*	Wood	Bitter-cool	4.0	Primary herbs
7	*Ficus racemosa*	Root	Astringent-cool	4.0	Primary herbs
8	*Gymnopetalum chinense*	Fruit	Bitter-cool	4.0	Primary herbs
9	*Harrisonia perforata*	Root	Bitter-intoxicating	4.0	Primary herbs
10	*Ligusticum sinense*	Rhizome	Oily-pungent hot	1.0	Primary herbs
11	*Mesua ferrea*	Flower	Aromatic-cool	4.0	Supportive herbs
12	*Nelumbo nucifera*	Stamen	Astringent-aromatic cool	2.0	Supportive herbs
13	*Phyllanthus emblica*	Fruit	Astringent-sour-sweet	8.0	Adjunct herbs
14	*Pinus kesiya*	Wood	Bitter-pungent hot	1.0	Primary herbs
15	*Terminalia bellirica*	Fruit	Sour-astringent-sweet	8.0	Adjunct herbs
16	*Terminalia chebula*	Fruit	Astringent-sour	8.0	Adjunct herbs
17	*Terminalia* sp. “Samo Thet”	Fruit	Sour-astringent	8.0	Adjunct herbs
18	*Tarenna hoaensis*	Wood	Bitter-sweet	4.0	Primary herbs
19	*Tiliacora triandra*	Root	Tasteless-bitter	4.0	Primary herbs
20	*Tinospora crispa*	Stem	Bitter-cool	4.0	Primary herbs
21	*Vetiveria zizanioides*	Root	Aromatic-cool	4.0	Supportive herbs

2.3.2 Herbal component of MHR remedy

2.3.2.1 *Azadirachta indica* A.Juss.

Common names: Neem Tree, Nim Tree, Indian Lilac, Margosa Tree

Thai names: Sadao (Central), Kadao (Peninsular), Cha-tang (Suai), Saliam (Northern)

Family: Meliaceae

Part used: Petioles

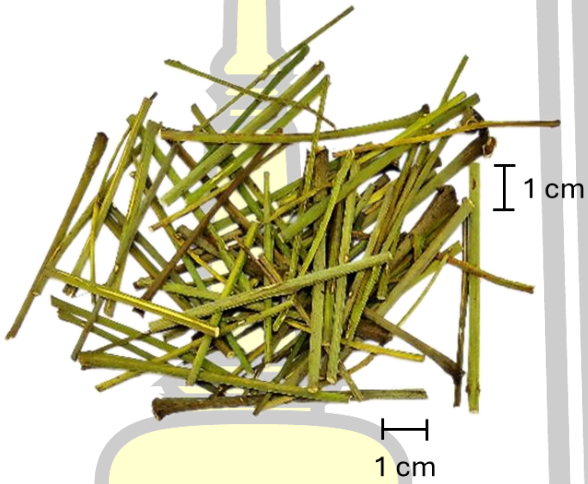

Figure 2 Petiole of *Azadirachta indica* A.Juss.

Botanical characteristics:

A. indica is a tree, up to 15 (-30) m tall, with round large crown and sometimes fluted buttress, all parts bitter. The newly formed young shoots are reddish-brown in color. The bark is gray and furrowed. Leaves are compound, alternate, and tends to cluster near the end of the branches. The compound leaf is pinnate (15 – 35 cm long) with 8-19 leaflets. Leaflet is lanceolate (3.5 – 10 cm long and 1.2 – 4 cm wide), sometimes slightly curved like sickle, with distinct toothed margin, tapering tips and distinctly asymmetric base. Leaves are red when young and gradually turn green. When injured, the leaves have a slight garlic scent. The leaf stalk (petiole) is 3 – 7 cm long, where the base is slightly swollen and have 2 pairs of small pit-like glands. Flowers are inflorescences at the ends of branches while young

leaves are budding, white petals. The fruit is fresh, oval, with 1 seed (Hashmat *et al*., 2012)

Traditional used:

The plant is used for the treatments of fever, malaria, internal heat and thirst, and tonic for bile (Wutthithammawet, 2002).

Chemical constituents:

The chemical constituents are found in the leaves of *A. indica* as nimbin, nimbanene, 6-desacetylnimbinene, nimban-diol, nimbolide, ascorbic acid, n-hexacosanol and aminoacid, 7 -desacetyl-7 -benzoylazadiradione, 7 -desacetyl-7 -benzoylgedunin, 1 7 -hydroxyazadiradione and nimbiol. The four best limnoids compounds are: azadirachtin, salannin, meliantriol, and nimbin (Mondal and Mondal, 2012). Flavonoid such as myricetin 3-O-galactoside, trifolin, isorhamnetin, quercetin and kaempferol. Phenolic such as *p*-coumaric acid, ferulic acid and phloretin. Aromatics such as 4-methoxy benzaldehyde, scopoletin, indole-3-acrylic acid and 2,4-quinolinediol (Maran *et al*., 2021).

2.3.2.2 *Bridelia ovata* Decne.

Common names: Burma Bridelia

Thai names: Maka (General), Kong kaep (Chiang Mai), Madka (Khon Kaen), Samsa, Makaton (Loei), Madka Madka (Nong Khai), Madka (Nakhon Ratchasima), Kong Kongkab (Northern), Salua Siwala (Karen-Mae Hong Son)

Family: Phyllanthaceae

Part used: Leaves

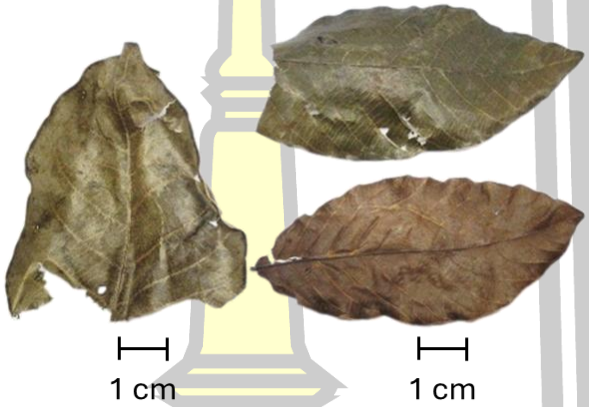

Figure 3 Leaf of *Bridelia ovata* Decne.

Botanical characteristics:

B. ovata is a scrambling shrub or small tree, up to 8 m high, crown flattened: branches hairless, with scattered warts. Bark is fissured, dark greyish brown. This species can be recognised by its rather large, broadly elliptic and papery leaves with more than 14 pairs of secondary veins and the often-prominent olive blackish color in dry state. The branches and leaves are entirely hairless, and the latter are blunt on both ends. Leaves are elliptic to slightly obovate, 5-18 by 2-8 cm. Flowers are borne in multibracteate glomerules of 1-20 nearly stalkless to shortly stalked yellowish green flowers. Bracts are ovate-triangular, up to 2 x 1-1.5 mm. Flowers are nearly stalkless to shortly stalked (male flowers mostly), flower-stalk up to 2 mm long, hairless, female flowers base or flower-stalk often shorter and stouter, up to 1.5 mm in diameter; sepals triangular, up to 2 x 1.5 mm, hairless, greenish cream tinged with red; petals elliptic, 0.5-1.2 x 0.7-1 mm, tip roundish or notched,

whitish yellow; disc hairless. Fruits are depressed ellipsoid, notched at tip, bilobate, sometimes obconical at base, 5-7 x 6-7.5 x 7-8 mm, fleshy, pale greenish purple when fresh, dry blackish (Thongkon, 1995).

Traditional used:

Folk medicine uses the leaves as a mild laxative, tonic for bile and lymph, treat fever, hiccups, carminative, indigestion, lymphatic loss and beriberi (Wutthithammawet, 2002).

Chemical constituents:

Thongkon (1995) reported chemical constituents of *B. ovata* leaves including friedelin, friedelin-3ß-ol and stigmasterol.

Chemical-physical quality requirements (Supatarawanich *et al*., 1995):

Loss on drying, not more than 7.0 per cent w/w after drying at 105° to constant weight.

Acid-insoluble ash, not more than 2.0 per cent w/w.

Total ash, not more than 12.0 per cent w/w.

Ethanol-soluble extractive, not less than 17.0 per cent w/w.

Water-soluble extractive, not less than 12.0 per cent w/w.

Ether-soluble extractive, not less than 3.0 per cent w/w.

2.3.2.3 *Capparis micracantha* DC.

Common names: Caper-Thorn, Thorn Caper, Melada, Jambol Merah, Thai Caper Flame Bean

Thai names: Kradat khao (Central), Kradat pa (Chon Buri), Krarok yai (Central), Khon klong (Phetchabun), Khon khong (Saraburi), Chingcho (Central), Chai chu (Chaiyaphum), Chingchi (Central), Si so (Prachin Buri), Phaya chom pluak (Central), Phuang ma rado (Pattani), Meng so (Pattani), Ram (Songkhla), Samae so (Central), Sae ma thalai (Chiang Rai), Nuat maeo daeng (Chiang Mai), Mak mok (Chaiyaphum)

Family: Capparaceae

Part used: Roots

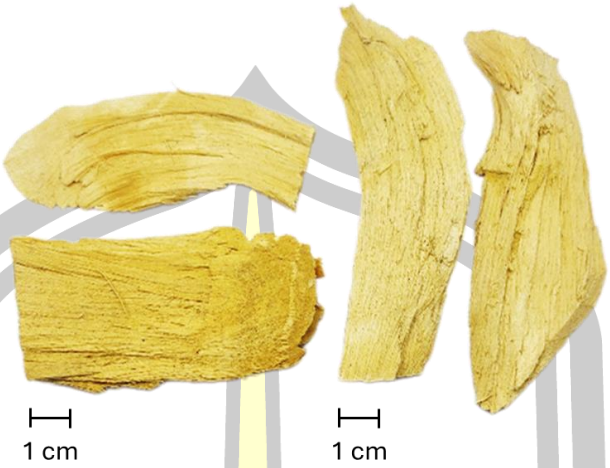

Figure 4 Root of *Capparis micracantha* DC.

Botanical characteristics:

C. micracantha is a thorny shrub (1–6 m tall) or, rarely, a climber, and is quite common. It is easily recognized by its axillary inflorescence with a series of 2–6 flowers arranged in a row. The flowers have petals and stamens up to 1.6 cm and 3.4 cm long, respectively. It can also be differentiated from the other subspecies by its greenish sepals, 15–25 stamens, and globose to ellipsoid fruits versus the dull, greyish purple sepals, numerous (up to 100) stamens, and oblong fruits.

Traditional used:

The root has been used for the treatments of gastrointestinal diseases, carminative, toxic fever, eye disease, stomach disease, cancer, helps the uterus to enter the garage, cough, and asthma (Wutthithammawet, 2002).

Chemical constituents:

C. micracantha roots has been reported to contain alkaloids such as capparine B and stachydrine. Terpenoids such as capparisditerpenol. Flavonoid such as rutin (Singharachai *et al*., 2011).

Chemical-physical quality requirements (Department of Medical Sciences, 2018)**:**

Loss on drying, not more than 8.0 per cent w/w after drying at 105° to constant weight**.**

Acid-insoluble ash, not more than 1.0 per cent w/w.

Total ash, not more than 4.0 per cent w/w**.**

Ethanol-soluble extractive, not less than 2.0 per cent w/w.

Water-soluble extractive, not less than 7.0 per cent w/w.

2.3.2.4 *Cassia fistula* L.

Common names: Golden Shower, Indian Laburnum, Pudding-pipe Tree

Thai names: Khun (Central, Northern), Ku-phe-ya (Karen-Kanchanaburi), Chaiya phruek (Central), Pue-yu (Karen-Mae Hong Son), Pu-yo (Karen-Mae Hong Son), Poe-so (Karen-Mae Hong Son), Mae-la-yu (Karen-Mae Hong Son), Ratcha phrik (Southeastern), Ratcha phruek (Central), Lom laeng (Northern), Lak khoei lak kluea (Peninsular)

Family: Fabaceae

Part used: Pulps

Figure 5 Pulp of *Cassia fistula* L.

Botanical characteristics:

C. fistula is a deciduous tree, up to 20 m tall; bark smooth, greenish grey when young, becoming rough and dark brown when mature. Leaves pinnately compound, 10 to 60 cm long; petiole 7 to 10 cm long; rachis 15 to 25 cm long; stipule small and caducous; leaflets opposite, 3-to 8-paired, ovate to ovate-oblong, 6 to 20 cm long, 3.5 to 9 cm wide, apex acute to acuminate, base cuneate, upper surface glabrous,

lower surface silvery pubescent when young, becoming glabrous when mature. Inflorescence raceme, axillary, drooping, lax, many-flowered, 20 to 40(-60) cm long; pedicel 1.5 to 5 cm long; bract about 1 cm long, caducous. Flower: sepals 5, ovate-elliptic, about 1 cm long, velutinous, reflexed at anthesis; petals 5, subequal, broadly ovate or obovate, 2 to 3.5 cm long, 1 to 2 cm wide, distinctly veined, short-clawed, yellow, stamens 10,3 long, 4 short and 3 reduced forms, long stamens much curled and bearing large oblong anther, opening by apical and basal slits, short stamens with straight filaments, reduced stamens with minute anther, ovary stalked, velutinous, style velutinous, stigma small. Fruit pod, terete, 20 to 60 cm long, 1 to 2.5 cm wide, pendulous, indehiscent, blackish brown to black. Seeds numerous, elliptic, 7.5 to 9 mm long, 5 to 7 mm wide, flat, glossy brown, embedded in blackish pulp, separated by spongy septa (Department of Medical Sciences, 2021).

Traditional used:

Folk medicine uses the pulps as a laxative, use to treat constipation, fever for mucous, phlegm, colic, poisoning from fever, cough, sore throat, chest tightness, malaria, and dysentery as an anthelmintic (Wutthithammawet, 2002).

Chemical constituents:

Lee *et al*. (2001) reported chemical constituents of *C. fistula* arils including 1-hexacosanol, 1-octacosanol, palmitic acid, stearic acid, oleic acid, linoleic acid, heptacosyl eicosanate, chrysophanol, physcion, rhein methyl ester, ziganein, β-sitosterol, stigmasterol, lupeol, rhein, emodin, citreorosein, 1,4,5-trihydroxyanthraquinone, isoscopoletin, scopoletin, dimethyl-7-hydroxychromone, isovanillic acid, 2,5-dimethyl-7- meth oxy chromone, vanillic acid, 2,4-dihydroxybenzaldehyde, glyceryl-1-tetracosanoate and β-sitosterol-*D*-glucoside.

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 14.0 per cent w/w after drying at 105° to constant weight.

Foreign matter, not more than 2.0 per cent w/w.

Total ash, not more than 4.0 per cent w/w.

Ethanol-soluble extractive, not less than 66.0 per cent w/w.

Water-soluble extractive, not less than 67.0 per cent w/w.

2.3.2.5 Chan Khao

"Chan Khao" is a medicinal material that can be derived from at least three different plant species: Chantana (*Tarenna hoaensis* Pit.), Chan (*Diospyros decandra* Lour.), and Chan Hom (*Santalum album* L.) (Srisopon *et al*., 2015; Picheansoonthon *et al*., 2017). Additionally, "Chantana" is also the name of another medicinal substance sold in Thai pharmacies. According to a study by Srisopon *et al*. (2015), most of the "Chan Khao" currently available in the market is derived from the plant scientifically known as *T. hoaensis*.

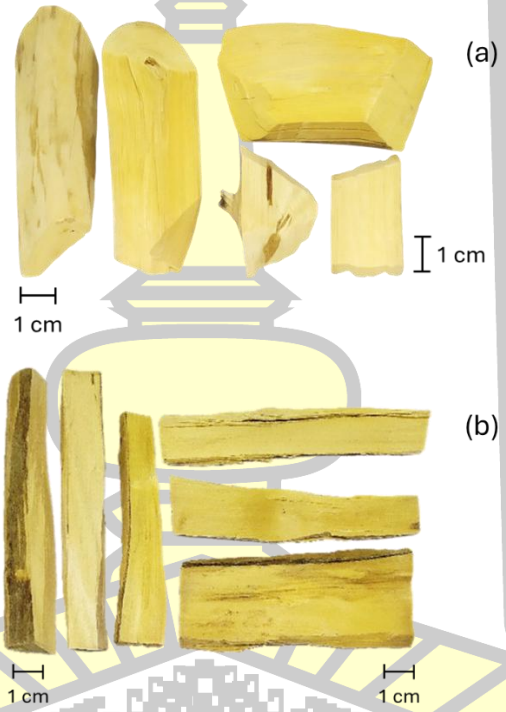

Figure 6 Wood of Chan Khao from different sources (a) Bangkok and (b) Nakhon Pathom.

1) *Tarenna hoaensis* Pit.

Common names: -

Thai names: Chantana, Chan Kao (common), Chan Hom (Rayong), Chan Bai Lek (Prachuap Khiri Khan), and Chan Tabia (Eastern).

Family: Rubiaceae

Part used: Woods

Botanical characteristics:

T. hoaensis is a shrub or small tree, 5-10 m height and oval frutescent crown. Bark is light grey, entire or small fissure nook along stem length. Wood is white or pale yellow. Leaves are simple, obtuse shape or oblong margin, thick lamina, glabrous and shiny surface and flat petiole with stipule. Flowers are inflorescence. Floret is sympetalous. It is white with 4 sepals and 4 petals. Fresh fruit is ellipse with sepals and ripe fruit is black-purple.

Traditional used:

The plant is used as a nourishes blood and heart, supports the fire element, strengthens the nervous system, alleviates internal heat and thirst, treats lung, liver, and bile disorders, reduces high fever, relieves kamdao fever, and combats fatigue (Wutthithammawet, 2002).

Chemical constituents:

The major chemical constituents found in *T. hoaensis* wood are geniposidic acid and α-santalol (Srisopon *et al*., 2015).

2) *Diospyros decandra* Lour.

Common names: Gold Apple

Thai names: Chan (General), Chan luuk hom, Chan khao, Chan In, Chan O (Central).

Family: Ebenaceae

Part used: Woods

Botanical characteristics:

D. decandra is a mid-size tree with 10-20 m height, straight stem and brown-black bark. Leaves are simple, alternate, oblong or ovate shape. The margin is ovate with 2.5-3 cm width and 7-10 cm length. Apex is acute. Base is obtuse or acute. Surface is pubescent. Flower is dioecious. Staminate flowers are inflorescence. Floret is cream color. It has 5 petals and 5 sepals with brown-red hair. Pistillate flower is solitary. It is creamy-white color. It has round ovary with hair. The character of pistillate flower similar to staminate flower but the size is bigger. Fruit is

fleshy berry. It is round or flat shape and green color. Mature fruit is yellow, fragrant and edible. It has 3-4 round-ovate brown seeds

Traditional used:

The plant is used to nourish blood and heart, supports the fire element, strengthens the nervous system, alleviates internal heat and thirst, treats lung, liver, and bile disorders, reduces high fever, relieves kamdao fever, and combats fatigue (Wutthithammawet, 2002).

Chemical constituents:

Chemical constituents found in *D. decandra* stem bark include triterpenes such as 2-Oxo-3β,19α-dihydroxy-24-nor-urs-12-en-28-oic acid, 2-Oxo-3β,19α,22α-trihydroxy-24-nor-urs-12-en28-oic acid, 3-Oxo-2,19α,22α-trihydroxy-24-nor-urs-1,4,12-trien-28-oic acid, 4-Oxo-19α,22α-dihydroxy-3,24-dinor-2,4-secours-12-en-2,28-dioic acid, 19α,22α-Dihydroxy-24-nor-2,3-seco-urs-12-en2,3,28-trioic acid trimethyl ester (Nareeboon *et al*., 2006).

3) *Santalum album* L.

Common names: Sandalwood tree

Thai names: Chan khao, Chan Himalia, Chan Hom (Thai)

Family: Santalaceae

Part used: Woods

Botanical characteristics:

S. album is a hemiparasitic tree up to 20 m tall; glabrous; bark rough, cracked, dark grey or brownish black. Leaves simple, opposite or sub-opposite, elliptic-lanceolate or ovate, coriaceous, 4 to 11 cm long, 1.5 to 3.5 cm wide, apex acute or shortly acuminate, base obtuse, attenuate, margin undulate; petiole slender, about 1 cm long. Inflorescence paniculate, terminal or axillary, pedunculate; peduncle slender, tortuous. Flowers 9 to 15, receptacular, pedicellate, pedicel slender, angular, 1 to 3 mm long; perianth tube campanulate, 4- to 5-lobed, deltoid, 1.5 to 3.5 mm long, 1 to 1.5 mm wide, reflexed, whitish, turning reddish then crimson, stamens 4 or 5, opposite to perianth lobes, filament narrow, slightly dilated at the base, covered with white hair-tuft; ovary semi-inferior, style angular, stigma 3-lobed; nectary concave, deeply 5-lobed, protruding between the perianth segments, brownish, turning reddish

then crimson. Fruit drupe, globose or subglobose, about 1 cm in diameter, green, turning red then purplish black, juicy when ripe; exocarp smooth; endocarp ribbed. Seed 1, globose or obovoid (Department of Medical Sciences, 2021).

Traditional used:

The plant is used as a stomachic stimulant, carminative and to treat intestinal catarrh and colic to stimulate appetite, and aromatic (Wutthithammawet, 2002).

Chemical constituents:

The major constituents were found in the essential oil of *S. album* wood is santalol (90% or more) a mixture of two primary sesquiterpene alcohols, $C_{15}H_{24}O$ viz, α-santalol (b.p.-166-167 ºC) and β-santalol (b.p-177-178 ºC). More than hundred constituents of sandalwood oil in categories of tannins, terpenes, resins and waxes have been reported which include such as hydrocarbons- santene, nortricyclo-ekasantalene, α- and β- santalenes, alcohols-santenol, teresantalol, aldehydes- nor-tricyclo-kasantalal 3,7,8 and the acids α-and β- santalic acids and teresantalic acids.

Two minor components namely cyclosantalal (0.21-2.26%) and isocyclo-santalal (0.11-1.47%) new sesquiterpene aldehyde were reported. Also a new acid– ketosantalic (as methyl ester) & gamma – L –glutamyl-S-(trans-1-propenyl)-L-cysteine sulfoxide, aninteresting natural sulfoxide diastereoisomers, have been isolatedfrom sandal. Some authors also report the presence of Tricyclosantalal, α-santalene, trans-β-bergamotene, β-santalene (S & E), α-curcumine, α-santalol, beta-santalol(S&E), nuciferol, α-santalal and β-santalal (Sindhu *et al*., 2010).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Water, not more than 9.0 per cent v/w.

Foreign matter, not more than 0.5 per cent w/w**.**

Total ash, not more than 1.5 per cent w/w**.**

Ethanol-soluble extractive, not less than 3.0 per cent w/w.

Water-soluble extractive, not less than 3.0 per cent w/w.

Volatile oil, not less than 1.0 per cent v/w.

2.3.2.6 *Clerodendrum indicum* (L.) Kuntze

Common names: Turks Turban, Tube Flower, Skyrocket, Champagne Clerodenrum, Clerodendron

Thai names: Thao yai mom

Family: Lamiaceae

Part used: Roots

Figure 7 Root of *Clerodendrum indicum* (L.) Kuntze

Botanical characteristics:

C. indicum is a subshrub to shrub 1 to 2 m tall; suffrutescent or herbaceous, stoloniferous; stem usually erect, sometimes bent, mostly unbranched, hollowed; branchlets purple to purplish, channelled, smooth. Leaves simple, whorled with 3 to 5 per node or opposite, decussate; leaf blade narrowly lanceolate to oblong-lanceolate, 10 to 21 cm long, 1.3 to 2.5 cm wide, apex short acuminate, base attenuate, margin entire or sinuate, membranous, glabrous, midrib prominent, lateral veins 10 to 12 pairs, sessile or petiolate, petiole up to 8 mm long, with nodal hairs at petiole base. Inflorescence terminal leafy thyrse, 20 to 45 cm long, 10 to 15 cm wide, cyme red, few flowered; peduncle up to 3 cm; bract linear-lanceolate to lanceolate, 1 to 2 cm; bracteole awl-shaped. Flower: calyx 1 to 1.5 cm long, divided 3/4 to base, densely minute round glandular, lobes 5, ovate-lanceolate, 0.8 to 1.5 cm long, 3 to 6 mm wide, apex acute; corolla white, becoming cream-coloured, tube funnelform, curved, 5 to 9 cm, lobes 5, spreading, lanceolate, elliptic or ovate-oblong, 0.8 to 1.5

cm long, 3 to 6 mm wide, apex obtuse; stamens 4, free, long exserted, reddish towards the end; ovary superior, glabrous, 2-loculed, ovules 2 in each locule, style 9.5 to 15 cm long, longer than stamens, stigma 2-branched, 0.3 to 1.5 cm long. Fruit drupe, subglobose to globose, 0.7 to 1.2 cm in diameter, 1- to 4-lobed, dark blue, black when ripe; surrounded at base by accrescent leathery red calyx, up to 3.8 cm in diameter. Seed 1 per locule (Department of Medical Sciences, 2021).

Traditional used:

The root has been used for the treatments of fever, cold, cough, vomiting, abscess and poisonous bites (Wutthithammawet, 2002).

Chemical constituents:

C. indicum roots has been reported to contain 3b-hydroxy-D:B-friedo-olean-5-ene, oleanolic acid-3-acetate, taraxerol, lupeol, (22E)-stigmasta-4,22,25-trien-3-one, stigmasta-4,25-dien-3-one, stigmasta-4,22-dien-3-one, 22-dehydroclerosterol, clerosterol, stigmasterol, 22-dehydroclerosterol-3-O-b-D-glucopyranoside, clerosterol-3-O-b-D-glucopyranoside, stigmasterol-3-O-b-D-glucopyranoside, pectolinarigenin and hispidulin (Somwong *et al*., 2015).

Chemical-physical quality requirements (Department of Medical Sciences, 2021)**:**

Loss on drying, not more than 9.0 per cent w/w after drying at 105° to constant weight**.**

Foreign matter, not more than 2.0 per cent w/w**.**

Acid-insoluble ash, not more than 1.0 per cent w/w.

Total ash, not more than 5.0 per cent w/w**.**

Ethanol-soluble extractive, not less than 1.5 per cent w/w.

Water-soluble extractive, not less than 4.0 per cent w/w.

2.3.2.7 *Dracaena cochinchinensis* (Lour.) S.C.Chen

Common names: Dragon's blood tree, Cinnabaris

Thai names: Chan par, Chan daeng (Thai)

Family: Asparagaceae

Part used: Woods

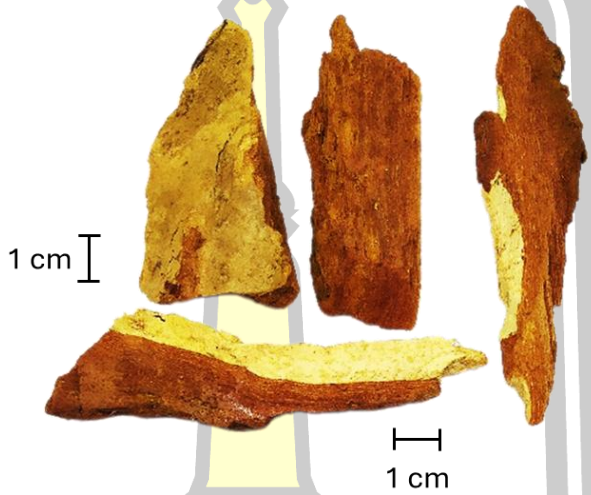

Figure 8 Wood of *Dracaena cochinchinensis* (Lour.) S.C.Chen

Botanical characteristics:

D. cochinchinensis is a tree 5 to 15 m tall; stem more or less branched, internodes short, bark smooth, greyish white, becoming greyish brown with age. Leaves simple, spirally arranged, crowded at the top, sword-shaped, 30 to 100 cm long, 2 to 5 cm wide, apex acute, base completely covering internodes, margin entire, blade leathery. Inflorescence terminal, panicle, more than 40 cm long, drooping; rachis densely papillosepubescent. Flower whitish, in clusters of 2 to 5, pedicel 3 to 6 mm long, articulate dis tally; perianth campanulate, 6 to 8 mm long, tube 1.5 to 2 mm long, lobes 6, 5 to 6 mm long; s tamens 6, inserted in tube of perianth, filament reddish, flat, 0.5 to 0.7 mm wide, anther versatile; ovary superior, 3-loculed, ovule(s) 1 or 2 per locule, style slender, stigma capitate. Fruit berry, subglobose, 8 to 12 mm wide, orange when ripe. Seeds 1 to 3 (Department of Medical Sciences, 2021).

Traditional used:

It has been used as a folk medicine such as relieves internal and external fever, treats all types of fevers, including fevers due to toxins and bile imbalances, alleviates childhood fevers, reduces restlessness, alleviates heat-related fevers and all types of fevers, relieves internal heat and thirst, reduces excessive sweating, relieves coughs caused by toxins and bile, treats fever due to bile disorders, nourishes the heart, reduces inflammation and pain associated with infected and swollen abscesses, aids in wound healing and helps prevent bleeding gums (Wutthithammawet, 2002).

Chemical constituents:

The chemical constituents of *D. cochinchinensis* stem are loureiriol, stillbenoids including 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone, 4,4'-dihydroxy-2,6-dimethoxydihydro- chalcone, 2,4'-dihydroxy-4,6-dimethoxydihydrochalcone, 4'-hydroxy-2,4,6-trimethoxy dihydrochalcone, 4,6,4'-trihydroxy-2-methoxydihydrochal cone, 4,3',5'-trihydroxy- stilbene, 4,3'-dihydroxy-5'- methoxystilbene and 4-hydroxy-3',5'-dimethoxystilbene (Likhitwitayawuid *et al*., 2002).

Other reported constituents are Dracaenogenins A and B, loureirin A, loureirin B and loureirin C (Zheng *et al*., 2006).

Chemical-physical quality requirements (Department of Medical Sciences, 2021)**:**

Loss on drying, not more than 8.0 per cent w/w after drying at 105° to constant weight**.**

Foreign matter, not more than 0.5 per cent w/w.

Total ash, not more than 1.0 per cent w/w.

Ethanol-soluble extractive, not less than 12.0 per cent w/w.

Water-soluble extractive, not less than 1.0 per cent w/w.

2.3.2.8 *Ficus racemosa* L.

Common names: Cluster Fig Tree, Indian Fig Tree

Thai names: Ma duea utum phon, Ma duea chumphon (Central), Ku-sae (Karen-Mae Hong Son), Duea kliang (Central, Northern), Duea nam (Peninsular), Ma duea (Lampang)

Family: Moraceae

Part used: Roots

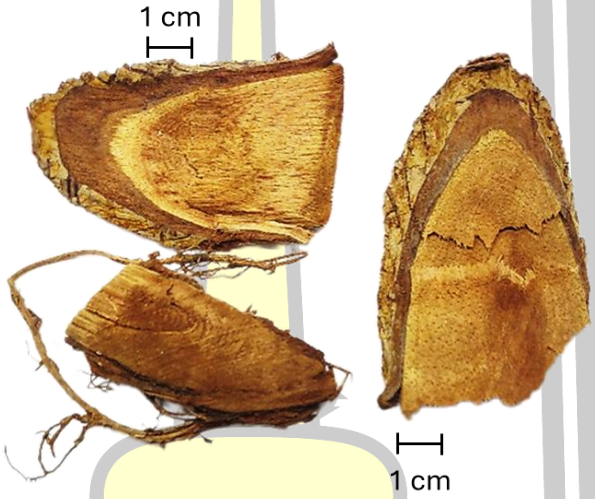

Figure 9 Root of *Ficus racemosa* L.

Botanical discription:

F. racemosa is an evergreen or deciduous tree up to 30 m tall, monoecious; becoming buttressed, often with irregular crown, bark whitish to pinkish brown, smooth when young, cracked when aged, exudating ivory or pinkish latex; young twig solid, finely pubescent. Leaves simple, spirally arranged, elliptic, obovate, oblong, lanceolate or subovate, 5 to 20 cm long, 3 to 10(-12) cm wide, apex acute or acuminate, base obtuse, oblique or cuneate, margin entire, sometimes irregularly dentate or sublobate, coriaceous, sparsely pilose or glabrescent on both surfaces, lateral nerves 4 to 9 pairs, conspicuous on the lower surface; petiole 1.5 to 7.5 cm long, grooved, brownish, minutely hairy, stipule triangular ovate, 1 to 1.5 cm long, about 5 mm wide, brownish, subpersistent or caducous. Inflorescence and fruit known

as syconium or fig, cauliflorous or borne on leafless branches, up to 25 cm long; peduncle 0.3 to 1.2 cm long; basal bracts 3, 1 to 2 mm long, persistent; receptacle subpyriform to globose, puberulous, without lateral bracts. Flowers 3 types: male flower sessile, ostiolar bracts many, tepals 3- to 4-connated, lobes dentate-lacerate, reddish, stamens 1 to 3, pistillode present; female flower sessile or subsessile, tepal as in male, ovary with reddish dots, substipitate, style lateral, glabrous, stigma simple; gall flower dispersed among females, pedicellate, ovary dark red and glabrous. Fruit pyriform or depressed subglobose, 3 to 5 cm in diameter when fresh, 1.5 to 3 cm in diameter when dry, green when young, pinkish to purple-red or orange at maturity, usually streaked, puberulous, apex flat to slightly concave, ostiole about 3 mm in diameter, prominent: internal hairs absent (Department of Medical Sciences, 2021).

Traditional used:

The root has been used for the treatments of fever, toxic fever or severe fever, alleviates internal heat and cools excessive heat, expels fever-related toxins, expels phlegm and stagnant blood, treats seasonal fevers and anti-diarrhea (Wutthithammawet, 2002).

Chemical constituents:

F. racemosa roots has been reported to contain bergenin, bergapten, triterpenes polypodatetraene, α-amyrin acetate, gluanol acetate, lupeol acetate, beta-sitosterol, cycloartenol, and euphorbol (Joseph and Raj, 2010; Jain *et al*., 2013).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 10.0 per cent w/w after drying at 105° to constant weight**.**

Foreign matter, not more than 2.0 per cent w/w**.**

Total ash, not more than 7.0 per cent w/w**.**

Ethanol-soluble extractive, not less than 1.0 per cent w/w.

Water-soluble extractive, not less than 2.0 per cent w/w.

2.3.2.9 *Gymnopetalum chinense* (Lour.) Merr.

Thai names: Kra dom, Ma noi cha, Ma noi hok, Khi ka liam (Thai)

Family: Cucurbitaceae

Part used: Fruits

Figure 10 Fruit of *Gymnopetalum chinense* (Lour.) Merr.

Botanical characteristics:

G. chinense is an annual climber. Stem and branches slender, hispid or villous, glabrescent. Petiole 2-4 cm; leaf blade ovate-cordate, 4-8 × 4-8 cm, membranous, 5-angular or 3-5-lobed; middle lobe larger, triangular, both surfaces scabrous, base cordate, apex acuminate. Plants monoecious. Male flowers solitary, or 3-8 in a raceme; peduncle slender, 10-15 cm; bracts leaflike, 1-2.5 cm, yellow-brown villous, 3-lobed; calyx tube tubular, elongate, ca. 2 cm; segments linear, ca. 7 mm; corolla white; segments oblong-ovate, 15-20 × 10-12 mm, ± villous; filaments ca. 0.5 mm; anthers ca. 7 mm. Female flowers solitary; pedicels 1-4 cm; ovary oblong, 10-12 × ca. 5 mm, yellow-brown villous, acute at both ends; style 5-8 mm; stigmas 3. Fruit orange, oblong-ovoid, 4-5 cm, smooth, 10-ribbed, acute at both ends. Seeds oblong, ca. 7 × 3-3.5 mm, both ends obtuse (Zhengyi *et al*., 2012).

Traditional used:

The plant is used as a tonic for bile, blood and uterus, appetite, treat delirium, hiccups, quench blood poisoning, fever, uterine inflammation and detoxify (Wutthithammawet, 2002).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 9.0 per cent w/w after drying at 105° to constant weight**.**

Acid-insoluble ash, not more than 1.0 per cent w/w.

Total ash, not more than 11.0 per cent w/w.

Ethanol-soluble extractive, not less than 11.0 per cent w/w.

Water-soluble extractive, not less than 26.0 per cent w/w.

2.3.2.10 *Harrisonia 56erforate* (Blanco) Merr.

Thai names: Khontha, Kalantha, Seefan, Seefan khontai, Seefan khontha (Central), Chee, Chee nam, Seetoh, Nam chee (Northern), Mee-chee (Karen-Mae Hong Son)

Family: Rutaceae

Part used: Roots

Figure 11 Root of *Harrisonia 56erforate* (Blanco) Merr.

Botanical characteristics:

H. 56erforate is an erect or straggling shrub, up to 5 m tall, rarely small tree: thorn conical, straight or slightly curved, woody cylindrical-shaped in old stem. Leaves spirally arranged, imparipinnate, with 1 to 7 pairs of leaflets, up to 20

cm long; petiole reddish, 0.5 to 4.5 cm long; rachis narrowly winged; leaflet subsessile, lanceolate or ovate-oblong, 1 to 5 cm long, 0.5 to 2 cm wide, apex acute or obtuse, base acute or obtuse, unequal-sided, margin crenate-serrate, glabrous, sometimes pubescent on nerves. Inflorescence cymose, 8-to 20-flowered, in upper leaf axils and end of twigs, 12 to 20 cm long, rarely solitary flower, peduncle 7 to 11 cm long. Flower purplish red outside, creamy white inside, pubescent; pedicel 3 to 4 mm long, pubescent; calyx small, 4- or 5-lobed, lobe broadly triangular, apex obtuse, pubescent outside, petals 4 to 5, oblong-obovate or lanceolate, 5 to 9 mm long, 2 to 4 mm wide, apex acute, pubescent on both surfaces, stamens 8 or 10, filament 6 to 8 mm long, whitish, glabrous or pubescent, attached to edge of cup-shaped disc; ovary superior, 0.5 to 1 mm long, 4- or 5-loculed, ovule 1 per locule, style 5 to 8 mm long, pubescent, stigma knob-like. Fruit berry, subglobose, 4 to 9 mm long, 1 to 1.5 cm wide, slightly lobed, glabrous, endocarp hard, with 3 to 5 seeds (Department of Medical Sciences, 2021).

Traditional used:

The root has been used for the treatments of diarrhea, dysentery, reduce body heat, detoxify, fever, internal heat and thirst (Wutthithammawet, 2002).

Chemical constituents:

H. 57erforate roots were shown to contain several chromones, limonoids, triterpenoids, and prenylated polyketides including harrisotone A–E, haperforine A-E, haperforine E, 12-desacetylhaperforine A, haperforine C2, haperforine F, haperforine G, foritin, harrisonol A, peucenin-7-methyl ether, *O*-methylalloptaeroxylin, perforatic acid, eugenin, saikochromone A, greveichromenol, perforamone A–D. Other reported constituents are *β*-sitosterol, obacunone, herteropeucenin-7-methyl ether, perforatic acid and harrisonin, harperforatin, harperfolide, and harperamone (Juckmeta *et al*., 2014), pectolinarigenin and perforatinolone (Somwong *et al*., 2015).

Chemical-physical quality requirements (Department of Medical Sciences, 2021)**:**

Loss on drying, not more than 9.0 per cent w/w after drying at 105° to constant weight**.**

Foreign matter, not more than 2.0 per cent w/w**.**

Total ash, not more than 4.0 per cent w/w**.**

Ethanol-soluble extractive, not less than 2.0 per cent w/w.

Water-soluble extractive, not less than 3.0 per cent w/w.

2.3.2.11 *Ligusticum sinense* Oliv. cv. *Chuanxiong*

Common names: Szechuan lovage, Selinum

Thai names: Kot huabua

Family: Apiaceae

Part used: Rhizomes

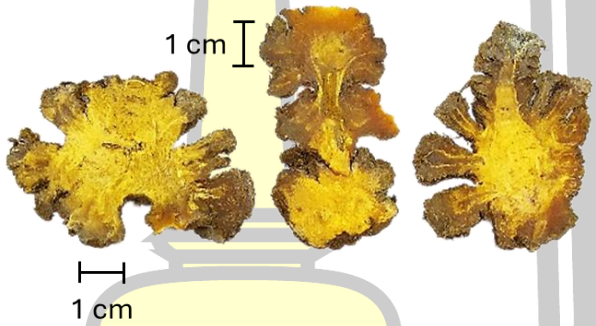

Figure 12 Rhizome of *Ligusticum sinense* Oliv. cv. *Chuanxiong*

Botanical characteristics:

L. sinense is a perennial herb, 30 to 100 cm tall; rhizome thick, apparently swollen at nodes, internodes short; stem erect, striated and branched. Leaves alternate, bipinnate or tripinnate; petiole sheathing at base, clasping the stem; basal one 10 to 20 cm long; blade triangular-ovate in outline, 15 to 20 cm long, 10 to 15 cm wide, ternate to 1- or 2-pinnated, primary pinnae 4 to 6 pairs, proximal pinnae remote, ultimate segments ovate or oblong-ovate, 2 to 3 cm long, 1 to 2 cm wide, margin irregularly serrate, glabrous on both surfaces except for the nerves; cauline leaves similar to basal, reduced, sessile, 1-pinnated. Inflorescence compound umbel, terminal and axillary, 6 to 8 cm across when anthesis, bracts 5 to 6(-10); rays 15 to 30, subequal, 3 to 5 cm; bracteoles 5 to 8, linear, shorter than pedicel, reflexed. Flower

small, white; calyx teeth obsolete; petals obovate, base cuneate; style reflexed. Fruit schizocarp, oblong-ovoid, 2 to 3 mm long, 1.5 to 2 mm wide, dorsal and intermediate ribs prominent, filiform; lateral rib narrowly winged; vittae 1 to 3(-4) in each furrow, 4 to 6 on commissure. Seed smooth (Department of Medical Sciences, 2021).

Traditional used:

Folk doctors used the rhizomes to treat hemorrhoids, carminative, headache, joint pain, bone pain, cold, fever, cough, anemia, menstrual pain, and irregular menstruation (Wutthithammawet, 2002).

Chemical constituents:

L. sinense has about 2% essential oil and has a sour taste of resin. Essential oil contains ligustilide, cnidilid, neocnidilide, senkyunolid, 3-butyl phthalide, butylidenephthalide, sabinene, α-firpene, myrcene, alkaloids, chuanxiongzine and pelolyfine.

Anhydride including 1 -ß-ethyl acrylate-7 -carboxy-carboline, 1 -acetylß-carboline, valine anhydride, trimethylamine, choline, uracil, adenine, adenosine, scopoletin.

Phenolic acids including ferulic acid, sedanonic acid, chrysophanol, 4 -hydroxy-3 -methoxy-phenylethylene, 1 -hydroxyl-1 -(3 -methoxy-4 -hydroxyphenyl)-ethanol, 4 -hydroxybenzoic acid, caffeic acid, vanillic acid, protocatechuic acid, linoleic acid, palmitic acid, 5 ,5 ′-double-*O*-methyl furfural, *n*-hexadecanoic, protocatechuic acid, caffeic acid, 4-pentylcyclohex-3-ene-1α, 2ß-diol.

Phthalide lactones including ligustilide, neoligustilide, senkyunolide, 3-butyl phthalide, butylidenephthalide, 4-hydroxy-3-butyl phthalide, 3-butyl-3-hydroxyl-4,5-dihydro phthalide, 4,7-dihydroxy-3-butyl phthalide, senkyunolide B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, ligustilide glycol, Z,Z´-6,6’-7,3’α-diligustiide,Z-6,8’,7,3’-diligustilide, Z´-3,8-Dihydro-6,6′,7,3′α-diligustilide, Chuanxiongnolide A, Chuanxiongnolide B, Ligusticoside A, Alkaloids, phenolic acids, ceramides, cerebrosides.

Other constituents including Spathulenol, ß-Sitosterol, Sucrose, A glycerin ester (contained two linoleic acids and a palmitic acid), 5-Methylol-6-endo-3′-methoxy-4’-hydroxyphenyl-8-ox-bis-(3, 2, 1)-octa-ene-2-ketone, Senkyunon, Xiongterpene, (4S)-p-Menth-1-ene4,7-diol, Aromadendrane-4α, 10 ß-diol,

Aromadendrane-4ß, 10α-diol, Aromadendrane-4ß, 10ß-diol, Augustic acid, Apigenin, Quercetin and Cosmosiin.

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Water, not more than 12.0 per cent v/w.

Foreign matter, not more than 2.0 per cent w/w.

Acid-insoluble ash, not more than 2.0 per cent w/w.

Total ash, not more than 6.0 per cent w/w.

Ethanol-soluble extractive, not less than 18.0 per cent w/w.

Water-soluble extractive, not less than 35.0 per cent w/w.

2.3.2.12 *Mesua ferrea* L.

Common names: Iron wood, Ceylon Ironwood, Na Tree, Diya Na, Nagchampa, Nagacuram, Nagasari, Ironwood Tree, Indian Rose Chestnut, Poached Egg Tree, Cobra's Saffron, Penaga Lilin, Penaga, Lenggapus

Thai names: Bunnak (General), Ka-ko (Karen-Mae Hong Son), Kam-ko (Shan-Mae Hong Son), Pa-na-kho (Malay-Pattani), Saraphi doi (Chiang Mai)

Family: Calophyllaceae

Part used: Flowers

Figure 13 Flower of *Mesua ferrea* L.

Botanical characteristics:

M. ferrea is a Tree up to 30 m tall; trunk upright, cylindrical, often buttressed at base; young twig slender, exuding aromatic white resin when wounded. Leaves simple, opposite, elliptic, oblong or lanceolate, 6 to 13 cm long, 1 to 4 cm wide, apex acute, base acute or obtuse, margin entire, blade leathery, midrib faint and depressed on both surfaces, lateral veins numerous, very fine, almost invisible, lower surface whitish glaucous; young leaves reddish to pinkish; petiole 0.4 to 1.2 cm long. Flower solitary or fascicled, fragrant, axillary, peduncle 0.8 to 2.3 cm long, slender, sepals 4, light green, orbicular, 1 to 1.5 cm long, arranged in 2 rows, outer pair small, inner pair larger, densely velvety puberulous outside, fleshy, petals 4, white or pinkish, obovate or obcordate, 1.5 to 4 cm long, base cuneate, margin curled, brown or purple s triations, caducous; stamens numerous, anthers orange to golden yellow, linear, 0.4 to 1 cm long; ovary superior, ovoid, up to 5 mm long, 2-loculed, each locule 2-ovuled, style 1, about 1 cm long, stigma peltate. Fruit ovoid to ellipsoid, 2.5 to 3.5 cm long, with conical apex, striated, sepal enlarged up to 4 cm long, persistent, dark orange or purplish brown; pericarp tough. Seeds 1 to 4, up to 2.4 cm long, woody, smooth, glossy, brown, oily (Department of Medical Sciences, 2021).

Traditional used:

Folk doctors used the flowers of *M.ferrea* use to treat astringent, carminative, blood tonic and cardiac tonic (Wutthithammawet, 2002).

Chemical constituents:

Zhang *et al*. (2019) reported constituents of *M. ferrea* flower are flavonoid and phenolic compounds including *O*-rhamnoside, quercitrin, quercetin, rhusflavanone, mesuaferrone B, 5,6,6'-trihydroxy[1.1'-biphenyl]-3,3'-dicarboxylic acid, 3-amino-4-hydroxybenzoic acid, procatechuic acid, gallic acid and procatechuic acid ethyl ester.

Other reported constituents of *M. ferrea* stamen are α-amyrin, β-amyrin, β-sitosterol, mesuaferrones A, mesuaferrones B, mesuanic acid, 1,5-dihydroxyxanthone, euxanthone 7-methyl ether and β-sitosterol (Chahar *et al*., 2013).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 11.0 per cent w/w after drying at 105° to constant weight.

Foreign matter, not more than 2.0 per cent w/w.

Acid-insoluble ash, not more than 1.5 per cent w/w.

Total ash, not more than 5.0 per cent w/w.

Ethanol (80 per cent)-soluble extractive, not less than 22.5 per cent w/w.

Water-soluble extractive, not less than 12.5 per cent w/w.

2.3.2.13 *Nelumbo nucifera* Gaertn.

Common names: Lotus, Sacred lotus, Egyptian lotus

Thai names: Bua luang, Bua, Ko kra not, Bua ubon, Bua chat khao, Bua chat chomphu, Bua chat si chomphu, Buntharik, Puntharik, Pathum, Patthama, Sattabongkot, Sattabut

Family: Nelumbonaceae

Part used: Stamens

Figure 14 Stamen of *Nelumbo nucifera* Gaertn.

Botanical characteristics:

N. nucifera is a perennial aquatic herb with milky latex and stout creeping rhizomes. Leaves simple, alternate, arising above the water surface, circular, 10 to 100 cm in diameter, margin entire or slightly undulate, papery, greyish green below, green on the upper, peltate, veins radiate from its centre in all directions, become forked near the margin; petiole terete, stout, up to 1 m or more long, about 1 cm in diameter, smooth or prickly. Flower solitary, large and showy, on thick peduncle rising several centimetres above the leaves; flower bud ovate, acute, 5 to 8 cm long, flower hemispheric at anthesis, 8 to 25 cm in diameter, perianth with 4 or 5 outermos t sepals, elliptic to ovate, 1.5 to 5 cm long, 0.8 to 3.5 cm wide, free, green or pinkish green, to white or pink with age, petal-like, incurved; petals 5 to numerous, elliptic. 4 to 15 cm long, 2 to 8 cm wide, obtuse, incurved, white to dark pink or reddish; stamens numerous, 2.2 to 4.5 cm long, anther linear, 1.5 to 2 cm long, up to 2 mm wide, golden yellow, with white clavate connective appendage up to 7 mm long at the apex, incurved; ovary apocarpous, 12 to 30 free carpels, receptacle expanded to cone-shape, flat upper surface, 3 to 5 cm long, about 2.5 cm wide with individual carpels sunken into it; style short. Fruit indehiscent nutlets, embedded in an accrescent spongy conical receptacle, 9 to 13 cm long, 4 to 7 cm wide, nutlet ovoid, 1.5 to 2 cm long; pericarp thick, hard, brownish or greyish black when dry, endocarp thin, whitish pulp. Seed 1 (Department of Medical Sciences, 2021).

Traditional used:

Folk doctors used the stemen of *N. nucifera* to treat cardiac tonic, vertigo and faintness (Wutthithammawet, 2002).

Chemical constituents:

The chemical constituents of *N. nucifera* stamen are linalool, luteolin glucoside, dehydroanonaine, anonaine, armepavine, kaempferol-3-*O*-*β*-D-glucuronide, *β*-sitosterol, asimilobine, demethylcoclaurine, lirinidine, dehydro nuciferine, isoliensinine, liensinine, quercetin, liriodenine, dehydroemerine, isoquercitrin (hirsutrin), nornuciferine, *N*-methylasimilobine, *N*-methylcoclaurine, *N*-methylisococlaurine, *N*-norarmepavine, roemerin, kaempferol, kaempferol-3-*O*-*α*-L-rhamnopyranosyl-1(1→6)-*β*-D-glucopyranoside, kaempferol-3-*O*-*α*-L-rhamnopyra nosyl-(1→2)-*β*-D-glucopyranoside, Kaempferol-3-*O*-*β*-D-glucopyranoside,

kaempferol-3-O-β-D-glucoronopyranoside (Paudel and Panth, 2015), β-cyclogeraniol diglycoside, cycloartenol, *p*-hydroxybenzoic acid, vanilloloside and 5́-*O*-methyladenosine (Jung *et al*., 2010).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 12.0 per cent w/w after drying at 105° to constant weight.

Foreign matter, not more than 2.0 per cent w/w.

Acid-insoluble ash, not more than 1.0 per cent w/w.

Total ash, not more than 6.0 per cent w/w.

Water-soluble extractive, not less than 10.5 per cent w/w.

2.3.2.14 *Phyllanthus emblica* L.

Common names: Emblic myrabolan, Malacca tree, Indian gooseberry, Amla, Amalaki

Thai names: Ma kham pom (General), Kan-tot (Khamer-Chanthaburi), Kam thuat (Ratchaburi), Mang-lu, San-ya-sa (Karen-Mae Hong Son)

Family: Phyllanthaceae

Part used: Fruits

Figure 15 Fruit of *Phyllanthus emblica* L.

Botanical characteristics:

P. emblica is a small or medium-sized tree, up to 20 m tall, deciduous, with crooked trunk and spreading branches; bark greenish grey, peeling off in conchoidal flakes; branchlets glabrous or finely pubescent, 10 to 20 cm long. Leaves imbricate when young, subsessile, 0.5 to 2.5 cm long, 1.5 to 5.5 mm wide, closely set along the branchlets, distichous, light green, glabrous, narrowly linear, obtuse, having appearance of pinnate leaves, stipule minute, ovate, finely acute. Flower small, monoecious, apetalous, greenish yellow, in axillary fascicles on the leaf-bearing branchlets, often on the naked portion below the leaves, with fimbricate bracts at the base. Male flowers numerous, on short slender pedicel; calyx-lobes 6, oblong, obtuse, 1.2 mm long, anthers 3, filaments united in a short central column, disk-glands 6, alternating with the calyx-segments. Female flowers few, subsessile or sessile, calyx as in the male, ovary 3-celled, half immersed in the lacerate, cup-shaped disc, style connate at the base, stigmas 3, bilobed, lobes dilated, recurved. Fruit sessile, 1.3 to 2.7 cm in diameter, fleshy, globose or depress globose, with 6 longitudinal faint lines, glabrous, lucid, pale yellow, endocarp of triangular cocci, bony, dehiscent, with 3 short bundles of vascular tissue at the base. Seeds 6, trigonous (Department of Medical Sciences, 2021).

Traditional used:

Folk medicine uses the fruits as an expectorant and moisten the throat, used to treat cough, sore throat, hemorrhoids, diarrhea, dysentery, and dyspepsia (Wutthithammawet, 2002).

Chemical constituents:

Ascorbic acid (vitamin C) is the most common constituent of the *P. emblica* fruit. Beside this, other phytochemicals isolated from this plant include tannins, flavonoids, phenolics, terpenoids, sterols, alkaloids, minerals, vitamins, amino acids, fatty acids, glycosides etc.

Emblicanin A and emblicanin B, pedunculagin and punigluconin are the major tannins reported from this plant (Gaire and Subedi, 2014). Other constituents of tannins are chebulagic acid, chebulinic acid (Ellagitannin), punigluconin, emblicanin A-B, chebulagic acid (Benzopyran tannin), corilagin (Ellagitannin), geraniin (Dehydroellagitannin), ellagotannin, ellagitannins,

pedunculagin, 1-*O*-galloyl-b-D-glucose, 3-ethylgallic acid (3-ethoxy-4,5-dihydroxy benzoic acid), trigallayl glucose, 3,6-di-*O*-galloyl-D-glucose, isostrictiniin, 1,6-di-O-galloyl-b-Dglucose (Ahmad *et al*., 2021), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose, carpinusnin, chebulanin, corilagin, furosin, geraniin, isostrictinin, isocorilagin, isomallotusinin, mallotusinin, mallonin neochebulagic acid, phyllanemblinin A, phyllanemblinin B, phyllanemblinin C, phyllanemblinin D, phyllanemblinin E, phyllanemblinin F, phyllanthunin, punicafolin, putranjivain A, putranjivain B, tercatain, epicatechin-(4β → 8)-epigallocatechin, phyllemtannin, prodelphinidin B1, prodelphinidin B2 and prodelphinidin B-2,3´-O-gallate (Taya *et al*., 2021).

Phenolic and flavonoid compounds isolated from *P. emblica* fruit such as chebulic acid, ellagic acid, ethyl gallate, flavogallonic acid bislactone, gallic acid, methyl gallate, gallic acid 3-O-(6´-O-galloyl)-β-D-glucoside, pyrogallol, syringaldehyde, vanillic acid, 3-ethylgallic acid, methyl-4-hydroxybenzoate, kaempferol, isoquercitrin, quercetin, apigenin-7-O-(6´´-butyryl-β-glucopyranoside), (-)-epiafzelechin, (-)-epigallocatechin, (+)-gallocatechin, (-)-epicatechin and avicularin (Taya *et al*., 2021).

Terpenoids such as 3,20-dioxo-dinorfriedelane, lupeol, phyllaemblic acid, phyllaemblic acid B, C, phyllaemblicin A, B, C, D, E, F, G1, G2, G3, G4, G5, G6, G7, G8 and 4´-Hydroxyphyllaemblicin B (Taya *et al*., 2021).

Sterols such as β-sitosterol, 7-ketositosterol, 7α-hydroxysitosterol, 7α-acetoxysitosterol, 7β-ethoxysiterol, daucosterol, stigmast-4-en-3-one, stigmast-4-ene-3β,6α-diol and β-daucosterol (Taya *et al*., 2021).

Alkaloids such as (2S)-1-[2-(furan-2-yl)-2-oxoethyl]-5-oxopyrrolidine-2-carboxylate, 5-hydroxy-isoquinoline (Taya *et al*., 2021), phyllantidine and phyllantine (Ahmad *et al*., 2021).

Fatty acids such as linoleic acid, stearic acid, palmitic acid, linolenic acid, myristicacid, and oleic acid (Ahmad *et al*., 2021).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 9.0 per cent w/w after drying at 105° to constant weight.

Acid-insoluble ash, not more than 1.0 per cent w/w.

Total ash, not more than 4.0 per cent w/w.

Ethanol-soluble extractive, not less than 16.0 per cent w/w.

Water-soluble extractive, not less than 26.0 per cent w/w.

Tannins content, not less than 20.0 per cent w/w. Use 4 g of Emblic Myrobalan, in fine powder, accurately weighed.

2.3.2.15 *Pinus kesiya* Royle ex Gordon

Common names: Khasiya Pine

Thai names: Son (General), Kia plueak bang (Chiang Mai), Pak lom (Chaiyaphum), Pak (Shan - Mae Hong Son, Phetchabun), Kia plueak daeng (Northern), Chuang (Northern, Northeastern), Son khao (Central), Chiang bang (Karen- Mae Hong Son)

Family: Pinaceae

Part used: Woods

Figure 16 Wood of *Pinus kesiya* Royle ex Gordon

Botanical characteristics:

P. kesiya is a large tree up to 45 m tall with a bole free of branches for 15-20 m and up to 100 cm in diameter, a thick, reticulately and deeply fissured bark, and often pruinose branchlets with a waxy bloom. Needles in bundles of (2-)3(-4), very slender and flexible, (10-)12-21(-25) cm long, bright grass green. Mature

cones up to 3 together, pendulous, ovoid to ovoid-conical, (4-)5-8(-10) cm long, subsessile or on a short stalk up to 10 mm long; apophysis beaked or flattened with a short, blunt, deciduous umbo. Seed small with a short, 1.5-2.5 cm long wing.

Traditional used:

Folk doctors used the decoction of roots to treat hemorrhoids, carminative, headache, joint pain, bone pain, cold, fever, cough, anemia, menstrual pain and irregular menstruation (Wutthithammawet, 2002).

Chemical constituents:

The major chemical constituents found in the essential oil of *P. kesiya* are β-pinene (38.9%), α-pinene (21.8%), myrcene (11.6%), germacrene D (7.9%), β-caryophyllene (2.8%) and limonene (2.2%) (Govindarajan *et al*., 2016).

Chemical-physical quality requirements (Soonthornchareonnon and Ruangwises, 2008):

Loss on drying, not more than 5.04 per cent w/w after drying at 105° to constant weight.

Acid-insoluble ash, not more than 0.07 per cent w/w.

Total ash, not more than 0.39 per cent w/w.

Ethanol-soluble extractive, not less than 31.71 per cent w/w.

Water-soluble extractive, not less than 1.19 per cent w/w.

Hexane-soluble extractive, not less than 30.22 per cent w/w.

Dichloromethane-soluble extractive, not less than 46.68 per cent w/w.

2.3.2.16 Samo Thet

Samo Thet is a medicinal material imported from India and sold in Thai pharmacies. The fruits of Samo Thet are smaller than those of Samo Thai (*Terminalia chebula* Retz.) found in Thailand, but the fruits have more pronounced ridges compared to Samo Thai. In some texts, it is also referred to as Samo Chit (Picheansoonthon *et al*., 2017).

Thai names: Samo Thet

Botanical names: *Terminalia* sp.

Family: Combretaceae

Part used: Fruits

Figure 17 Fruit of Samo Thet

Botanical characteristics:

Samo Thet, the scientific name of this herb, has not been clearly identified. It is only known to be derived from plants in the genus *Terminalia* of the family Combretaceae. Deciduous trees, to 25 m high, bark 5-6 mm thick, surface dark brown to black, fissures shallow, vertical, exfoliating in thick scales; blaze yellowish-brown; young shoots densely pubescent; branchlets brownish or greyish, glabrous. Leaves simple, opposite to alternate, exstipulate; petiole 12-25 mm long, stout, grooved above, pubescent, 2 sessile glands at the top; lamina 9.5-28 x 4-13 cm, ovate, elliptic, obovate or elliptic-obovate, base round, obtuse, oblique or subtruncate, apex acute, acuminate, obtuse or apiculate, margin entire, glabrous above tawny villous

beneath, coriaceous; lateral nerves 6-12 pairs, pinnate, ascending, prominent, arched towards the margin, intercostae reticulate, prominent. Flowers bisexual, greenish-white, 5-6 mm across, in terminal and axillary spikes with offensive smell; bracts 2-3 mm long; calyx tube 1.5-2.5 × 0.8-1 mm, villous, constricted above the ovary, lobes 5, creamy, triangular, 1.5 mm; petals 0; stamens 10 in 2 rows; filaments 4-6 mm; disc 5-lobed, villous; ovary 2 mm long, inferior, densely villous, 1-celled; style 5 mm, subulate; stigma terminal. Fruit a drupe 3-4 x 2-2.5 cm, obovoid, woody, obscurely 5 angled, glabrous, greenish-yellow; seed one (Sasidharan, 2008). Dried fruit, ellipsoid to obovoid or sometimes ovoid, (2–) 3–5 × (1.5–) 2–3 cm, 5-angled and ribbed or ridged, glabrous, rounded to emarginated or occasionally apiculate at apex, often narrowing at base into a stipe (up to 5 mm long), outline of cross section undulate, 5-pointed, star-like (Chakrabarty *et al*., 2019).

Traditional used:

Folk medicine uses the fruits as a mild laxative with stimulating the excretion and then stopping by itself, expectorant and moisten the throat, use to treat cough, sore throat, and improve blood circulation (Wutthithammawet, 2002).

Chemical constituents:

The major chemical constituents of the *T. chebula* fruit are tannins, phenolic acids and flavonoids. Main compounds among tannins (hydrolysable tannins) are terflavin A, terchebulin, punicalagin, chebulagic acid (CA), chebulinic acid, corilagin, casuarinin, chebulanin, tercatain, gemin, tellimagrandin I, punicacortein C-D, chebulic acid, methyl chebulagate, neochebulagic acid, eschweilenol C, phyllanemblinin E-F.

The main constituents of phenolic acids are gallic acid, digallic acid, ellagic acid, ethyl gallate, methyl gallate, 4-*O*-methylgallic acid, ferulic acid, vanillic acid, *p*-coumaric acid, eugenol, caffeic acid, melilotic acid, phloroglucinol and pyragallol.

The main constituents of flavonoids include rutin, quercetin, luteolin, isoquercetin, 3-methoxy quercetin and 3,4́-dimethoxy quercetin (Nigam *et al*., 2020).

Chemical-physical quality requirements (Jirawattanapong *et al*., 1997):

Loss on drying, not more than 8.89 ± 1.59 per cent w/w after drying at 105° to constant weight.

Acid-insoluble ash, not more than 0.10 ± 0.03 per cent w/w.

Total ash, not more than 2.46 ± 0.23 per cent w/w.

Ethanol-soluble extractive, not less than 45.61 ± 2.34 per cent w/w.

Water-soluble extractive, not less than 40.22 ± 2.81 per cent w/w.

2.3.2.17 *Terminalia bellirica* (Gaertn.) Roxb.

Common names: Chebulic myrobalan

Thai names: Samo phi phek, Samo haen (Central), Si-ba-du (Karen-Chiang Mai), Lan (Chiang Rai), Sa-khu (Karen-Mae Hong Son), Haen, Haen khao, Haen ton (Northern)

Family: Combretaceae

Part used: Fruits

Figure 18 Fruit of *Terminalia bellirica* (Gaertn.) Roxb.

Botanical characteristics:

T. bellirica is a large tree up to 50 m tall, deciduous, up to 2 m in girth, usually with large buttresses; bark blackish, brittle, longitudinally fissured and cracked, thick, cut yellow. Leaves coriaceous, obovate, 4 to 20 cm long, 2 to 15 cm wide, glabrous, nerves widely spaced, 6 to 8 pairs, petiole glabrous, 3 to 9 cm long,

usually with a pair of dotted glands at about the middle or near leaf-base, occasionally inconspicuous or hardly observed when dry. Inflorescence spike or raceme, 3 to 15 cm long, often crowded at the ends of branchlets without leaves so as to form terminal panicles, flowers andromonoecious, male on the upper part, tomentose; calyx 1 to 2 mm long, 4 to 5 mm in diameter, calyx segments recurved, deltoid, 1.5 mm long; stamen 3 to 3.5 mm long, ovary ellipsoid, 2 to 3.5 mm long, 1.5 to 3 mm wide, s tyle 4 mm long; disc densely, rusty villous. Fruit drupe, subglobose to broadly ellipsoid, 2 to 3.5 cm long, 1.5 to 3 cm wide, slightly 5-ridged, densely velvety pubescent, very hard when dry. Seed 1, ellipsoid, rough, 1.2 cm long, 0.5 cm wide (Department of Medical Sciences, 2021).

Traditional used:

Folk medicine uses the fruits as a mild laxative with stimulating the excretion and then stopping by itself, expectorant and moisten the throat, use to treat fever, cough, sore throat, hemorrhoids, diarrhea, dysentery, ascites and eye disease (Wutthithammawet, 2002).

Chemical constituents:

The chemical constituents of *T. belerica* fruits showed the presence of ellagic acid, ethyl gallate, galloyl glucose, lignans (termilignan and thannilignan), chebulagic acid, phyllemblin, 7-hydroxy 3’4’ (methylenedioxy) flavone, anolignan B, β-sitosterol, glucoside (bellericanin), gallo-tannic acid, benzoyl-β-D-(4′→10″ geranilanoxy)–xylopyranosides, mannitol, glucose, fructose, rhamnose, coloring matter, resins, and greenish yellow oil (Ansari *et al*., 2016; Deb *et al*., 2016).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 11.0 per cent w/w after drying at 105° to constant weight.

Acid-insoluble ash, not more than 0.6 per cent w/w.

Total ash, not more than 5.0 per cent w/w.

Ethanol-soluble extractive, not less than 17.0 per cent w/w.

Ethanol (70 per cent)-soluble extractive, not less than 29.0 per cent w/w.

Water-soluble extractive, not less than 24.0 per cent w/w.

Tannins content, not less than 16.0 per cent w/w. Use 4 g of Belleric Myrobalan, in powder, accurately weighed.

2.3.2.18 *Terminalia chebula* Retz.

Common names: Chebulic myrobalan

Thai names: Samo thai, Ma-nae (Karen-Chiang Mai), Mak-nae (Karen-Mae Hong Son), Sommo (Northeastern)

Family: Combretaceae

Part used: Fruits

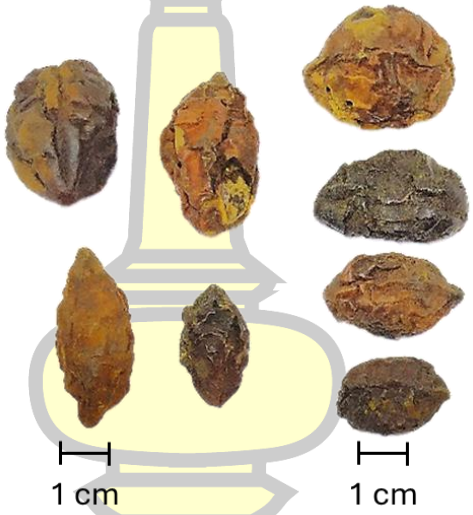

Figure 19 Fruit of *Terminalia chebula* Retz.

Botanical characteristics:

T. chebula is a medium-sized or large tree up to 30 m tall, up to 1.3 m in girth; bark rough, scaly; shoots and young leaves usually rusty villous. Leaves simple, opposite, coriaceous, broadly ovate to ovate- elliptic, 8 to 15 cm long, 6 to 10 cm wide, glabrescent, nerves obscure above, slightly raised and usually brownish pubescent beneath, apex acute or abruptly acuminate, base cuneate, slightly cordate or rounded: petiole 1 to 3 cm long, glabrous or sparsely pubescent with a pair of nodular glands near leaf-base. Inflorescence axillary or terminal panicle, usually with 3 to 6 spikes, spike 3 to 6 cm long; rachis pubescent; flower 2 mm long, 3 to 4 mm in

diameter; bract nearly glabrous, 1.5 to 2 mm long; calyx outside glabrous, inside densely villous, calyx-segment triangular, stamen 3 to 4 mm long; ovary glabrous, ovoid, about 1 mm long, style glabrous, 2.5 to 3 mm long, disc lobed, densely villous. Fruit drupe, glabrous, subglobose to ellipsoid, 2.5 to 5 cm long, 1.5 to 2.5 cm wide, usually smooth or frequently 5-angulate ridged, wrinkled, turning blackish when dry. Seed 1, rough, ellipsoid, 1.5 to 2 cm long, 0.5 to 0.7 cm wide, without ridges (Department of Medical Sciences, 2021).

Traditional used:

Folk medicine uses fruits as a mild laxative that stimulates excretion and then stops by itself, used to treat fever for mucous, phlegm, colic, poisoning from fever, cough, sore throat, lymphatic drainage, and vomiting. It is an astringent, cures diarrhea and dysentery. It also nourishes the body (Wutthithammawet, 2002).

Chemical constituents:

The major chemical constituents of the *T. chebula* fruit are tannins, phenolic acids and flavonoids. Main compounds among tannins (hydrolysable tannins) are terflavin A, terchebulin, punicalagin, chebulagic acid (CA), chebulinic acid, corilagin, casuarinin, chebulanin, tercatain, gemin, tellimagrandin I, punicacortein C-D, chebulic acid, methyl chebulagate, neochebulagic acid, eschweilenol C, phyllanemblinin E-F.

The main constituents of phenolic acids are gallic acid, digallic acid, ellagic acid, ethyl gallate, methyl gallate, 4-*O*-methylgallic acid, ferulic acid, vanillic acid, *p*-coumaric acid, eugenol, caffeic acid, melilotic acid, phloroglucinol and pyragallol.

The main constituents of flavonoids include rutin, quercetin, luteolin, isoquercetin, 3-methoxy quercetin and 3,4́-dimethoxy quercetin (Nigam *et al*., 2020).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 11.0 per cent w/w after drying at 105° to constant weight.

Acid-insoluble ash, not more than 0.6 per cent w/w.

Total ash, not more than 3.5 per cent w/w.

Ethanol-soluble extractive, not less than 20.0 per cent w/w.

Ethanol (70 per cent)-soluble extractive, not less than 29.0 per cent w/w.

Water-soluble extractive, not less than 28.0 per cent w/w.

Tannins content, not less than 14.0 per cent w/w. Use 4 g of Chebulic Myrobalan, in powder, accurately weighed.

Foaming index, not less than 170, when determined by the following method.

2.3.2.19 *Tiliacora triandra* (Colebr.) Diels

Common names: Bamboo grass, Ya-nang

Thai names: Ya-nang (General), Choi Nang (Chiang Mai), Thaowan-Khiao (Central), Yat Nang, Wan Yo (Surat Thani), Thao Roi Pla, Puchao Khao Khiao, Thao Yanang, Yanang Khao, Yan Nang, Ya Phakhini

Family: Menispermaceae

Part used: Roots

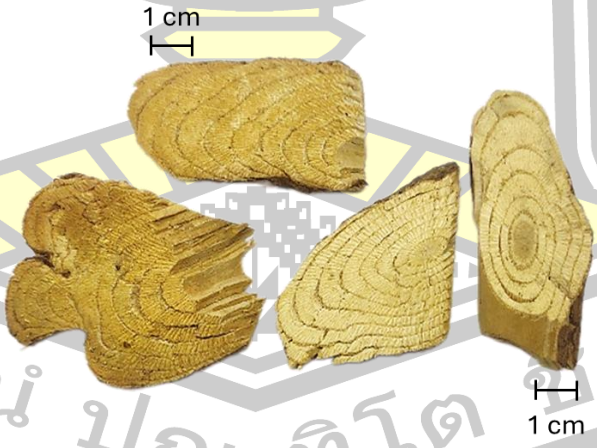

Figure 20 Root of *Tiliacora triandra* (Colebr.) Diels

Botanical characteristics:

T. triandra is a Climber or straggling shrub, stem usually slender, glabrous or pubescent, striate. Leaves simple, alternate, ovate, elliptic or lanceolate, 5 to 11(-17) cm long, 2 to 6(-10) cm wide, apex obtuse, acute or acuminate, base attenuate, obtuse or subcordate, margin entire, glabrous, papyraceous, pinnately nerved, often with steeply ascending basal nerves, seemingly sub-palmately nerved, midrib of lower surface rugulose near base; petiole 0.5 to 2 cm long, rugulose. Inflorescence paniculate, 1- to few-flowered, axillary or cauliflorous, 2 to 8(-17) cm long; peduncle about 5 mm long. Male flower yellowish, about 2 mm in diameter, sepals 6,2seriate, 3 outer ones smaller, 3 inner ones broadly elliptic, about 2 mm long, subglabrous; petals 3 or 6, minute glabrous, stamens 3, clavate, about 2 mm long. Female flower reddish; sepal as in male flower, petals 6, oblongelliptic, about 1 mm long, carpels 8 or 9, less than 1 mm long, borne on short gynophore, glabrous, stigma sessile; staminode absent. Fruit drupe, obovoid, 0.7 to 1 cm long, 6 to 7 mm wide, subcompressed, glabrous, green when young becoming yellowish or reddish when ripe; stalk 3 to 4 mm long; endocarp transversely and irregularly ridged. Seed 1, horseshoe-shaped, curved, rugulose (Department of Medical Sciences, 2021).

Traditional used:

The root has been used for the treatment of drunkenness, detoxification, chickenpox, rash, toxic or severe fever and constipation (Wutthithammawet, 2002).

Chemical constituents:

T. triandra roots have been reported to contain alkaloids including tiliacolinine, tiliacorine, nortiliacorinine A, tiliacorinin 2,-N-oxide tiliandrine , tetraandrine and D-isochondendrine (Somwong *et al*., 2015).

Chemical-physical quality requirements (Department of Medical Sciences, 2021)**:**

Loss on drying, not more than 9.0 per cent w/w after drying at 105° to constant weight**.**

Foreign matter, not more than 2.0 per cent w/w**.**

Total ash, not more than 8.0 per cent w/w**.**

Ethanol-soluble extractive, not less than 4.0 per cent w/w.

Water-soluble extractive, not less than 6.0 per cent w/w.

2.3.2.20 *Tinospora crispa* (L.) Hook. f. & Thomson

Common Name: Petawali, Makabuhai, Liane-quinine

Thai names: Bora phet (General), Khruea khao ho, Chung ching (Northern), Chettamun nam (Nongkhai), Tua chettamun yan, Thao hua duan (Saraburi), Hang nu (Saraburi, Ubon Ratchathani)

Family: Menispermaceae

Part used: Stems

Figure 21 Stem of *Tinospora crispa* (L.) Hook. f. & Thomson

Botanical characteristics:

T. crispa is a woody climber with tuberous roots; young stems smooth, older ones very prominently tuberculate with exceedingly bitter sap, aerial root filiform, very long. Leaves broadly ovate to orbicular, 5 to 14 cm long, 4 to 12 cm wide, apex acuminate, base cordate, palmately 5- to 7-nerved at the base; petiole 5 to 15 cm long. Inflorescence pseudoracemose, not coetaneous with the leaves. Male inflorescence very slender, a few in groups. Male flower small, on filiform pedicel; sepals pale green, 3 outer ones ovate, 3 inner ones obovate; petals 3, stamens 6. Female inflorescence similar to male onebut shorter. Female flower with sepals and petals as in male; s taminodes 6; carpels 3. Drupe orange, ellipsoid, up to 2 cm long (Department of Medical Sciences, 2021).

Traditional used:

The plant is used for the treatment of fever, smallpox, appetite, indigestion, stomach disease, hiccups, malaria, thirst, aphthous ulcer, cholera, and diarrhea, reduce blood sugar, tonic for bile and the body. It also helps to sweat (Wutthithammawet, 2002).

Chemical constituents:

Ahmad *et al*. (2016) reported constituents *T. crispa* stem are flavonoids including apeginin, diosmetin, genkwanin, luteolin 4′-methyl ether 7-glucoside, genkwanin 7-glucoside, and luteolin 4′-methyl ether 3′-glucoside.

The triterpenoids, cycloeucalenol and cycloeucalenone were also isolated from the stem. Diterpenoids and their glycosides are the main terpenoids in *T. crispa* and the most common are the clerodane-type furanoditerpenoids. Diterpenoids, tinocrispol A, borapetol A, borapetols B, were isolated from the ethanol extract of *T. crispa* vines.

Diterpenoid glycosides, 2-*O*-lactoylborapetoside B, 6′-*O*-lactoylborapetoside B, borapetoside A, borapetoside B, borapetoside C, borapetoside D, borapetoside E, borapetoside F, borapetoside G, borapetoside H, rumphioside A, rumphioside B, rumphioside C, rumphioside F, rumphioside I, syringin, columbin, tinocrisposide A, tinocrisposide B, tinocrisposide C, and tinocrisposide D were isolated from the methanol extract of *T. crispa* (Chung, 2011; Lam *et al*., 2012).

Alkaloids are important secondary metabolites from the plant. The most common alkaloids found in *T. crispa* are aporphines. These include *N*-formylasimilobine 2-*O*-β-D-glucopyranoside, *N*-formylasimilobine 2-*O*-β- D-glucopyranosyl-(1→2)-β-D-glucopyranoside (tinoscorside A), magnoflorine, *N*-demethyl-*N*-formyldehydronornuciferine (Fukuda *et al*., 1983; Choudhary *et al*., 2010a), *N*-formylanonaine (39), *N*-acetylanonaine, *N*-formylnornuciferine, *N*-acetylnornuciferine (Pachaly *et al*., 1992; Na *et al*., 2005), and lysicamine. The furquinolone alkaloids isolated from *T. crispa* comprise tyramine, higenamine (Praman *et al*., 2012), *N*-cis-feruloyltyramine, N-trans-feruloyltyramine, paprazine, and N-trans-caffeoyltyramine (Naomichi *et al*., 1983; Chung, 2011). The protoberberine alkaloids include 4,13-dihydroxy-2,8,9-trimethoxydibenzo[a,g]quinolizinium, columbamine, dihydrodiscretamin (Yusoff *et*

al., 2014), palmatine, jatrorrhizine, and berberine (Bisset and Nwaiwu, 1983). Salsolinol (a tetrahydroisoquinoline) and (−)-Litcubinine (a dibenzopyrrocoline type alkaloid) were identified from n-butanol fraction of *T. crispa* stem (Praman *et al*., 2012).

Lignans are group of compounds that arise from the shikimic acid pathway. Secoisolariciresinol and syringaresinol are lignans isolated from the methanol extract of *T. crispa* (Chung, 2011). Adenosine, uridine, and adenine are the nucleosides isolated from the *n*-butanol fraction of *T. crispa* stem (Praman *et al*., 2012). Sterols like β-sitosterol, stigmasterol and makisterone C have also been isolated from *T. crispa* (Lin, 2009).

Chemical-physical quality requirements (Department of Medical Sciences, 2021):

Loss on drying, not more than 11.0 per cent w/w after drying at 105° to constant weight.

Foreign matter, not more than 2.0 per cent w/w.

Acid-insoluble ash, not more than 0.5 per cent w/w.

Total ash, not more than 7.0 per cent w/w.

Ethanol-soluble extractive, not less than 5.0 per cent w/w.

Water-soluble extractive, not less than 10.0 per cent w/w.

Determination of bitterness, not less than 210 units per g when determined by the following method.

2.3.2.21 *Vetiveria zizanioides* (L.) Nash

Common names: Vetiver grass

Thai names: Faek hom, Faek, Faek lum, Kaeng hom, Khaem hom

Family: Poaceae

Part used: Roots

Figure 22 Root of *Vetiveria zizanioides* (L.) Nash

Botanical characteristics:

V. zizanioides is a coarse, erect, tufted perennial, growing 1 to 2 m high. Roots are fibrous and fragrant. Leaves are arranged in two rows, about 1 m long, 1 cm or less in width, and folded. Panicles are terminal, erect, purple or greenish, about 20 cm long; the branches are slender, whorled, spreading or ascending, 5 to 12 cm long. Sessile spikelets are about 4 mm long and muricate.

Traditional used:

Folk doctors used the decoction of roots to dissolve or break kidney stones and treat fever (Wutthithammawet, 2002).

Chemical constituents:

The volatiles from *V. zizanioides* in the roots was valencene, while in the shoots and leaves were 9-octadecenamide, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene and 1,2-benzendicarboxylic acid, diisooctyl ester. (Huang *et al*., 2004)

Chemical-physical quality requirements (Techadamrongsin and Pecharaply, 2005):

Loss on drying, not more than 8.0 per cent w/w after drying at 105° to constant weight.

Foreign matter, not more than 2.0 per cent w/w.

Acid-insoluble ash, not more than 5.0 per cent w/w.

Total ash, not more than 9.0 per cent w/w.

Ethanol-soluble extractive, not less than 2.0 per cent w/w.

Water-soluble extractive, not less than 2.0 per cent w/w.

Volatile oil, not less than 0.25 per cent v/w.

2.4 Biological activities of plant components in MHR remedy

In general, the mechanism of fever is due to the induction of cytokines, such as interleukin (IL)-1β, IL-6, interferons, and tumor necrosis factor (TNF), and subsequent generation of prostaglandins (PGs) in the CNS, particularly prostaglandin E_2 (PGE_2), thought to act as a proximal mediator of fever. Nitric oxide (NO) participates in several systems that are involved in body temperature regulation under euthermic conditions. Therefore, initial treatment of fever involves lowering body temperature, reducing cytokine secretion and the production of proximal mediators of fever (anti-inflammatory), and modulating immunity. Even though the Mo-Ha-Rak (MHR) have not been tested on reduced body temperature (antipyretic activity) directly, but many herbal components have been tested on the antipyretic, anti-inflammatory, analgesic, antioxidant, antimicrobial and immunomodulatory activity, which are related to the treatment model of fever. Details and references are shown in Appendix A.

To evaluate the potential pharmacological effects of MHR, the activities of individual herbal components of MHR were reviewed. The herbal components of MHR, which have been tested on antipyretic, anti-inflammatory and immunomodulatory are summarized in **Table 4**. In these reviews, the result showed that many herbal components of MHR have been tested on one or all antipyretic, anti-inflammatory, analgesic, antioxidant, antimicrobial and immunomodulatory activity. Details and references are shown in Appendix A.

Ha-Rak or Benjalokawichian remedy is a Thai traditional preparation which is in the list of Herbal Medicinal Products A.D 2011 Thailand. It consists of five plant roots, including *Capparis micracantha* DC., *Clerodendrum indicum* (L.) Kuntze, *Ficus racemosa* L., *Harrisonia perforata* (Blanco) Merr. and *Tiliacora triandra* (Colebr.) Diels. It has been commonly used to reduce fever related to the inflammatory mechanism (Traditional Thai Medicine Rehabilitation Foundation, 2015). These were used as a component of the primary herb group in the MHR remedy. Ha-Rak remedy had antipyretic, anti-inflammatory, analgesic, antioxidant, antimalarial, antibacterial, anticancer, antiproliferative and safety in humans. Details and references are shown in Appendix A.

Almost components of primary herbs showed antipyretic, anti-inflammatory, analgesic, antioxidant, antimalarial, antibacterial, antiviral and anticancer. Details and references are shown in Appendix A.

Various components of adjunct herbs showed antipyretic, anti-inflammatory, analgesic, antioxidant, antimalarial, antibacterial, antiviral, anticancer, laxative and spasmogenic. *Bridelia ovata* leaves and *Cassia fistula* pulps exert a stimulant laxative effect by promoting intestinal contraction. The fruits of *Phyllanthus emblic*, *Terminalia bellirica*, *Terminalia chebula* “Samo Thai” and *Terminalia chebula* “Samo Thet” have a mild laxative effect, functioning as stool softeners. Details and references are shown in Appendix A.

Some components of the supportive herbs exhibit antipyretic, anti-inflammatory, analgesic, antimicrobial, anticonvulsant and anxiolytic. Details and references are shown in Appendix A.

Table 4 Summary of biological activities of plant components in MHR remedy.

Group	Material	Biological activities									
		Antipyretic	Anti-inflammatory	Analgesic	Antioxidant	Antimalarial	Antibacterial	Antivirals	Anticancer	Immunomodulatory	Toxicity
1.1	*C. micracantha* (ชิงชี่)	+++	++	++	++	n/a	n/a	n/a	n/a	n/a	-
	C. indicum (เท้ายายม่อม)	+	+++	+++	n/a	+++	n/a	+++	+++	n/a	-
	F. racemosa (มะเดื่อชุมพร)	+++	+++ (bark)	+++	+++	+++	++	n/a	n/a	n/a	-
	H. perforata (คนทา)	+++	+++	+++	+++	+++	++	+++	n/a	n/a	-
	T. triandra (ย่านาง)	+++	++	+++	+++	+++	+++	++	+++	n/a	-
1.2	*A. indica* (สะเดา)	++ (leaf)	+++ (leaf)	+++ (leaf)	++ (leaf)	n/a	++ (leaf)	+++ (leaf)	++ (leaf)	n/a	- (leaf)
	G. chinense (กระดอม)	n/a	n/a	n/a	n/a	n/a	++ (leaf)	n/a	n/a	n/a	n/a
	T. crispa (บอระเพ็ด)	++	++	+++	+++	n/a	++	+++	++	+++	++ (hepatitis)
1.3	*D. cochinchinensis* (จันทน์แดง)	++	+++	+++	+++	n/a	+++	+++	+++	n/a	-
	T. hoaensis (จันทน์ขาว)	-	-	-	-	-	-	-	-	-	-
1.4	*L. sinense* (โกฐหัวบัว)	n/a	+++	n/a	++	n/a	++	n/a	++	n/a	-
	P. kesiya (สน)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	++	n/a	-

Note: 1 = Primary herbs; 1.1 Reduced-toxic fever herbs, 1.2 Anti-kamdao and lohit fever herbs, 1.3 Anti-di fever herbs and 1.4 Anti-semha and lom fever herbs, + is effective, - is not reported, n/a is not available.

Table 4 Summary of biological activities of plant components in MHR remedy (Continued).

Group	Material	Biological activities												
		Antipyretic	Anti-inflammatory	Analgesic	Antioxidant	Antimalarial	Antibacterial	Antivirals	Anticancer	Immunomodulatory	Laxative	Anticonvulsant	Anxiolytic	Toxicity
2.1	*B. ovata* (มะกา)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	+++	n/a	+++ (Stimulant)			-
	C. fistula (ดูน)	+++	++	+++	+++	n/a	++	++	++	n/a	+++ (Stimulant)			-
2.2	*T. bellirica* (สมอพิเภก)	++	+++	+++	+++	+++	+++	n/a	++	++	+++ (Stool softeners or wetting agents)			-
	T. chebula (สมอไทย)	++	+++	+++	+++	+++	++	+++	++	++	+++ (Stool softeners or wetting agents)			-
	Terminalia sp. "Samo Thet" (สมอเทศ)	+++	++	n/a	++	n/a	+++	n/a	n/a	n/a	+++ (Stool softeners or wetting agents)			-
	P. emblica (มะขามป้อม)	n/a	+++	+++	+++	+++	++	++	++	+++	+++ (Stool softeners or wetting agents)			-
3	*M. ferrea* (บุนนาค)	n/a	+++	n/a	+++	n/a	++	n/a	n/a	+++		+++	+++	-
	N. nucifera (บัวหลวง)	n/a	n/a	n/a	+++	n/a	n/a	n/a	n/a	n/a		n/a	n/a	
	V. zizanioides (แฝกหอม)	++	+++	+++	+++	n/a	+++	n/a	n/a	n/a		+++	+++	-

Note: 1 = Adjunct herbs; 2.1 Stimulant laxative, 2.2 Sour-astringent laxative and 3 = Supportive herbs, + is effective, - is not reported, n/a is not available.

2.5 Related research

Nutmakul (2020) classified remedy in the National List of Essential Medicine B.E. 256 using herbal taste. Nineteen herbal formulas were selected and divided into 3 groups depending on the herbal taste of the remedy or Ya Rot Prathan: Cold medicine (6 formulas), Sukhum medicine (5 formulas), and Hot medicine (8 formulas). The herbal taste of each herbal component was scored to calculate the ratio of herbal taste in the formula. The result showed that most of the formulas in the Cold medicine had a ratio of bitter taste and tasteless higher than the others. While the Sukhum group had a bitter, hot and aromatic-cool taste with a ratio of approximately 20-30% in the formula and a slightly sweet taste. The hot medicine had a ratio of hot taste of more than 40% in the formula. Each score of these tastes was replaced in Fisher's linear discriminant equations. These established equations can discriminate principal tastes from the selected herbal formulas, and the accuracy is as high as 94.7%.

In addition, High-performance liquid chromatography (HPLC) has been widely used as a tool for the development of quality control of Thai traditional preparations.

Nualkaew *et al*. (2004) analyzed the chemical composition of Prasaplai remedy by HPLC. The gradient of 0.5% trifluoroacetic acid in water and acetonitrile was used as a mobile phase with stationary phase of 5 µ C18, 250 x4 mm, Phenomenex. It was found that 13 chemical compounds could be analyzed. In addition, chemicals from *Z. cassumunar* and *N. sativa* reacted to form three new compounds: (E)-4-(3,4-dimethoxyphenyl) but-3-en-1-yl linoleate, (E)-4-(3,4-dimethoxyphenyl) but-3-en-1-yloleate and (E)-4-(3,4-dimethoxyphenyl) but-3-en-1-ylpalmitate.

Itharat and Sakpakdeejaroen (2010) determined cytotoxic compounds of Thai traditional preparation, Benjakul using HPLC. Piperine has been identified as the main compound in the extract. In addition, plumbagin was found as the most cytotoxic compound. The reversed-phase HPLC was performed with a gradient mobile phase of water and acetonitrile, and peaks were detected at 256 nm. Based on validation results, this analytical method is precise, accurate and stable for the quantitative determination of piperine and plumbagin, which are cytotoxic compounds isolated from the ethanolic extract of Benjakul.

Mukkasombut *et al*. (2020) developed a validated method for Prasaprohyai remedy extract by HPLC. The developed HPLC system was specific for the detection of ethyl-*p*-methoxycinnamate (EPMC) and eugenol content in Prasaprohyai remedy. All other analysis parameters complied with standard requirements. For EPMC, the analytical range was 25-450 µg/mL with linearity (r^2 = 0.9999), LOD 0.1 µg/mL and LOQ 0.5 µg/mL. The analytical range of eugenol was 2.5-30 µg/mL with linearity (r^2 = 0.9998), LOD 0.25 µg/mL, LOQ 0.5 µg/mL.

Piwngam *et al*. (2020) developed a validated HPLC method for the determination of markers in the Lom-Am-Ma-Preuk (LAP) remedy. The developed method was specific to these markers with retention times of 45.7, 56.6 and 53.9 min for eugenol, myristicin and piperine, respectively, with a total analysis time of 70 min. The coefficient of determination (R^2) was more than 0.999. The accuracy and precision were in an acceptable range with good recovery verified by the coefficient of variation of less than 2%. The limits of detection (LOD) of eugenol, myristicin and piperine were 0.5, 0.2 and 1.3 µg/mL and limits of quantitation (LOQ) were 1.9, 0.6 and 5 µg/mL, respectively. The contents of eugenol, myristicin and piperine in LAP could be obtained in one analysis for the first time.

Anekchai and Sakunphueak (2021) developed a method for quality control of Chantaleela (CTL) remedy by establishing a simple but comprehensive method using HPLC. HPLC fingerprints of CTL were developed to assess qualitative parameters and comprehensive chemical profiling. This method is highly precise and accurate in quantifying the chemical constituents of CTL including eurycomanone, loureirin A, imperatorin and atractylodin.

Kakatum *et al*. (2021) developed a validated method for Sahastara remedy extract by HPLC. The developed HPLC system was specific for the detection of gallic acid, ellagic acid, plumbagin, piperine, and β-asarone content in Sahastara remedy. All validated parameters of HPLC method complied with standard requirements. Each analyzed peak showed good selectivity with a baseline resolution greater than 1.51. The linearity of all compounds was > 0.999, % recovery of all compounds was within 98.0-102.0% and the precision of all compounds was less than 2.0% CV.

Klangprapun (2021) developed a quality control method of Prab – Chom – Poo – Tha – Weeb remedy and their components by HPLC. The stationary phase, C18

Kinetex 2.6 micrometer Columns, 100 x 4.6 millimeter was used while 0.05 % trifluoroacetic acid in water and acetonitrile was used as a mobile phase with the detector of PDA. Nineteen herbal components and major compounds were identified in the HPLC chromatogram.

Umpanth *et al*. (2022) developed a method for quantifying the content of the markers in the Feethanuthorawat remedy by HPLC. The major compound, 6 - gingerol was detected and quantified. It presented good linearity within the test range (r^2 = 0.9994). The relative standard deviation of intraday precision and intraday precision were 1.21and 1.45 %, respectively. The per cent recovery was in the range of 99.61 to 100.84%. The limit of detection and the limit of quantitation was 0.13 and 0.39 µg/mL, respectively.

CHAPTER III
MATERIALS AND METHODS

This study is experimental research to scientifically prove the formulation theory of Thai traditional preparation (polyherbal medicine). The chemical profile, pharmacological activities, and toxicity of ethanolic and aqueous extracts from each modified formula, and the original formula of Mo-Ha-Rak (MHR) will be investigated. The relationship between the number and ratio of herbs with pharmacological activity and toxicity will be analyzed.

3.1 Materials

3.1.1 Plant materials

3.1.1.1 Source of plant materials

The Mo-Ha-Rak (MHR) remedy consists of 21 herbal components. These dried plant materials were purchased from Thai traditional pharmacies, and authentic samples were personally collected, with details on plant part use and sources provided in **Table 5** and **Table 6**.

1) Purchased from Thai traditional pharmacies

The 11 dried plant materials, including *D. cochinchinensis, L. sinense, M. ferrea, N. nucifera, P. emblica, P. kesiya, T. hoaensis, T. chebula*, *Terminalia* sp. “Samo Thet”, *T. crispa* and *V. zizanioides*, were purchased from Thai traditional pharmacies in Bangkok and Nakhon Pathom. The five dried plant materials, including *C. micracantha, C. indicum, F. racemosa, H. perforata* and *T. triandra*, were purchased from Thai traditional pharmacies in Bangkok. *B. ovata* and *C. fistula* were purchased from Thai traditional pharmacies in Phichit, Bangkok, Nakhon Pathom and Krabi. *G. chinense* was purchased from Thai traditional pharmacies in Bangkok. Additionally, *A. indica* was purchased from 12 traditional pharmacies across Thailand.

2) Self-collection of plant materials

Some fresh plant materials were collected by the researchers in collaboration with local traditional healers and were subsequently identified by experts. These plant samples were designated as authentic reference samples. The five

fresh plant materials, including *C. micracantha, C. indicum, F. racemosa, H. perforata* and *T. triandra,* were collected from Roi Et. *B. ovata* and *C. fistula* were collected from Maha Sarakham. *G. chinense* was collected from Chiang Mai. Lastly, *A. indica* was collected from Lampang, Maha Sarakham and Phetchaburi.

3.1.1.2 Plant identification

1) Medicinal material inspection

The voucher specimens were deposited at the Faculty of Pharmacy, Mahasarakham University, Maha Sarakham, Thailand. The crude drug samples and the voucher specimens were authenticated by specialist and compare with Reference herbarium as follows: Department of Medical Sciences Herbarium (DMSC), the Department of Medical Sciences, Nonthaburi, Thailand, or other recognized herbaria such as the Development of Herbal Health Products (TT-OC-SK), Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand; the Mahidol University Herbarium, Department of Pharmaceutical Botany (PBM), Faculty of Pharmacy, Mahidol University, Nakhon Pathom, Thailand; the herbarium of Thai Medicinal Plants at Faculty of Pharmaceutical Science (SKP), Prince of Songkla University, Songkhla Province, Thailand. In addition, the botanical identification of the medicinal materials was conducted based on physical characteristics using the macroscopical method, as well as chemical characteristics analyzed through TLC and HPLC techniques.

2) Verify the names of herbal medicinal substances and their properties based on the reference textbook by Picheansoonthon *et al*. (2017) and the Department of Medical Sciences (2021).

3) Verify the scientific names of plants (Plant Identification) using the Flora of Thailand and The Plant Names of Thailand by Smitinan (2011).

Monographs for twenty of these are available in the Thai herbal pharmacopeia (THP) or other references except for *Azadirachta indica* A. Juss. The extractive value, one of the specifications, will be determined for 20 herbs based on THP methods. Only plant materials meeting or exceeding the average extractive value or standard requirement will be selected for this study. *Azadirachta indica* A. Juss. was used to determine extractive values for specification development.

Table 5 Source of plant materials and reference voucher specimens.

No.	Material / Voucher specimen number	Reference voucher specimen number	Thai name	Part used	Source
1	*Bridelia ovata* Decne. / CC.MSU-PH-01	TT-OC-SK-1253	มะกา (Ma Ka)	Leaf	Phichit Bangkok Nakhon Pathom Krabi Maha Sarakham (Authentic)
2	*Capparis micracantha* DC. / CC.MSU-PH-03	PBM05097	ชิงชี่ (Chingchi)	Root	Roi Et (Authentic) Bangkok
3	*Cassia fistula* L. / CC.MSU-PH-02	DMSC5162, DMSc1142,	คูน (Khun)	Pulp	Phichit Bangkok Nakhon Pathom Krabi Maha Sarakham (Authentic)
4	*Clerodendrum indicum* (L.) Kuntze / CC.MSU-PH-04	DMSC5243, DMSc1078	เท้ายายม่อม (Thaoyaimom)	Root	Roi Et (Authentic) Bangkok
5	*Dracaena cochinchinensis* (Lour.) S.C.Chen	DMSC5179, DMSc0934,	จันทน์แดง (Chan Daeng)	Wood	Bangkok Nakhon Pathom
6	*Ficus racemosa* L. / CC.MSU-PH-05	DMSC5221, DMSc1077	มะเดื่อชุมพร (Maduea Chumpon)	Root	Roi Et (Authentic) Bangkok
7	*Gymnopetalum chinense* (Lour.) Merr. / CC.MSU-PH-08	-	กระดอม (Kra Dom)	Fruit	Bangkok Chiang Mai (Authentic)
8	*Harrisonia perforata* (Blanco) Merr. / CC.MSU-PH-06	DMSC5218, DMSc1080	คนทา (Khon Tha)	Root	Roi Et (Authentic) Bangkok
9	*Ligusticum sinense* Oliv.	DMSc1148	โกฐหัวบัว (Kot Huabua)	Rhizome	Bangkok Nakhon Pathom
10	*Mesua ferrea* L.	DMSC5165, DMSc0763,	บุนนาค (Bunnak)	Flower	Bangkok Nakhon Pathom
11	*Nelumbo nucifera* Gaertn.	DMSC5156, DMSc0891, 0892	บัวหลวง (Bua Luang)	Stamen	Bangkok Nakhon Pathom
12	*Phyllanthus emblica* L.	DMSC904	มะขามป้อม (Makhampom)	Fruit	Bangkok Nakhon Pathom
13	*Pinus kesiya* Royle ex Gordon	TT-OC-SK-910	สน (Son)	Wood	Bangkok Nakhon Pathom
14	*Tarenna hoaensis* Pit.	-	จันทน์ขาว (Chan Khao)	Wood	Bangkok Nakhon Pathom
15	*Terminalia bellirica* (Gaertn.) Roxb.	DMSC92, 171	สมอพิเภก (Samo Pipek)	Fruit	Bangkok Nakhon Pathom

Table 5 Source of plant materials and reference voucher specimens (Continued).

No.	Material / Voucher specimen number	Reference voucher specimen number	Thai name	Part used	Source
16	*Terminalia chebula* Retz.	DMSC899	สมอไทย (Samo Thai)	Fruit	Bangkok Nakhon Pathom
17	*Terminalia* sp. "Samo Thet"	-	สมอเทศ (Samo Thet)	Fruit	Bangkok Nakhon Pathom
18	*Tiliacora triandra* (Colebr.) Diels / CC.MSU-PH-07	DMSC5210, DMSc1071	ย่านาง (Yanang)	Root	Roi Et (Authentic) Bangkok
19	*Tinospora crispa* (L.) Hook. f. & Thomson	DMSC354, 355	บอระเพ็ด (Borapet)	Stem	Bangkok Nakhon Pathom
20	*Vetiveria zizanioides* (L.) Nash	DMSc792	แฝกหอม (Faek Hom)	Root	Bangkok Nakhon Pathom

Table 6 Sources of *Azadirachta indica* were purchased from traditional pharmacies in Thailand.

Material/ Voucher specimen number	Reference voucher specimen number	Thai name	Part used	Source	
				Region	Province
Azadirachta indica A. Juss. / CC.MSU-PH-09, CC.MSU-PH-10, CC.MSU-PH-11	SKP 095 13 03 01	สะเดา (Sadao)	Petiole	Northern	Lampang (Authentic)
					Phitsanulok
				Central	Nakhon Sawan
					Phichit
					Nakhon Pathom
					Bangkok
				Northeastern	Sakon Nakhon
					Maha Sarakham (Authentic)
					Yasothon
				Eastern	Chanthaburi
					Chonburi
				Western	Phetchaburi (Authentic)
					Kanchanaburi
				Southern	Krabi
					Songkhla

3.1.2 Chemicals and reagents

3.1.2.1 Extraction

1) 95% Ethanol, commercial grade (ITALMAR company, Thailand)

2) Deionized water (Faculty of Pharmacy, Mahasarakham University, Thailand)

3.1.2.2 Extractive value

1) Ethanol, AR grade (RCI-Labscan, Thailand)

2) Chloroform ($CHCl_3$), analytical grade (RCI-Labscan, Thailand)

3) Deionized water (Faculty of Pharmacy, Mahasarakham University, Thailand)

3.1.2.3 High Performance Liquid Chromatography (HPLC)

1) Acetonitrile, HPLC grade (RCI-Labscan, Thailand)

2) Methanol, HPLC grade (RCI-Labscan, Thailand)

3) 2-Propanol, HPLC grade (RCI-Labscan, Thailand)

4) Trifluoroacetic acid 99.9% (Acros Organics, Belgium)

5) Deionized water (Faculty of Pharmacy, Mahasarakham University, Thailand)

3.1.2.4 Thin-Layer Chromatography (TLC)

1) Methanol, AR Grade (RCI Labscan Ltd., Thailand)

2) Chloroform ($CHCl_3$), analytical grade (RCI-Labscan, Thailand)

3) n-Hexane 99%, AR Grade (RCI Labscan Ltd., Thailand)

4) Ethyl Acetate, AR Grade (RCI Labscan Ltd., Thailand)

5) Toluene, AR Grade (RCI Labscan Ltd., Thailand)

6) Formic acid, AR Grade (RCI Labscan Ltd., Thailand)

7) Acetic acid, AR Grade (RCI Labscan Ltd., Thailand)

8) Sulfuric acid 98%, AR Grade (RCI Labscan Ltd., Thailand)

9) Diethylamine, AR Grade (RCI Labscan Ltd., Thailand)

10) Anisaldehyde (Merck KGaA, Germany)

3.1.2.5 Anti-inflammatory activities

1) Dimethyl sulfoxide (DMSO) (RCL Labscan, Thailand)

2) Distilled water (Faculty of Pharmacy, Mahasarakham University, Thailand)

3) Fetal bovine serum (FBS) (Gibco™, USA)

4) Antibiotic-Antimycotic (penicillin, streptomycin, and Gibco ampho tericin B) (Gibco™, USA)

5) Dulbecco's Modified Eagle Medium (DMEM) (Gibco™, USA)

6) Lipopolysaccharide from *E. coli* O55:B5 (LPS) (Sigma, USA)

7) Trypsin-EDTA (Gibco™, USA)

8) Hydrochloric acid (HCl) (Univar, Australia)

9) N-(1-Naphthyl) ethylenediamine dihydrochloride (Sigma, USA)

10) Phosphase buffered saline tablet (PBS) (Amresco, USA)

11) Phosphoric acid 85% (H_3PO_4) (Sigma, USA)

12) Sodium bicarbonate ($NaHCO_3$) (BHD, England)

13) Sodium hydroxide (NaOH) (Univar, Australia)

14) Sulfanilamide ($H_2NC_6H_4SO_2NH_2$) (Sigma, USA)

15) Thiazolyl blue tetrazolium bromide (MTT) (Sigma, USA)

16) Trypan blue 0.4% (Gibco™, USA)

3.1.2.6 Antioxidant activities

1) Sodium nitroprusside ($C_5FeN_6Na_2O$) (RCL Labscan, Thailand)

2) Sodium chloride (NaCl) (RCL Labscan, Thailand)

3) Potassium chloride (KCl) (RCL Labscan, Thailand)

4) Sodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$) (KemAus™ , Australia)

5) Potassium dihydrogen phosphate (KH_2PO_4) (Sigma, USA)

6) Dipotassium phosphate (K_2HPO_4) (Sigma, USA)

7) N-(1-Naphthyl) ethylenediamine dihydrochloride (Sigma, USA)

8) Phosphoric acid 85% (H_3PO_4) (Sigma, USA)

9) Sulfanilamide ($H_2NC_6H_4SO_2NH_2$) (Sigma, USA)

10) Riboflavin (Sigma, China)

11) Nitro tetrazolium blue chloride (NBT) (Sigma, USA)

12) Ethylenediaminetetraacetic acid (EDTA) (Univar, Australia)

13) Dimethyl sulfoxide (DMSO) (RCL Labscan, Thailand)

14) Distilled water (Faculty of Pharmacy, Mahasarakham University, Thailand)

3.1.2.7 Reference standard

1) Indomethacin (Sigma, USA)
2) Ascorbic acid (Sigma, USA)
3) Trolox (Sigma, USA)
4) Chebulic acid (Chengdu Alfa Biotechnology, China)
5) Gallic acid (Sigma, USA)
6) Protocatechuic acid (Wuhan ChemNorm Biotech, China)
7) Bergenin (Chengdu Alfa Biotechnology, USA)
8) Chlorogenic acid (Sigma, USA)
9) Chebulanin (Wuhan ChemFaces Biochemical, China)
10 Corilagin (Sigma, USA)
11) Chebulagic acid (Chengdu Alfa Biotechnology, China)
12) Ellagic acid (Sigma, USA)
13) Resveratrol (Sigma, USA)
14) Rhein (Sigma, USA)
15) Loureirin A (Wuhan ChemFaces Biochemical, China)
16) Pectolinarigenin (Wuhan ChemFaces Biochemical, China)
17) ß-sitosterol (Sigma, USA)
18) Stigmasterol (Sigma, USA)
19) Lupeol (Sigma, USA)

3.1.3 Instruments and equipment

1) 75 cm^2 plastic tissue culture flasks (Costar Corning, USA)
2) 96-well microplates flat, bottom with lid (Costar Corning, USA)
2) 96-well microplates flat, bottom without lid (Costar Corning, USA)
3) 96-well microplates U, bottom with lid (Costar Corning, USA)
4) Autoclave (Hirayama, Japan)
5) Balance 0.01 mg-41 g (Mettler-Toledo, Swizerland)
6) Balance 0.01 g-220 g (Precica, Switzerland)
7) Balance 0.5 g-3100 g (Mettler-Toledo, Swizerland)
8) Buchner Funnel (Schott Duran, Germany)
9) Cell culture flask, canted neck 75 cm^3 (Costar Corning, USA)

10) Centrifugulation (Beckman Coulter, USA)

11) Centrifuge tube 15, 50 ml (Costar Corning, USA)

12) CO_2 humidified incubator (Shel lab, USA)

13) Crusibles (Coorstex, USA)

15) Disposable pipatte 2, 5, 10, 25 mL (Costar Corning, USA)

16) Erlenmeyer flasks (Schott Duran, Germany)

17) Eppendrofs (Costar Corning, USA)

18) Examination glove (Sritrang gloves, Thailand)

19) Filter paper no.1 (110 mmØ) (Whatman, USA)

20) Filter paper no.4 (110 mmØ) (Whatman, USA)

21) Freezer (Sanyo, Japan)

22) Glass bottle 50, 250, 500, 1000 mL (Schott Duran, Germany)

23) Glasswares 10, 25, 50, 100, 250, 600, 1000 mL (Schott Duran, Germany Pyrex, USA)

24) Hematocytometer (Boeco, Germany)

25) Hot air oven (Memmert, Germany)

26) Hot plate (Thermolyne, USA)

27) Incubated tabletop orbital shaker (Thermo Scientific, USA)

28) Inverted microscope (Nikon, Japan)

29) Laminar air flow (Boss tech, Thailand)

30) Liquid nitrogen tank (Taylor-Wharton, USA)

31) Lipophilizer (Telster, Spain)

32) Litmus paper pH-fix 4.5-10.0 (Macherey-Nagel, Germany)

33) McFarland densitometer (Grant-Bio, England)

34) Membran filter with pore-size rating of 0.22 µm (Millipore, Germany)

35) Membran filter with pore-size rating of 0.45 µm (Millipore, Germany)

36) Micropipettes 20 µl, 200 µl, 1000 µL (Thermo Scientific, USA)

37) Microplate reader (Bio Tek, USA)

38) Moisture analyzer (Scaltec instrument, Germany)

39) Muffle furnace (Nabertherm, Germanny)

40) Multi-channels pipette (Thermo Scientific, USA)

41) pH buffer (Thermo Scientific, USA)

42) pH meter (WTW inolab, Germany)

43) Pipette tips (Costar Corning, USA)

44) Pipetteboy (Integra biosciences, Switzerland)

45) Reagent reservoir (Sterile) (Costar Corning, USA)

46) Refrigerrator (4ºC) (Sharp, Japan)

47) Refrigerrator (-20ºC) (Sanyo, Japan)

48) Rotary evaporator (Buchi, Switzerland)

49) Shaking incubator (Vision Scientific, Korea)

50) Sonicator (Elma, Germany)

51) Lyophilizer

52) Syringes (Nipro, Thailand)

53) Vacuum desiccator (Simax, USA)

54) Vacuum pump (Rocker, Taiwan)

55) Vortex mixer (Scientific industries, USA)

56) Water bath (Memmert, Germany)

57) Water purification machine (Elga, UK)

58) Column: Luna C18 (5µ 100Å, 250x4.60 mm) (Phenomenex, USA)

59) Guard cartridge: Security Guard (Phenomenex, USA)

60) Analytical HPLC: Agilent 1260 Infinity II Prime HPLC system (Agilent, USA)

61) TLC plate, siliga gel 60 F_{254}, per-coated on aluminium sheets 20x20 cm, layer thickness 0.25 mm (Merck KGaA, Germany)

62) PLC plate, siliga gel 60 F_{254}, per-coated on glass plate 20x20 cm, layer thickness 0.5 mm (Merck KGaA, Germany)

63) TLC spotter and densitometer (Camag, Switzerland)

64) Microplate spectrofluorometer (Camag, Switzerland)

65) Needle syringe 100 µL (Camag, Switzerland)

66) TLC tank (Camag, Switzerland)

67) TLC glass reagent sprayer (Camag, Switzerland)

3.2 Methods

3.2.1 Experimental design

The Thai traditional formulation theory suggests that each group of herbs within a formula serves distinct roles, including the primary herbs, adjunct herbs, supportive herbs and flavoring herbs, all working together to address primary symptoms and complications in disease treatment (Nithetsukkit, Khun, 1973; Khrupanyamat, 2016). In this study, the effects of removing the number of herbs in the various modified formulas will be examined compared to the original Mo-Ha-Rak (MHR) formula in terms of chemical composition, pharmacological activities (anti-inflammatory and antioxidant effects), and toxicity. This investigation aims to provide further insights into the formulation theory behind Thai traditional polyherbal medicine. Various factors, including adjustments in the quantity and ratio of herbs, will be analyzed to better understand their impact on efficacy and safety.

3.2.1.1 Formula analysis of MHR remedy

Herb components of the MHR remedy can be classified by a theory of Thai traditional medicine into 3 major groups, which are shown in **Table 7**.

1) The primary herbs (PH)

This herbal group is used to treat conditions such as severe fever (toxic fever), internal heat and excessive thirst. The herbs in this group are further categorized into 4 sub-groups as follows:

1.1) Reduced-toxic fever herbs. These herbs have a distinctly bitter and cooling taste. They are effective in treating toxic fevers accompanied by rashes, helping to expel toxins from the body. Key herbs in this category include the roots of *Capparis micracantha, Clerodendrum indicum, Ficus racemosa, Harrisonia perforata*, and *Tiliacora triandra*.

1.2) Anti-*kamdao* fever[1] (internal body heat) and *lohit* fever[2] (related to the blood disorder) herbs, both of which cause high fever and rashes, have

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***Note:** In the theory of Thai traditional medicine, fever are classified into two main types, namely common fever, and severe fever (toxic fever), a fever with high temperature that is divided into two sub-types consisting of rash fever (exanthematous fever) and fever without rash. The causes of fever can be attributed to *kamado*, *lohit*, *di*, *semha*, or *lom*, either individually or in combination.

a bitter, cooling taste. Key herbs in this category include the petioles of *Azadirachta indica*, fruits of *Gymnopetalum chinense*, and stems of *Tinospora crispa*.

1.3) Anti-*di* fever[3] (related to bile disorder) herbs, as well as to relieve internal heat and excessive thirst. They have a bitter and cooling taste. Key herbs in this category include the wood of *Dracaena cochinchinensis* and *Tarenna hoaensis*.

1.4) Anti-*semha* fever[4] (related to water element disorder) and *lom* fever[5] (related to wind element disorder) herbs. These herbs have a bitter aromatic taste. Key herbs in this category include the rhizomes of *Ligusticum sinense* and the wood of *Pinus kesiya* (Royle ex Gordon).

2) The adjunct herbs (AH)

Herbal groups in this category are used as laxatives, for detoxifying fevers, as expectorants, and to treat coughs and colds. This group is further divided into two sub-groups as follows:

2.1) Stimulant laxative, to detoxify fevers related to *semha* (secretions) and *lohit* (blood), and to reduce body heat. The herbs responsible for this group include the leaves of *Bridelia ovata* and the pulp of *Cassia fistula*.

2.2) Sour-astringent laxative, detoxifying fevers, and helping treat coughs and colds. The herbs responsible for this group include the fruits of

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[1] Kamdao fever (fever with internal body heat): The symptom of this fever include headache, body heat, chills and shivering, no tears, vomiting, insomnia, dry mouth, thirst, pain in the mouth and throat, and blood-red eyes.

[2] Lohit fever (fever from blood disorder): The symptom of this fever include headache, body heat, red face, blood-red eyes, tears, red eyes, sore feet and heat all over the body, anxious mind.

[3] Semha fever (fever from water element disorder): The symptom of this fever includes cold, hairlessness, goosebumps, yellow eyes, dreaminess, excessive saliva in the mouth, cold hands and feet, craving for sweet and savory food, weakness, shivering hot and cold.

[4] Di fever (fever from bile disorder): The symptom of this fever includes body heat, delirium, sleep over, headache, thirst, bitterness in the mouth, body aches, green eye rims.

[5] Lom fever (fever from wind disorder): The symptom of this fever includes dizziness, lightheadedness, not hot, cloudy and cloudy eyes. In addition, red eyes (fading red), body pain, colic, hiccups, vomiting, coughing, thirst, mouth ulcers, chest pains, and shortness of breath might be found.

Phyllanthus emblic, *Terminalia bellirica*, *Terminalia chebula*, and various *Terminalia* species.

3) The supportive herbs (SH)

The herbals in this group serve as heart tonics to help relieve fatigue, reduce body heat, and support recovery after fever. The herbs responsible for this group include the flowers of *Mesua ferrea*, the stamens of *Nelumbo nucifera*, and the roots of *Vetiveria zizanioides*.

Based on the analysis of herbal components in MHR remedy and its alignment with the etiology, pathogenesis, and symptoms of fever, the suitability of this formula for patients can be assessed according to the type of fever as follows:

1) Multi-cause and chronic fevers: These fevers persist for an extended period, leading to severe endogenous inflammation. Patients often experience symptoms such as weight loss, fatigue, loss of appetite, blurred vision, altered consciousness, and constipation.

2) Fevers with extreme heat: This group is characterized by high fever, intense thirst, and widespread rashes on the body.

Table 7 Formula analysis of MHR remedy.

Structure	Function	Sub-group	Sub-function	Material	Part used	Taste	Ratio (% w/w)
1. Primary herbs	Treat of fever, severe fever (toxic fever), internal heat and excessive thirst.	1.1 Reduced-toxic fever herbs	They are effective in treating toxic fevers accompanied by rashes, helping to expel toxins from the body.	*C. micracantha* (ชิงชี่)	Root	Bitter	4.0
				C. indicum (เท้ายายม่อม)	Root	Tasteless-intoxicating	4.0
				F. racemosa (มะเดื่อชุมพร)	Root	Astringent - cool	4.0
				H. perforata (คนทา)	Root	Bitter-intoxicating	4.0
				T. triandra (ย่านาง)	Root	Tasteless-bitter	4.0
		1.2 Anti-kamdoa and lohit fever herbs	Treat of kamdao fever (internal body heat) and lohit fever (related to the blood disorder) that cause high fever and rash	*A. indica* (สะเดา)	Petiole	Bitter	4.0
				G. chinense (กระดอม)	Fruit	Bitter-cool	4.0
				T. crispa (บอระเพ็ด)	Stem	Bitter-cool	4.0
		1.3 Anti-di fever herbs	Relieve internal heat and excessive thirst.	*D. cochinchinensis* (จันทน์แดง)	Wood	Bitter-cool	4.0
				T. hoaensis (จันทน์ขาว)	Wood	Bitter-sweet	4.0
		1.4 Anti-semha and lom fever herbs	Treat of semha fever (water element disorder) and lom fever (wind element disorder).	*L. sinense* (โกฐหัวบัว)	Rhizome	Oily-pungent hot	1.0
				P. kesiya (สน)	Wood	Bitter-pungent hot	1.0

Table 7 Formula analysis of MHR remedy (Continued).

Structure	Function	Sub-group	Sub-function	Material	Part used	Taste	Ratio (% w/w)
2. Adjunct herbs	Laxatives, detoxify from fever, expectorant and treat cough and cold.	2.1 Stimulant laxative	Laxatives, detoxifying from fever for semha (secretions) and lohit (blood), and reducing body heat	*B. ovata* (มะกา)	Leaf	Bitter-intoxicating	4.0
				C. fistula (คูน)	Pulp	Sweet	12.0
		2.2 Sour-astringent laxative	Laxatives, expectorant and detoxify from fever, and cure cough and cold	*T. bellirica* (สมอพิเภก)	Fruit	Sour-astringent-sweet	8.0
				T. chebula (สมอไทย)	Fruit	Astringent - sour	8.0
				Terminalia sp. “Samo Thet” (สมอเทศ)	Fruit	Sour-astringent	8.0
				P. emblica (มะขามป้อม)	Fruit	Astringent - sour- sweet	8.0
3. Supportive herbs	Serve as heart tonics to help relieve fatigue, reduce body heat, and support recovery after fever.			*M. ferrea* (บุนนาค)	Flower	Aromatic-cool	4.0
				N. nucifera (บัวหลวง)	Stamen	Astringent-aromatic cool	2.0
				V. zizanioides (แฝกหอม)	Root	Aromatic-cool fragrant	4.0

3.2.1.2 Experimental design

As mentioned above, the objective of this study is to prove the formulation theory of Thai traditional drugs while aiming to reduce the number of herbs used in remedies. The reduction of herbs in each group will be designed based on various factors and compared to the chemical profiles and pharmacological activities of each assignment.

1) Formula design criteria

1.1) To vary the number and ratio of herbal components in the MHR remedy.

1.2) To vary the number and ratio of adjunct herbs in the MHR remedy.

1.3) To vary the number and ratio of supportive herbs in the MHR remedy.

1.4) To vary the number and ratio of herbal components in only primary herbs.

1.5) To fix toxic fever herbs and vary the number of herbal components in MHR.

1.6) To vary the number and ratio of toxic fever herbs.

2) Experimental design for preparation of original MHR and modified MHR

2.1) Original formula

- FG01: MHR remedy.

2.2) Modified MHR remedies by removing the adjunct herbs or supportive herbs.

- FG02: MHR remedy without adjunct herbs and supportive herbs.

- FG03: MHR remedy without supportive herbs.

2.3) Modified MHR remedies by changing the number of supportive herbs.

- FG04: MHR remedy without two herbs of *N. nucifera* stamens and *V. zizanioides* roots.

- FG05: MHR remedy without two herbs of *M. ferrea* flowers and *V. zizanioides* roots.

- FG06: MHR remedy without two herbs of *M. ferrea* flowers and *N. nucifera* stamens.

- FG07: MHR remedy without *V. zizanioides* roots.

- FG08: MHR remedy without *N. nucifera* stamens.

- FG09: MHR remedy without *M. ferrea* flowers.

2.4) Modified MHR remedies by changing the number of herbs in the adjunct herbs.

- FG10: MHR remedy without adjunct herbs.

- FG11: MHR remedy without sour-astringent laxative herbs.

- FG12: MHR remedy without sour-astringent laxative herbs and supportive herbs.

- FG13: MHR remedy without stimulant laxative herbs.

- FG14: MHR remedy without stimulant laxative herbs and supportive herbs.

2.5) Modified MHR remedies by fixing the primary herbs and changing the number of adjunct herbs.

- FG15: exclude three herbs of *T. chebula* fruits, *Terminalia* sp. “Samo Thet” fruits and *P. emblica* fruits.

- FG16: exclude three herbs of *T. bellirica* fruits, *Terminalia* sp. “Samo Thet” fruits and *P. emblica* fruits.

- FG17: exclude three herbs of *T. bellirica* fruits, *T. chebula* fruits and *P. emblica* fruits.

- FG18: exclude three herbs of *T. bellirica* fruits, *T. chebula* fruits and *Terminalia* sp. “Samo Thet” fruits.

- FG19: exclude two herbs of *Terminalia* sp. “Samo Thet” fruits and *P. emblica* fruits.

- FG20: exclude two herbs of *T. chebula* fruits and *P. emblica* fruits.

- FG21: exclude two herbs of *T. chebula* fruits and *Terminalia* sp. “Samo Thet” fruits.

- FG22: exclude two herbs of *T. bellirica* fruits and *P. emblica* fruits.

- FG23: exclude two herbs of *T. bellirica* fruits and *Terminalia* sp. “Samo Thet” fruits.

- FG24: exclude two herbs of *T. bellirica* fruits and *T. chebula* fruits.

- FG25: exclude one herb of *P. emblica* fruits.

- FG26: exclude one herb of *Terminalia* sp. “Samo Thet” fruits.

- FG27: exclude one herb of *T. chebula* fruits.

- FG28: exclude one herb of *T. bellirica* fruits.

- FG29: exclude five herbs of *T. bellirica, T. chebula and Terminalia sp., P. emblic* and *C. fistula* pulps.

- FG30: exclude five herbs of *T. bellirica, T. chebula and Terminalia sp., P. emblic* and *B. ovata* leaves.

2.6) Modified MHR remedies by changing the number of primary herbs only

- FG31: exclude herbs for the anti-semha and lom fever.

- FG32: exclude herbs for the anti-semha and lom fever, and anti-di fever.

- FG33: exclude herbs for the anti-semha and lom fever, anti-di fever, anti-kamdao and lohit fever.

- FG34: exclude herbs for the anti-di fever.

- FG35: exclude herbs for the anti-kamdao and lohit fever.

- FG36: exclude herbs for the anti-kamdao and lohit fever, anti-semha and lom fever.

- FG37: exclude herbs for the anti-kamdao and lohit fever and anti-di fever.

- FG38: exclude one herb for anti-semha and lom fever, *P. kesiya* woods.

- FG39: exclude one herb for anti-semha and lom fever, *L. sinense* rhizomes.

2.7) Modified MHR remedies by excluding herbs for anti-semha and lom fever and one herb for anti-di fever

- FG40: exclude herbs for anti-semha and lom fever and one herb for anti-di fever, *T. hoaensis* woods.

- FG41: exclude herbs for anti-semha and lom fever and one herb for anti-di fever, *D. cochinchinensis* woods.

2.8) Modified MHR remedies by excluding herbs for anti-semha and lom fever, anti-di fever and one herb for anti-kamdao and lohit fever

- FG42: exclude one herb for anti-kamdao and lohit fever, *T. crispa* stems.

- FG43: exclude one herb for anti-kamdao and lohit fever, *G. chinense* fruits.

- FG44: exclude one herb for anti-kamdao and lohit fever, *A. indica* petioles.

2.9) Modified MHR remedies by fixing reduced-toxic fever herbs and plus one of the other components.

- FG45: plus one herb for anti-kamdao and lohit fever, *A. indica* petioles.

- FG46: plus one herb for anti-kamdao and lohit fever, *G. chinense* fruits.

- FG47: plus one herb for anti-kamdao and lohit fever, *T. crispa* stems.

- FG48: plus one herb for anti-di fever, *D. cochinchinensis* woods.

- FG49: plus one herb for anti-di fever, *T. hoaensis* woods.

- FG50: plus one herb for anti-samha and lom fever, *L. sinense* rhizomes.

- FG51: plus one herb for anti-samha and lom fever, *P. kesiya* woods.

- FG52: plus one herb for stimulant laxative, *B. ovata* leaves.

- FG53: plus one herb for a stimulant laxative, *C. fistula* pulps.

- FG54: plus one herb for sour-astringent laxative, *T. bellirica* fruits.

- FG55: plus one herb for sour-astringent laxative, *T. chebula* fruits.

- FG56: plus one herb for sour-astringent laxative, *Terminalia* sp. “Samo Thet” fruits.

- FG57: plus one herb for sour-astringent laxative, *P. emblica* fruits.

- FG58: plus one herb for hear tonic, *M. ferrea* flowers.

- FG59: plus one herb for hear tonic, *N. nucifera* stamens.

- FG60: plus one herb for hear tonic, *V. zizanioides* roots.

2.10) Modified MHR remedies by changing reduced-toxic fever herbs only.

- FG61: exclude four herbs, *C. indicum* roots, *F. racemosa* roots, *H. perforata* roots and *T. triandra* roots.

- FG62: exclude four herbs, *C. micracantha* roots, *F. racemosa* roots, *H. perforata* roots and *T. triandra* roots.

- FG63: exclude four herbs, *C. micracantha* roots, *C. indicum* roots, *H. perforata* roots and *T. triandra* roots.

- FG64: exclude four herbs, *C. micracantha* roots, *C. indicum* roots, *F. racemosa* roots and *T. triandra* roots.

- FG65: exclude four herbs, *C. micracantha* roots, *C. indicum* roots, *F. racemosa* roots and *H. perforata* roots.

- FG66: exclude three herbs, *F. racemosa* roots, *H. perforata* roots and *T. triandra* roots.

- FG67: exclude three herbs, *C. indicum* roots, *H. perforata* roots and *T. triandra* roots.

- FG68: exclude three herbs, *C. indicum* roots, *F. racemosa* roots and *T. triandra* roots.

- FG69: exclude three herbs, *C. indicum* roots, *F. racemosa* roots and *H. perforata* roots.

- FG70: exclude three herbs, *C. micracantha* roots, *H. perforata* roots and *T. triandra* roots.

- FG71: exclude three herbs, *C. micracantha* roots, *F. racemosa* roots and *T. triandra* roots.

- FG72: exclude three herbs, *C. micracantha* roots, *F. racemosa* roots and *H. perforata* roots.

- FG73: exclude three herbs, *C. micracantha* roots, *C. indicum* roots and *T. triandra* roots.

- FG74: exclude three herbs, *C. micracantha* roots, *C. indicum* roots and *H. perforata* roots.

- FG75: exclude three herbs, *C. micracantha* roots, *C. indicum* roots and *F. racemosa* roots.

- FG76: exclude two herbs, *H. perforata* roots and *T. triandra* roots.

- FG77: exclude two herbs, *F. racemosa* roots and *T. triandra* roots.

- FG78: exclude two herbs, *F. racemosa* roots and *H. perforata* roots.

- FG79: exclude two herbs, *C. indicum* roots and *T. triandra* roots.

- FG80: exclude two herbs, *C. indicum* roots and *H. perforata* roots.

- FG81: exclude two herbs, *C. indicum* roots and *F. racemosa* roots.

- FG82: exclude two herbs, *C. micracantha* roots and *T. triandra* roots.

- FG83: exclude two herbs, *C. micracantha* roots and *H. perforata* roots.

- FG84: exclude two herbs, *C. micracantha* roots and *F. racemosa* roots.

- FG85: exclude two herbs, *C. micracantha* roots and *C. indicum* roots.

- FG86: exclude one herb, *T. triandra* roots.
- FG87: exclude one herb, *H. perforata* roots.
- FG88: exclude one herb, *F. racemosa* roots.
- FG89: exclude one herb, *C. indicum* roots.
- FG90: exclude one herb, *C. micracantha* roots.

2.11) Modified MHR remedies by using only adjunct herbs or supportive herbs.

- FG91: only adjunct herbs.
- FG92: only supportive herbs.

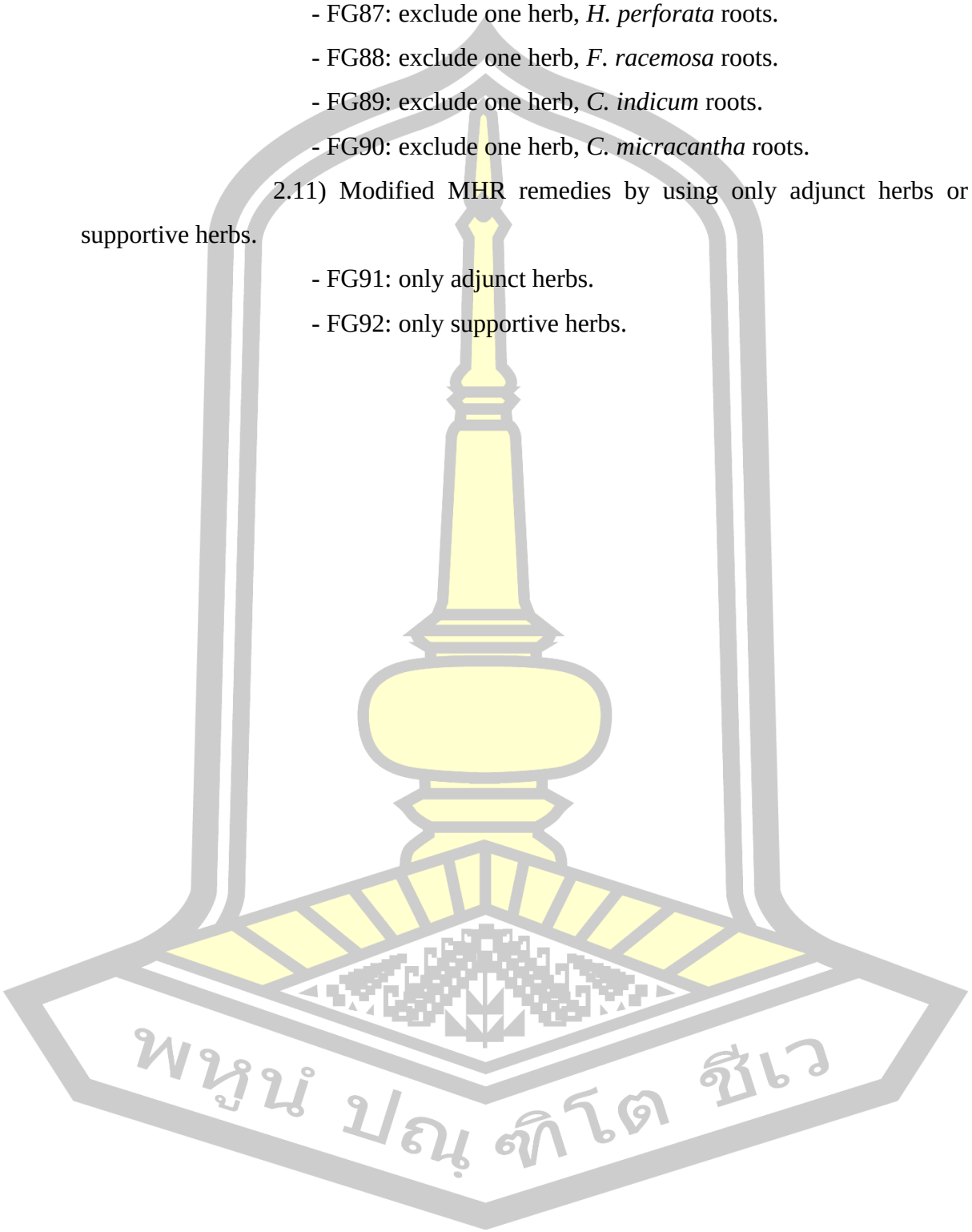


Table 8 Experimental design to decrease the number of herbs in various factors.

Structure	Sub-group	Material	Ratio (% w/w)	Formula groups (FG)															
				01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16
1. Primary herbs	**1.1 Reduced-toxic fever herbs**	C. micracantha (ชิงชี่)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		C. indicum (เท้ายายม่อม)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		F. racemosa (มะเดื่อชุมพร)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		H. perforata (คนทา)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		T. triandra (ย่านาง)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1.2 Anti-kamdao and lohit fever herbs	*A. indica* (สะเดา)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		G. chinense (กระดอม)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		T. crispa (บอระเพ็ด)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1.3 Anti-di fever herbs	*D. cochinchinensis* (จันทน์แดง)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		T. hoaensis (จันทน์ขาว)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1.4 Anti-semha and lom fever herbs	*L. sinense* (โกฐหัวบัว)	1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		P. kesiya (สน)	1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
2. Adjunct herbs	**2.1 Stimulant laxative**	*B. ovata* (มะกา)	4	■	□	■	■	■	■	■	■	■	□	■	■	□	□	■	■
		C. fistula (ลูน)	12	■	□	■	■	■	■	■	■	■	□	■	■	□	□	■	■
	2.2 Sour-astringent laxative	*T. bellirica* (สมอพิเภก)	8	■	□	■	■	■	■	■	■	■	□	□	□	■	■	■	□
		T. chebula (สมอไทย)	8	■	□	■	■	■	■	■	■	■	□	□	□	■	■	□	■
		Terminalia sp. (สมอเทศ)	8	■	□	■	■	■	■	■	■	■	□	□	□	■	■	□	□
		P. emblica (มะขามป้อม)	8	■	□	■	■	■	■	■	■	■	□	□	□	■	■	□	□
3. Supportive herbs		*M. ferrea* (บุนนาค)	4	■	□	□	■	□	□	■	■	□	■	■	□	■	□	□	□
		N. nucifera (บัวหลวง)	2	■	□	□	□	■	□	■	□	■	■	■	□	■	□	□	□
		V. zizanioides (แฝกหอม)	4	■	□	□	□	□	■	□	■	■	■	■	□	■	□	□	□

Note: ■ = Include, □ = Exclude

Table 8 Experimental design to decrease the number of herbs in various factors (Continued).

Structure	Sub-group	Material	Ratio (% w/w)	Formula groups (FG)															
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. Primary herbs	**1.1 Reduced-toxic fever herbs**	C. micracantha (ชิงชี่)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		C. indicum (เท้ายายม่อม)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		F. racemosa (มะเดื่อชุมพร)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		H. perforata (คนทา)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		T. triandra (ย่านาง)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1.2 Anti-kamdao and lohit fever herbs	*A. indica* (สะเดา)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		G. chinense (กระดอม)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		T. crispa (บอระเพ็ด)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1.3 Anti-di fever herbs	*D. cochinchinensis* (จันทน์แดง)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	□
		T. hoaensis (จันทน์ขาว)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	□
	1.4 Anti-semha and lom fever herbs	*L. sinense* (โกฐหัวบัว)	1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	□	□
		P. kesiya (สน)	1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	□	□
2. Adjunct herbs	**2.1 Stimulant laxative**	*B. ovata* (มะกา)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	□	□	□
		C. fistula (คูน)	12	■	■	■	■	■	■	■	■	■	■	■	■	□	■	□	□
	2.2 Sour-astringent laxative	*T. bellirica* (สมอพิเภก)	8	□	□	■	■	■	□	□	□	■	■	■	□	□	□	□	□
		T. chebula (สมอไทย)	8	□	□	■	□	□	■	■	□	■	■	□	■	□	□	□	□
		Terminalia sp. (สมอเทศ)	8	■	□	□	■	□	■	□	■	■	□	■	■	□	□	□	□
		P. emblica (มะขามป้อม)	8	□	■	□	□	■	□	■	■	□	■	■	■	□	□	□	□
3. Support herbs		*M. ferrea* (บุนนาค)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		N. nucifera (บัวหลวง)	2	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		V. zizanioides (แฝกหอม)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□

Note: ■ = Include, □ = Exclude

Table 8 Experimental design to decrease the number of herbs in various factors (Continued).

Structure	Sub-group	Material	Ratio (% w/w)	Formula groups (FG)															
				33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
1. Primary herbs	**1.1 Reduced-toxic fever herbs**	C. micracantha (ชิงชี่)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		C. indicum (เท้ายายม่อม)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		F. racemosa (มะเดื่อชุมพร)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		H. perforata (คนทา)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		T. triandra (ย่านาง)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1.2 Anti-kamdao and lohit fever herbs	*A. indica* (สะเดา)	4	□	■	□	□	□	■	■	■	■	■	■	□	■	□	□	□
		G. chinense (กระดอม)	4	□	■	□	□	□	■	■	■	■	■	□	■	□	■	□	□
		T. crispa (บอระเพ็ด)	4	□	■	□	□	□	■	■	■	■	□	■	■	□	□	■	□
	1.3 Anti-di fever herbs	*D. cochinchinensis* (จันทน์แดง)	4	□	□	■	■	□	■	■	■	□	□	□	□	□	□	□	■
		T. hoaensis (จันทน์ขาว)	4	□	□	■	■	□	■	■	□	■	□	□	□	□	□	□	□
	1.4 Anti-semha and lom fever herbs	*L. sinense* (โกฐหัวบัว)	1	□	■	■	□	■	■	□	□	□	□	□	□	□	□	□	□
		P. kesiya (สน)	1	□	■	■	□	■	□	■	□	□	□	□	□	□	□	□	□
2. Adjunct herbs	**2.1 Stimulant laxative**	*B. ovata* (มะกา)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		C. fistula (คูน)	12	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
	2.2 Sour-astringent laxative	*T. bellirica* (สมอพิเภก)	8	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		T. chebula (สมอไทย)	8	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		Terminalia sp. (สมอเทศ)	8	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		P. emblica (มะขามป้อม)	8	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
3. Support herbs		*M. ferrea* (บุนนาค)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		N. nucifera (บัวหลวง)	2	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		V. zizanioides (แฝกหอม)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□

Note: ■ = Include, □ = Exclude

Table 8 Experimental design to decrease the number of herbs in various factors (Continued).

Structure	Sub-group	Material	Ratio (% w/w)	Formula groups (FG)															
				49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
1. Primary herbs	**1.1 Reduced-toxic fever herbs**	C. micracantha (ชิงชี่)	4	■	■	■	■	■	■	■	■	■	■	■	■	■	□	□	□
		C. indicum (เท้ายายม่อม)	4	■	■	■	■	■	■	■	■	■	■	■	■	□	■	□	□
		F. racemosa (มะเดื่อชุมพร)	4	■	■	■	■	■	■	■	■	■	■	■	■	□	□	■	□
		H. perforata (คนทา)	4	■	■	■	■	■	■	■	■	■	■	■	■	□	□	□	■
		T. triandra (ย่านาง)	4	■	■	■	■	■	■	■	■	■	■	■	■	□	□	□	□
	1.2 Anti-kamdao and lohit fever herbs	*A. indica* (สะเดา)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		G. chinense (กระดอม)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		T. crispa (บอระเพ็ด)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
	1.3 Anti-di fever herbs	*D. cochinchinensis* (จันทน์แดง)	4	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		T. hoaensis (จันทน์ขาว)	4	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
	1.4 Anti-semha and lom fever herbs	*L. sinense* (โกฐหัวบัว)	1	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		P. kesiya (สน)	1	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□
2. Adjunct herbs	**2.1 Stimulant laxative**	*B. ovata* (มะกา)	4	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□
		C. fistula (คูน)	12	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□
	2.2 Sour-astringent laxative	*T. bellirica* (สมอพิเภก)	8	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□
		T. chebula (สมอไทย)	8	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□
		Terminalia sp. (สมอเทศ)	8	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□
		P. emblica (มะขามป้อม)	8	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□
3. Support herbs		*M. ferrea* (บุนนาค)	4	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□
		N. nucifera (บัวหลวง)	2	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□
		V. zizanioides (แฝกหอม)	4	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□

Note: ■ = Include, □ = Exclude

Table 8 Experimental design to decrease the number of herbs in various factors (Continued).

Structure	Sub-group	Material	Ratio (% w/w)	Formula groups (FG)															
				65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
1. Primary herbs	**1.1 Reduced-toxic fever herbs**	C. micracantha (ชิงชี่)	4		■	■	■	■							■	■	■	■	■
		C. indicum (เท้ายายม่อม)	4		■				■	■	■				■	■	■		
		F. racemosa (มะเดื่อชุมพร)	4			■			■			■	■		■			■	■
		H. perforata (คนทา)	4				■			■		■		■		■		■	
		T. triandra (ย่านาง)	4	■				■			■		■	■			■		■
	1.2 Anti-kamdao and lohit fever herbs	*A. indica* (สะเดา)	4																
		G. chinense (กระดอม)	4																
		T. crispa (บอระเพ็ด)	4																
	1.3 Anti-di fever herbs	*D. cochinchinensis* (จันทน์แดง)	4																
		T. hoaensis (จันทน์ขาว)	4																
	1.4 Anti-semha and lom fever herbs	*L. sinense* (ไกฐหัวบัว)	1																
		P. kesiya (สน)	1																
2. Adjunct herbs	**2.1 Stimulant laxative**	*B. ovata* (มะกา)	4																
		C. fistula (คูน)	12																
	2.2 Sour-astringent laxative	*T. bellirica* (สมอพิเภก)	8																
		T. chebula (สมอไทย)	8																
		Terminalia sp. (สมอเทศ)	8																
		P. emblica (มะขามป้อม)	8																
3. Support herbs		*M. ferrea* (บุนนาค)	4																
		N. nucifera (บัวหลวง)	2																
		V. zizanioides (แฝกหอม)	4																

Note: ■ = Include, □ = Exclude

Table 8 Experimental design to decrease the number of herbs in various factors (Continued).

Structure	Sub-group	Material	Ratio (% w/w)	Formula groups (FG)											
				81	82	83	84	85	86	87	88	89	90	91	92
1. Primary herbs	**1.1 Reduced-toxic fever herbs**	C. micracantha (ชิงชี่)	4	■	□	□	□	□	■	■	■	■	□	□	□
		C. indicum (เท้ายายม่อม)	4	□	■	■	■	□	■	■	■	□	■	□	□
		F. racemosa (มะเดื่อชุมพร)	4	□	■	■	□	■	■	■	□	■	■	□	□
		H. perforata (คนทา)	4	■	■	□	■	■	■	□	■	■	■	□	□
		T. triandra (ย่านาง)	4	■	□	■	■	■	□	■	■	■	■	□	□
	1.2 Anti-kamdao and lohit fever herbs	*A. indica* (สะเดา)	4	□	□	□	□	□	□	□	□	□	□	□	□
		G. chinense (กระดอม)	4	□	□	□	□	□	□	□	□	□	□	□	□
		T. crispa (บอระเพ็ด)	4	□	□	□	□	□	□	□	□	□	□	□	□
	1.3 Anti-di fever herbs	*D. cochinchinensis* (จันทน์แดง)	4	□	□	□	□	□	□	□	□	□	□	□	□
		T. hoaensis (จันทน์ขาว)	4	□	□	□	□	□	□	□	□	□	□	□	□
	1.4 Anti-semha and lom fever herbs	*L. sinense* (โกฐหัวบัว)	1	□	□	□	□	□	□	□	□	□	□	□	□
		P. kesiya (สน)	1	□	□	□	□	□	□	□	□	□	□	□	□
2. Adjunct herbs	**2.1 Stimulant laxative**	*B. ovata* (มะกา)	4	□	□	□	□	□	□	□	□	□	□	■	□
		C. fistula (คูน)	12	□	□	□	□	□	□	□	□	□	□	■	□
	2.2 Sour-astringent laxative	*T. bellirica* (สมอพิเภก)	8	□	□	□	□	□	□	□	□	□	□	■	□
		T. chebula (สมอไทย)	8	□	□	□	□	□	□	□	□	□	□	■	□
		Terminalia sp. (สมอเทศ)	8	□	□	□	□	□	□	□	□	□	□	■	□
		P. emblica (มะขามป้อม)	8	□	□	□	□	□	□	□	□	□	□	■	□
3. Support herbs		*M. ferrea* (บุนนาค)	4	□	□	□	□	□	□	□	□	□	□	□	■
		N. nucifera (บัวหลวง)	2	□	□	□	□	□	□	□	□	□	□	□	■
		V. zizanioides (แฝกหอม)	4	□	□	□	□	□	□	□	□	□	□	□	■

Note: ■ = Include, □ = Exclude

3.2.2 Quality control of plant materials

3.2.2.1 Physical determination

Physical examination using the macroscopical method and analysis of foreign matter, which is a key quality control method for medicinal plants in Thai Herbal Pharmacopoeia (THP), served as the criterion for selecting plant materials in this study. Vegetable drugs should be free from molds, insects and other animal contamination. Foreign matter is material consisting of any or all of the following:

1) Foreign organs: matter coming from the source plant but not defined as the drug.

2) Foreign elements: matter not coming from the source plant and of either vegetable or mineral origin.

Weigh 100 to 500 g of the substance being examined or the quantity specified in the monograph and spread it in a thin layer. Separate the foreign matter by hand as completely as possible, weigh it and calculate the percentage present (Department of Medical Sciences, 2021).

3.2.2.2 Extractive value

The extractive value, one of the methods for quality control of medicinal plants in THP, is used for the criteria of plant material selection in this study. The ethanol-soluble extractive and water-soluble extractive of every plant material will be analyzed. These methods were carried out in triplicate.

1) Ethanol-soluble extractive

The ethanol soluble (ethanolic extract) was evaluated for extractive value. Five grams of the dried powder (No. 70 mesh) was macerated in an Erlenmeyer flask. 100 mL of 95% ethanol was added for ethanolic extract. The flask was shaken for 6 hours and allowed to stand at room temperature for 18 hours, then filtered 20 mL of extract was evaporated and dried at 105ºC. This process was repeated until the weight was constant. Percentage yields of all extracts were calculated using the following equation (Department of Medical Sciences, 2021):

$$\%Yield = \frac{Weight\ of\ the\ extract\ (g)}{Weight\ of\ dried\ powder\ (g)} \times 100$$

2) Water-soluble extractive

The water soluble (aqueous extract) was evaluated for extractive value. Five grams of the dried powder (No. 70 mesh) was macerated in Erlenmeyer flask. 100 mL of chloroform water (Chloroform : Water = 2.5 : 997.5 v/v) added for aqueous extract. The flask was shaken for 6 hours and allowed to stand at room temperature for 18 hours, then filtered 20 mL of extract was evaporated and dried at 105ºC. This process was repeated until the weight was constant. Percentage yields of all extracts were calculated using the following equation (Department of Medical Sciences, 2021):

$$\%Yield = \frac{Weight\ of\ the\ extract\ (g)}{Weight\ of\ dried\ powder\ (g)} \times 100$$

3.2.4 Preparation of crude extracts

Plant materials were sourced from a single supplier of medicinal plants that met the extractive value standards outlined in the monograph. After washing with water, the materials were sliced into small pieces and dried in a hot air oven at 50°C before being ground into fine powder. The plant ingredients were then weighed and blended according to the original MHR remedy (FG01) and modified MHR remedies (FG02-92). Each herbal component, as well as MHR remedy and modified MHR remedies, was subjected to maceration in 70% ethanol and decoction in distilled water.

3.2.4.1 Maceration

The crude powder of the remedies and their ingredients were macerated in 70% ethanol (1:5 w/v) for 3 days (Nualkaew, 2020) and filtered through a Whatman No.1 filter paper. The filtrate was dried by a rotary evaporator. The maceration was repeated twice with residue and dried again by vacuum drying. Percentage yields of all the ethanolic extracts were calculated.

3.2.4.2 Decoction

The crude drug of remedies and their ingredients were boiled in distilled water (1:5 w/v) for 30 minutes (Nualkaew, 2020) in stainless steel pot and filtered through a Whatman No.1 filter paper. The filtrate was dried by lyophilizer.

Percentage yields of all the aqueous extracts were calculated using the following equation:

$$\%Yield = \frac{Weight\ of\ the\ extract\ (g)}{Weight\ of\ dried\ powder\ (g)} \times 100$$

The crude extracts were kept in a freezer (-20ºC) until use.

3.2.5 Anti-inflammatory and cytotoxicity activities

3.2.5.1 Animal cell lines

Murine leukemia macrophage cell line (RAW 264.7) was obtained from American Type Culture Collection (ATCC TIB-71). This cell line was cultured in DMEM medium containing 10% heat-inactivated fetal bovine serum, penicillin and streptomycin. The cells were incubated at 37º C in a 5% CO_2 incubator and subpassaged every 4-5 days.

3.2.5.2 Preparation of sample solution

The ethanolic extracts were dissolved in sterile dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/mL, but the aqueous extracts were dissolved in sterile distilled water to a final concentration of 20 mg/mL and filtered with Millipore filter 0.22 µm. Each extract was diluted with DMEM to obtain a final concentration range of 1–100 µg/mL for the ethanolic extract and 500 µg/mL for the aqueous extract.

3.2.5.3 Assay for nitric oxide (NO) inhibitory effects in RAW 264.7 cells (Tewtrakul and Itharat, 2007)

The cells (RAW 264.7) were cultured in a flask with DMEM medium containing 10% FBS, 1% Antibiotic-Antimycotic (penicillin, streptomycin, and Gibco amphotericin B), RAW 264.7 cells were washed by phosphate buffer saline (PBS) and suspended by 0.25% trypsin-EDTA. The cells were cultured in a sterile 96-well plate ($1x10^5$ cells/well) with 100 µL complete DMEM and incubated in 5% CO_2, 37º C overnight (24 hours). Complete DMEM (100 µL/well) containing 1 µg/mL of lipopolysaccharide (LPS) was replaced in control and only complete DMEM was replaced in normal. Next, 100 µL/well of each sample concentration were added but

100 µL/well of complete DMEM was added in control medium, 100 µL/well of 0.2% DMSO in control solvent, then incubated overnight (24 hours). Supernatant 100 µL was transferred to another sterile 96-well plate, followed by 100 µL of Griess reagent. The NO production was determined by measuring the accumulation of nitrite which interacted with Griess reagent. The absorbance was measured by microplate spectrophotometers at wavelength 520 nm. This method was carried out in triplicate. The inhibition (%) was calculated using the following equation and IC_{50} value was calculated using Prism program.

$$\%Inhibition = \frac{C - S}{C} \times 100$$

Control (C) : LPS (+), sample (-)

Sample (S) : LPS (+), sample (+)

3.2.5.4 Cytotoxicity by MTT assay (Tewtrakul and Itharat, 2007)

MTT assay was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromine (MTT) colorimetric method. This method continued from NO assay above. The plates were incubated at 37º C in 5% CO_2 incubator for 24 hours. MTT solution (10 µL, 5 mg/mL in PBS) was added in each well and incubated 2 hours. Supernatant was removed and 75 µL of DMSO was added to dissolve the formazan production in cells. The density of formazan solution was measured by microplate spectrophotometers at wavelength 570 nm. If the density of cell viability is less than 70% that sample was considered to toxic (ISO: 2015).

$$\%Cell\ viability = \frac{C - S}{C} \times 100$$

Control (C) : LPS (-), sample (-)

Sample (S) : LPS (-), sample (+)

3.2.6 Antioxidant activities

3.2.6.1 Preparation of sample solution

The ethanolic extracts were dissolved in ethanol to a final concentration of 10 mg/mL and the aqueous extracts were dissolved in distilled water to a final concentration of 10 mg/mL and filtered with Millipore filter 0.45 µm. Each extract was diluted to obtain a final concentration of 1-100 µg/mL.

3.2.6.2 Assay for nitric oxide free radical scavenging activity (Marcocci *et al*., 1994; Aktas *et al*., 2013)

Nitric oxide (NO) was generated from sodium nitroprusside and measured by the Griess reaction with slight modifications. Sodium nitroprusside in aqueous solution at physiological pH generates NO which interacts with oxygen to produce nitrite ions, estimated using Griess reagent. Scavengers of NO compete with oxygen, leading to reduced production of nitrite ions. To make an aqueous solution of sodium nitroprusside, 10 mM sodium nitroprusside was dissolved in 0.1 M phosphate buffer saline (PBS) (pH 7.4). To test the samples, 50 µL of a serially diluted sample was added to a 96-well flat-bottomed plate. Following this, 50 µL of 10 mM sodium nitroprusside, dissolved in phosphate buffered saline (PBS), was added to each well and the plate was incubated under light at room temperature for 120 min. Finally, 100 µL of the Griess reagent (a mixture of 1% sulfanilamide in 5% phosphoric acid, and 0.1% N-(1-naphthyl)-ethylene diamine hydrochloride in distilled water) was added to each well to measure the nitrite content. The absorbance of samples was measured after 10 min at 520 nm with a microplate spectrophotometer. Ascorbic acid and Trolox were used as a positive control. The IC_{50} values for each test compound as well as standard preparation were calculated.

$$\%Scavenging = \frac{Abs(control) - [Abs(sample) - Abs(blank)]}{Abs(control)} \times 100$$

Abs(control) : Absorbance of control

Abs(sample) : Absorbance of test sample

Abs(blank) : Absorbance of test sample without Griess reagent

3.2.6.3 Assay for super oxide free radical scavenging activity (Jaikong, 2021)

Superoxide anion ($O_2^{\bullet-}$) is an oxygen molecule with an extra electron that can damage mitochondria, DNA, and other molecules. Superoxide generated both *in vivo* and in foods can undergo several reactions including dismutation to give H_2O_2. Superoxide anions are generated in riboflavin-light-NBT system by the oxidation of riboflavin and assayed by the reduction of nitro-blue tetrazolium (NBT) resulting in the formation of blue formazan. To make an aqueous solution of NBT, 750 µM NBT was dissolved in 50 mM potassium phosphate buffer (PPB, pH 8.0). To test the samples, 40 µL of a serial diluted sample added into a 96-well flat-bottomed plate. Subsequently, 20 µL of 50 mM PPB, 20 µL of 1 mM EDTA in PPB, and 100 µL of 266 µM riboflavin in PPB were added to each well. Finally, 20 µL of the NBT solution was added to measure the $O_2^{\bullet-}$ radical, and the plate was incubated under a 15-watt light source (16 cm vertical) at room temperature for 10 minutes. The density of formazan solution was measured by microplate spectrophotometers at wavelength 590 nm. Ascorbic acid and Trolox were used as a positive control. The IC_{50} values for each test compound as well as standard preparation were calculated.

$$\%Scavenging = \frac{Abs(control) - [Abs(sample) - Abs(blank)]}{Abs(control)} \times 100$$

Abs(control) : Absorbance of control

Abs(sample) : Absorbance of test sample

Abs(blank) : Absorbance of test sample without riboflavin solution

3.2.7 Characterization and identification of chemical marker by HPLC

3.2.7.1 Identification of original formula of MHR and modified MHR formulas

The ethanolic extracts were dissolved in 70% ethanol to a final concentration of 10 mg/mL, while the aqueous extracts were dissolved in deionized water to a final concentration of 20 mg/mL. The solutions were filtered using a 0.45

µm Millipore filter and the filtrate (20 µL) was injected into HPLC. The HPLC chromatogram was determined at wavelength 190-800 nm. The mobile phase consisted of 0.1% v/v trifluoroacetic acid (TFA) in water (A) and acetonitrile (B) was used with Luna, 5µ 100Å C18, 250x4.6 mm (Phenomenex®) as the stationary phase. A suitable gradient mobile phase ratio of 0.1% v/v trifluoroacetic acid (TFA) in water (A) and acetonitrile (B) was developed. The flow rate was set at 0.8 mL/min. The analysis was performed using an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA). HPLC chromatogram was selected for determination in all experiments.

3.2.7.2 Identification of the component of MHR

Herbal component powders of MHR (0.5 g) were dissolved in 25 mL of 70% ethanol and sonicated for 30 min to obtain the ethanolic extract. For the aqueous extracts, a 0.5 g sample of each component powder was dissolved in 25 mL of deionized water and then boiled in distilled water for 30 min. The solution was filtered through 0.45 µm Millipore filter and the filtrate (20 µL) was injected into HPLC. The HPLC chromatogram was determined at wavelength 190-800 nm. The mobile phase consisted of 0.1% v/v trifluoroacetic acid (TFA) in water (A) and acetonitrile (B) was used with Luna, 5µ 100Å C18, 250x4.6 mm (Phenomenex®) as the stationary phase. The flow rate was set at 0.8 mL/min. The analysis was performed using an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA). Each chromatographic peak of MHR was identified by comparison of their retention time and superimposition of their normalized UV spectra with the individual reference compound. In addition, spiking 50 % (v/v) of each component to MHR was also used.

3.2.7.3 Identification of the chromatographic peak

All peaks of the HPLC chromatogram of MHR were identified in which they originated from each herbal component. The major peaks of MHR were identified by comparison of their retention time and superimposition of their normalized UV spectra against those corresponding to the reference standard or isolated compound.

3.2.8 Characterization and identification of chemical marker by TLC

The Thin-Layer Chromatography (TLC) analysis of extracts obtained from 70% ethanolic extraction of the MHR remedy, its herbal components, and modified MHR was performed using Silica Gel 60 F_{254} as the stationary phase. The ethanolic extracts were dissolved in 70% ethanol to a final concentration of 10 mg/mL, while the reference standard was dissolved in *n*-propanol to a final concentration of 1 mg/mL. System I used a mobile phase consisting of toluene, ethyl acetate, methanol, and formic acid in a 7:2:1:0.5 (v/v) ratio, while System II used a mobile phase with toluene, ethyl acetate, methanol, and formic acid in a 5:3:2:0.5 (v/v) ratio. The chromatographic plates were examined under ultraviolet light at two wavelengths (UV 254 nm and UV 366 nm), followed by spraying with anisaldehyde-sulfuric acid reagent. Distances between the spots were measured and the retention factor (R_f) values were recorded.

$$Rf = \frac{Sample\ distance}{Solvent\ distance}$$

3.2.9 Phytochemical studies and isolation of chemical constituents of MHR

3.2.9.1 Isolation of perforatic acid

Perforatic acid was isolated from the 70% ethanolic extract of *Harrisonia perforata* roots by preparative TLC on Silica Gel 60 F_{254} using mobile phase of chloroform : methanol : formic acid (95:5:0.5, v/v/v). The major band of R_f 0.40 was extracted with methanol under ultrasonication for 30 min in 3 repeats to obtain perforatic acid in the form of slightly yellow powder.

The structures of the isolated compounds were determined by their NMR data [^{1}H and ^{13}C on a Varian Unity Inova 500 spectrometer (400 MHz for ^{1}H and ^{13}C)]. ESI mass spectra were obtained from an Agilent Technologies 1200 Binary LC System coupled to a Bruker microTOF-Q mass spectrometer. The UV spectrum in methanol using an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA) with column C18 (Luna, 5µ 100Å, 250x4.6 mm, Phenomenex®) as the stationary phase.

3.2.9.2 Isolation of *O*-methyllaloptaeroxyrin

O-methyllaloptaeroxyrin (perforatin A) was isolated from the 70% ethanolic extract of *Harrisonia perforata* roots by preparative TLC on Silica Gel 60 F_{254} using mobile phase of chloroform : ethyl acetate (8:2, v/v). The major band of R_f 0.38 was extracted with methanol under ultrasonication for 30 min in 3 repeats to obtain *O*-methyllaloptaeroxyrin in the form of slightly yellow powder.

The structures of the isolated compounds were determined by their NMR data [^{1}H and ^{13}C on a Varian Unity Inova 500 spectrometer (400 MHz for ^{1}H; 100 MHz for and ^{13}C)]. ESI mass spectra were obtained from an Agilent Technologies 1200 Binary LC System coupled to a Bruker microTOF-Q mass spectrometer. The UV spectrum in methanol using an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA) with column C18 (Luna, 5µ 100Å , 250x4.6 mm, Phenomenex®) as the stationary phase.

3.2.9.3 Isolation of peucenin-7-methyl ether

Peucenin-7-methyl ether was isolated from the 70% ethanolic extract of *Harrisonia perforata* roots by preparative TLC on Silica Gel 60 F_{254} using mobile phase of toluene : ethyl acetate (9:1, v/v). The major band of R_f 0.60 was extracted with methanol under ultrasonication for 30 min in 3 repeats to obtain peucenin-7-methyl ether in the form of slightly yellow powder.

The structures of the isolated compounds were determined by their NMR data [^{1}H and ^{13}C on a Varian Unity Inova 500 spectrometer (400 MHz for ^{1}H; 100 MHz for and ^{13}C)]. ESI mass spectra were obtained from an Agilent Technologies 1200 Binary LC System coupled to a Bruker microTOF-Q mass spectrometer. The UV spectrum in methanol using an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA) with column C18 (Luna, 5µ 100Å , 250x4.6 mm, Phenomenex®) as the stationary phase.

3.2.10 Statistical analysis

All data are the means of three replications. The values of IC_{50} were evaluated by using GraphPad Prism v10.1.0 software. Values of different parameters were expressed as the mean ± standard deviation (standard deviation, SD) and mean ± standard error of the mean (SEM). A probability level of at least $p < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS statistical software. The details are as follows.

3.2.10.1 Anti-inflammatory activity test reported for % inhibition of NO production and IC_{50}, and cell viability (%). The % inhibition of NO and cell viability (%) are presented as mean ± SEM, while the IC_{50} values are expressed as mean ± SD. The differences in anti-inflammatory activity and cell viability between the modified MHR formulas and the original MHR formula were analyzed using one-way ANOVA followed by post-hoc tests.

3.2.10.2 Antioxidant activities test reported for % inhibition and IC_{50} of nitric oxide and superoxide radical scavenging. The % inhibition of nitric oxide and superoxide radical scavenging are presented as mean ± SEM, while the IC_{50} values are expressed as mean ± SD. The differences in antioxidant activities between the modified MHR formulas and the original MHR formula were analyzed using one-way ANOVA followed by post-hoc tests.

3.2.10.3 Identification and characterization of major compounds (markers) by HPLC of the extracts of each modified MHR formula and the original formula of MHR. The results were shown with mean ± SD.

3.2.10.4 The relationship analysis was conducted using parameters such as % inhibition of pharmacological effects, % cell viability, and peak area of chemical markers (metabolites) from HPLC analysis across 92 modified MHR formulas. The Bioinformatics website (http://www.bioinformatics.com.cn/) was used to visualize Pearson's rank correlations and Pearson's Rho analysis. The Pearson correlation coefficient (r) measures the linear relationship between two variables. It ranges from -1 to 1, where -1 indicates a perfect negative linear relationship, 1 indicates a perfect positive linear relationship, and 0 suggests no linear correlation. Principal component analysis (PCA) was visualized using GraphPad Prism v10.1.0 software. Additionally, Python v3.5 was employed for clustering heat map analysis.

CHAPTER IV
RESULTS

4.1 Standardization of MHR remedy

4.1.1 Quality control of herbal components of MHR

The monographs for twenty herbs are available in the Thai Herbal Pharmacopoeia (THP) or other references, except for *Azadirachta indica* A.Juss. Physical examination using the macroscopical method and foreign matter analysis served as criteria for selecting plant materials in this study. The selected plant materials were free from minerals, mold, insects, and other forms of animal contamination. The extractive value, one of the specifications in THP, was used to assess the quality of 20 herbal components. To establish the specification for *Azadirachta indica* A. Juss., samples were sourced from fifteen traditional pharmacies across Thailand for extractive value determination. Each experiment was performed in triplicate to ensure accuracy. The results for the extractive values of all plant materials are presented in **Table 9**.

A study of extractive value, ethanol-soluble and water-soluble extractives revealed that 18 plant materials met the standard requirements, including: *Capparis micracantha* roots, *Clerodendrum indicum* roots, *Dracaena cochinchinensis* woods, *Ficus racemosa* roots, *Gymnopetalum chinense* fruits, *Harrisonia perforata* roots, *Ligusticum sinense* rhizomes, *Mesua ferrea* flowers, *Nelumbo nucifera* stamens, *Phyllanthus emblica* fruits, *Pinus kesiya* woods, *Tarenna hoaensis* woods, *Terminalia bellirica* fruits, *Terminalia chebula* fruits, *Terminalia* sp. “Samo Thet” fruits, *Tiliacora triandra* roots, *Tinospora crispa* stems and *Vetiveria zizanioides* roots. However, two plant materials from a single source did not meet the standard requirements, *Bridelia ovata* leaves and *Cassia fistula* pulps. Both plant materials were sourced from five additional suppliers until their extractive values met the standard requirements.

The ethanol-soluble extractive percentage of *Azadirachta indica* petiole ranged from 2.49% to 6.17%, with an average of 4.30% ± 1.14%. The water-soluble extractive percentage ranged from 6.17% to 15.23%, with an average of 10.57% ±

2.65%. Detailed results for the extractive values of *A. indica* petiole are presented in **Table 10**.

Plant materials with extractive values in the average range or meeting standard requirements were selected for this study. The sources of raw materials used in preparing each designated formula, along with the original MHR formula, are listed in **Table 11**.

Table 9 The extractive value of plant materials of MHR compared to the specification in THP or other references.

No.	Material	Specification		Source	Extract method	Results*	Evaluation
		EtOH	Water				
1	*B. ovata* (มะกา) [a]	≥ 17.0%	≥ 12.0%	Phichit	EtOH	2.1076 ± 0.0398	Not Passed
					Water	8.8003 ± 0.2053	Not Passed
				Bangkok	EtOH	3.5360 ± 0.7221	Not Passed
					Water	11.5236 ± 0.1001	Not Passed
				Nakhon Pathom	EtOH	3.2333 ± 0.0927	Not Passed
					Water	6.8654 ± 0.2441	Not Passed
				Krabi	EtOH	2.7436 ± 0.1697	Not Passed
					Water	6.9789 ± 0.1951	Not Passed
				Maha Sarakham	EtOH	2.5005 ± 0.1319	Not Passed
					Water	11.2504 ± 0.1762	Not Passed
2	*C. micracantha* (ชิงชี่) [b]	≥ 2.0%	≥ 2.0%	Roi Et	EtOH	2.5005 ± 0.1319	Passed
					Water	11.2504 ± 0.1762	Passed
				Bangkok	EtOH	3.5360 ± 0.7221	Passed
					Water	11.5236 ± 0.1001	Passed

* All values are mean ± SD as obtained by triplicate (n=3) analyses, [a] Supatarawanich *et al*. (1995), [b] Department of Medical Sciences (2018), [c] Department of Medical Sciences (2021).

Table 9 The extractive value of plant materials of MHR compared to the specification in THP or other references (Continued).

No.	Material	Specification		Source	Extract method	Results*	Evaluation
		EtOH	Water				
3	*C. fistula* (คูน) [c]	≥ 66.0%	≥ 67.0%	Phichit	EtOH	6.5291 ± 0.0528	Not Passed
					Water	69.6483 ± 1.2739	Passed
				Bangkok	EtOH	14.2056 ± 0.1203	Not Passed
					Water	47.7865 ± 0.2613	Not Passed
				Nakhon Pathom	EtOH	16.9022 ± 0.0898	Not Passed
					Water	62.2258 ± 1.4845	Not Passed
				Krabi	EtOH	9.6103 ± 0.1409	Not Passed
					Water	64.3543 ± 1.9059	Not Passed
				Maha Sarakham	EtOH	3.7498 ± 0.0585	Not Passed
					Water	48.6836 ± 16.4933	Not Passed
4	*C. indicum* (เท้ายายม่อม) [c]	≥ 1.0%	≥ 4.0%	Roi Et	EtOH	2.2342 ± 0.0452	Passed
					Water	10.3214 ± 0.4822	Passed
				Bangkok	EtOH	3.2333 ± 0.0927	Passed
					Water	6.8654 ± 0.2441	Passed
5	*D. cochinchinensis* (จันทน์แดง) [c]	≥ 17.0%	≥ 2.0%	Bangkok	EtOH	21.7391 ± 0.3562	Passed
					Water	2.9799 ± 0.0417	Passed
				Nakhon Pathom	EtOH	29.3047 ± 0.6875	Passed
					Water	2.1076 ± 0.0457	Passed
6	*F. racemosa* (มะเดื่อชุมพร) [c]	≥ 1.0%	≥ 2.0%	Roi Et	EtOH	2.1076 ± 0.0398	Passed
					Water	8.8003 ± 0.2053	Passed
				Bangkok	EtOH	2.7436 ± 0.1697	Passed
					Water	6.9789 ± 0.1951	Passed
7	*G. chinense* (กระดอม) [d]	≥ 11.0%	≥ 26.0%	Bangkok	EtOH	18.2135 ± 0.2927	Passed
					Water	26.7693 ± 0.7197	Passed
				Nakhon Pathom	EtOH	17.3369 ± 0.2960	Passed
					Water	29.3541 ± 0.2073	Passed
8	*H. perforata* (คนทา) [c]	≥ 2.0%	≥ 3.0%	Roi Et	EtOH	8.9763 ± 0.1549	Passed
					Water	8.4972 ± 0.0252	Passed
				Bangkok	EtOH	6.8725 ± 0.0875	Passed
					Water	8.7504 ± 0.0868	Passed
9	*L. sinense* (โกฐหัวบัว) [c]	≥ 18.0%	≥ 35.0%	Bangkok	EtOH	28.4215 ± 0.3526	Passed
					Water	35.9818 ± 0.8284	Passed
				Nakhon Pathom	EtOH	22.2223 ± 0.9032	Passed
					Water	39.1658 ± 0.2904	Passed

* All values are mean ± SD as obtained by triplicate (n=3) analyses, [c] Department of Medical Sciences (2021), [d] Soonthornchareonnon and Ruangwises (2008).

Table 9 The extractive value of plant materials of MHR compared to the specification in THP or other references (Continued).

No.	Material	Specification		Source	Extract method	Results *	Evaluation
		EtOH	Water				
10	*M. ferrea* (บุนนาค) [c]	≥ 4.5%	≥ 2.5%	Bangkok	EtOH	28.9499 ± 0.5145	Passed
					Water	19.5742 ± 0.3856	Passed
				Nakhon Pathom	EtOH	32.5477 ± 0.1858	Passed
					Water	24.5924 ± 0.4773	Passed
11	*N. nucifera* (บัวหลวง) [c]	≥ 1.22 ± 0.02%	≥ 10.5%	Bangkok	EtOH	10.0490 ± 1.1447	Passed
					Water	19.7257 ± 0.1268	Passed
				Nakhon Pathom	EtOH	7.0393 ± 0.1606	Passed
					Water	15.9756 ± 0.2189	Passed
12	*P. emblica* (มะขามป้อม) [c]	≥ 16.0%	≥ 26.0%	Bangkok	EtOH	26.3715 ± 0.2816	Passed
					Water	43.6919 ± 0.1111	Passed
				Nakhon Pathom	EtOH	19.1555 ± 0.0236	Passed
					Water	34.1238 ± 0.2691	Passed
13	*P. kesiya* (สน) [d]	≥ 31.71%	≥ 1.19%	Nakhon Pathom	EtOH	34.6534 ± 0.6677	Passed
					Water	1.4949 ± 0.0549	Passed
				Krabi	EtOH	33.5273 ± 0.8450	Passed
					Water	2.0577 ± 0.0199	Passed
14	*T. hoaensis* (จันทน์ขาว) [c]	≥ 3.0%	≥ 3.0%	Bangkok	EtOH	4.0356 ± 0.0610	Passed
					Water	5.4875 ± 0.0404	Passed
				Nakhon Pathom	EtOH	3.1997 ± 0.0305	Passed
					Water	4.7981 ± 0.0650	Passed
15	*T. bellirica* (สมอพิเภก) [c]	≥ 17.0%	≥ 24.0%	Bangkok	EtOH	26.0611 ± 0.2755	Passed
					Water	42.5096 ± 0.2629	Passed
				Nakhon Pathom	EtOH	22.2695 ± 0.1866	Passed
					Water	33.3635 ± 0.2615	Passed
16	*T. chebula* (สมอไทย) [c]	≥ 20.0%	≥ 28.0%	Bangkok	EtOH	26.2844 ± 0.9678	Passed
					Water	36.4844 ± 0.9153	Passed
				Nakhon Pathom	EtOH	25.2430 ± 0.3985	Passed
					Water	31.0706 ± 0.2007	Passed
17	*Terminalia* sp. "Samo Thet" (สมอเทศ) [e]	≥ 45.61 ± 2.34%	≥ 40.22 ± 2.81%	Bangkok	EtOH	50.6402 ± 0.1833	Passed
					Water	67.0668 ± 1.1283	Passed
				Nakhon Pathom	EtOH	40.4311 ± 0.2806	Passed
					Water	54.4564 ± 2.7220	Passed
18	*T. triandra* (ย่านาง) [c]	≥ 4.0%	≥ 6.0%	Roi Et	EtOH	5.0977 ± 0.3172	Passed
					Water	13.1826 ± 0.1205	Passed
				Bangkok	EtOH	2.6503 ± 0.2234	Passed
					Water	9.0666 ± 0.6669	Passed

* All values are mean ± SD as obtained by triplicate (n=3) analyses, [c] Department of Medical Sciences (2021), [e] Jirawattanapong *et al*. (1997).

Table 9 The extractive value of plant materials of MHR compared to the specification in THP or other references (Continued).

No.	Material	Specification		Source	Extract method	Results *	Evaluation
		EtOH	Water				
19	*T. crispa* (บอระเพ็ด) [c]	≥ 5.0%	≥ 10.0%	Bangkok	EtOH	6.0103 ± 0.0987	Passed
					Water	14.5964 ± 0.3505	Passed
				Nakhon Pathom	EtOH	8.3941 ± 0.1965	Passed
					Water	19.7162 ± 0.3030	Passed
20	*V. zizanioides* (แฝกหอม) [f]	≥ 2.0%	≥ 2.0%	Bangkok	EtOH	6.5363 ± 0.1839	Passed
					Water	14.8830 ± 1.2521	Passed
				Nakhon Pathom	EtOH	7.2889 ± 0.1016	Passed
					Water	35.0699 ± 2.0679	Passed

* All values are mean ± SD as obtained by triplicate (n=3) analyses, [c] Department of Medical Sciences (2021), [e] Jirawattanapong *et al*. (1997), [f] Techadamrongsin and Pecharaply (2005).

Table 10 The extractive value of *Azadirachta indica* petiole from fifteen traditional pharmacies.

No.	Source	Ethanol-soluble, n = 3	Water-soluble, n = 3
1	Lampang (Authentic)	4.3715	10.7311
2	Phitsanulok	2.6937	6.1730
3	Nakhon Sawan	2.4907	6.2497
4	Phichit	3.3496	10.0319
5	Nakhon Pathom	5.8468	15.2263
6	Bangkok	4.9843	13.8075
7	Sakon Nakhon	4.3219	9.1313
8	Maha Sarakham (Authentic)	5.1077	9.0998
9	Yasothon	3.4995	9.4661
10	Chanthaburi	3.7058	9.8253
11	Chonburi	5.2344	13.3824
12	Phetchaburi (Authentic)	6.1699	12.9756
13	Kanchanaburi	4.8848	12.6798
14	Krabi	2.9101	8.7666
15	Songkhla	4.8746	11.0314
Mean		**4.2964**	**10.5718**
SD		**1.1415**	**2.6470**
Max		6.1699	15.2263
Min		2.4907	6.1730
Range		3.6791	9.0533

Table 11 Selected sources of plant materials for the preparation of original MHR and modified MHR.

No.	Material	Thai name	Part used	Source
1	*A. indica*	สะเดา (Sadao)	Petiole	Nakhon Pathom
2	*B. ovata*	มะกา (Ma Ka)	Leaf	Bangkok
3	*C. micracantha*	ชิงชี่ (Chingchi)	Root	Roi Et
4	*C. fistula*	คูน (Khun)	Pulp	Nakhon Pathom
5	*C. indicum*	เท้ายายม่อม (Thaoyaimom)	Root	Roi Et
6	*D. cochinchinensis*	จันทน์แดง (Chan Daeng)	Wood	Bangkok
7	*F. racemosa*	มะเดื่อชุมพร (Maduea Chumpon)	Root	Roi Et
8	*G. chinense*	กระดอม (Kra Dom)	Fruit	Bangkok
9	*H. perforata*	คนทา (Khon Tha)	Root	Roi Et
10	*L. sinense*	โกฐหัวบัว (Kot Huabua)	Rhizome	Bangkok
11	*M. ferrea*	บุนนาค (Bunnak)	Flower	Bangkok
12	*N. nucifera*	บัวหลวง (Bua Luang)	Stamen	Bangkok
13	*P. emblica*	มะขามป้อม (Makhampom)	Fruit	Bangkok
14	*P. kesiya*	สน (Son)	Wood	Krabi
15	*T. hoaensis*	จันทน์ขาว (Chan Khao)	Wood	Bangkok
16	*T. triandra*	ย่านาง (Yanang)	Root	Roi Et
17	*T. bellirica*	สมอพิเภก (Samo Pipek)	Fruit	Bangkok
18	*T. chebula*	สมอไทย (Samo Thai)	Fruit	Bangkok
19	*Terminalia sp.* "Samo Thet"	สมอเทศ (Samo Thet)	Fruit	Bangkok
20	*T. crispa*	บอระเพ็ด (Borapet)	Stem	Nakhon Pathom
21	*V. zizanioides*	แฝกหอม (Faek Hom)	Root	Nakhon Pathom

4.1.2 Characterization and identification of chemical marker by High-Performance Liquid Chromatograph (HPLC)

4.1.2.1 Identification of original MHR and its components

1) The ethanolic extract

1.1) MHR extract

The ethanolic extract of MHR was dissolved in 70% ethanol to a final concentration of 10 mg/mL and filtered through a 0.45 µm Millipore filter. The HPLC analysis was conducted on Agilent 1260 infinity II prime HPLC system (Agilent Technologies, USA). Chromatographic detection was performed at wavelengths 254 and 280 nm. The mobile phase consisted of 0.1% v/v TFA in water

and acetonitrile using a gradient elution profile detailed in **Table 12**. A Luna C18 column (5 µ, 100 Å, 250 x 4.6 mm, Phenomenex®) was employed for separation, with a flow rate set at 0.8 mL/min. The HPLC chromatograms of the MHR ethanolic extract, showing retention times over 160 minutes, are presented in **Figure 23**.

1.2) Herbal components of MHR

A 0.5 g sample of each component powder was dissolved in 25 mL of 70% ethanol in a volumetric flask and sonicated for 30 minutes. The solution was then filtered through a 0.45 µm Millipore filter, and a 20 µL aliquot of the filtrate was injected into the HPLC system. HPLC analysis was performed using an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA), with chromatographic detection at 254 nm and 280 nm. The mobile phase comprised 0.1% v/v TFA in water and acetonitrile, applied with a gradient elution as detailed in **Table 12**. A Luna C18 column (5 µ, 100 Å, 250 x 4.6 mm, Phenomenex®) was used for separation, with a flow rate of 0.8 mL/min. HPLC chromatograms of the MHR component, with retention times up to 160 minutes, are shown in Appendix B. Each chromatographic peak of MHR was identified by comparing retention times and overlaying normalized UV spectra with those of individual reference compounds.

Table 12 The mobile phase for HPLC analysis of MHR ethanolic extract and its components.

Time (min)	Mobile phase A (%), 0.1% TFA in water	Mobile phase B (%), acetonitrile	Flow rate (mL/min)
0	98	2	0.8
5	97	3	0.8
10	96	4	0.8
15	95	5	0.8
20	93	7	0.8
25	91	9	0.8
30	90	10	0.8
35	89	11	0.8
40	88	12	0.8
45	87	13	0.8
50	86	14	0.8
55	82	18	0.8
60	80	20	0.8

Table 12 The mobile phase for HPLC analysis of M H R ethanolic extract and its components (Continued).

Time (min)	Mobile phase A (%), 0.1% TFA in water	Mobile phase B (%), acetonitrile	Flow rate (mL/min)
65	79	21	0.8
70	78	22	0.8
75	75	25	0.8
80	73	27	0.8
85	70	30	0.8
90	65	35	0.8
95	60	40	0.8
100	55	45	0.8
105	50	50	0.8
110	50	50	0.8
115	45	55	0.8
120	40	60	0.8
125	35	65	0.8
130	30	70	0.8
135	18	82	0.8
140	18	82	0.8
145	10	90	0.8
150	5	95	0.8
155	0	100	0.8
160	0	100	0.8

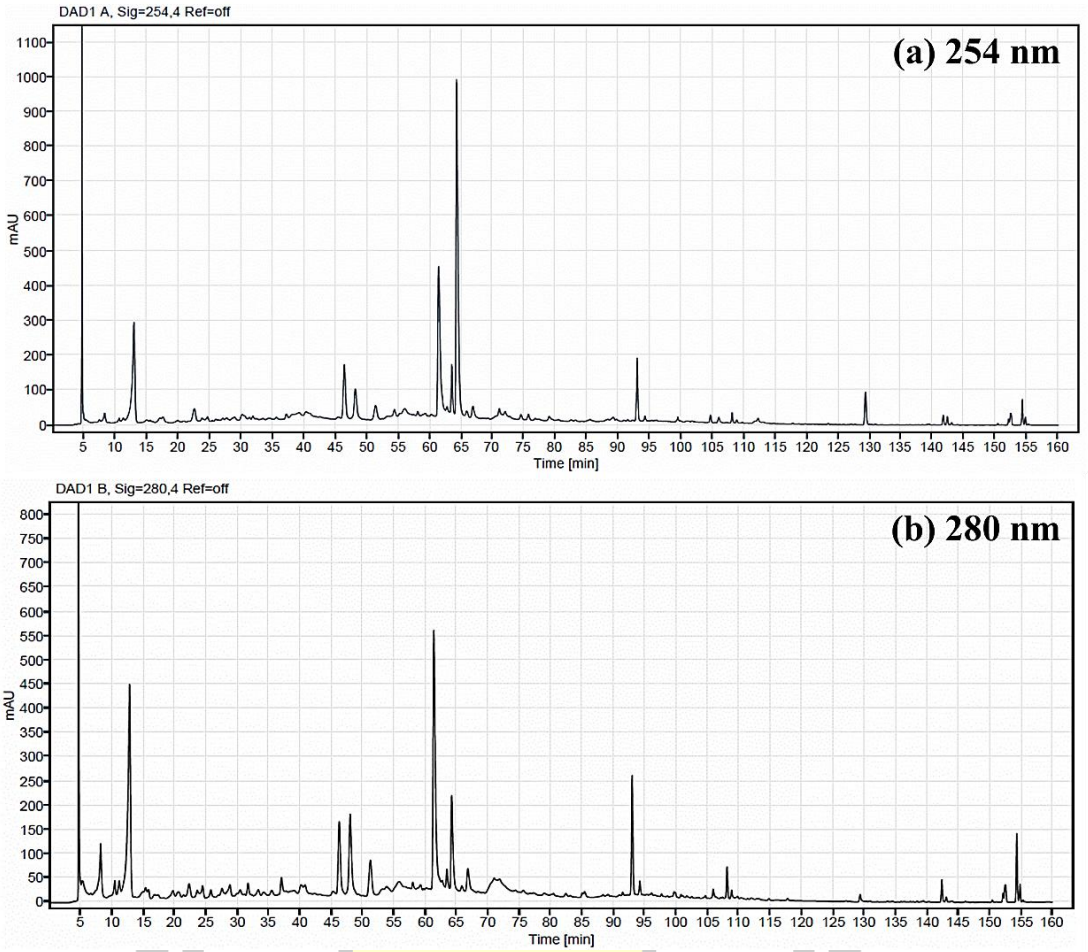


Figure 23 HPLC chromatograms of MHR ethanolic extract (FG01E) detected at wavelengths 254 nm (a) and 280 nm (b).

2) The aqueous extract

2.1) MHR extract

The aqueous extract was dissolved in deionized water to a final concentration of 20 mg/mL and filtered through a 0.45 µm Millipore filter. HPLC analysis was conducted on an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA), with detection wavelengths set at 254 nm and 280 nm. The mobile phase, consisting of 0.1% v/v TFA in water and acetonitrile, was applied using a gradient elution detailed in **Table 13**. A Luna C18 column (5 µ, 100 Å, 250 x 4.6 mm, Phenomenex®) was used for separation, with a flow rate of 0.8 mL/min. HPLC chromatograms of the MHR aqueous extract, with retention times extending to 160 min, are presented in **Figure 24**.

2.2) Herbal components of MHR

A 0.5 g sample of each component powder was dissolved in 25 mL of deionized water and then boiled in distilled water for 30 minutes. The resulting solution was filtered through a 0.45 µm Millipore filter, and a 20 µL aliquot of the filtrate was injected into the HPLC system. HPLC analysis was performed using an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, California, USA), with detection wavelengths set at 254 nm and 280 nm. The mobile phase, consisting of 0.1% v/v TFA in water and acetonitrile, was applied using a gradient elution as detailed in **Table 13**. A Luna C18 column (5 µ, 100 Å, 250 x 4.6 mm, Phenomenex®) was used for reversed-phase separation, with a flow rate of 0.8 mL/min. HPLC chromatograms of the MHR component, with retention times up to 160 minutes, are shown in Appendix C. Each chromatographic peak of MHR was identified by comparing retention times and overlaying normalized UV spectra with those of individual reference compounds.

Table 13 The mobile phase for HPLC analysis of MHR aqueous extract and its components.

Time (min)	Mobile phase A (%), 0.1% TFA in water	Mobile phase B (%), acetonitrile	Flow rate (mL/min)
0	99	1	0.8
10	99	1	0.8
15	98	2	0.8
20	97	3	0.8
25	96	4	0.8
30	95	5	0.8
35	94	6	0.8
40	93	7	0.8
45	92	8	0.8
50	91	9	0.8
55	90	10	0.8
65	88	12	0.8
70	87	13	0.8
75	86	14	0.8
80	85	15	0.8
85	84	16	0.8
90	83	17	0.8

Table 13 The mobile phase for HPLC analysis of MHR aqueous extract and its components (Continued).

Time (min)	Mobile phase A (%), 0.1% TFA in water	Mobile phase B (%), acetonitrile	Flow rate (mL/min)
95	82	18	0.8
100	80	20	0.8
105	75	25	0.8
110	75	25	0.8
115	70	30	0.8
120	65	35	0.8
125	60	40	0.8
130	55	45	0.8
135	50	50	0.8
140	40	60	0.8
145	30	70	0.8
150	20	80	0.8
155	10	90	0.8
160	0	100	0.8

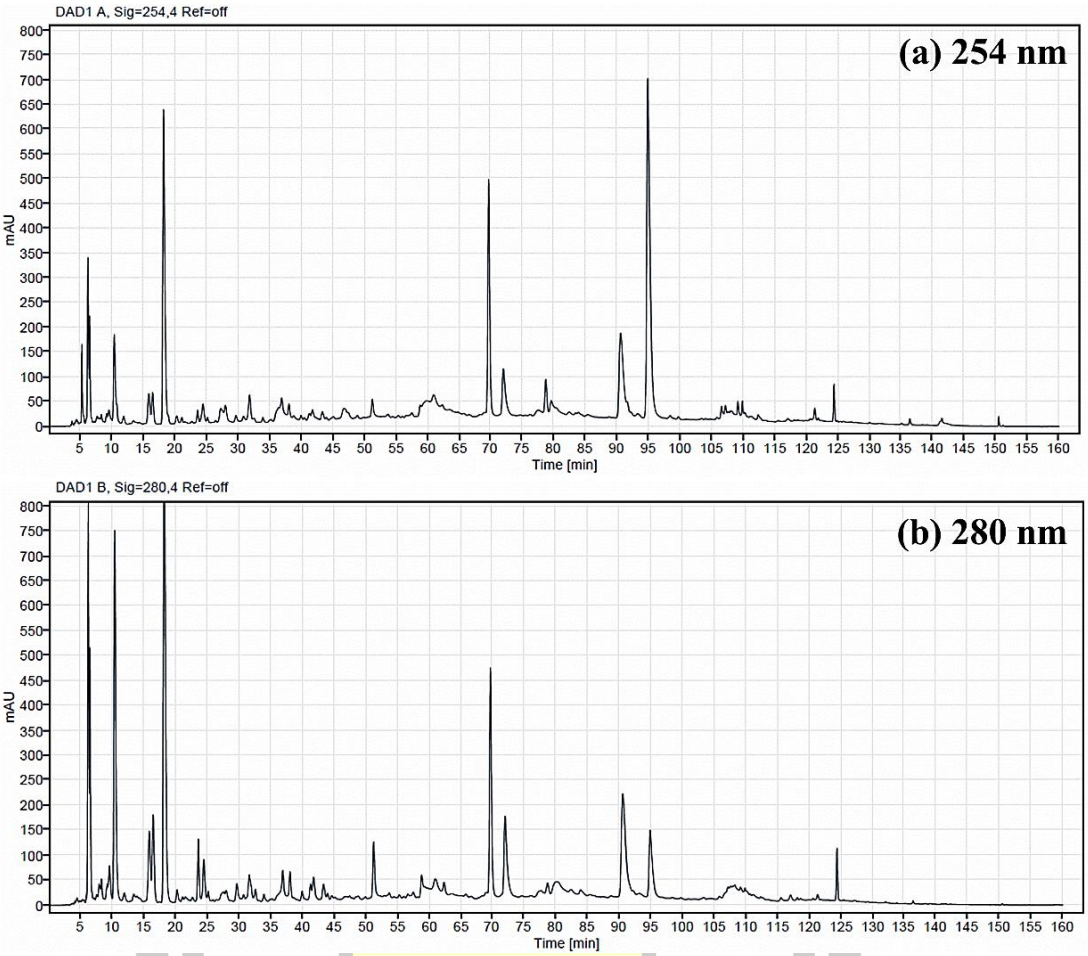


Figure 24 HPLC chromatograms of MHR aqueous extract (FG01A) detected at wavelengths 254 nm (a) and 280 nm (b).

4.1.2.2 Peak identification in HPLC chromatogram of MHR

The peaks in the HPLC chromatogram of MHR were identified by comparing their retention times and overlaying their normalized UV spectra with those of the HPLC chromatogram of herbal components, reference standards or isolated compounds.

1) The ethanolic extract

Eighteen components were identified in the HPLC chromatogram of MHR (FG01E) at both wavelengths (254 nm and 280 nm), including *Azadirachta indica*, *Bridelia ovata*, *Capparis micracantha*, *Cassia fistula*, *Clerodendrum indicum*, *Dracaena cochinchinensis*, *Gymnopetalum chinense*, *Harrisonia perforata*, *Ligusticum sinense*, *Mesua ferrea*, *Nelumbo nucifera*, *Phyllanthus emblica*, *Pinus*

kesiya, *Terminalia bellirica*, *Terminalia chebula*, *Terminalia* sp. “Samo Thet”, *Tinospora crispa*, and *Vetiveria zizanioides* while *Tarenna hoaensis* was identified at 254 nm only and *Ficus racemose* and *Tiliacora triandra* could be detected only at 280 nm, as shown in **Figure 25**.

The major peaks in the HPLC chromatogram of the aqueous extract of MHR were identified by comparing their retention times and overlaying their normalized UV spectra with those of reference standards and isolated compounds including spiking. The chebulic acid, gallic acid, protocatechuic acid, bergenin, chlorogenic acid, chebulanin, corilagin, chebulagic acid, ellagic acid, resveratrol, rhein, and loureirin A, pectolinarigenin, along with isolated compounds perforatic acid, *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether and TT01 were used as reference standards. The chemical assignments in the HPLC of MHR for quality control are presented in **Figure 26**, and the UV spectra of the major compounds are shown in **Figure 27**.

The chemical profile of the HPLC chromatogram of MHR and their corresponding assigned names of herbal components and chemical markers in each peak are shown in **Figure 28**, with the list in **Table 14**.

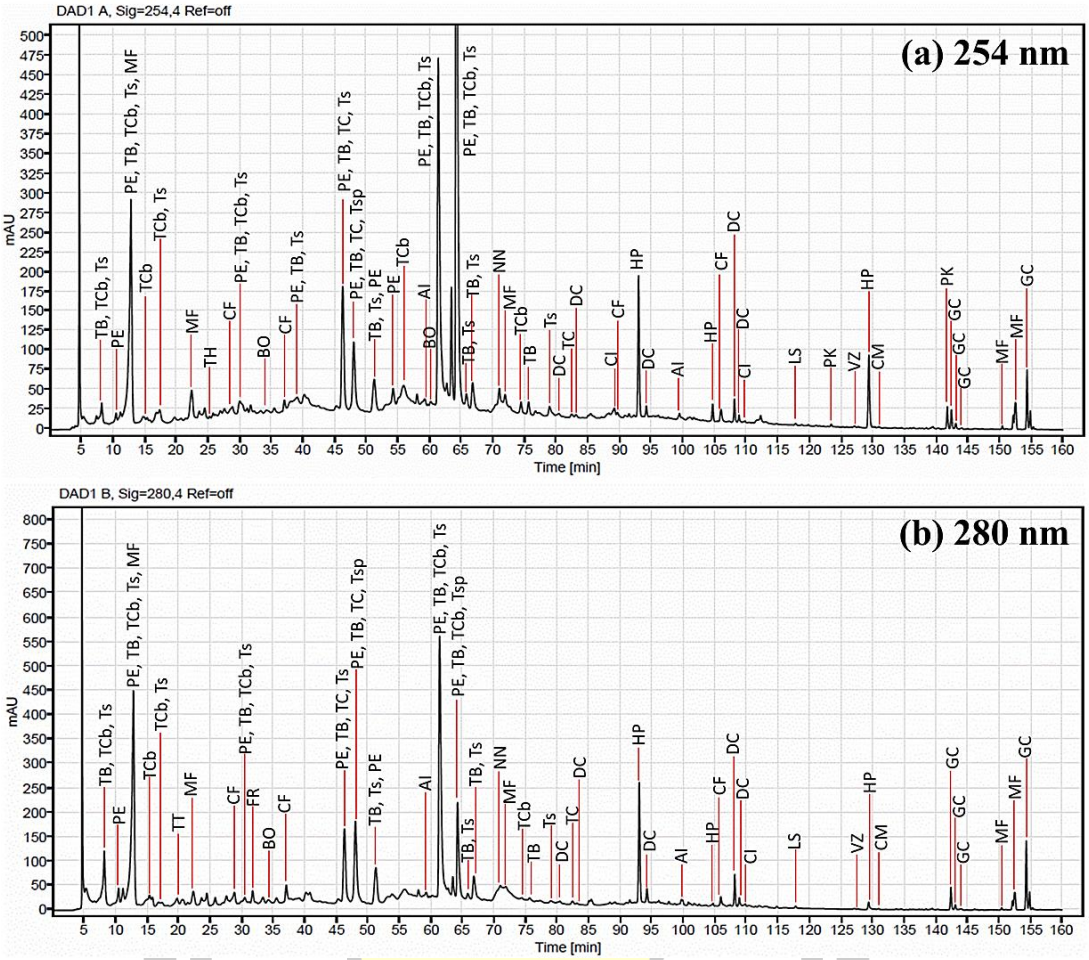


Figure 25 Identification of chromatographic peaks for ethanolic extract of MHR (FG01E) detected at wavelengths 254 nm (a) and 280 nm (b).

AI = *A. indica* (สะเดา), BO = *B. ovata* (มะกา), CF = *C. fistula* (คูน), CI = *C. indicum* (เท้ายายม่อม), CM = *C. micracantha* (ชิงชี่), DC = *D. cochinchinensis* (จันทน์แดง), FR = *F. racemosa* (มะเดื่อชุมพร), GC = *G. chinense* (กระดอม), HP = *H. perforata* (คนทา), LS = *L. sinense* (โกฐหัวบัว), MF = *M. ferrea* (บุนนาค), NN = *N. nucifera* (บัวหลวง), PE = *P. emblica* (มะขามป้อม), PK = *P. kesiya* (สน), TB = *T. bellirica* (สมอพิเภก), TC = *T. crispa* (บอระเพ็ด), TCb = *T. chebula* (สมอไทย), TH = *T. hoaensis* (จันทน์ขาว), Ts = *Terminalia* sp. “Samo Thet” (สมอเทศ), TT = *T. triandra* (ย่านาง), VZ = *V. zizanioides* (แฝกหอม).

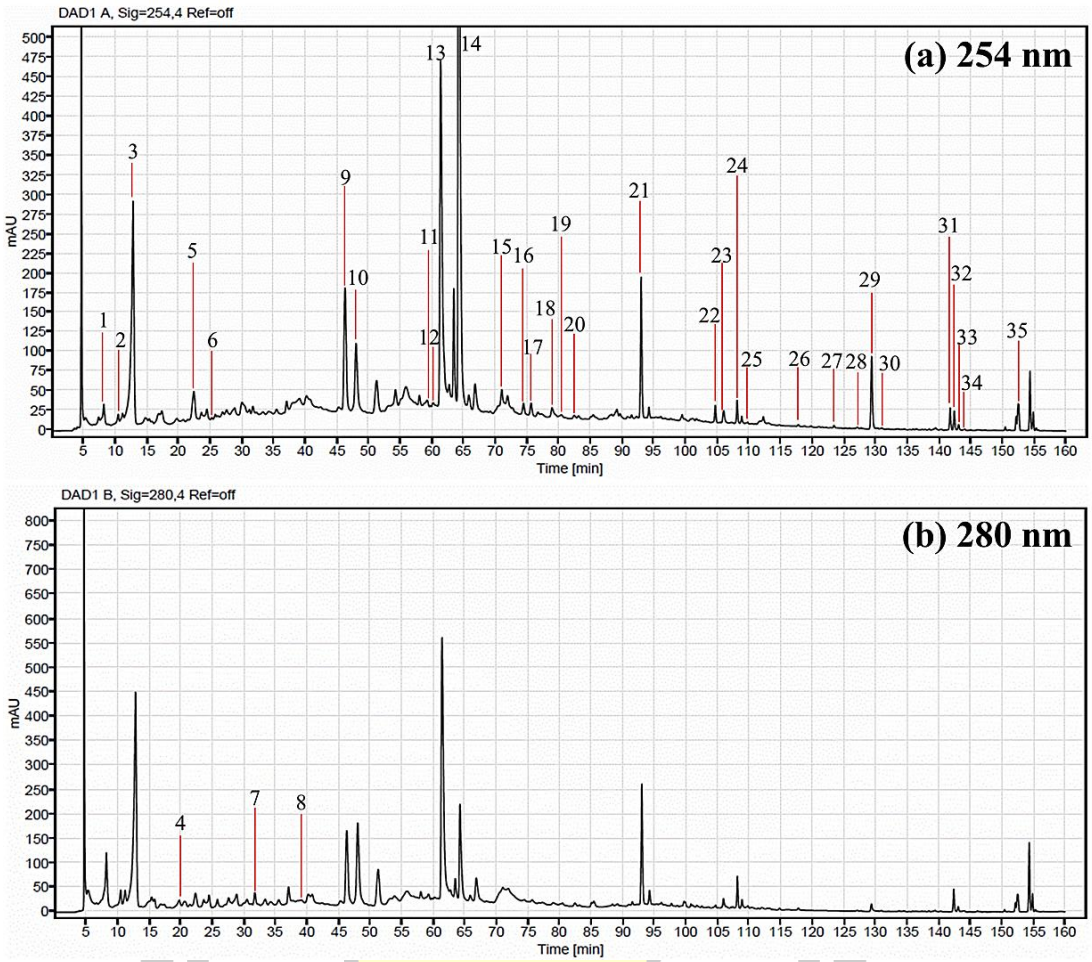


Figure 26 The chemical assignments in HPLC chromatogram for ethanolic extract of MHR (FG01E) detected at wavelengths 254 nm (a) and 280 nm (b).
(1) Chebulic acid, (2) PE01, (3) Gallic acid, (4) TT01, (5) Protocatechuic acid, (6) SA01, (7) Bergenin, (8) Chlorogenic acid, (9) Chebulanin, (10) Corilagin, (11) AI01, (12) BO01, (13) Chebulagic acid, (14) Ellagic acid, (15) NN01, (16) TCb01, (17) TB01, (18) Ts01, (19) Resveratrol, (20) TC01, (21) Perforatic acid, (22) *O*-methyllaloptaeroxyrin, (23) Rhein, (24) Loureirin A, (25) Pectolinarigenin, (26) LS01, (27) PK01, (28) VZ01, (29) Peucenin-7-methyl ether, (30) CM01, (31) PK02, (32) GC01, (33) GC02, (34) GC03, (35) MF02.

Table 14 The major compounds in the HPLC chromatogram of MHR ethanolic extract.

No.	RT (min)	Chemical assignments	Wavelength (nm)	Plant materials
1	8.2	Chebulic acid	254	*T. bellirica, T. chebula, Terminalia* sp. “Samo Thet”
2	10.5	PE01	254	*P. emblica*
3	12.8	Gallic acid	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. “Samo Thet”, *M. ferrea*

Table 14 The major compounds in the HPLC chromatogram of MHR ethanolic extract (Continued).

No.	RT (min)	Chemical assignments	Wavelength (nm)	Plant materials
4	20.4	TT01	280	*T. triandra*
5	22.5	Protocatechuic acid	254	*M. ferrea*
6	25.3	TH01	254	*T. hoaensis*
7	31.8	Bergenin	280	*F. racemosa*
8	38.1	Chlorogenic acid	280	*F. racemosa*
9	46.3	Chebulanin	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
10	48.1	Corilagin	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
11	59.7	AI01	254	*A. indica*
12	60.1	BO01	254	*B. ovata*
13	61.3	Chebulagic acid	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
14	64.2	Ellagic acid	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
15	71.9	NN01	254	*N. nucifera*
16	74.4	TCb01	254	*T. chebula*
17	75.6	TB01	254	*T. bellirica*
18	78.9	Ts01	254	*Terminalia* sp. "Samo Thet"
19	80.4	Resveratrol	254	*D. cochinchinensis*
20	82.4	TC01	254	*T. crispa*
21	92.9	Perforatic acid	254	*H. perforata*
22	104.6	*O*-methyllaloptaeroxyrin	254	*H. perforata*
23	105.9	Rhein	254	*C. fistula*
24	108.1	Loureirin A	254	*D. cochinchinensis*
25	109.7	Pectolinarigenin	254	*C. indicum*
26	117.7	LS01	254	*L. sinense*
27	123.3	PK01	254	*P. kesiya*
28	127.0	VZ01	254	*V. zizanioides*
29	129.3	Peucenin-7-methyl ether	254	*H. perforata*
30	130.8	CM01	254	*C. micracantha*
31	141.6	PK02	254	*P. kesiya*
32	142.3	GC01	254	*G. chinense*
33	143.0	GC02	254	*G. chinense*
34	144.0	GC03	254	*G. chinense*
35	152.4	MF02	254	*M. ferrea*

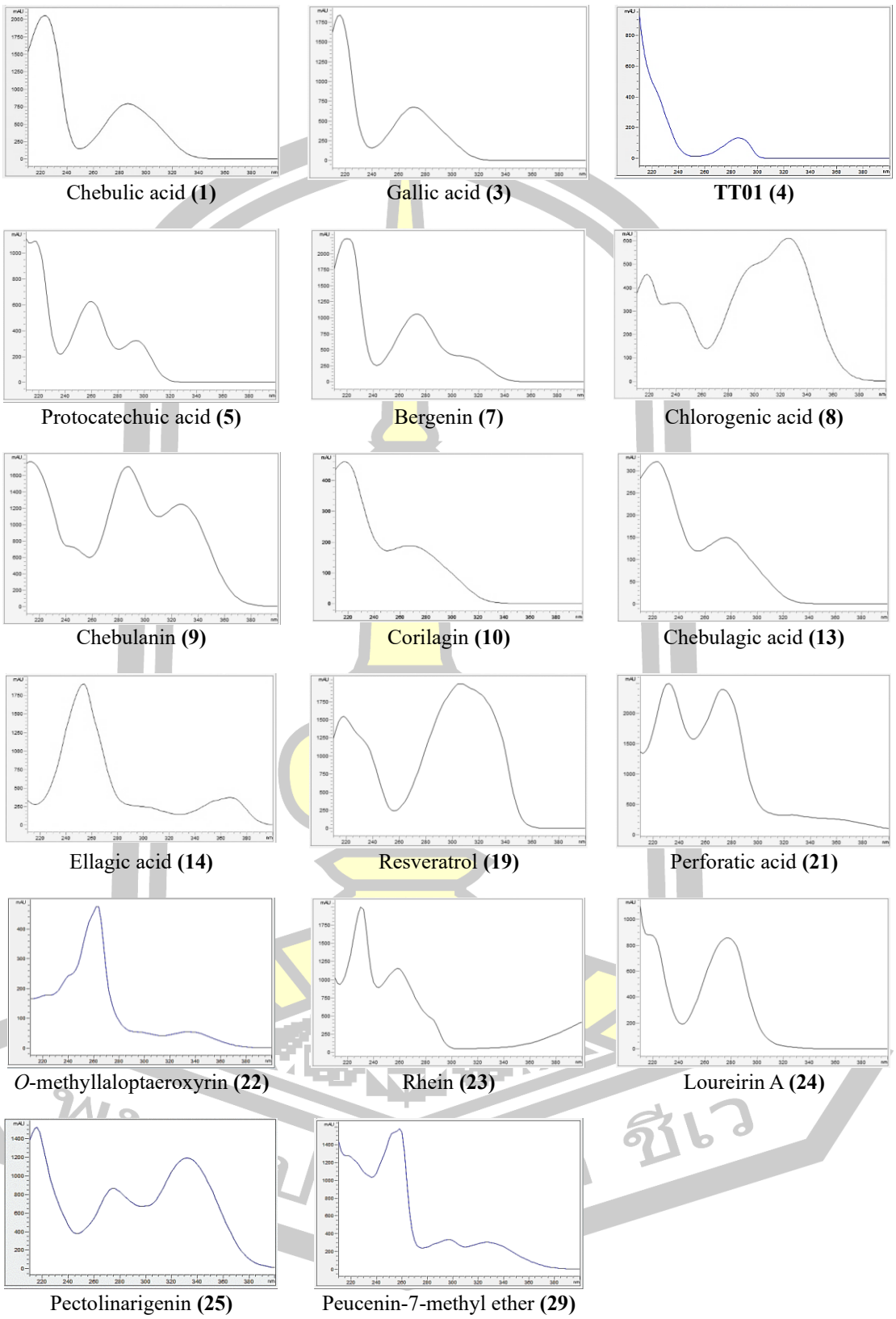


Figure 27 The UV spectrum of major compounds in MHR ethanolic extract.

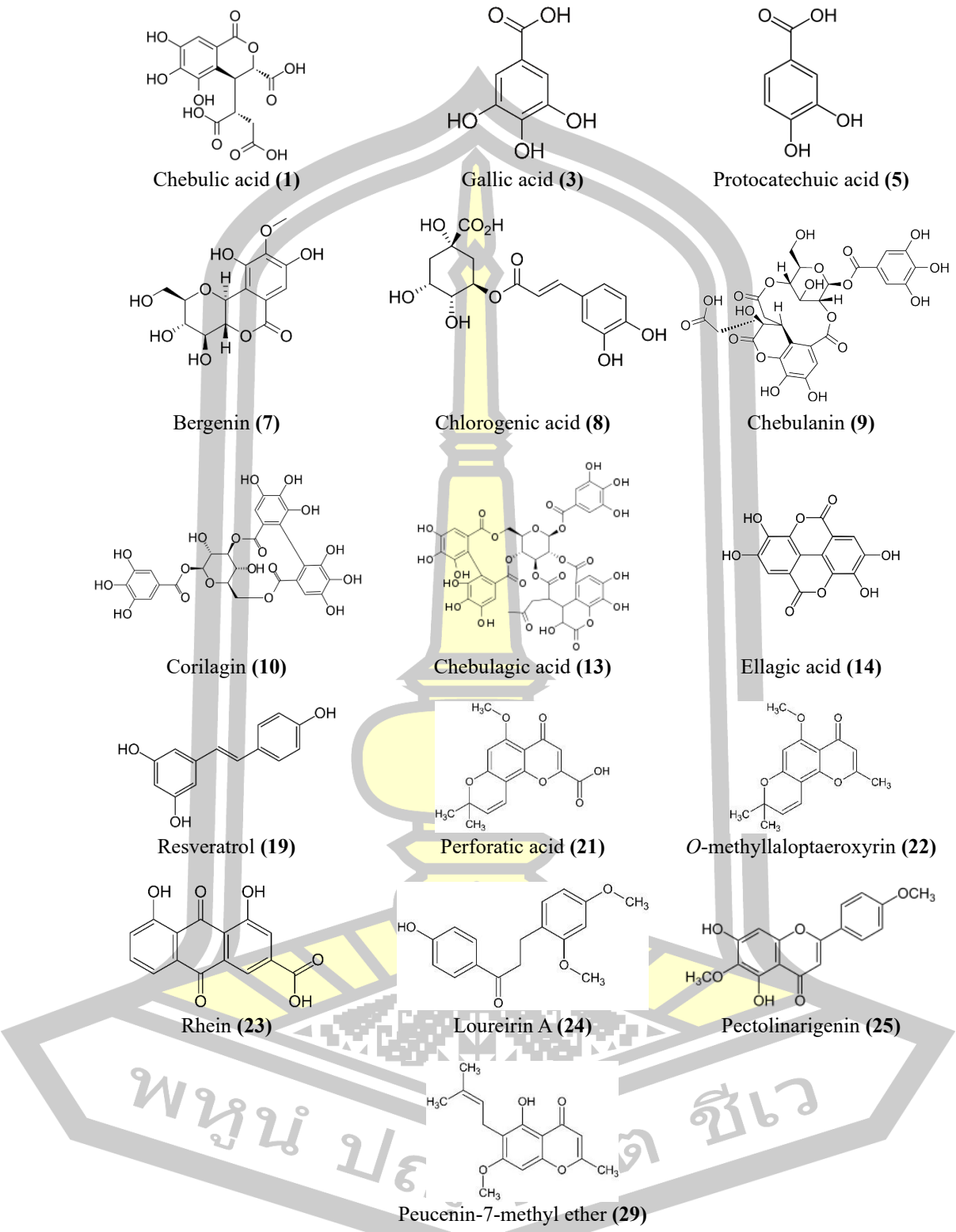


Figure 28 The chemical structure of major compounds in MHR ethanolic extract.

2) The aqueous extract

The aqueous extract of MHR (FG01A) successfully identified all components at wavelengths of 254 nm and 280 nm, as shown in **Figure 29**. A total of 21 components were detected: *Azadirachta indica*, *Bridelia ovata*, *Capparis micracantha*, *Cassia fistula*, *Clerodendrum indicum*, *Dracaena cochinchinensis*, *Ficus racemose*, *Gymnopetalum chinense*, *Harrisonia perforata*, *Ligusticum sinense*, *Mesua ferrea*, *Nelumbo nucifera*, *Phyllanthus emblica*, *Pinus kesiya*, *Tarenna hoaensis*, *Terminalia bellirica*, *Terminalia chebula*, *Terminalia* sp., *Tinospora crispa*, *Tiliacora triandra* and *Vetiveria zizanioides.*

The major peaks in the HPLC chromatogram of aqueous extract of MHR were identified by comparing retention times and overlaying normalized UV spectra with reference standards and isolated compounds. Confirmation was achieved through spiking the extract with reference standards. Identified peaks included those of reference standards, chebulic acid, gallic acid, protocatechuic acid, bergenin, chebulanin, corilagin, chebulagic acid, ellagic acid, rhein, loureirin A, as well as isolated chemical compounds, perforatic acid, *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether and TT01. **Figure 30** presents the HPLC chemical assignments of the aqueous MHR extract. **Figure 31** shows the UV spectrum of major compounds. The names of chemical constituents in the aqueous extract of MHR, corresponding to each peak in the HPLC chromatogram, are listed in **Table 15**, with their chemical structures shown in **Figure 32**.

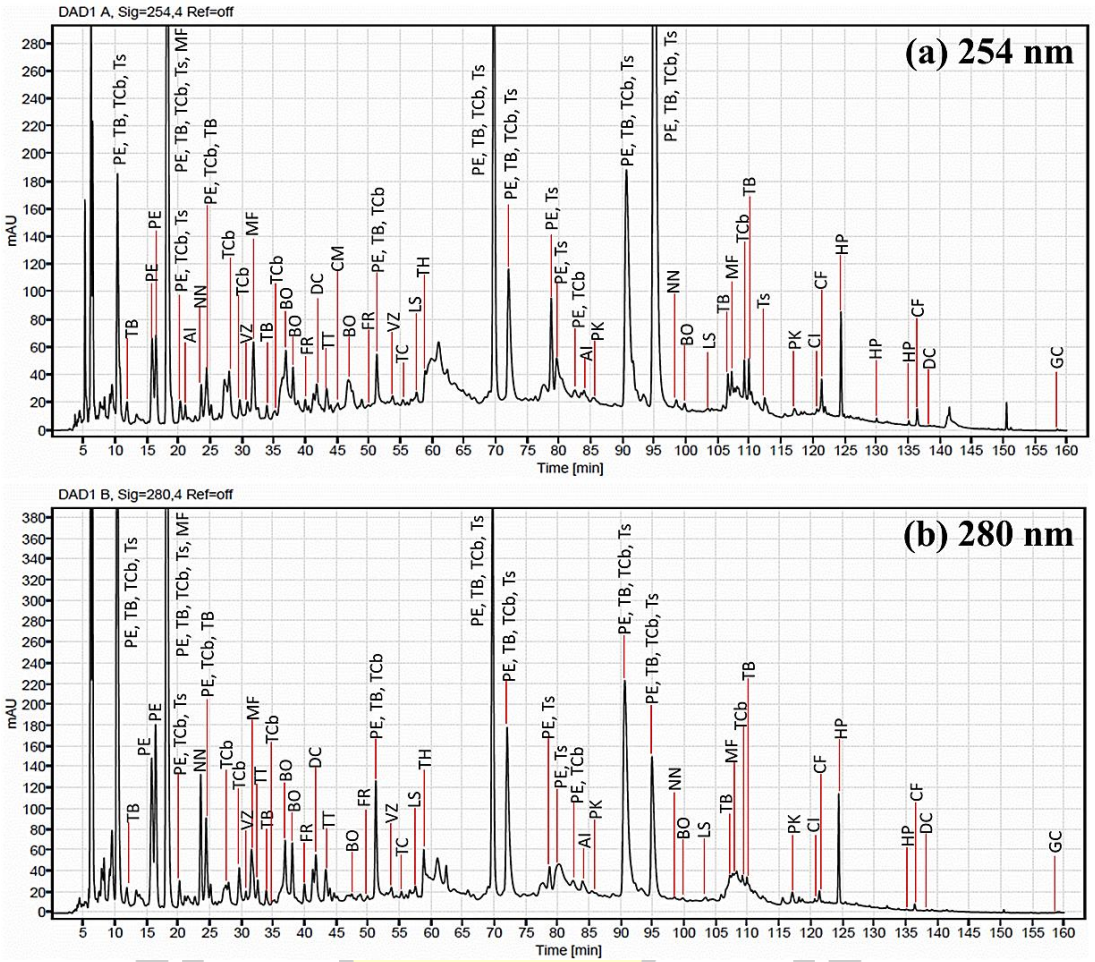


Figure 29 HPLC chromatograms of MHR aqueous extract (FG01A) detected at wavelengths 254 nm (a) and 280 nm (b).

AI = *A. indica* (สะเดา), BO = *B. ovata* (มะกา), CF = *C. fistula* (คูน), CI = *C. indicum* (เท้ายายม่อม), CM = *C. micracantha* (ชิงชี่), DC = *D. cochinchinensis* (จันทน์แดง), FR = *F. racemosa* (มะเดื่อชุมพร), GC = *G. chinense* (กระดอม), HP = *H. perforata* (คนทา), LS = *L. sinense* (โกฐหัวบัว), MF = *M. ferrea* (บุนนาค), NN = *N. nucifera* (บัวหลวง), PE = *P. emblica* (มะขามป้อม), PK = *P. kesiya* (สน), TB = *T. bellirica* (สมอพิเภก), TC = *T. crispa* (บอระเพ็ด), TCb = *T. chebula* (สมอไทย), TH = *T. hoaensis* (จันทน์ขาว), Ts = *Terminalia* sp. “Samo Thet” (สมอเทศ), TT = *T. triandra* (ข่อย), VZ = *V. zizanioides* (แฝกหอม).

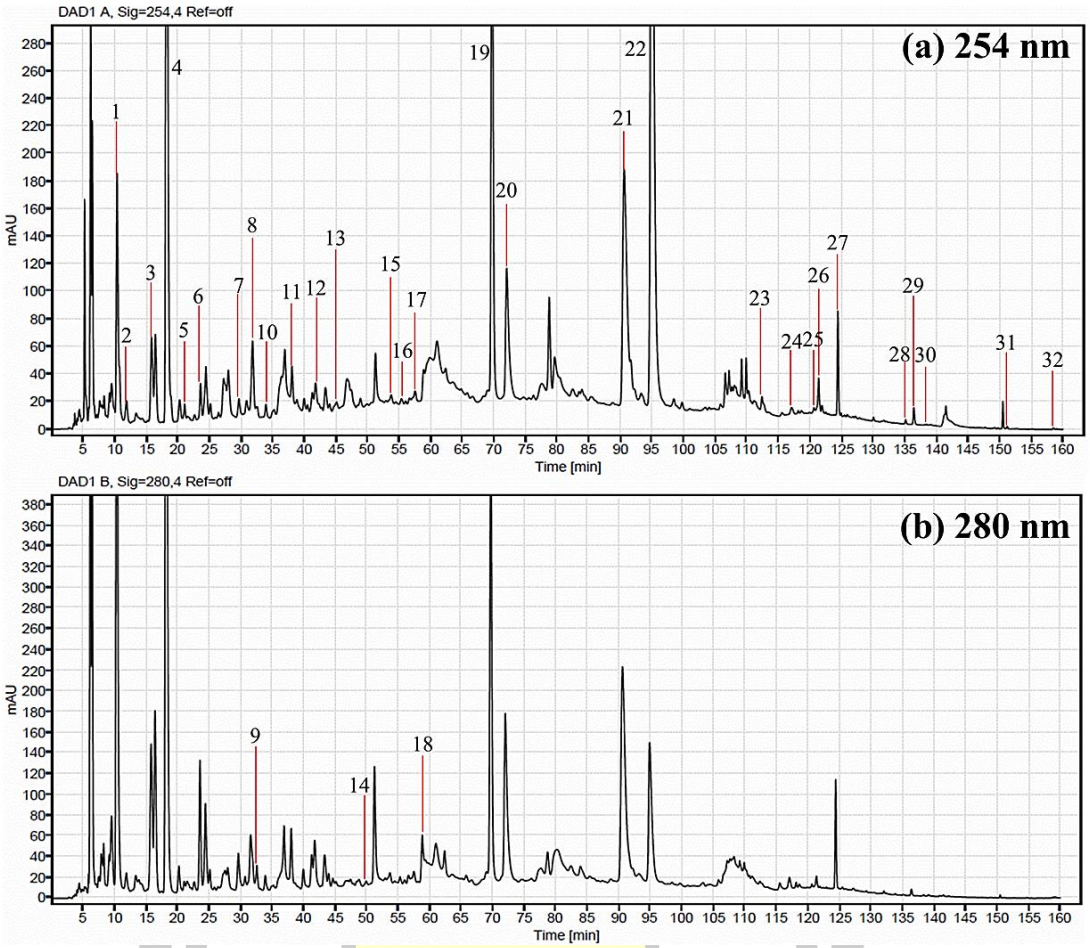


Figure 30 The chemical assignments in HPLC chromatogram of MHR aqueous extract (FG01A) detected at wavelengths 254 nm (a) and 280 nm (b).
(1) Chebulic acid, (2) TB01, (3) PE01, (4) Gallic acid, (5) AI01, (6) NN01, (7) TCb01, (8) Protocatechuic acid, (9) TT01, (10) TB02, (11) BO01, (12) DC01, (13) CM01, (14) Bergenin, (15) VZ01, (16) TC01, (17) LS01, (18) SA01, (19) Chebulanin, (20) Corilagin, (21) Chebulagic acid, (22) Ellagic acid, (23) Ts01, (24) PK01, (25) CI01, (26) CF01, (27) Perforatic acid, (28) *O*-methyllaloptaeroxyrin, (29) Rhein, (30) Loureirin A, (31) Peucenin-7-methyl ether, (32) GC01.

Table 15 The major compounds in the HPLC chromatogram of MHR aqueous extract.

No.	RT (min)	Chemical assignments	Wavelength (nm)	Plant materials
1	10.2	Chebulic acid	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
2	10.6	TB01	254	*T. bellirica*
3	15.7	PE01	254	*P. emblica*

Table 15 The major compounds in the HPLC chromatogram of MHR aqueous extract (Continued).

No.	RT (min)	Chemical assignments	Wavelength (nm)	Plant materials
4	18.0	Gallic acid	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet", *M. ferrea*
5	20.9	AI01	254	*A. indica*
6	24.3	NN01	254	*N. nucifera*
7	29.5	TCb01	254	*T. chebula*
8	31.7	Protocatechuic acid	254	*M. ferrea*
9	32.5	TT01	280	*T. triandra*
10	33.8	TB02	254	*T. bellirica*
11	37.9	BO01	254	*B. ovata*
12	41.6	DC01	254	*D. cochinchinensis*
13	45.0	CM01	254	*C. micracantha*
14	49.8	Bergenin	280	*F. racemosa*
15	53.6	VZ01	254	*V. zizanioides*
16	55.2	TC01	254	*T. crispa*
17	57.4	LS01	254	*L. sinense*
18	59.7	TH01	254	*T. hoaensis*
19	69.6	Chebulanin	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
20	71.9	Corilagin	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
21	90.5	Chebulagic acid	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
22	94.8	Ellagic acid	254	*P. emblica, T. bellirica, T. chebula, Terminalia* sp. "Samo Thet"
23	112.3	Ts01	254	*Terminalia* sp. "Samo Thet"
24	117.0	PK01	254	*P. kesiya*
25	120.5	CI01	254	*C. indicum*
26	121.2	CF01	254	*C. fistula*
27	124.3	Perforatic acid	254	*H. perforata*
28	135.0	*O*-methyllaloptaeroxyrin	254	*H. perforata*
29	136.3	Rhein	254	*C. fistula*
30	138.3	Loureirin A	254	*D. cochinchinensis*
31	151.1	Peucenin-7-methyl ether	254	*H. perforata*
32	158.4	GC01	254	*G. chinense*

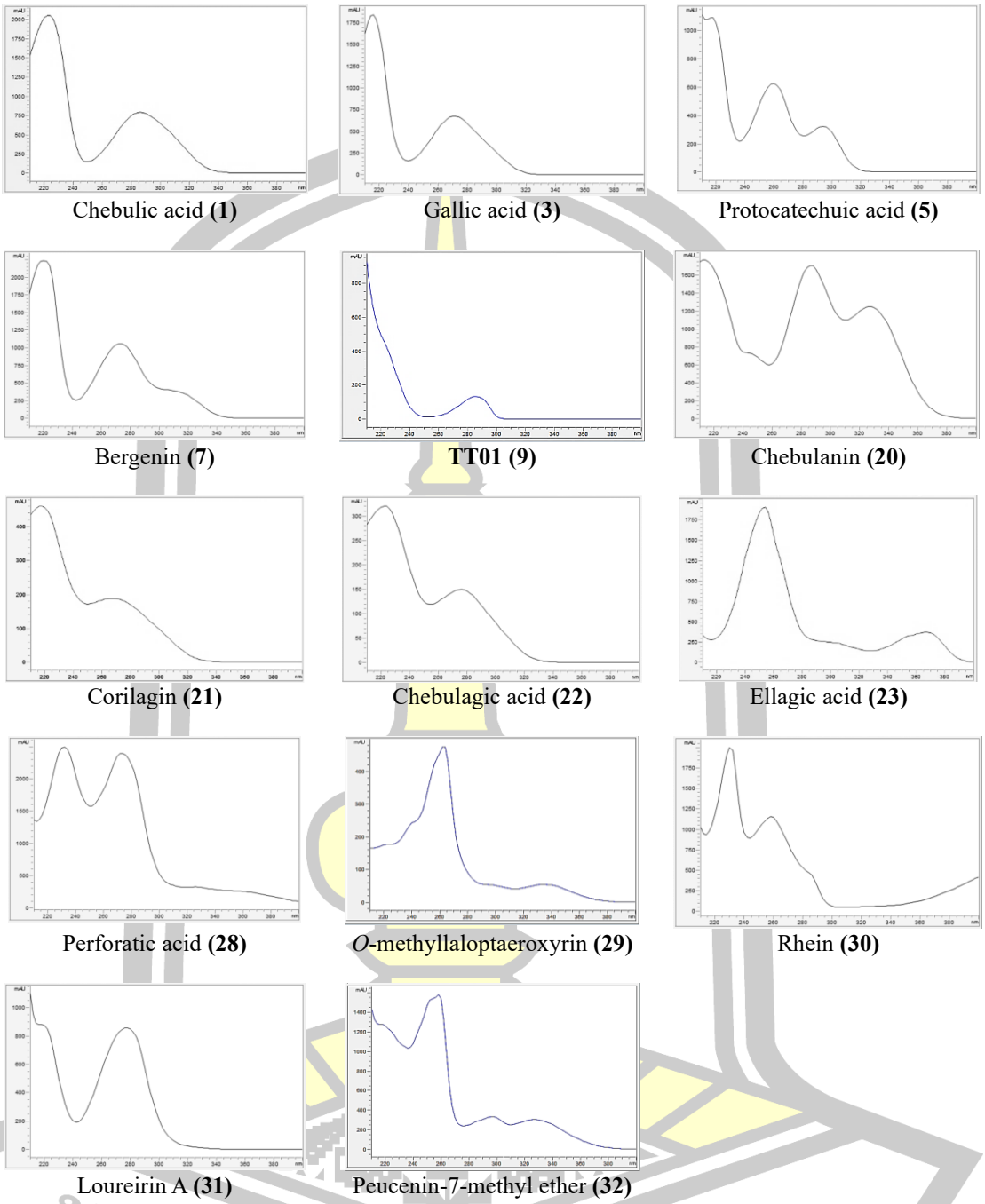
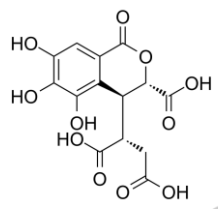
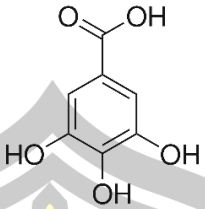
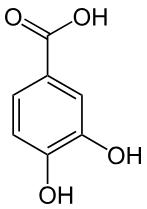
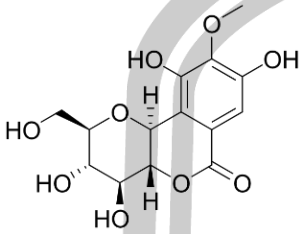


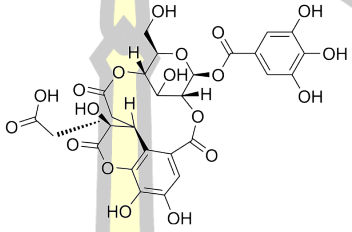
Figure 31 The UV spectrum of major compounds in MHR aqueous extract.

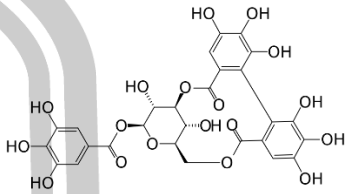

Chebulic acid **(1)**

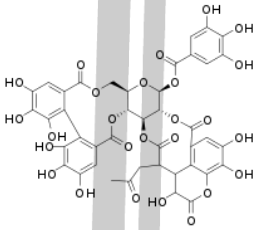

Gallic acid **(3)**

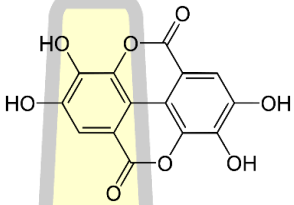

Protocatechuic acid **(5)**

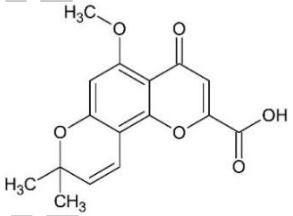

Bergenin **(7)**

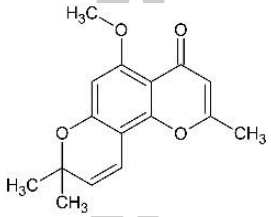

Chebulanin **(20)**

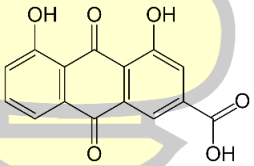

Corilagin **(21)**

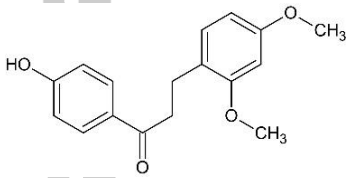

Chebulagic acid **(22)**


Ellagic acid **(23)**


Perforatic acid **(28)**


O-methyllaloptaeroxyrin **(29)**


Rhein **(30)**


Loureirin A **(31)**

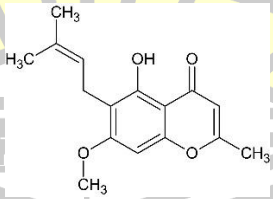

Peucenin-7-methyl ether **(32)**

Figure 32 The chemical structure of MHR aqueous extract.

4.1.3 Characterization and identification of chemical marker by Thin-Layer Chromatography (TLC)

The TLC analysis of extracts obtained from 70% ethanolic extraction of the Mo-Ha-Rak (MHR) remedy, its herbal components, and modified MHR was performed using Silica Gel 60 F_{254} as the stationary phase. System I used a mobile phase consisting of toluene, ethyl acetate, methanol, and formic acid in a 7:2:1:0.5 (v/v) ratio, while System II used a mobile phase with toluene, ethyl acetate, methanol, and formic acid in a 5:3:2:0.5 (v/v) ratio. The chromatographic plates were examined under ultraviolet light at two wavelengths (UV 254 nm and UV 366 nm), followed by spraying with anisaldehyde-sulfuric acid reagent. Distances between the spots were measured and the retention factor (Rf) values were recorded.

4.1.3.1 Identification of original MHR and its components

In System I at 254 nm, the TLC chromatogram of the original MHR reference batches showed a total of 10 quenched bands as shown in **Table 22-23**, **Figure 33A** and **Figure 34A**. *H. perforata* exhibited the highest correspondence with seven matching bands, followed by *M. ferrea,* which matched six bands. In contrast, *C. micracantha, F. racemosa, T. triandra, A. indica, T. crispa, T. hoaensis, N. nucifera,* and *V. zizanioides* showed no matching with the quenched bands of MHR.

At 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 19 bands, with the following Rf values and colors: Rf 0.04 (blue), 0.10 (blue), 0.15 (dark blue), 0.20 (green), 0.27 (blue), 0.29 (quenching), 0.32 (blue), 0.35 (light blue), 0.40 (blue), 0.45 (blue), 0.47 (red), 0.50 (blue), 0.55 (blue), 0.59 (yellow), 0.64 (blue), 0.71 (red), 0.76 (blue), 0.79 (blue), and 0.89 (blue), as shown in **Table 24-25**, **Figure 33B** and **Figure 34B**. Among these, bands at Rf 0.15 and Rf 0.29 matched those observed at 254 nm. Rf 0.16 was detected in *T. bellirica, T. chebula, Terminalia* sp., *P. emblica*, and *M. ferrea*, while Rf 0.30 was observed in *H. perforata*. Additionally, *H. perforata* exhibited the highest correspondence with nine matching bands, followed by *D. cochinchinensis*, which matched seven bands.

Detection with anisaldehyde-sulphuric acid under UV 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 19 bands, with the following Rf values and colors: Rf 0.04 (blue), 0.10 (blue), 0.15 (dark blue), 0.20 (green), 0.27 (blue), 0.29 (quenching), 0.32 (blue), 0.35 (light blue), 0.40

(blue), 0.45 (blue), 0.47 (red), 0.50 (blue), 0.55 (blue), 0.59 (yellow), 0.64 (light purple), 0.71 (purple), 0.76 (light purple), 0.79 (blue), and 0.89 (blue), as shown in **Table 26-27**, **Figure 33D** and **Figure 34D**. Among these, bands at Rf 0.16 and Rf 0.30 matched those observed at 254 nm. Rf 0.16 was detected in *T. bellirica, T. chebula, Terminalia* sp., *P. emblica*, and *M. ferrea*, while Rf 0.30 was observed in *H. perforata*. Additionally, *H. perforata* exhibited the highest correspondence with ten matching bands.

In System II at 254 nm, the TLC chromatogram of the original MHR reference batches showed a total of 14 quenched bands as shown in **Table 28-29**, **Figure 35A** and **Figure 36A**. *T. chebula* and *P. emblica* exhibited the highest correspondence with nine matching bands, followed by *H. perforata,* which matched eight bands. In contrast, *V. zizanioides* showed no matching with the bands of MHR.

At 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 23 bands, with the following Rf values and colors: Rf 0.04 (blue), 0.08 (blue), 0.13 (dark blue), 0.18 (blue), 0.22 (blue), 0.24 (dark blue), 0.28 (blue), 0.38 (blue), 0.42 (green), 0.45 (blue), 0.47 (dark blue), 0.52 (blue), 0.56 (green), 0.58 (quenching), 0.63 (light blue), 0.66 (light blue), 0.68 (red), 0.69 (red), 0.71 (yellow), 0.79 (red), 0.87 (red), 0.90 (blue) and 0.96 (blue), as shown in **Table 30-31**, **Figure 35B** and **Figure 36B**. Among these, bands at Rf 0.47 and Rf 0.58 matched those observed at 254 nm. Rf 0.47 was detected in *T. bellirica, T. chebula, Terminalia* sp., *P. emblica*, and *M. ferrea*, while Rf 0.58 was observed in *H. perforata*. Additionally, *C. indicum, L. sinense, B. ovata* and *T. bellirica,* exhibited the highest correspondence with nine matching bands.

Detection with anisaldehyde-sulphuric acid under UV 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 24 bands, with the following Rf values and colors: Rf 0.04 (blue), 0.08 (orange), 0.13 (dark blue), 0.18 (blue), 0.22 (blue), 0.24 (dark blue), 0.28 (blue), 0.38 (blue), 0.42 (green), 0.45 (blue), 0.47 (dark blue), 0.52 (blue), 0.56 (green), 0.58 (quenching), 0.63 (light blue), 0.66 (light blue), 0.68 (red), 0.69 (red), 0.71 (yellow), 0.78 (light purple), 0.82 (purple), 0.85 (light purple), 0.90 (blue) and 0.96 (blue), as shown in **Table 32-33**, **Figure 35D** and **Figure 36D**. Among these, bands at Rf 0.47 and Rf 0.58 matched those observed at 254 nm. Rf 0.47 was detected in *T. bellirica, T. chebula, Terminalia*

sp., *P. emblica*, and *M. ferrea*, while Rf 0.58 was observed in *H. perforata*. Additionally, *T. chebula* and *M. ferrea* exhibited the highest correspondence with eleven matching bands.

4.1.3.2 Identification of original MHR and modified MHR of primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs

In System I at 254 nm, the TLC chromatogram of the original MHR reference batches showed a total of 10 quenched bands as shown in **Table 23** and **Figure 34A**. The primary herbs (PH, 02E) displayed a total of seven quenched bands, the adjunct herbs (AH, 91E) exhibited three quenched bands, and the supportive herbs (SH, 92E) showed five quenched bands. The Rf value of 0.19 was observed exclusively in AH and SH, corresponding to bands identified in T. *bellirica, T. chebula, Terminalia sp., P. emblica*, and *M. ferrea*. Meanwhile, Rf values of 0.33 and 0.72 were detected only in SH, corresponding to bands found in *M. ferrea*. Modified MHR remedies by fixing reduced toxic fever herbs, displayed major bands at Rf 0.30, 0.46, and 0.74, which correspond to bands found in *H. perforata*. The modified MHR formula without supportive herbs (03E) exhibited seven quenched bands, including Rf 0.19 but lacking Rf 0.89, which was present in PH. In the modified MHR without the adjunct herbs (10E) showed five quenched bands, including Rf 0.19 but lacking Rf 0.35, 0.50 and 0.89, which was present in PH.

At 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 19 bands, as shown in **Table 25** and **Figure 34B**. The primary herbs (PH, 02E) displayed a total of 14 color bands, the adjunct herbs (AH, 91E) exhibited 8 color bands, and the supportive herbs (SH, 92E) showed 4 color bands. The Rf values of 0.04, 0.10, 0.20, and 0.59 were detected exclusively in CH. The Rf values of 0.04 (blue), 0.10 (blue), and 0.20 (green) correspond to bands identified in *T. bellirica, T. chebula, Terminalia* sp., and *P. emblica*. The Rf value of 0.59 (yellow) corresponds to bands identified in *C. fistula*. Additionally, the Rf value of 0.15 (dark blue) was observed only in CH and SH, corresponding to bands identified in *T. bellirica, T. chebula, Terminalia sp., P. emblica*, and *M. ferrea*.

Detection with anisaldehyde-sulphuric acid under UV 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 19

bands, as shown in **Table 27** and **Figure 34D**. The primary herbs (PH, 02E) displayed a total of 15 color bands, the adjunct herbs (AH, 91E) exhibited 11 color bands, and the supportive herbs (SH, 92E) showed 8 color bands. The Rf values of 0.04, 0.10, and 0.59 were detected exclusively in AH. The Rf values of 0.04 (blue) and 0.10 (blue) correspond to bands identified in *T. bellirica, T. chebula, Terminalia* sp., and *P. emblica*. The Rf value of 0.59 (yellow) corresponds to bands identified in *C. fistula*. Additionally, the Rf value of 0.15 (dark blue) was observed only in AH and SH, corresponding to bands identified in *T. bellirica, T. chebula, Terminalia sp., P. emblica*, and *M. ferrea*.

In System II at 254 nm, the TLC chromatogram of the original MHR reference batches showed a total of 14 quenched bands as shown in **Table 29** and **Figure 36A**. The primary herbs (PH, 02E) displayed a total of 10 quenched bands, the adjunct herbs (AH, 91E) exhibited 10 quenched bands, and the supportive herbs (SH, 92E) showed 8 quenched bands. The Rf value of 0.46 and 0.50 was observed exclusively in AH and SH, corresponding to bands identified in *T. bellirica, T. chebula, Terminalia sp., P. emblica*, and *M. ferrea*. Meanwhile, Rf values of 0.22 and 0.26 were detected only in SH, corresponding to bands found in *T. bellirica, T. chebula, Terminalia* sp. "Samo Thet" and *P. emblica*. Modified MHR remedies by fixing reduced toxic fever herbs, displayed major bands at Rf 0.57, 0.68, and 0.88, which correspond to bands found in *H. perforata*. The modified MHR formula without supportive herbs (03E) exhibited seven quenched bands, including Rf 0.22, 0.26, 0.46 and 0.50 but lacking Rf 0.76 and 0.97, which was present in MH. In the modified MHR without the adjunct herbs (10E) showed five quenched bands, including Rf 0.46 and 0.50 but lacking Rf 0.57, 0.76, 0.88 and 0.97, which was present in PH.

At 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 23 bands, as shown in **Table 31** and **Figure 36B**. The primary herbs (PH, 02E) displayed a total of 12 color bands, the adjunct herbs (AH, 91E) exhibited 15 color bands, and the supportive herbs (SH, 92E) showed 7 color bands. The Rf values of 0.13, 0.22, 0.45, 0.68, 0.71 and 0.79 were detected exclusively in AH. The Rf values of 0.13 (dark blue), 0.22 (blue), and 0.45 (blue) 0.20 correspond to bands identified in *T. bellirica, T. chebula, Terminalia* sp., and *P.*

emblica. The Rf value of 0.68 (red) and 0.79 (red) corresponds to bands identified in *B. ovata*. The Rf value of 0.71 (yellow) corresponds to bands identified in *C. fistula*. Additionally, the Rf value of 0.47 (dark blue) was observed only in AH and SH, corresponding to bands identified in *T. bellirica, T. chebula, Terminalia sp., P. emblica*, and *M. ferrea*.

Detection with anisaldehyde-sulphuric acid under UV 366 nm, the TLC chromatogram of the original MHR reference batches displayed a total of 24 bands, as shown in **Table 33** and **Figure 36D**. The primary herbs (PH, 02E) displayed a total of 17 color bands, the adjunct herbs (AH, 91E) exhibited 16 color bands, and the supportive herbs (SH, 92E) showed 10 color bands. The Rf values of 0.22, and 0.45 were detected exclusively in AH. The Rf values of 0.22 (blue) correspond to bands identified in *Terminalia* sp. "Samo Thet". The Rf values of 0.45 (blue) correspond to bands identified in *T. bellirica*. Additionally, the Rf value of 0.47 (dark blue) was observed only in AH and SH, corresponding to bands identified in *T. bellirica, T. chebula, Terminalia sp., P. emblica*, and *M. ferrea*.

4.1.2.3 Chemical marker identification in TLC chromatogram

The TLC analysis of extracts obtained from the 70% ethanolic extraction of the MHR remedy, its herbal components, and modified MHR revealed the presence of chemical markers, including β-sitosterol, lupeol, and stigmasterol. In both System I and System II, the TLC analysis was conducted using anisaldehyde-sulfuric acid under UV 366 nm. It was found that β-sitosterol and stigmasterol matched a light purple band with the MHR (MHR) remedy, displaying an Rf value of 0.64 in System I and 0.78 in System II, as shown in **Table 26-27**, **Figure 33D** and **Figure 34D**. These bands corresponded to most samples, except for *G. chinense*. Lastly, lupeol was identified as a light purple band in the MHR remedy, showing an Rf value of 0.75 in System I and 0.85 in System II, as shown in **Table 32-33**, **Figure 35D** and **Figure 36D**. These bands corresponded to bands identified in *A. indica, T. crispa, D. cochinchinensis, L. sinense, B. ovata, M. ferrea, N. nucifera*, and in the modified formulations 02E, 31E, 32E, 33E, 03E, 91E, 12E, 14E, 10E, and 92E.

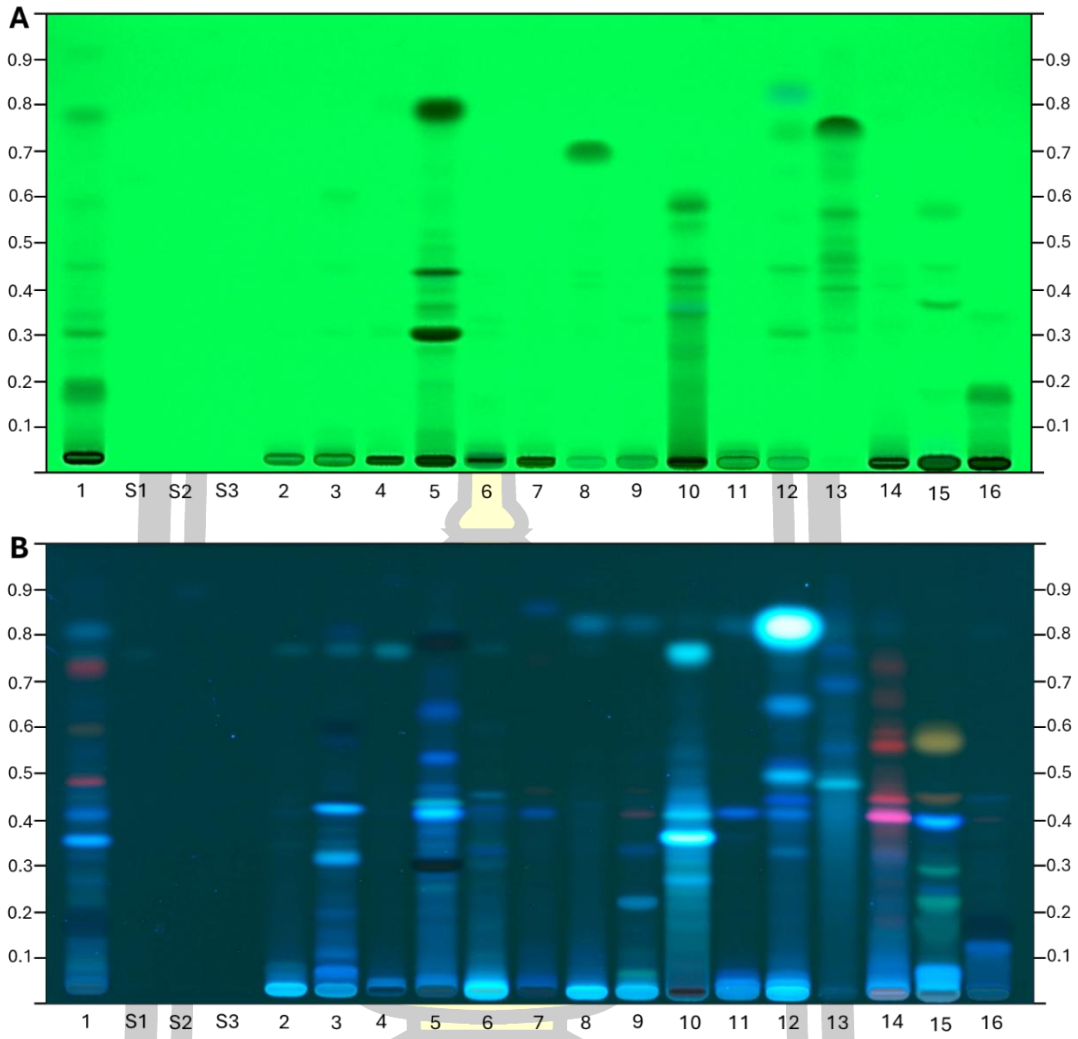


Figure 33 TLC chromatograms of MHR and its plant components compared to chemical markers.

TLC was performed using silica gel 60 plates developed in a mobile phase of toluene, ethyl acetate, methanol, and formic acid (7:2:1:0.5 v/v) (System I) . Detection was conducted under UV 254 nm (A) and 366 nm (B). Samples: (1) MHR, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ข่าน้าง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (คูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

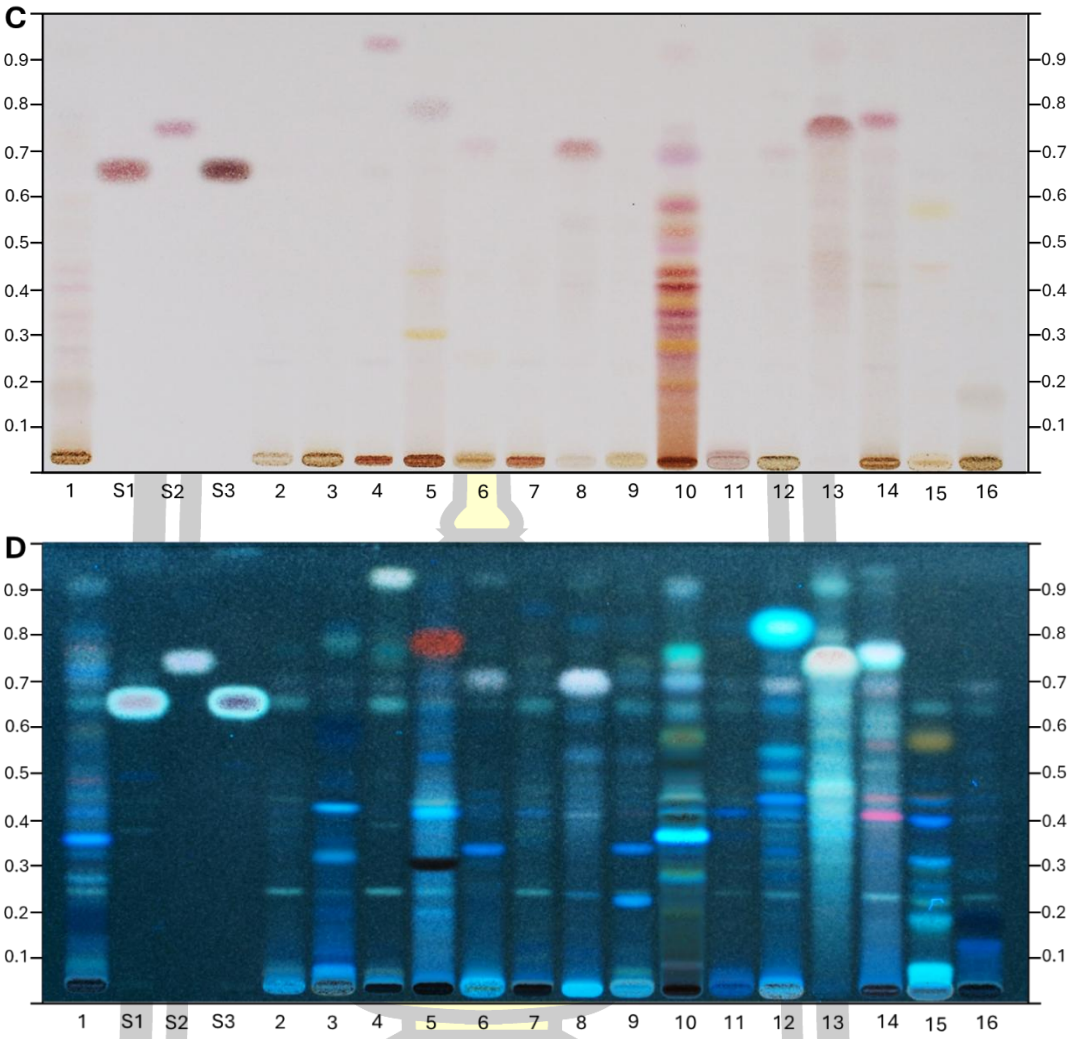


Figure 33 TLC chromatograms of MHR and its plant components compared to chemical markers (Continued).

TLC was performed using silica gel 60 plates developed in a mobile phase of toluene, ethyl acetate, methanol, and formic acid (7:2:1:0.5 v/v) (System I). Detection was conducted using anisaldehyde-sulphuric acid reagent (C) and anisaldehyde-sulphuric acid under UV 366 nm (D). Samples: (1) MHR, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (ลูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

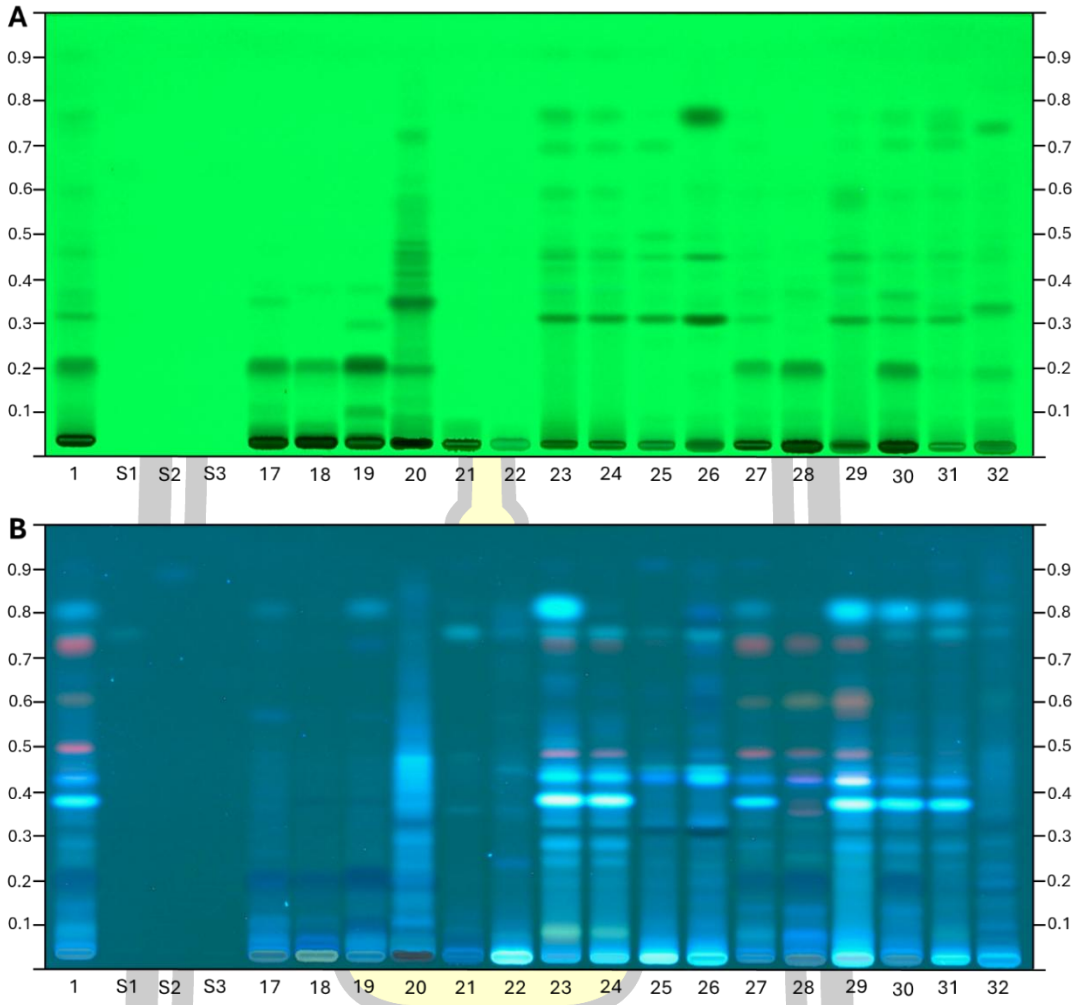


Figure 34 TLC chromatograms of MHR, its plant components and modified MHR compared to chemical markers.

TLC was performed using silica gel 60 plates developed in a solvent system of toluene, ethyl acetate, methanol, and formic acid (7:2:1:0.5 v/v) (System I). Detection was conducted under UV at 254 nm (A) and 366 nm (B). Samples: (1) MHR, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. "Samo Thet" (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

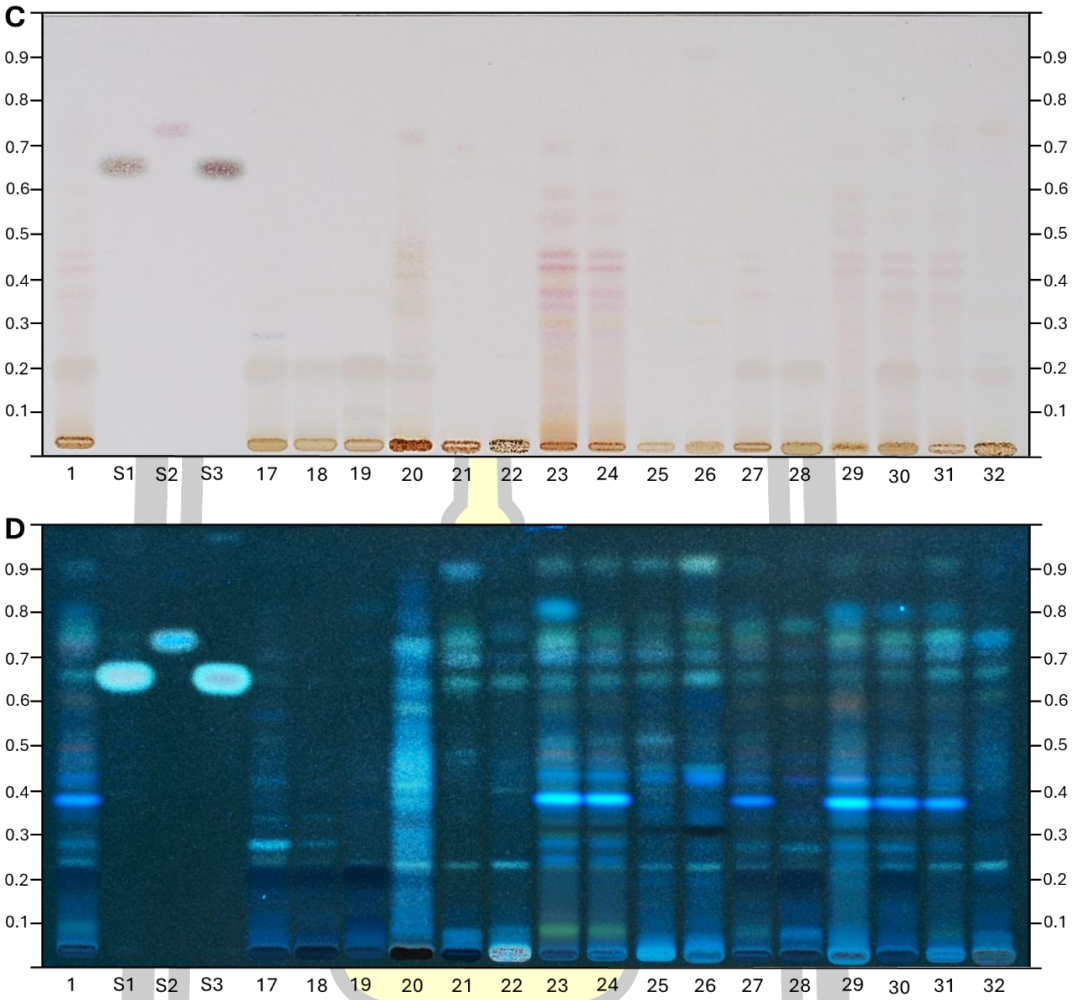


Figure 34 TLC chromatograms of MHR, its plant components and modified MHR compared to chemical markers (Continued).

TLC was performed using silica gel 60 plates developed in a solvent system of toluene, ethyl acetate, methanol, and formic acid (7:2:1:0.5 v/v) (System I). Detection was conducted using anisaldehyde-sulphuric acid reagent (C) and anisaldehyde-sulphuric acid under 366 nm UV (D). Samples: (1) MHR, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. "Samo Thet" (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

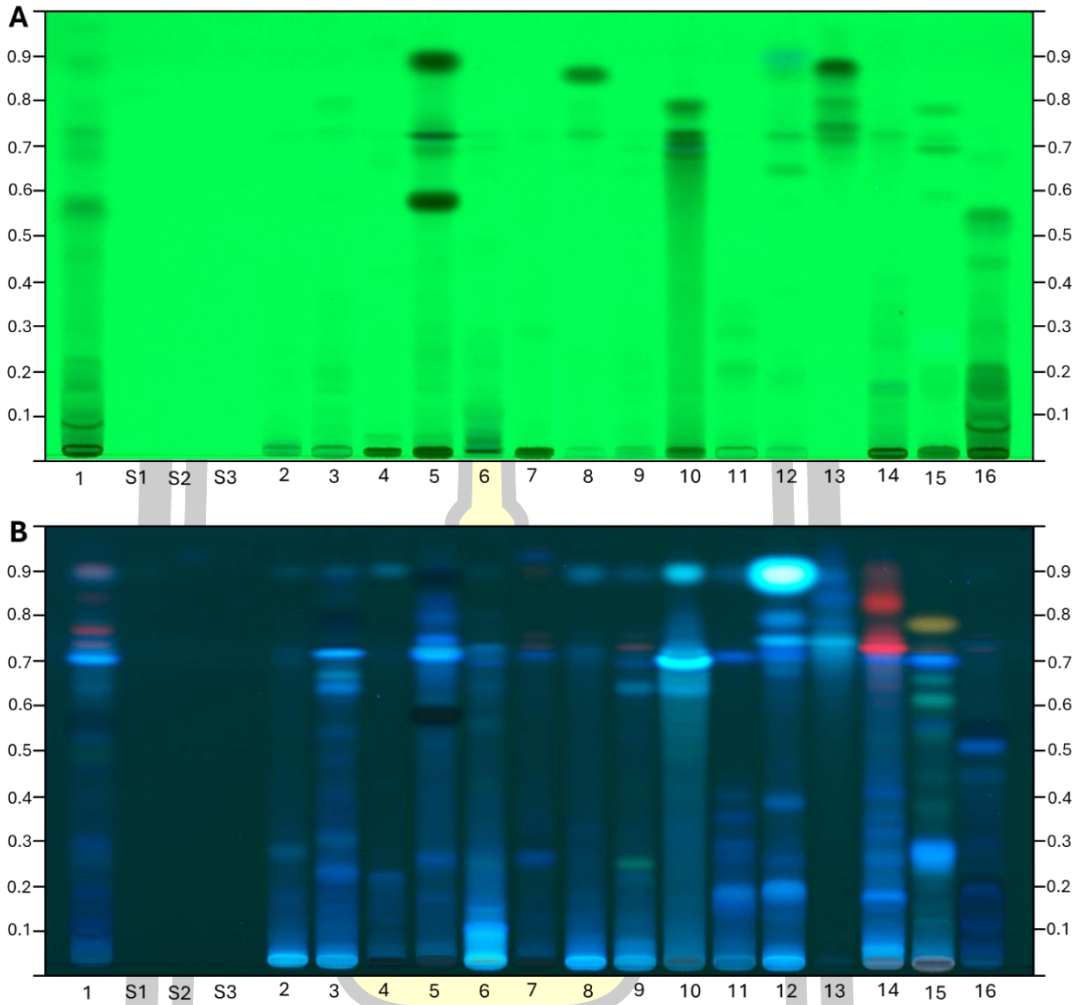


Figure 35 TLC chromatograms of MHR and its plant components compared to chemical markers.

TLC was performed using silica gel 60 plates developed in a mobile phase of toluene, ethyl acetate, methanol, and formic acid (5:3:2:0.5 v/v) (System II) . Detection was conducted under UV at 254 nm (A) and 366 nm (B). Samples: (1) MHR, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (คูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

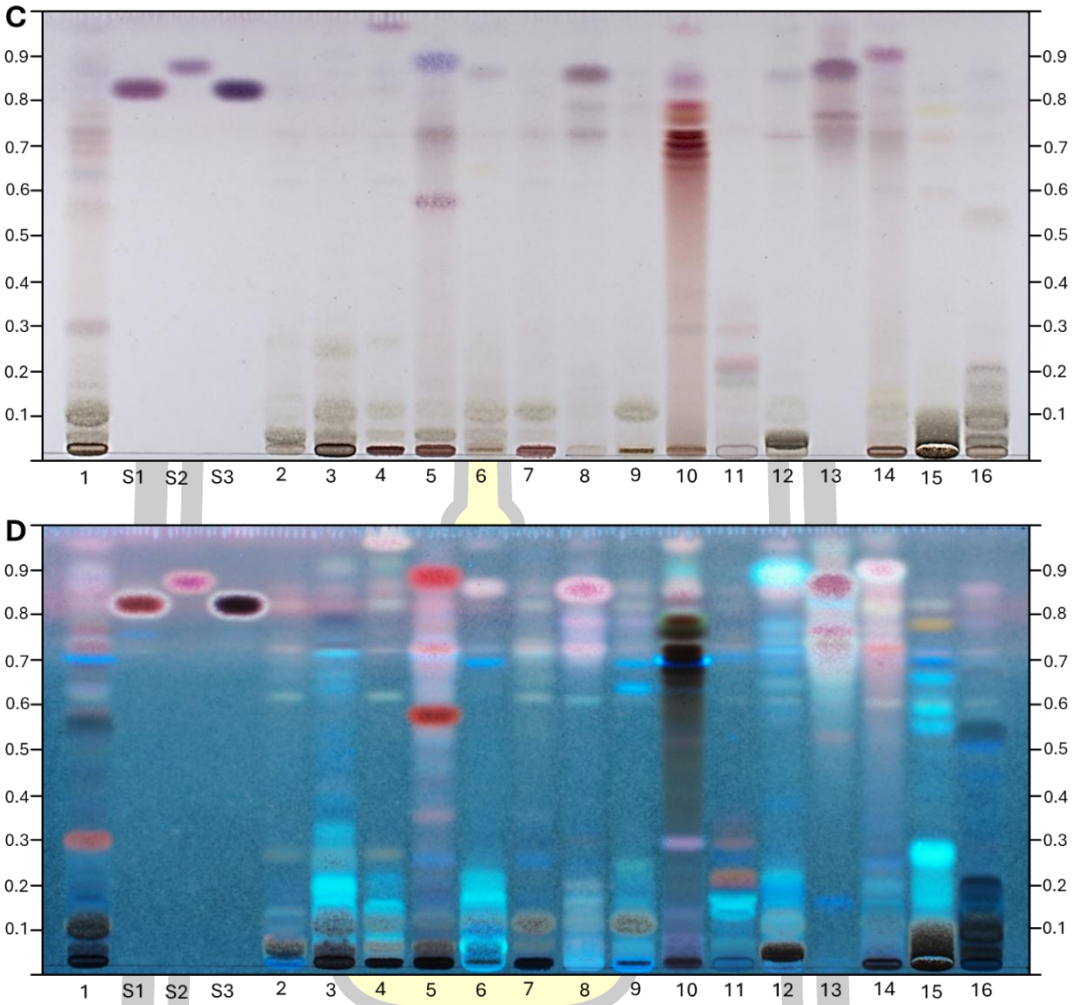


Figure 35 TLC chromatograms of MHR and its plant components compared to chemical markers (Continued).

TLC was performed using silica gel 60 plates developed in a mobile phase of toluene, ethyl acetate, methanol, and formic acid (5:3:2:0.5 v/v) (System II). Detection was conducted using anisaldehyde-sulphuric acid reagent (C) and anisaldehyde-sulphuric acid under 366 nm UV (D). Samples: (1) MHR, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้าขายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (ลูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

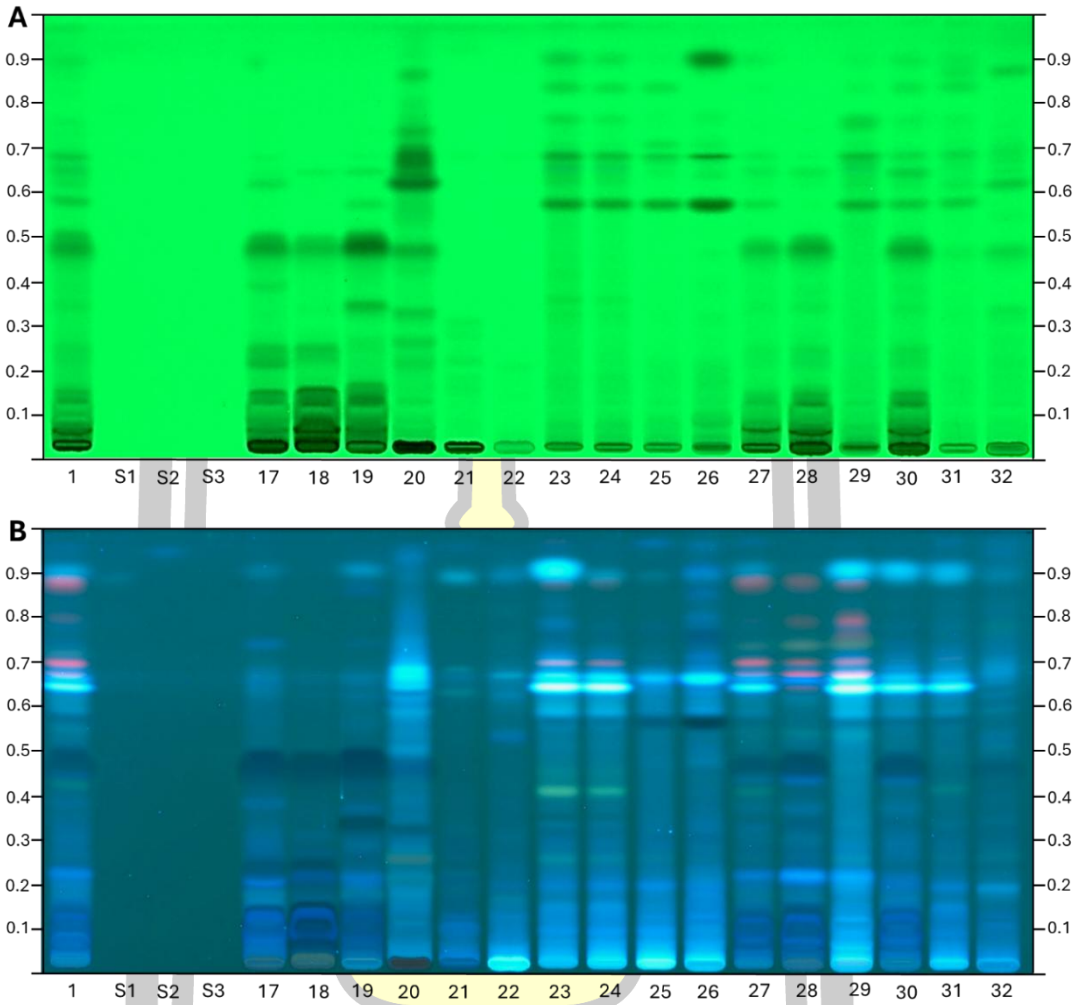


Figure 36 TLC chromatograms of MHR, its plant components and modified MHR compared to chemical markers.

TLC was performed using silica gel 60 plates developed in a solvent system of toluene, ethyl acetate, methanol, and formic acid (5:3:2:0.5 v/v) (System II). Detection was conducted under UV at 254 nm (A) and 366 nm (B). Samples: (1) MHR, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. "Samo Thet" (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

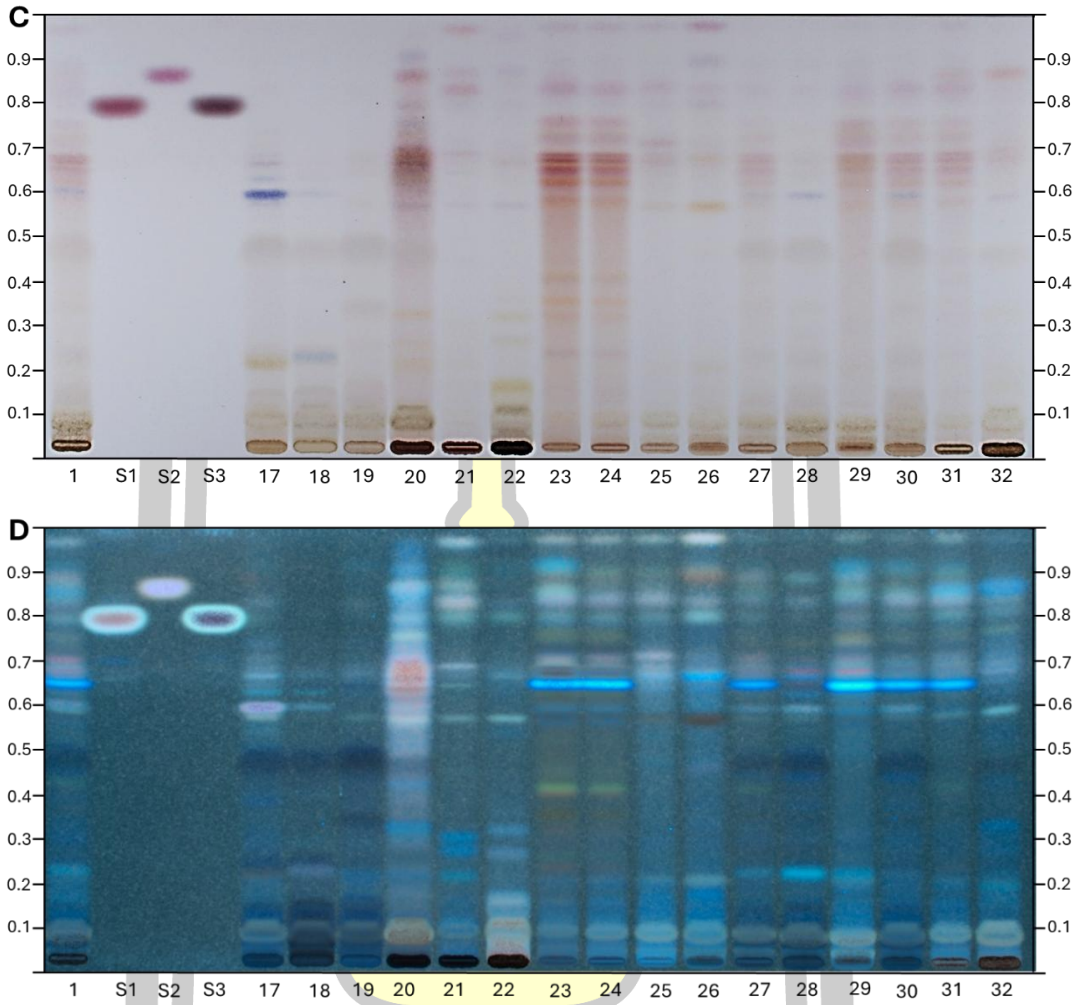


Figure 36 TLC chromatograms of MHR, its plant components and modified MHR compared to chemical markers (Continued).

TLC was performed using silica gel 60 plates developed in a solvent system of toluene, ethyl acetate, methanol, and formic acid (5:3:2:0.5 v/v) (System II). Detection was conducted using anisaldehyde-sulphuric acid reagent (C) and anisaldehyde-sulphuric acid under 366 nm UV (D). Samples: (1) MHR, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. "Samo Thet" (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

4.2 Phytochemical studies of some components of MHR

Three chromones, perforatic acid, *O*-methyllaloptaeroxyrin, and peucenin-7-methyl ether, were identified as major compounds isolated from the root of *Harrisonia perforata* (Blanco) Merr. These compounds represent major peaks in the HPLC fingerprints of both the modified and original MHR formulations. They were used as marker compounds for qualitative and quantitative analysis of the chemical profiles by HPLC, despite the absence of corresponding reference standards.

4.2.1 Perforatic acid

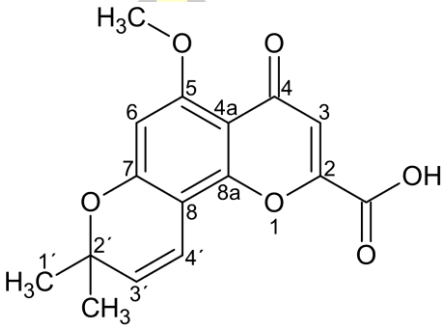


Figure 37 The chemical structure of perforatic acid.

Perforatic acid is a chromone and was isolated from the 70% ethanolic extract of *Harrisonia perforata* roots by preparative TLC on Silica Gel 60 F_{254} using mobile phase of chloroform : methanol : formic acid (95:5:0.5, v/v/v). The major band of Rf 0.40 was extracted with methanol under ultrasonication for 30 min in 3 repeats to obtain perforatic acid in the form of slightly yellow powder. The UV spectrum in methanol using HPLC analysis showed a maximum absorption (λ_{max}) at 232 and 274 nm. ESI-MS m/z 303.0877 $[M+H]^+$ and calculated for $C_{16}H_{14}O_6$ as 302.28 g/mol.

The ^{1}H-NMR spectrum (**Table 16**) showed signals of 4 aromatic protons δ_H 7.04 (1H, d, J=8.0 Hz, H-3), δ_H 6.42 (1H, s, H-6), δ_H 5.67 (1H, d, J = 7.9 Hz, H-3′) and δ_H 6.75 (1H, s, H-4′), methoxy group (δ_H 3.89, 3H, s) and two methyl group (δ_H 1.46, 3Hx2, s) which corresponding to Thadaniti *et al*. (1994) .

The ^{13}C-NMR spectra (**Table 16**) revealed the presence of a lactone group (δ_C 179.7), one carboxyl group (δ_C 165.0), eleven aromatic carbons (δ_C 78.1, 96.6,

102.9, 108.4, 112.5, 114.9, 127.2, 153.0, 154.1, 158.8, 160.3), two secondary methyl carbon (δ_C 27.1, 29.3) and one methoxy carbon (δ_C 55.3) which corresponding to Thadaniti *et al*. (1994).

Table 16 The NMR data of perforatic acid.

Position	Thadaniti *et al*. (1994)[a]		Our result[b]	
	δ_C	δ_H (mult, *J* in Hz)	δ_C[c]	δ_H (mult, *J* in Hz)
2	159.8		160.3	
3	113.1	6.89 (*s*, 1H)	112.5	6.75 (*s*, 1H)
4	179.5		179.7	
4a	102.5		102.9	
5	153.6		153.0	
6	96.4	6.29 (*s*, 1H)	96.6	6.42 (*s*, 1H)
7	155.5		154.1	
8	108.2		108.4	
8a	158.4		158.8	
1′	27.4	1.43 (*s*, 3H)	27.1	1.46 (*s*, 3H)
2′	77.9		78.1	
3′	114.6	5.54 (*d*, *J* =10.0, 1H)	114.9	5.67 (*d*, *J* =7.9, 1H)
4′	126.8	6.88 (*d*, *J* =10.0, 1H)	127.2	7.04 (*d*, *J* =8.0, 1H)
2′-CH_3	27.4	1.43 (*s*, 3H)	29.3	1.46 (*s*, 3H)
5-OCH_3	55.4	3.89 (*s*, 3H)	55.3	3.89 (*s*, 3H)
2-COOH	165.5		165.0	

[a, b] Spectra were recorded in CD_3OD, ^{13}C (400 MHz), ^{1}H (400 MHz)

4.2.2 *O*-methyllaloptaeroxyrin

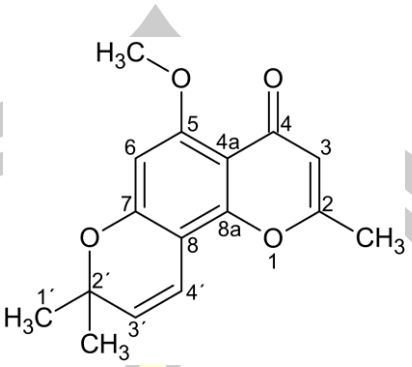


Figure 38 The chemical structure of *O*-methyllaloptaeroxyrin.

O-methyllaloptaeroxyrin (perforatin A) is a chromone and was isolated from the 70% ethanolic extract of *Harrisonia perforata* roots by preparative TLC on Silica Gel 60 F_{254} using mobile phase of chloroform : ethyl acetate (8:2, v/v). The major band of Rf 0.38 was extracted with methanol under ultrasonication for 30 min in 3 repeats to obtain *O*-methyllaloptaeroxyrin in the form of slightly yellow powder. The UV spectrum in methanol using HPLC analysis showed a maximum absorption (λ_{max}) at 264 and 338 nm. ESI-MS m/z 273.1147 $[M+H]^+$ and calculated for $C_{16}H_{16}O_4$ as 272.1047 g/mol.

The ^{1}H-NMR spectrum (**Table 17**) showed signals of 4 aromatic protons δ_H 6.73 (1H, d, J = 8.00 Hz, H-4′), δ_H 6.45 (1H, s, H-6), δ_H 5.71 (1H, d, J = 8.00 Hz, H-3′) and δ_H 6.05 (1H, s, H-3), methoxy group (δ_H 3.89, 3H, s) and three methyl group (δ_H 1.47, 2.38, 3Hx2, s) which corresponding to Thadaniti *et al*. (1994).

The ^{13}C-NMR spectra (**Table 17**), supported by the HSQC and DEPTQ-135 data, revealed the presence of a lactone group (δ_C 178.7), eleven aromatic carbons (δ_C 78.0, 96.4, 102.3, 107.3, 110.4, 114.4, 127.7, 154.2, 158.4, 160.4, 164.8), three secondary methyl carbon (δ_C 18.2, 22.81, 27.0) and one methoxy carbon (δ_C 55.2) which corresponding to Thadaniti *et al*. (1994).

2D NMR spectra studies revealed the existence of a secondary methyl moiety due to the ^{1}H-^{1}H COSY correlation of –CH(3')–CH_2(4')–, combined with the HMBC correlations of CH_3-1'/C-2', CH_3-2'/C-1', CH_3-2'/C-2', and CH_3-2'/C-4' as shown in **Figure 39**. The position of the methoxy at C-5 was supported by HMBC

correlations between methoxy methyl at δ_H 3.89 and C-5. Three secondary methyl at C-2, C-1', and C-2', were supported by HMBC correlations between the secondary methyl at δ_H 2.38 and C-2, δ_H 1.47 and C-1', and δ_H 1.47 and C-2', respectively.

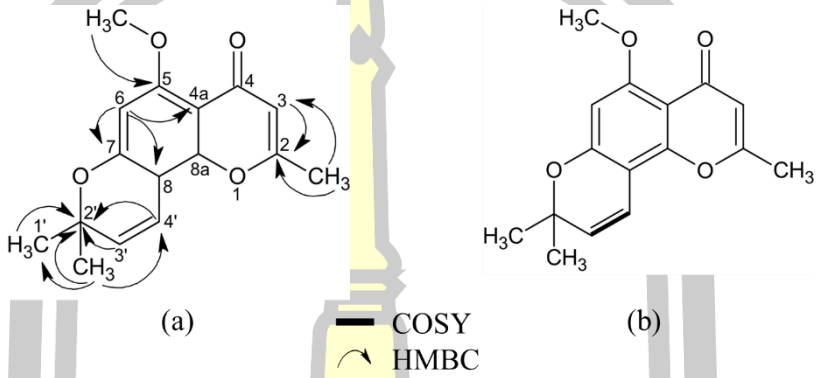


Figure 39 HMBC (a) and ^{1}H-^{1}H COSY (b) correlations of *O*-methyllaloptaeroxyrin.

Table 17 The NMR data of *O*-methyllaloptaeroxyrin.

Position	Thadaniti *et al*. (1994)[a]		Our result[b]	
	δ_C	δ_H (mult, *J* in Hz)	δ_C[c]	δ_H (mult, *J* in Hz)
2	163.3		164.8	
3	112.3	6.00 (*s*, 1H)	110.4	6.05 (*s*, 1H)
4	178.3		178.7	
4a	103.3		102.3	
5	155.0		154.2	
6	97.0	6.29 (*s*, 1H)	96.4	6.45 (*s*, 1H)
7	158.0		158.4	
8	109.0		107.3	
8a	161.0		160.4	
1′	28.8	1.48 (*s*, 3H)	22.8	1.47 (*s*, 3H)
2′	78.5		78.0	
3′	115.8	5.57 (*d*, *J* =10.3, 1H)	114.4	5.71 (*d*, *J* =8.0, 1H)
4′	127.9	6.70 (*d*, *J* =10.3, 1H)	127.7	6.73 (*d*, *J* =8.0, 1H)
2′-CH_3	28.8	1.48 (*s*, 3H)	27.0	1.47 (*s*, 3H)
2- CH_3	20.2	2.29 (*s*, 3H)	18.2	2.38 (*s*, 3H)
5-OCH_3	57.0	3.92 (*s*, 3H)	55.2	3.89 (*s*, 3H)

[a] Spectra were recorded in CD_3OD, ^{13}C (400 MHz), ^{1}H (400 MHz)

[b] Spectra were recorded in CD_3OD, ^{13}C (100 MHz), ^{1}H (400 MHz)

4.2.3 Peucenin-7-methyl ether

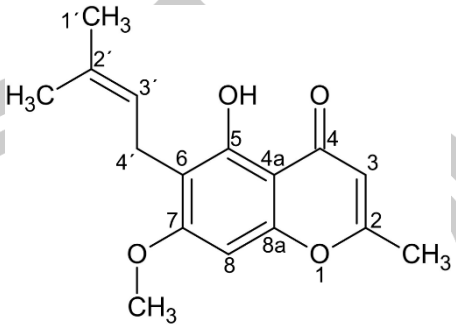


Figure 40 The chemical structure of peucenin-7-methyl ether.

Peucenin-7-methyl ether is a chromone and was isolated from the 70% ethanolic extract of *Harrisonia perforata* roots by preparative TLC on Silica Gel 60 F_{254} using mobile phase of toluene : ethyl acetate (9:1, v/v). The major band of Rf 0.60 was extracted with methanol under ultrasonication for 30 min in 3 repeats to obtain peucenin-7-methyl ether in the form of slightly yellow powder. The UV spectrum in methanol using HPLC analysis showed a maximum absorption (λ_{max}) at 258, 297 and 330 nm. ESI-MS m/z 275.1304 $[M+H]^+$ and calculated for $C_{16}H_{18}O_4$ as 274.1204 g/mol.

The ^{1}H-NMR spectrum (**Table 18**) showed signals of two aromatic protons δ_H 6.08 (1H, s, H-3) and δ_H 6.45 (1H, s, H-8), methoxy group (δ_H 3.92, 3H, s) and three methyl group (δ_H 1.68, δ 1.82, δ 2.43, 3H, s) which corresponded to Thadaniti *et al*. (1994). The ^{13}C-NMR spectra (**Table 18**), supported by the HSQC and DEPTQ-135 data, revealed the presence of a lactone group (δ_C 183.2), eight aromatic carbons (δ_C 94.6, 103.9, 107.3, 107.7, 154.6, 160.2, 163.0, 168.0), one secondary methyl carbon (δ_C 19.1), two tertiary methyl carbon (δ_C 16.7, 25.3), one methoxy carbon (δ_C 55.3), one methylene carbon (δ_C 21.0) and two methine carbon (δ_C 121.7, 132.2) which corresponding to Thadaniti *et al*. (1994).

In addition, its 2D NMR spectra revealed the existence of a 2-methylbutyl moiety due to the ^{1}H-^{1}H COSY correlation of –CH(3')–CH_2(4')– as shown in **Figure 41**. Two tertiary methyls (δ_H 1.68 and δ_H 1.82) were assigned to CH_3-1' and CH_3-2' by the HMBC correlations of CH_3-1'/C-2', CH_3-1'/C-3', CH_3-2'/C-1', and CH_3-2'/C-3'. The attachment of this unit at C-6 of the chromone nucleus was confirmed by HMBC

correlations from H_2-4' (doublet protons) to C-5, C-6 and C-7. The position of the methoxy at C-7 and another secondary methyl at C-2 was supported by HMBC correlations between methoxy methyl at δ_H 3.92 and C-7, and between the secondary methyl at δ_H 2.41 and C-2, respectively.

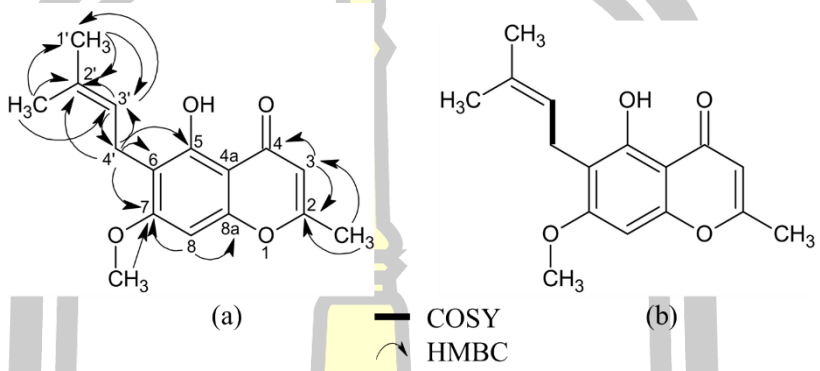


Figure 41 HMBC (a) and COSY (b) correlations of peucenin-7-methyl ether.

Table 18 The NMR data of peucenin-7-methyl ether.

Position	Thadaniti *et al*. (1994)[a]		Our result[b]	
	δ_C	δ_H (mult, *J* in Hz)	δ_C[c]	δ_H (mult, *J* in Hz)
2	167.4		168.0	
3	108.8	6.00 (*s*, 1H)	107.7	6.08 (*s*, 1H)
4	183.6		183.2	
4a	108.2		107.3	
5	155.3		154.6	
6	105.2		103.9	
7	161.1		160.2	
8	95.5	6.36 (*s*, 1H)	94.6	6.45 (*s*, 1H)
8a	163.2		163.0	
1′	21.2	1.79 (*s*, 3H)	16.7	1.82 (*s*, 3H)
2′	132.3		132.2	
3′	122.6	5.15 (*t*, *J* =9.0, 1H)	121.7	5.15 (*t*, *J* =6.6, 1H)
4′	22.2	3.38 (*d*, *J* =9.0, 2H)	21.0	3.41 (*d*, *J* =7.2, 2H)
2′-CH_3	26.4	1.67 (*s*, 3H)	25.3	1.68 (*s*, 3H)
2- CH_3	18.4	2.36 (*s*, 3H)	19.1	2.41 (*s*, 3H)
7-OCH_3	58.5	3.68 (*s*, 3H)	55.3	3.92 (*s*, 3H)
5-OH		12.78 (*s*, 1H)		-

[a] Spectra were recorded in CD_3OD, ^{13}C (400 MHz), ^{1}H (400 MHz)

[b] Spectra were recorded in CD_3OD, ^{13}C (100 MHz), ^{1}H (400 MHz)

4.3 Extractive value

The ethanolic and aqueous extracts of the original MHR and each modified MHR were prepared by maceration in 70% ethanol and decoction in distilled water, respectively. These extraction methods align with Thai traditional medicine practices. Most crude ethanolic and aqueous extracts were obtained as powders. The percentage yield by weight for the original MHR and each modified MHR are presented in **Figure 42**.

The percentage yields of all ethanolic extracts ranged from 5.75% to 35.33%, with the highest yield observed in FG91E (35.33%) and the lowest in FG69E (5.75%). For aqueous extracts, the yields ranged from 3.54% to 24.55%, with FG91A providing the highest yield (24.55%) and FG64A the lowest (3.54%). The percentage yields the ethanolic and aqueous extracts of MHR remedy (Mo-Ha-Rak), were 25.18% and 14.03%, respectively.

Most ethanolic extracts had a higher percentage yield than aqueous extracts, as the different chemical constituents in the plants respond uniquely to each extraction method and solvent used.

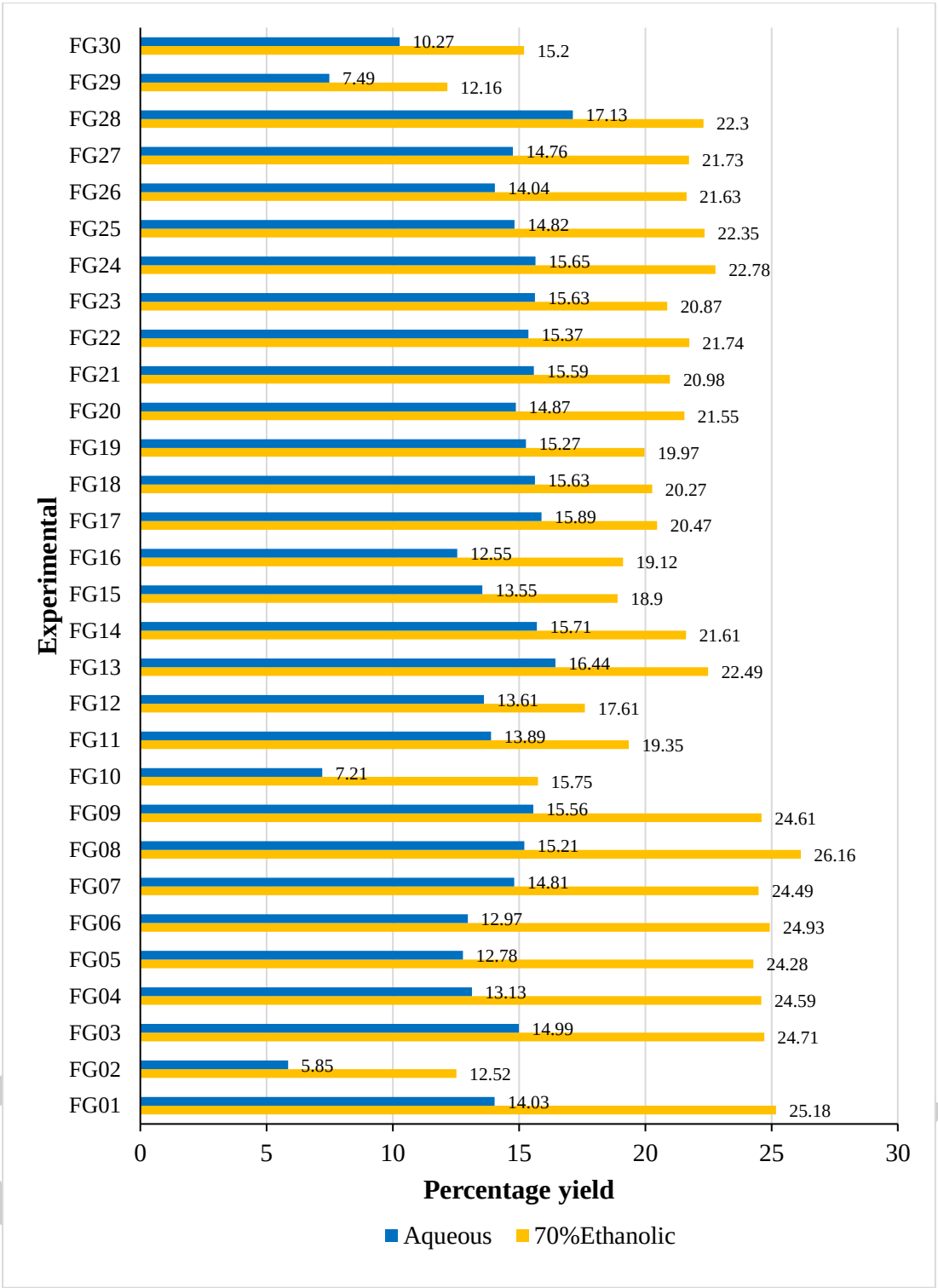


Figure 42 The percentage yields of the ethanolic and aqueous extracts of the original MHR and each modified MHR.

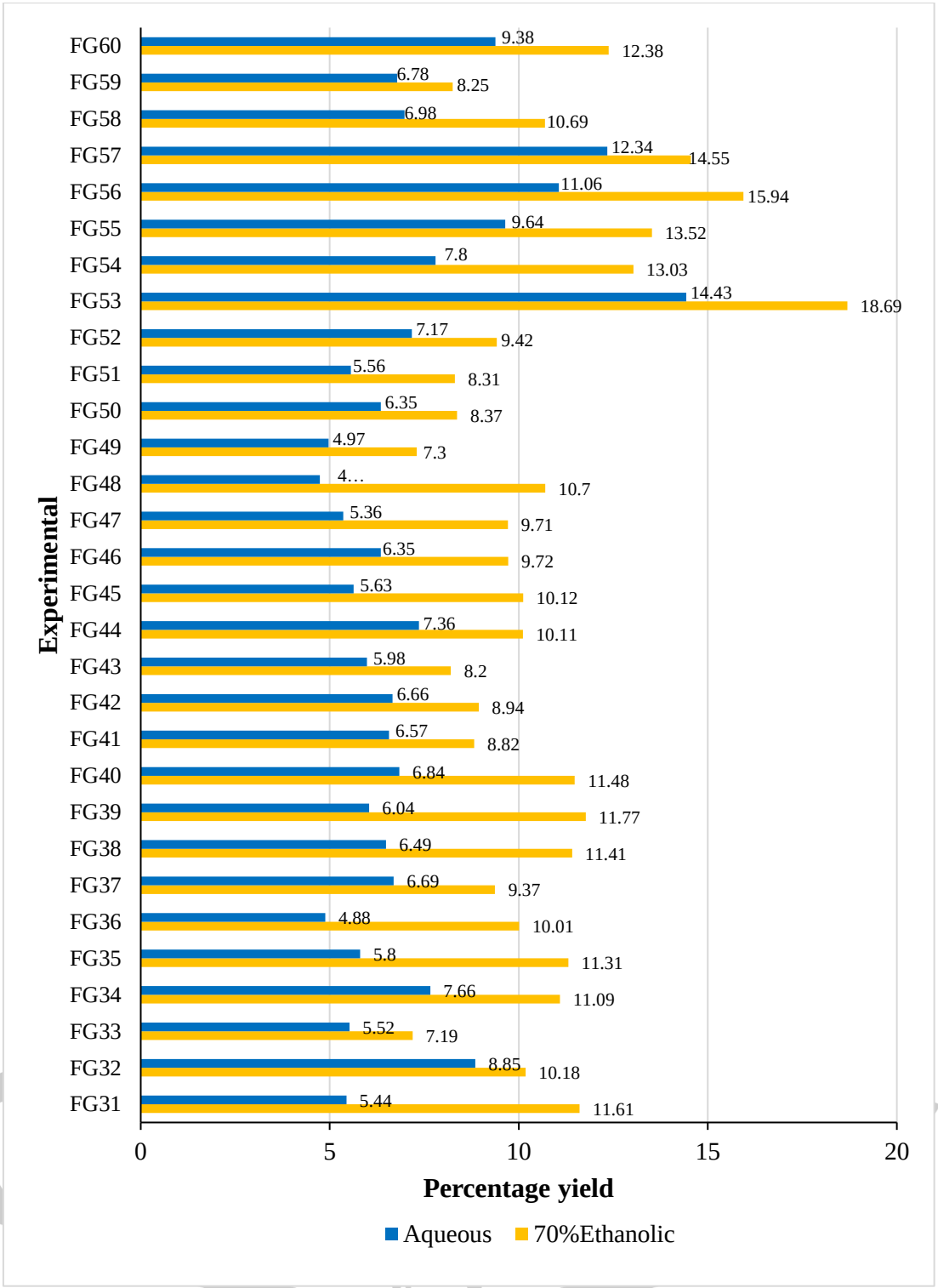


Figure 42 The percentage yields of the ethanolic and aqueous extracts of the original MHR and each modified MHR (Continued).

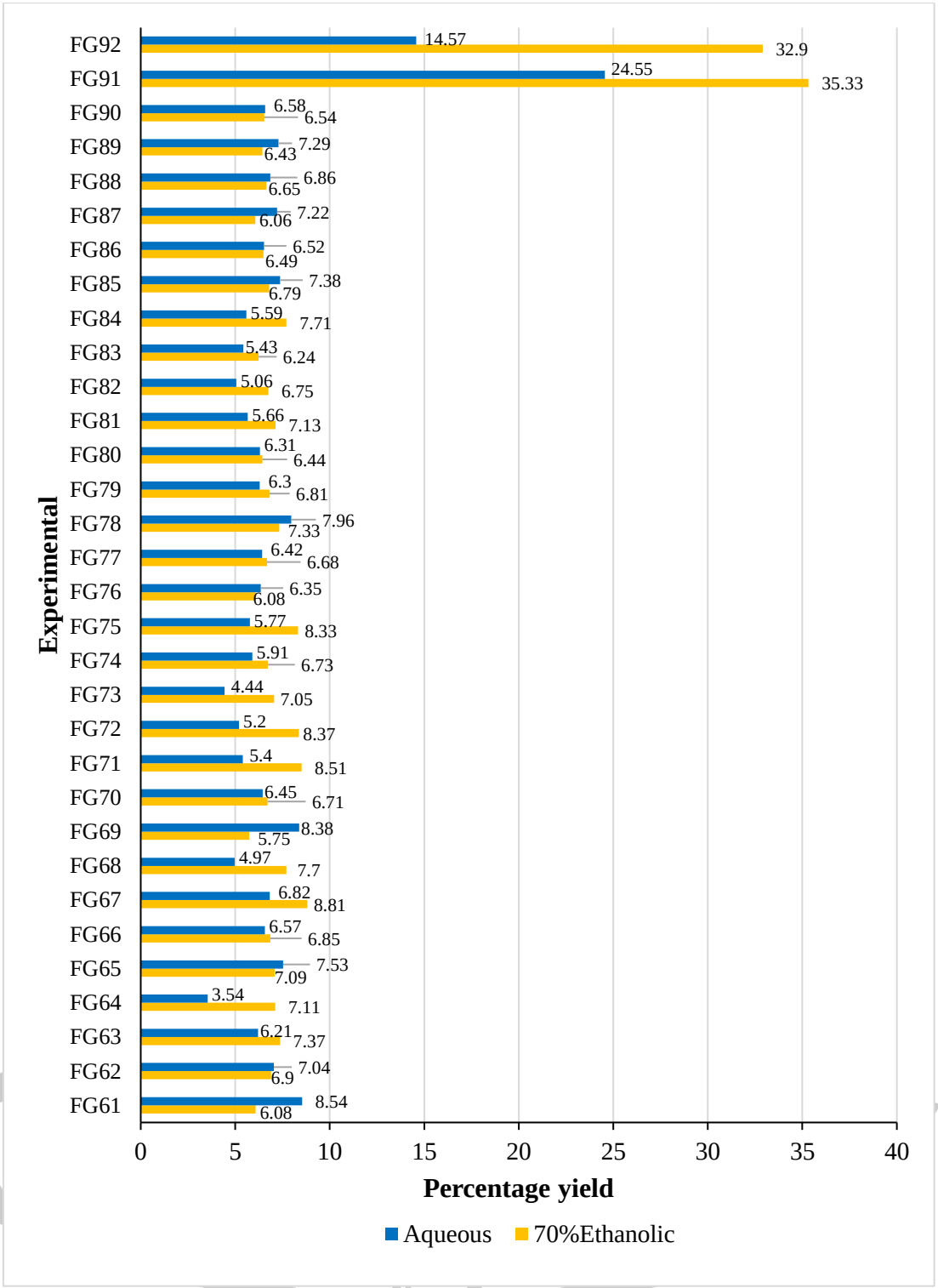


Figure 42 The percentage yields of the ethanolic and aqueous extracts of the original MHR and each modified MHR (Continued).

4.4 Establishment of HPLC fingerprints of MHR

In this study, the ethanolic and aqueous extracts of 92 modified MHR and original MHR were qualitatively and quantitatively analyzed for their chemical profiles using HPLC. The ethanolic extracts were prepared to a final concentration of 10 mg/mL, while the aqueous extracts were prepared at 20 mg/mL. The mobile phase consisted of 0.1% v/v TFA in water and acetonitrile, with gradient elution over 160 minutes as described in section 4.1.2. Results indicated that wavelengths of 254 and 280 nm were optimal for detecting chemical compounds across various modified MHR formulas, within the 190–800 nm range. Chromatographic patterns varied across formulars in terms of the number of peaks and peak areas, reflecting differences in raw materials influenced by the quantity and proportions of herbs used.

4.4.1 The ethanolic extract

4.4.1.1 The qualitative analysis

1) HPLC chromatogram of original MHR and modified MHR of primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs

In the ethanolic extract at 254 nm, HPLC chromatogram of the 92 modified MHR and original MHR showed 129-159 peaks, with at least 35 peaks identified as common peaks. Peaks number 1 - 15 were selected as major peaks for MHR (Mo-Ha-Rak, 01E), as shown in **Figure 43**. For the primary herb group alone (PH, 02E), most peaks appeared in the latter half of the chromatogram, with major peaks identified as 7, 8, 10, 11, 12, 13, and 15, compared to the MHR. In the modified MHR without the supportive herbs (03E), peak numbers 2 and 15 were absent compared to MHR. In the adjunct herbs (AH, 91E), most peaks appeared in the first half of the chromatogram, with strong peaks identified as 1, 3, 4, 5, 6, and 8. In the modified MHR without the adjunct herbs (10E) resulted in the absence of peak 1, 3, 4, 5, 6, and 9 compared to MHR. Lastly, in the supportive herb group alone (SH, 92E), peaks appeared throughout the chromatogram, with prominent peaks identified as 1, 2, and 14.

Modified MHR remedies by fixing reduced toxic fever herbs with one of the other components, 45E-60E, were analyzed compared to original MHR (01E) and modified MHR without herbs for the treatment of semha, lom, di, kamdoa

and lohit fever (only reduced toxic fever herbs) (33E). It was observed that peak 1, 2, 3, 4, 5, and 6 appeared in the AH group (54E-57E), while peak 9 was found in formula 53E. Peaks 1, 2, and 14 were present in the SH group (58E). In the AH group, peak number 10 appeared in formula 48E, peak 12 in formula 51E, and peak 13 and 15 in formula 46E. Additionally, peak 7, 8, and 11, which are representative of the primary herbs, were consistently observed across all modified MHR extracts (33E, 45E-51E), as shown in **Figure 44**.

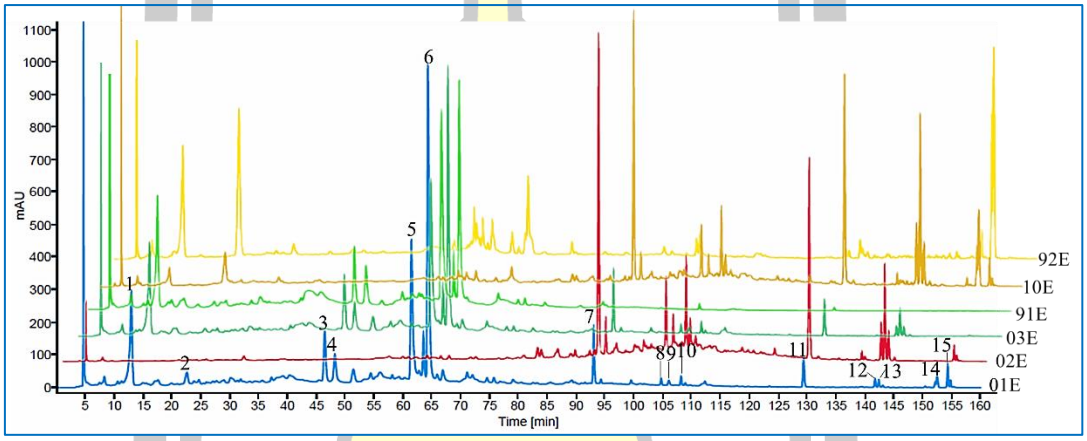


Figure 43 HPLC chromatogram of original MHR (01E) and modified MHR remedies, without adjunct and supportive herbs (02E), without supportive herbs (03E), only adjunct herbs (91E), without adjunct herbs (10E), and only supportive herbs (92E).

Major peak: (1) gallic acid, (2) protocatechuic acid, (3) chebulanin, (4) corilagin, (5) chebulagic acid, (6) ellagic acid, (7) perforatic acid, (8) *O*-methyllaloptaeroxyrin, (9) rhein, (10) lourierin A, (11) peucenin-7-methyl ether, (12) PK02, (13) GC01, (14) MF02, (15) GC04.

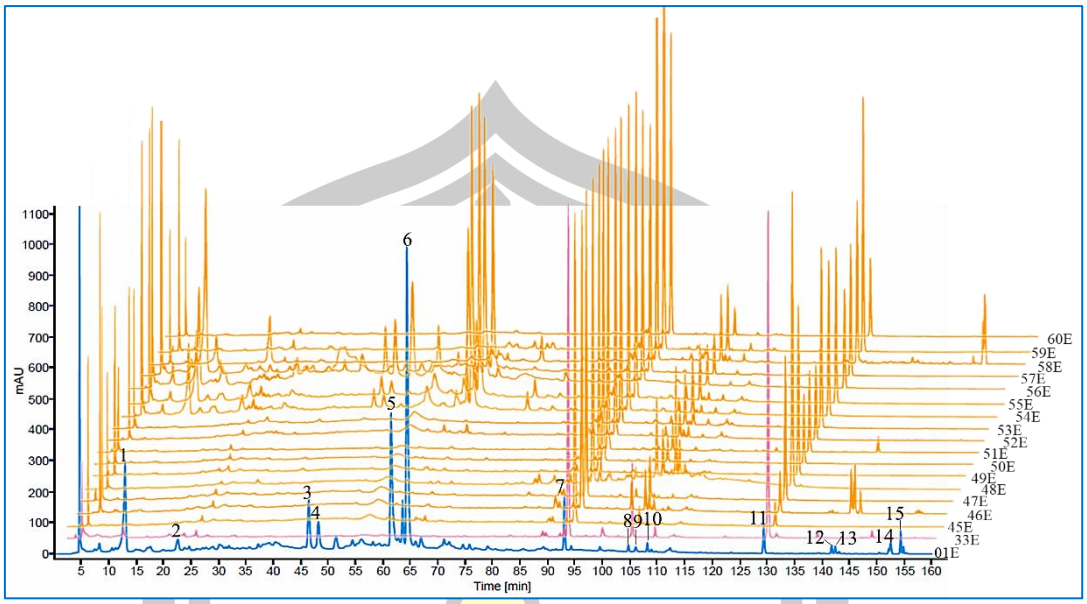


Figure 44 HPLC chromatogram of original MHR (01E) and modified MHR remedies by fixing reduced toxic fever herbs with one of the other components (45E-60E). Major peak: (1) gallic acid, (2) protocatechuic acid, (3) chebulanin, (4) corilagin, (5) chebulagic acid, (6) ellagic acid, (7) perforatic acid, (8) O-methyllaloptaeroxyrin, (9) rhein, (10) lourierin A, (11) peucenin-7-methyl ether, (12) PK02, (13) GC01, (14) MF02, (15) GC04.

2) HPLC chromatogram of modified MHR specifically modification of primary herbs

In the HPLC chromatogram of the primary herb, most peaks appeared in the latter half of the HPLC fingerprint, with major peaks identified as 7, 8, 10, 11, 12, 13, and 15. These peaks were assigned as characteristic markers corresponding to perforatic acid, *O*-methyllaloptaeroxyrin, lourierin A, peucenin-7-methyl ether, PK02, GC01 and GC04, respectively. Modified MHR 02E was the primary herbs, while formulas 31E-37E that included variations in the primary herbs. Formulas 38E-39E involved adjustments the number of herbs for treating semha and lom fever, formulas 40E-41E adjusted the herbs for treating di fever and formulas 42E-44E involved changing in the number of herbs for treating kamado and lohit fever. Formulas 45E-51E were modified MHR remedies by fixing reduced toxic fever herbs and plus one of the other components compared to only reduced toxic fever herbs (33E). Major peaks 7, 8, and 11, were present in the formulas containing herbs

for reduced toxic fever (33E) including formulas 31E-51E. Peaks 10 was observed in formulas containing *D. cochinchinensis,* specifically in formulas 02E, 31E, 35E, 36E, 38E-40E and 48E. Peak 12 appeared in the formulas containing *P. kesiya* including formulas 02E, 34E, 35E, 37E, 39E and 50E. Meanwhile, peaks 13 and 15 were found in the formulas containing *G. chinense*, namely 02E, 31E, 32E, 34E, 38E-42E, 44E and 46E, as shown in **Figure 45**.

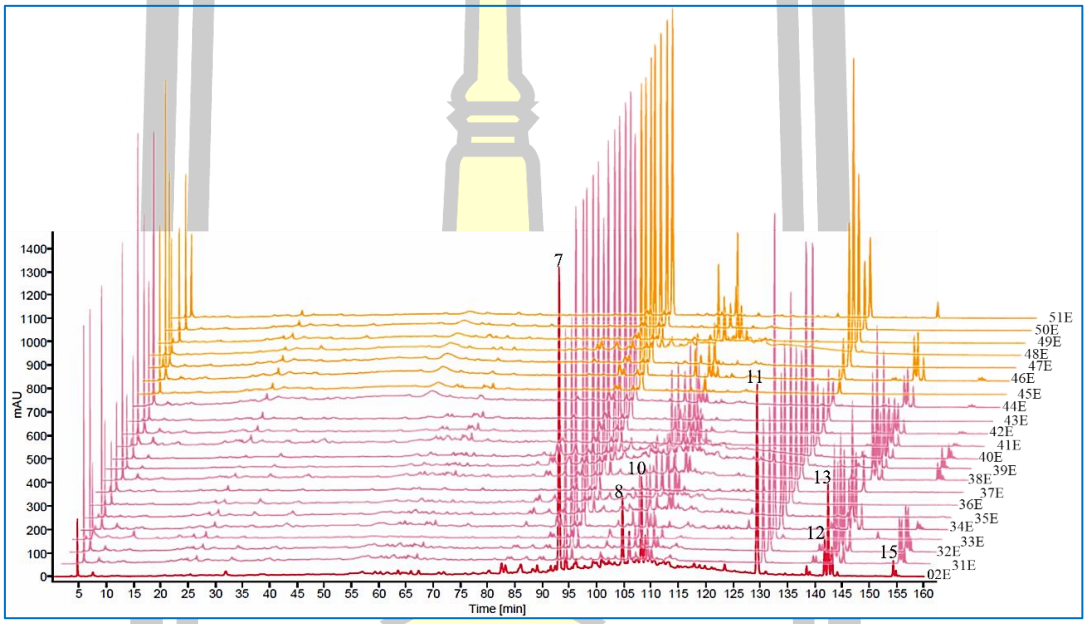


Figure 45 HPLC chromatogram of modified MHR showing only the modifications of primary herbs (31E-51E) compared to primary herbs (02E).
Major peak: (7) perforatic acid, (8) *O*-methyllaloptaeroxyrin, (10) lourierin A, (11) peucenin-7-methyl ether, (12) PK02, (13) GC01, (15) GC04.

Formulas 61E-90E involved modifications to the number of herbs that reduced toxic fever within the primary herbs. Specifically, formulas 61E-65E removed four herbs, formulas 66E-75E removed three herbs, formulas 76E-85E removed two herbs, and formulas 86E-90E removed one herb. Major peaks 7, 8, and 11, were found in the formulas containing *H. perforate*, specifically in formulas 64E, 68E, 71E, 73E, 75E, 77E, 79E, 81E, 82E, 84E, 85E, 86E, 88E, 89E, and 90E. Peak 16 was observed in the formulas containing *T. triandra*, including 65E, 69E, 72E, 74E, 75E, 78E, 80E, 81E, 83E, 84E, 85E, 87E, 88E, 89E, and 90E. Peak 17 was present in

the formulas containing *F. racemose,* as shown in formulas 63E, 67E, 70E, 73E, 74E, 76E, 79E, 80E, 82E, 83E, 85E, 86E, 87E, 89E, and 90E. Lastly, peak 18 was detected in the formulas with *C. indicum* specifically 62E, 66E, 70E, 71E, 72E, 76E, 77E, 78E, 82E, 83E, 84E, 86E, 87E, 88E, and 90E, as shown in **Figure 46-47**.

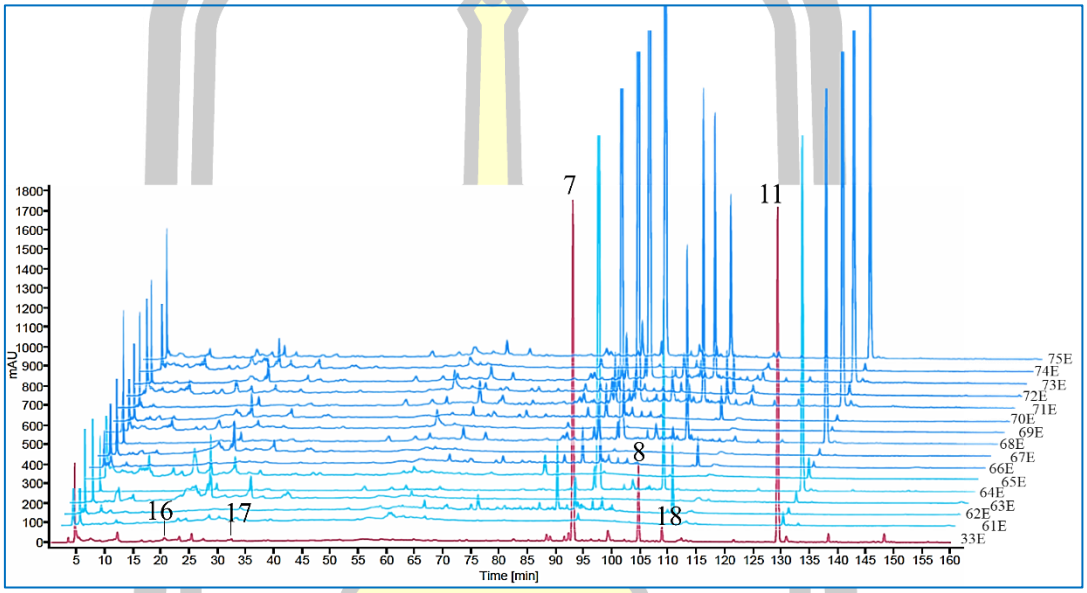


Figure 46 HPLC chromatogram of modified MHR showing reduced toxic herbs (33E) and their modified reduced toxic herbs by removing 4 herbs (61E-65E) and 3 herbs (66E-75E).

Major peak: (7) perforatic acid, (8) *O*-methyllaloptaeroxyrin, (11) peucenin-7-methyl ether, (16) TT01, (17) bergenin, (18) pectolinarigenin.

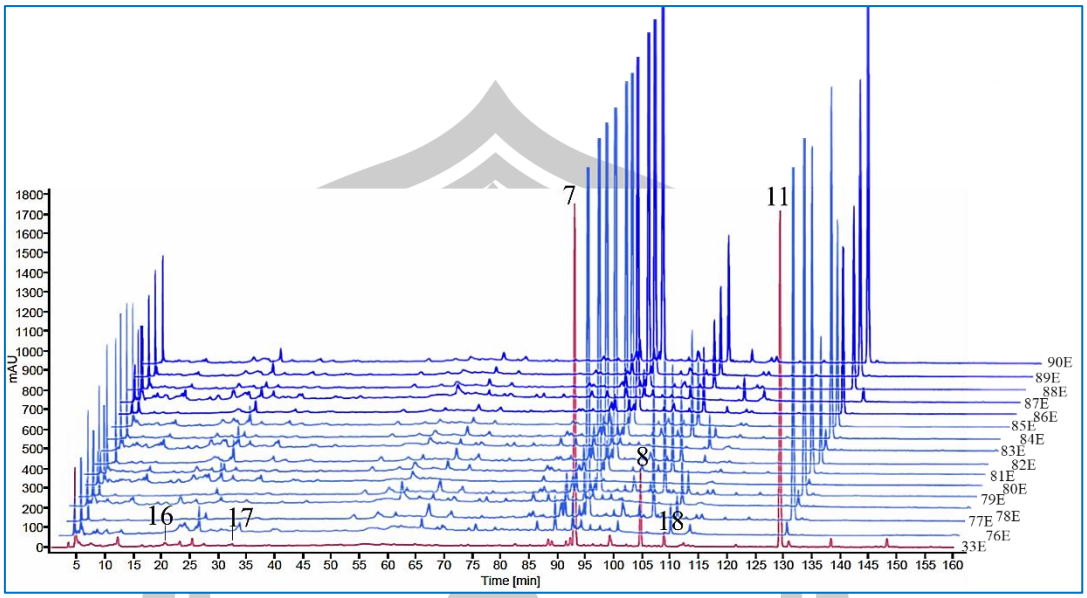


Figure 47 HPLC chromatogram of modified MHR showing reduced toxic herbs (33E) and their modified reduced toxic herbs by removing 2 herbs (76E-90E). Major peak: (7) perforatic acid, (8) *O*-methyllaloptaeroxyrin, (11) peucenin-7-methyl ether, (16) TT01, (17) bergenin, 18 pectolinarigenin.

3) HPLC chromatogram of modified MHR specifically modification of adjunct herbs

The modification of adjunct herbs by changing the number of herbs while fixing primary herbs, led to the identification of peaks within the same group as the primary herbs, specifically peaks 7, 8, 10, 11, 12, 13, and 15. Meanwhile, most peaks in the CH appeared in the first half of the HPLC fingerprint, with major peaks identified as 1, 3, 4, 5, 6, and 9. Peaks number 1, 3, 4, 5, 6, and 9 were assigned as characteristic peaks that correspond to gallic acid, chebulanin, corilagin, chebulagic acid, ellagic acid and rhein, respectively, as shown in **Figure 48**.

Formula 03E involves removing the supportive herbs, formula 91E contains only the adjunct herbs, while formulas 11E-14E involve changes in the number of herbs within the adjunct herbs and supportive herbs. Formulas 15E-28E were modified by removing the number of sour-astringent laxative herbs in adjunct herbs. Specifically, formulas 15E-18E removed three herbs, formulas 19E-24E removed two herbs, and formulas 25E-28E removed one herb. Formulas 29E-30E involved changes in the number of stimulant laxative herbs in adjunct herbs. Formulas

52E-57E fixed the herbs for reduced toxic fever by changing the adjunct herbs. The major peaks, numbers 1, 3, 4, 5, and 6, were found in the formulas containing *T. bellirica*, *T. chebula*, *Terminalia* sp. “Samo Thet” or *P. emblica* including formulas 03E, 91E, 13E-28E, 54E-57E. Peak 9 was observed in the formulas containing *C. fistula* including formulas 03E, 91E, 11E, 12E, 15E-28E and 30E. Lastly, the formulas 11E and 13E showed peaks at numbers 2 and 14, due to modification of adjunct herbs, as shown in **Figure 48-49**.

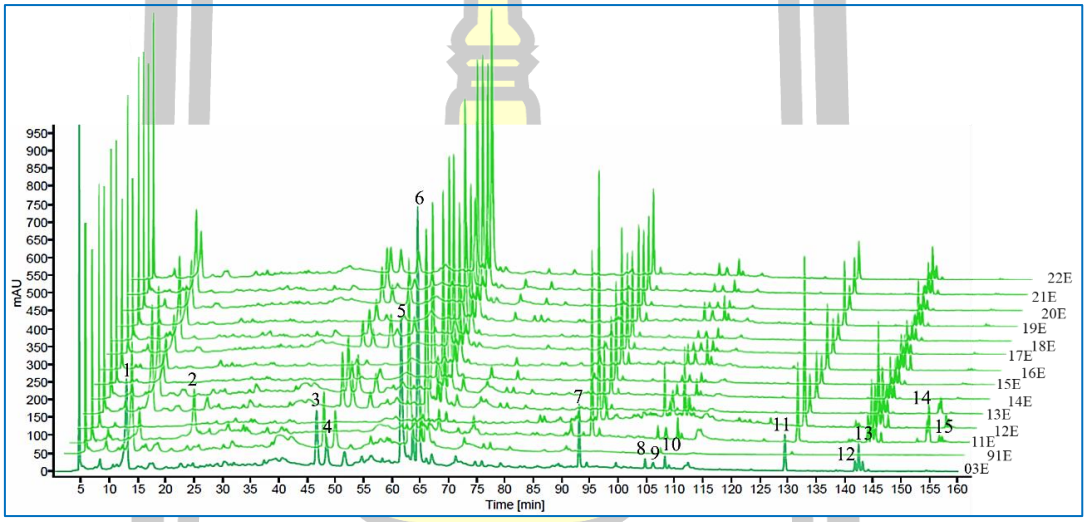


Figure 48 HPLC chromatogram of modified MHR by modification of adjunct herbs. Major peak: (1) gallic acid, (2) protocatechuic acid, (3) chebulanin, (4) corilagin, (5) chebulagic acid, (6) ellagic acid, (7) perforatic acid, (8) *O*-methyllaloptaeroxyrin, (9) rhein, (10) lourierin A, (11) peucenin-7-methyl ether, (12) PK02, (13) GC01, (14) MF02, (15) GC04.

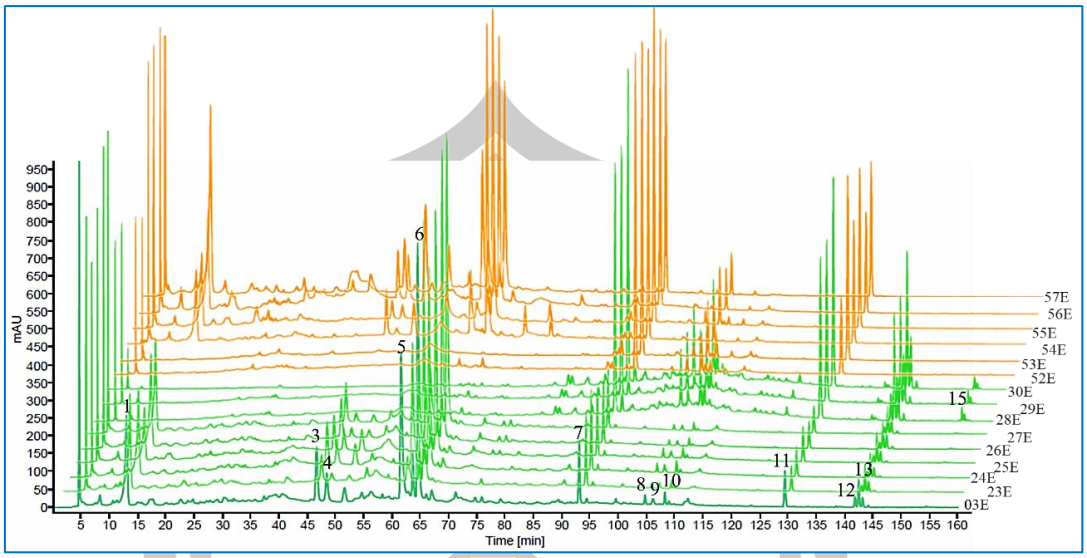


Figure 49 HPLC chromatogram of modified MHR by modification of adjunct herbs, focus on removing the number of sour-astringent laxative herbs.

Major peak: (1) gallic acid, (2) protocatechuic acid, (3) chebulanin, (4) corilagin, (5) chebulagic acid, (6) ellagic acid, (7) perforatic acid, (8) *O*-methyllaloptaeroxyrin, (9) rhein, (10) lourierin A, (11) peucenin-7-methyl ether, (12) PK02, (13) GC01, (14) MF02, (15) GC04.

4) The supportive herbs

The modification of adjunct herbs by changing the number of herbs while fixing the primary herbs, led to the identification of peaks within the same group as the primary herbs, specifically peaks 7, 8, 10, 11, 12, 13, and 15. Moreover, major peak numbers 1, 3, 4, 5, 6, and 9 were the same group as the adjunct herbs, peak of supportive herbs appeared throughout the HPLC fingerprint, with strong peaks identified as 1, 2, and 14. Peaks number 1, 2, and 14 were assigned as characteristic peaks that correspond to gallic acid, protocatechuic acid and MF02, respectively, as shown in **Figure 50**.

Formula 10E involves removing the adjunct herbs, formula 92E contained only supportive herbs, while formulas 04E-09E involve changes in the number of herbs within the SH subgroup. Formulas 58E-60E showed the modification of supportive herbs by fixing reduced toxic fever herbs. The major peaks, numbers 1, 2, and 14 were found in the formulas containing *M. ferrea* including formulas 10E, 92E, 04E, 07E, and 08E, as shown in **Figure 50**.

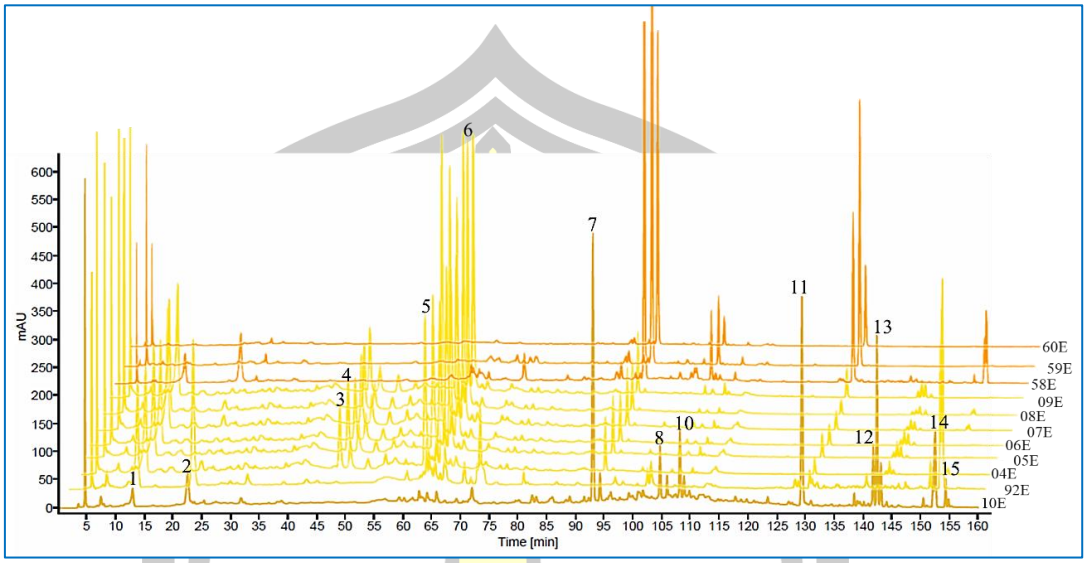


Figure 50 HPLC chromatogram of modified MHR by modification of supportive herbs.

Major peak: (1) gallic acid, (2) protocatechuic acid, (3) chebulanin, (4) corilagin, (5) chebulagic acid, (6) ellagic acid, (7) perforatic acid, (8) *O*-methyllaloptaeroxyrin, (9) rhein, (10) lourierin A, (11) peucenin-7-methyl ether, (12) PK02, (13) GC01, (14) MF02, (15) GC04.

4.4.1.2 The quantitative analysis of major components in MHR

In the ethanolic extract at 254 nm of HPLC chromatogram, a total of 15 major peaks were identified. Peaks number 1-15 were assigned as characteristic peaks corresponding to gallic acid, protocatechuic acid, chebulanin, corilagin, chebulagic acid, ellagic acid, perforatic acid, *O*-methyllaloptaeroxyrin, rhein, lourierin A, peucenin-7-methyl ether, PK02, GC01, MF02, and GC04, respectively. According to the quantitative analysis of the peak areas of these components, gallic acid (**Figure 51**) was observed in the original MHR formula, the adjunct herbs, and the supportive herbs. The highest amount of gallic acid was found in formula 57E, with a value of 18,416.39 ± 2.13 mAU, which is a formula containing reduced toxic fever herbs and *P. emblica*.

Protocatechuic acid (**Figure 52**) was a major compound shown in the original MHR formula, and the supportive herbs. The highest amount of

protocatechuic acid was found in formula 58E, with a value of 5,160.27 ± 0.62 mAU, which is a formula containing reduced toxic fever herbs and *M. ferrea*.

Chebulanin (**Figure 53**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of chebulanin was found in formula 14E, with a value of 4,211.25 ± 0.83 mAU, which is a formula containing primary herbs with sour-astringent laxative.

Corilagin (**Figure 54**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of corilagin was found in formula 56E, with a value of 6,806.12 ± 1.16 mAU, which is a formula containing reduced toxic fever herbs with *Terminalia sp.*

Chebulagic acid (**Figure 55**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of chebulagic acid was found in formula 14E, with a value of 14,879.31 ± 0.84 mAU, which is a formula containing primary herbs with sour-astringent laxative.

Ellagic acid (**Figure 56**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of ellagic acid was found in formula 54E, with a value of 21,832.85 ± 2.13 mAU, which is a formula containing reduced toxic fever herbs with *T. bellirica*.

Perforatic acid (**Figure 57**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of perforatic acid was found in formula 64E, with a value of 95,500.35 ± 0.11 mAU, which is a formula containing *H. perforata*.

O-methyllaloptaeroxyrin (**Figure 58**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of HP02 was found in formula 64E, with a value of 21,290.26 ± 2.46 mAU, which is a formula containing *H. perforata*.

Rhein (**Figure 59**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of rhein was found in formula 53E, with a value of 988.86 ± 3.12 mAU, which is a formula containing reduced toxic fever herbs with *C. fistula*.

Lourierin A (**Figure 60**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The

highest amount of lourierin A was found in formula 48E, with a value of 12378.27 ± 4.44 mAU, which is a formula containing reduced toxic fever herbs with *D. cochinchinensis*.

Peucenin-7-methyl ether (**Figure 61**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of HP03 was found in formula 64E, with a value of 56,259.26 ± 1.70 mAU, which is a formula containing *H. perforata*.

PK02 (**Figure 62**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of PK02 was found in formula 02E, with a value of 2,196.32 ± 1.00 mAU, which is the primary herbs alone.

GC01 (**Figure 63**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of GC01 was found in formula 02E, with a value of 5,175.78 ± 1.90 mAU, which is the primary herbs alone.

MF02 (**Figure 64**) was a major compound shown in the original MHR formula, and the supportive herbs. The highest amount of MF02 was found in formula 58E, with a value of 3364.26 ± 2.21 mAU, which is a formula containing reduced toxic fever herbs with *M. ferrea*.

Lastly, GC04 (**Figure 65**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of GC04 was found in formula 31E, with a value of 1,435.35 ± 8.54 mAU, which is a formula exclude herbs for the treatment of semha fever and lom fever.

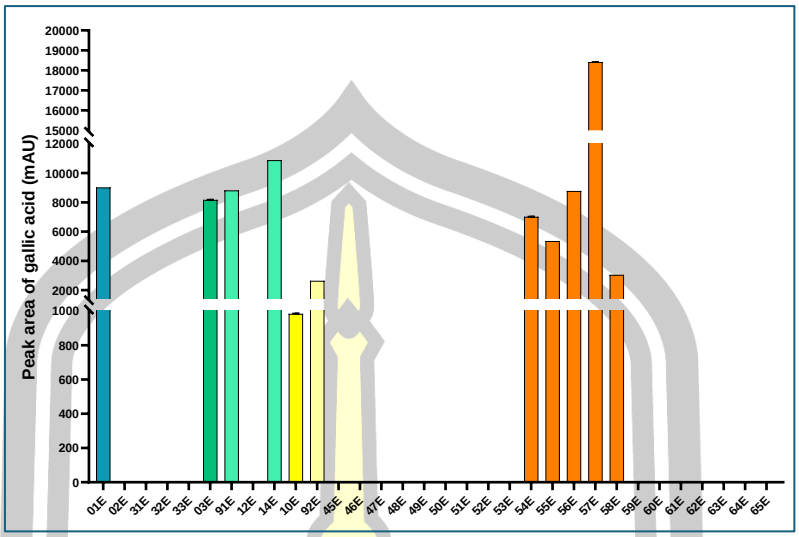


Figure 51 Peak area of gallic acid in modified MHR remedies.

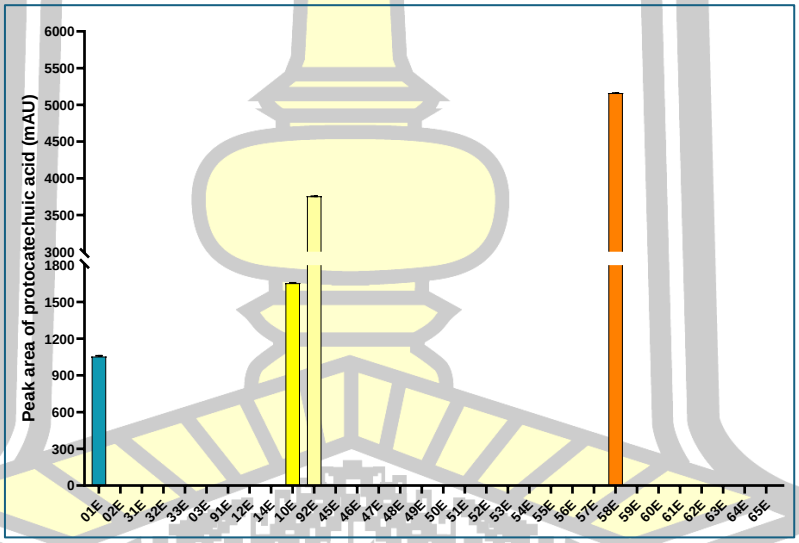


Figure 52 Peak area of protocatechuic acid in modified MHR remedies.

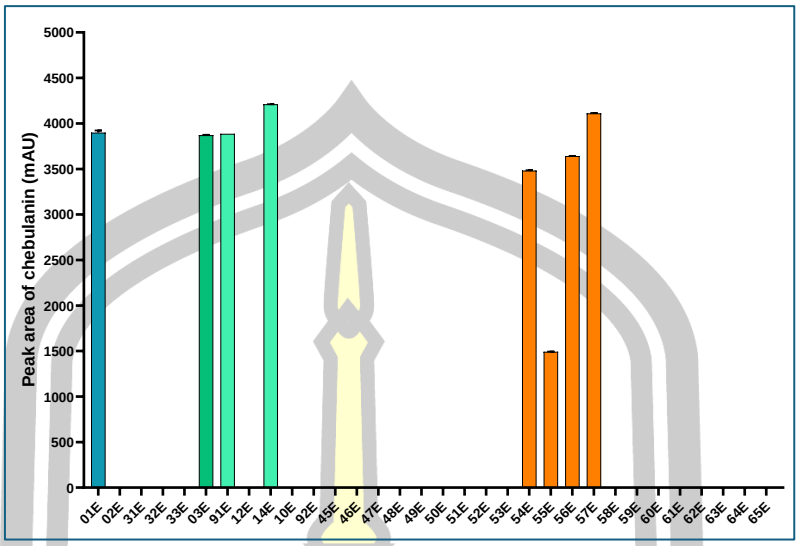


Figure 53 Peak area of chebulanin in modified MHR remedies.

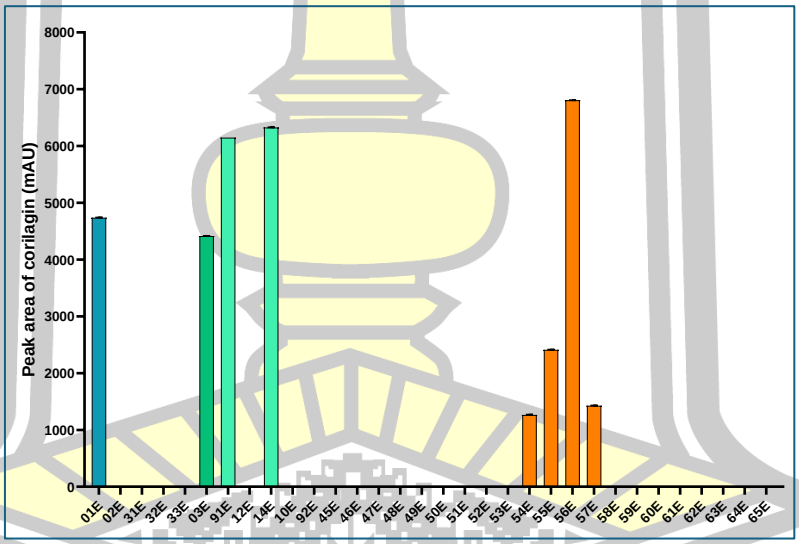


Figure 54 Peak area of corilagin in modified MHR remedies.

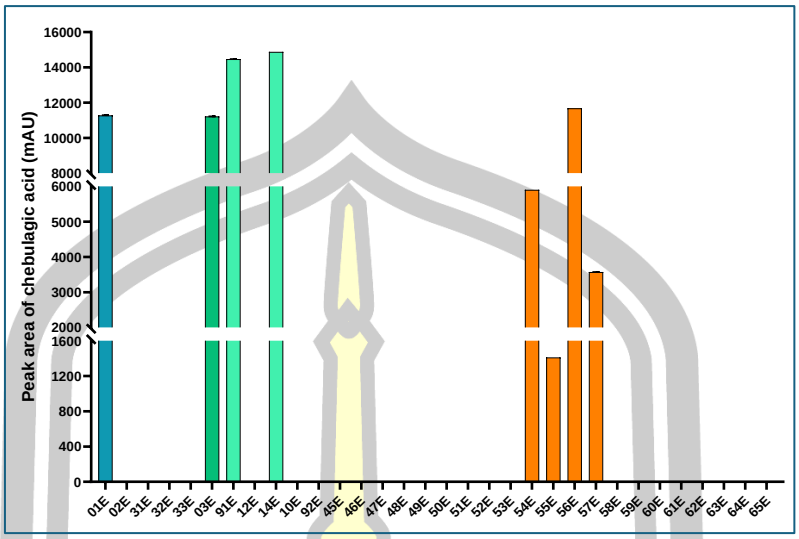


Figure 55 Peak area of chebulagic acid in modified MHR remedies.

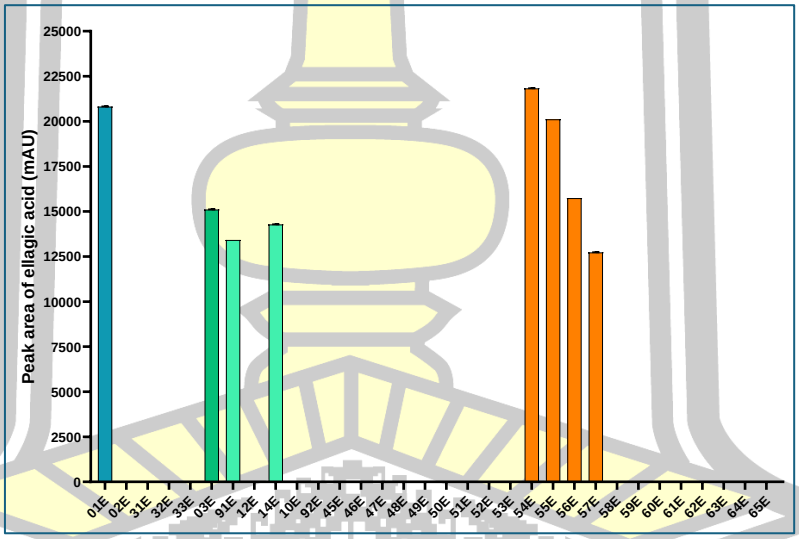


Figure 56 Peak area of ellagic acid in modified MHR remedies.

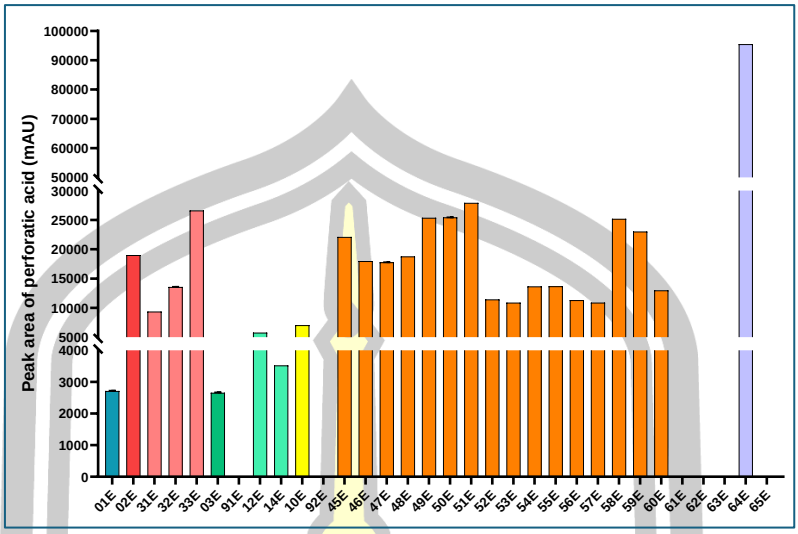


Figure 57 Peak area of perforatic acid in modified MHR remedies.

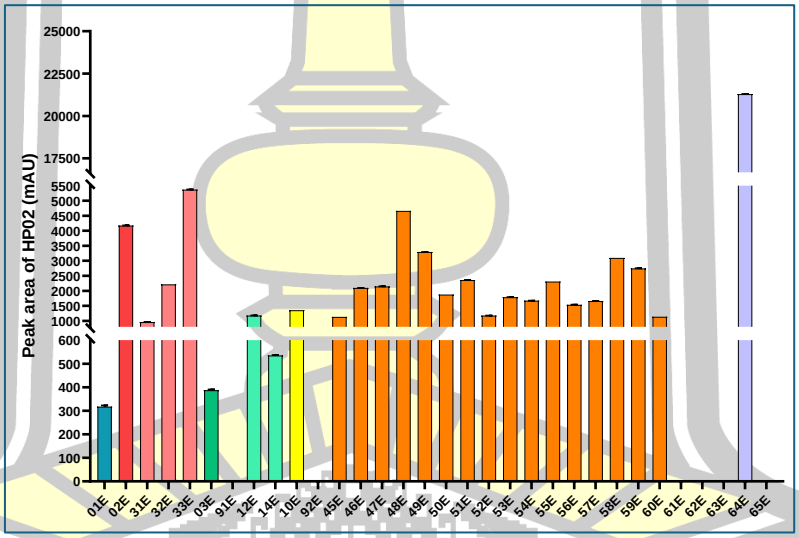


Figure 58 Peak area of *O*-methyllaloptaeroxyrin in modified MHR remedies.

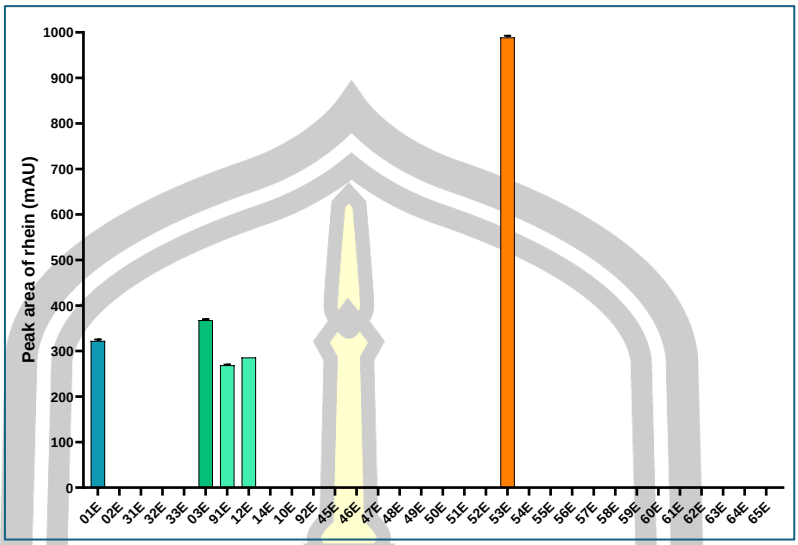


Figure 59 Peak area of rhein in modified MHR remedies.

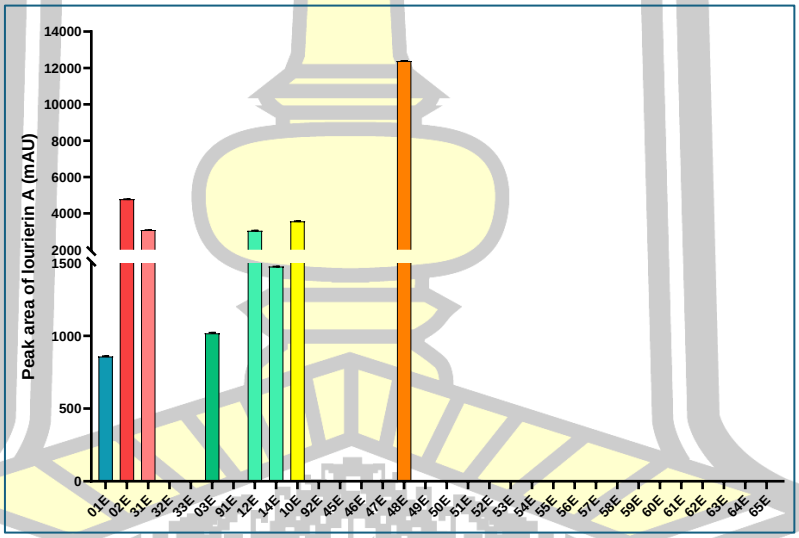


Figure 60 Peak area of loureirin A in modified MHR remedies.

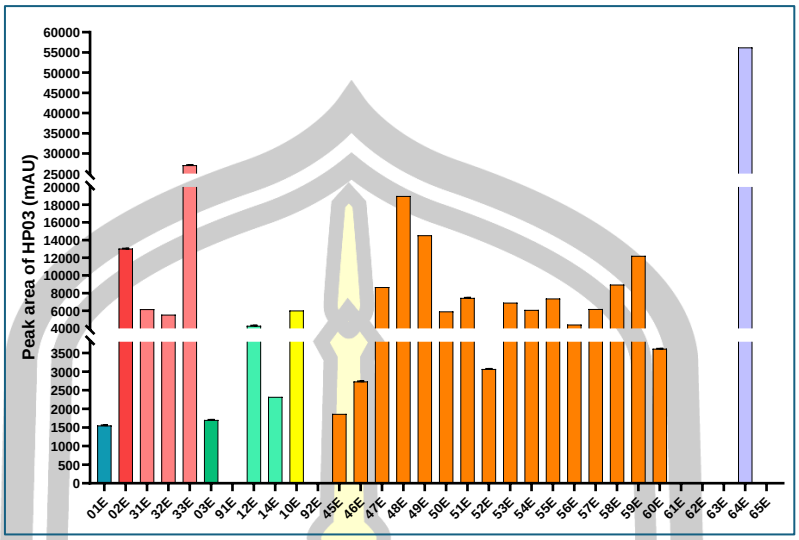


Figure 61 Peak area of peucenin-7-methyl ether in modified MHR remedies.

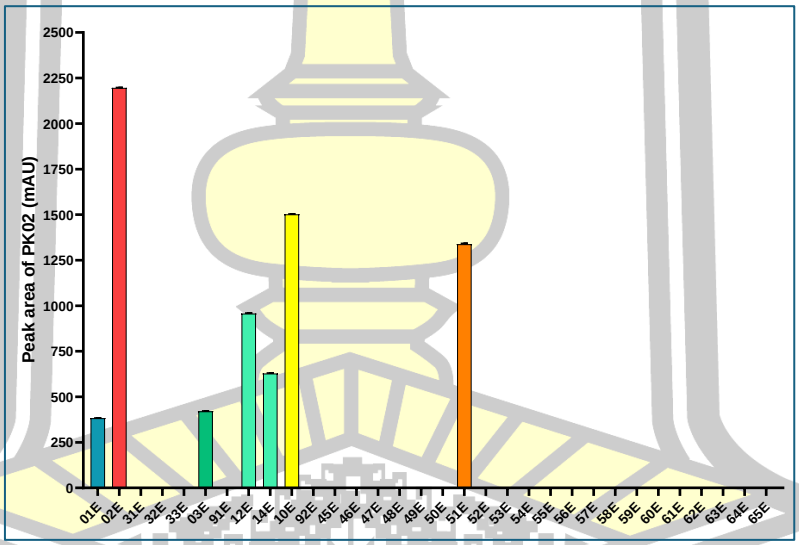


Figure 62 Peak area of PK02 in modified MHR remedies.

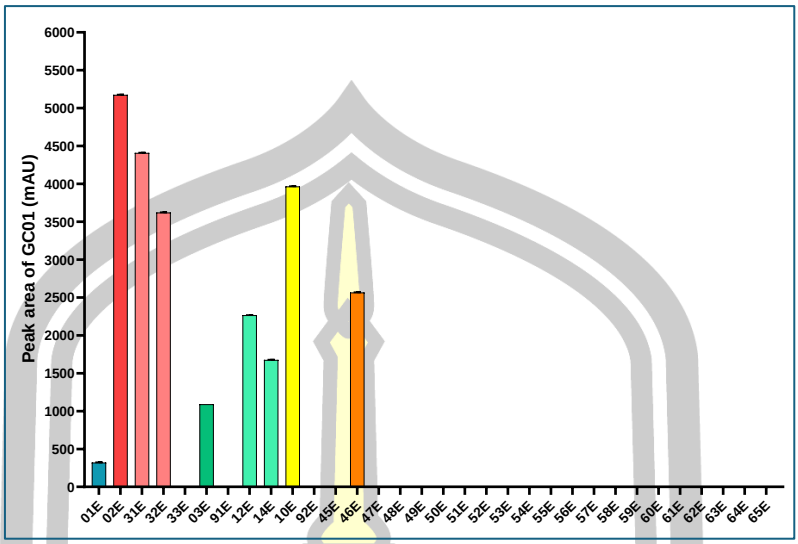


Figure 63 Peak area of GC01 in modified MHR remedies.

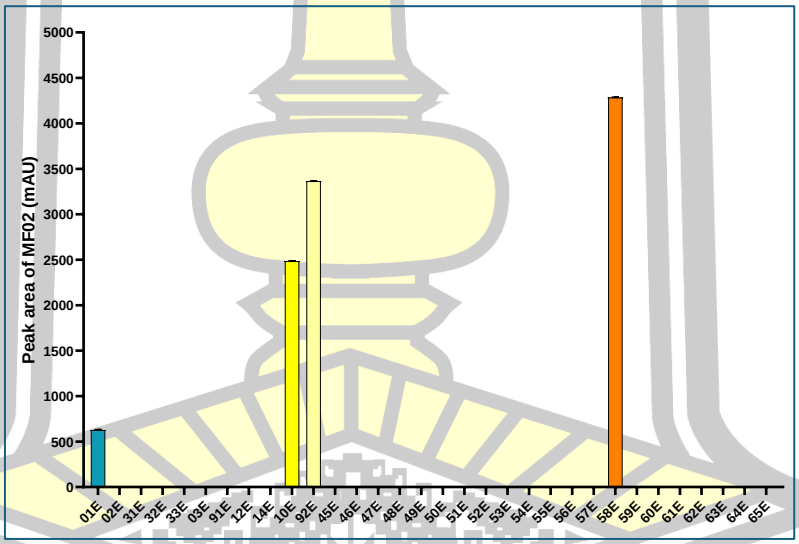


Figure 64 Peak area of MF02 in modified MHR remedies.

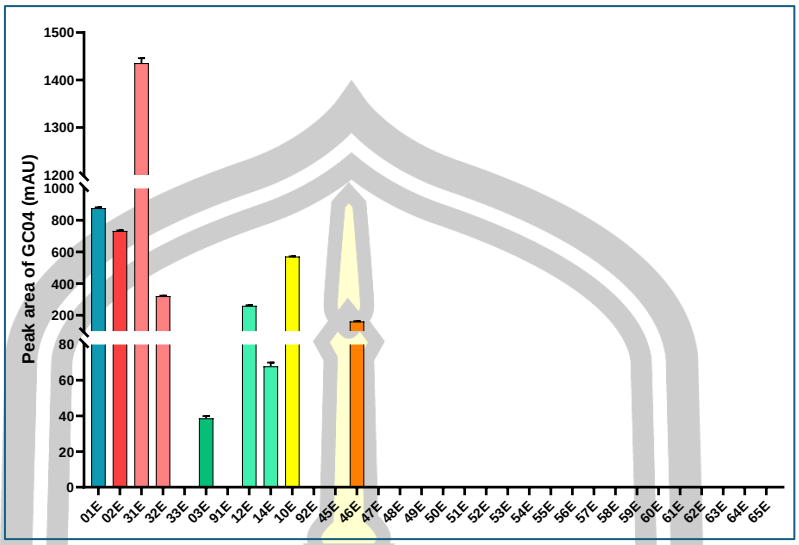


Figure 65 Peak area of GC04 in modified MHR remedies.

4.4.2 The aqueous extract

4.4.2.1 The qualitative analysis

1) HPLC chromatogram of original MHR and modified MHR of primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs

In the HPLC analysis of aqueous extract detected at 254 nm, HPLC chromatogram of original and modified MHR remedies showed 150-158 peaks, with at least 34 peaks identified as common peaks. Peaks number 1-11 were selected as major peaks for MHR remedy (Mo-Ha-Rak, 01A), which are shown in **Figure 66**. Most peaks of the primary herbs (PH, 02A) appeared in the front of the HPLC chromatogram, with major peaks identified as 4, 9, and 10, compared to the MHR. When the supportive herbs were removed (03A), peak number 3 did not show in the HPLC chromatogram compared to MHR. Only the HPLC chromatogram of the adjunct herbs (AH, 91A), peaks appeared throughout the chromatogram, with major peaks identified as 1, 2, 5, 6, 7, 8, and 11. Removing the number of herbs in the adjunct herbs (10A) resulted in the absence of peak numbers 1, 5, 6, 7, 8, and 11 compared to MHR. Lastly, only the supportive herbs (SH, 92A), peaks appeared throughout the chromatogram, with major peaks identified as 1, 2, and 14. Formulas 45A-60A were modified MHR remedies by fixing reduced toxic fever herbs and plus

one of the other components. It was concluded that peaks number 1, 2, 5, 6, 7, and 8 were found in the herbs of AH group (54A-57A), while peaks number 11 was found in formula 53A. Additionally, peaks 2 and 3 were found in the herbs of the SH group (58A), while peaks 4, 9, and 10 were observed in all formulas (33A, 45A-60A), as shown in **Figure 67**.

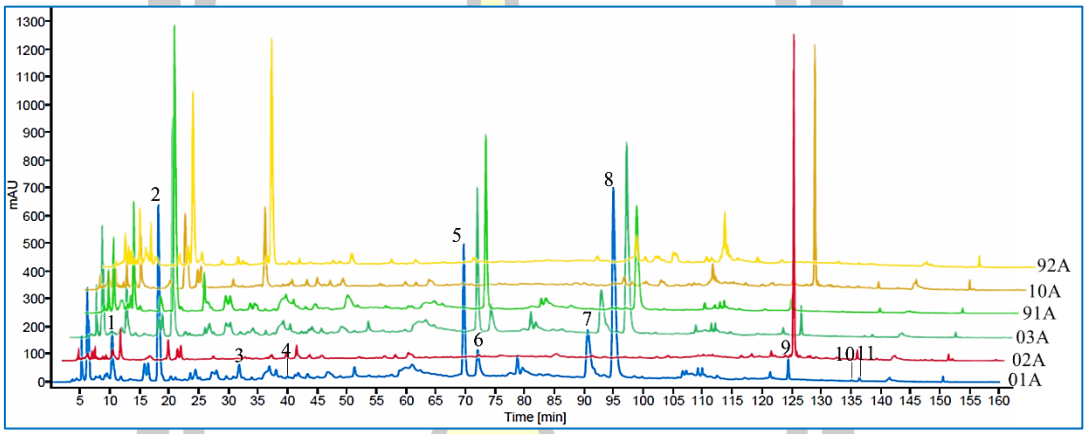


Figure 66 HPLC chromatogram of original MHR (01A) and modified MHR remedies, without adjunct and supportive herbs (02A), without supportive herbs (03A), only adjunct herbs (91A), without adjunct herbs (10A), and only supportive herbs (92A).

Major peak: (1) chebulic acid, (2) gallic acid, (3) protocatechuic acid, (4) FR02, (5) chebulanin, (6) corilagin, (7) chebulagic acid, (8) ellagic acid, (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin, (11) rhein.

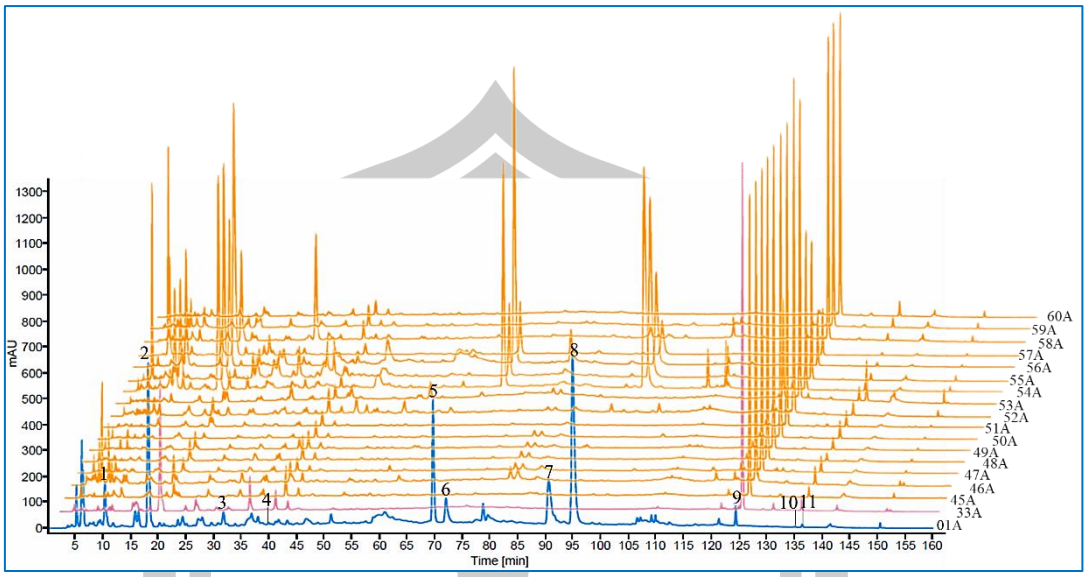


Figure 67 HPLC chromatogram of original MHR (01A) and modified MHR remedies by fixing reduced toxic fever herbs with one of the other components (45A-60A). Major peak: (1) chebulic acid, (2) gallic acid, (3) protocatechuic acid, (4) FR02, (5) chebulanin, (6) corilagin, (7) chebulagic acid, (8) ellagic acid, (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin, (11) rhein.

2) HPLC chromatogram of modified MHR specifically modification of primary herbs

In the HPLC chromatogram of primary herb group, most peaks appeared in the latter half of the HPLC fingerprint, with major peaks identified as 4, 9 and 10. Peaks number 4, 9, and 10 were assigned as characteristic peaks that correspond to FR02, perforatic acid and *O*-methyllaloptaeroxyrin, respectively. Formula 02A was the primary herbs, formulas 31A-37A involved changes in the primary herbs, formulas 38A-39A involved adjustments in the number of herbs for the treatment of semha and lom fever, formulas 40A-41A involved changes in the number of herbs for the treatment of di fever, formulas 42A-44A involved changes in the number of herbs for the treatment of kamado and lohit fever, and formulas 45A-51A were modified MHR remedies by fixing reduced toxic fever herbs and plus one of the other components compared to only reduced toxic fever herbs (33A). The major peaks number 4, 9 and 10, were found in the formulas containing only reduced toxic fever herbs (33A) including formulas 31A-51A, as shown in the **Figure 68**.

Formulas 61A-90A involved modifications to the number of herbs that reduced toxic fever within the primary herbs. Specifically, formulas 61A-65A removed four herbs, formulas 66A-75A removed three herbs, formulas 76A-85A removed two herbs, and formulas 86A-90A removed one herb. The major peaks numbers 9 and 10, were found in the formulas containing *H. perforata* specifically in formulas 64A, 68A, 71A, 73A, 75A, 77A, 79A, 81A, 82A, 84A, 85A, 86A, 88A, 89A, and 90A. Peak 4 was found in the formulas containing *F. racemosa* which is present in formulas 63A, 67A, 70A, 73A, 74A, 76A, 79A, 80A, 82A, 83A, 85A, 86A, 87A, 89A, and 90A. Peaks number 12 was found in the formulas containing *T. triandra* which is present in in formulas 65A, 69A, 72A, 74A, 75A, 78A, 80A, 81A, 83A, 84A, 85A, 87A, 88A, 89A, and 90A. And peaks number 13 was found in the formulas containing *C. indicum* which is present in formulas 62A, 66A, 70A, 71A, 72A, 76A, 77A, 78A, 82A, 83A, 84A, 86A, 87A, 88A, and 90A, as shown in the **Figure 69-70**.

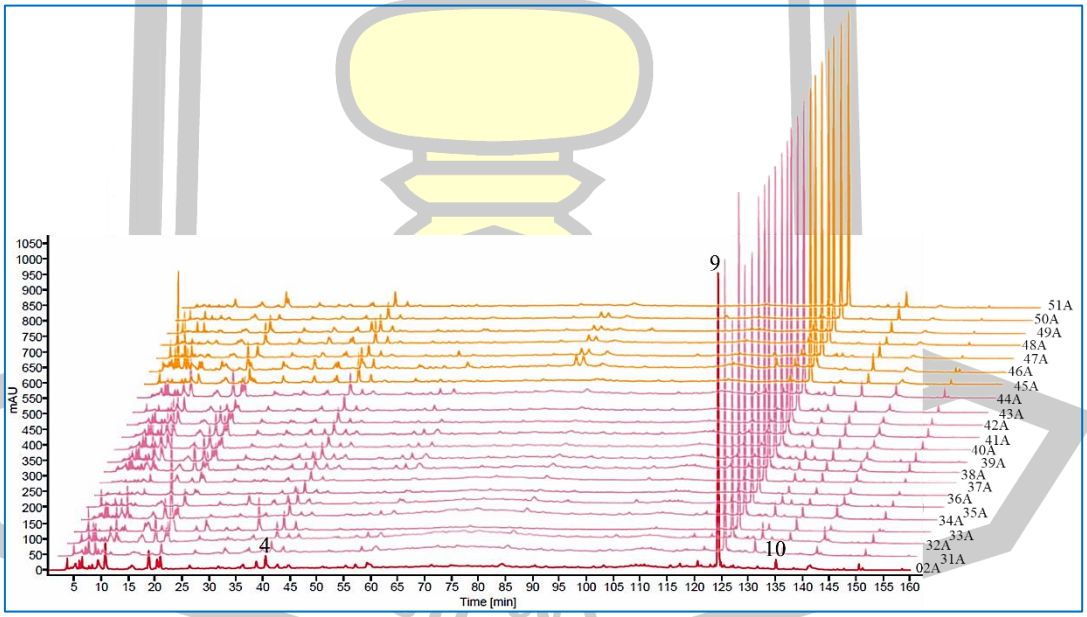


Figure 68 HPLC chromatogram of modified MHR showing only the modifications of primary herbs (31E-51A) compared to primary herbs (02A).
Major peak: (4) FR02 (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin, (12) TT02, (13) pectolinarigenin.

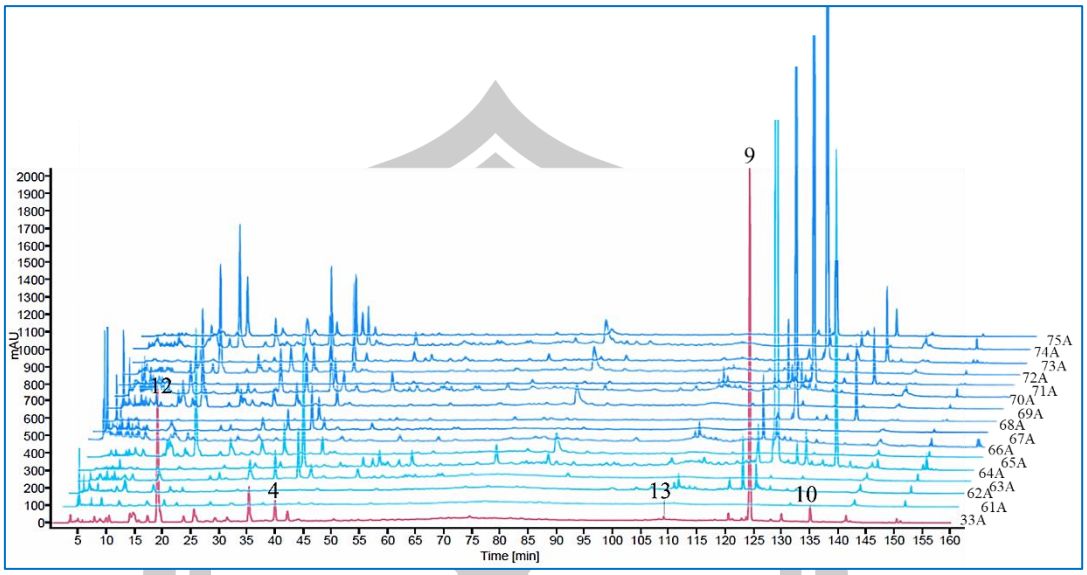


Figure 69 HPLC chromatogram of modified MHR showing reduced toxic herbs (33A) and their modified reduced toxic herbs by removing 4 herbs (61A-65A) and 3 herbs (66A-75A).
Major peak: (4) FR02 (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin, (12) TT02, (13) pectolinarigenin.

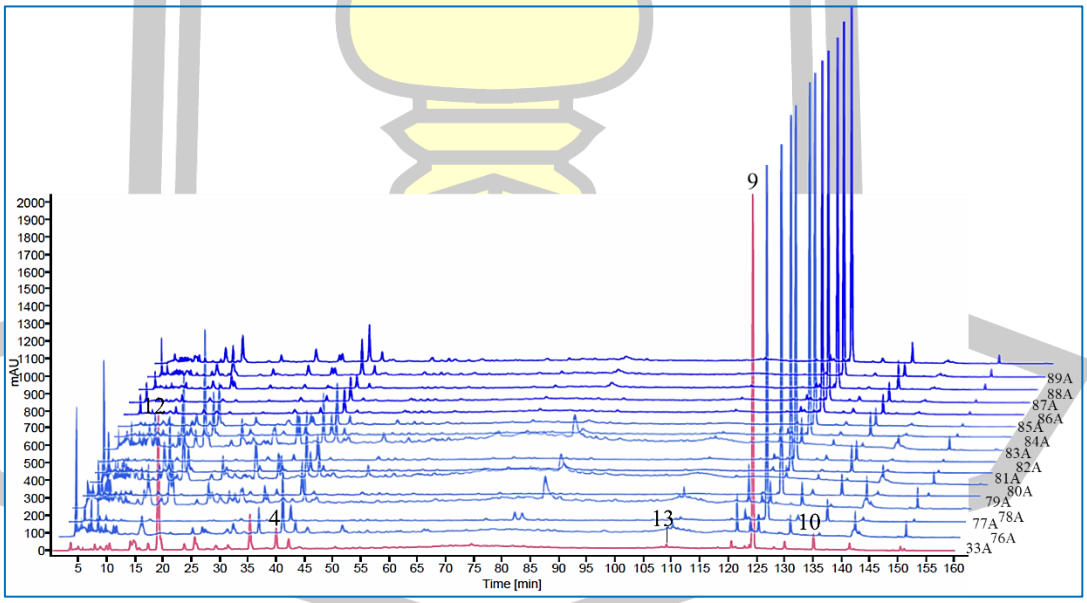


Figure 70 HPLC chromatogram of modified MHR showing reduced toxic herbs (33A) and their modified reduced toxic herbs by removing 2 herbs (76A-90A).
Major peak: (4) FR02, (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin, (12) TT02, (13) pectolinarigenin.

3) HPLC chromatogram of modified MHR specifically modification of adjunct herbs

The modification of adjunct herbs by changing the number of herbs while fixing primary herbs, led to the identification of peaks within the same group as the primary herbs, specifically peaks 4, 9 and 10. Meanwhile, most peaks in the AH appeared in the first half of the HPLC fingerprint, with major peaks identified as 1, 2, 5, 6, 7, 8, and 11. Peaks number 1, 2, 5, 6, 7, 8, and 11 were assigned as characteristic peaks that correspond to chebulic acid, gallic acid, chebulanin, corilagin, chebulagic acid, ellagic acid and rhein, respectively, as shown in **Figure 71**.

Formula 03A involve removing the supportive herbs, formula 91A contained only the adjunct herbs, while formulas 11A-14A involve changes in the number of herbs within the adjunct herbs and supportive herbs. Formulas 15A-28A were modified by removing the number of sour-astringent laxative herbs in adjunct herbs. Specifically, formulas 15A-18A removed three herbs, formulas 19A-24A removed two herbs, and formulas 25A-28A removed one herb. Formulas 29A-30A involved changes in the number of stimulant laxative herbs in adjunct herbs. Formulas 52A-57A fixed the herbs for reduced toxic fever by changing the adjunct herbs. The major peaks number 1, 2, 5, 6, 7 and 8, were found in the formulas containing *T. bellirica*, *T. chebula*, *Terminalia* sp. “Samo Thet” or *P. emblica* including formulas 03A, 91A, 13A-28A, 54A-57A. Peak number 11 was observed in the formulas containing *C. fistula* including formulas 03A, 91A, 11A, 12A, 15A-28A and 30A. Lastly, the formulas 11A and 13A showed peaks number 3, due to modification of adjunct herbs, as shown in the **Figure 71-72**.

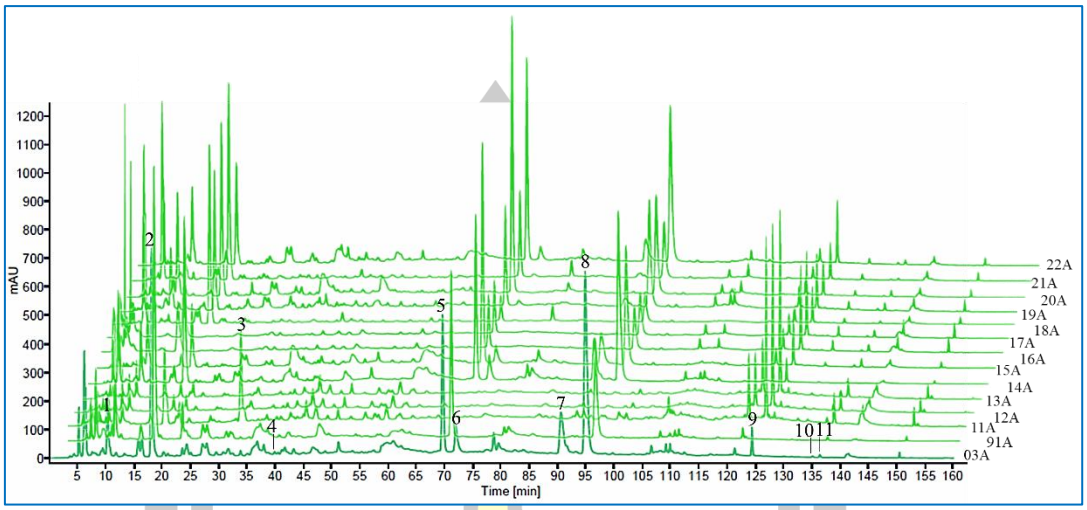


Figure 71 HPLC chromatogram of modified MHR by modification of adjunct herbs. Major peak: (1) chebulic acid, (2) gallic acid, (3) protocatechuic acid, (4) FR02, (5) chebulanin, (6) corilagin, (7) chebulagic acid, (8) ellagic acid, (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin, (11) rhein.

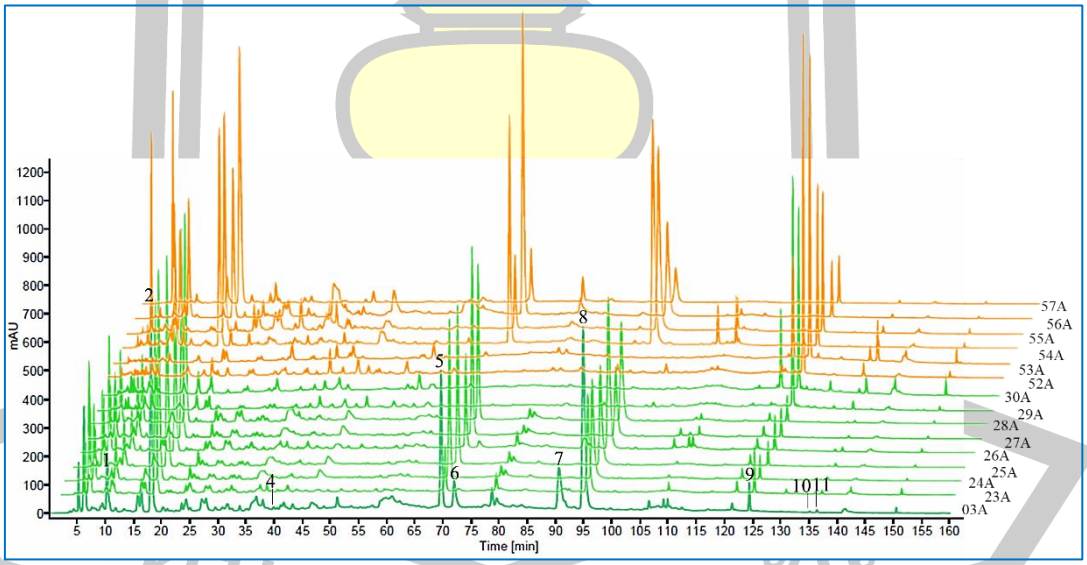


Figure 72 HPLC chromatogram of modified MHR by modification of adjunct herbs, focus on removing the number of sour-astringent laxative herbs.
Major peak: (1) chebulic acid, (2) gallic acid, (3) protocatechuic acid, (4) FR02, (5) chebulanin, (6) corilagin, (7) chebulagic acid, (8) ellagic acid, (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin, (11) rhein.

4) The supportive herbs

The modification of adjunct herbs by changing the number of herbs while fixing the primary herbs, led to the identification of peaks within the same group as the primary herbs, specifically peaks 4, 9, and 10. Moreover, major peaks number 1, 2, 5, 6, 7, 8, and 11 were the same group as the adjunct herbs, peak of supportive herbs appeared throughout the HPLC fingerprint, with strong peaks identified as 2 and 3. Peaks number 2 and 3 were assigned as characteristic peaks that correspond to gallic acid and protocatechuic acid, respectively, as shown in **Figure 73**.

Formula 10A involve removing the adjunct herbs, formula 92A contained only supportive herbs, while formulas 04A-09A involve changes in the number of herbs within the SH subgroup. Formulas 58A-60A showed the modification of supportive herbs by fixing reduced toxic fever herbs. The major peaks, numbers 2 and 3 were found in the formulas containing *M. ferrea* including formulas 10A, 92A, 04A, 07A, and 08A, as shown in **Figure 73**.

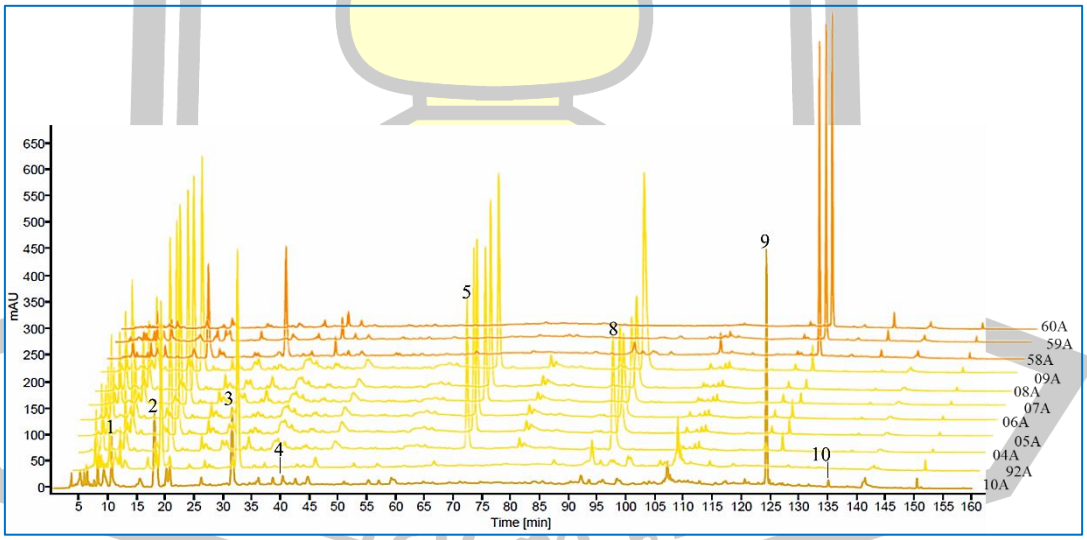


Figure 73 HPLC chromatogram of modified MHR by modification of supportive herbs.

Major peak: (1) chebulic acid, (2) gallic acid, (3) protocatechuic acid, (4) FR02, (5) chebulanin, (6) corilagin, (7) chebulagic acid, (8) ellagic acid, (9) perforatic acid, (10) *O*-methyllaloptaeroxyrin.

4.4.2.2 The quantitative analysis of major components in MHR

In the aqueous extract at 254 nm of HPLC chromatogram, a total of 11 major peaks were identified. Peaks number 1-11 were assigned as characteristic peaks corresponding to chebulic acid, gallic acid, protocatechuic acid, FR02, chebulanin, corilagin, chebulagic acid, ellagic acid, perforatic acid, *O*-methyllaloptaeroxyrin and rhein, respectively. According to the quantitative analysis of the peak areas of these components, chebulic acid (**Figure 74**) was observed in the original MHR formula and the adjunct herbs. The highest amount of chebulic acid was found in formula 56A, with a value of 16,103.79 ± 4.29 mAU, which is a formula containing reduced toxic fever herbs and *Terminalia* sp.

Gallic acid (**Figure 75**) was a major compound shown in the original MHR formula, the adjunct herbs, and the supportive herbs. The highest amount of gallic acid was found in formula 57A, with a value of 50,916.95 ± 28.41 mAU, which is a formula containing reduced toxic fever herbs and *P. emblica*.

Protocatechuic acid (**Figure 76**) was a major compound shown in the original MHR formula, and the supported primary herbs. The highest amount of protocatechuic acid was found in formula 92A, with a value of 5,160.27 ± 0.62 mAU, which is a formula for only supportive herbs.

FR02 (**Figure 77**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of FR02 was found in formula 33A, with a value of 2,059.95 ± 4.46 mAU, which is a formula for only reduced toxic fever herbs.

Chebulanin (**Figure 78**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of chebulanin was found in formula 56A, with a value of 51261.09 ± 2.69 mAU, which is a formula containing reduced toxic fever herbs and *Terminalia* sp.

Corilagin (**Figure 79**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of corilagin was found in formula 14A, with a value of 5228.76 ± 13.08 mAU, which is a formula containing primary herbs with sour-astringent laxative.

Chebulagic acid (**Figure 80**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of chebulagic acid

was found in formula 14A, with a value of 10,694.92 ± 3.57 mAU, which is a formula containing primary herbs with sour-astringent laxative.

Ellagic acid (**Figure 81**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of ellagic acid was found in formula 14A, with a value of 33,723.69 ± 22.06 mAU, which is a formula containing primary herbs with sour-astringent laxative.

Perforatic acid (**Figure 82**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of perforatic acid was found in formula 64A, with a value of 38,084.87 ± 40.62 mAU, which is a formula containing *H. perforata*.

HP02 (**Figure 83**) was a major compound shown in the original MHR formula, the primary herbs, the adjunct herbs, and the supportive herbs. The highest amount of HP02 was found in formula 64A, with a value of 13,320.46 ± 11.81 mAU, which is a formula containing *H. perforata*.

Lastly, rhein (**Figure 84**) was a major compound shown in the original MHR formula, and the adjunct herbs. The highest amount of rhein was found in formula 53A, with a value of 736.31 ± 15.95 mAU, which is a formula containing reduced toxic fever herbs and *C. fistula*.

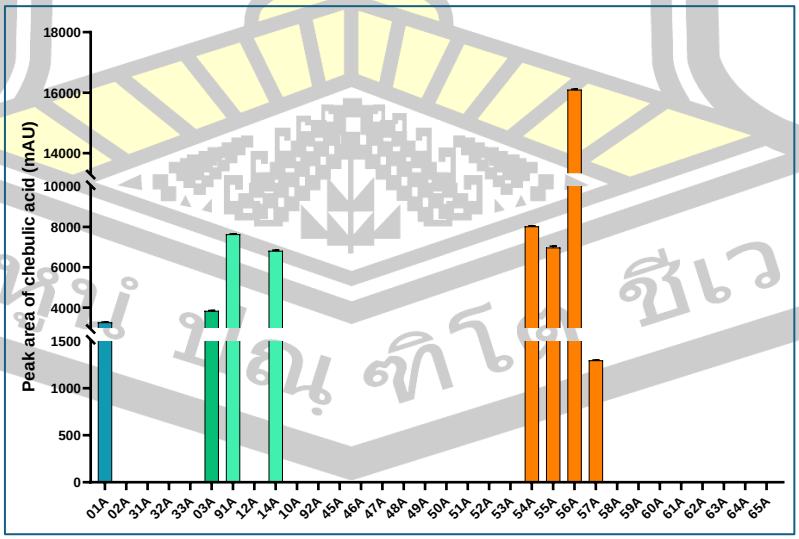


Figure 74 Peak area of chebulic acid in modified MHR remedies.

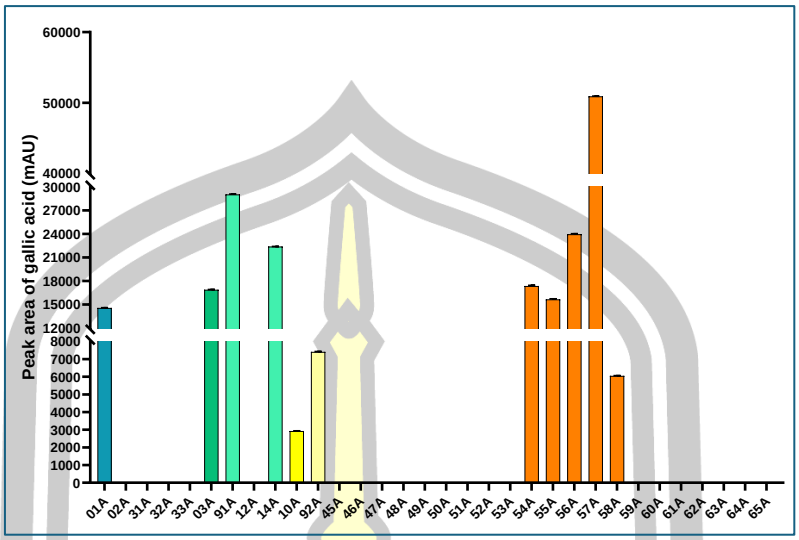


Figure 75 Peak area of gallic acid in modified MHR remedies.

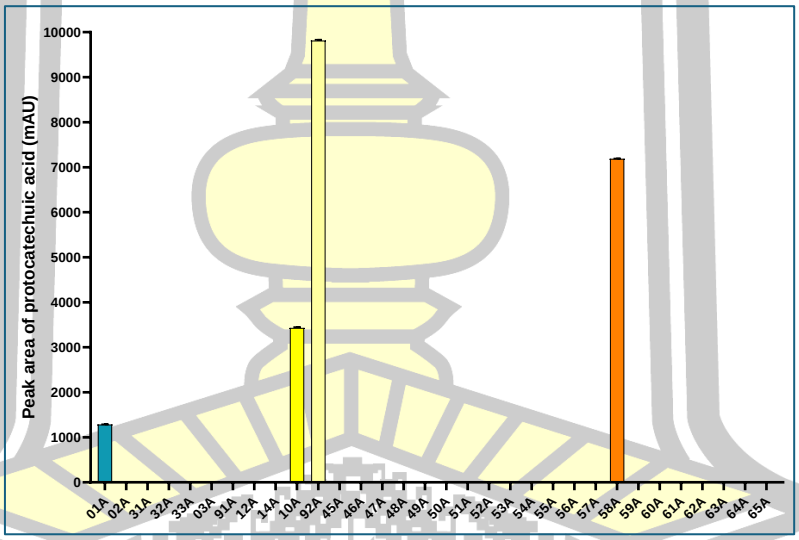


Figure 76 Peak area of protocatechuic acid in modified MHR remedies.

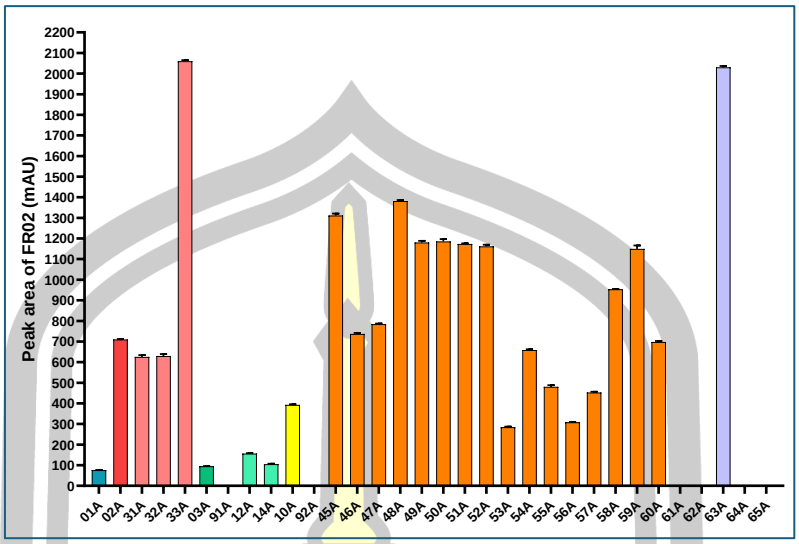


Figure 77 Peak area of FR02 in modified MHR remedies.

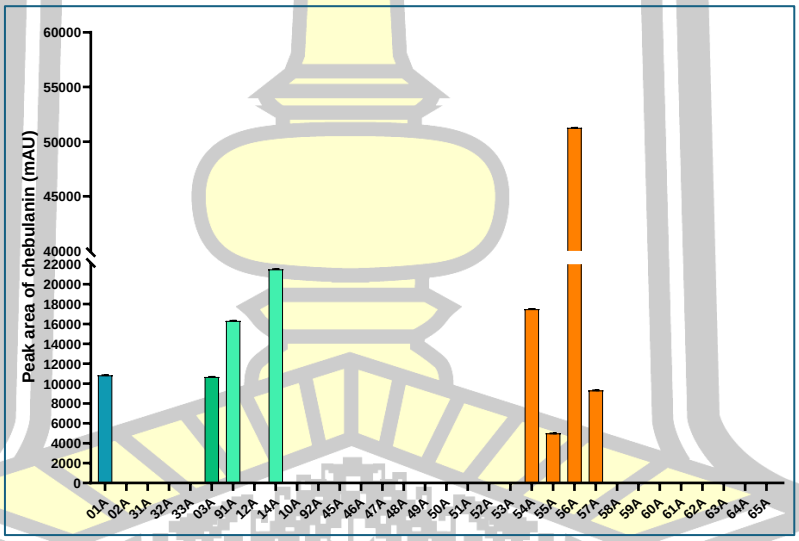


Figure 78 Peak area of chebulanin in modified MHR remedies.

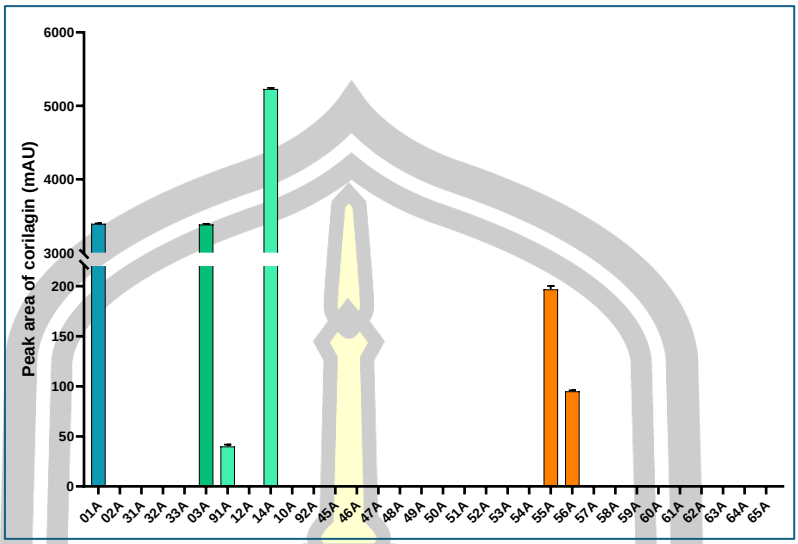


Figure 79 Peak area of corilagin in modified MHR remedies.

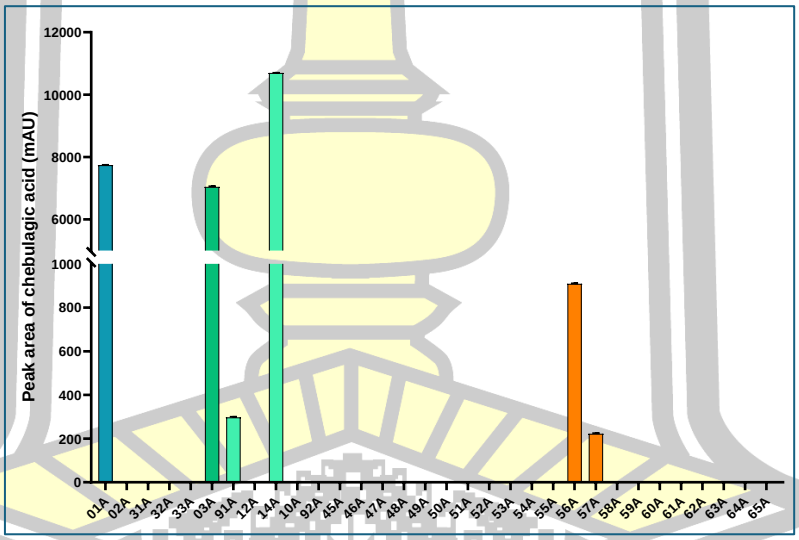


Figure 80 Peak area of chebulagic acid in modified MHR remedies.

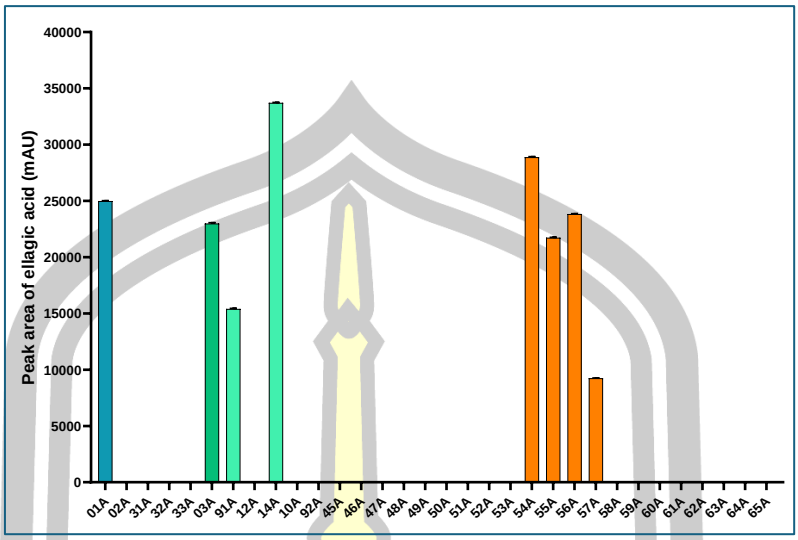


Figure 81 Peak area of ellagic acid in modified MHR remedies.

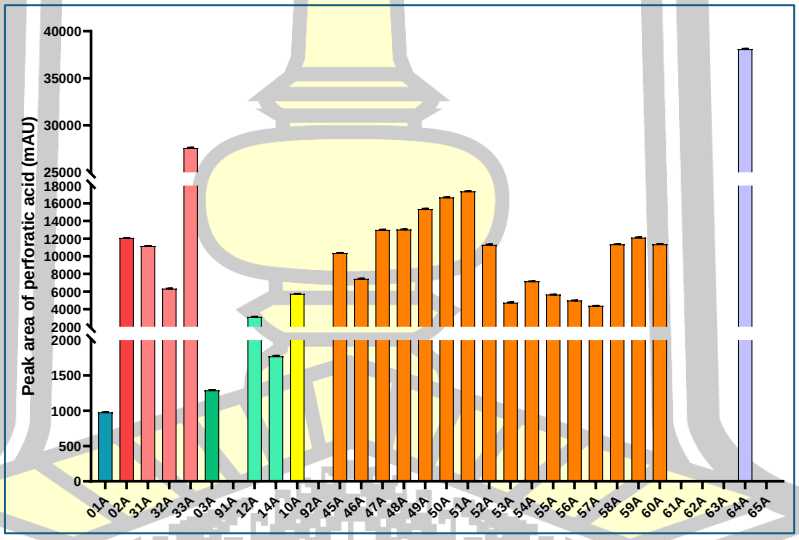


Figure 82 Peak area of perforatic acid in modified MHR remedies.

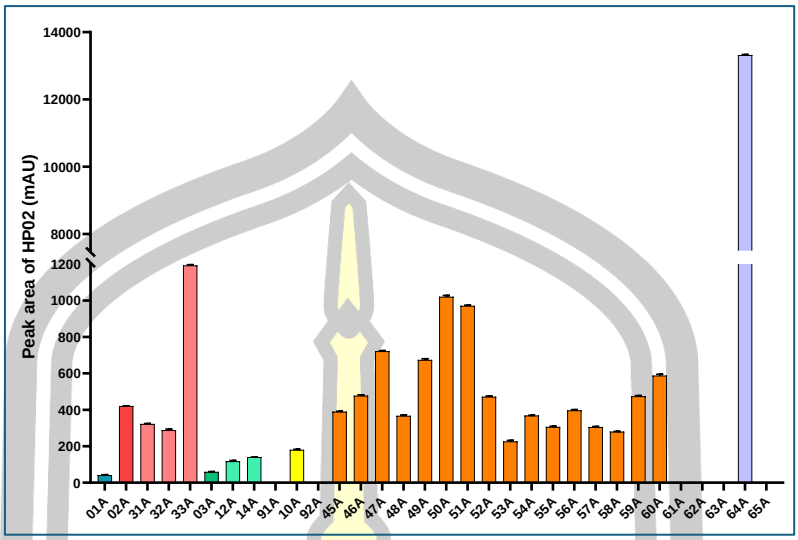


Figure 83 Peak area of *O*-methyllaloptaeroxyrin in modified MHR remedies.

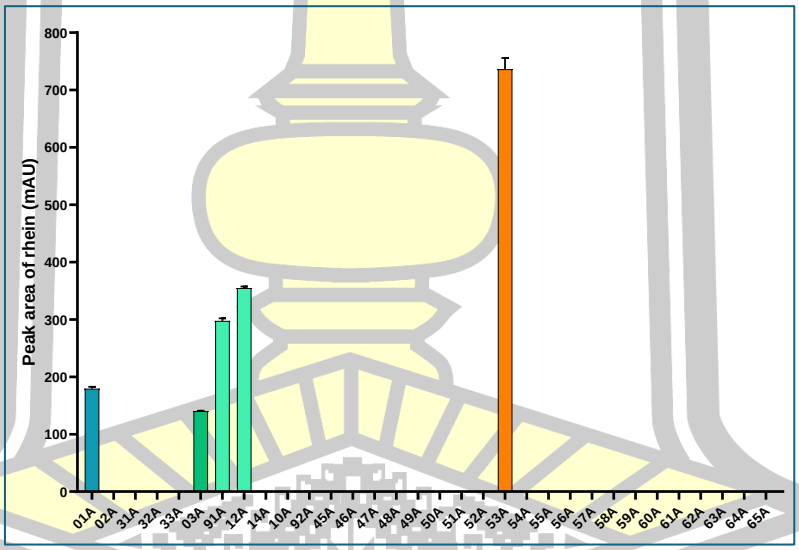


Figure 84 Peak area of rhein in modified MHR remedies.

4.5 Anti-inflammatory activity and toxicity of original MHR and modified MHR

Anti-inflammatory activity of the ethanolic and aqueous extracts of original MHR and 92 modified MHR remedies were tested by measuring their inhibitory effects on LPS-induced nitric oxide (NO) release from murine macrophages cell lines (RAW 264.7). Measurement of NO production was performed by using Griess reaction and cytotoxicity was performed by MTT assay.

4.5.1 Anti-inflammatory activity of original MHR and modified MHR of primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs

4.5.1.1 Anti-inflammatory activity and toxicity of original MHR and modified MHR ethanolic extract

In the ethanolic extract (**Figure 85A**) at a concentration of 100 µg/mL, the MHR remedy (Mo-Ha-Rak, 01E) showed anti-inflammatory activity of 31.32 ± 0.80%, which was significantly lower than that of indomethacin at a concentration of 100 µg/mL (positive control) at 56.27 ± 0.98%. The modified formulas that included only the primary herbs (PH, 02E) increased the anti-inflammatory effect (72.26 ± 0.52%) compared to MHR, and the effect was significantly higher than that of only the adjunct herbs (AH, 91E) (23.39 ± 1.49%) and only supportive herbs (SH, 92E) (20.08 ± 0.33%). Removing the supportive herbs in MHR (03E) (52.80 ± 1.50%) or the adjunct herbs in MHR (10E) (53.47 ± 0.95%) did not reduce the anti-inflammatory effect compared to MHR, as both formulas 03E and 10E showed no significant difference.

The IC_{50} values (**Figure 86A**) of formulas 02E, 03E, 10E and indomethacin were 63.52 ± 1.05, 74.75 ± 0.47, 75.95 ± 1.51 and 73.42 ± 1.51 µg/mL, respectively. In contrast, formulas 01E, 91E and 92E exhibited no measurable activity (IC_{50} > 100 µg/mL).

Toxicity studies (**Figure 85B**) showed that the MHR (01E) had a cell viability of 104.64 ± 2.43%, which was significantly lower than that of indomethacin (positive control) at 121.70 ± 1.00%. The removal of supportive herbs (03E) resulted in a cell viability of 100.31 ± 3.27%, not significantly different from MHR. However, removing the herb in the adjunct herbs (10E) led to a higher cell viability of 118.42 ± 2.55%, compared to MHR. Similarly, the formula containing only the adjunct herbs

(91E) yielded a cell viability of 93.64 ± 1.25%, while the formula with only the supportive herbs (92E) showed a viability of 101.67 ± 1.04%, both of which were not significantly different from MHR. The formula with only the primary herbs (PH) resulted in a lower cell viability (89.47 ± 2.67%) compared to MHR, though it was not significantly different from the formulas 91E and 92E. Additionally, the concentration of primary herbs above 50 µg/mL, increased cytotoxicity was observed (**Figure 86B**).

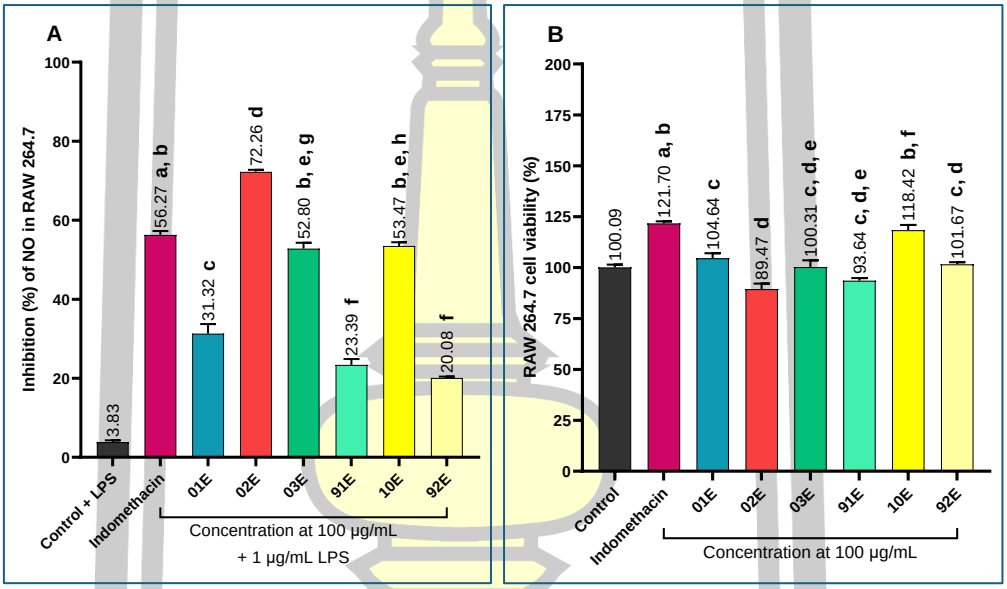


Figure 85 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01E) and modified MHR (Primary herbs (02E), without supportive herbs (03E), adjunct herbs (91E), without adjunct herbs (10E) and supportive herbs (92E)).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

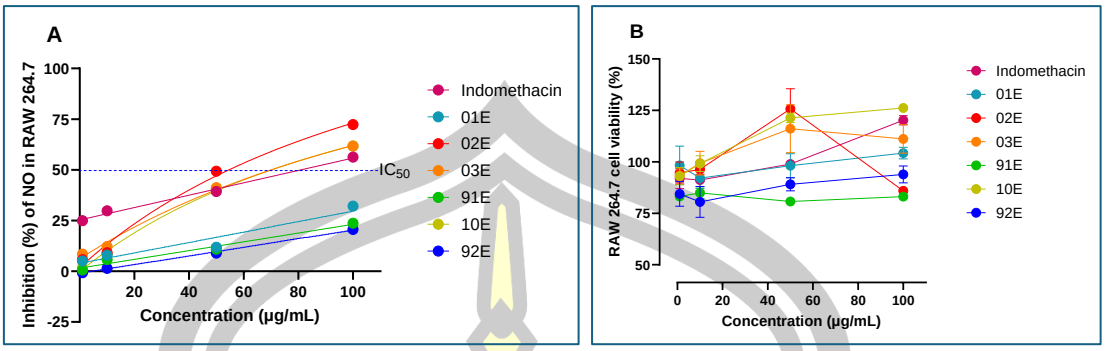


Figure 86 Comparison of nitric oxide inhibition (A) and cell viability (B) of original MHR (01E) and modified MHR (Primary herbs (02E), without supportive herbs (03E), adjunct herbs (91E), without adjunct herbs (10E) and supportive herbs (92E)). Values are expressed as mean ± SD (n=3).

Formulas 45E-60E were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of the other components of MHR compared to only reduced-toxic fever herbs (33E). Formulas 61E-65E were modified MHR remedies using only one herb of reduced-toxic fever herb. Anti-inflammatory activity studies (**Figure 87A**) revealed that formulas 45E-65E exhibited a range of anti-inflammatory effects from 6.66% to 76.90%. Among these, formulas 48E (33E plus *D. cochinchinensis*, 73.76 ± 1.60%), 63E (only *F. racemose*, 72.32 ± 0.42%), and 65E (only *T. triandra*, 76.90 ± 0.86%) demonstrated the highest activity, with no significant differences between them. These formulas also showed anti-inflammatory activity comparable to that of 33E (75.92 ± 0.49%) and significantly higher than the original MHR (31.32 ± 0.80%).

Toxicity studies (**Figure 87B**) indicated that formulas 45E-65E showed cell viability ranging from 4.23% to 122.90%. Formulas 45E-57E and 60E exhibited cell viability greater than 100%. Formulas 45E-50E, 53E-57E, and 60E showed no significant difference in cell viability compared to the original MHR and formulas 45E-57E also showed no significant differences among themselves. In contrast, formula 65E (only *T. triandra*) displayed high cytotoxicity, with a cell viability of 4.23 ± 0.80%, falling below the 70% survival threshold. However, at a concentration of 50 µg/mL of 65E (only *T. triandra*), no toxicity was observed (% cell viability =

76.45 ± 1.46), with no significant difference in its anti-inflammatory effect (73.84 ± 1.10%) compared to a concentration of 100 µg/mL.

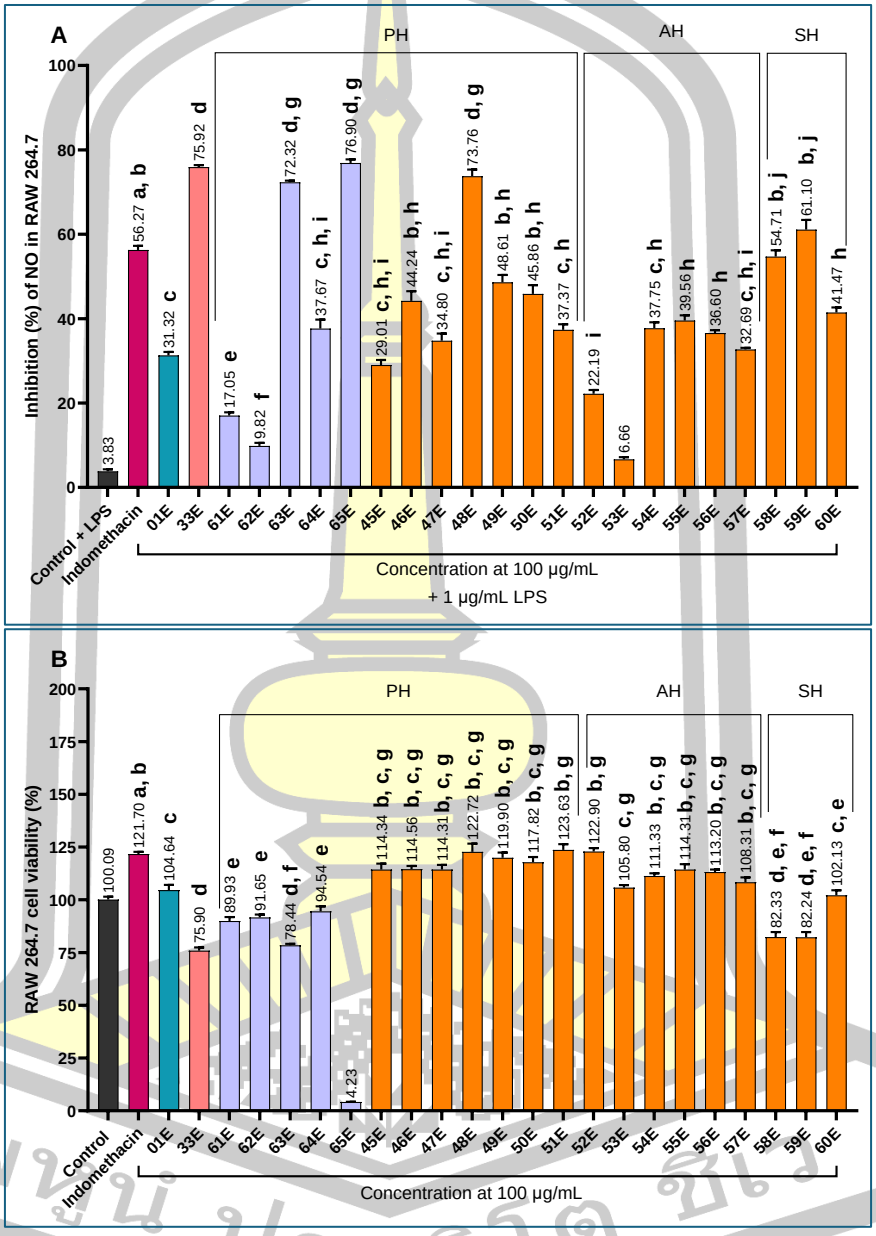


Figure 87 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01E) and modified MHR by fixing reduced-toxic fever herbs plus one of the other components (45E-60E) compared to only one of the reduced-toxic fever herbs (61E-65E) and only reduced-toxic fever herbs (33E).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.5.1.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract

In the aqueous extract at a concentration of 500 µg/mL, most formulas exhibited only mild anti-inflammatory activity. Therefore, this study selected key representative formulas from each group for further evaluation including original MHR (Mo-Ha-Rak, 01A) and modified MHR (primary herbs (02A), without supportive herbs (03A), adjunct herbs (91A), without adjunct herbs (10A) and supportive herbs (92A)). The original MHR (01A) showed an anti-inflammatory activity of 12.03 ± 0.59%, which was significantly lower than that of the positive control, indomethacin at 56.27 ± 0.98%. The formula containing only the primary herbs (PH, 02A) demonstrated a similar level of anti-inflammatory activity (8.65 ± 1.13%) with no significant difference compared to MHR. Furthermore, the anti-inflammatory effect of the formula containing only the adjunct herbs (91A) (13.72 ± 0.55%) was not significantly different from MHR. Removing the number of herbs in either the supportive herbs formula (03A) (10.94 ± 0.80%) or the adjunct herb formula (10A) (8.01 ± 1.17%) did not significantly diminish the anti-inflammatory effect compared to MHR, as both formulas 03A and 10A showed no significant differences. The formula containing only the supportive herbs (92A) showed no measurable activity, as indicated in **Figure 88A**.

Toxicity studies (**Figure 88B**) found that original MHR (MHR, 01A) had a cell viability of 89.72 ± 1.55%, significantly lower than that of positive control, indomethacin which showed 121.70 ± 1.00%. All formulas showed no significant differences compared to MHR, and no significant differences among themselves.

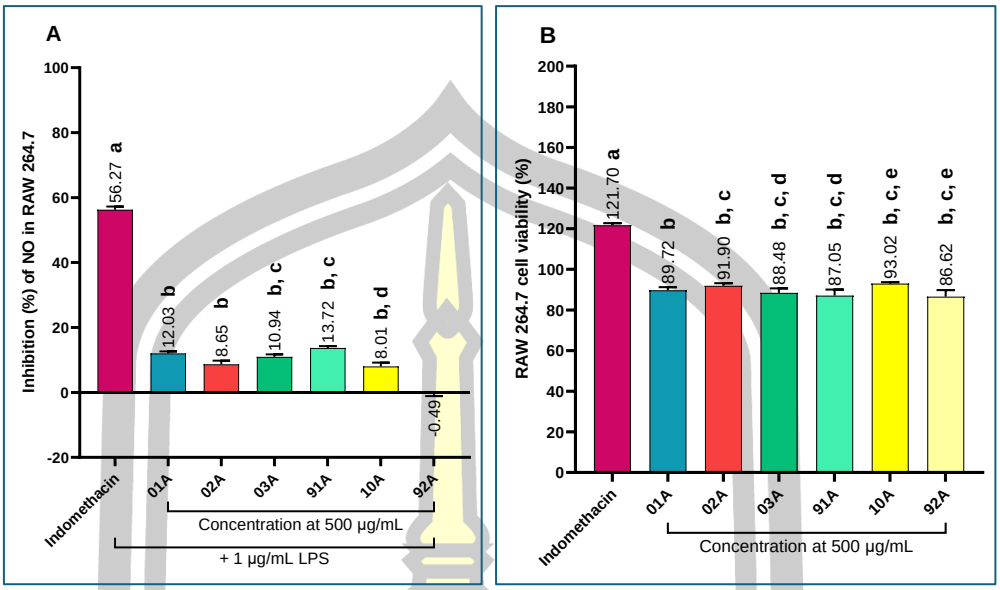


Figure 88 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01A) and modified MHR (Primary herbs (02A), without supportive herbs (03A), adjunct herbs (91A), without adjunct herbs (10A) and supportive herbs (92A)).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

Formulas 61A-65A, each consisting of single herbs from reduced-toxic fever herbs were evaluated for anti-inflammatory activity (**Figure 89A**). Results showed that these formulas exhibited anti-inflammatory effects ranging from -4.73% to 77.41%. Formula 65A (only *T. triandra*) (77.41 ± 0.48%) demonstrated the highest anti-inflammatory activity at 77.41 ± 0.48%, significantly surpassing both formula 33E (71.62 ± 0.25%) and the original MHR (12.03 ± 0.59%). It also showed significantly greater activity than 33E (71.62 ± 0.25%) and MHR (12.03 ± 0.59%). In contrast, formula 62A (only *C. indicum*) showed no measurable activity.

Toxicity studies (**Figure 89B**) found that the modified formulas 61A-65A showed cell viability ranging from 5.30% to 88.47%. Formulas 61A, 63A, and 64A showed no significant differences in cell viability compared to MHR and 33A, as well as among themselves. In contrast, formulas 62A (only *C. indicum*) and 65A (only *T. triandra*) showed cytotoxicity, with a cell viability value of 54.96± 0.59% and 4.23 ± 0.80%, respectively, both falling below the 70% survival threshold.

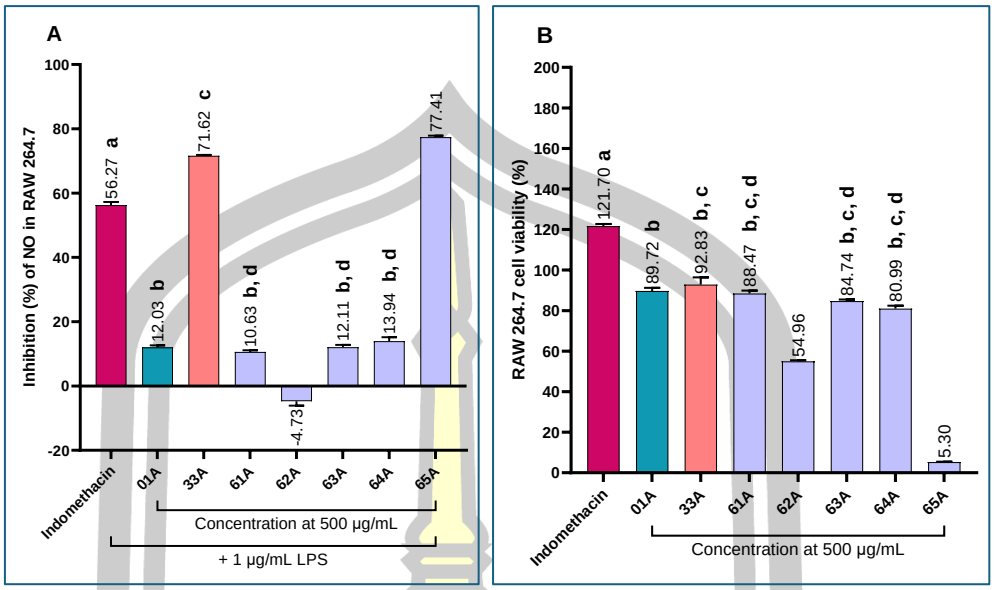


Figure 89 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01A) compared to only one of the reduced-toxic fever herbs (61A-65A) and only reduced-toxic fever herbs (33A).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.5.2 Anti-inflammatory activity and toxicity of primary herbs

Formula 02 contained only the primary herbs (PH), formulas 31-37 involved modifications of primary herbs, formulas 38-39 involved adjustments in the number of herbs for anti-semha and lom fever, formulas 40-41 involved changes in the number of herbs for anti-di fever, formulas 42-44 involved changes in the number of herbs for anti-kamado and lohit fever, and formulas 45-51 were modified MHR remedies by fixing reduced-toxic fever herbs and plus one of the other components compared to only reduced toxic fever herbs (33).

4.5.2.1 Anti-inflammatory activity and toxicity of primary herbs ethanolic extract

1) Anti-inflammatory activity and toxicity of modified MHR by primary components

This study found that (**Figure 90A**) modifying the primary herbs showed significantly different anti-inflammatory effects compared to PH. Removing anti-semha and lom fever herbs (subgroup 1.4) (formula 31E, 62.16 ± 0.99%),

removing anti-di fever herbs (subgroup 1.3) (formula 34E, 41.13 ± 2.08%), or removing anti- kamdoa and lohit fever herbs (subgroup 1.2) (formula 35E, 62.28 ± 2.28%) significantly reduced the anti-inflammatory effect compared to PH (72.26 ± 0.52%). Formulas 31E and 35E showed similar effectiveness, with no significant difference between them, while formula 34E was significantly less effective than both. Cell viability for these formulas was not significantly different from PH, and no significant differences were observed among the formulas themselves, as shown in **Figure 90B**.

Excluding herbs from two subgroups, as in formulas 32E (reduction in anti-di fever herbs, subgroup 1.3) and reduction in anti-semha and lom fever herbs (subgroup 1.4, 39.87 ± 1.52%) and 37E (reduction in anti- kamdoa and lohit fever herbs and reduction in anti-di fever herbs, 58.61 ± 2.25%), significantly reduced the anti-inflammatory effect compared to PH. In contrast, excluding the anti-kamdoa and lohit fever herbs (subgroup 1.2) and anti-semha and lom fever herbs (subgroup 1.4), (formula 36E, 75.92 ± 1.23%) did not significantly impact the anti-inflammatory effect compared to PH. Additionally, cell viability for these formulas remained similar to that of PH, with no significant differences observed among them.

Moreover, excluding herbs from all three subgroups, anti-kamdoa and lohit fever herbs (subgroup 1.2), anti-di fever herbs (subgroup 1.3) and anti-semha and lom fever herbs (subgroup 1.4) (formula 33E, 75.92 ± 0.49%) did not significantly reduce the anti-inflammatory effect compared to PH. This formula also maintained similar cell viability to PH. However, excluding all three subgroups (33E) resulted in the lowest cell viability compared to formulas where only one or two subgroups were reduced.

Removing single herb from the group of anti-semha and lom fever herbs (subgroup 1.4) as in formulas 38E (excluding *P. kesiya*) and 39E (excluding *L. sinense*), significantly reduced the anti-inflammatory effect compared to PH, with no significant differences between these two formulas. Cell viability for both formulas remained comparable to that of PH, with no significant differences observed among the formulas.

Removing a single herb from the group of anti-di fever herbs (subgroup 1.3), formula 40E (excluding *T. hoaensis*) was significantly more effective

than 31E, while formula 41E (excluding *D. cochinchinensis*) was significantly less effective than 31E. Both formulas showed similar cell viability to 31E, though formula 41E demonstrated significantly higher cell viability than 40E.

Removing a single herb from the group of anti-kamdoa and lohit fever herbs (subgroup 1.2) led to a weak anti-inflammatory effect, with inhibition below 50%. Removing one herb in this subgroup formula 42E (excluding *T. crispa*) did not significantly reduce the anti-inflammatory effect compared to formula 32E. In contrast, formulas 43E (excluding *G. chinense*) and 44E (excluding *A. indica*) significantly reduced the anti-inflammatory effect compared to 32E. These formulas also demonstrated cell viability significantly higher than that of the primary herbs (PH), with no significant differences observed among them.

Removing two herbs in the group of anti-kamdoa and lohit fever herbs (subgroup 1.2), formula 45E (excluding *G. chinense* and *T. crispa*) significantly reduced the anti-inflammatory effect compared to 32E. Meanwhile, formulas 46E (excluding *A. indica* and *T. crispa*) and 47E (excluding *A. indica* and *G. chinense*) did not show a significant reduction in anti-inflammatory effect compared to 32E. These formulas also maintained cell viability similar to that of the primary herbs (PH), with no significant differences observed among them.

The IC_{50} value (**Figure 91A**) of 02E, 31E, 33E, 35E, 36E, 37E and indomethacin were 63.52 ± 1.05, 77.84 ± 0.86, 69.10 ± 0.55, 80.39 ± 2.08, 66.85 ± 0.58, 88.42 ± 1.44 and 73.42 ± 1.51 µg/mL, respectively. In contrast, formulas 01E, 32E, and 34E showed no measurable activity, with IC_{50} values exceeding 100 µg/mL. Additionally, as shown in **Figure 91B**, the primary herbs exhibited increased cytotoxicity at higher concentrations.

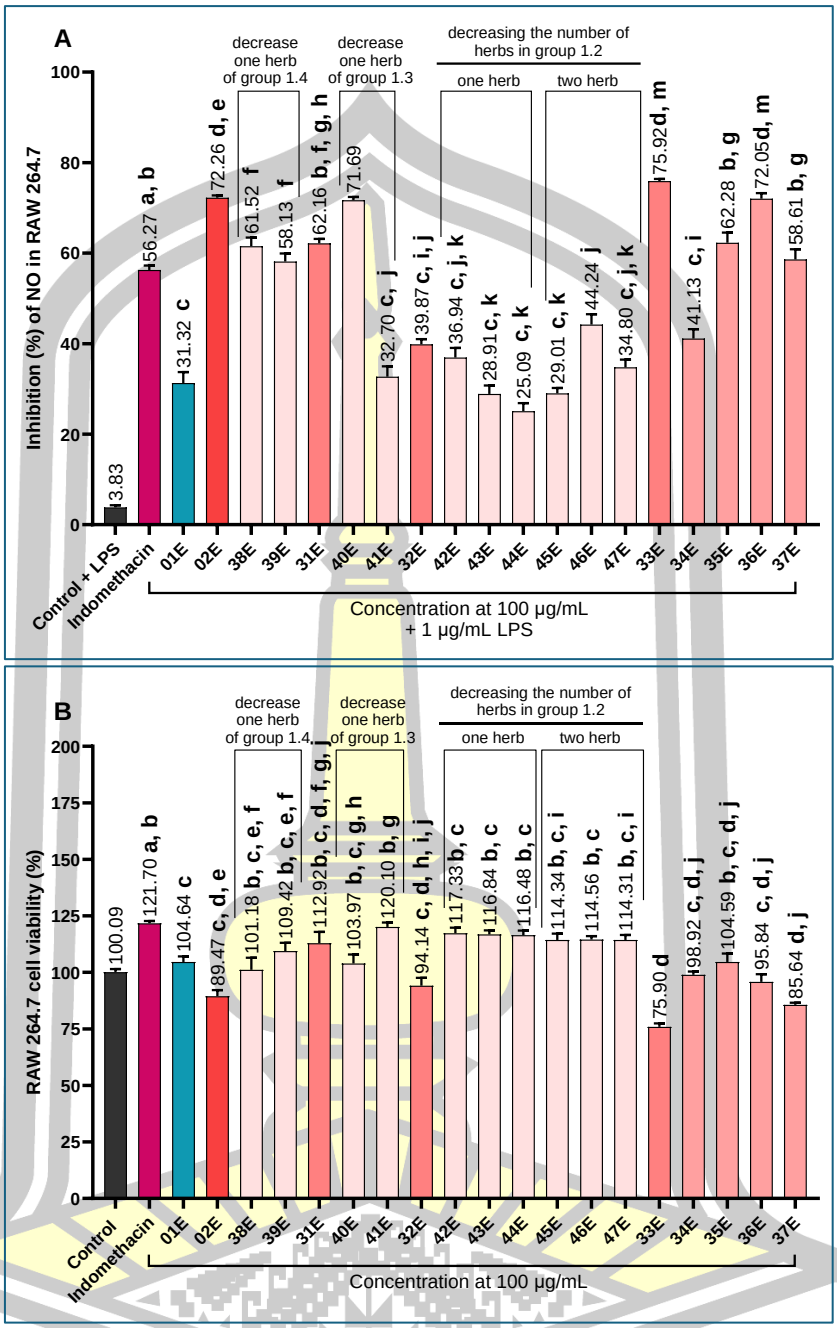


Figure 90 Anti-inflammatory activity (A) and toxicity (B) of original MHR (01E) and modified MHR (decrease one herb of group 1.4, decrease one herb of group 1.3 and decrease one and two herb of group 1.2).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

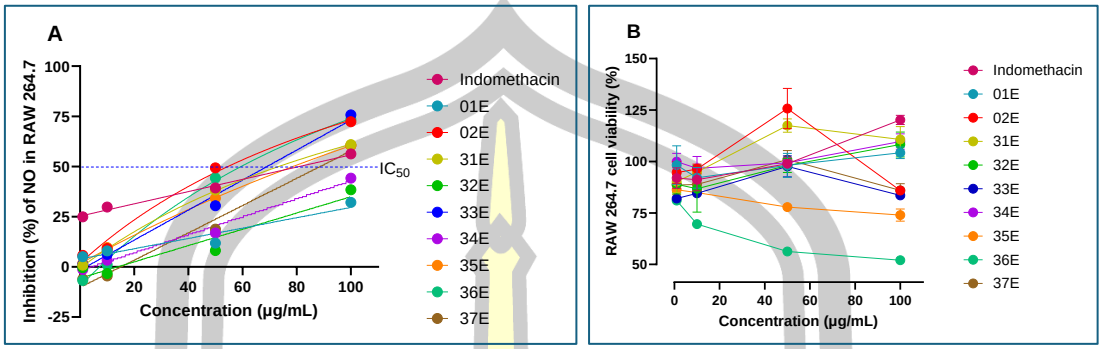


Figure 91 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in different modifying primary herbs (formulas 01E-37E) at various concentrations.
Values are expressed as mean ± SD (n=3).

2) Anti-inflammatory activity and toxicity of modified MHR by fixing reduced-toxic fever herbs and plus one of the primary components

Formulas 45E-51E were modified MHR remedies by fixing reduced-toxic fever herbs and plus one of the primary herbs compared to only reduced-toxic fever herbs (33E). Formulas 61E-65E consisted of single herbs from reduced-toxic fever herbs. The results (**Figure 92A**) revealed that formulas 45E-65E exhibited anti-inflammatory activity ranging from 9.82% to 76.90%. Among them, formulas 48E (33E combined with *D. cochinchinensis*, 73.76 ± 1.60%, 63E (*F. racemose*, 72.32 ± 0.42%), and 65E (*T. triandra*, 76.90 ± 0.86%) exhibited the highest activity, with no significant differences among them. Their activity was also comparable to that of formula 33E (75.92 ± 0.49%) and the primary herbs (PH, 72.26 ± 0.52%). In contrast, formulas 61E (*C. micracantha*) and 62E (*C. indicum*) showed only a weak anti-inflammatory effect.

Toxicity studies (**Figure 92B**) found that formulas 45E-51E and 61E-62E had cell viability ranging from 4.23% to 123.63%. Formulas 45E-51E exhibited cell viability greater than 100%, with no significant differences among them, and showed significantly greater viability compared to 33E and PH. However, formula 65E (only *T. triandra*) showed high cytotoxicity, with cell viability of 4.23 ± 0.80%, falling below the 70% survival threshold.

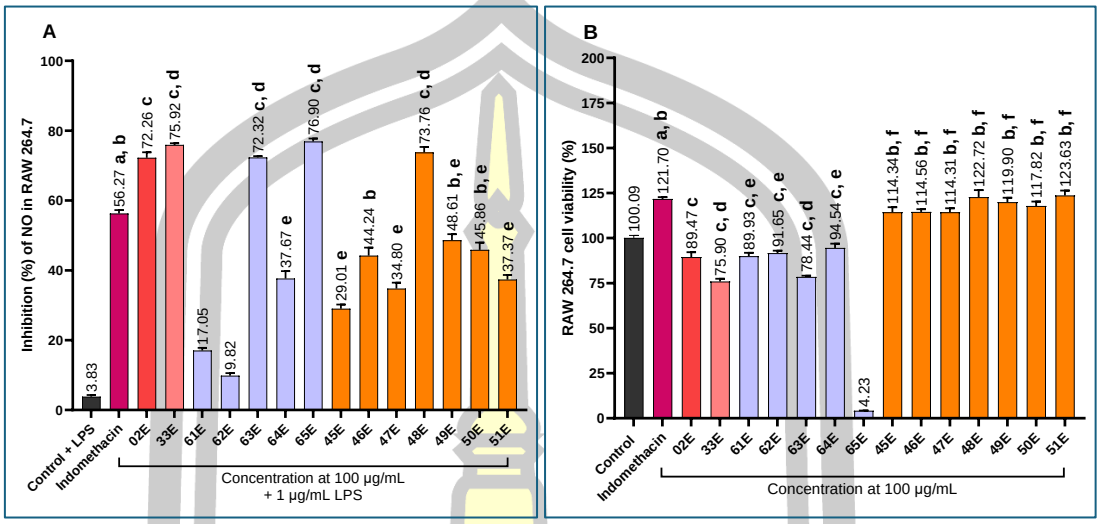


Figure 92 Anti-inflammatory activity (A) and toxicity (B) of modified MHR by fixing reduced-toxic fever herbs and plus one of the primary components. Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

3) Anti-inflammatory activity and toxicity of modified MHR by modification of reduced-toxic fever herbs

Formulas 61E-90E (**Figure 93A**) were developed by varying the number of herbs in the reduced-toxic fever herb subgroup compared to formula 33E. Removing a single herb, formulas 87E (excluding *H. perforata*, 75.20 ± 0.75%, 89E (excluding *C. indicum,* 74.01 ± 0.34% and 90E (excluding *C. micracantha*, 77.19 ± 0.33%), did not significantly reduce the anti-inflammatory effect compared to 33E (75.92 ± 0.49%), with no significant differences among them. Meanwhile, formulas 86E (excluding *T. triandra*, 37.99 ± 2.12%) and 88E (excluding *F. racemose*, 54.92 ± 1.34%) showed a significant reduction in the anti-inflammatory effect compared to 33E. Formulas 86E, 88E and 90E exhibited significantly higher cell viability than 33E, with no significant differences among them. Meanwhile, formulas 87E and 89E showed cell viability similar to 33E, with no significant differences among the formulas (**Figure 93B**).

With the removal of two herbs, only formula 80E (excluding *C. indicum* and *H. perforate*, 72.61 ± 0.77%) retained an anti-inflammatory effect comparable to 33E, with no significant differences between 80E, 83E (excluding *C. micracantha* and *H. perforata*, 70.01 ± 0.64%), and 85E (excluding *C. micracantha* and *C. indicum*, 67.60 ± 1.06%). Formulas 76E (excluding *H. perforata* and *T. triandra*), 78E (excluding *F. racemosa* and *H. perforata*), 79E (excluding *C. indicum* and *T. triandra*), 81E (excluding *C. indicum* and *F. racemosa*) and 84E (excluding *C. micracantha* and *F. racemosa*) showed moderate effects with no significant differences among them. Meanwhile, formulas 77E (excluding *F. racemosa* and *T. triandra*) and 82E (excluding *C. micracantha* and *T. triandra*) showed weaker effects, with no significant differences between the two. In terms of cell viability, formulas 76E and 77E showed significantly higher viability than 33E, while formulas 78E, 79E, 81E, 82E, 84E, and 85E were comparable to 33E. Additionally, Formulas 80E and 83E, however, had significantly lower cell viability than 33E, and formulas 80E, 83E, and 85E demonstrated cytotoxicity.

With the removal of three herbs, formulas 67E (excluding *C. indicum, H. perforata* and *T. triandra*, 70.58 ± 1.00%), 69E (excluding *C. indicum, F. racemosa* and *H. perforate*, 73.80 ± 0.86%), 74E (excluding *C. micracantha C. indicum* and *H. perforate,* 76.01 ± 0.16%) and 75E (excluding *C. micracantha C. indicum* and *F. racemose,* 72.62 ± 0.38%) retained an anti-inflammatory effect comparable to 33E, with no significant differences observed. Formula 72E (excluding *C. micracantha, F. racemosa* and *H. perforate*, 69.12 ± 0.56%) also showed similar efficacy. Formulas 70E (excluding *C. micracantha, H. perforate* and *T. triandra*) and 73E (excluding *C. micracantha C. indicum* and *T. triandra*) showed moderate effects, with no significant differences among them. Meanwhile, formulas 66E (excluding *F. racemosa, H. perforata and T. triandra*), 68E (excluding *C. indicum, F. racemosa* and *T. triandra*) and 71E (excluding *C. micracantha, F. racemosa* and *T. triandra*) exhibited weaker effects, with no significant differences among them. Most formulas did not show a significant reduction in cell viability compared to 33E. However, formulas 71E and 74E had significantly lower cell viability than 33E, with only formula 74E exhibiting cytotoxicity upon the exclusion of three herbs.

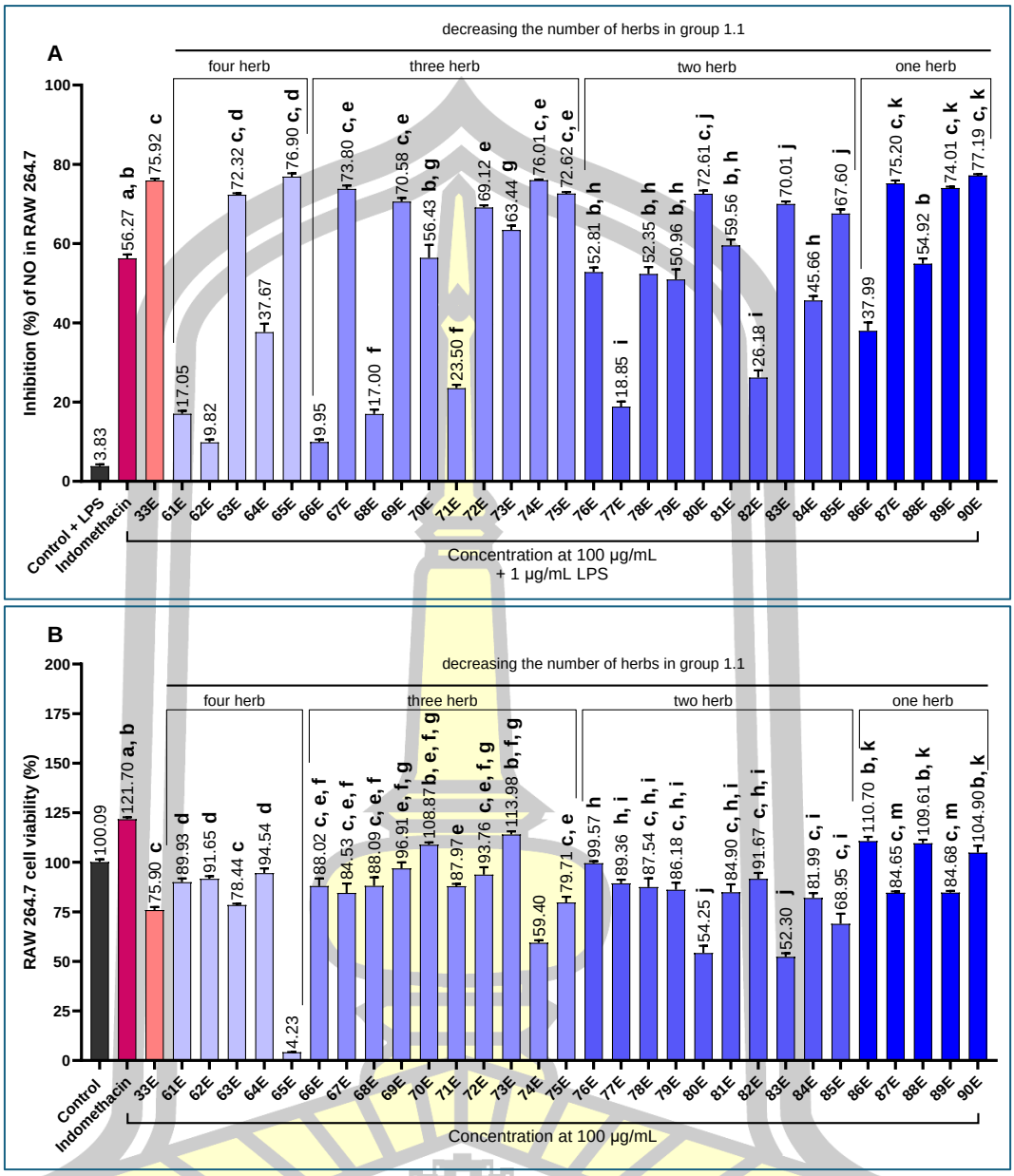


Figure 93 Anti-inflammatory activity (A) and toxicity (B) of modified MHR by decreasing the number of herbs in reduced-toxic fever herbs (subgroup 1.1). Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

With the removal of four herbs or inclusion of a single herb, formulas 63E (*F. racemose*, 72.32 ± 0.42%) and 65E (*T. triandra*, 76.90 ± 0.86%) exhibited the highest anti-inflammatory activity, with no significant differences between them and comparable efficacy to 33E. In contrast, formulas 61E (*C.*

micracantha) and 62E (*C. indicum*) exhibited weak anti-inflammatory effect. Among these, only formula 65E (*T. triandra*) displayed notable cytotoxicity, with cell viability of 4.23 ± 0.80%, falling below the survival threshold of 70%.

4.5.2.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract by modification of herbs in primary herbs

In the aqueous extract found that (**Figure 94A**) modifications to the primary herbs did not diminish the anti-inflammatory effect compared to MHR and PH. Specifically, excluding herbs from subgroup 1.4 (anti-semha and lom fever herbs, 31A) (29.82 ± 1.36%) and subgroup 1.3 (anti-di fever herbs, 34A) (31.48 ± 0.73%) showed no significant differences between them, both of which displayed more effective anti-inflammatory activity than 35A (12.12 ± 0.50). When herbs in two subgroups were excluded, formula 36A (removing subgroups 1.2 and 1.4) achieved an anti-inflammatory effect of 53.57 ± 2.19% and formula 37A (removing subgroups 1.2 and 1.3) achieved an anti-inflammatory effect of 63.97 ± 2.96%. These two formulas did not significantly differ in activity, though both were more effective than 32A (removing subgroups 1.3 and 1.4), which had an activity of 31.09 ± 0.72%. Finally, herbs from all three subgroups 1.2, 1.3, and 1.4 (formula 33A) exhibited a high anti-inflammatory effect of 75.92 ± 0.49%, which did not significantly reduce the anti-inflammatory effect compared to PH.

Cytotoxicity studies (**Figure 94B**) showed that formulas 31A-37A had cell viability ranging from 87.00% to 93.44%, indicating no toxicity. Modifying the number of herbs in the primary group across these formulas did not significantly impact cell viability compared to PH and MHR.

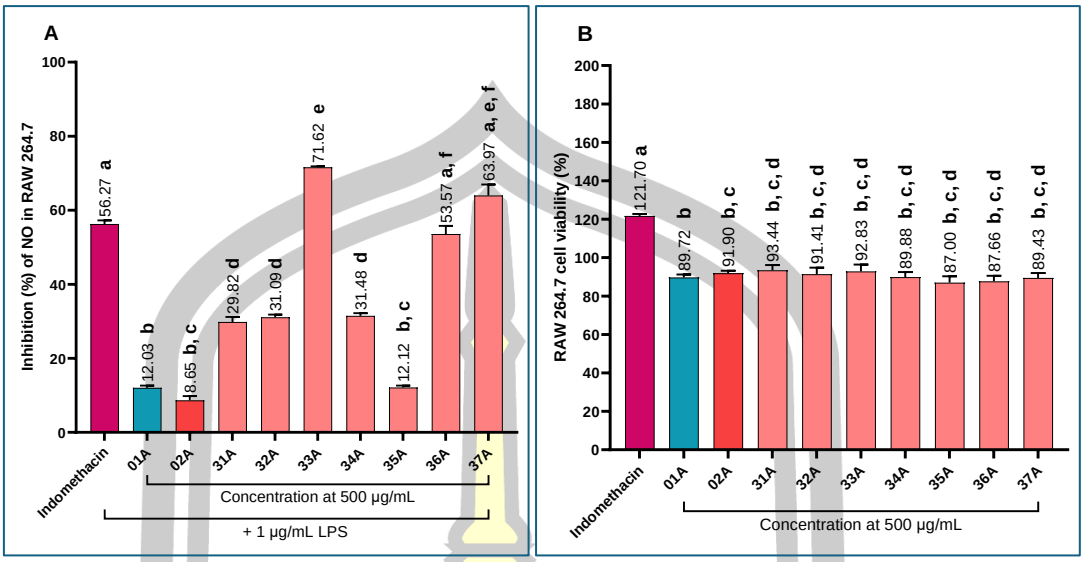


Figure 94 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR aqueous extract by modification of herbs in primary herbs. Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

Formulas 61A-65A consisting of a single herb from the subgroup of reduced-toxic fever herbs were evaluated for anti-inflammatory activity (**Figure 95A**). These formulas showed a range of anti-inflammatory effects, from -4.73% to 77.41%. Notably, formula 65A (*T. triandra*) displayed the highest activity (77.41 ± 0.48%), significantly outperforming both formula 33A (71.62 ± 0.25%) and MHR (12.03 ± 0.59%). In contrast, formula 62A (*C. indicum*) showed no detectable anti-inflammatory activity.

Toxicity studies (**Figure 95B**) revealed that cell viability for formulas 61A-65A ranged from 5.30% to 88.47%. Formulas 61A, 63A, and 64A demonstrated cell viability comparable to MHR and 33A, with no significant differences observed among them. In contrast, formulas 62A (*C. indicum*) and 65A (*T. triandra*) exhibited cytotoxicity, with cell viability values of 54.96± 0.59% and 4.23 ± 0.80%, respectively, which is lower than the 70% survival threshold.

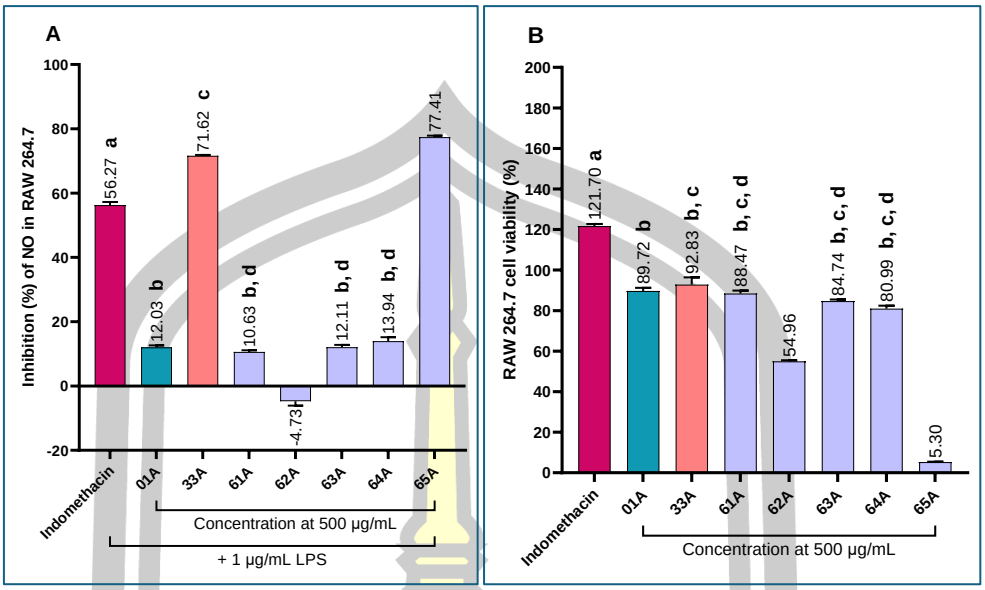


Figure 95 Anti-inflammatory activity (A) and toxicity (B) of original MHR compared to subgroup 1.1 (reduced-toxic fever herbs) and their single herbs.
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.5.3 Anti-inflammatory activity and toxicity of adjunct herbs

4.5.3.1 Anti-inflammatory activity and toxicity of adjunct herbs ethanolic extract

In the adjunct herbs (AH), modifications were made to the number of herbs while fixing the primary herbs. Formula 03E involved removing the supportive herbs, formula 91E contained only the adjunct herbs, while formulas 11E-14E introduced variations in the number of herbs within the AH subgroup. Formulas 15E-28E focused on modifying the number of herbs in subgroup 2.2 (sour-astringent laxative) within the AH. Specifically, formulas 15E-18E reduced three herbs, formulas 19E-24E reduced two herbs, and formulas 25E-28E reduced one herb. Formulas 29E-30E involved changes in the number of herbs for the subgroup 2.1 (stimulant laxative) within the AH. Additionally, formulas 52E-57E were modified by fixing reduced-toxic fever herbs and plus one of adjunct herbs.

1) Anti-inflammatory activity and toxicity of modified MHR by removing the number of adjunct herbs

In the ethanolic extract (**Figure 96A**) at a concentration of 100 µg/mL, removing the number of herbs in the supportive herbs (formula 03E) achieved an anti-inflammatory effect of 52.80 ± 1.50%), which was not significantly different from MHR (01E) at 31.32 ± 0.80%. In contrast, formula 03E showed a significantly reduced anti-inflammatory effect compared to the primary herbs alone (PH, 02E), which reached 72.26 ± 0.52%. Furthermore, the formula containing only the adjunct herbs (91E) demonstrated a significantly lower anti-inflammatory effect (23.39 ± 1.49%) compared to MHR, PH, and 03E.

The IC_{50} value (**Figure 97A**) of 03E and indomethacin were 74.75 ± 0.47 and 73.42 ± 1.51 µg/mL, respectively, indicating comparable effectiveness. In contrast, formulas 01E, 91E, 12E, and 14E showed no measurable activity, with IC_{50} values exceeding 100 µg/mL.

Toxicity studies (**Figure 96B**) found that formulas 03E, 91E, 11E-28E and 52E-57E had cell viability values ranging from 92.73% to 122.90%, indicating no toxicity. Removing herbs in the supportive herbs (03E) resulted in a cell viability of 100.31 ± 3.27%, which was not significantly different from MHR (104.64 ± 2.43%). Similarly, the formula containing only the adjunct herbs (91E) achieved a cell viability of 93.64 ± 1.25%, comparable to that the primary herbs alone (02E) at 89.47 ± 2.67%. Moreover, formulas 52E-57E showed significantly greater cell viability compared to 33E. However, **Figure 97B** also showed that most primary herbs led to decreased cell viability at higher concentrations.

Formula 11E (8.24 ± 1.17%), which excluded herbs from subgroup 2.2 (sour-astringent laxative) while retaining the supportive herbs, demonstrated a significantly reduced anti-inflammatory effect compared to MHR, though cell viability remained comparable to MHR. In contrast, formula 13E (33.27 ± 1.34%), which excluded herbs from subgroup 2.1 (stimulant laxative) while maintaining the supportive herbs, showed no significant difference in anti-inflammatory effect and cell viability when compared to MHR.

Excluding herbs in subgroup 2.2 (formula 12E) resulted in an anti-inflammatory effect of 46.08 ± 1.21%, which was not significantly different from that

of formula 03E. In contrast, excluding herbs from subgroup 2.1 (14E) produced a significantly lower anti-inflammatory effect (41.53 ± 0.98%) compared to 03E, though cell viability was not significantly affected relative to 03E.

Removing a single herb from subgroup 2.1, formulas 29E (excluding *C. fistula*, 61.53 ± 0.99%) and 30E (excluding *B. ovata*, 59.77 ± 1.70%, neither of which showed a significant reduction in anti-inflammatory effect compared to 03E. There were also no significant differences in anti-inflammatory activity between 29E and 30E. Similarly, both formulas exhibited cell viability comparable to 03E, with no significant differences between them in this regard.

Removing one herb from subgroup 2.2, formulas 25E (excluding *P. emblica,* 35.36 ± 1.82%, 26E (excluding *Terminalia* sp.,19.68 ± 0.87%, 27E (excluding *T. chebula*, 30.27 ± 0.87%) and 28E (excluding *T. bellirica*, 21.29 ± 1.57%) showed a significant reduction in the anti-inflammatory effect compared to 03E. They also showed no significantly different cell viability from 03E.

Removing two herbs in subgroup 2.2, led to significant reductions in anti-inflammatory effects for formulas 19E (excluding *Terminalia* sp. “Samo Thet” and *P. emblica*, 24.63 ± 0.88%, 20E (excluding *T. chebula* and *P. emblica*, 25.73 ± 1.23%, 21E (excluding *T. chebula* and *Terminalia* sp., 32.28 ± 1.47%), 22E (excluding *T. bellirica* and *P. emblica*, 38.34 ± 1.56%, 23E (excluding *T. bellirica* and *Terminalia* sp., 26.81 ± 1.37%) and 24E (excluding *T. bellirica* and *T. chebula*, 39.22 ± 1.29% compared to 03E. They also showed no significantly different cell viability from 03E.

Removing three herbs in subgroup 2.2, resulted in significant reductions in anti-inflammatory effects for formula 15E (excluding *T. chebula, Terminalia* sp. “Samo Thet” and *P. emblica*, 44.74 ± 1.14%, 16E (excluding *T. bellirica, Terminalia* sp. “Samo Thet” and *P. emblica*, 30.04 ± 0.74%, 17E (excluding *T. bellirica, T. chebula and P. emblica*, 26.90 ± 1.17%, and 18E (excluding *T. bellirica, T. chebula* and *Terminalia* sp., 20.24 ± 0.70% compared to 03E. They also showed no significantly different cell viability from 03E.

2) Anti-inflammatory activity and toxicity of modified MHR by fixing reduced-toxic fever herbs and varying of adjunct herbs

In the ethanolic extract, formulas 52E-57E were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) and varying of adjunct herbs compared to only reduced-toxic fever herbs (33E). These formulas exhibited a significant reduction in anti-inflammatory effects compared to 33E. Specifically, formulas 54E (33E combined with *T. bellirica*), 55E (33E combined with *T. chebula*), 56E (33E combined with *Terminalia* sp.) and 57E (33E combined with *P. emblica*) showed moderate anti-inflammatory effects, with no significant differences among them. Meanwhile, formulas 52E (33E combined with *B. ovata*) and 53E (33E combined with *C. fistula*) had a weak anti-inflammatory effect, as shown in **Figure 96A**. Toxicity studies found that formulas 52E-57E showed cell viability ranging from 92.73% to 122.90%, indicating no toxicity and no significant reduction in cell viability was observed compared to 33E, as shown in **Figure 96B**.

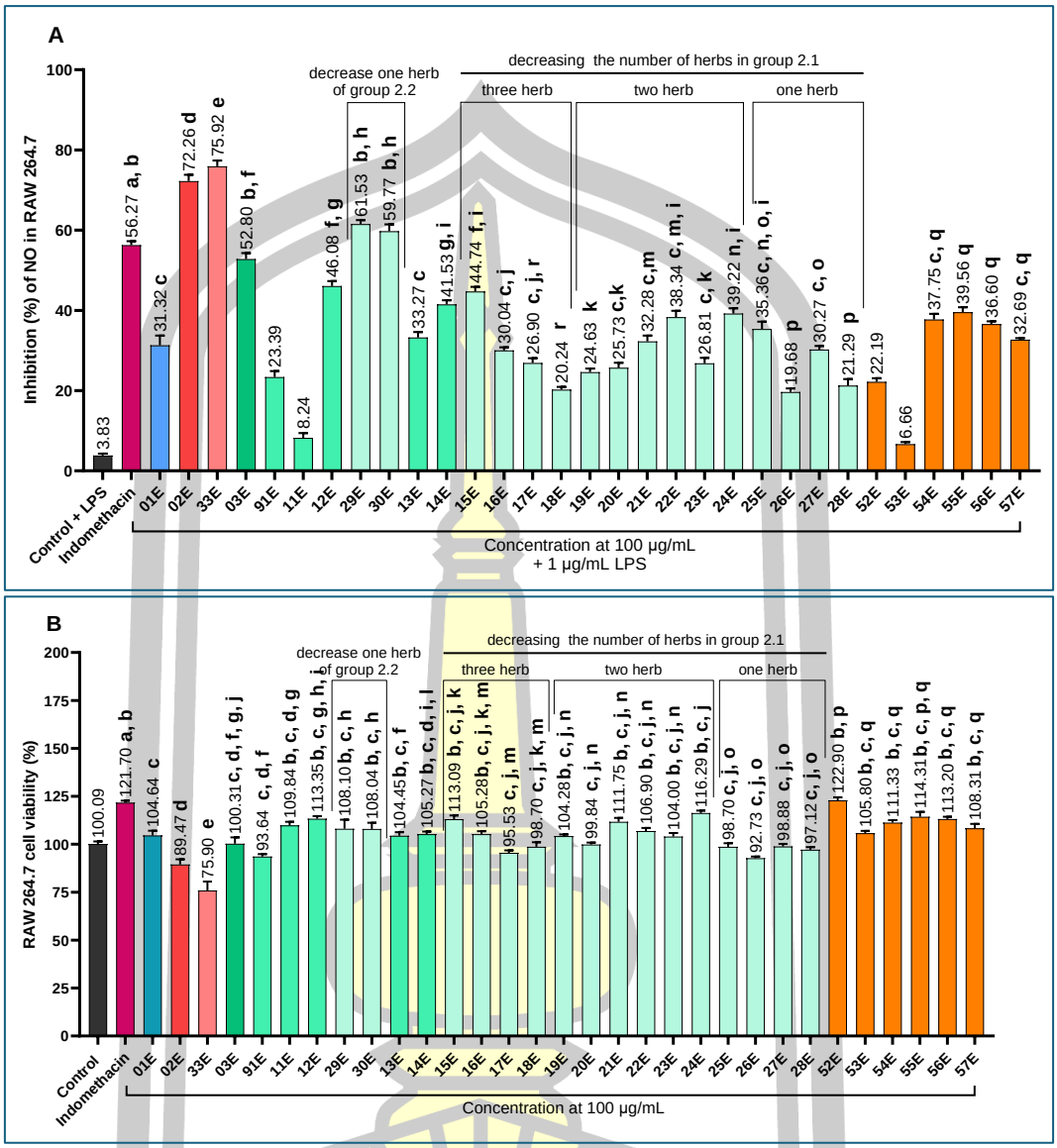


Figure 96 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR by modification of adjunct herbs.
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

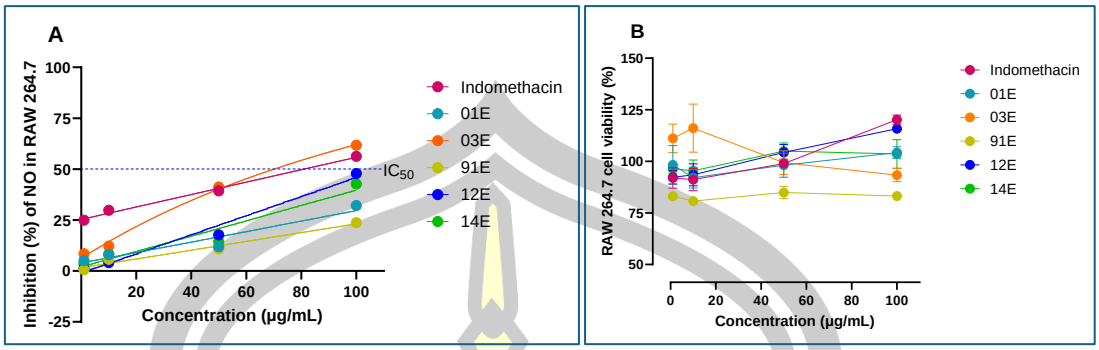


Figure 97 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in the different modifying adjunct herbs at various concentrations. Values are expressed as mean ± SD (n=3).

4.5.3.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract by modification of adjunct herbs

In the aqueous extract (**Figure 98A**) at a concentration of 500 µg/mL, most formulas exhibited weak anti-inflammatory activity. The reduction of herbs in the supportive herbs (formula 03A) resulted in an anti-inflammatory effect of 10.94 ± 0.80%, which was not significantly different from that of MHR (01A) at 12.03 ± 0.59%. In contrast, formula 03A showed a significant reduction in anti-inflammatory effect compared to the primary herbs alone (PH, 02A), which had an effect of 8.65 ± 1.13%. Additionally, the formula containing only the adjunct herbs (91A) exhibited a significantly lower anti-inflammatory effect (13.72 ± 0.55%) compared to MHR, PH, and 03A. Excluding herbs in subgroup 2.2 (12A) led to a significant reduction in anti-inflammatory activity (5.73 ± 0.38%) compared to 03A. In contrast, excluding herbs in subgroup 2.1 (14A) resulted in an anti-inflammatory effect of 22.26 ± 1.11%, which was not significantly different from that of 03A.

Toxicity studies (**Figure 98B**) found that formulas 03A, 91A, 12A and 14A showed cell viability ranging from 87.05% to 104.77%, indicating no toxicity. Removing herbs in the supportive herbs (03A) resulted in cell viability of 88.48 ± 2.14%, which was not significantly different from that of MHR (89.72 ± 1.55%). Similarly, the formula containing only the adjunct herbs (91A) exhibited a cell viability of 87.05 ± 3.01%, showing no significant difference compared to the primary herbs alone (02A), which had a viability of 91.90 ± 1.31%. Excluding the herbs in

subgroup 2.2 (12A) resulted in a cell viability of 97.14 ± 0.64%, which was not significantly different from 03A. Meanwhile, excluding the herbs in subgroup 2.1 (14A) yielded a significantly higher cell viability of 104.77 ± 1.72% compared to 03A.

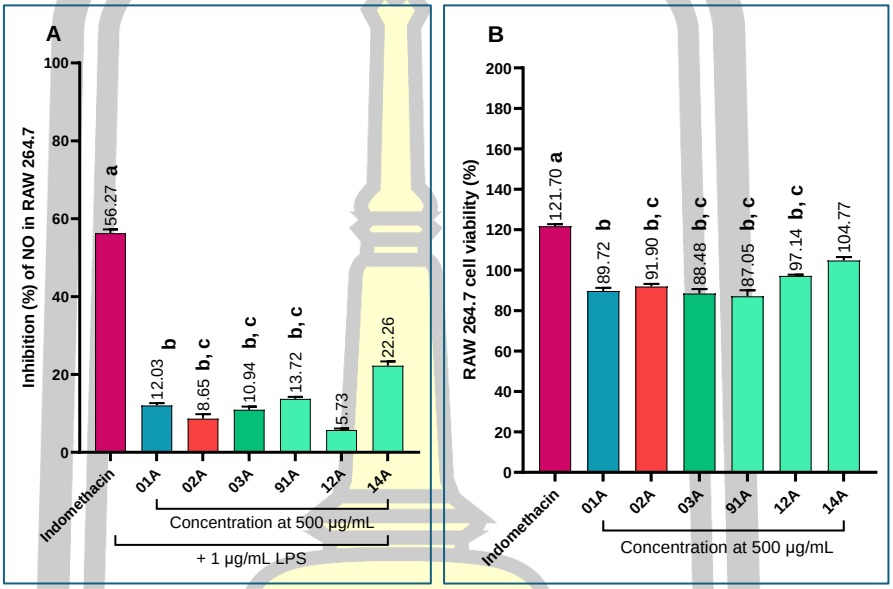


Figure 98 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR by modification of adjunct herbs.
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.5.4 Anti-inflammatory activity and toxicity of original MHR and modified MHR by modification of supportive herbs

4.5.4.1 Anti-inflammatory activity and toxicity of original MHR and modified MHR ethanolic extract by modification of supportive herbs

The supportive herbs (SH), modifications were made in the number of herbs while fixing the primary herbs and the adjunct herbs. Formula 10E involve removing the adjunct herbs, formula 92E contains only the supportive herbs, while the formulas 04E-09E adjust the number of herbs within the SH group. Additionally, formulas 58E-60E were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of supportive herbs compared to only reduced-toxic fever herbs (33E).

In the ethanolic extract (**Figure 99A**) at a concentration of 100 µg/mL, removing the number of herbs in the adjunct herbs (10E, 53.47 ± 0.95%) did not significantly reduce the anti-inflammatory effect compared to MHR (01E, 31.32 ± 0.80%), and there was also no significant difference observed when removing the herbs in the supported primary herbs (formula 03E, 52.80 ± 1.50%). Additionally, the formula containing only the supportive herbs (92E) demonstrated a markedly lower anti-inflammatory effect (23.39 ± 1.49%), compared to the primary herbs alone (PH, 02E, 72.26 ± 0.52%).

Removing one herb in SH, resulted in a significantly reduced anti-inflammatory effect compared to MHR: formula 07E (excluding *V. zizanioides*) had an effect of 25.80 ± 0.68%, formula 08E (excluding *N. nucifera*) showed 16.06 ± 1.05% and formula 09E (excluding *M. ferrea*) showed 22.33 ± 0.72%. When two herbs were removed, formulas 05E (excluding *M. ferrea* and *V. zizanioides*, 28.19 ± 2.74% and 06E (excluding *M. ferrea* and *N. nucifera*, 27.60 ± 2.28% did not showed a significant reduction in anti-inflammatory effect compared to MHR. In contrast, formula 04E (excluding *N. nucifera* and *V. zizanioides*, 18.98 ± 0.57%) showed a significant reduction in the anti-inflammatory effect compared to MHR.

Additionally, formulas 58E-60E were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of the supportive herbs compared to only reduced-toxic fever herbs (33E). These formulas exhibited a significant reduction in anti-inflammatory effect compared to

33E alone. Formulas 58E (33E plus *M. ferrea*) and 59E (33E plus *N. nucifera*) showed moderate effects, with no significant differences among them. Meanwhile, formula 60E (33E plus *V. zizanioides*) displayed a weaker anti-inflammatory effect.

The IC_{50} values (**Figure 100A**) of 10E and indomethacin were 75.95 ± 1.51 and 73.42 ± 1.51 µg/mL, respectively, indicating comparable potency. In contrast, MHR and formula 92E did not exhibit measurable activity, with IC_{50} values exceeding 100 µg/mL.

Toxicity effects (**Figure 99B**) revealed that formulas 03E-10E, 58E-60E and 92E showed cell viability ranging from 82.24% to 118.42%, indicating no toxicity. Formulas 03E-10E, 60E and 92E exhibited cell viability greater than 100%. Specifically, removing herbs in the adjunct herbs (10E) (118.42 ± 2.55%) significantly increased cell viability compared to MHR (104.64 ± 2.43%) and to the formula with removing the supportive herbs (03E) (100.31 ± 3.27%). Meanwhile, the formula containing only the adjunct herbs (92E) (101.67 ± 1.04%) showed cell viability similar to that of the primary herbs alone (02E) (89.47 ± 2.67%). Removing one and two herbs in SH did not significantly impact cell viability compared to MHR.

Moreover, formula 60E showed significantly greater cell viability compared to 33E, while formulas 58E and 59E showed no significant difference from 33E. However, as shown in **Figure 100B**, increasing the concentration of the primary herbs generally led to reduced cell viability.

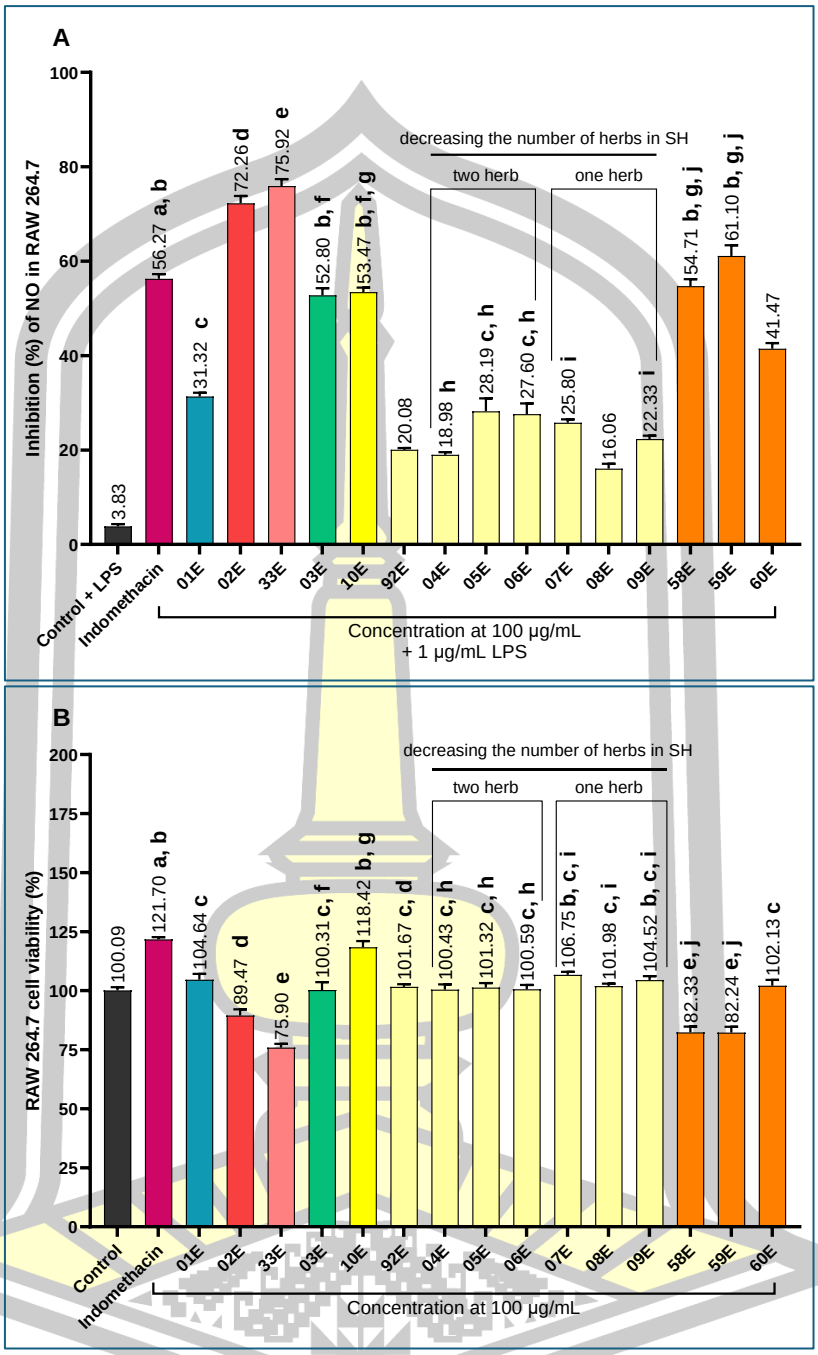


Figure 99 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR by modification of supportive herbs.
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

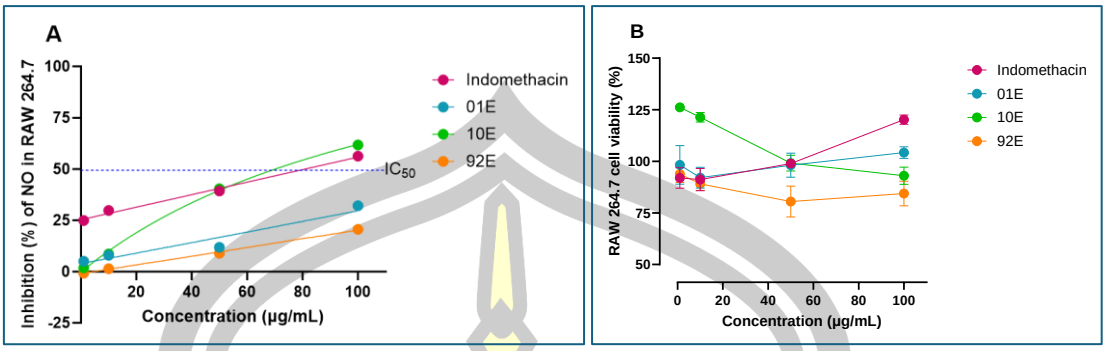


Figure 100 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in the supportive herbs at various concentrations. Values are expressed as mean ± SD (n=3).

4.5.4.2 Anti-inflammatory activity and toxicity of original MHR and modified MHR aqueous extract by modification of supportive herbs

In the aqueous extract (**Figure 101A**) at a concentration of 500 µg/mL, most formulas exhibited only weak anti-inflammatory activity. Removing the number of herbs in the adjunct herbs, formula (10A) (8.01 ± 1.17%) did not significantly reduce the anti-inflammatory effect compared to MHR. Similarly, removing the number of herbs in the supported primary herb formula, 03A (10.94 ± 0.80%), showed no significant difference in anti-inflammatory effect. The formula containing only the supportive herbs (92A) showed no measurable activity.

Toxicity effects (**Figure 101B**) indicated that removing herbs from the adjunct herbs in formula 10A (93.02 ± 0.70%) did not result in a significant difference in cell viability compared to MHR (89.72 ± 1.55%) or the formula with reduced supportive herbs, 03A (88.48 ± 2.14%). Similarly, the formula containing only the adjunct herbs (92A) (86.62 ± 3.19%), showed cell viability comparable to the primary herbs alone, 02A (91.90 ± 1.31%). This suggests no significant impact on cell viability from these herb modifications.

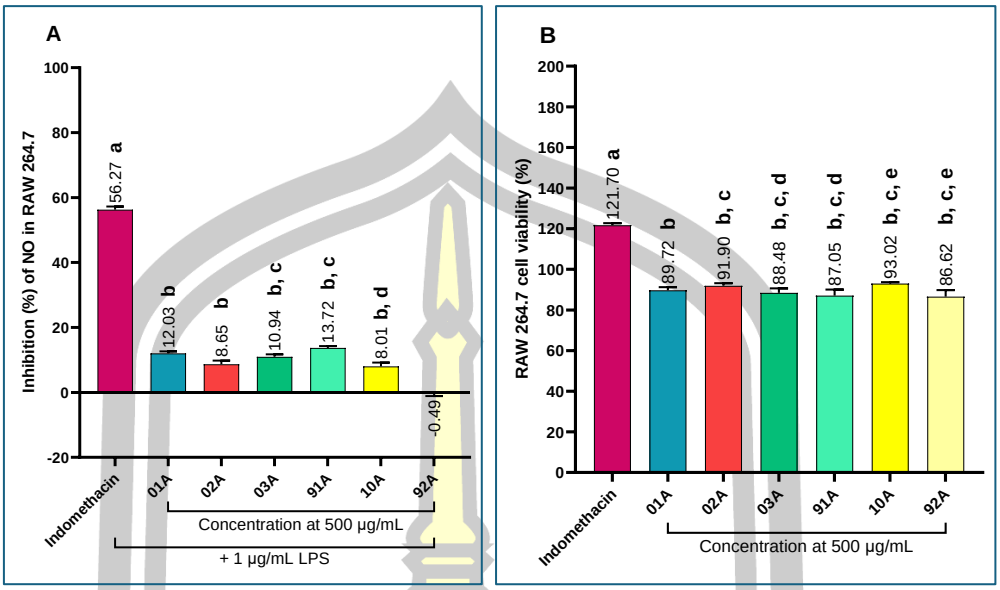


Figure 101 Anti-inflammatory activity (A) and toxicity (B) of original MHR and modified MHR aqueous extract by modification of supportive herbs.
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.5.5 Anti-inflammatory activity and toxicity of major compounds of MHR

The marker compounds in MHR, including bergenin, chebulagic acid, chebulanin, chebulic acid, chlorogenic acid, corilagin, ellagic acid, gallic acid, lourierin A, pectolinarigenin, protocatechuic acid, resveratrol, rhein, and the isolated compound perforatic acid, *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether and TT01, were identified by comparing their retention times and UV spectra with reference standards and isolated compounds, as well as by spiking in the HPLC analysis of the MHR remedy.

TT01 exhibited the highest anti-inflammatory activity, with an IC_{50} value of 9.75 ± 0.25 µg/mL, followed by resveratrol and rhein with an IC_{50} value of 17.83 ± 0.70 µg/mL and 19.68 ± 0.09 µg/mL, respectively. Moderate anti-inflammatory activity was observed for ellagic acid and lourierin A, with IC_{50} values of 42.13 ± 1.87 and 72.42 ± 1.40 µg/mL, respectively, both of which were more effective than indomethacin (positive control), which had an IC_{50} value of 73.42 ± 0.48 µg/mL. Conversely, 12 marker compounds, including bergenin, chebulagic acid, chebulanin, chebulic acid, chlorogenic acid, corilagin, gallic acid, *O*-methyllaloptaeroxyrin,

perforatic acid, pectolinarigenin, peucenin-7-methyl ether and protocatechuic acid, showed no measurable activity (IC_{50} > 100 µg/mL), as shown in the **Table 19** and **Figure 102A**.

However, as shown in **Figure 102B**, the marker compounds TT01, resveratrol, rhein, and ellagic acid exhibited decreased cell viability at higher concentrations, falling below the survival threshold of 70%.

Table 19 Anti-inflammatory activity of the major compounds in MHR

Marker compounds	**% Inhibition of NO production / (% Cell viability)**				**IC_{50} (µg/mL)**
	100 µg/mL	**50 µg/mL**	**10 µg/mL**	**1 µg/mL**	
Bergenin	8.68 ± 0.86 (79.59 ± 1.37)	5.51 ± 1.14 (78.09 ± 3.52)	-0.80 ± 0.61 (75.15 ± 0.72)	-2.37 ± 0.21 (78.45 ± 4.73)	>100
Chebulagic acid	41.43 ± 1.15 (92.83 ± 6.42)	31.43 ± 0.94 (89.09 ± 1.79)	23.56 ± 2.68 (86.41 ± 1.01)	20.99 ± 3.55 (87.68 ± 6.01)	>100
Chebulanin	33.56 ± 0.41 (97.36 ± 1.63)	26.79 ± 1.54 (95.28 ± 0.48)	21.42 ± 1.11 (94.97 ± 5.60)	20.85 ± 2.96 (88.13 ± 3.65)	>100
Chebulic acid	36.11 ± 1.72 (75.99 ± 0.39)	23.42 ± 0.32 (78.68 ± 1.43)	4.16 ± 0.61 (84.83 ± 3.34)	-2.30 ± 0.40 (90.10 ± 3.84)	>100
Chlorogenic acid	21.11 ± 1.25 (72.40 ± 1.60)	12.86 ± 1.29 (73.46 ± 4.06)	5.58 ± 0.48 (75.40 ± 2.99)	7.02 ± 0.79 (77.07 ± 2.09)	>100
Corilagin	20.90 ± 1.18 (86.30 ± 2.98)	12.65 ± 2.22 (89.48 ± 1.77)	8.38 ± 2.09 (86.15 ± 4.39)	5.66 ± 1.12 (97.88 ± 4.63)	>100
Ellagic acid	56.01 ± 2.16 (56.45 ± 3.79)	45.05 ± 1.80 (63.79 ± 2.56)	21.17 ± 2.07 (85.94 ± 3.02)	9.68 ± 1.06 (90.60 ± 5.47)	42.13 ± 1.87
Gallic acid	32.76 ± 1.78 (60.08 ± 0.50)	10.49 ± 1.07 (79.55 ± 1.68)	1.85 ± 0.62 (90.08 ± 4.18)	4.43 ± 1.34 (89.90 ± 5.40)	>100
Lourierin A	63.86 ± 0.96 (102.95 ± 1.70)	39.62 ± 0.48 (112.70 ± 6.54)	16.19 ± 0.96 (103.23 ± 5.31)	12.96 ± 1.35 (100.81 ± 5.68)	72.42 ± 1.40
O-methyllalop taeroxyrin	14.34 ± 0.00 (117.86 ± 0.11)	8.19 ± 0.21 (117.30 ± 1.19)	4.15 ± 0.33 (88.29 ± 0.32)	4.34 ± 1.10 (84.95 ± 0.29)	>100
Perforatic acid	33.94 ± 0.27 (79.11 ± 0.90)	27.73 ± 3.38 (86.59 ± 1.97)	16.37 ± 2.82 (94.85 ± 8.00)	11.22 ± 2.19 (99.10 ± 3.88)	>100
Pectolinarigenin	35.49 ± 1.71 (64.60 ± 0.84)	31.94 ± 0.92 (86.90 ± 2.30)	17.21 ± 0.49 (96.07 ± 4.29)	-9.62 ± 1.05 (87.29 ± 1.82)	>100
Peucenin-7-methyl ether	24.74 ± 1.44 (52.48 ± 2.12)	4.83 ± 1.58 (52.94 ± 1.84)	1.32 ± 0.74 (59.82 ± 1.80)	4.36 ± 1.20 (73.91 ± 2.86)	>100
Protocatechuic acid	30.76 ± 1.13 (100.65 ± 5.38)	23.48 ± 0.95 (97.27 ± 6.42)	20.16 ± 1.25 (91.32 ± 1.86)	12.93 ± 2.02 (89.68 ± 4.11)	>100
Resveratrol	75.37 ± 0.52 (7.14 ± 0.68)	55.59 ± 1.76 (65.34 ± 82.56)	35.32 ± 0.70 (82.56 ± 1.60)	6.47 ± 0.35 (84.90 ± 2.34)	17.83 ± 0.70
Rhein	64.86 ± 0.91 (53.82 ± 0.83)	69.18 ± 0.40 (54.66 ± 0.01)	8.98 ± 0.56 (90.75 ± 2.79)	-15.94 ± 1.31 (85.34 ± 3.07)	19.68 ± 0.09
TT01	74.18 ± 0.58 (26.76 ± 1.56)	74.05 ± 0.19 (54.55 ± 0.94)	30.57 ± 0.24 (94.51 ± 2.90)	14.06 ± 0.54 (97.79 ± 2.19)	9.75 ± 0.25
Indomethacin (Positive control)	56.31 ± 1.08 (120.20 ± 1.83)	39.29 ± 0.74 (98.91 ± 0.35)	29.80 ± 2.64 (91.19 ± 4.45)	24.93 ± 2.94 (91.92 ± 4.08)	73.42 ± 0.48

Note: All data represents the mean ± SD in triplicate experiments.

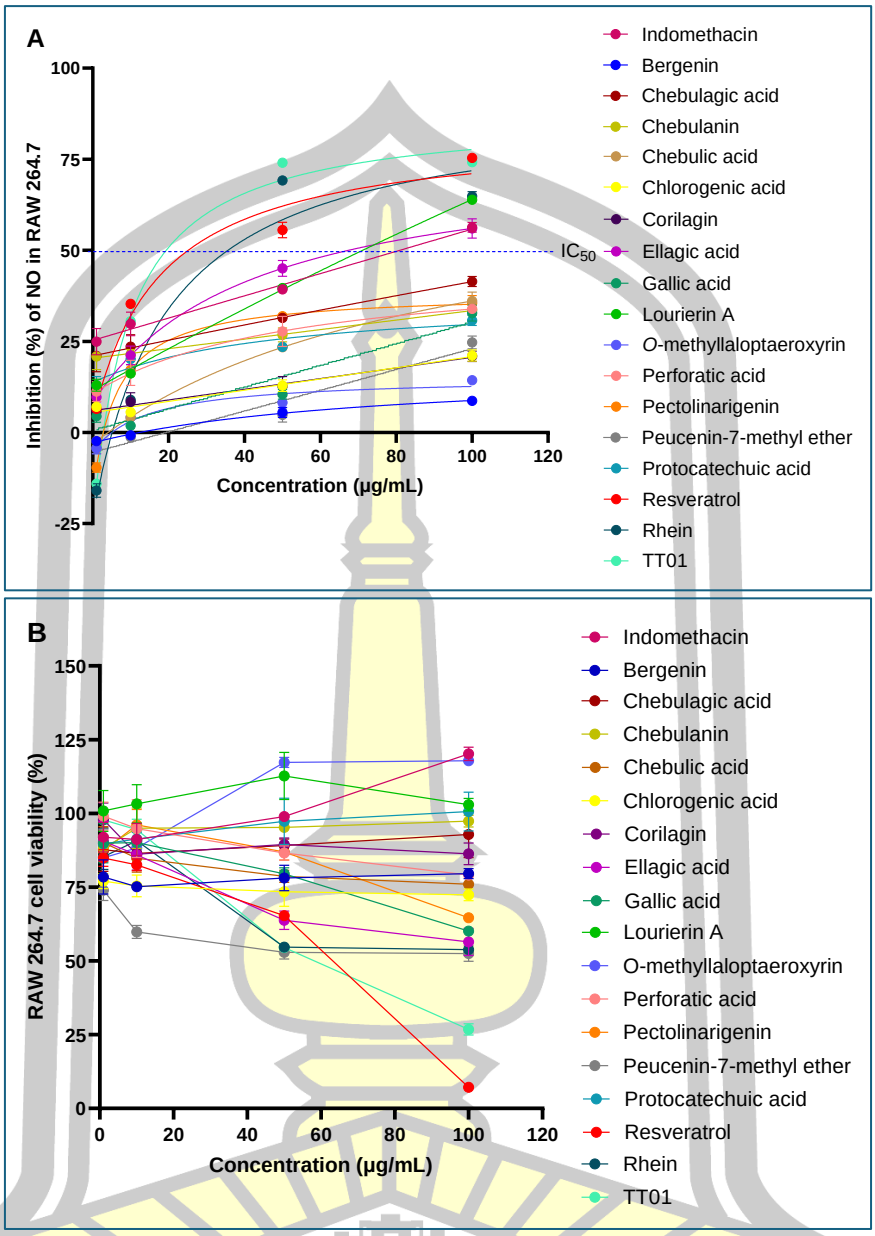


Figure 102 Comparison of nitric oxide inhibition (A) and cell viability (B) of the ethanolic extract in the major compounds at various concentrations. Values are expressed as mean ± SD (n=3).

4.6 Antioxidant activity of original MHR and modified MHR

The antioxidant activity of the ethanolic and aqueous extracts of original MHR and 92 modified MHR remedies were tested by measuring their inhibitory effects on free radicals. The nitric oxide radical scavenging activity was assessed using a modified Griess reaction, while the superoxide anion ($O_2^{\bullet-}$) scavenging activity was determined using a riboflavin-light-NBT system with modifications to inhibit formazan formation.

4.6.1 Antioxidant activity of original MHR and modified MHR (primary herbs, primary herbs plus adjunct herbs, primary herbs plus supportive herbs, adjunct herbs and supportive herbs)

4.6.1.1 Antioxidant activity of original MHR and modified MHR ethanolic extract

In the ethanolic extract (**Figure 103A**) at a concentration of 100 µg/mL, the MHR remedy (Mo-Ha-Rak, 01E) exhibited nitric oxide (NO) radical scavenging activity of 61.93 ± 0.93%, which was significantly higher than that of ascorbic acid (45.91 ± 1.02%) and trolox (26.62 ± 0.76%), both positive controls at the same concentration. The primary herbs (PH, 02E) demonstrated similar NO radical scavenging activity (56.23 ± 0.75%) to MHR, while significantly outperforming the supportive herbs (92E) (28.00 ± 0.75%). However, PH activity was significantly lower than that of the adjunct herbs formula (91E) (62.54 ± 0.16%). Removing the supportive herbs in MHR (03E) (60.40 ± 0.64%) did not significantly reduce the NO radical scavenging effect compared to MHR, whereas reduction in the adjunct herbs (10E) (33.00 ± 0.74%) led to a significantly lower effect than MHR.

As shown in **Figure 103B**, MHR exhibited superoxide anion ($O_2^{\bullet-}$) scavenging activity of 64.91 ± 0.72%, which was significantly lower than that of ascorbic acid (90.37 ± 1.86%) but significantly higher than that of trolox (25.71 ± 1.64%). MA (46.33 ± 1.36%) showed a notably reduced $O_2^{\bullet-}$ radical scavenging effect compared to MHR and 91E (76.76 ± 0.66%), though it remained significantly higher than that of 92E (28.05 ± 0.76%). Removing the number of herbs in the supportive herbs (03E) (66.80 ± 0.61%) did not significantly reduce the $O_2^{\bullet-}$ radical scavenging effect compared to MHR. However, removing the number of herbs in the adjunct

herbs (10E) (28.74 ± 1.35%) led to a significantly lower scavenging effect compared to MHR.

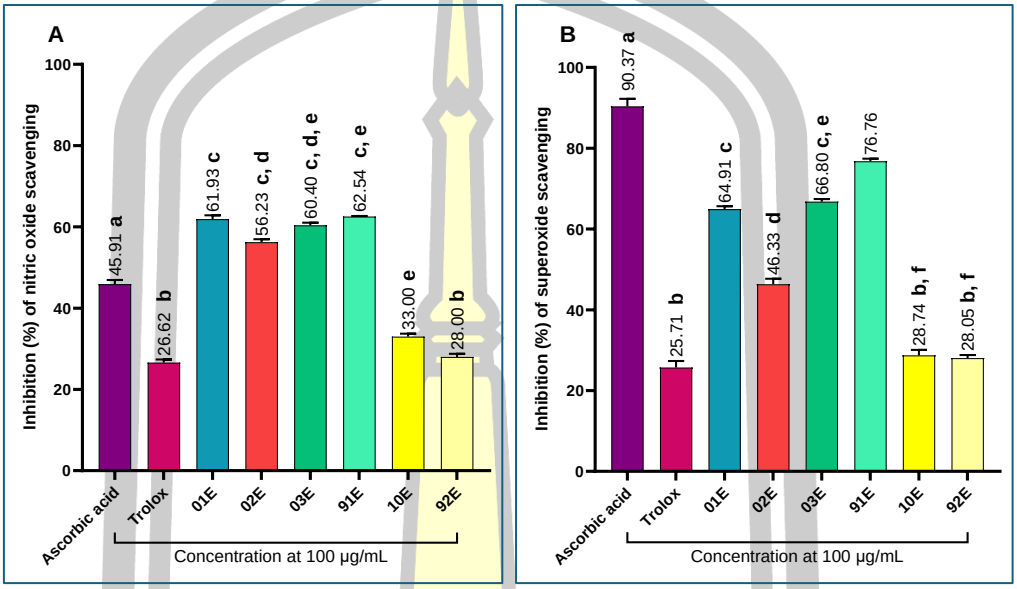
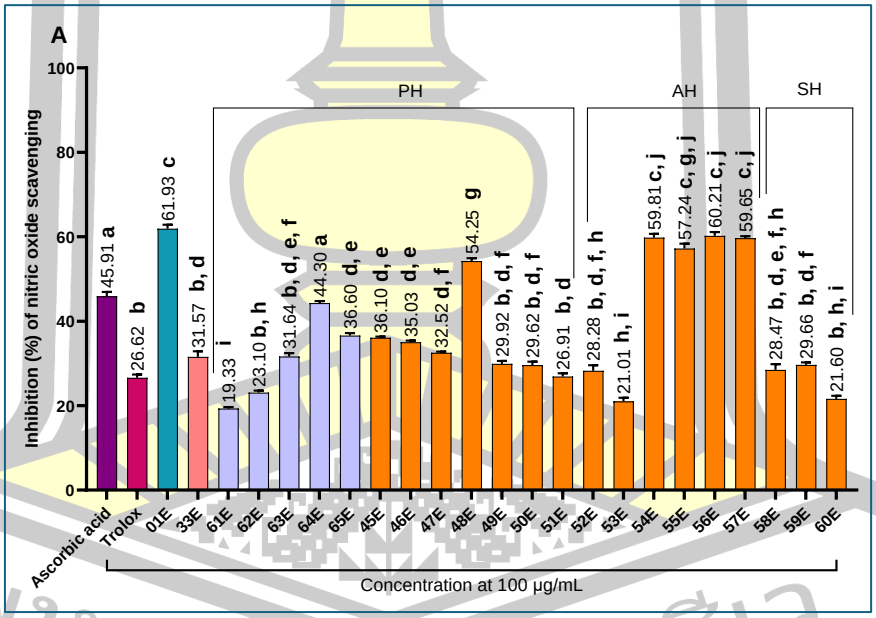


Figure 103 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01E) and modified MHR (Primary herbs (02E), without supportive herbs (03E), adjunct herbs (91E), without adjunct herbs (10E) and supportive herbs (92E)).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

The formulas 45E-60E were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of the other components of MHR compared to only reduced-toxic fever herbs (33E). Formulas 61E-65E were modified MHR remedies using only one herb of reduced-toxic fever herb. NO radical scavenging effect (**Figure 104A**) revealed that formulas 45E-65E exhibited a range of NO radical scavenging effects from 19.33% to 59.81%. Among these, formulas 48E (33E plus *D. cochinchinensis*) (54.25 ± 0.70%), 54E (33E plus *T. bellirica*) (59.81 ± 0.87%), 55E (33E plus *T. chebula*) (57.24 ± 1.12%), 56E (33E plus *Terminalia* sp.) (60.21 ± 0.88%) and 57E (33E plus *P. emblica*) (59.65 ± 0.47%) showed moderate effects significantly higher than 33E (31.57 ± 1.31%). However, formulas 54E, 55E, 56E, and 57E did not show significant differences compared to

MHR (31.32 ± 0.80%). Meanwhile, the other formulas displayed weaker effects, with a significantly lower NO radical scavenging effect compared to MHR.

The superoxide anion ($O_2^{\bullet-}$) scavenging studies (**Figure 104B**) found that the formulas 45E-65E exhibited $O_2^{\bullet-}$ radical scavenging ranging from 21.14% to 78.89%. Formulas 63E (only *F. racemosa*) (75.56 ± 0.64%), 54E (33E plus *T. bellirica*) (73.01 ± 1.64%), 56E (33E plus *Terminalia* sp.) (78.89 ± 0.64%) and 57E (33E plus *P. emblica*) (75.45 ± 0.44%) showed high effects, with no significant differences observed among them. Moderate $O_2^{\bullet-}$ radical scavenging activity was observed in 65E (only *T. triandra*) (61.67 ± 0.79%), 55E (33E plus *T. chebula*) (67.99 ± 1.17%), and 58E (33E plus *M. ferrea*) (50.09 ± 0.94%), with no significant differences compared to MHR (64.91 ± 0.72%). In contrast, other formulas showed weak effects.



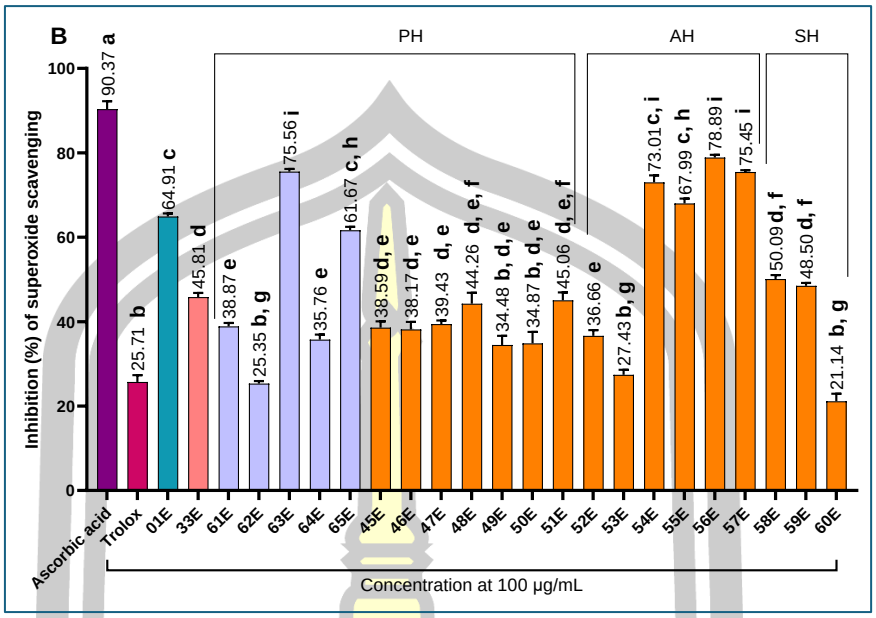


Figure 104 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01E) and modified MHR by fixing reduced-toxic fever herbs plus one of the other components (45E-60E) compared to only one of the reduced-toxic fever herbs (61E-65E) and only reduced-toxic fever herbs (33E). Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.6.1.2 Antioxidant activity of original MHR and modified MHR aqueous extract

In the aqueous extract (**Figure 105A**) at a concentration of 100 µg/mL, the MHR remedy (Mo-Ha-Rak, 01A) exhibited nitric oxide (NO) radical scavenging activity of 56.47 ± 0.29%, significantly higher than that of the positive controls, ascorbic acid (45.91 ± 1.02%) and trolox (26.62 ± 0.76%). The formula containing only the primary herbs (PH, 02A) (22.87 ± 0.53%) showed a significantly reduced NO radical scavenging effect compared to MHR, with no significant difference from the supportive herbs formula (92A) (27.30 ± 0.70%). However, the PH formula exhibited significantly lower activity compared to the formula containing only the adjunct herbs (AH, 91A) (35.06 ± 0.23%). Removing the number of herbs in the supportive herbs (03A) (57.61 ± 0.36%) or in the adjunct herbs (10A) (54.03 ± 0.46%) did not significantly reduce the NO radical scavenging effect compared to MHR.

As shown in **Figure 105B**, MHR exhibited superoxide anion ($O_2^{\bullet-}$) scavenging activity of 92.47 ± 0.40%, which was not significantly different from that of ascorbic acid (90.37 ± 1.86%) but significantly higher than that of trolox (25.71 ± 1.64%). MA (86.32 ± 0.31%) showed a significantly reduced $O_2^{\bullet-}$ radical scavenging effect compared to MHR, with significantly lower than that of 91A (94.71 ± 0.20%) and 92A (92.71 ± 0.33%). Removing the number of herbs in the supportive herbs (03A) (93.36 ± 0.37%) did not significantly affect the $O_2^{\bullet-}$ radical scavenging effect compared to MHR. However, removing the number of herbs in the adjunct herbs (10A) (75.47 ± 2.14%) led to a significantly lower effect compared to MHR.

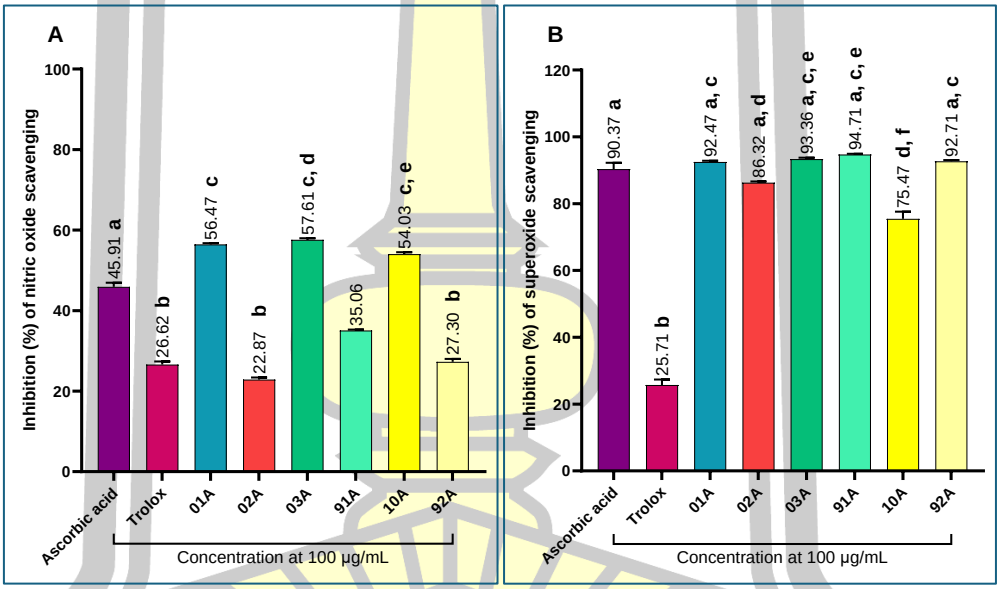
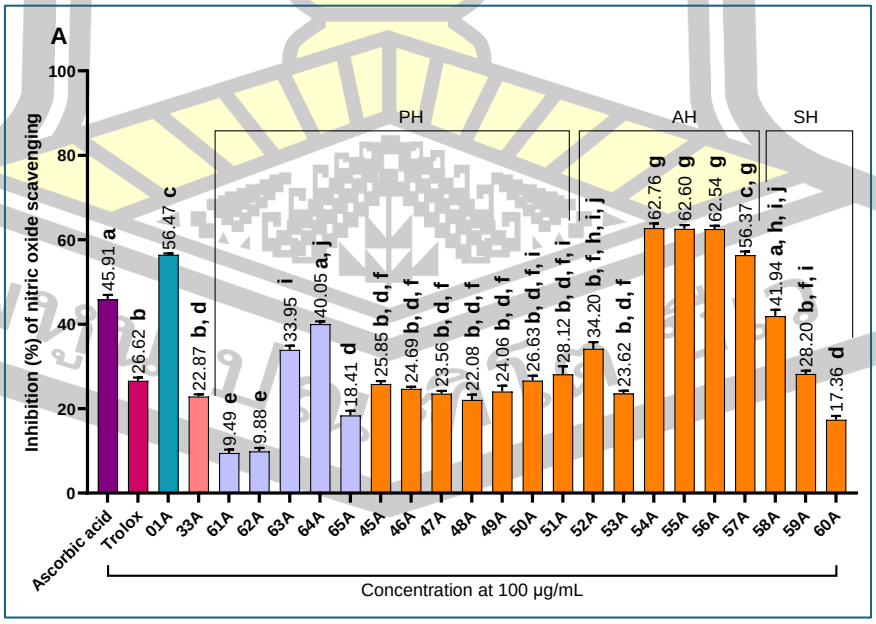


Figure 105 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01A) and modified MHR (Primary herbs (02A), without supportive herbs (03A), adjunct herbs (91A), without adjunct herbs (10A) and supportive herbs (92A)).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

The formulas 45A-60A were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of the other components of MHR compared to only reduced-toxic fever herbs (33A). Formulas 61A-65A were modified MHR remedies using only one herb of reduced-toxic fever

herb. The NO radical scavenging effect (**Figure 106A**) for formulas 45A-65A ranged from 9.49% to 62.76%. The formulas 54A (33A plus *T. bellirica*) (62.76 ± 1.10%), 55A (33A plus *T. chebula*) (62.60 ± 0.90%), 56A (33A plus *Terminalia* sp.) (62.54 ± 0.75%) and 57A (33A plus *P. emblica*) (56.37 ± 0.85%) showed moderate NO radical scavenging effects, all significantly greater than 33A (22.87 ± 0.90%). However, only formula 57A did not show significant differences compared to MHR (56.47 ± 0.29%). The remaining formulas displayed weak effects, with significantly lower NO radical scavenging activity compared to MHR.

Superoxide anion ($O_2^{\bullet-}$) scavenging studies (**Figure 106B**) found that formulas 45A-65A exhibited $O_2^{\bullet-}$ radical scavenging ranging from 35.07% to 93.24%, with most formulas showing strong effects. Formulas 65A (*T. triandra*) (92.60 ± 0.30%), 49A (33A plus *T. bellirica*) (88.13 ± 0.88%), 51A (33A plus *T. bellirica*) (89.22 ± 1.57%), 52A (33A plus *T. bellirica*) (88.88 ± 1.13%), 54A (93.24 ± 0.31%), 55A (92.32 ± 0.30%), 56A (92.89 ± 0.29%) and 57A (89.81 ± 0.44%) showed no significant differences compared to MHR (92.47 ± 0.40%). Moderate $O_2^{\bullet-}$ radical scavenging activity was observed in formula 61A (only *C. micracantha*) (67.78 ± 0.95%). Only formula 62A (only *C. indicum*) (35.07 ± 1.50%) exhibited a weak scavenging effect.



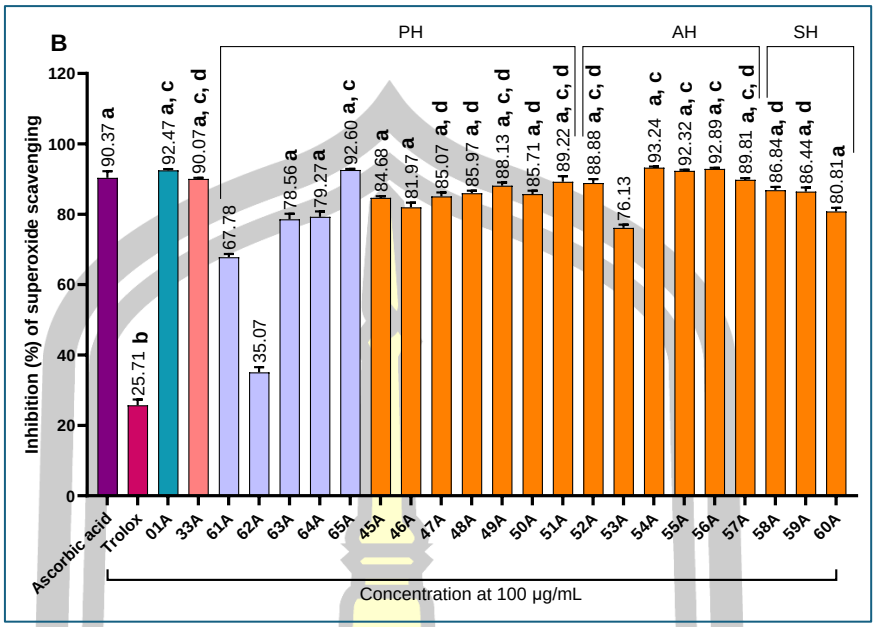


Figure 106 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of original MHR (01A) and modified MHR by fixing reduced-toxic fever herbs plus one of the other components (45A-60A) compared to only one of the reduced-toxic fever herbs (61A-65A) and only reduced-toxic fever herbs (33A). Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.6.2 Antioxidant activity of primary herbs

4.6.2.1 Antioxidant activity of primary herbs ethanolic extract

Formula 02E contained the primary herbs (MA). Formulas 31E-37E involved modifications of primary herbs, while formulas 38E-39E involved adjustments in the number of herbs for anti-semha and lom fever. Formulas 40E-41E focused on changes in the number of herbs for anti-di fever, and formulas 42E-44E involved changes in the number of herbs for anti-kamdao and lohit fever. Finally, formulas 45E-51E were modified MHR remedies by fixing reduced-toxic fever herbs and adding one of the other components compared to only reduced-toxic fever herbs (33E) were studied on antioxidant activity.

1) Antioxidant activity of primary components

This study found that modifications to primary herbs (**Figure 107A**) led to a significantly reduced NO radical scavenging effect compared to MHR. Specifically, removing herbs for anti-semha and lom fever (subgroup 1.4) (31E)

(38.99 ± 1.42%) or removing herbs for anti-di fever (subgroup 1.3) (34E) (31.65 ± 1.22%) significantly reduced the NO radical scavenging effect compared to PH (56.23 ± 0.75%). In contrast, removing herbs for anti-kamdao and lohit fever (subgroup 1.2) (35E) (53.19 ± 1.67%) did not significantly reduce NO radical scavenging effect compared to MA. Similarly, modifications to primary herbs (**Figure 107B**) resulted in most formulas showing a significantly reduced $O_2^{\bullet-}$ radical scavenging effect compared to MHR. Removing herbs in subgroup 1.4 (31E) (47.46 ± 0.80%) or subgroup 1.2 (35E) (50.89 ± 1.72%) did not significantly reduce the $O_2^{\bullet-}$ radical scavenging effect compared to PH (46.33 ± 1.36%). However, removing the herbs in subgroup 1.3 (34E) (25.59 ± 0.70%) led to a significantly reduced $O_2^{\bullet-}$ radical scavenging effect compared to PH.

Removing herbs in two subgroups (**Figure 107A**), including formulas 32E (removing subgroups 1.3 and 1.4) (26.55 ± 1.02%) and 37E (removing subgroups 1.2 and 1.3) (29.79 ± 0.50%) resulted in significantly reduced the NO radical scavenging effect compared to MA. In contrast, removing herbs in subgroups 1.2 and 1.4 (36E) (51.70 ± 1.01%) did not significantly reduce the NO radical scavenging effect compared to MA. Additionally, excluding herbs in all three subgroups, herbs for anti-kamdao and lohit fever (subgroup 1.2), herbs for anti-di fever (subgroup 1.3) and herbs for anti-semha and lom fever (subgroup 1.4) (33E) (31.57 ± 1.31%) significantly reduced the NO radical scavenging effect compared to PH.

Additionally, excluding herbs in two subgroups (**Figure 107B**) in formulas 32E (removing subgroups 1.3 and 1.4) (50.08 ± 1.14%), 36E (removing subgroups 1.2 and 1.4) (55.86 ± 1.45%) and 37E (removing subgroups 1.2 and 1.3) (43.98 ± 1.20%), did not significantly reduce $O_2^{\bullet-}$ radical scavenging effect compared to MA. Similarly, excluding herbs across all three subgroups, herbs for anti-kamdao and lohit fever (subgroup 1.2), herbs for anti-di fever (subgroup 1.3) and herbs for anti-semha and lom fever (subgroup 1.4) (33E) (45.81 ± 1.01%) did not significantly reduce the $O_2^{\bullet-}$ radical scavenging effect compared to PH. Among formulas with single-herb exclusions, 39E (excluding *L. sinense*) and 40E (excluding *T. hoaensis*) exhibited moderate antioxidant activity, while other formulas involving herb exclusions showed weaker effects.

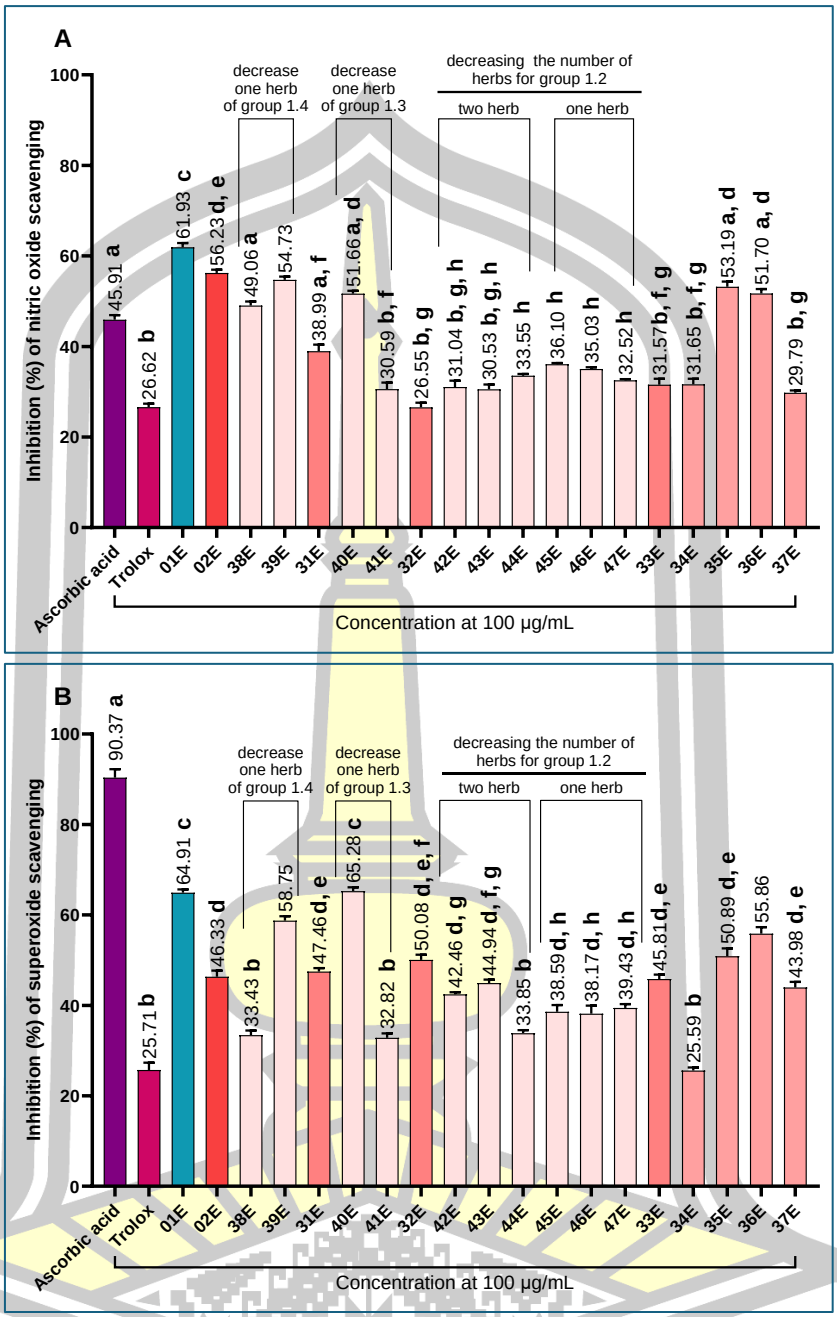


Figure 107 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of original MHR (01E) and modified MHR (decrease one herb of group 1.4, decrease one herb of group 1.3 and decrease one and two herb of group 1.2).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

2) Antioxidant activity of modified MHR by fixing reduced-toxic fever herbs and plus one of the primary components

Formulas 45E-51E were modified MHR remedies by fixing reduced-toxic fever herbs and plus one of the primary components compared to only reduced-toxic fever herbs (33E). Formulas 61E-65E consisted of single herbs from reduced-toxic fever herbs. NO radical scavenging studies (**Figure 108A**) revealed that formulas 45E-65E exhibited NO radical scavenging activity ranging from 9.33% to 54.25%. Among these, 48E (33E plus *D. cochinchinensis*) (54.25 ± 0.70%) exhibited the highest activity, with no significant difference from PH (56.23 ± 0.75%), and significantly higher activity than 33E (31.57 ± 1.31%). Other formulas displayed weaker effects.

In the $O_2^{\bullet-}$ radical scavenging study (**Figure 108B**), formulas 45E-65E exhibited $O_2^{\bullet-}$ radical scavenging activity ranging from 25.35% to 75.56%. Formula 63E (only *F. racemosa*) exhibited the highest activity with 75.56 ± 0.64%, followed by 65E (only *T. triandra*) with 61.67 ± 0.79%. Both formulas exhibited significantly higher O2•– scavenging activity compared to PH (46.33 ± 1.36%) and 33E (45.81 ± 1.01%).

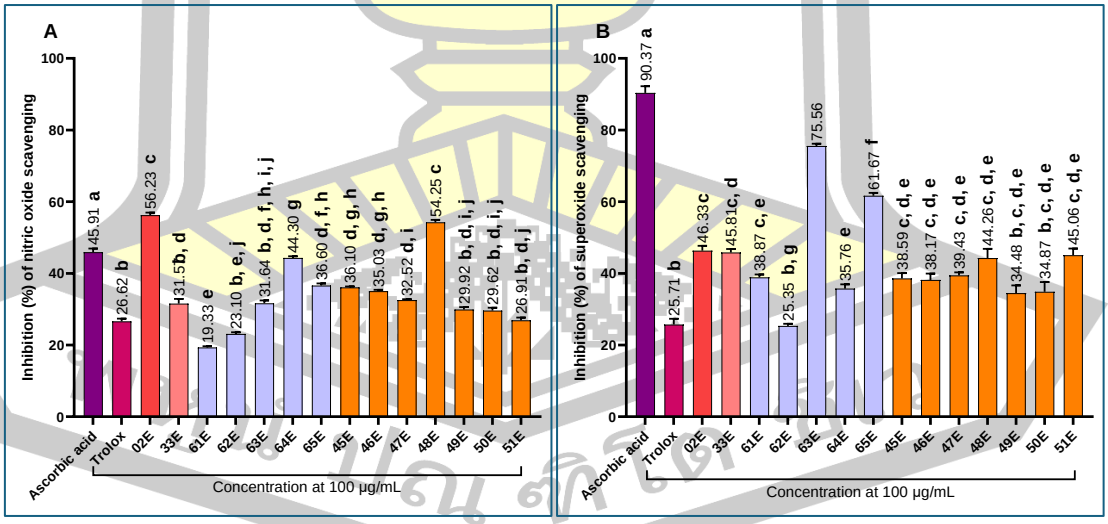


Figure 108 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of modified MHR by fixing reduced-toxic fever herbs and plus one of the primary components (45E-51E).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

3) Antioxidant activity of modified MHR by modification of reduced-toxic fever herbs

Formulas 61E-90E were modified by varying the number of herbs in the reduced-toxic fever herb subgroup compared to formula 33E. In the NO radical scavenging assay (**Figure 109A**) at a concentration of 100 µg/mL, most formulas exhibited weak NO radical scavenging activity ranging from 13.07% to 44.30%. Notably, formula 64E (containing only *H. perforata*) showed a significantly greater NO radical scavenging effect at 44.30 ± 0.46% compared to 33E (31.57 ± 1.31%).

In the $O_2^{\bullet-}$ radical scavenging assay (**Figure 109B**), formulas 61E-90E exhibited $O_2^{\bullet-}$ radical scavenging activity ranging from 18.31% to 75.56%. By removing a single herb, formulas 86E (excluding *T. triandra*) (54.73 ± 0.92%) and 88E (excluding *F. racemosa*) (54.68 ± 1.24%) showed moderate effects, with significantly greater $O_2^{\bullet-}$ radical scavenging activity compared to 33E (45.81 ± 1.01%).

With the removal of two herbs, formulas 77E (excluding *F. racemosa* and *T. triandra*) (53.73 ± 2.22%), 80E (excluding *C. indicum* and *H. perforata*) (56.08 ± 1.61%), 81E (excluding *C. indicum* and *F. racemosa*) (52.96 ± 0.44%), 82E (excluding *C. micracantha* and *T. triandra*) (60.50 ± 1.32%) and 83E (excluding *C. micracantha* and *H. perforata*) (51.43 ± 1.26%) showed moderate effects, with significantly higher $O_2^{\bullet-}$ radical scavenging activity than 33E.

With the removal of three herbs, formulas 68E (excluding *C. indicum, F. racemosa* and *T. triandra*) (54.42 ± 1.04%) and 75E (excluding *C. micracantha C. indicum* and *F. racemosa*) (59.76 ± 1.58%) showed moderate effects, with significantly higher $O_2^{\bullet-}$ radical scavenging activity than 33E.

With the removal of four herbs or inclusion of a single herb, formulas 63E (*F. racemosa*) exhibited the highest activity with a 75.56 ± 0.64%, followed by 65E (*T. triandra*) with a 61.67 ± 0.79%. Both formulas showed higher $O_2^{\bullet-}$ radical scavenging activity compared to 33E (45.81 ± 1.01%). Other formulas displayed weak effects.

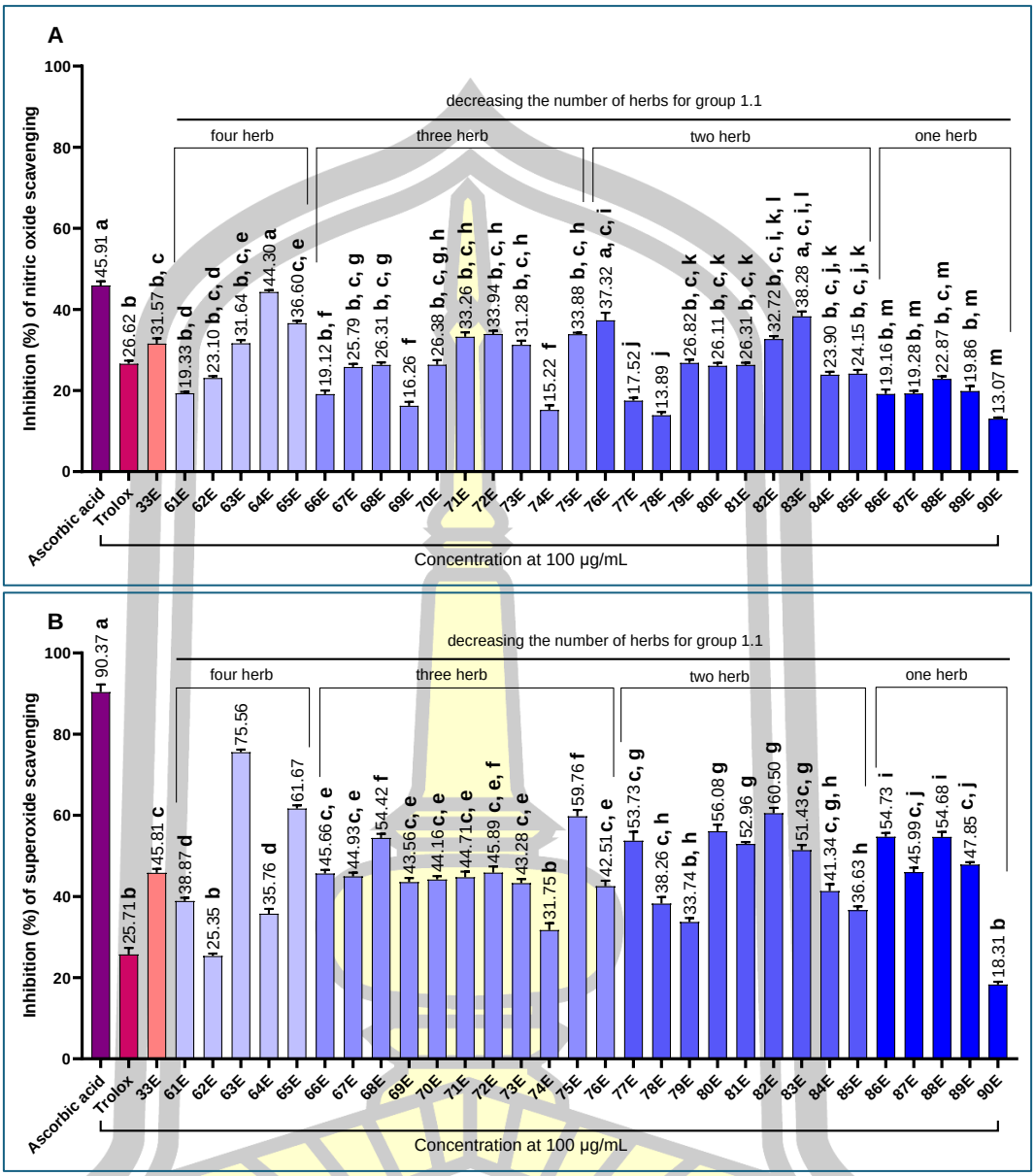
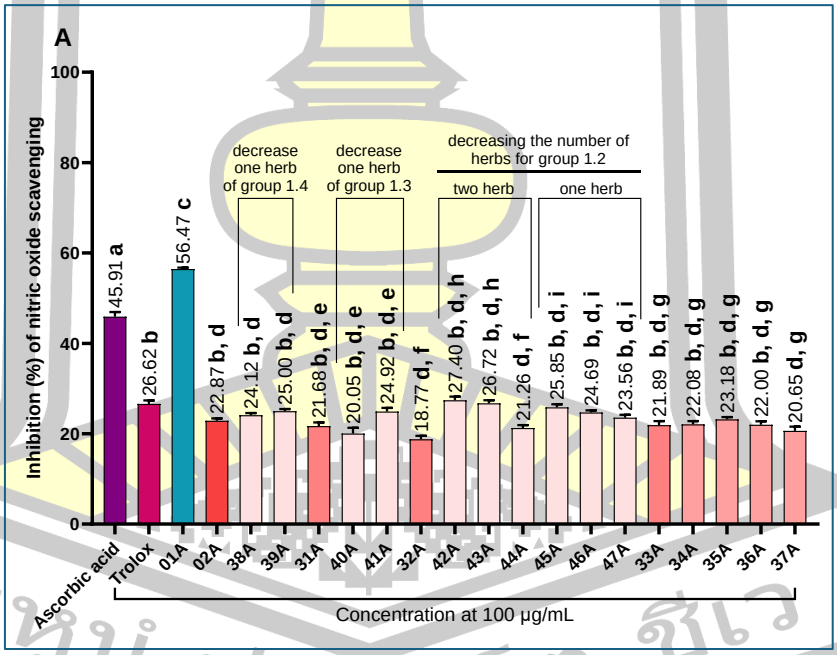


Figure 109 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of modified MHR by decreasing the number of herbs in reduced-toxic fever herbs (subgroup 1.1, 61-90E).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.6.2.2 Antioxidant activity of primary herbs aqueous extract

1) Antioxidant activity of modified MHR by primary components

In the NO radical scavenging assay (**Figure 110A**), formulas 31A-47A at a concentration of 100 µg/mL, all formulas exhibited weak NO radical scavenging activity ranging from 18.77% to 27.40%. Modification of the primary herbs resulted in a significantly reduced NO radical scavenging effect compared to MHR. In contrast, all modified formulas 31A-47A exhibited high $O_2^{\bullet-}$ radical scavenging activity, ranging from 81.97% to 92.44%, as shown in **Figure 110B**. Formulas 32A (removing subgroups 1.3 and 1.4) exhibited the highest activity with 92.44 ± 0.37%, followed by 33A (removing subgroups 1.2, 1.3, and 1.4) with 90.07 ± 0.29%. Both formulas showed higher $O_2^{\bullet-}$ radical scavenging activity compared to PH (86.32 ± 0.31%).



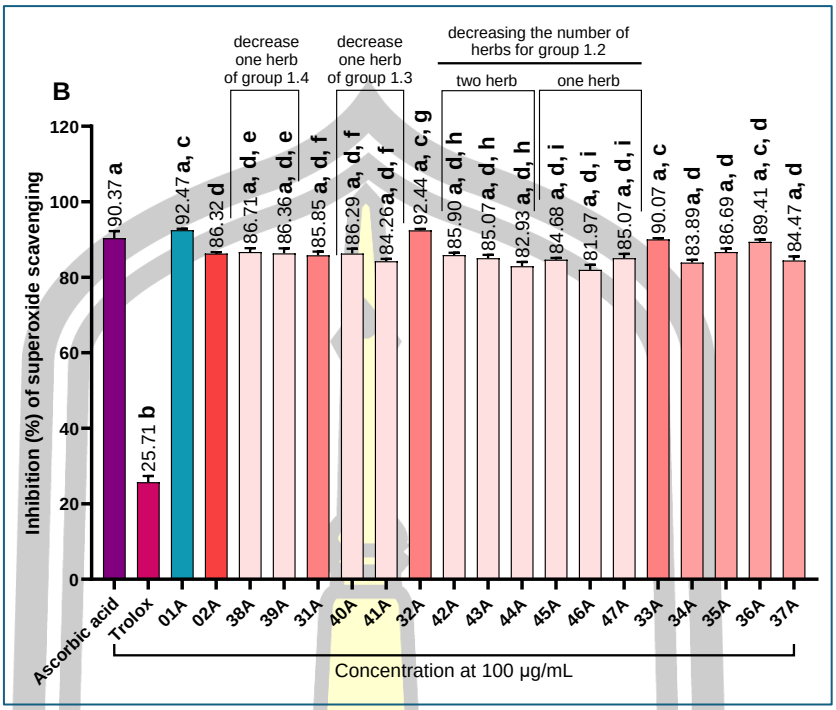


Figure 110 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of aqueous extract of original MHR (01A) and modified MHR (decrease one herb of group 1.4, decrease one herb of group 1.3 and decrease one and two herbs of group 1.2).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

2) Antioxidant activity of modified MHR by fixing reduced-toxic fever herbs and plus one of the primary components

Formulas 45A-51A were modified MHR remedies by fixing reduced-toxic fever herbs and plus one of the primary components compared to only reduced-toxic fever herbs (33A). Formulas 61A-65A consisted of single herbs from reduced-toxic fever herbs. NO radical scavenging studies (**Figure 111A**) revealed that formulas 45A-65A exhibited weak NO radical scavenging ranging from 9.49% to 40.05%. Among them, formula 64A (only *H. perforata*) exhibited the highest activity with 40.05 ± 0.61%, followed by 63A (only *F. racemosa*) with 33.95 ± 0.95%. Both showed higher NO radical scavenging activity compared to PH (22.87 ± 0.53%) and 33A (21.89 ± 0.90%). In contrast, the $O_2^{\bullet-}$ radical scavenging effects (**Figure 111B**) found that formulas 45A-65A exhibited $O_2^{\bullet-}$ radical scavenging activity ranging from 35.07% to 92.60%. Most formulas exhibited high $O_2^{\bullet-}$ radical scavenging activity.

Formula 65A (only *T. triandra*) exhibited the highest activity with a 92.60 ± 0.30%, with significantly higher $O_2^{\bullet-}$ radical scavenging activity than both PH (86.32 ± 0.31%) and 33A (90.07 ± 0.29%).

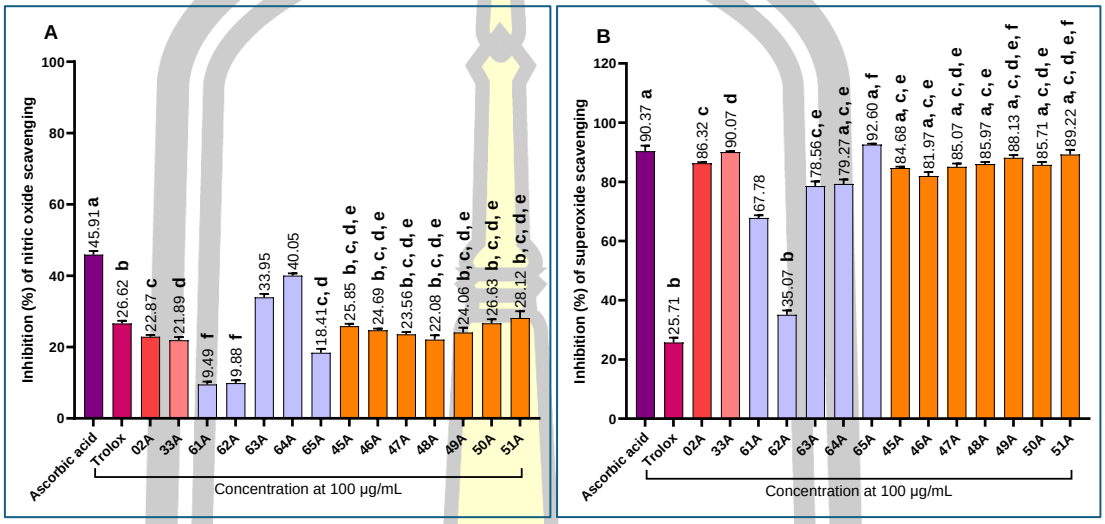


Figure 111 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of modified MHR aqueous extract by fixing reduced-toxic fever herbs and plus one of the primary components (45E-51E).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

3) Antioxidant activity of modified MHR by modification of reduced-toxic fever herbs

Formulas 61E-90E were modified by varying the number of herbs in the reduced-toxic fever herb subgroup compared to formula 33E. In the NO radical scavenging assay (**Figure 112A**) at a concentration of 100 µg/mL, most formulas exhibited weak NO radical scavenging activity ranging from 6.04% to 40.05%. Removing four herbs or inclusion of a single herb, formula 64A exhibited the highest activity at 40.05 ± 0.61%, a significantly greater NO radical scavenging effect compared to 33A (21.89 ± 0.90%).

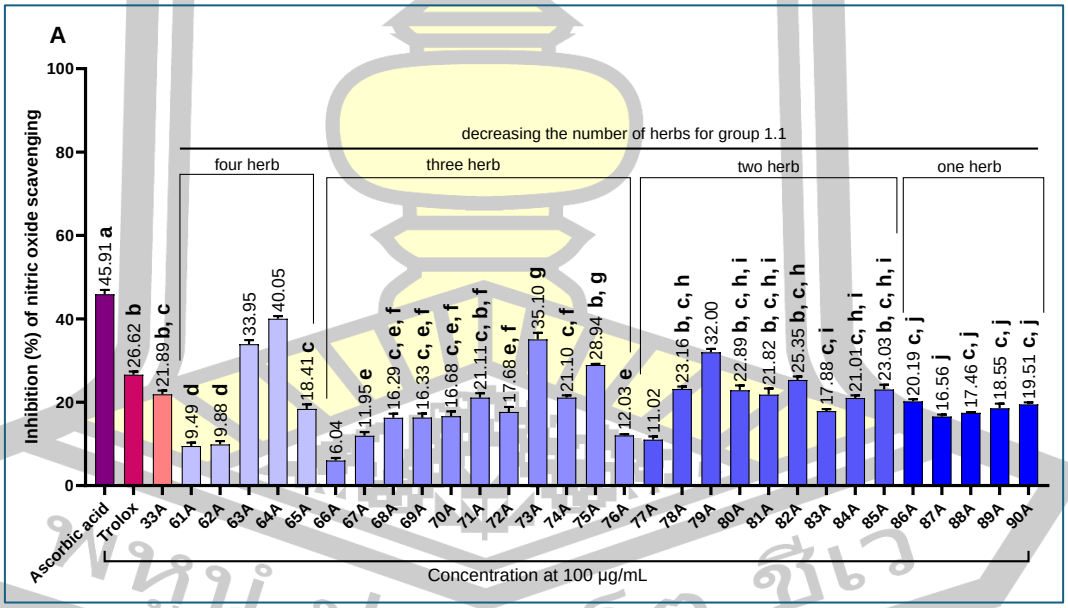
In the $O_2^{\bullet-}$ radical scavenging assay (**Figure 112B**), formulas 61A-90A exhibited $O_2^{\bullet-}$ radical scavenging activity ranging from 35.07% to 92.60%. Removing

a single herb, formulas 87A, 88A, 89A and 90A showed high scavenging effects. Moderate $O_2^{\bullet-}$ radical scavenging activity was observed for 86A.

With the removal of two herbs, formulas 77A, 78A, 79A, 82A, 83A, 84A and 85A showed high effects. Moderate $O_2^{\bullet-}$ radical scavenging activity was observed for 70A and 71A.

With the removal of three herbs, formulas 67A, 68A, 69A, 72A, 73A, 74A ,75A and 76A showed high effects. Moderate $O_2^{\bullet-}$ radical scavenging activity was observed for 80A and 81A. Formula 66A showed weak $O_2^{\bullet-}$ radical scavenging activity.

With the removal of four herbs or inclusion of a single herb, formulas 63A (*F. racemosa*), 64A (*H. perforate*) and 65A (*T. triandra*) showed high effects. Moderate $O_2^{\bullet-}$ radical scavenging activity was observed for 61A (*C. micracantha*). Formula 62A (*C. indicum*) showed weak $O_2^{\bullet-}$ radical scavenging activity.



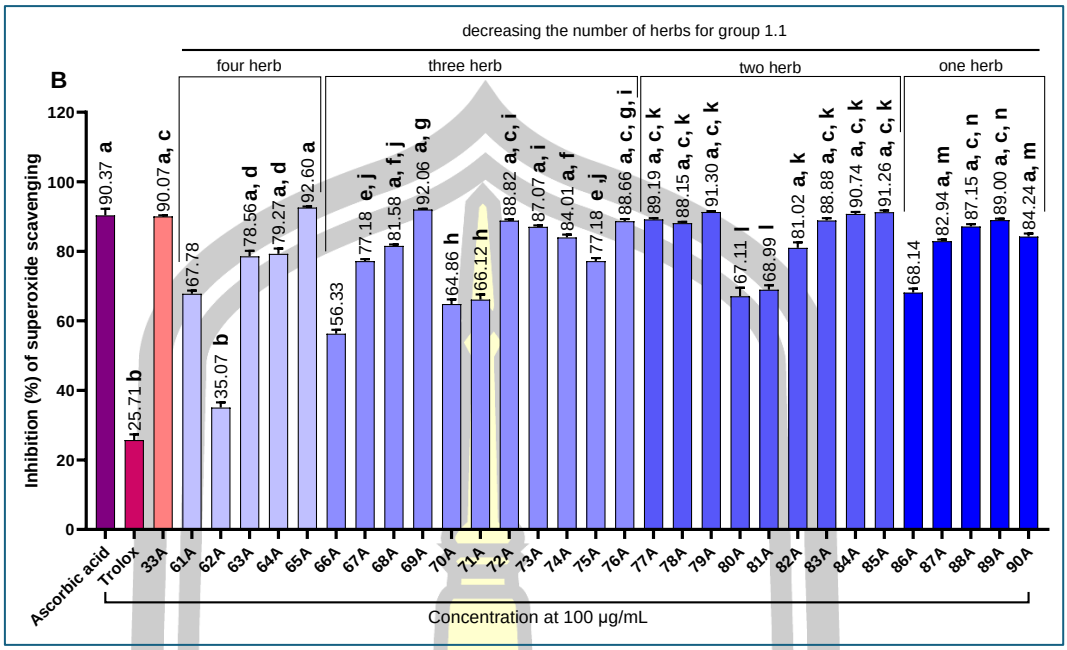


Figure 112 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of modified MHR aqueous extract by decreasing the number of herbs in reduced-toxic fever herbs (subgroup 1.1, 61-90A).
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.6.3 Antioxidant activity of adjunct herbs

4.6.3.1 Antioxidant activity of adjunct herbs ethanolic extract

In the adjunct herbs (AH), modifications were made to the number of herbs while fixing the primary herbs. Formula 03E involved a reduction in the supportive herbs, while formula 91E contained only the adjunct herbs. Formulas 11E-14E introduced variations in the number of herbs within the AH subgroup. Formulas 15E-28E focused on modifying the number of herbs in subgroup 2.2 (sour-astringent laxative) within the AH. Specifically, formulas 15E-18E reduced three herbs, formulas 19E-24E reduced two herbs, and formulas 25E-28E reduced one herb. Formulas 29E-30E involved changes in the number of herbs for the subgroup 2.1 (stimulant laxative) within the AH. Additionally, formulas 52E-57E were modified by fixing reduced-toxic fever herbs and adding one of the adjunct herbs.

1) Antioxidant activity of modified MHR by removing the number of adjunct herbs

In the NO radical scavenging assay (**Figure 113A**) at a concentration of 100 µg/mL, removing the number of herbs in the supportive herbs (03E) (60.40 ± 0.64%) did not significantly reduce the NO radical scavenging effect compared to MHR (01E) (61.93 ± 0.93%) and the primary herbs alone (PH, 02E) (56.23 ± 0.75%). Moreover, the formula containing only the adjunct herbs (91E) (62.54 ± 0.16%) demonstrated a significantly greater NO radical scavenging effect compared to PH. Similarly, in the $O_2^{\bullet-}$ radical scavenging assay (**Figure 113B**) at the same concentration, removing the number of herbs in the supportive herbs (03E) (66.80 ± 0.61%) did not significantly reduce the $O_2^{\bullet-}$ radical scavenging effect compared to MHR (01E) (64.91 ± 0.72%) and the primary herbs alone (PH, 02E) (46.32 ± 1.36%). Additionally, the formula containing only the adjunct herbs (91E) (76.76 ± 0.66%) demonstrated a significantly greater $O_2^{\bullet-}$ radical scavenging effect compared to PH.

Formula 11E, which excluded herbs from subgroup 2.2 (sour-astringent laxative) while retaining the supportive herbs, demonstrated a significantly reduced antioxidant effect compared to MHR. Meanwhile, formula 13E, which excluded herbs from subgroup 2.1 (stimulant laxative) while maintaining the supportive herbs, did not show a significantly different antioxidant effect compared to MHR in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities.

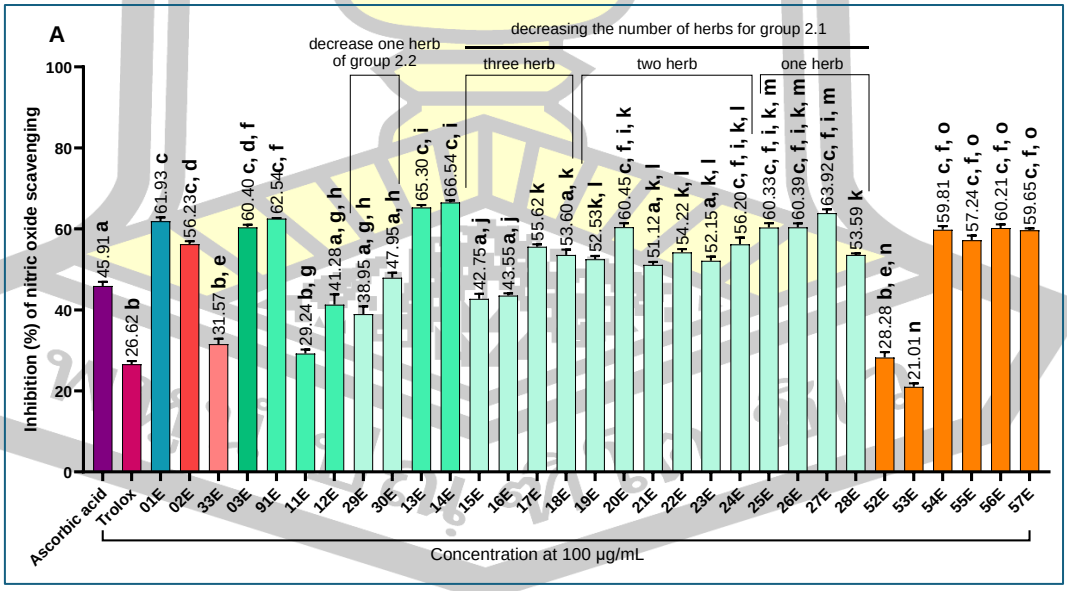
Excluding the herbs in subgroup 2.2 (12E) resulted in a significantly reduced antioxidant effect compared to 03E in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities. In contrast, excluding herbs from subgroup 2.1 (14E) did not significantly reduce the antioxidant effect compared to 03E in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities.

Removing a single herb from subgroup 2.1, formulas 29E (excluding *C. fistula*) and 30E (excluding *B. ovata*) resulted in significantly reduced NO radical scavenging and $O_2^{\bullet-}$ radical scavenging effects compared to 03E, with no significant differences among them. Most formulas with herbs removed from subgroup 2.2 showed a significant reduction in the NO radical scavenging effect compared to 03E. However, formulas 20E and 24E-27E did not exhibit a significant

reduction in NO radical scavenging effect compared to 03E. Moreover, most formulas with herbs removed from subgroup 2.2 showed a significant reduction in the $O_2^{\bullet-}$ radical scavenging effect compared to 03E. Meanwhile, formulas 20E, 24E and 25E did not exhibit a significant reduction in $O_2^{\bullet-}$ radical scavenging effect compared to 03E.

2) Antioxidant activity of modified MHR by fixing reduced-toxic fever herbs and varying of adjunct herbs

In the ethanolic extract, formulas 52E-57E (**Figure 113A-B**) were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) and varying of adjunct herbs compared to only reduced-toxic fever herbs (33E). The formulas 54E (33E plus *T. bellirica*), 55E (33E plus *T. chebula*), 56E (33E plus *Terminalia* sp.) and 57E (33E plus *P. emblica*) showed moderate effects in NO radical scavenging and high effects in $O_2^{\bullet-}$ radical scavenging activity. They also did not significant reduction in the antioxidant effect compared to 33E. Meanwhile, formulas 52E (33E plus *B. ovata*) and 53E (33E plus *C. fistula*) resulted in a weak antioxidant effect.



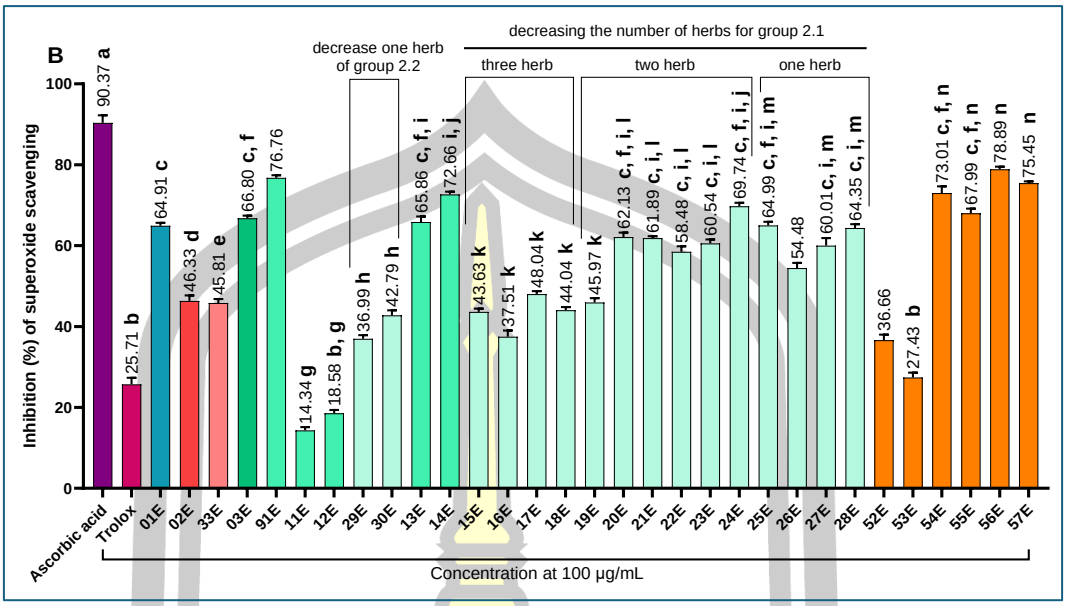


Figure 113 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of original MHR and modified MHR by modification of adjunct herbs.
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.6.3.2 Antioxidant activity of original MHR and modified MHR aqueous extract by modification of adjunct herbs

1) Antioxidant activity of modified MHR by removing the number of adjunct herbs

In the NO radical scavenging assay (**Figure 114A**) at a concentration of 100 µg/mL, removing the number of herbs in the supportive herbs (03A) (60.40 ± 0.64%) did not significantly reduce the NO radical scavenging effect compared to MHR (01A) (61.93 ± 0.93%) and the primary herbs alone (PH, 02A) (56.23 ± 0.75%). Additionally, the formula containing only the adjunct herbs (91A) (62.54 ± 0.16%) demonstrated a significantly greater NO radical scavenging effect compared to PH. Similarly, in the $O_2^{\bullet-}$ radical scavenging assay (**Figure 114B**) at the same concentration, removing the number of herbs in the supportive herbs (03A) (66.80 ± 0.61%) did not significantly reduce the $O_2^{\bullet-}$ radical scavenging effect compared to MHR (01A) (64.91 ± 0.72%) and the primary herbs alone (PH, 02A) (46.32 ± 1.36%). Additionally, the formula containing only the adjunct herbs (91A)

(76.76 ± 0.66%) demonstrated a significantly greater $O_2^{\bullet-}$ radical scavenging effect compared to PH.

Formula 11A, which excluded herbs from subgroup 2.2 (sour-astringent laxative) while retaining the supportive herbs, showed a significantly reduced antioxidant effect compared to MHR. In contrast, formula 13A, which excluded herbs from subgroup 2.1 (stimulant laxative) while maintaining the supportive herbs, did not show a significantly different antioxidant effect compared to MHR in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities.

Removing herbs from subgroup 2.2 (12A) led to a significantly reduced antioxidant effect compared to 03A in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities. In contrast, removing the herbs in subgroup 2.1 (14A) did not significantly reduce the antioxidant effect compared to 03A in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities.

Removing a single herb from subgroup 2.1, formulas 29A (excluding *C. fistula*) and 30A (excluding *B. ovata*) resulted in a significantly reduced NO radical scavenging and $O_2^{\bullet-}$ radical scavenging effects compared to 03A. In contrast, formulas with herbs removed from subgroup 2.2 did not show a reduction in the NO radical scavenging effect compared to 03A. However, most formulas with herbs removed from subgroup 2.2 showed a significant reduction in the $O_2^{\bullet-}$ radical scavenging effect compared to 03A. Notably, formulas 20A, 21A and 25A-27A did not exhibit a significant reduction in $O_2^{\bullet-}$ radical scavenging effect compared to 03A.

2) Antioxidant activity of modified MHR by fixing reduced-toxic fever herbs and varying of adjunct herbs

Formulas 52A-57A (**Figure 114A-B**) were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) and varying of adjunct herbs compared to only reduced-toxic fever herbs (33A). The formulas 54A (33A plus *T. bellirica*), 55A (33A plus *T. chebula*), 56A (33A plus *Terminalia* sp.) and 57A (33A plus *P. emblica*) showed moderate effects in NO radical scavenging and high effects in $O_2^{\bullet-}$ radical scavenging activity. They also did not significant reduction in the antioxidant effect compared to 33A. Meanwhile, formula 52A (33A plus *B. ovata*) showed a significantly reduced NO radical scavenging effect but did not significantly reduce the $O_2^{\bullet-}$ radical scavenging effect compared to 33A.

Lastly, formula 53A (33A plus *C. fistula*) did not significantly reduce the antioxidant effect compared to 33A in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities.

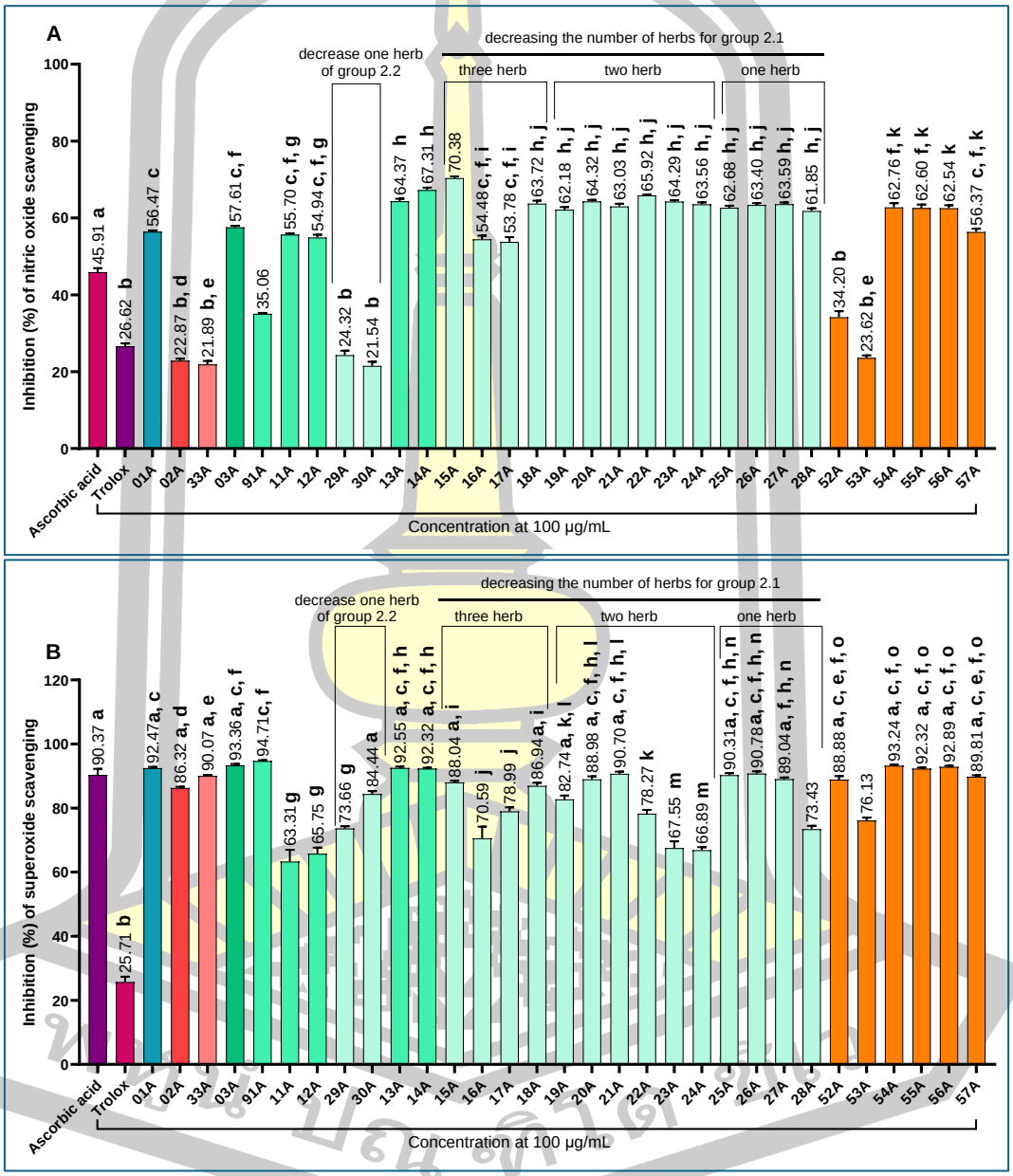


Figure 114 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of aqueous extract of original MHR and modified MHR by modification of adjunct herbs.
Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

4.6.4 Antioxidant activity of original MHR and modified MHR by modification of supportive herbs

4.6.4.1 Antioxidant activity of original MHR and modified MHR ethanolic extract by modification of supportive herbs

Modifications to the supportive herbs (SH) involved adjusting the number of herbs while keeping the primary and adjunct herbs constant. Formula 10E involved removing the adjunct herbs, formula 92E contains only the supportive herbs, while formulas 04E-09E adjust the number of herbs within the SH group. Additionally, formulas 58E-60E were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of supportive herbs compared to only reduced-toxic fever herbs (33E).

In the ethanolic extract (**Figure 115A**) at a concentration of 100 µg/mL, removing the number of herbs in the adjunct herbs (10E) (33.00 ± 0.74%) resulted in a significantly reduced nitric oxide (NO) radical scavenging effect compared to MHR (61.93 ± 0.93%). This reduction was also significantly greater than that observed with the reduction of herbs in the supportive herbs (03E) (60.40 ± 0.64%). The formula containing only the supportive herbs (92E) (28.00 ± 0.75%) showed a significantly lower effect than the primary herbs alone (PH, 02E) (56.23 ± 0.75%).

As shown in **Figure 115B**, removing the number of herbs in the adjunct herbs (10E) (28.74 ± 1.35%) resulted in a significantly reduced superoxide anion ($O_2^{\bullet-}$) scavenging effect compared to MHR (64.91 ± 0.72%), with a significantly lower effect than that of removing the number of herbs in the supportive herbs (03E) (66.80 ± 0.61%). The formula containing only the supportive herbs (92E) (28.05 ± 0.76%) showed a significantly lower effect than the primary herbs alone (PH, 02E) (46.33 ± 1.36%).

Removing herbs from SH, formulas 04E-09E showed moderate effects with no significant reduction in antioxidant activity compared to MHR in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities. However, formulas 08E and 09E exhibited a significantly lower $O_2^{\bullet-}$ radical scavenging effect compared to MHR, while also demonstrating a significantly higher antioxidant effect than 03E.

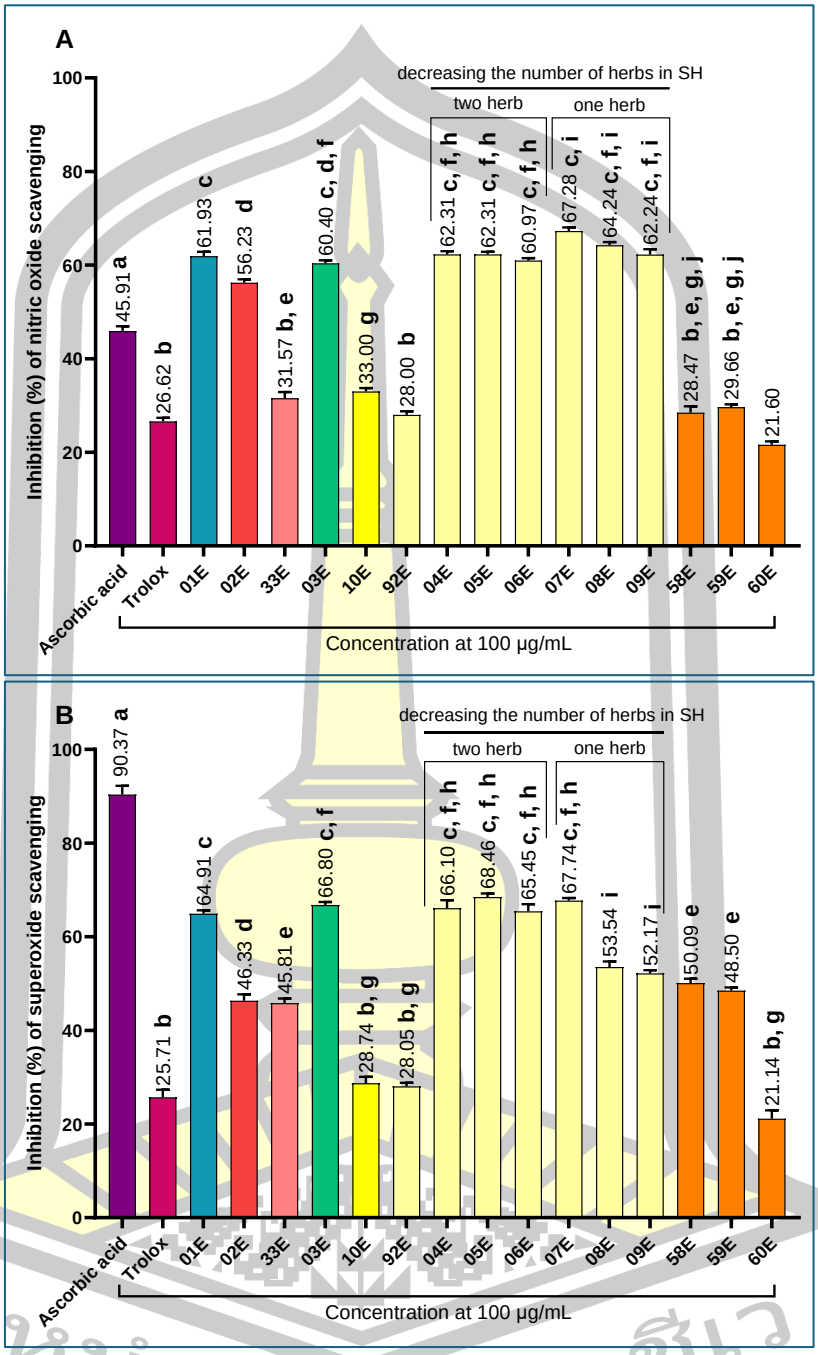


Figure 115 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of ethanolic extract of original MHR and modified MHR by modification of supportive herbs.

Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

Additionally, formulas 58E-60E were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of the supportive herbs compared to only reduced-toxic fever herbs (33E). The formulas 58E (33E plus *M. ferrea*) and 59E (33E plus *N. nucifera*) did not show a significant reduction in antioxidant activity compared to 33E in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities. Meanwhile, formula 60E (33E plus *V. zizanioides*) resulted in significantly reduced NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities compared to 33E.

4.6.4.2 Antioxidant activity of original MHR and modified MHR aqueous extract by modification of supportive herbs

In the aqueous extract (**Figure 116A**) at a concentration of 100 µg/mL, removing the number of herbs in the adjunct herbs (10A) (54.03 ± 0.46%) did not result in a significantly reduced nitric oxide (NO) radical scavenging effect compared to MHR (56.47 ± 0.29%) and 03A (supportive herbs reduction) (57.61 ± 0.36%). The formula containing only the supportive herbs (92A) (35.06 ± 0.23%) showed a significantly higher effect than the primary herbs alone (PH, 02A) (22.87 ± 0.53%).

As shown in **Figure 116B**, removing the number of herbs in the adjunct herbs (10A) (75.47 ± 2.14%) resulted in a significantly reduced superoxide anion ($O_2^{\bullet-}$) scavenging effect compared to MHR (92.47 ± 0.40%), with a significantly lower effect than that of removing the number of herbs in the supportive herbs (03A) (93.36 ± 0.37%). The formula containing only the supportive herbs (92A) (92.71 ± 0.33%) showed a significantly higher effect than the primary herbs alone (PH, 02A) (86.32 ± 0.31%).

Removing herbs from SH, formulas 04A-09A showed moderate effects with no significant reduction in NO radical scavenging activity compared to MHR. Meanwhile, formulas 04A-09A showed high effects with no significant reduction in $O_2^{\bullet-}$ radical scavenging activity compared to MHR.

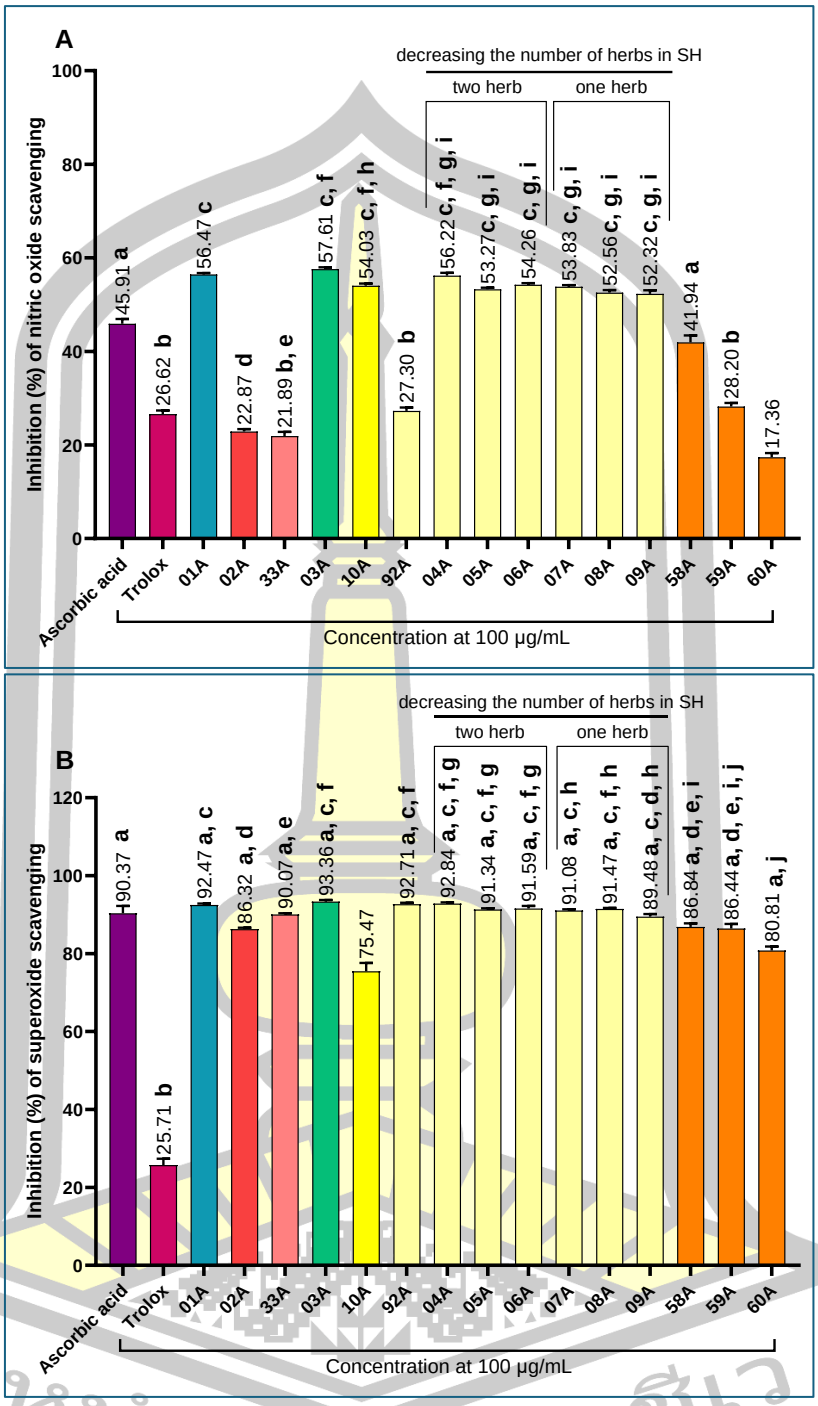


Figure 116 Nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities of aqueous extract of original MHR and modified MHR by modification of supportive herbs.

Values are expressed as mean ± SEM (n=9). Bars with the same letters indicate no significant differences ($p<0.05$).

Additionally, formulas 58A-60A were modified MHR remedies by fixing a subgroup of primary herbs (reduced-toxic fever herbs) plus one of the supportive herbs compared to only reduced-toxic fever herbs (33A). The formulas 58A (33A plus *M. ferrea*) and 59A (33A plus *N. nucifera*) did not significant reduction in antioxidant activity compared to 33A in both NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities. Lastly, formula 60A (33A plus *V. zizanioides*) resulted in significantly reduced NO radical scavenging and $O_2^{\bullet-}$ scavenging activities compared to 33A.

4.6.5 Antioxidant activity of major compounds of MHR

The marker compounds in (Mo-Ha-Rak) MHR, include bergenin, chebulagic acid, chebulanin, chebulic acid, chlorogenic acid, corilagin, ellagic acid, gallic acid, lourierin A, pectolinarigenin, protocatechuic acid, resveratrol, rhein, as well as the isolated compound, perforatic acid, *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether and TT01.

In nitric oxide radical scavenging activity, corilagin demonstrated the highest potency with an IC_{50} value of 5.42 ± 0.25 µg/mL, followed by resveratrol and gallic acid with IC_{50} values of 5.99 ± 0.38 µg/mL and 8.01 ± 0.56 µg/mL, respectively. The second-highest nitric oxide radical scavenging activities were observed for chlorogenic acid, chebulagic acid, bergenin, rhein, and pectolinarigenin, with IC_{50} values of 11.51 ± 0.35, 12.15 ± 0.31, 14.74 ± 0.16, 20.05 ± 0.58, and 27.33 ± 1.56 µg/mL, respectively, all of which were more effective than ascorbic acid (positive control), which had an IC_{50} > 100 (102.41 ± 0.49 µg/mL). In contrast, nine marker compounds, including chebulanin, chebulic acid, ellagic acid, lourierin A, *O*-methyllaloptaeroxyrin, perforatic acid, peucenin-7-methyl ether, protocatechuic acid, and TT01, showed no measurable activity (IC_{50} > 100 µg/mL), as shown in **Table 20** and **Figure 117A**.

In superoxide radical scavenging activity, ellagic acid exhibited the highest potency, with an IC_{50} value of 6.89 ± 0.03 µg/mL, followed by protocatechuic acid at 6.99 ± 0.26 µg/mL. The second highest superoxide radical scavenging activities were observed for chebulic acid, chebulagic acid, chlorogenic acid, pectolinarigenin, corilagin, gallic acid, and lourierin A, with IC_{50} values of 24.80 ± 0.89, 25.20 ± 0.46, 28.35 ± 1.79, 28.89 ± 1.69, 29.55 ± 1.33, 34.97 ± 0.61, and 36.11 ± 2.53 µg/mL,

respectively. These compounds were more effective than ascorbic acid (positive control), which had an IC_{50} of 40.92 ± 0.38 µg/mL. Moderate superoxide radical scavenging activity was observed for rhein, with an IC_{50} value of 54.73 ± 1.92 µg/mL. Two major compounds, chebulanin and TT01, showed weak superoxide radical scavenging activity with IC_{50} values of 72.75 ± 0.80 and 83.52 ± 1.09 µg/mL, respectively. Four marker compounds, *O*-methyllaloptaeroxyrin, perforatic acid, peucenin-7-methyl ether, and resveratrol, showed no measurable activity (IC_{50} > 100 µg/mL), as shown in **Table 21** and **Figure 117B**.

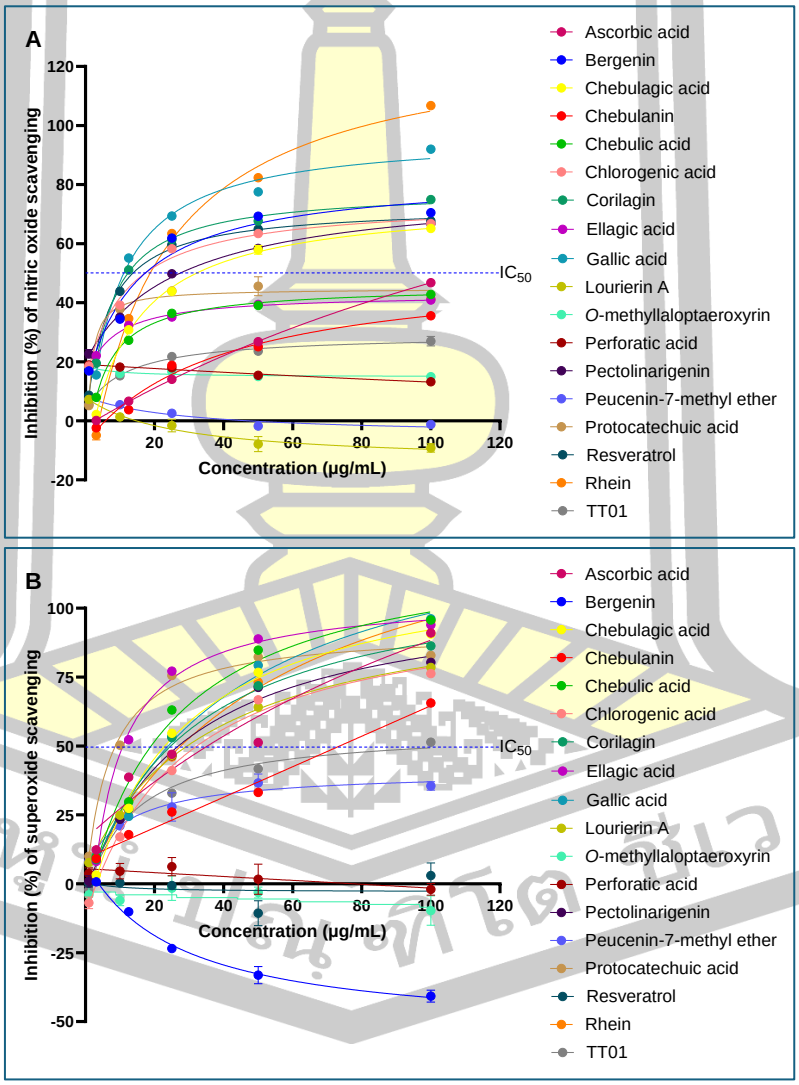


Figure 117 Comparison of nitric oxide radical scavenging (A) and superoxide radical scavenging (B) activities in the major compounds at various concentrations. Values are expressed as mean ± SD (n=3).

Table 20 Nitric oxide radical scavenging activity of the major compounds in MHR.

Marker compounds	**% Inhibition of nitric oxide radical**							**IC_{50} (µg/mL)**
	100 µg/mL	**50 µg/mL**	**25 µg/mL**	**12.5 µg/mL**	**10 µg/mL**	**3.125 µg/mL**	**1 µg/mL**	
Bergenin	70.48 ± 0.63	69.32 ± 0.47	61.91 ± 0.26	-	34.42 ± 0.68	-	16.92 ± 0.65	14.74 ± 0.16
Chebulagic acid	65.12 ± 0.49	57.89 ± 1.24	43.85 ± 0.73	30.84 ± 0.43	-	2.16 ± 0.35	-	12.15 ± 0.31
Chebulanin	35.57 ± 0.42	25.09 ± 0.35	18.77 ± 0.33	3.76 ± 0.13	-	-2.36 ± 0.02	-	> 100
Chebulic acid	42.83 ± 0.22	39.08 ± 0.14	36.42 ± 0.24	27.29 ± 0.07	-	7.95 ± 0.27	-	> 100
Chlorogenic acid	66.79 ± 0.38	63.44 ± 0.31	58.27 ± 0.28	-	39.19 ± 0.39	-	18.55 ± 0.42	11.51 ± 0.35
Corilagin	74.90 ± 0.22	67.68 ± 0.58	61.33 ± 0.33	51.12 ± 0.32	-	19.50 ± 0.07	-	5.42 ± 0.25
Ellagic acid	40.88 ± 0.38	39.40 ± 0.78	35.14 ± 0.17	32.29 ± 0.22	-	22.08 ± 0.22	-	> 100
Gallic acid	91.99 ± 0.96	77.52 ± 0.72	69.33 ± 0.26	55.12 ± 0.21	-	15.54 ± 0.30	-	8.01 ± 0.56
Lourierin A	-9.18 ± 1.17	-7.90 ± 2.08	-1.60 ± 1.73	-	1.33 ± 0.93	-	7.24 ± 0.15	> 100
O-methyllalop taeroxyrin	14.96 ± 0.77	14.98 ± 0.74	16.73 ± 0.55	-	15.99 ± 0.80	-	18.33 ± 0.40	> 100
Pectolinarigenin	66.76 ± 0.49	58.37 ± 0.49	49.80 ± 0.77	-	35.10 ± 0.61	-	22.73 ± 1.16	27.33 ± 1.56
Perforatic acid	13.19 ± 0.13	15.42 ± 0.10	17.98 ± 0.79	-	18.21 ± 0.73	-	18.93 ± 0.21	> 100
Peucenin-7-methyl ether	-1.26 ± 0.36	-1.77 ± 0.38	2.53 ± 0.71	-	5.44 ± 0.70	-	7.38 ± 0.77	> 100
Protocatechuic acid	41.31 ± 1.12	45.58 ± 2.59	43.97 ± 0.72	-	37.81 ± 0.42	-	5.18 ± 0.64	> 100
Resveratrol	67.72 ± 0.16	64.93 ± 0.24	59.62 ± 0.49	-	43.84 ± 0.80	-	8.63 ± 0.46	5.99 ± 0.38
Rhein	106.75 ± 0.26	82.34 ± 0.22	63.42 ± 0.46	34.60 ± 0.26	-	-4.92 ± 1.28	-	20.05 ± 0.58
TT01	26.98 ± 1.33	23.61 ± 0.40	21.74 ± 0.47	-	15.26 ± 0.20	-	5.55 ± 0.61	> 100
Ascorbic acid (Positive control)	46.79 ± 0.95	26.79 ± 0.78	14.05 ± 0.14	6.64 ± 0.17	-	0.14 ± 0.24	-	> 100 (102.41 ± 0.49)

Note: All data represents the mean ± SD in triplicate experiments.

Table 21 Superoxide radical scavenging activity of the major compounds in MHR.

Marker compounds	**% Inhibition of superoxide radical**							**IC_{50} (μg/mL)**
	100 μg/mL	**50 μg/mL**	**25 μg/mL**	**12.5 μg/mL**	**10 μg/mL**	**3.125 μg/mL**	**1 μg/mL**	
Bergenin	-40.76 ± 1.79	-33.17 ± 2.54	-23.48 ± 0.88	-	-10.16 ± 0.92	-	0.74 ± 0.75	> 100
Chebulagic acid	90.61 ± 0.64	76.75 ± 0.22	54.63 ± 0.22	27.38 ± 0.05	-	3.21 ± 0.29	-	25.20 ± 0.46
Chebulanin	65.61 ± 0.36	33.17 ± 0.39	26.05 ± 0.33	17.86 ± 0.44	-	9.14 ± 0.56	-	72.75 ± 0.80
Chebulic acid	95.73 ± 0.19	84.79 ± 0.48	63.10 ± 0.37	29.71 ± 0.33	-	9.69 ± 0.53	-	24.80 ± 0.89
Chlorogenic acid	76.26 ± 0.59	66.78 ± 0.33	41.08 ± 0.63	-	17.05 ± 0.62	-	6.94 ± 1.73	28.35 ± 1.79
Corilagin	86.34 ± 0.43	71.86 ± 0.41	53.02 ± 0.98	27.07 ± 0.40	-	8.80 ± 0.29	-	29.55 ± 1.33
Ellagic acid	93.90 ± 0.16	88.86 ± 0.18	77.17 ± 0.42	52.26 ± 0.19	-	8.13 ± 0.40	-	6.89 ± 0.03
Gallic acid	96.17 ± 0.30	79.29 ± 0.62	53.49 ± 1.06	24.48 ± 0.71	-	10.53 ± 0.53	-	34.97 ± 0.61
Lourierin A	78.49 ± 0.60	64.08 ± 0.47	46.64 ± 0.19	-	24.89 ± 1.10	-	7.70 ± 1.02	36.11 ± 2.53
O-methyllalop taeroxyrin	-9.72 ± 4.38	-3.15 ± 1.62	-1.58 ± 3.62	-	-6.01 ± 1.56	-	-3.97 ± 2.49	> 100
Pectolinarigenin	80.35 ± 0.56	71.29 ± 0.31	46.49 ± 0.94	-	23.40 ± 0.92	-	0.88 ± 1.65	28.89 ± 1.69
Perforatic acid	-2.05 ± 1.72	1.66 ± 4.46	6.26 ± 2.69	-	4.60 ± 2.32	-	3.77 ± 1.67	> 100
Peucenin-7-methyl ether	35.50 ± 1.27	36.56 ± 2.67	27.91 ± 4.22	-	21.05 ± 0.51	-	8.30 ± 1.10	> 100
Protocatechuic acid	82.88 ± 0.35	82.28 ± 0.14	75.59 ± 0.31	-	50.34 ± 0.94	-	9.95 ± 0.43	6.99 ± 0.26
Resveratrol	2.98 ± 3.76	-10.65 ± 3.37	-0.61 ± 0.79	-	0.35 ± 1.15	-	0.12 ± 0.36	> 100
Rhein	95.31 ± 0.73	73.19 ± 0.18	46.02 ± 0.43	26.49 ± 0.53	-	9.17 ± 0.15	-	54.73 ± 1.92
TT01	51.42 ± 0.51	41.70 ± 0.58	32.91 ± 0.62	-	23.59 ± 0.18	-	-2.22 ± 1.77	83.52 ± 1.09
Ascorbic acid (Positive control)	91.03 ± 0.37	51.24 ± 1.17	46.98 ± 0.72	38.67 ± 0.53	-	12.36 ± 0.40	-	40.92 ± 0.38

Note: All data represents the mean ± SD in triplicate experiments.

4.7 Investigation of the relationship between experimental parameters

To assess relationships between various parameters, this study employed ethanolic extracts for consistency. Parameters included inhibition of pharmacological effects, cell viability, and peak area of chemical markers in HPLC analysis of original MHR (Mo-Ha-Rak) and modified MHR formulas. Pearson's correlation coefficients (r) were calculated using a bioinformatics website (http://www.bioinformatics.com.cn/) to assess linear relationships between all parameter pairs. As a reminder, r ranges from -1 (perfect negative correlation) to 1 (perfect positive correlation), with 0 indicating no linear association. Principal component analysis (PCA) was visualized using GraphPad Prism v10.1.0 software for dimensionality reduction and data exploration. PCA is a dimensionality reduction and machine learning method used to simplify a large data set into a smaller set while still maintaining significant patterns and trends. Additionally, Python v3.5 was employed to generate a clustering heat map for further visualization of relationship patterns.

4.7.1 Relationship between pharmacological effects

The relationship between pharmacological effects, including anti-inflammatory activities, cell viability, nitric oxide scavenging activities, and superoxide scavenging activities, was analyzed using a combination of a Pearson rank correlation and the loading plot from principal component analysis (PCA) (**Figure 118A-B**). Results indicated an inverse relationship between anti-inflammatory activity and cell viability. In contrast, nitric oxide scavenging activity was positively correlated with superoxide scavenging activity. However, neither nitric oxide scavenging activity nor superoxide scavenging activity showed a significant correlation with anti-inflammatory activity or cell viability.

The hierarchical clustering heat map (**Figure 119**) of the 92 modified MHR formulas effectively distinguished patterns in cell viability, anti-inflammatory, nitric oxide scavenging activities, and superoxide scavenging. Based on the dissimilarity in % inhibition of pharmacological effects and % cell viability, three distinct clusters emerged. Cluster 1 (**Figure 119B1**) highlighted the primary herbs and included 31 formulas, along with two additional formulas featuring both adjunct and supportive herbs. This cluster demonstrated high anti-inflammatory activity and

moderate cell viability but relatively low nitric oxide and superoxide scavenging activities, as shown in **Figure 119A**.

Cluster 2 (**Figure 119B2**) emphasized the primary herbs, consisting of 21 formulas, along with 6 formulas featuring adjunct herbs and 3 formulas with supportive herbs. This cluster exhibited high cell viability, but weak anti-inflammatory, nitric oxide scavenging, and superoxide scavenging activities, as shown in **Figure 119A**.

Cluster 3 (**Figure 119B3**) highlighted the adjunct herbs, consisting of 19 formulas, along with 6 formulas of supportive herbs and the original MHR. This cluster exhibited high cell viability, moderate nitric oxide scavenging, but weak superoxide scavenging activities, as shown in **Figure 119A**.

However, formula 65E (*T. triandra*) was an outlier, exhibiting high anti-inflammatory activity and moderate superoxide scavenging activity, but weak cell viability and nitric oxide scavenging, as shown in **Figure 119A**.

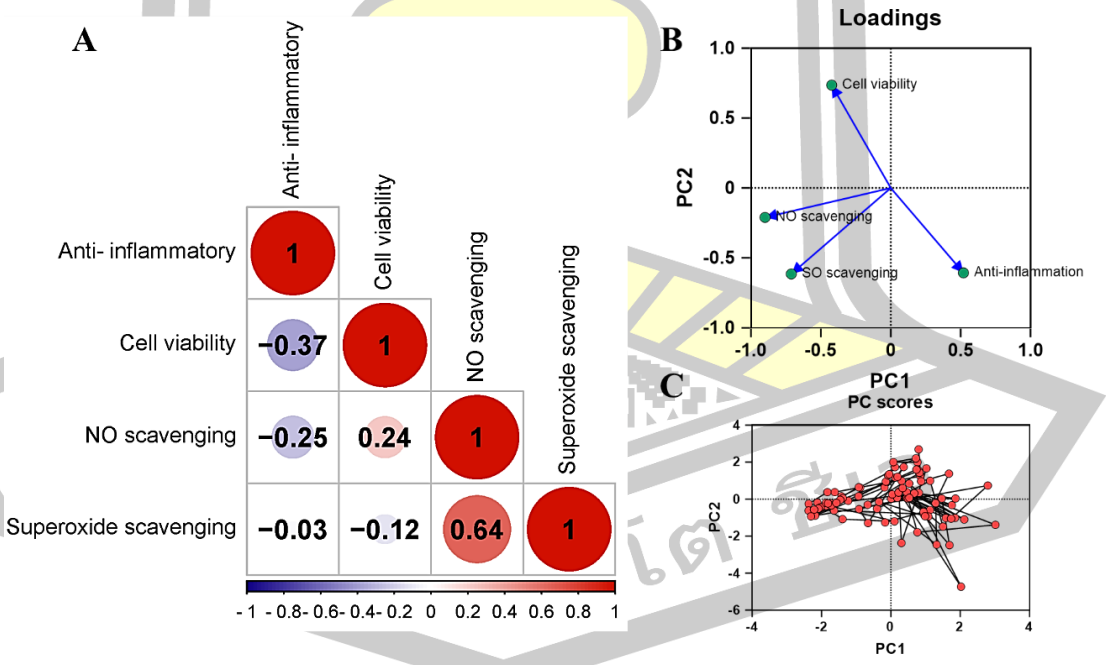


Figure 118 Correlation analysis between pharmacological effects. (A) Pearson's rank correlation plot, (B) PCA loading plot and (C) PCA score plot (n=92).

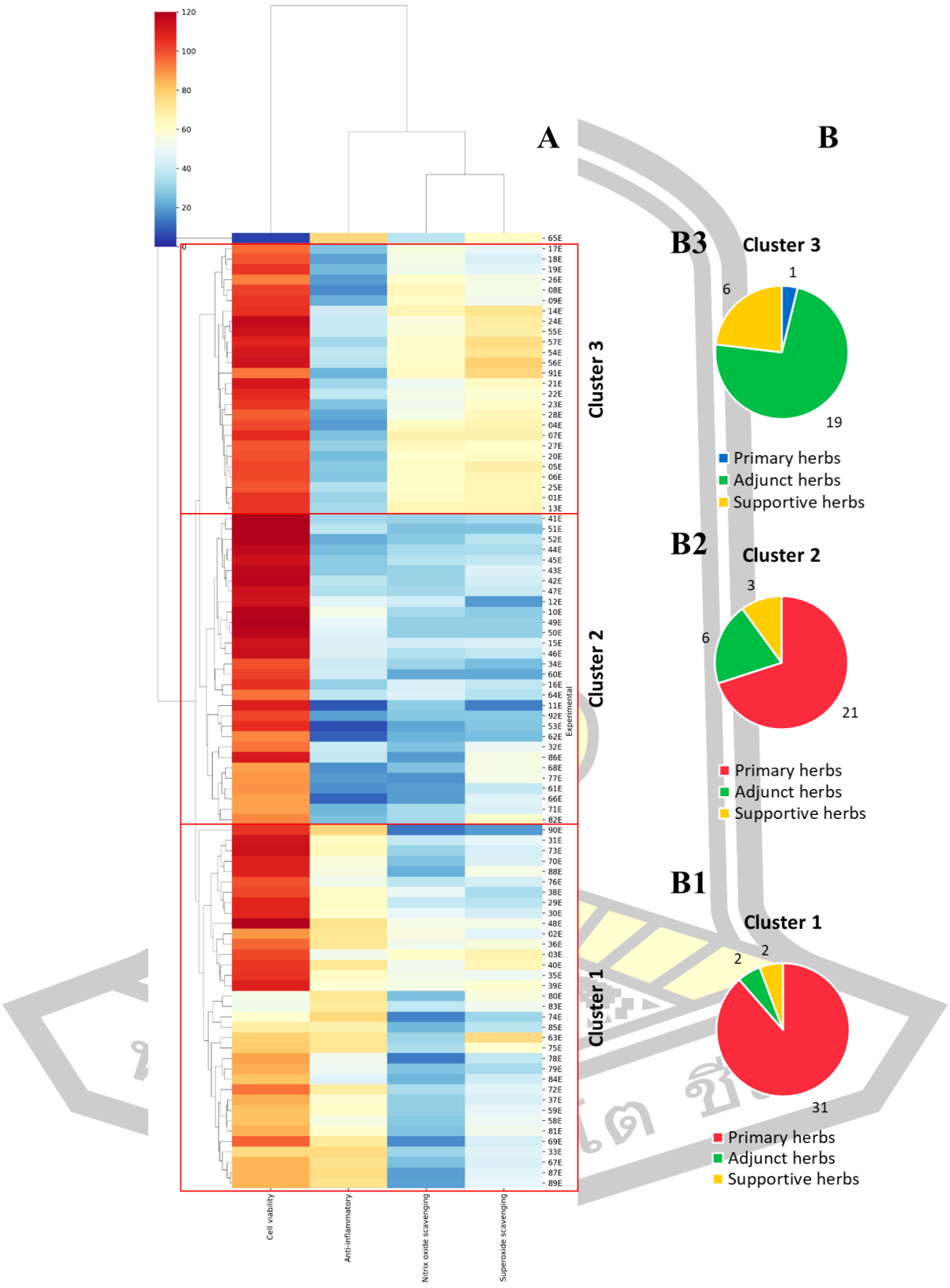


Figure 119 Heatmap cluster analysis for exploring the role of 92 modified MHR formulas in relation to their pharmacological effects.

4.7.2 Relationship between chemical markers

The chemical markers were identified by comparing their retention times and UV spectra of all components with MHR remedy using HPLC analysis. All reference standards were confirmed by the spiking method. The chemical markers of the primary herbs (PH) included 19 markers, namely TH01, AI01, TC01, LS01, PK01, PK02, CM01, GC01, GC02, GC03, TT01, *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether, pectolinarigenin, perforatic acid, bergenin, chlorogenic acid, resveratrol, and lourierin A. For the adjunct herbs (AH), a total of 12 markers were identified, including PE01, BO01, TCb01, TB01, Ts01, chebulagic acid, chebulanin, chebulic acid, corilagin, ellagic acid, gallic acid, and rhein. Additionally, the supportive herbs (SH) showed five chemical markers: MF02, NN01, VZ01, gallic acid, and protocatechuic acid.

Pearson's rank correlation analysis between the primary herbs (PH) and the adjunct herbs (AH) revealed that most chemical markers were negatively correlated. However, rhein from AH showed positively correlated between some chemical markers from PH including AI01 (r=0.26), TC01 (r=0.33), PK01 (r=0.39), PK02 (r=0.23), GC01 (r=0.33), GC02 (r=0.22), resveratrol (r=0.31), and lourierin A (r=0.28), as shown in **Figure 120**.

Correlation analysis between the primary herbs (PH) and the supportive herbs (SH) revealed that most chemical markers were negatively correlated, as shown in **Figure 121**.

Additionally, the correlation analysis between the adjunct herbs (AH) and the supportive herbs (SH) indicated that most chemical markers were not correlated, as shown in **Figure 122**. However, gallic acid, a chemical marker found in both AH and SH, showed a positive correlation with some chemical markers from AH. Lastly, the chemical markers within each group (PH, AH, and SH) showed positive correlations among themselves.

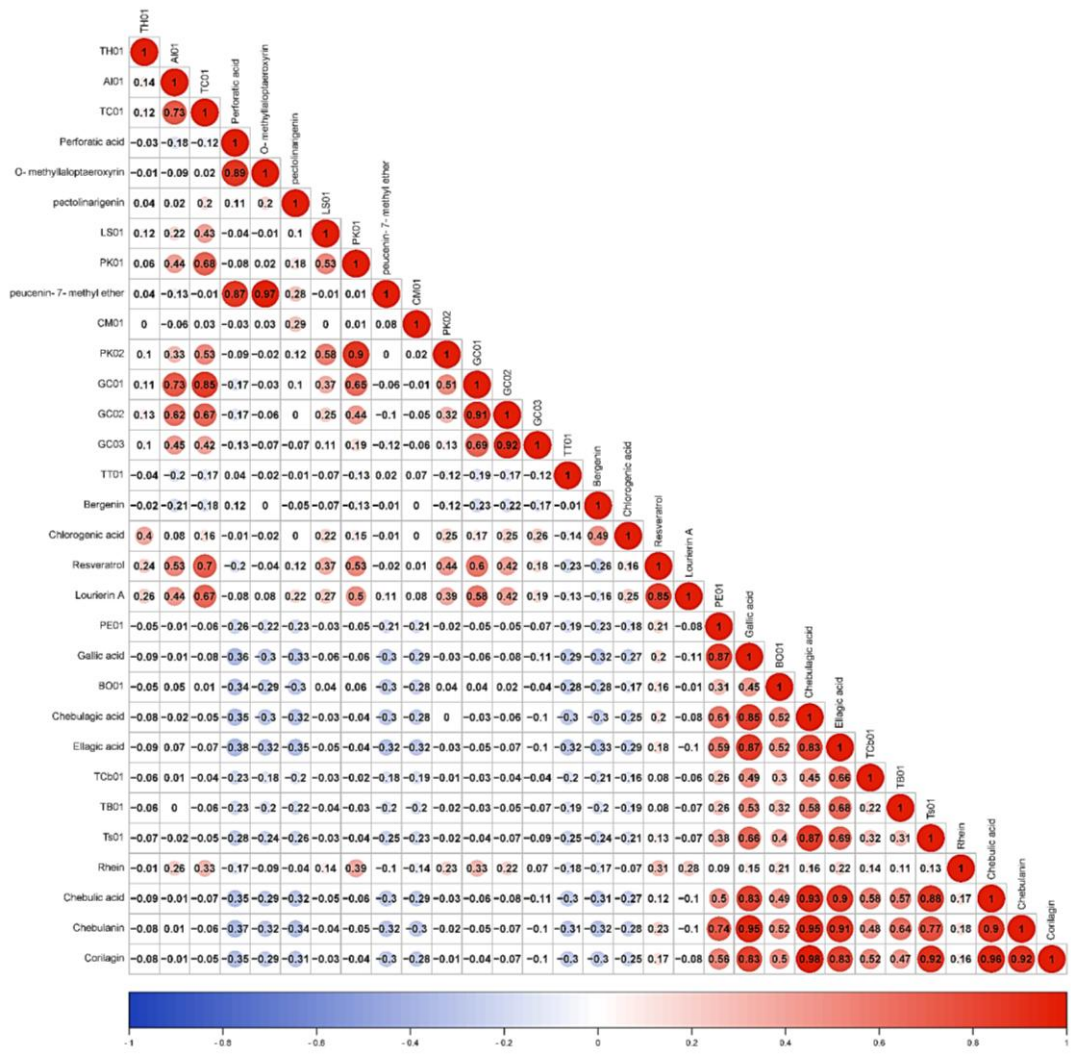


Figure 120 Pearson's rank correlation analysis between chemical markers of the primary herbs (PH) and the adjunct herbs (AH).

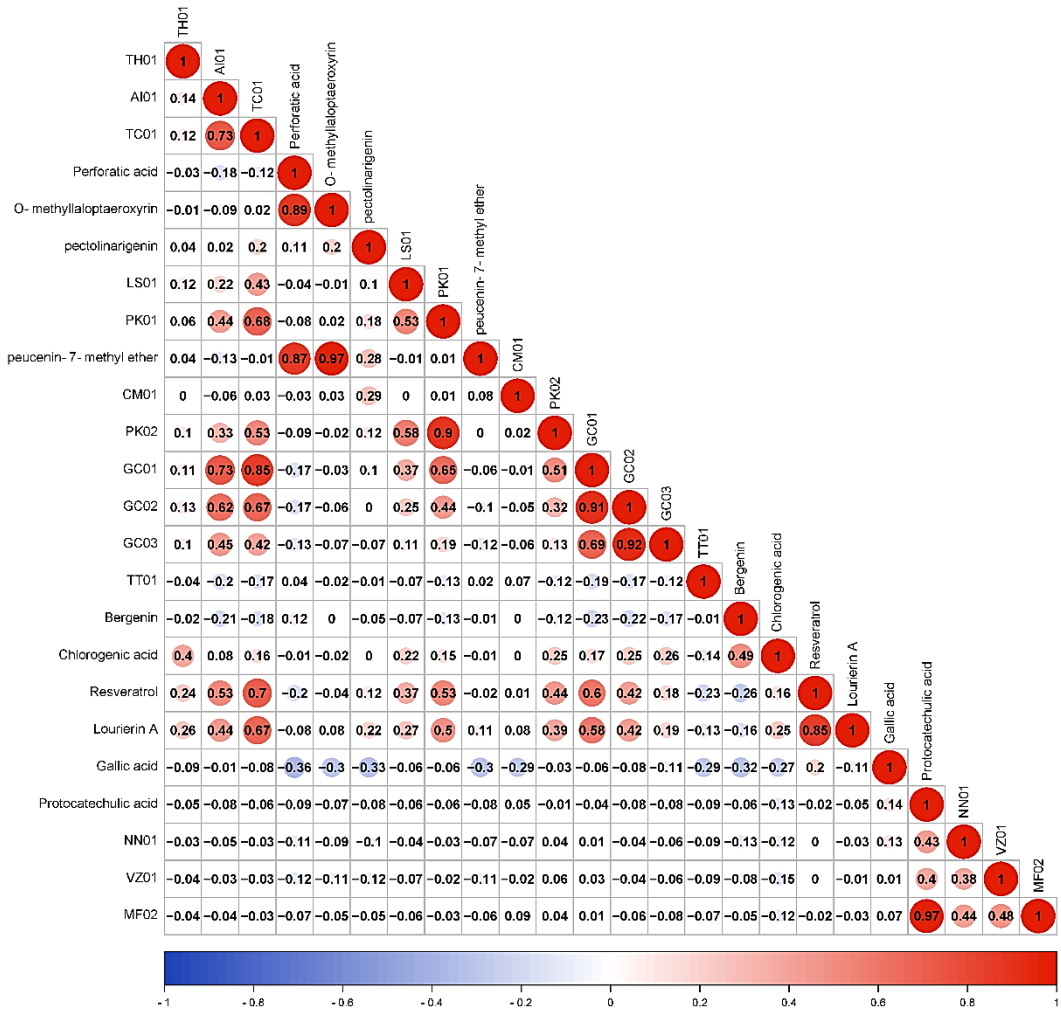


Figure 121 Pearson's rank correlation analysis between chemical markers of the primary herbs (PH) and the supportive herbs (SH).

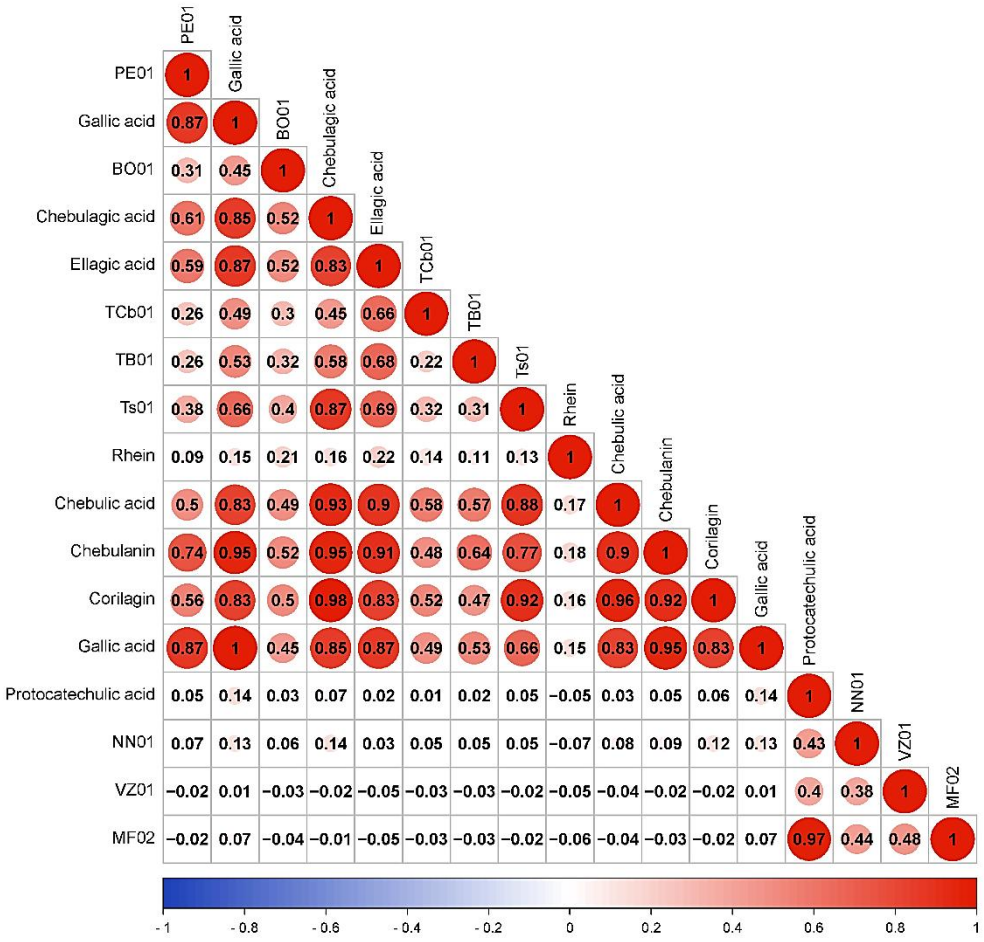


Figure 122 Pearson's rank correlation analysis between chemical markers of the adjunct herbs (AH) and the supportive herbs (SH).

4.7.3 Relationship between pharmacological effects and chemical markers

4.7.3.1 Relationship between pharmacological effects and chemical markers in the primary herbs

The primary herbs (PH) contained 19 chemical markers, including TH01, AI01, TC01, LS01, PK01, PK02, CM01, GC01, GC02, GC03, TT01, as well as *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether, pectolinarigenin, perforatic acid, bergenin, chlorogenic acid, resveratrol, and lourierin A.

Pearson's rank correlation analysis between the amount of chemical markers of PH and anti-inflammatory activity (**Figure 123**) revealed that most chemical markers were positively correlated with anti-inflammatory effects. Among these, TC01 exhibited the highest positive correlation with anti-inflammatory activity, with an r-value of 0.52, followed by bergenin, which showed a positive correlation with an r-value of 0.36. Meanwhile, Pearson's Rho analysis indicated that the %inhibition in anti-inflammatory activity increased with the amount of chemical markers. Among these, lourierin A and GC01 exhibited the highest positive correlation with anti-inflammatory activity, with rho-values of 0.82, followed by PK02 with a rho-value of 0.79, as shown in **Figure 124**.

In cell viability, Pearson's rank correlation analysis between the amount of chemical markers of PH and cell viability (**Figure 123**) revealed that several chemical markers were positively correlated with cell viability, including SA01, AI01, TC01, LS01, PK01, PK02, GC01, GC02, GC03, resveratrol, and lourierin A. In contrast, the correlation analysis showed that some chemical markers were negatively correlated with cell viability, including *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether, pectolinarigenin, CM01, TT01, perforatic acid, chlorogenic acid, and bergenin. Among these, TT01 exhibited the highest negative correlation with cell viability, with an r-value of -0.82. According, Pearson's Rho analysis indicated that TT01 show the % cell viability decreased with the amount of chemical markers. TT01 also exhibited the highest negative correlation with cell viability, with a rho-value of -0.75, as shown in **Figure 125**.

In nitric oxide scavenging, Pearson's rank correlation analysis between the amount of chemical markers of PH and nitric oxide scavenging (**Figure 123**) revealed that several chemical markers were positively correlated with nitric oxide

scavenging, including TH01, AI01, TC01, LS01, PK01, PK02, GC01, GC02, GC03, resveratrol, and lourierin A. Among these, resveratrol exhibited the highest positive correlation with nitric oxide scavenging, with an r-value of 0.56, followed by lourierin A, which showed a positive correlation with an r-value of 0.34. In contrast, the correlation analysis showed that some chemical markers were negatively correlated with cell viability, including *O*-methyllaloptaeroxyrin, peucenin-7-methyl ether, pectolinarigenin, CM01, TT01, perforatic acid, chlorogenic acid, and bergenin. However, Pearson's Rho analysis indicated that the %inhibition in nitric oxide scavenging activity decreased with the amount of chemical markers, as shown in **Figure 126**.

In superoxide scavenging, Pearson's rank correlation analysis between the amount of chemical markers of PH and superoxide scavenging (**Figure 123**) revealed that most chemical markers were negatively correlated with superoxide scavenging effects. Pearson's Rho analysis indicated that the %inhibition in superoxide scavenging activity decreased with the amount of chemical markers. Only resveratrol was responsible for the increase in %inhibition of superoxide scavenging activity, as its effect increased with the amount of chemical markers, as shown in **Figure 127**.

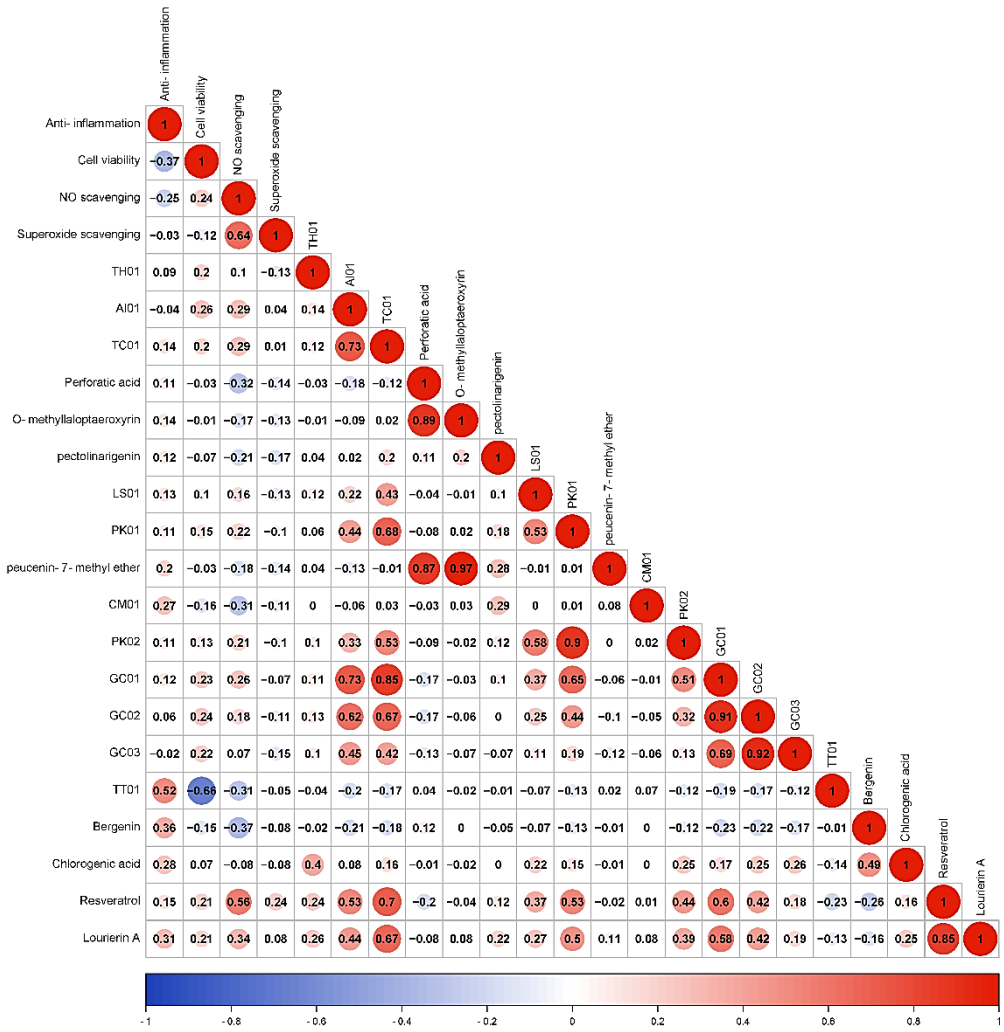


Figure 123 Pearson's rank correlation analysis between pharmacological effects and chemical markers of the primary herbs (PH).

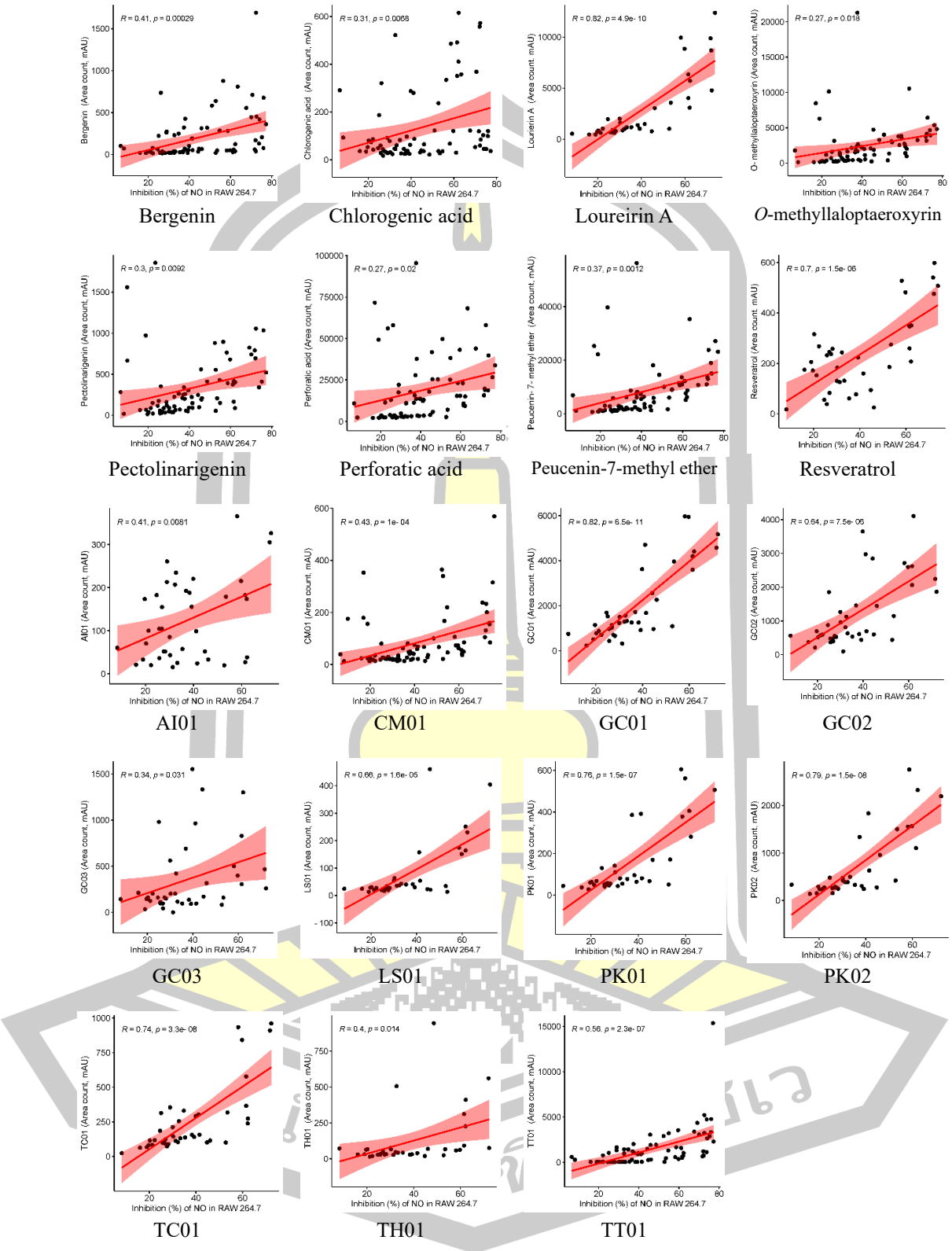


Figure 124 Pearson's Rho analysis between anti-inflammatory activity and chemical marker of the primary herbs (PH).

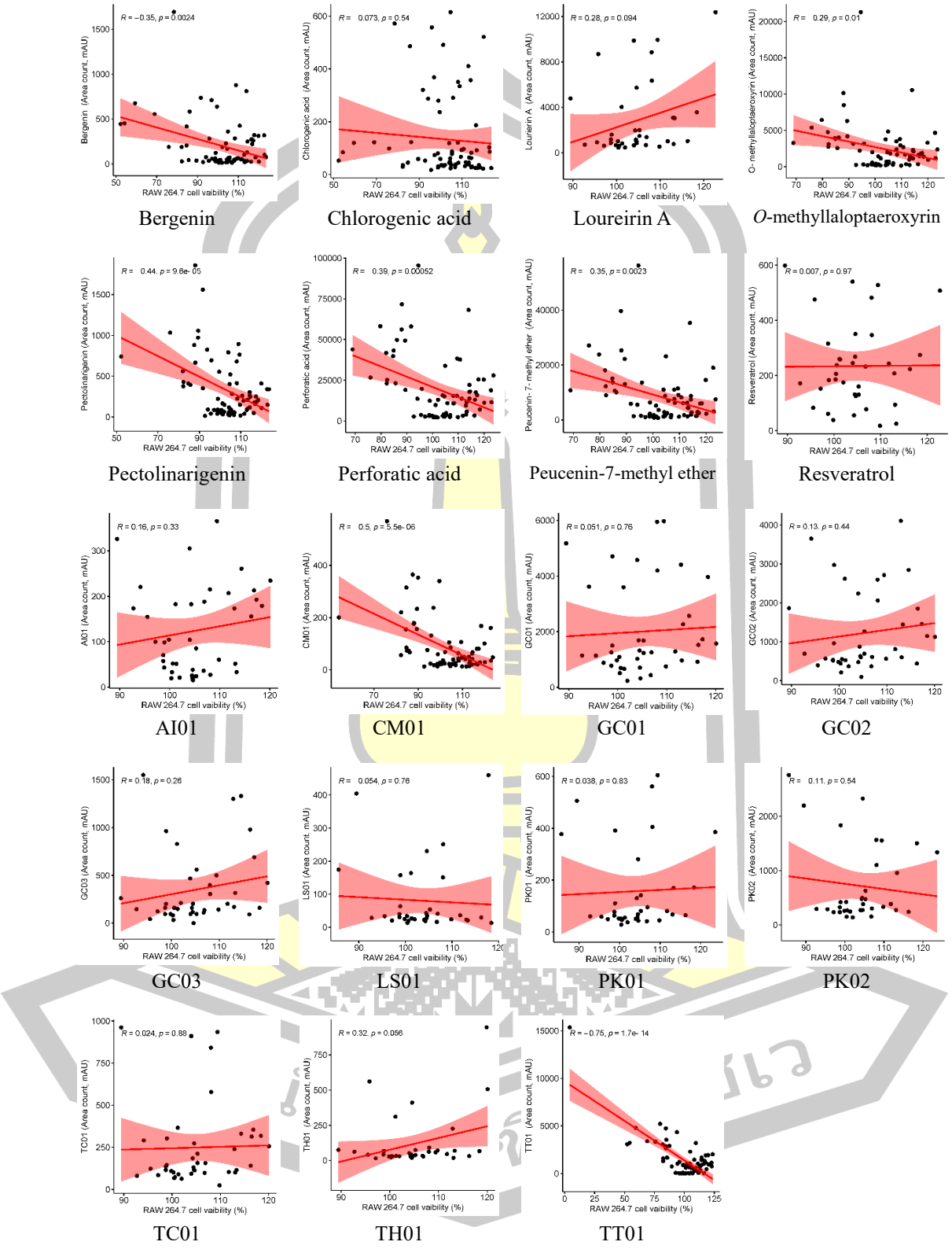


Figure 125 Pearson's Rho analysis between cell viability effects and chemical marker of the primary herbs (PH).

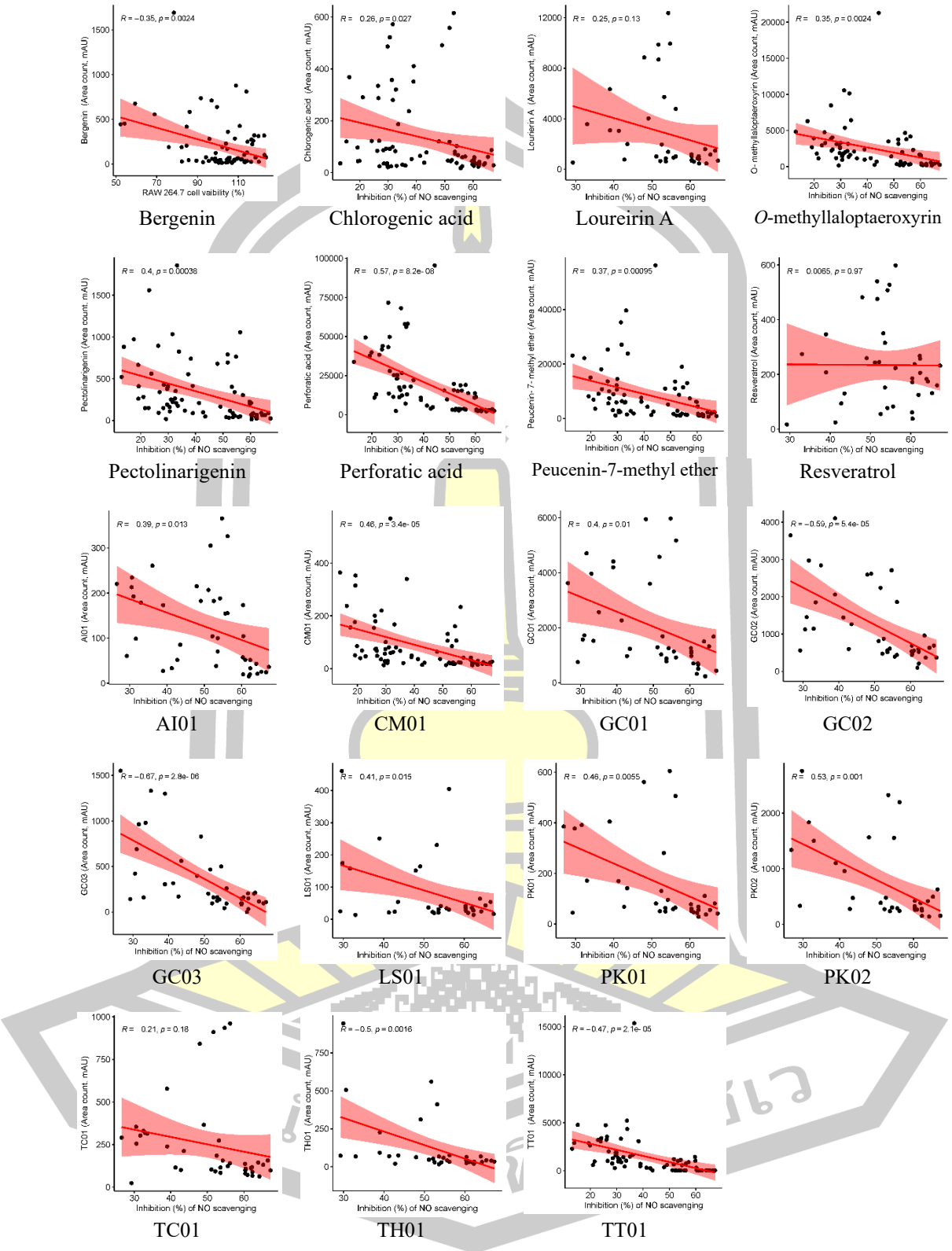


Figure 126 Pearson's Rho analysis between nitric oxide scavenging activity and chemical marker of the primary herbs (PH).

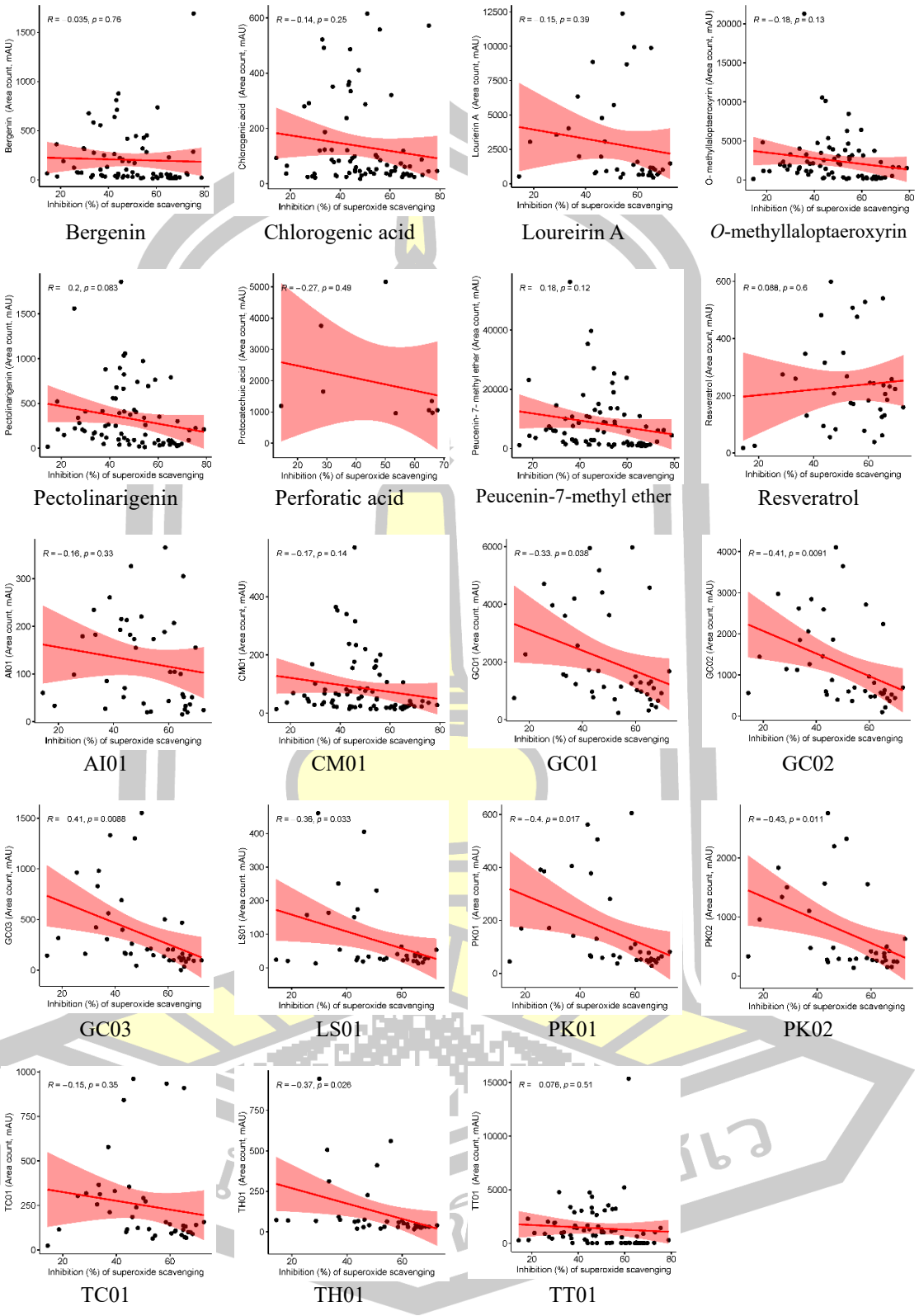


Figure 127 Pearson's Rho analysis between superoxide scavenging activity and chemical marker of the primary herbs (PH).

4.7.3.2 Relationship between pharmacological effects and chemical markers in the adjunct herbs

In the adjunct herbs (AH), a total of 12 chemical markers were identified, including PE01, BO01, TCb01, TB01, Ts01, chebulagic acid, chebulanin, chebulic acid, corilagin, ellagic acid, gallic acid, and rhein.

Pearson's rank correlation analysis between the amount of chemical markers of AH and anti-inflammatory activity (**Figure 128**) revealed that most chemical markers were negatively correlated with anti-inflammatory effects. Among these, ellagic acid, gallic acid, and chebulanin exhibited the highest negative correlation with anti-inflammatory activity, with an r-value of -0.43. Meanwhile, Pearson's Rho analysis revealed that only rhein showed a significant positive correlation with anti-inflammatory activity, with a rho-value of 0.47 and $p < 0.05$, as shown in **Figure 129**.

In cell viability, Pearson's rank correlation analysis between the amount of chemical markers of AH and cell viability (**Figure 128**) revealed that most chemical markers were positively correlated with cell viability, with r-values ranging from 0.12 to 0.22. Meanwhile, Pearson's Rho analysis revealed that several chemical markers were positively correlated with cell viability, including PE01, BO01, ellagic acid, TCb01, TB01, Ts01, rhein, chebulic acid and corilagin. In contrast, Pearson's Rho analysis showed that some chemical markers were negatively correlated with cell viability, including gallic acid, chebulagic acid, and chebulanin, as shown in **Figure 130**.

In nitric oxide scavenging, Pearson's rank correlation analysis between the amount of chemical markers of MA and nitric oxide scavenging (**Figure 128**) revealed that most chemical markers were positively correlated with nitric oxide scavenging. Among these, chebulanin exhibited the highest positive correlation with nitric oxide scavenging, with an r-value of 0.81, followed by ellagic acid, which showed a positive correlation with an r-value of 0.80. Meanwhile, Pearson's Rho analysis revealed that several chemical markers were positively correlated with nitric oxide scavenging, including PE01, gallic acid, chebulagic acid, ellagic acid, chebulic acid, chebulanin and corilagin. In contrast, Pearson's Rho analysis showed that some

chemical markers were negatively correlated with nitric oxide scavenging, including BO01, TCb01, TB01, Ts01 and rhein, as shown in **Figure 131**.

In superoxide scavenging, Pearson's rank correlation analysis between the amount of chemical markers of MA and superoxide scavenging (**Figure 128**) revealed that most chemical markers were positively correlated with superoxide scavenging. Among these, chebulic acid exhibited the highest positive correlation with superoxide scavenging, with an r-value of 0.67, followed by chebulanin, which showed a positive correlation with an r-value of 0.66. According to Pearson's Rho analysis, the %inhibition in superoxide scavenging activity increased with the amount of chemical markers. However, BO01 and rhein showed a decrease in %inhibition in superoxide scavenging activity as the amount of chemical markers increased, as shown in **Figure 132**.

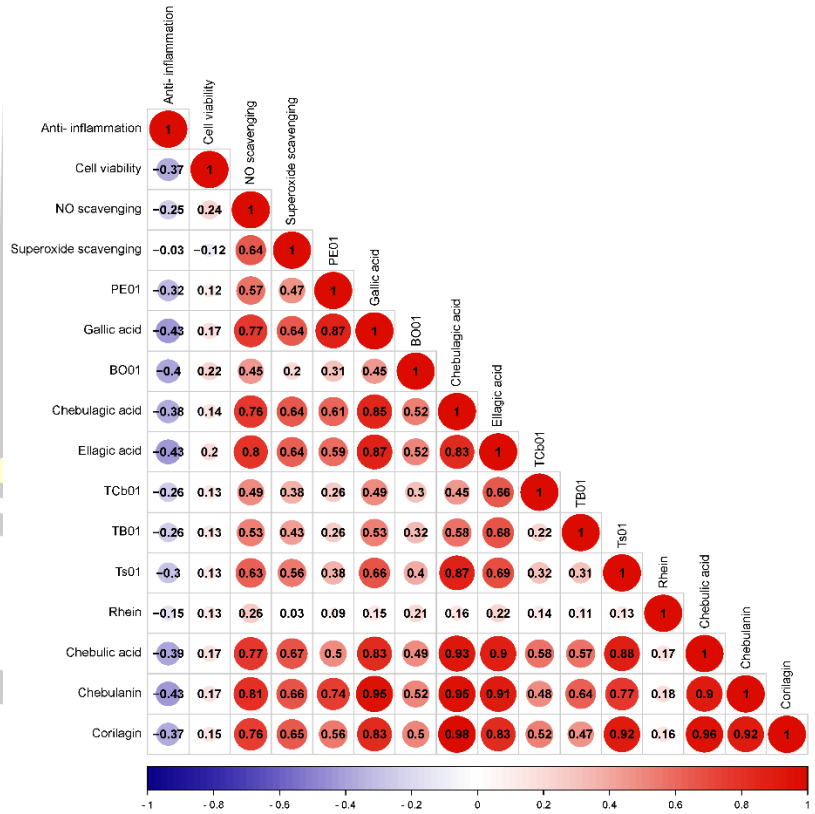


Figure 128 Pearson's rank correlation analysis between pharmacological effects and chemical markers of the adjunct herbs (AH).

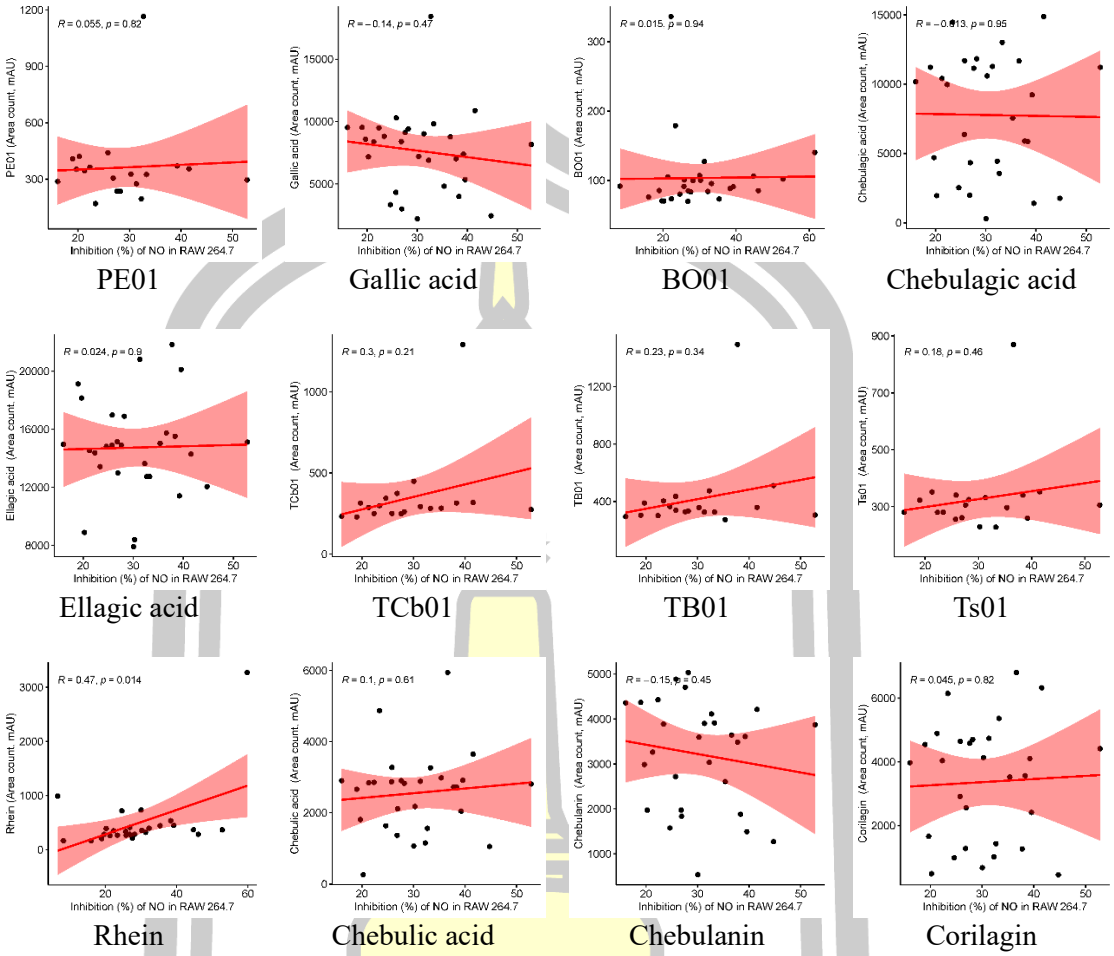


Figure 129 Pearson's Rho analysis between anti-inflammatory activity and chemical markers of the adjunct herbs (AH).

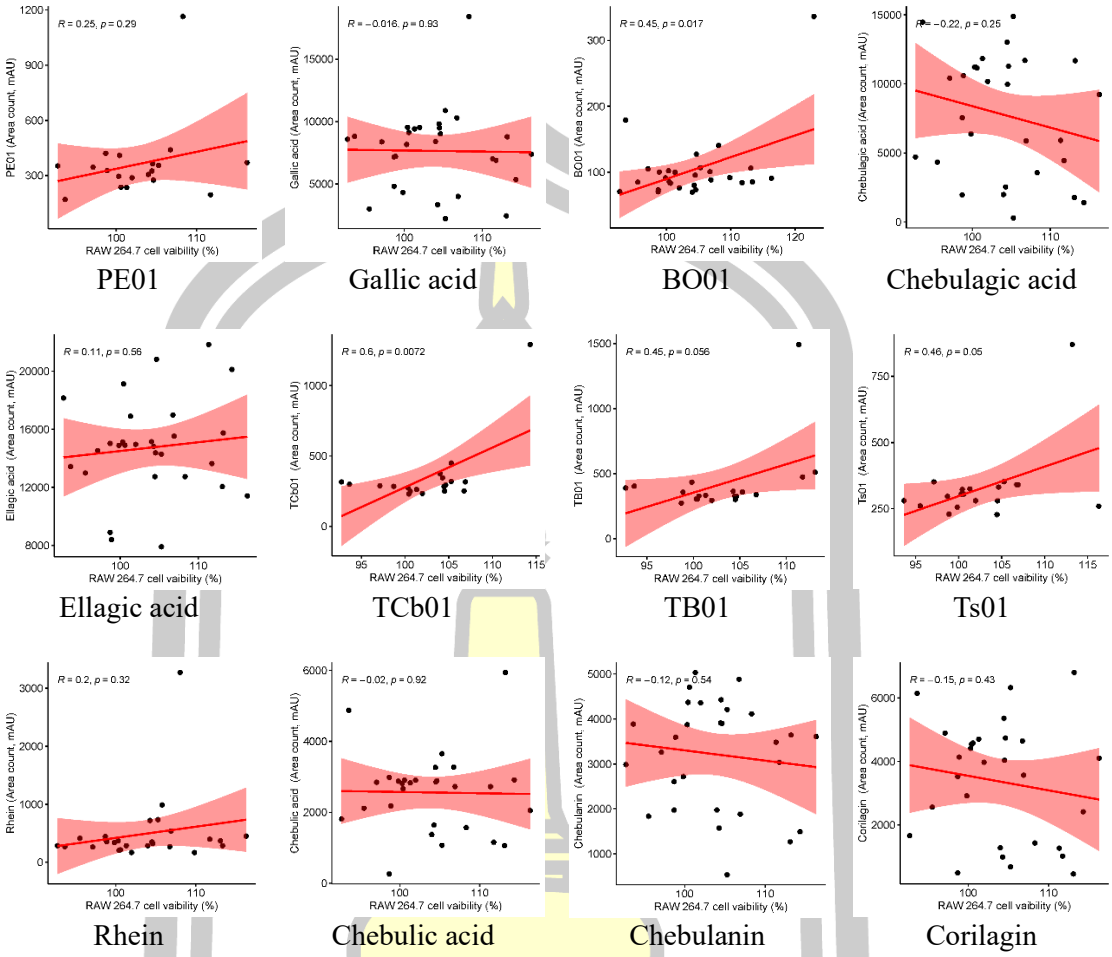


Figure 130 Pearson's Rho analysis between cell viability effects and metabolites of the chemical markers of the adjunct herbs (AH).

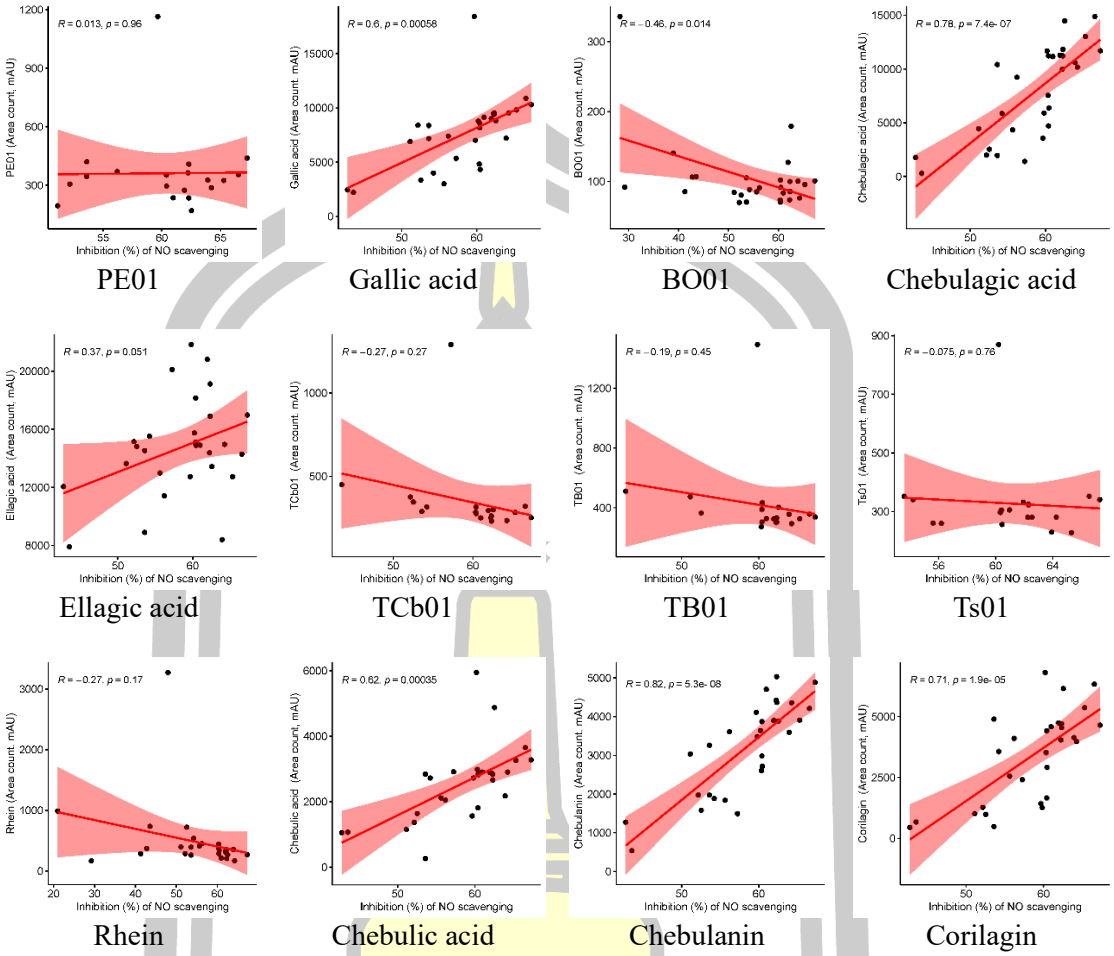


Figure 131 Pearson's Rho analysis between nitric oxide scavenging activity and chemical markers of the adjunct herbs (AH).

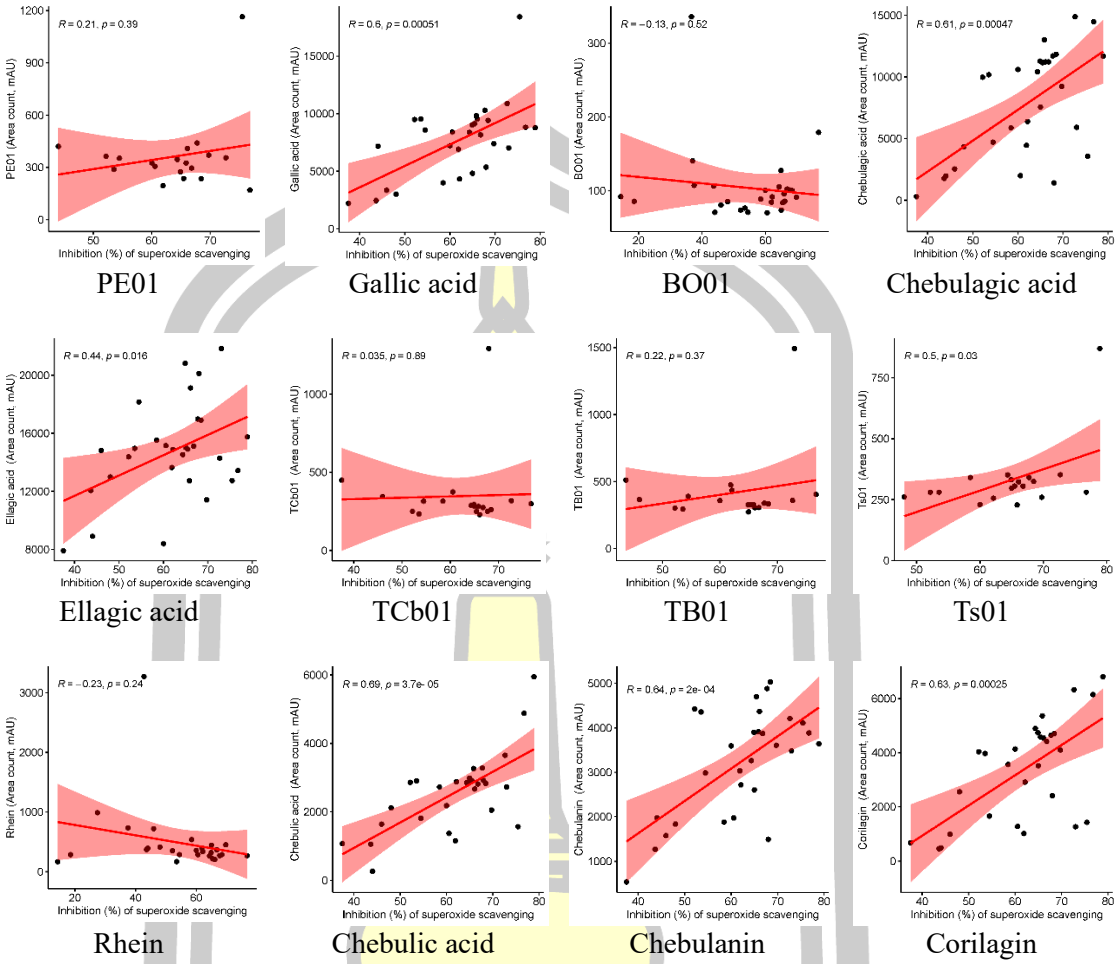


Figure 132 Pearson's Rho analysis between superoxide scavenging activity and chemical markers of the adjunct herbs (AH).

4.7.3.3 Relationship between pharmacological effects and chemical markers in the supportive herbs

A total of five metabolites were identified in the supportive herbs (SH), including MF02, NN01, VZ01, gallic acid, and protocatechuic acid.

Pearson's rank correlation analysis between the amount of chemical markers of SH and anti-inflammatory activity (**Figure 133**) revealed that most chemical markers were negatively correlated with anti-inflammatory effects. Among these, gallic acid exhibited the highest negative correlation with anti-inflammatory activity, with an r-value of -0.43. In contrast, Pearson's Rho analysis revealed that most chemical markers were positively correlated with anti-inflammatory effects. Only gallic acid showed a negative correlation with anti-inflammatory activity, with a rho-value of -0.24, as shown in **Figure 134**.

In cell viability, Pearson's rank correlation analysis between the amount of chemical markers of SH and cell viability (**Figure 133**) revealed that most chemical markers showed no significant correlation with cell viability. Meanwhile, Pearson's Rho analysis revealed that most chemical markers were negatively correlated with cell viability. Only VZ01 showed no significant correlation with cell viability, as shown in **Figure 135**.

In nitric oxide scavenging, Pearson's rank correlation analysis between the amount of chemical markers of SH and nitric oxide scavenging (**Figure 133**) revealed that most chemical markers showed no significant correlation with nitric oxide scavenging. However, only gallic acid showed a positive correlation with nitric oxide scavenging, with an r-value of 0.77. According to Pearson's Rho analysis, only gallic acid showed an increase in %inhibition of nitric oxide scavenging activity with the amount of chemical markers, exhibiting a strong positive correlation with a rho-value of 0.97. Meanwhile, other chemical markers showed a negative correlation with nitric oxide scavenging activity, as shown in **Figure 136**.

In superoxide scavenging, Pearson's rank correlation analysis between the amount of chemical markers of SH and superoxide scavenging (**Figure 133**) revealed that most chemical markers were negatively correlated with superoxide scavenging. In contrast, only gallic acid showed a positive correlation with nitric oxide scavenging, with an r-value of 0.64. According to Pearson's Rho analysis, only

gallic acid showed an increase in %inhibition of superoxide scavenging activity with the amount of chemical markers, exhibiting a strong positive correlation with a rho-value of 0.92. Meanwhile, other chemical markers showed a negative correlation with nitric oxide scavenging activity, as shown in **Figure 137**.

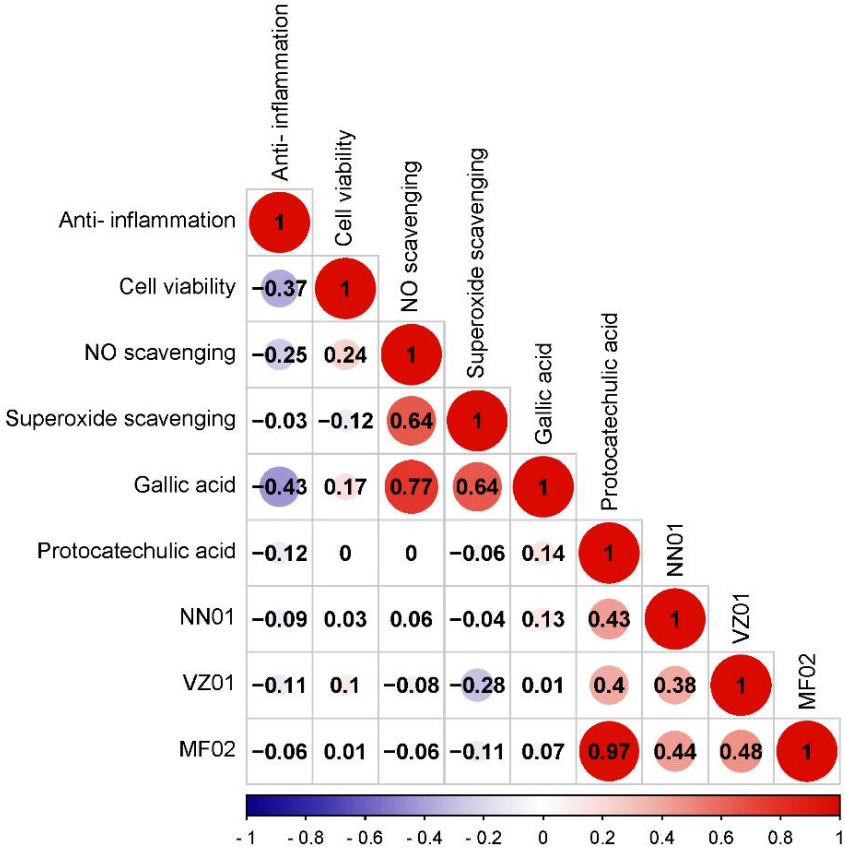


Figure 133 Pearson's rank correlation analysis between pharmacological effects and chemical markers of the supportive herbs (SH).

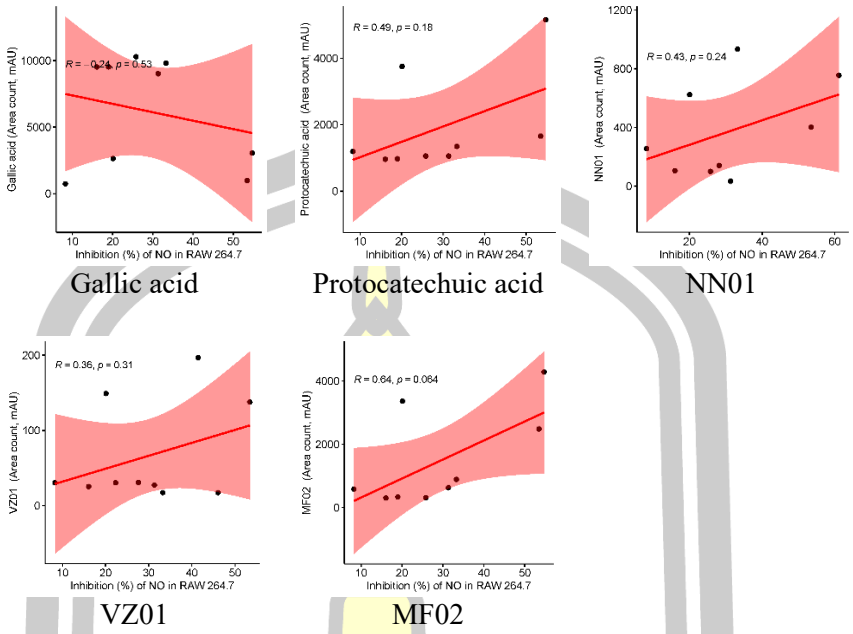


Figure 134 Pearson's Rho analysis between anti-inflammatory activity and chemical markers of the supportive herbs (SH).

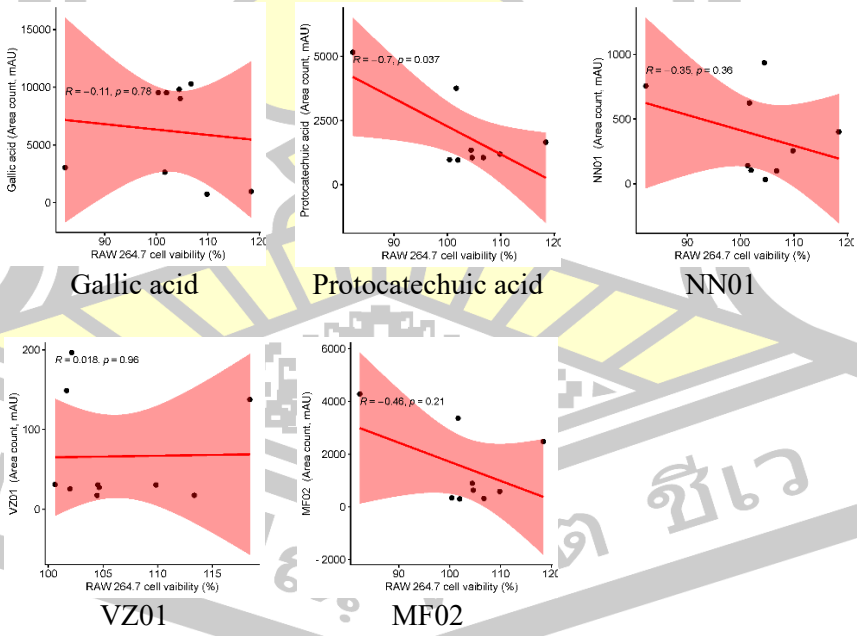


Figure 135 Pearson's Rho analysis between cell viability effects and the chemical markers of the supportive herbs (SH).

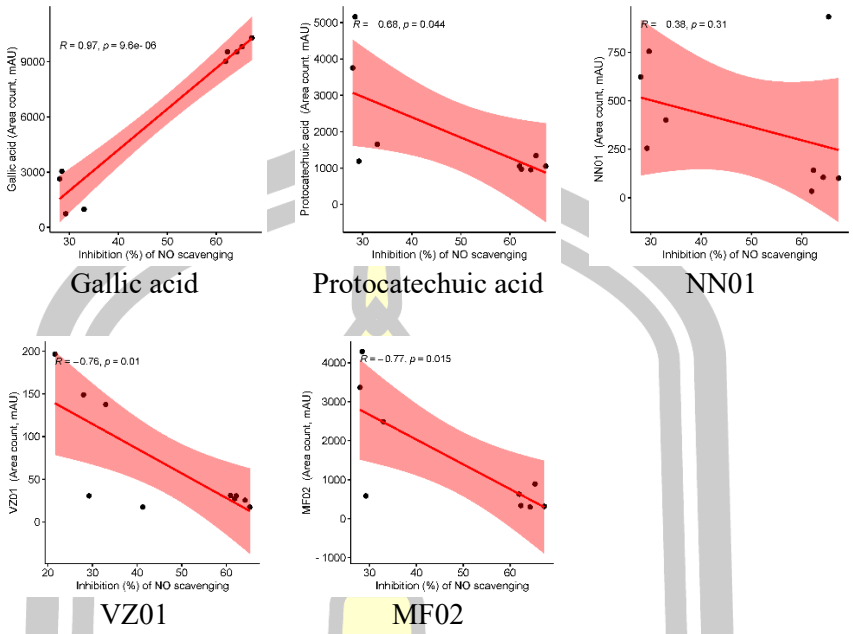


Figure 136 Pearson's Rho analysis between nitric oxide scavenging activity and chemical markers of the supportive herbs (SH).

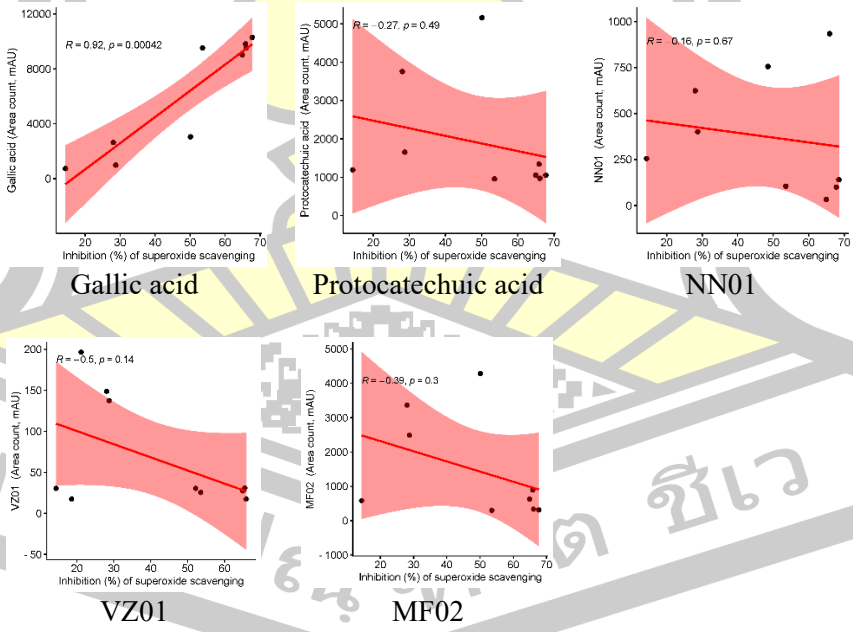


Figure 137 Pearson's Rho analysis between superoxide scavenging activity and chemical markers of the supportive herbs (SH).

CHAPTER V
CONCLUSIONS, DISCUSSIONS AND SUGGESTIONS

Thai Traditional medicine (polyherbal medicine) often consists of a large number of herbs, with some recipes containing nearly 50 herbs, aiming to treat various diseases and symptoms within a single remedy. Nithetsukkit, who wrote *Ayurvedhsuksa* textbook, mentioned that the structure of Thai traditional polyherbal medicine comprises four distinct groups of herbs: primary herbs (PH), adjunct herbs (AH), supportive herbs (SH), and flavoring herbs (FH). These groups are designed to address the primary symptoms and any complications in the treatment of diseases (Nithetsukkit, Khun, 1973). However, there is no detailed explanation about the number or quantity of herbs that should be included in each group within a remedy.

This study aims to provide a further explanation of the formulation theory underlying Thai traditional polyherbal medicine. Mo-Ha-Rak (MHR), a Thai traditional polyherbal medicine comprising 21 medicinal plants, was selected for a case study of this study. It is used by Thai traditional practitioners for the treatment of severe fever with drowsiness, internal heat with thirst, restlessness, delirium and unconsciousness.

The herbal components of MHR were classified into 3 groups (PH, AH and SH) following the Thai traditional medicine theory in the *Ayurvedhsuksa* textbook. The functions of each herbal group are different; PH is responsible for anti-fever, AH is for laxative and SH is for body and heart tonic. The PH was classified into 4 subgroups: (PH1) reduced-toxic fever herbs (PH2) anti-kamdoa and lohit fever herbs (PH3) anti-di fever herbs (PH4) anti-semha and lom fever herbs. The AH was also classified into 2 subgroups (AH1) stimulant laxative (AH2) sour-astringent laxative.

The modifications of herbs in various factors of the 92 modified and original MHR were investigated in terms of relation to their chemicals, pharmacological activities (anti-inflammatory and antioxidant), and toxicity. Due to MHR being used for the treatment of fever, anti-inflammatory activity was selected as a major activity to explain the relation in each group of herbs. From all experiments in this study, the conclusions can be summarized as follows.

5.1 Conclusions

1) When comparing the anti-inflammatory effect between each herbal group of MHR, the result showed that PH exhibited the significantly highest anti-inflammatory effect (72.26%) followed by PH+AH (52.80%) and PH+SH (53.47%) > MHR (31.32%) > AH (21.39%), SH (20.08%). The anti-inflammatory effects of PH+AH and PH+SH were not significantly different, as well as that of AH and SH. The studies indicated that PH exhibits a direct anti-inflammatory effect. The combination of PH with other herbal groups displayed a lower anti-inflammatory activity. MHR showed a medium anti-inflammatory effect when compared to PH, AH and SH.

2) The anti-inflammatory activity of PH was significantly higher than PH+AH (52.80%) and PH plus AH subgroups, PH+AH1 (46.08%) and PH+PH2 (41.53%) including AH (23.39%). The anti-inflammatory effect of AH was relatively low compared to PH and MHR. The anti-inflammatory effect of PH decreases when PH is combined with AH.

3) The anti-inflammatory activity of PH1 (75.92%) was not significantly different from PH (72.26%) and PH1+PH3 (72.05%) but significantly higher than PH1+PH4 (58.61%) and PH1+PH2 (39.87%). The combination of PH1 with other PH subgroups displayed different anti-inflammatory activity, PH1+PH3+PH4 (62.28%), PH1+PH2+PH3 (62.16%) and PH1+PH2+PH4 (41.13%). This study showed that PH1 is a major active herbal group and herbs within the PH subgroup exhibit both synergistic and antagonistic effects.

4) Five herbs in PH1 showed different anti-inflammatory activity, *F. racemose* (72.32%) and *T. triandra* (76.90%) exhibited the highest anti-inflammatory activity followed by *H. Perforate* (37.67%), *C. micracantha* (17.05%) and *C. indicum* (9.82%), respectively. The anti-inflammatory effects of *C. micracantha* and *C. indicum* were not significantly different. The anti-inflammatory activity of PH1 was 75.92%; this result indicated that herbs in PH1 may express synergistic anti-inflammatory effects.

5) The anti-inflammatory effect of PH1 decreased when combined with other herbs in the formula especially combined with low anti-inflammatory effect herbs, ranging from a slight reduction (PH1 + *D. cochinchinensis*), a moderate reduction (PH1 + *M. ferrea* or *N. nucifera*), to a significant reduction (PH1+*B. ovata* or *C.*

fistula). The greater the number of herbs without anti-inflammatory effects in the formula, the more the anti-inflammatory effect of the formula decreases.

6) Although the indication of herbs in PH1 to PH4 is used for anti-fever, only some herbs possess strong anti-inflammatory effects, such as *F. racemose*, *T. triandra*, *D. cochinchinensis*, *L. sinense*, *P. kesiya*, while *G. chinense, T. crispa* and *T. hoaensis* showed weak anti-inflammatory effects.

7) The antioxidant activity (% inhibition of NO radical scavenging) of herbal groups and their mixture were listed in the following order: AH (62.54%), MHR (61.93%), PH+ AH (60.40%), PH (56.23%), PH+SH (33.00%) and SH (28. 00%). Antioxidant activity is not directly related to PH but is directly related to AH. It may support other effects of the remedy.

8) The % cell viability in each herbal group was listed in the following order: PH+SH (118.42%), MHR (104.64%), SH (101.67%), AH (100.31%), PH+AH (93.64%) and PH (89.47%). PH is relatively toxic to cells, especially in the PH subgroup; PH1 showed a significantly lowest % cell viability of 75.90%. SH has the potential to reduce the toxicity of PH.

9) The correlation analysis between major compounds and anti-inflammatory activity indicated that most major compounds of PH were positively correlated with anti-inflammatory effects. PH revealed nine major compounds, including bergenin, chlorogenic acid, lourierin A, *O*-methyllaloptaeroxyrin, pectolinarigenin, perforatic acid, peucenin-7-methyl ether, resveratrol, and TT01. The TT01, resveratrol, lourierin A are major compounds in PH and demonstrated high anti-inflammatory activity. The TT01 and resveratrol showed a negative correlation to cell viability, while only lourierin A showed a positive correlation to cell viability.

10) For overall studies, it could be concluded that only primary herbs exhibit a direct anti-inflammatory activity. The combination of primary herbs with other herbal groups displayed a lower anti-inflammatory activity. Increasing the number or ratio of adjunct and supportive herbs or even the number of primary herbs might reduce the activity of the primary herbs. The greater the number of herbs without anti-inflammatory effects in the formula, the more the anti-inflammatory effect of the formula decreases. In contrast, adjunct herbs may support other effects of the remedy, as well as, supportive herbs tend to reduce the toxicity of primary herbs.

The study for a scientific-based explanation of Thai traditional medicine theory for Thai traditional herbal remedies involves numerous factors that need to be studied, including chemical composition, pharmacological effects, toxicity, and quality control. The data obtained from this study not only provide information to explain the formulation theory in Thai traditional medicine but also offer general information related to various aspects as follows.

1) This study obtained the HPLC fingerprint of MHR which can be used for qualitative quality control of this preparation under the following conditions: the mobile phase consisted of 0.1% v/v TFA in water and acetonitrile, applied with gradient elution at a flow rate of 0.8 mL/min, with retention times extending up to 160 minutes. A Luna C18 column (5 µm, 100 Å, 250 x 4.6 mm, Phenomenex®) was used for separation, performed on an Agilent 1260 Infinity II Prime HPLC system (Agilent Technologies, USA), with detection wavelengths set at 254 nm and 280 nm. In this HPLC fingerprint, seventeen major compounds were identified, including bergenin, chebulagic acid, chebulanin, chebulic acid, chlorogenic acid, corilagin, ellagic acid, gallic acid, lourierin A, *O*-methyllaloptaeroxyrin, perforatic acid, pectolinarigenin, peucenin-7-methyl ether, protocatechuic acid, resveratrol, rhein, and TT01.

2) Three major compounds, perforatic acid, *O*-methyllaloptaeroxyrin and peucenin-7-methyl ether were isolated from the root of *Harrisonia perforata* for reference compounds.

3) The specification of *Azadirachta indica* petiole, ethanolic and water extract was established for quality control of raw material.

4) TLC fingerprint of MHR was established using Silica Gel 60 F_{254} as a stationary phase with 2 mobile, phases toluene, ethyl acetate, methanol, and formic acid (7:2:1:0.5 v/v) and toluene, ethyl acetate, methanol, and formic acid (5:3:2:0.5 v/v).

5.2 Discussions

5.2.1 General discussions

5.2.1.1 Quality control of herbal components of MHR

There are two medicinal materials in MHR, Chan Khao and Samo Thet, that may originate from multiple plant species.

"Chan Khao" is a medicinal material that can be derived from at least three different plant species: Chantana (*Tarenna hoaensis* Pit.), Chan (*Diospyros decandra* Lour.), and Chan Hom (*Santalum album* L.) (Srisopon *et al*., 2015; Picheansoonthon *et al*., 2017). Additionally, "Chantana" is also the name of another medicinal substance sold in Thai traditional pharmacies. According to a study by Srisopon *et al*. (2015), most of the "Chan Khao" currently available in the market is derived from the plant scientifically known as *T. hoaensis*. In this study, the physical characteristics of the medicinal materials were analyzed by the macroscopical method as well as the chemical characteristics analyzed by TLC technique were found to be similar to *T. hoaensis*.

"Samo Thet" is a medicinal material imported from India and sold in Thai Traditional pharmacies. The fruits of Samo Thet are smaller than those of Samo Thai (*Terminalia chebula* Retz.) found in Thailand, but the fruits have more pronounced ridges compared to Samo Thai. In some texts, it is also referred to as Samo Chit (Picheansoonthon *et al*., 2017). Currently, the scientific name of this herb has not been clearly identified. It is only known to be derived from plants in the genus *Terminalia* of the family Combretaceae.

5.2.1.2 Characterization and identification of chemical marker

Sixteen major compounds were identified by HPLC, including bergenin, chebulagic acid, chebulanin, chebulic acid, chlorogenic acid, corilagin, ellagic acid, gallic acid, lourierin A, *O*-methyllaloptaeroxyrin, perforatic acid, pectolinarigenin, peucenin-7-methyl ether, protocatechuic acid, resveratrol and rhein. HPLC was optimal for detecting chemical compounds across various modified MHR formulas with a UV absorbance range from 190–800 nm. In the ethanolic extract at 254 nm, HPLC chromatogram of the 92 modified MHR and original MHR showed 129-159 peaks. However, the HPLC-photodiode array detector used in this study is specific for detecting compounds with double bonds and aromatic rings in their

chromophores. The compounds without their chromophore could not be detected by a photodiode array detector. Therefore, TLC and specific reagents were also used in this study. The TLC chromatograms of the modified and original MHR, visualized at UV 254 nm, UV 366 nm, and under anisaldehyde-sulfuric acid with UV 366 nm, revealed 10–14, 19–23, and 19–23 bands, respectively. Detection with anisaldehyde-sulfuric acid under UV 366 nm confirmed the presence of β-sitosterol, lupeol, and stigmasterol in MHR. The substances found in TLC mostly correspond to those found in HPLC. These findings indicate that the HPLC method can detect a broader range of compounds compared to TLC.

5.2.1.3 *O*-methyllaloptaeroxyrin and peucenin-7-methyl ether were identified in the HPLC chromatogram of aqueous extract

The chemical structure of *O*-methyllaloptaeroxyrin and peucenin-7-methyl ether are classified as non-polar compounds, they should not be present in aqueous extract. However, non-polar compounds may be found in water extracts, even though they dissolve more readily in non-polar solvents like organic solvents. This can be attributed to the following factors:

1) Micelle formation: Some non-polar compounds can aggregate into micelles in water due to the presence of surfactants or similar components found in plants, such as saponins. These enable non-polar compounds to partially disperse in water (Rai *et al*., 2021).

2) Emulsion formation: Water and oil can form emulsions during the extraction process, with plant components such as phospholipids acting as emulsifiers. This allows non-polar compounds to exist in water extracts as emulsions (Pichot *et al*., 2013).

3) Partial solubility of compounds: While some compounds are primarily non-polar, they may contain functional groups that impart slight polarity, such as hydroxyl or carboxyl groups in terpenoids. These groups allow the compounds to dissolve partially in water (Rai *et al*., 2023).

4) Temperature and pressure during extraction: High temperatures or pressures during water extraction can enhance the solubility of certain non-polar compounds in water. These conditions may alter the structure of water, increasing its solubility capacity in supercritical or near-supercritical states. This finding is

consistent with Cheng *et al*. (2021), who reported that under high-pressure conditions (e.g., 100–374°C) without reaching the critical point, water remains in a liquid state with reduced polarity. This state enables water to dissolve non-polar or low-polar compounds more effectively.

5) Binding to plant components: Non-polar compounds may bind to water-soluble plant components such as proteins or carbohydrates, allowing them to appear in water extracts (Rai *et al*., 2021).

5.2.1.4 Models for pharmacological study

Anti-inflammatory activity was assessed by evaluating the inhibitory effects on LPS-induced nitric oxide (NO) release from murine macrophage cell lines (RAW 264.7), which represents the primary function of the MHR remedy. NO is one of the inflammatory mediators causing inflammation in many organs and it is an inorganic free radical that has been implicated in physiological and pathological processes, such as vasodilation, body temperature regulation, non-specific host defense and acute or chronic inflammation (Kou and Schroder, 1995; Lantz *et al*., 2005). In fever or inflammatory reactions, pro-inflammatory cytokines lead to the expression of the inducible nitric oxide synthase (iNOS) in monocyte/macrophages, neutrophil granulocytes and many other cells; in the case of bacterial infection, endotoxin is another strong inducer of expression. In this sequence, large amounts of NO are synthesized, exceeding the physiological NO production by up to 1000-fold (Forstermann *et al*., 1994; Knowles and Moncada, 1994; Weinberg *et al*., 1995; Cook and Cattell,1996).

The antioxidant activity was tested by measuring their inhibitory effects on free radicals to represent the secondary or minor function of the MHR remedy. The nitric oxide radical (NO) scavenging activity was assessed using a modified Griess reaction, while the superoxide anion ($O_2^{\bullet-}$) scavenging activity was determined using a riboflavin-light-NBT system with modifications to inhibit formazan formation. Nitric oxide (NO) and superoxide anions are reactive oxygen species (ROS) produced by iNOS, which can cause damage to mitochondria, DNA, and other cellular molecules. NO reacts with superoxide anions to form peroxynitrite, a potent oxidant that can decompose into hydroxyl radicals (OH) and NO.

Additionally, superoxide generated both *in vivo* and in foods can undergo several reactions including dismutation to give H_2O_2 (Dontha, 2016).

However, this research was conducted using only one cell line and *in vitro* methods, which do not account for digestion, absorption, active metabolites, or the mechanism of action within the body.

5.2.1.5 Selection of solvent extraction

The ethanol extract and aqueous extract of the original and modified MHR were used in this study. The result showed that the aqueous extract demonstrated weak activity even using a high concentration. Therefore, the ethanol extract of the original and modified MHR was selected for the validation of formulation theory in different factors. Water extracts typically exhibit weaker effects in invitro study compared to extracts obtained using other methods, such as organic solvents, due to several limitations:

1) Solubility of active compounds: Water, being highly polar, is suitable for dissolving compounds that are highly water-soluble (e.g., polyphenols). However, it is less effective for extracting compounds that dissolve better in organic solvents or those with low polarity, such as essential oils or certain alkaloids that are crucial for biological activity (Chompoo *et al*., 2019; Rucksakaew *et al*., 2019; Adeeyo *et al*., 2023).

2) Loss of active compounds: Water extraction often involves high heat, which can cause the degradation of some active compounds, such as vitamins or certain antioxidants, thereby reducing the concentration of bioactive substances (Borges *et al*., 2020; Suriyaphan *et al*., 2023).

3) Quantity of extracted compounds: Water extraction methods often cannot yield as much of the active compounds as extraction methods using suitable solvents like ethanol or methanol, which are more effective at extracting a broader range of compounds (Chompoo *et al*., 2019; Borges *et al*., 2020).

5.2.2 Validation of the formulation theory for Thai traditional polyherbal medicine

5.2.2.1 The anti-inflammatory effect of each herbal group and their mixture of original and modified MHR

The result showed that PH exhibited a significantly higher anti-inflammatory effect (72.26%) than AH (21.39%) and SH (20.08%). The anti-inflammatory effects of AH and SH were not significantly different. The studies indicated that PH exhibits a direct anti-inflammatory effect.

The results of this study on anti-inflammatory effects are consistent with numerous previous studies. The anti-inflammatory effects of herbs in PH, *F. racemosa* (72.32%) and *T. triandra* (76.90%) exhibited the highest anti-inflammatory activities, followed by *H. perforata* (37.67%), *C. micracantha* (17.05%), and *C. indicum* (9.82%). Other herbs in the formula, particularly when combined with PH1, also showed notable anti-inflammatory activities, with *D. cochinchinensis* (73.76%) exhibiting the highest activity, followed by *T. hoaensis* (48.61%), *L. sinense* (45.86%), *G. chinense* (44.24%), *P. kesiya* (37.37%), *T. crispa* (34.80%), and *A. indica* (29.01%). Previous studies on 95% ethanol extracts of these herbs against NO production in LPS-stimulated RAW 264.7 cells reported the following IC_{50} values: *L. sinense*, 3.77 µg/mL (Itharat *et al*., 2009); *D. cochinchinensis*, 40.73 µg/mL (Sukkasem, 2015); *C. indicum*, 46.55 µg/mL; *H. perforata*, 53.16 µg/mL; *T. triandra*, 54.65 µg/mL; *C. micracantha*, 61.1 µg/mL (Juckmeta and Itharat, 2012); and *F. racemosa*, > 100 µg/mL (Juckmeta and Itharat, 2012). Meanwhile, the inhibitory effects of *A. indica*, *G. chinense*, *T. crispa*, *T. hoaensis*, and *P. kesiya* on NO production in LPS-stimulated RAW 264.7 cells have not been reported in previous studies.

The anti-inflammatory effects of herbs in AH, when combined with PH1, revealed that *T. chebula* (39.56%) exhibited the highest activity, followed by *T. bellirica* (37.75%), *Terminalia* sp. “Samo Thet” (36.60%), *P. emblica* (32.69%), *B. ovata* (22.19%), and *C. fistula* (6.66%). Previous studies on 95% ethanol extracts of these herbs against nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells reported that *T. bellirica*, *T. chebula*, and *P. emblica* were not measurable, with IC_{50} values > 100 µg/mL (Nuaeissara et al., 2022). Meanwhile, the inhibitory effects of *B.*

ovata, *C. fistula*, and *Terminalia* sp. “Samo Thet” on NO production in LPS-stimulated RAW 264.7 cells have not been reported in previous studies.

The anti-inflammatory effects of herbs in SH, when combined with PH1, revealed that *N. nucifera* (61.10%) exhibited the highest anti-inflammatory activity, followed by *M. ferrea* (54.71%) and *V. zizanioides* (41.47%). Previous studies on 95% ethanol extracts of these herbs against nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells reported IC_{50} values of 65.71 µg/mL for *M. ferrea*, and > 100 µg/mL for both *N. nucifera* and *V. zizanioides* (Sukkasem, 2015).

When comparing the anti-inflammatory effect between each herbal group and their mixture, the result showed that PH exhibited the significantly highest anti-inflammatory effect (72.26%) followed by PH+AH (52.80%) and PH+SH (53.47%) > MHR (31.32%) > AH (21.39%), SH (20.08%). The anti-inflammatory effects of PH+AH and PH+SH were not significantly different. The studies revealed that the combination of PH with other herbal groups displayed a lower anti-inflammatory activity. MHR showed a medium anti-inflammatory effect when compared to PH, AH and SH. The anti-inflammatory effect of PH+AH is less than that of PH due to the reduced number of anti-inflammatory herbs in PH within the PH+AH when compared to the equivalent total weight of PH only. PH consisted of 12 medicinal plants in MHR, accounting for a total ratio of 42% w/w in HMR, while the total ratio of PH in PH+AH and PH+SH was 46.67 and 80.77%, respectively. Although the proportion of PH in PH+AH is lower than in PH+SH, there was no significant difference in the anti-inflammatory effect between PH+AH and PH+SH. This finding supports the idea that increasing the number of herbs in AH or SH may not enhance the efficacy of PH.

In correlation analysis, most major compounds of PH were positively correlated with anti-inflammatory effects. This indicates that the percentage of inhibition in anti-inflammatory activity increased with the amount of chemical markers. In contrast, most chemical markers of PH were negatively correlated with cell viability. Additionally, the correlation analysis also indicated that major compounds in AH were negatively correlated with anti-inflammatory effects and showed a positive correlation with cell viability.

Consistently, the TT01 major compounds in PH demonstrated the highest anti-inflammatory activity, with an IC_{50} value of 9.75 ± 0.25 µg/mL. This was followed by resveratrol, which also exhibited high anti-inflammatory activity with an IC_{50} value of 17.83 ± 0.70 µg/mL. Additionally, lourierin A, another major compounds, showed moderate anti-inflammatory activity, with an IC_{50} value of 72.42 ± 1.40 µg/mL.

The anti-inflammatory activity of PH1 (75.92%) was not significantly different from PH (72.26%) and PH1+PH3 (72.05%) but significantly higher than PH1+PH4 (58.61%) and PH1+PH2 (39.87%). The combination of PH1 with other PH subgroups displayed different anti-inflammatory activity, PH1+PH3+PH4 (62.28%), PH1+PH2+PH3 (62.16%) and PH1+PH2+PH4 (41.13%). This study showed that PH1 is a major active herbal group and herbs within the PH subgroup exhibit both synergistic and antagonistic effects. The herbs in PH include all herbs of PH1, but the anti-inflammatory activity of PH did not differ significantly from PH1. This suggests that the herbs in PH may exhibit both synergistic and antagonistic anti-inflammatory activity. However, when comparing the anti-inflammatory activity of PH1 combined with other herbs in PH to PH1 alone, no herb mixed with PH1 was found to have a higher anti-inflammatory effect than PH1. The only herb that, when combined with PH1, resulted in an anti-inflammatory effect comparable to PH1 was *D. cochinchinensis*. All other herbs, when mixed with PH1, exhibited lower anti-inflammatory effects than PH1, including *A. indica*, *G. chinense*, *T. crispa*, *T. hoaensis*, *L. sinense*, *P. kesiya*, *B. ovata*, *C. fistula*, *T. bellirica*, *T. chebula*, *T. sp.*, *P. emblica*, *M. ferrea*, *N. nucifera*, and *V. zizanioides*. When comparing the ratio of PH1 to PH, it was found that PH1 accounts for 47.62%, therefore, the quantity of herbs in PH1 is reduced by 52.38%. Based on the anti-inflammatory effects of the herbs in PH and the ratio of PH1 in PH, this supports the possibility that certain herbs in PH may enhance the anti-inflammatory effects of PH1.

The anti-inflammatory effect of PH1+PH2 is relatively low, indicating that the herbs in PH2 may counteract the anti-inflammatory effect of PH1. Alternatively, this could be due to the increased proportion of herbs in PH2, which reduced the proportion of herbs in PH1 from 100% to 62.5%. In contrast, PH1+PH3 demonstrates a relatively high anti-inflammatory effect, comparable to PH and PH1,

suggesting that PH3 has a relatively strong anti-inflammatory effect or the proportion of PH1 in PH1+PH3 was higher than in PH1+PH2 (71.73%).

Consistent with previous studies, the ethanolic extract of the Benjalokawichian or Ha-Rak remedy, when composed of five mixed herbs, exhibited the highest anti-inflammatory (NO inhibitory) activity (IC_{50} = 40.36 µg/mL), surpassing that of individual herbs (*Clerodendrum petasites*, *Harrisonia perforata*, *Tiliacora triandra*, and *Capparis micracantha*, with IC_{50} values of 46.55, 53.16, 54.65, and 61.35 µg/mL, respectively). In contrast, *Ficus racemosa* showed no significant anti-inflammatory activity (IC_{50} > 100 µg/mL) (Juckmeta, 2011). Similar to the study of 95% ethanolic extract from Prasachandaeng remedy, the formulation composed of 12 mixed herbs exhibited prostaglandin E2 inhibitory activity with an IC_{50} of 4.64 µg/mL, which was comparable to *Dracaena cochinchinensis* (IC_{50} = 3.06 µg/mL) at a proportion of 50%. An additional six herbs exhibited prostaglandin E2 inhibitory activity, with IC_{50} values ranging from 4.05 to 8.97 µg/mL, whereas five other herbs showed no measurable activity (Prommee *et al*., 2020).

In contrast, the ethanolic extract of the Kheaw-Hom remedy, composed of 21 mixed herbs, exhibited moderate anti-inflammatory (NO inhibitory) activity with an IC_{50} of 59.77 µg/mL, while *Mammea siamensis* demonstrated the highest effect with an IC_{50} of 11.55 µg/mL. Fourteen herbs showed NO inhibitory activity with IC_{50} values ranging from 14.26 to 88.67 µg/mL, whereas six herbs had no measurable activity (Sukkasem, 2015). Similarly, the ethanolic extract of the Leard-Ngam remedy, composed of 20 mixed herbs, exhibited moderate anti-inflammatory (NO inhibitory) activity with an IC_{50} of 28.18 µg/mL, with *Piper nigrum* showing the highest effect (IC_{50} = 1.31 µg/mL). Seventeen herbs demonstrated NO inhibitory activity with IC_{50} values ranging from 2.87 to 97.82 µg/mL, while one herb showed no measurable activity (Threrapanithan *et al*., 2015). Furthermore, the ethanolic extract of the Sa-Tri-Lhung-Klod remedy, composed of 17 mixed herbs, exhibited anti-inflammatory (NO inhibitory) activity with an IC_{50} of 20.59 µg/mL, with *Curcuma comosa* showing the highest effect (IC_{50} = 5.21 µg/mL). Eleven herbs displayed NO inhibitory activity with IC_{50} values ranging from 2.87 to 97.82 µg/mL, whereas five herbs showed no measurable activity (Inprasit *et al*., 2020).

The anti-inflammatory effect of individual herbs in PH1 exhibited different effect levels. *T. triandra* had the highest anti-inflammatory effect, followed by *F. racemosa*, C. micracantha, *H. perforata*, and *C. indicum*, respectively. This indicates that herbs in PH1 exhibited both synergistic and antagonistic effects.

5.2.2.2 The antioxidant activity of each herbal group and their mixture of original and modified MHR

The antioxidant activity (% inhibition of NO radical scavenging) of AH (62.54%) was significantly higher than PH (56.23%) MHR (61.93%), PH+ AH (60.40%), PH (56.23%), PH+SH (33.00%) and SH (28. 00%). Antioxidant activity is not directly related to PH but is directly related to AH. It may support other effects of the remedy. Removing herbs in AH significantly reduced the antioxidant effect compared to the original MHR. Meanwhile, formulas containing only AH increased the antioxidant effect compared to the original MHR, and this effect was significantly stronger than that of formulas containing only SH. Additionally, removing herbs in SH or formulas containing only SH did not enhance the antioxidant effect of PH in the ethanolic extract, but it also did not reduce the antioxidant effect compared to PH in the aqueous extract. This result confirmed that the antioxidant effect is directly related to AH. Literature reviews confirm that most herbal components in AH showed high antioxidant activity.

This corroborates previous studies on the methanolic extract of *C. fistula* demonstrated potent antioxidant activity, with EC_{50} values of 0.91 µg/mL for DPPH radical scavenging and 0.88 µg/mL for hydroxyl radical scavenging (Irshad *et al*., 2012). The ethanolic extract of *P. emblica* exhibited antioxidant activity with IC_{50} values of 3.49 mg/mL in the DPPH radical assay and 4.95 mg/mL in the ABTS radical assay (Arjin *et al*., 2020). Similarly, ellagic acid (IC_{50} = 1.71 µg/mL) showed higher antioxidant activity compared to the ethyl acetate extract (IC_{50} = 11.78 µg/mL) and aqueous extracts (IC_{50} = 14.44 µg/mL) of *T. bellirica* in the ABTS radical scavenging assay (Gupta *et al*., 2021). The IC_{50} values of methanol, chloroform, ethyl acetate, *n*-butanol, organic aqueous, and water extracts of *T. chebula* ranged from 4.05 to 8.97 µg/mL for lipid peroxidation, 0.48 to 2.42 µg/mL for superoxide radical scavenging, and 0.004 to 0.021 µg/mL for free radical scavenging (Cheng *et al*., 2003). Furthermore, chebulanin, isolated from the fruit of *T. chebula*, displayed potent anti-

lipid peroxidation, anti-superoxide radical formation, and free radical scavenging activities with IC_{50} values of 3.96 µg/mL, 0.04 µg/mL, and 0.031 µg/mL, respectively (Cheng *et al*., 2003).

The adjunct herbs (AH) exhibited a synergistic effect on the antioxidant activity of the primary herbs. Modified MHR remedies by fixing reduced-toxic fever herbs and plus *T. bellirica, T. chebula, Terminalia* sp. "Samo Thet" or *P. emblica* resulted in increased NO radical scavenging and $O_2^{\bullet-}$ radical scavenging activities. Many chemical markers consistently exhibited strong antioxidant activity. Corilagin showed the highest nitric oxide (NO) radical scavenging effect, with an IC_{50} value of 5.42 ± 0.25 µg/mL, followed by gallic acid, chebulagic acid, and rhein, with IC_{50} values of 8.01 ± 0.56, 12.15 ± 0.31, and 20.05 ± 0.58 µg/mL, respectively. For superoxide radical scavenging activity, ellagic acid demonstrated the highest potency, with an IC_{50} value of 6.89 ± 0.03 µg/mL, followed by chebulic acid, chebulagic acid, corilagin, and gallic acid, with IC_{50} values of 24.80 ± 0.89, 25.20 ± 0.46, 29.55 ± 1.33, and 34.97 ± 0.61 µg/mL, respectively. These chemical markers were derived from *T. bellirica, T. chebula, Terminalia* sp. "Samo Thet" and *P. emblica*, except for rhein, which was derived from *C. fistula*.

5.2.2.3 The toxicity of each herbal group and their mixture of original and modified MHR

The % cell viability in each herbal group was listed in the following order: PH+SH (118.42%), MHR (104.64%), SH (101.67%), AH (100.31%), PH+AH (93.64%) and PH (89.47%). PH is relatively toxic to cells, especially in the PH subgroup; PH1 showed a significantly lowest % cell viability of 75.90% while the % cell viability of PH+SH exhibited pretty high. The result indicated that SH has the potential to reduce the toxicity of PH. *T. triandra* is the only herb in PH1 that exhibited high toxicity (% cell viability = 4.23) at a concentration of 100 µg/mL. However, at a concentration of 50 µg/mL, it showed no toxicity (% cell viability = 76.45), with no significant difference in its anti-inflammatory effect between the two concentrations.

5.3 Suggestions

5.3.1 The study for a scientific-based explanation of Thai traditional medicine theory for Thai traditional herbal remedy should include further investigation into other mechanisms of fever, as well as expanded studies in animal models or at the clinical level.

5.3.2 In order to obtain empirical scientific evidence supporting the Thai traditional medicine formulation theory, studies should be conducted on other remedies for the treatment of different diseases.

5.3.3 The result of this study confirms the explanation of the formulation theory and definition of Thai traditional polyherbal medicine by Nithetsukkit, Khun (1973) in the Ayurvedhsuksa textbook: PH is responsible for primary symptoms, AH is responsible for enhancing the efficacy of the treatment of the minor symptoms, and SH is for helping regulate the potency of PH and AH, ensuring their effects are stable, not excessive, and within desired limits. The result of this study also confirms the criticism of Nithetsukkit, Khun (1973) that Thai traditional remedies should not consist of numerous herbal ingredients in a single formula. The intention is to treat various conditions with one remedy leads to minor effectiveness because individual herbs tend to have mild potency, and when combined with numerous other herbs, the potency of each herb becomes even weaker.

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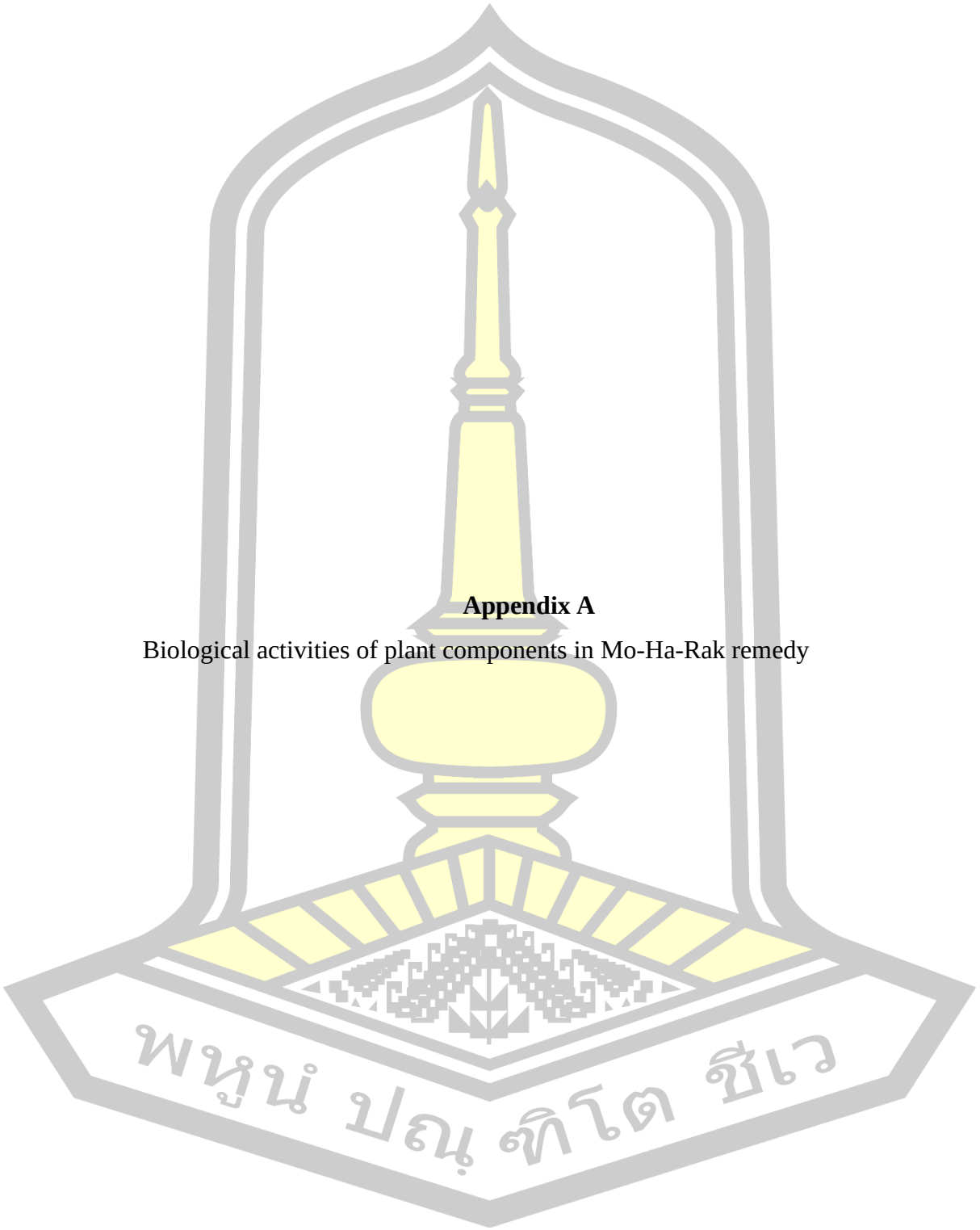
APPENDIX



Appendix A

Biological activities of plant components in Mo-Ha-Rak remedy

Biological activities of plant components in Mo-Ha-Rak remedy

Botanical Names	Activities	Part of used / Bioactive compounds	Biological activities	Reference
Ha-Rak remedy	Antipyretic	Root of five plants	Powder of Ha-Rak remedy at various doses (100, 200, 400 mg/kg, p.o.) significantly reduced rectal temperature from the first hour after brewer's yeast induced pyxeria in rats. Doses of 200 mg/kg was the most potent.	Okpanyi and Ezeukwu (1981)
		Root of five plants	The ethanolic extract of Ha-Rak remedy at various doses (25-400 mg/kg) significantly ($p < 0.05$) reduced body temperature on lipopolysaccaride (LPS) in rats and were found to be as potent as acetylsalicylic acid (300 mg/kg). Doses of 400 mg/kg was the most potent.	Kumar *et.al.* (2015)
	Analgesic	Root of five plants	The ethanolic extract of Ha-Rak remedy at various doses (25-400 mg/kg p.o.) were evaluated for their analgesic activity using the hot-plate, tail-flick and acetic acid-induced writhing models in mice. The ethanolic extract (400 mg/kg) produced a significant analgesic response in the hot-plate test, while all doses of extract, except the lowest dose, produced significant analgesic responses in the tail-flick test. The ethanolic extract doses of 200 and 400 mg/kg significantly ($p<0.05$) decreased the mean writhing response compared to vehicle controls.	Dinda *et al*. (2011)
	Anti-inflammatory	Root of five plants	The 80% ethanol extract of Ayurved Siriraj Ha-Rak remedy at 1 and 10 µg/mL inhibited COX-2 protein expression induced by IL-1β 1 ng/mL in the human umbilical vein endothelial cell (HUVEC) at the level of pre-translational level and has a biphasic dose-dependent effect on COX activity.	Okpanyi and Ezeukwu (1981)
		Root of five plants	The 80% ethanol extract of Ayurved Siriraj Ha-Rak remedy at 1 and 10 µg/mL and indomethacin (5 mg/kg) attenuated the increases of iNOS and COX-2 mRNA expression in homogenates of the heart collected from rats receiving LPS. Ha-Rak remedy did not aggravate LPS-induced organ injuries and expression of inflammatory cytokines, TNF-α, IL-1β and IL-6.	Dinda *et al*. (2011)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Ha-Rak remedy	Anti-inflammatory	Root of five plants	The 95% ethanol extract of Ha-Rak remedy showed activity against the over-expression of NO in LPS-stimulated RAW 264.7 with IC_{50} values of 40.36 ± 1.99 µg/mL (standard dose of Indomethacin has IC_{50} value of 20.32 ± 3.23 µg/mL).	Kumar *et.al.* (2015)
		Root of five plants	The 80% ethanol extract of Ha-Rak remedy (300, 1000, 3000 mg/kg) for 14 days before they were induced with LPS (6 mg/kg, i.v.) in rat. The markers of organ injury/dysfunction and pro-inflammatory cytokines were measured at 6 hours after LPS administration. The extract of Ha-Rak remedy trends to attenuate the plasma AST, ALT, CK, TNF-a, and IL-1β, although these effects were not statistically significant.	Booranasubkajorn *et al.* (2017)
		Root of five plants	The 80% ethanol extract of Ha-Rak remedy showed inhibition of COX-2 protein expression induced by IL-1β 1 ng/mL in HUVEC at concentrations under 10 μg/mL, but was no affected the expression of COX-1. Despite the significantly increased levels of exogenous PGE_2, Ha-Rak remedy had no effect on COX-2 mRNA expression.	Palo *et al.* (2017)
		Root of five plants	The aqueous extract of Ha-Rak remedy showed activity against the over-expression of NO in LPS-stimulated RAW 264.7 with IC_{50} values of 16.56 µg/mL.	Kwanhian and Bunluepuech (2018)
		Root of five plants	The ethanolic extract of Ha-Rak remedy showed moderate activity against the over-expression of NO in LPS-stimulated RAW 264.7 with IC_{50} values of 40.4 ± 2.0 µg/mL, but the aqueous extract was slightly active with IC_{50} values above 100 μg/mL (standard dose of Indomethacin has IC_{50} value of 20.3 ± 3.7 µg/mL).	Juckmeta *et al*. (2019)
	Antioxidant	Root of five plants	The ethanol extract of Ha-Rak remedy showed antioxidant activity in DPPH radical scavenging with IC_{50} value of 83.53 µg/mL (standard quercetin and BHT had IC_{50} value of 0.45 and 3.47 µg/mL).	Singharachai *et al.* (2011)
		Root of five plants	The 95% ethanol extract of Ha-Rak remedy showed antioxidant activity in DPPH radical scavenging with EC_{50} value of 40.93 ± 1.25 µg/mL (standard BHT had EC_{50} value of 12.75 µg/mL).	Juckmeta and Itharat (2012)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Ha-Rak remedy	Antioxidant	Root of five plants	The 80% ethanol extract of Ayurved Siriraj Ha-Rak remedy protected against increased tyrosinase activity and melanin in association with inhibition of cellular oxidative stress and upregulation of antioxidant defense system including GSH content as well as activities and mRNA level of catalase, GPx and GST in irradiated B16 cells.	Tripatara (2013)
		Root of five plants	The aqueous extract of Ha-Rak remedy showed antioxidant activity in DPPH and ABTS radical scavenging with SC_{50} value of 120.17 ± 0.07 and 73.27 ± 0.08 µg/mL, respectively.	Kwanhian and Bunluepuech (2018)
		Root of five plants	The 70% and 95% ethanolic extract of Ha-Rak remedy showed slightly activity against DPPH radical with IC_{50} value of 526.09 ± 0.26 and 586.89 ± 0.36 µg/mL, respectively (standard ascorbic acid and trolox had IC_{50} value of 5.42 ± 0.56 and 7.97 ± 0.43 µg/mL, respectively).	Noysang and Pummarin (2019)
	Antimalarials	Root of five plants	The dichloromethane extract of Ha-Rak remedy showed antimalarials effect by against the chloroquine-sensitive (Pf3D7) and -resistant (PfW2) strains *Plasmodium falciparum* using flow cytometry with IC_{50} values of 2.58 ± 0.39 (SI = 5.60) and 6.72 ± 1.46 (SI = 2.15) µg/mL, respectively.	Nutmakul *et al*. (2016)
		Root of five plants	The dichloromethane extract of Ha-Rak remedy showed antimalarials effect by against chloroquine-resistant *Plasmodium falciparum* (PfW2) strains at the ring, trophozoite, and schizont stages were exposed to the extracts or compounds for 2, 4, 6, 8, 10, 12, 24 or 48 h. The antiplasmodial activity of extract possessed a slow onset of action and was the most effective against ring-stage parasites. After 48 h of extracts, most of the treated parasites, at all stages, turned to the pyknotic form and could not recover even after extracts or compounds removal.	Nutmakul *et al*. (2020)
	Antibacterial	Root of five plants	The ethanolic extract of Ha-Rak remedy and standard gentamicin showed antimicrobial activities by against *Streptococcus pyogenes* using disc diffusion assay with zone of inhibition were 13.0 ± 1.4 and 16.0 ± 1.2 mm, respectively.	Nuaeissara *et al*. (2011)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Ha-Rak remedy	Antibacterial	Root of five plants	The 70% and 95% ethanolic extract of Ha-Rak remedy, and standard clindamycin showed antimicrobial activities by against *Propionibacterium acnes* using disc diffusion assay with MIC value were 10.10 ± 0.45 mg/mL, 5.05 ± 0.01 mg/mL and 0.55 ± 0.50 µg/mL, respectively.	Noysang and Pummarin (2019)
	Antiproliferative	Root of five plants	The ethanolic extract of Ha-Rak remedy exhibited antiproliferative activity against doxorubicin-sensitive (K562) and doxorubicin-resistant (K562/adr) erythromyelogenous leukemic cells with IC_{50} values of 79.33 ± 1.33 and 139.00 ± 0.99, respectively.	Suttana *et al.* (2021)
	Anticancer	Root of five plants	The ethanolic extract of Ha-Rak remedy showed cytotoxic activity against five cancer cell lines of the respiratory tract, KB, Hep2, A549, COR-L23 and NCI-H226 with IC_{50} values in the range of 10.1 ± 2.8 to 33.6 ± 0.9 µg/mL.	Juckmeta *et al.* (2019)
	Toxicity	Root of five plants	The ethanol extract of Ha-Rak remedy showed non-toxic on *Artemia salina* L. (Brine shrimp larva) eggs with LC_{50} of 265 µg/mL.	Singharachai *et al*. (2011)
		Root of five plants	The sub-chronic oral toxicity of 80% ethanol extract of Ha-Rak remedy (300, 1000, 3000 mg/kg) for 14 days did not show organ toxicity in Wistar rats.	Tripatara (2013)
		Root of five plants	The sub-chronic oral toxicity of 80% ethanol extract of Ha-Rak remedy (300, 1000, 3000 mg/kg) for 14 days did not increase plasma urea, creatinine, AST, ALT, CK, and lipase, when compared to sham before they were induced with LPS (6 mg/kg, i.v.) in rat.	Booranasubkajorn *et al.* (2017)
		Root of five plants	A randomized, placebo, controlled trial was carried out in 46-healthy Thai volunteers, both male and female of Ha-Rak remedy powder (1,500 mg/day) or placebo. Adverse events occurred including abdominal pain, loose stools in Ha-Rak remedy group and vomiting, common cold and pancytopenia in placebo group. The results of the laboratory blood test indicate the completeness of the blood cells.	Chandranipapongse *et al.* (2017)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Ha-Rak remedy	Toxicity	Root of five plants	The 80% ethanol extract of Ha-Rak remedy was no cytotoxicity in the human umbilical vein endothelial cell (HUVEC) with extract at up to 100 μg/mL.	Palo *et al.* (2017)
		Root of five plants	The aqueous extract of Ha-Rak remedy showed cytotoxicity effect with CD_{50} value of 44.32 ± 3.25 µg/mL in LPS-stimulated RAW 264.7 by MTT assay.	Kwanhian and Bunluepuech (2018)
Azadirachta indica A.Juss.	Antipyretic	Leaves	The methanolic extract at dose of 400 mg/kg, p.o. on pyrogenic lipopolysaccharid induced fever in rats. It shows that indomethacin 4 mg/kg, within 3 hours after oral administration, reduced an existing fever in rabbits by 30.6 %, acetylsalicylic acid by 24 % and *A. indica* composite extract 400 mg/kg body weight by 15.7 %.	Okpanyi and Ezeukwu (1981)
		Leaves	The extract of leaves at 62.5 mg, 125 mg, 250 mg, and 500 mg/kg, i.p. on brewer's yeast induced fever in rats. The extract in the dose of 125 mg/kg showed significant antipyretic effect only 4th hour of initial pyrexia.	Kumar *et.al.* (2015)
	Analgesic	Leaves	The hydro-alcoholic extract, ethyl acetae and n-butanol fractions at 100 mg/kg were evaluated using acetic acid induced writhing, hot plate test and tail flick method in mice. In the acetic acid-induced writhing model, the extract and the fractions had a good analgesic effect ($P<0.01$) characterized by a reduction in the number of writhes when compared to the control. In tail flick method, the extract and all the fractions at 100 mg/kg showed significant activity ($P<0.01$) after 30 min.	Dinda *et al.* (2011)
		Leaves	The extract of leaves at 62.5 mg, 125 mg, 250 mg, and 500 mg/kg, i.p. increase in tail flick latency (TFL) in rats. The extract in the dose of 250 mg/kg body weight showed significant rise in TFL from 60 minutes to 90 min. TFL increased significantly from 45 min to 90 min in 500 mg/kg and in the dose of 62.5 mg and 125 mg/kg body weight, it produced no significant effect.	Kumar *et.al.* (2015)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Azadirachta indica A.Juss.	Anti-inflammatory	Leaves	The methanolic extract at dose of 400 and 800 mg/kg, p.o. in carrageenin-induced rat paw edema were reduced more than indomethacin 4 mg/kg (19%), with 20.6% and 31.8%, respectively.	Okpanyi and Ezeukwu (1981)
		Leaves	The hydro-alcoholic extract, ethyl acetae and n-butanol fractions at 100 mg/kg body weight significantly reduced the formation of oedema induced by carrageenan in rats.	Dinda *et al.* (2011)
		Leaves	The extract at dose of 62.5 mg, 125 mg, 250 mg, and 500 mg/kg, i.p. in carrageenin-induced rat paw edema showed a percentage inhibition of edema at 4 hours, 2.7%, 26. %, 52.32% and 63.01%, respectively.	Kumar *et.al.* (2015)
		Leaves	The antioxidant activity of the methanolic extract of *A. indica* was also determined and it was found that maximum inhibition obtained was 71.23% when 500 µg methanolic extract was used.	Pokhrel *et al.* (2015)
	Antibacterial	Leaves	The hexane, chloroform and methanol extracts of *A. indica* leaves were expressed antibacterial activity by against *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Micrococcus luteus, Bacillus subtilis, Enterococcus faecalis* and *Streptococcus faecalis.* Methanol extract was the most effective against all the tested bacteria. The chloroform extract showed good to moderate whereas hexane extract showed low antibacterial activity. The inhibition zone values were interpreted as sensitive (18 mm), intermediate (14-17 mm) and resistant (<14 mm).	Koona *et al.* (2011)
		Leaves	The antibacterial activity was performed using six different bacterial strains: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhi* (ATCC14028), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 35659). It was found that the maximum zone of inhibition of 22±3 mm was shown against *S. aureus* using 700 µg methanolic extract.	Pokhrel *et al.* (2015)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Azadirachta indica A.Juss.	Antiviral	Leaves	The aqueous extract of *A. indica* leaves at its maximum non-toxic concentration of 1.897 mg/ml completely inhibited 100–10,000 $TCID_{50}$ of Dengue virus type-2 as indicated by the absence of cytopathic effects.	Parida *et al*. (2002)
		Leaves	The crude acidic extract of leaves (90.38%) possesses very remarkable antiviral activity against herpes simplex virus type 1 compared with acyclovir (54.33%) at concentration 20 µg/mL.	Amer *et al.* (2010)
	Anticancer	Leaves	The cytotoxic activity of crude aqueous extracts from leaves revealed significant inhibition of Ehrlich ascites carcinoma cell line growth and had anticancer activity at different concentrations (250, 500, 750 and 1000 µg/mL). The acidic extract from leaves inhibited Ehrlich ascites carcinoma cell line growth and had anticancer activity. IC_{50} values of acidic extracts from leaves was 669.43 µg/mL.	Amer *et al.* (2010)
		Leaves	The purified protein extract was found to be active against HELA cervical cancer cell, BT-549 breast cancer cell and Neuro-2a cell lines with IC_{50} value of 74.03 ± 2.31, 64.82 ± 1.64, 238.32 ± 2.12 and 109.94 ± 2.96, 59.61 ± 0.75 µg/mL for 24 h and 48 h, respectively.	Saiqali *et al.* (2018)
	Cytotoxic	Leaves	The acute toxicity, a total of 70 NMRI mice of mixed sexes, weighing between 23 and 30 grams were used. 10 mice were used for each of the 6 different doses of methanolic extract as also for the control. The result of the acute oral toxicity of methanolic extract indicates a very low toxicity with an LD_{50} in the range of 13 g/kg in mice.	Okpanyi and Ezeukwu (1981)
		Leaves	The acute oral toxicity of the methanolic extract at a dose of 20, 200 and 2000 g/kg body weight in mice. No significant changes were observed in the behavioural or autonomic responses in mice after treatment with different dosses of *A. indica* leaf extract. There was no mortality in these animals during the observational period of 14 days.	Kanagasanthosh *et al*. (2015)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Bridelia ovata Decne.	Laxative	Leaves	A clinical study of bolus and infusion *B. ovata* leaf at doses of 1500 – 2000 mg in 24 healthy males and females. Found that tamarind leaves act as a mild laxative. In the constipated population, the effect was more pronounced than in the normal population, and the infusion was more effective than the bolus.	Iamsa-ard and Sakkankoson (1978)
	Anticancer	Leaves	The ethanolic extract of *B. ovata* showed cytotoxic activity against COR L-23 lung cancer and PC3 prostate cancer cell lines with IC_{50} value of 7.11 and 6.29 µg/mL.	Saetung *et al.* (2004)
		Leaves	The ethyl acetate extract of *B. ovata* exhibited cytotoxicity in a dose-dependent manner upon the human A549 lung cancer cell line with IC_{50} value of 139.10 ± 34.60 µg/mL.	Poofery *et al.* (2020a)
		Leaves	The ethanolic extract of *B. ovata* showed cytotoxic activity against HepG2 human liver cancer cell lines at 50% survival (IC_{50}) with 92.8 ± 6.7 µg/mL.	Baig *et.al.* (2015)
		Leaves	The ethyl acetate extract of *B. ovata* showed greater toxicity against MDA-MB-231 breast cancer cells compared with their effect on MCF10A normal epithelial mammary cells with IC_{50} were 40.0±3.6 and 393.8±4.3 µg/mL, respectively.	Poofery *et al.* (2020b)
	Cytotoxic	Leaves	The ethanolic extract of *B. ovata* showed cytotoxic activity against normal fibroblast (10FS) with IC_{50} = 9.11 µg/mL.	Saetung *et al.* (2004)
		Leaves	The ethyl acetate extract of *B. ovata* showed cytotoxic activity against normal lung cell line with IC_{50} value of 143.78 ± 75.85 µg/mL.	Poofery *et al.* (2020)
Capparis micracantha DC.	Antipyretic	Roots	Powder of *C. micracantha* at 40 mg/kg, p.o. significantly reduced rectal temperature from the first hour after brewer's yeast induced pyxeria in rats.	Konsue *et al.* (2008)
		Roots	The ethanolic extract of *C. micracantha* at various doses (25-400 mg/kg) significantly ($p < 0.05$) reduced body temperature on lipopolysaccaride (LPS) in rats.	Jongchanapong *et al.* (2010)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Capparis micracantha DC.	Analgesic	Roots	The ethanolic extract of *C. micracantha* at various doses (25-400 mg/kg p.o.) were evaluated for their analgesic activity using the hot-plate, tail-flick and acetic acid-induced writhing models in mice. The ethanolic extract produced a significant analgesic response compared to vehicle controls.	Jongchanapong *et al*. (2010)
	Anti-inflammatory	Roots	The 95% ethanol extract of *C. micracantha* showed anti-inflammatory effect by against NO with IC_{50} values of 61.1 ± 4.3 µg/mL.	Juckmeta *et al.* (2019)
	Antioxidant	Roots	The ethanolic extract of *C. micracantha* showed antioxidant activity, with EC_{50} values of 61.37 µg/mL in DPPH radical.	Juckmeta and Itharat (2012)
	Toxicity		The ethanolic and water extracts of *C. micracantha* showed non-toxic on *Artemia salina* L. (Brine shrimp larva) eggs (LC_{50} > 1000 and 10000 µg/mL).	Singharachai *et al.* (2011)
Cassia fistula L.	Antipyretic	Fruits	The ethanolic extract at 250 and 500 mg/kg, p.o. dose showed significant ($p < 0.05$) antipyretic activity by reduced pyrexia on brewer's yeast induced fever in rats. Methanolic extract (500 mg/kg) showed the effect to the same degree as paracetamol (20 mg/kg, i.p.).	Singh *et.al.* (2012)
	Analgesic	Pods	The methanolic extract of *C. fistula* at doses of 250 mg/kg and 500 mg/kg, i.p. in Wister albino rats and Swiss Albino mice showed significant ($p<0.01$) inhibition in pain response induced by thermal, mechanical, and writhing stimuli in dose dependent manner.	Sheikh *et al.* (2010)
	Anti-inflammatory	Fruits	The aqueous extract of *C. fistula* at 300, 400 and 500 mg/kg dose inhibited swelling of the paw edema induced by carrageenin in rats with % inhibition of 43.81, 48.74 and 56.90%, respectively.	Anwikar and Bhitre (2010)
	Immunomodulatory	Fruits	The aqueous extract of *C. fistula* energizes the immune system by stimulating large number of anti-RBC producing cells in the spleen that suggest its therapeutic efficacy.	Ali *et al*. (2008)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Cassia fistula L.	Antioxidant	Fruits	The methanolic and hexane extracts of *C. fistula* showed potent antioxidant activity in DPPH radical scavenging, hydroxyl radical scavenging, FRAP and Fe^{3+} reducing power. EC_{50} of methanolic and hexane extracts against DPPH radical were 0.915 and 1.865 µg/mL, whereas against hydroxyl radical were 0.889 and 1.723 µg/mL, respectively, were less potent than ascorbic acid (EC_{50} = 0.102 and 0.105 µg/mL, respectively). Methanolic extract showed greater FRAP value as 136.05 equivalent mmol of Fe^{2+}/g sample, whereas the hexane extract showed FRAP value 75.09 equivalent mmol of Fe^{2+}/g sample. Other than, hexane extract showed less degree of Fe^{3+} reduction than the methanolic extracts.	Irshad *et.al.* (2012)
	Antibacterial	Fruits	The methanolic extract of *C. fistula* at 100 mg/mL had antibacterial activity namely, *Bacillus subtilis, Pseudomonas eurogenosa, Streptococcus faecalis, Salmonella typhi* and *Staphylococcus aureus* with zone of inhibition ranged from 3.00±0.10 to 6.02±0.23 mm. Maximum zone formation was against *Proteus mirabilis.*	Kadhim *et.al.* (2016)
		Fruit pulp	The hydroalcoholic and chloroform extracts of *C. fistula* at concentration 25, 50, 100 and 250 µg/mL were found to inhibit *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* with zone of inhibition ranging from 10 to 20 mm.	Bhalodia *et.al.* (2012)
	Antiviral	Fruit pulp	Antiviral activity of *C. fistula* was assayed by using different concentrations of non-toxic doses of the extract against 10$TCID_{50}$ dose of infectious bovine rhinotracheitis (IBR) virus in MDBK cell line by using MTT assay. Maximum non-toxic dose of extract using MDBK cell line was determined and found to be 5 mg/mL. In this preliminary study, it has been concluded that pod aqueous hot extract of *C. fistula* pods showed dose dependent anti IBR virus activity.	Anubhuti *et.al.* (2010)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Cassia fistula L.	Anticancer	Fruit pulp	The ethyl acetate and n-butanol extract of *C. fistula* were active against human cervical cancer (SiHa) and breast cancer (MCF-7) cell lines. IC_{50} of ethyl acetate and n-butanol extract against SiHa cells were 415.5±0.19 and 535.3±0.32 µg/mL, whereas against MCF-7 cells were 422.2±0.32 and 564.5±0.21 µg/mL respectively ($p < 0.01$).	Irshad *et.al.* (2014)
	Laxative (Stimulant laxatives)	Fruit pulp	Aqueous suspension of Sun-dried (SD) and non-Sun dried (NSD) fruit pulps were administered orally 60 min before experiment in rats. Both SD and NSD in the dose of 1.0 g/kg showed an increase in the number of defecations and fecal output during 4 hours after treatment but stool was semisolid with SD and semisolid and watery with NSD. Both SD and NSD treated rats showed increase in the intestinal intraluminal fluid (ILF) accumulation and motility but the accumulation of ILF was less marked in SD group compared to NSD group. The stimulatory effect of SD on ILF accumulation and intestinal motility could be due to its predominant action on NO formation as only L-NAME a NOS inhibitor blocked both ILF accumulation and intestinal motility per se and in SD-treated rats while atropine (anti-cholinergic), loperamide (µ and ê receptor inhibitor) and indomethacin (PGs synthesis blocker) partially blocked them.	Agrawal *et.al.* (2012)
	Toxicity	Fruits	The acute oral toxicity of the ethanolic extract in six wistar rats (200-220 g) of either sex was dosed with extracts in different concentrations and were observed for any symptoms of toxicity for 48 hrs as per guidelines no. 425 (OECD 2001) and LD_{50} was estimated > 5000 mg/kg.	Singh *et.al.* (2012)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Cassia fistula L.	Toxicity	Pods	The acute oral toxicity of the ethyl acetate fraction obtained from a hydroalcoholic extraction at the doses of 500 and 2000mg/kg, p.o and 10 ml/1kg of CMC 1% (vehicle, p.o., control group) in mice. The sedative and motor toxicity were evaluated by a phenobarbitone induced sleep test and rotarod behavior respectively. The extract in the doses used in this experiment did not produce sedation or motor toxicity.	Kalaiyarasi *et.al*. (2015)
		Fruit pulp	The acute oral toxicity of the aqueous suspension of Sun-dried (SD) fruit pulp at the doses of 10 g/kg oral dose (10 times of optimal effective dose) did not show any acute toxic effect in mice.	Agrawal *et.al.* (2012)
		Pods	The acute oral toxicity of aqueous extract of the pods at the doses of 800, 1600, 3200, 6400, 12800 mg/kg, p.o.in swiss albino mice. The results obtained for *C. fistula* infusion when compared with senokot tablet showed that the infusion of *C. fistula* pods possessed very low levels of toxicity, having the LD_{50} of 6600 mg/kg and without any pathological effects on the organs examined microscopically.	Akanmu *et.al.* (2004)
		Pods	The sub-chronic oral toxicity of aqueous extract of the pods at the doses of 250, 500 and 1000 mg/kg, p.o.in wister albino rats. The results showed not to produce any change in behaviour, in food consumption and body weight during the six weeks of experiments. Macroscopic inspection of the liver, kidney, testis and brain did not indicate any change in the organs of the treated groups compared to the control rats.	Akanmu *et.al.* (2004)
Clerodendrum indicum (L.) Kuntze	Antipyretic	Roots	Powder of *C. indicum* at 40 mg/kg, p.o. significantly reduced rectal temperature from the seventh hour after brewer's yeast induced pyxeria in rats.	Konsue *et al.* (2008)
		Roots	The ethanolic extract of *C. indicum* at various doses (25-400 mg/kg) significantly ($p < 0.05$) reduced body temperature on lipopolysaccaride (LPS) in rats.	Jongchanapong *et al*. (2010)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Clerodendrum indicum (L.) Kuntze	Analgesic	Roots	The ethanolic extract of *C. indicum* at various doses (25-400 mg/kg p.o.) were evaluated for their analgesic activity using the hot-plate, tail-flick and acetic acid-induced writhing models in mice. The ethanolic extract produced a significant analgesic response compared to vehicle controls.	Jongchanapong *et al.* (2010)
	Anti-inflammatory	Roots	The 70% methanol extract of *C. indicum* at various doses 100, 200, 300, 400 and 500 µg/mL showed anti-inflammatory by against protien denaturation using egg albumin. Doses of 500 µg/mL (66.4%) was more potent than diclofenac sodium (59.3%).	Sushma *et al.* (2021)
	Antimalarials	Roots	The dichloromethane extract of *C. indicum* showed antimalarials effect by against *Plasmodium falciparum* (Pf3D7) *in vitro* with IC_{50} values of 10.92 ± 2.02 µg/mL.	Nutmakul *et al.* (2016)
	Antivirals	Roots	The water extract of *C. indicum* showed inhibitory activity against HIV-1 integrase (IN) using the multiplate integration assay (MIA) with an IC_{50} value of 43.5 µg/mL.	Bunluepuech and Tewtrakul (2009)
	Anticancer	Roots	The petroleum ether and ethyl acetate extracts of *C. indicum* showed antiproliferative activity with IC_{50} values of 72.83 µg/mL and 31.33 µg/mL respectively on HCT116 colon cancer cell line.	Keerthi *et al.* (2020)
	Toxicity	Roots and stems	The acute oral toxicity of 50% ethanolic extract of *C. indicum* in mice at dose 10 g/kg (5,000-fold the size of the treatment in humans). The results showed not toxicity in mice.	Department of Medical Sciences (2003)
Dracaena cochinchinensis (Lour.) S.C.Chen	Antipyretic	Stems	The methanolic fraction of *D. cochinchinensis* exhibited antipyretic effect on brewer's yeast induced fever in rats.	Reanmongkol *et al.* (2003)
	Analgesic	Red resin	The red resin (dragon's blood) obtained from the wood of *D. cochinchinensis* at 1.72 g/kg and 3.44 g/kg for five consecutive days significantly inhibit mice's acetic acid-induced writhing.	Xiang *et al.* (2001)
		Cochinchinenin B	Cochinchinenin B isolated form *D. cochinchinensis* wood showed inhibition capsaicin (CAP) starting current (ICAP) of the dorsal root ganglia in isolated mice.	Wang *et al.* (2008)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Dracaena cochinchinensis (Lour.) S.C.Chen	Analgesic	Flavonoid-rich extracts	Flavonoid-rich extracts from the wood of *D. cochinchinensis* at various doses (100, 200, 400 mg/kg) alleviated significantly SNI-induced mechanical hypersensitivity, as paw withdrawal mechanical threshold (PMWT) increased in a dose-dependent manner. Moreover, the extract not only reduced the level of NO, NOS, TNF-α and IL-1β, but also upregulated the level of IL-10 in the spinal dorsal horn of SNI rats.	Chen *et al*. (2015)
	Anti-inflammatory	Stems	The 95% ethanolic extract of *D. cochinchinensis* showed anti-inflammatory effect by inhibition NO production (IC_{50} = 38.37±1.66 µg/ml).	Makchuchit (2010)
		Stillbenoids	The stillbenoids including 4,3',5'-trihydroxystilbene, 4,3'-dihydroxy-5'-methoxystilbene and 4-hydroxy-3', 5'-dimethoxystilbene were isolated form *D. cochinchinensis* wood. The result found these compounds exhibited the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) with IC_{50} value of 1.29 - 4.92 microM.	Likhitwitayawuid *et al*. (2002)
		Wood	The 95% ethanolic extract of *D. cochinchinensis* exhibited the highest NO, TNF-α and IL-6 inhibitory activity in LPS-stimulated RAW 264.7 with IC_{50} of 9.42 ± 1.81, 55.27 ± 5.47 and 12.02 ± 0.30 µg/mL, respectively.	Dechayont *et al*. (2021)
	Antioxidant	Wood	The 95% ethanolic and aqueous extracts of *D. cochinchinensis* showed antioxidant activity in $ABTS^{\bullet+}$ and DPPH radical scavenging. IC_{50} of 95% ethanolic extract against $ABTS^{\bullet+}$ and DPPH radical was 8.38 and 16.95 µg/mL, respectively. IC_{50} of aqueous extract against $ABTS^{\bullet+}$ and DPPH radical was 10.92 and 19.65 µg/mL, respectively.	Dechayont *et al*. (2021)
	Antibacterial	Wood	The ethanolic extract of wood inhibited *S. pyogenes, S. aureus, MRSA* and *P. aeruginosa* with MIC of 0.156, 0.625, 0.625 and 1.25 µg/mL, respectively.	Dechayont *et al*. (2021)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Dracaena cochinchinensis (Lour.) S.C.Chen	Antibacterial	Flavonoid	New flavonoid derivatives 6,7- and (2S)-4',7-dihydroxy-8-methylflavan were isolated from *D. cochinchinensis* woods and very effective against *H. pylori* with MIC values of 29.5, 29.5, and 31.3 μM, respectively	Zhu *et al*. (2007)
	Antivirals	Heart wood	The ethanolic and water extracts of heart wood showed inhibitory activity against HIV-1 integrase (IN) using the multiplate integration assay (MIA) with an IC_{50} value of 28.0 and 22.1 μg/mL, respectively.	Bunluepuech and Tewtrakul (2009)
	Anticancer	Cholest-4α-methyl-7-en-3β-ol	Cholest-4α-methyl-7-en-3β-ol was isolated from *D. cochinchinensis* woods, has potent inhibitory activity against PC12 tumors with a ratio of 0.5043 (10 μg/mL). The synthesized derivatives were tested on human cancer cell lines including colon (HCT-8), liver (BEL-7402) and nasopharyngeal cancer (KB) cells. The results showed that cholest-4α-methyl-8-en-3β,7α-diol 6α inhibits KB cells significantly with an IC_{50} value of 1.32×10^{-9} μg/mL. In addition, the cytotoxic properties of this compound against HCT-8 and BEL-7402 cells are excellent, with an IC_{50} of 1.2 μg/mL.	Fan *et al*. (2014)
	Toxicity	Wood	The 95% ethanolic and water extracts of *D. cochinchinensis* at concentration of 100 μg/mL showed non-toxic effect on the RAW 264.7 cell by MTT assay.	Dechayont *et al*. (2021)
		Wood	The acute toxicity of 50% ethanolic extract of *D. cochinchinensis* in mice at dose 10 g/kg, s.c. (1,111-fold the size of the treatment in humans). The results showed not toxicity in mice.	Department of Medical Sciences (2003)
		Wood	The chronic toxicity, rabbits were given extract of *D. cochinchinensis* at rates of 3 g/kg body weight and 1.5 g/kg body weight, once daily, for 90 days. The extract did not cause changes in the animal's pathological state, and had no significant effect on blood erythrocytes, leukocytes number, alanine aminotransferase, urea nitrogen, or weight. There was no functional damage to the liver or kidney.	Lin (1986)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Ficus racemosa L.	Antipyretic	Roots	The 95% ethanolic extract of *F. racemosa* at various doses (50, 100, 200 and 400 mg/kg) significantly ($p < 0.05$) reduced body temperature on lipopolysaccaride (LPS) and brewer's yeast induced pyxeria in rats. Doses of 200 and 400 mg/kg were equally potent as aspirin.	Chomchuen *et al*. (2010)
		Roots	Powder of *F. racemose* at 40 mg/kg, p.o. significantly reduced rectal temperature from the first hour after brewer's yeast induced pyxeria in rats.	Konsue *et al.* (2008)
		Roots	The ethanolic extract of *F. racemose* at various doses (25-400 mg/kg) significantly ($p < 0.05$) reduced body temperature on lipopolysaccaride (LPS) in rats.	Jongchanapong *et al*. (2010)
	Analgesic	Roots	The ethanolic extract of *F. racemosa* at various doses (25-400 mg/kg p.o.) were evaluated for their analgesic activity using the hot-plate, tail-flick and acetic acid-induced writhing models in mice. The ethanolic extract produced a significant analgesic response compared to vehicle controls.	Jongchanapong *et al*. (2010)
	Anti-inflammatory	Stem barks	Ethanol extract of *F. racemose* also inhibited COX-1 with IC_{50} value of 100 ng/mL proves that the drug is used in the treatment of inflammatory conditions.	Li *et al.* (2003)
		Barks	The 95% ethanolic extract of *F. racemosa* showed anti-inflammatory effect by against COX-1 and 5-LOX in vitro (IC_{50} = 83 and 58 µg/mL, respectively).	Li *et al.* (2004)
		Racemosic acid	Racemosic acid isolated from *F. racemosa* bark showed potent inhibitory activity against COX-1 and 5-LOX in vitro with IC_{50} values of 90 and 18 µM, respectively.	Li *et al.* (2004)
	Antioxidant	Roots	The ethanolic extract of *F. racemosa* showed potent antioxidant activity in DPPH radical scavenging with IC_{50} value of 4.87 µg/mL, was more potent than standard ascorbic acid (IC_{50} = 5.27 µg/mL). All concentrations (10 - 80 µg/mL) were more potent than standard ascorbic acid in reducing Fe^{3+} to Fe^{2+} by FRAP method.	Jain *et al.* (2013)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Ficus racemosa L.	Antioxidant	Roots	The ethanolic extract of *F. racemosa* showed potent antioxidant activity in DPPH radical scavenging with EC_{50} value of 4.87 µg/mL, was more potent than standard BHT (EC_{50} = 12.75 µg/mL).	Juckmeta and Itharat (2012)
	Antimalarials	Roots	The dichloromethane extract of *F. racemose* showed antimalarials effect by against *Plasmodium falciparum* (Pf3D7) *in vitro* with IC_{50} values of 19.35 ± 1.36 µg/mL.	Nutmakul *et al.* (2016)
	Antibacterial	Roots	The ethanolic extract of *F. racemose* at various doses showed antimicrobial activities by disc diffusion assay. The highest concentration of ethanol extract at dose of 8 mg/disc against *E. coli, B. subtilis, P. aeruginosa* and *E. cloacae* with zone of inhibition were 24.4, 7.2, 9.1 and 16.1 mm, respectively.	Goyal (2012)
		Roots	The ethanol and aqueous extracts of root at various doses 25, 50 and 75 mg/mL showed antimicrobial activities by disc diffusion assay. The highest concentration of ethanol and water extracts at dose of 75 mg/mL against *S. aureus*, *E. coli* and *K. pneumonia* with zone of inhibition were 35, 30, 25 mm and 35, 25, 10 mm, respectively. Standard drug ampicillin showed maximum antimicrobial activity with zone of inhibition were 40, 35 and 30 mm, respectively.	Murti and Kumar (2011)
	Cytoxicity	Roots	The ethanolic and water extracts of *F. racemosa* showed non-toxic on *Artemia salina* L. (Brine shrimp larva) eggs with LC_{50} more than 10,000 µg/mL.	Singharachai *et al.* (2011)
Gymnopetalum chinense (Lour.) Merr.	Antibacterial	Leaves	The methanolic extract showed highest zone of inhibition (16.66 mm) against *S. pyogenes* using DD assay. Similar results were examined as lowest MIC values were found with methanol leaf extract against *S. pyogenes* and *S. mutans* (200 µg/mL).	Kumar *et al.* (2017)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Harrisonia perforata (Blanco) Merr.	Antipyretic	Roots	Powder of *H. perforata* at 40 mg/kg, p.o. significantly reduced rectal temperature from the first hour after brewer's yeast induced pyxeria in rats.	Konsue *et al.* (2008)
		Roots	The ethanolic extract of *H. perforata* at various doses (25-400 mg/kg) significantly ($p < 0.05$) reduced body temperature on lipopolysaccaride (LPS) in rats.	Jongchanapong *et al*. (2010)
	Analgesic	Roots	The ethanolic extract of *H. perforata* at various doses (25-400 mg/kg p.o.) were evaluated for their analgesic activity using the hot-plate, tail-flick and acetic acid-induced writhing models in mice. The ethanolic extract produced a significant analgesic response compared to vehicle controls.	Jongchanapong *et al*. (2010)
	Anti-inflammatory	Roots	The 95% ethanol extract of *H. perforata* at various doses (50, 100, 200 and 400 mg/kg) significantly ($p < 0.05$) reduced the effect of acute inflammation in rat paw edema by 28.49-65.05% at 2 hours after carrageenan injection.	Somsil *et al.* (2012)
		Roots	The 95% ethanol extract of *H. perforata* showed the maximum inhibitory effect in LPS-stimulated J774A.1 cell for TNF-α, IL-1ß and IL-6 were 49.83, 47.27 and 32.16% respectively. The concentration of the extract produced maximum effect were 50 µg/mL for both TNF-α and IL-1ß but for IL-6, it was 12.5 µg/ml.	Somsil *et al.* (2010)
		Roots	The 95% ethanol extract of *H. perforata* showed activity against the over-expression of NO in LPS-stimulated RAW 264.7 with IC_{50} values of 53.16 ± 5.21 µg/mL.	Juckmeta and Itharat (2012)
		Harperfolide	Harperfolide was isolated from *H. perforata* root showed potent anti-inflammatory activity *in vitro*. It inhibited the generation of nitric oxide (NO) in LPS-stimulated RAW 264.7 with an IC_{50} value of 6.51±2.10 µM, was more potent than standard indomethacin with an IC_{50} value of 28.42±3.51 µM. It reduces the expression of iNOS protein that produces NO in the inflammation process.	Choodej *et al.* (2013)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Harrisonia perforata (Blanco) Merr.	Antimalarials	Roots	The dichloromethane and methanol extracts of *H. perforata* inhibited *Plasmodium falciparum* (Pf3D7) with IC_{50} values were 4.14 ± 1.59 and 13.09 ± 4.08 µg/mL, respectively.	Nutmakul *et al.* (2016)
	Antibacterial	Roots	The ethanolic extract of root inhibited *S. dysenteriae*, *A. buamannei*, *S. aureus*, MRSA, *S. pyogenes* and *B. subtilis* with inhibition zone of 7.2, 6.2, 7.8, 7.4, 12.0 and 7.2 mm, respectively. The water extract of root inhibited *S. dysenteriae*, *A. buamannei*, *S. pyogenes* and *B. subtilis* with inhibition zone of 7.8, 11.0, 8.0 and 11.7 mm, respectively.	Nuaeissara *et al.* (2011)
	Antivirals	Roots	The water extract of *H. perforata* showed inhibitory activity against HIV-1 integrase (IN) using the multiplate integration assay (MIA) with an IC_{50} value of 2.3 µg/mL.	Bunluepuech and Tewtrakul (2009)
	Antioxidant	Roots	The ethanolic extract of *H. perforata* showed antioxidant activity, with EC_{50} values of 16.91 µg/mL in DPPH radical.	Juckmeta and Itharat, 2012
	Toxicity	Roots	The ethanolic and water extracts of *H. perforata* showed LC_{50} of 600 and 560 µg/mL on *Artemia salina* L. eggs, respectively.	Singharachai *et al*. (2011)
Ligusticum sinense Oliv.	Anti-inflammatory	Rhizomes	The ethanolic extract of *L. sinense* showed activity against the over-expression of NO in LPS-stimulated RAW 264.7 with IC_{50} of 3.769 µg/mL.	Itharat *et al.* (2009)
	Antioxidant	Rhizomes	The essential oil of *L. sinense* to DPPH radical scavenging and inhibiting β-carotene bleaching was determined based on their concentrations, with IC_{50} values of 407.38 µg/mL and 224.64 µg/mL, respectively, less potent than BHT (IC_{50} = 25.66 ± 0.42 and 31.46 ± 0.68 µg/mL, respectively).	Wang *et.al.* (2011)
	Antibacterial	Rhizomes	The MIC values of the essential oil of *L. sinense* on test bacteria ranged from 62.5 µg/mL to 200 µg/mL, MBC values from 150 µg/mL to 250 µg/mL, and IC_{50} values from 43.53 µg/mL to 135.58 µg/mL. Of them, both *B. subtilis* and *A. tumefaciens* were the most sensitive with their IC_{50} values as 43.53 µg/mL and 49.35 µg/mL, respectively.	Wang *et.al.* (2011)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Ligusticum sinense Oliv.	Anticancer	Rhizomes	The methanolic extracts of L. sinense exhibited antimelanogenesis activity in B16-F10 cells with an IC_{50} value of 50 µg/mL.	Chu (2011)
	Toxicity	Rhizomes	The acute toxicity of aqueous extract of the rhizomes by intraperitoneal and muscle injection in mice. It was found that evealed that median lethal dose (LD_{50}) were 65.86 and 66.42 g/kg, respectively. Acute toxicity study in mice of 50% ethanolic extract from rhizomes found that LD_{50} values were greater than 10 g/kg when given by feeding or subcutaneous injection. The results showed not to produce any change in behavior, in food consumption and body weight during the six weeks of experiments. Macroscopic inspection of the liver, kidney, testis and brain did not indicate any change in the organs of the treated groups compared to the control mice.	Department of Medical Sciences (2003)
Mesua ferrea L.	Anti-inflammatory	Flowers	The 95% ethanolic extract of *M. ferrea* showed anti-inflammatory effect by inhibition NO production (IC_{50} = 26.23±3.42 µg/ml).	Makchuchit (2010)
			The ethanol extract of *M. ferrea* at dose of 400 mg/kg showed the maximum inhibit the paw edema induced by carrageenan in rats.	Tiwari and Nandy (2012)
		Flowers	The 95% ethanolic extract of *M. ferrea* showed anti-inflammatory effect by inhibition cyclooxygenase-2 enzyme (COX-2) in LPS-stimulated RAW 264.7 with IC_{50} = 8.38±0.68 µg/mL.	Mokmued *et al*. (2017)
	Immunomodulatory	Flower buds	ACII contain *M. ferrea* flower buds showed immunomodulatory activity on radiation induced immunosuppression. The lowered total white blood cell count was significantly increased. There was no significant change in the hemoglobin content of irradiated animals when compared with drug treated or normal animals.	Tharaka *et al.*, 2006

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Mesua ferrea L.	Antioxidant	Flowers	Ethanol extract from *M. ferrea* had antioxidant activity by DPPH assay with IC_{50} = 14.38 µg/mL was less potent than gallic acid (IC_{50} = 0.72 µg/mL).	Thongmak *et al.* (2021)
	Anticonvulsant	Flowers	Administration of ethanolic extract of *M. ferrea* in albino mice at dose 200, 400 and 600 mg/kg p.o. by MES assessed using albino mice against Maximum Electroshock Seizure (MES) test. It was found that extracts inhibited convulsions and significantly reduced the duration of seizures, with dose of 200, 400 and 600 mg/kg inhibited by 100% ($p < 0.01$.), 60% ($p < 0.01$) and 100% ($p < 0.001$), respectively.	Tiwari *et al.* (2012)
	Antiamnesic	Flowers	Administration of ethanolic extract of *M. ferrea* in rats at dose 100, 200 and 400 mg/kg p.o. for a period of 14 days, after which amnesia was induced by giving scopolamine (1 mg/kg, s.c) by assess memory using the T-maze continuous alternation task (T-CAT) and novel object recognition test (NORT). Pretreatment with extracts ameliorated the memory deficit caused by scopolamine;which was evidenced by a significantly greater relative proportion of spontaneous alternation percentage in the T-CAT, and a significant increase of discrimination index in the NORT. Further, extracts significantly inhibited anticholinesterase activity in the brain, elevated the levels of reduced glutathione and catalase, and decreased malondialdehyde and nitrite levels in the brain.	Shirsat-John *et al.* (2022)
	Antibacterial	Flowers	The ethanol extract at concentration 50 µg/mL showed against *Staphylococcus aureus, Bacilllus spp., Streptococcus pneumoniae, Sarcina lutea, Escherichia coli, Salmonella* spp., *Shigella* spp., *Klebsiella pneumoniae, Proteus mirabilis, Lactobacillus arabinosus, Vibrio cholerae* and *Pseudomonas* spp. with MIC values of 30, 3, 1, 1, 6, 3, 12, 1, 1, 1, 11 and 2 µg/mL, respectively.	Mazumder *et al.* (2004)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Mesua ferrea L.	Toxicity	Flowers	The ethanolic extract of *M. ferrea* showed cytotoxicity effect on the RAW 264.7 cell by MTT assay with % viability of 87.43±2.28%.	Mokmued *et al*. (2017)
		Flowers	The subacute oral toxicity of the ethanolic extract of *M. ferrea* in rats at the doses of 100, 500 and 1000 mg/kg, over a period of 28 days. Repeated administration of extract had no adverse effect on the growth rate and hematological parameters of the animals. There were no changes in the biochemical parameters.	Shirsat *et al*. (2020)
Nelumbo nucifera Gaertn.	Antioxidant	Stamens	Ethanol extract from *N. nucifera* had antioxidant activity by DPPH assay with IC_{50} = 0.82 µg/mL was less potent than gallic acid (IC_{50} = 0.72 µg/mL).	Thongmak *et al*. (2021)
		Isorhamnetin glycosides	Isorhamnetin glycosides including nelumboroside A, nelumboroside B, isorhamnetin glucoside and isorhamnetin rutinoside were isolated form stamen. The result found these compounds showed ONOO- scavenging activity.	Hyun *et al.* (2006)
Phyllanthus emblica L.	Analgesic	Fruits	The aqueous extract at the doses of 150, 300 and 600 mg/kg elicited a significant analgesic activity in a dose-dependent manner on both the early and late phase of formalin test in mice.	Jaijoy *et al*. (2010)
	Anti-inflammatory	Fruits	The aqueous extract of *P. emblica* significantly reduced the number of nodes on the lung surface and attenuated B(a)P-induced levels of proinflammatory cytokines MIP-2, TNF-α, IL-6, and IL-1ß in lung tissue. Moreover, the extract significantly decreased protein expressions of COX-2 and HIF-α.	Wang *et al*. (2017)
		Phenolics	The free and bounded phenolic compounds were assessed by HPLC technique. These phenolic compounds at dose level of 20 and 40 mg/kg decreased carrageenan-induced paw edema in rat.	Muthuraman *et al.* (2011)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Phyllanthus emblica L.	Anti-inflammatory	Fruits	Anti-inflammation activities of aqueous extract were evaluated using ethyl phenylpropiolate (EPP)- and arachidonic acid (AA)-induced ear edema, carrageenan-induced paw edema as well as cotton pellet-induced granuloma models in mice. The extract at 1 mg/ear exhibited anti-inflammatory effect on EPP-induced ear edema, but not on AA-induced ear edema. Oral administration of *P. emblica* at the doses of 150, 300 and 600 mg/kg caused dose-dependent inhibition of carrageenan-induced rat paw edema. *P. emblica* at 600 mg/kg did reduce neither transudative and proliferative phases nor body weight gain and thymus weight in cotton pelletinduced granuloma formation.	Jaijoy *et al.* (2010)
		Fruits	The 95% ethanol and hot water extracts of *P. emblica* showed similar COX-2 inhibition (53.4% and 51.0%). Moreover, at 100 µg/mL concentration. at 50 and 100 µg/mL concentration, the 95% ethanolic extract showed significantly higher NO inhibition (up to 49.1%) than other extracts.	Li *et al.* (2022)
	Antioxidant	Fruits	The ethanolic extract of fruit showed antioxidant activity, with IC_{50} values of 3.49 ± 0.17 mg/mL in DPPH radical and 4.95 ± 0.11 mg/mL in ABTS radical and a reducing power of 94.17 ± 0.62 mM Fe^{2+}/g in FRAP assay.	Arjin *et al*. (2020)
		Fruits	The aqueous extract of *P. emblica* showed potent antioxidant activity in DPPH radical scavenging, $ABTS^{\bullet+}$ radical scavenging, and cellular antioxidant status. IC_{50} of aqueous extract against DPPH radical, $ABTS^{\bullet+}$ radical and cellular radical status was 51.3±16.5, 295±5.4 and 0.65±0.04 µg/mL, respectively, was less potent than standard with IC_{50} value of 8.5±0.7 (gallic acid), 177±3.22 (trolox) and 4.48 ±1.32 (gallic acid) µg/mL, respectively.	Charoenteeraboon *et al*. (2010)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Phyllanthus emblica L.	Immunomodulatory	Fruits	The 95% methanol extract of *P. emblica* and co-treatment with arsenic decreased the levels of lipid peroxidation, ROS production, activity of caspase-3, apoptosis and increased cell viability, levels of antioxidant enzymes, cytochrome c oxidase and mitochondrial membrane potential as compared to mice treated with arsenic alone.	Singh *et al*. (2013)
		Fruits	The 90% ethanol extract of *P. emblica* inhibited apoptosis and DNA fragmentation induced by Cr. Moreover, the extract relieved the immunosuppressive effects of Cr on lymphocyte proliferation and even restored the IL-2 and g-IFN production considerably.	Ram *et al*. (2005)
		Phenolics	Effects of geraniin, quercetin 3-β-D-glucopyranoside, kaempferol 3-β-D-glucopyranoside, isocorilagin, quercetin, kaempferol and rutin compounds were isolated form *P. emblica* fruits on splenocyte proliferation were determined by the MTT method. Significantly stimulatory effects ($P < 0.05$) were found for geraniin and isocorilagin. The concentration of geraniin, quercetin 3-b-D-glucopyranoside, kaempferol 3-b-D-glucopyranoside, isocorilagin, quercetin, kaempferol and rutin to obtain 50% of stimulatory effect was 56, 123, 242, 42, 73, 93 and 92 µg/mL, respectively.	Liu *et.al*. (2012)
	Antimalarials	Fruits	The water extract of *P. emblica* fruits showed antimalarials effect by against *Plasmodium falciparum* (K1 strain) *in vitro* by assessing their ability to inhibit the uptake of [^{3}H] hypoxanthine with IC_{50} values of 14.37 ± 0.17 µg/mL.	Pinmai *et.al*. (2010)
	Antibacterial	Fruits	The alcohol extract of *P. emblica* fruit exhibited superior activity against *S. aureus* at 20 mg/mL, 29 mm was recorded as diameter zone of inhibition. This was followed by 18 mm *B. subtilis*, 15 mm *P. aeruginosa* and *E. coli* 12 mm respectively.	Dhale and Mogle (2011)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Phyllanthus emblica L.	Antiviral	Fruits	The ethanolic extract of *P. emblica* extract at a low concentration of 78 µg/mL could inhibit porcine reproductive and respiratory syndrome virus (PRRSV) infectivity in MARC-145 cells [virus titer = 4.5 $TCID_{50}$/ml (log10)].	Arjin *et al.* (2020)
		Fruits	The *n*-hexane (HX), carbon tetrachloride (CT), chloroform (CF), and aqueous (AQ) fractions from methanolic extract of *P. emblica* showed HIV reverse transcriptase inhibitory activity by MTT assay. AQF and HXF fractions show highest inhibition of recombinant HIV-RT (91% and 89% respectively) at 1 mg/mL concentration. CFF fraction shows highest inhibition of HIV-RT at 0.5 mg/mL and CTF fraction at 0.12 mg/mL concentration.	Estari *et.al*. (2012)
	Anticancer	Phenolics	Effects of geraniin, quercetin 3-β-D-glucopyranoside, kaempferol 3-β-D-glucopyranoside, isocorilagin, quercetin, kaempferol and rutin compounds were isolated form *P. emblica* fruits on cytotoxicity to human breast cancer cell (MCF-7) and human embryonic lung fibroblast cell (HELF) and were determined by the MTT method. The assay of anticancer activities suggested that geraniin and isocorilagin exhibited higher cytotoxicities than other compounds against MCF-7 with IC_{50} of 13.2 and 80.9 µg/mL, respectively. Isocorilagin exhibited a strong cytotoxicity to HELF cell with IC_{50} of 51.4 µg/mL. Geraniin, quercetin, kaempferol and their glycosides had weak cytotoxicity against HELF cells. Paclitaxel showed a strong cytotoxicity to MCF-7 and HELF with IC_{50} of 6.8 and 14.5 µg/mL, respectively.	Liu *et.al*. (2012)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Phyllanthus emblica L.	Laxative (Stool softeners or wetting agents)	Fruits	Administration of methanolic extract to mice produced 35.7% and 44.1% (n = 6) wet feces at 100 and 300 mg/kg, respectively. The positive control, carbachol (1 mg/kg) produced 48.2% wet feces, while the saline-treated group formed only 10.5% wet feces. When ethanolic extract (100 and 300 mg/kg) was studied in mice pretreated with atropine, the production of wet feces declined to 25.8% and 34.4%, respectively. Moreover, the methanolic extract dose dependently (100–300 mg/kg) propelled charcoal meal through the small intestine of mice. The methanolic extract at the doses of 100 and 300 mg/kg moved the charcoal meal to the respective levels of 77.1±1.6% ($p<0.001$) and 90.3±4.5% ($p<0.001$), when compared with the saline-treated group.	Mehmood *et.al*. (2012)
	Toxicity	Fruits	The acute oral toxicity of the methanolic extract at a dose of 3, 5 and 10 g/kg body weight in mice. The extract administration neither caused any significant change in the behaviors nor the death of animal(s) in all the test groups.	Mehmood *et.al*. (2011)
		Fruits	The acute toxicity, a single oral administration of the water extract at a dose of 5,000 mg/kg body weight in Sprague Dawley rats (5 females, 5 males) was performed and the results showed no toxicity in terms of general behavior change, mortality, or change in gross appearance of internal organs (LD_{50} > 5,000 mg/kg). Chronic toxicity was studied by daily oral dose (ten females, ten males) of 300, 600 and 1,200 mg/kg for 270 days. The results showed slightly significant differences in the body and organ weights between the control and treatment groups.	Jaijoy *et.al.* (2010)
		Fruits	The cytotoxic activity was exhibited by water extracts on Vero cells with IC_{50} values of 157.86 ± 14.90 µg/mL.	Pinmai *et.al.* (2010)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Pinus kesiya Royle ex Gordon	Anticancer	Branch	The 50% ethanolic extract of *P. kesiya* exhibited cytotoxicity in a dose-dependent manner upon the human leukemic U937-cell line with IC_{50} value of 299.0±5.2 µg/mL.	Machana *et al.* (2012)
	Cytotoxic	Branch	The cytotoxicity of the 50% ethanolic extract of *P. kesiya* was determined in Vero cell. It no cytotoxicity was found with IC_{50} > 500 µg/mL.	Machana *et al.* (2012)
Santalum album L.	Antipyretic	Sandalwood oil	The antipyretic effect of sandalwood oil as well as HESP oil was investigated against yeast induced pyrexia in albino rat in dose of 200 mg/kg using 0.2% of tween 80 as control and 100 mg/kg paracetamol as standard. A significantly high antipyretic effect observed in case of sandalwood oil and HESP.	Sindhu *et al.* (2010)
	Analgesic	Wood	The methanolic extract of wood was also screened for analgesic activity at various doses (100, 250 and 500 mg/kg) and compared with Diclofenac sodium (7 mg/ kg) taken as standard. The extract showed maximum effect at 500 mg/kg.	Saneja *et al*. (2009)
	Anti-inflammatory	Sandalwood oil	The sandalwood oil showed against formalin induced paw edema in albino rat in dose of 200 mg/kg.	Sindhu *et al.* (2010)
	Antioxidant	Wood	The 70% ethanolic extract of *S. album* showed antioxidant activity by DPPH assay with IC_{50} = 18.6 mg/mL was more potent than L-ascorbic acid (IC_{50} = 28.7 µg/mL).	Kim (2008)
	Antibacterial	Stems	The water extract of *S. album* inhibited *E. coli, S. aureus*, and *Pseudomonas* with inhibition zone of 0.6±0.002, 0.4±0.001 and 1.0±0.030 mm, respectively.	Mohana *et al.* (2006)
	Antivirals	Sandalwood oil	The sandalwood oil showed against Herpes simplex viruses-1 and -2 using MTT assay with an IC_{50} value of 25 and > 60 µg/mL, respectively.	Benencia and Courreges (1999)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Santalum album L.	Anticancer	Sandalwood oil	Topical application of sandalwood oil (5% in acetone, w/v) prevented skin carcinogenesis in CD-1 mice development initiated by 7,12-dimethylbenz [a]anthracene (DMBA) and promoted by 12-O-tetradecanoyl phorbol-13-acetate (TPA) and TPA-induced ornithine decarboxylase (ODC) activity in CD-1 mice. It significantly reduced papilloma incidence by 67%, multiplicity by 96%, and TPA-induced ODC activity by 70%	Dwivedi and Abu-Ghazaleh (1997)
		Neolignan	Neolignan including (7R,8R)-5-O-demethylbilagrewin and bilagrewin were isolated form *S. album* stem. Showed exhibited cytotoxicity against HL-60 human promyelocytic leukemia cells with IC_{50} values of 1.5±0.02 and 4.3±0.13mM, and against A549 human lung adenocarcinoma cells with IC_{50} values of 13.6±0.32 and 19.9±1.27mM, respectively.	Matsuo and Mimaki (2010)
	Toxicity	Sandalwood oil	The acute oral toxicity (LD_{50}) of sandalwood oil in rats was reported as 5.58 g/kg of body weight moreover the major constituent of sandalwood oil, a-santalol, in rats was reported as 3.8 g/kg. The acute dermal toxicity (LD_{50}) of sandalwood oil in rabbits was reported as >5 g/kg of body weight and a-santalol in rabbit was reported as >5 g/kg.	Burdock and Carabin (2008)
Terminalia bellirica (Gaertn.) Roxb.	Antipyretic	Fruits	The aqueous and ethanol extracts at 200 mg/kg, p.o. dose showed significant antipyretic activity by reduced pyrexia on brewer's yeast induced fever in albino rats.	Sharma *et al*. (2010)
	Analgesic	Fruits	The methanolic extract dose-dependently (50 - 100 mg/kg) reduced the number of writhes evoked by acetic acid in mice. At the doses of 50 and 100 mg/kg, the extract reduced the number of writhes to 40 ± 3.0 ($P < 0.01$, n = 5) and 18 ± 2.0 ($P < 0.001$, n= 5) respectively. Diclofenic at the dose of 20 mg/kg decreased the number of acetic acid-mediated writhes to 10 ± 1.0 ($P < 0.001$, n = 5).	Khan and Gilani (2010)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia bellirica (Gaertn.) Roxb.	Analgesic	Fruits	Ethanol extractive at doses of 100, 200 and 400 mg/kg exhibited significant analgesic activity in comparison to the control group and maximum increase in tail withdrawal time was observed at 800 mg/kg which was comparable to that of the standard drug pentazocine. Maximum analgesic response was observed on 14th day of the study on chronic administration of *T. bellirica* fruits for 15 days which can be due to cumulative effect of the drug.	Kaur and Jaggi (2010)
		Fruits	The ethanolic and aqueous extracts of fruits (200 mg/kg, p.o.) in acetic acid-induced writhing, Eddy's hot plate method and brewer's yeast-induced fever models in mice and rats. Both extracts showed a significant decrease in the number of the writhes in acetic acid-induced writhing and increase in paw licking time to heat stimuli in the hot plate method.	Sharma *et al*. (2010)
	Anti-inflammatory	Fruits	The ethyl acetate fraction isolated from *T. bellerica* (100 µg/mL) inhibited the over-expression of (COX), 5-lipoxygenase (5-LOX) activity, nitrate and inducible nitric oxide synthase (iNOS) level, reactive oxygen species (ROS) production, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and nuclear factor-κB (NF-κB) in LPS stimulated RAW 264.7 cells.	Jayesh *et al.* (2017)
		Fruits	The hydroalcoholic extract at 100, 200 and 400 mg/kg, p.o. dose in carrageenin-induced rat paw edema. The extract showed a significant anti-inflammatory activity in carrageenan-induced paw edema model at 1, 3, and 5 h. A significant inhibition ($P < 0.01$) of paw edema as compared to control group was observed at doses of 100, 200, and 400 mg/kg at 1, 3, and 5 h. The extract showed comparable efficacy to indomethacin at 200 mg/kg. Maximum percentage inhibition was observed with TBE 200 mg/kg at 3 h (57.6%).	Chauhan *et al.* (2018)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia bellirica (Gaertn.) Roxb.	Anti-inflammatory	Fruits	The aqueous acetone extracts isolated from *T. bellirica* significantly diminished the elevated levels of inflammatory markers were COX, 5-LOX, nitrate, reactive oxygen species (ROS) production, mRNA level expression of COX-2, TNF-α and IL-6 in LPS stimulated RAW 264.7 cells.	Jayesh *et al.* (2020)
		Fruits, Ellagic acid	Antiinflammatory activity of ethyl acetate and aqueous extracts and ellagic acid was investigated by inhibition of heat-induced albumin denaturation. Both the extracts and ellagic acid exhibited concentration-dependent anti-inflammatory activity. Ellagic acid exhibited superior activity (IC_{50} = 7.64 µg/mL) as compared to aqueous extract (IC_{50} = 36.22 µg/mL) and ethyl acetate extract (IC_{50} = 28.03 µg/mL) extracts by inhibiting the heat-induced denaturation of albumin more effectively. Diclofenac, a standard drug, showed comparatively higher anti-inflammatory activity with lower IC_{50} value (5.66 µg/mL).	Gupta *et al.* (2021)
		Fruits	Anti-inflammation activities of aqueous extract were evaluated using ethyl phenylpropiolate (EPP)- and arachidonic acid (AA)-induced ear edema models, a cotton pellet-induced granulation formation model, and a carrageenan-induced hind paw edema model. The aqueous extract exhibited significant anti-inflammatory effects against EPP-induced ear edema and carrageenan-induced hind paw edema in rats. However, the extract showed insignificant inhibitory activity against AA-induced ear edema and cotton pellet-induced granuloma.	Takuathung *et al*. (2022)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia bellirica (Gaertn.) Roxb.	Immunomodulatory	Fruits	The 95% ethanol extract at 150 and 350 mg/kg, p.o. dose showed significantly ($p < 0.01$) potentiated the DTH reaction by facilitating the footpad thickness response to SRBC's in sensitized mice. Moreover, pretreatment with ethanolic extract of *T. bellirica* (350 mg/kg, p.o.) showed significant ($p < 0.01$) increase in phagocytic index and significant ($p < 0.05$) protection against cyclophosphamide induced neutropenia.	Manjunatha *et al*. (2011)
	Antioxidant	Fruits	The ethyl acetate and aqueous extracts of *T. bellirica* fruit showed potent anti-DPPH radical and hydroxy radical scavenging activities. EC_{50} of ethyl acetate and aqueous extracts against DPPH radical were 5.79 and 14.67 µg/mL, whereas against hydroxy radical were 8.79 and 24.23 µg/mL, respectively. The reducing power of the extract increased with increasing concentration of extracts in the reaction mixture (1.3 - 13.15 mg/mL).	Gupta *et al.* (2020)
		Fruits, Ellagic acid	*T. bellirica* fruit extracts (ethyl acetate and aqueous) and ellagic acid were found to be significant scavengers ($p < 0.05$) of the ABTS radical and this activity was comparable to standard solution ascorbic acid (IC_{50} value 1.46 µg/mL). Ellagic acid (IC_{50} = 1.71 µg/mL) showed higher antioxidant activity as compared to ethyl acetate extract (IC_{50} = 11.78 µg/mL) and aqueous extracts (IC_{50} = 14.44 µg/mL) in ABTS radical scavenging assay. The activities of test extracts were comparatively lower than the activity shown by ascorbic acid. During FRAP assay, ellagic acid showed considerably higher reducing ability (99,708\±63.08 µmol $FeSO_4.7H_2O$ equivalent/mg) in comparison to ethyl acetate extract (6527.05±87.18 µmol $FeSO_4.7H_2O$ equivalent/mg). The result was statistically significant ($p < 0.05$).	Gupta *et al.* (2021)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia bellirica (Gaertn.) Roxb.	Antimalarials	Fruits	The water extract of *T. bellirica* fruits showed antimalarials effect by against *Plasmodium falciparum* (K1 strain) *in vitro* by assessing their ability to inhibit the uptake of [^{3}H] hypoxanthine with IC_{50} values of 14.33 ± 0.25 µg/mL.	Pinmai *et.al*. (2010)
	Antibacterial	Fruits	The aqueous and methanol extracts displayed antibacterial activity (MIC 0.25–4 mg/mL) against all strains of MRSA, MDR *Acinetobacter* spp. and MDR *P. aeruginosa*. The sequential aqueous extracts (MIC, 4 mg/mL) inhibited ESBL producing-*E. coli*. None of the extracts exhibited activity against MDR *K. pneumoniae* (MIC > 5 mg/mL). The sequential methanol extract (Soxhlet) recorded high antibacterial activity.	Dharmaratne *et al*. (2018)
	Anticancer	Fruits	An anticarcinogenesis effect was investigated using a 7,12-dimethylbenz(a) anthracene (DMBA) and 12-Otetradecanoylphorbol-13-acetate (TPA)-induced tumorigenesis model. The aqueous extract at concentrations of 1, 2, and 4 mg significantly suppressed the number of tumors per mouse at week 20, indicating a reduced multiplicity of skin papilloma formation of 37%, 12%, and 18%, respectively, compared with the DMBA/TPA-induced group	Takuathung *et al*. (2022)
	Antispasmodic	Fruits	The 70% methanol extract of *T. bellirica* caused relaxation of spontaneous contractions in isolated rabbit jejunum at 0.1–3.0 mg/mL. The extract inhibited the carbachol (CCh, 1 µM) and K^+ (80 mM)-induced contractions in a pattern similar to that of dicyclomine, but different from nifedipine and atropine.The extract exhibited protective effect against castor oil-induced diarrhea. These results indicate that *T. bellerica* fruit possess a combination of anticholinergic and Ca^{++} antagonist effects, which explain its folkloric use in the colic and diarrhea.	Gilani *et al*. (2008)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia bellirica (Gaertn.) Roxb.	Stimulatory and inhibitory effects on gastrointestinal	Fruits	The crude extract of Tri-sa-maw recipe (including *Terminalia chebula*, *Terminalia* sp. and *Terminalia bellirica*) showed both stimulatory (two *in vivo* models were used gastric emptying and gastrointestinal transit in male Sprague-Dawley rats) and inhibitory effects (*in vitro* isolated guinea pig ileum experiment) on the gastrointestinal function. Not only did the extract at the dose of 1,000 mg/kg inhibit the gastric emptying time, but also stimulate the movement of the digestive tract by increasing the mobility of charcoal. In the isolated guinea pig ileum experiment, the extract at low concentration (0.1 ng/mL) induced the contraction of isolated guinea pig ileum. However, the stimulation effect on contractions of isolated guinea pig ileum was very much decreased at the high concentration (0.2-1 ng/mL) of the extract.	Wannasiri *et al*. (2015)
	Toxicity	Fruits	The acute toxicity, a single oral administration of the water extract at a dose of 5,000 mg/kg body weight in rat (10 female, 10 male) was performed and the results showed no signs of toxicity such as general behavior changes, morbidity, mortality, changes on gross appearance or histopathological changes of the internal organs of rats.	Sireeratawong *et al*. (2013)
		Fruits	The chronic toxicity of the water extract was determined by oral feeding both female and male rats (10 female, 10 male) daily with the test substance at the dose of 300, 600 and 1,200 mg/kg body weight continuously for 270 days. The examinations of signs of toxicity showed no abnormalities in the test groups compared to the controls. In addition, these rats were analyzed for final body and organ weights, necropsy, as well as hematological, blood chemical and histopathological parameters.	Sireeratawong *et al*. (2013)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia bellirica (Gaertn.) Roxb.	Toxicity	Fruits	Acute and subacute oral toxicity of hydroalcoholic extract were evaluated in Wistar albino rats at doses of 2000 and 1000 mg/kg in acute and subacute toxicity studies, respectively. No mortality and signs of toxicity were observed in both acute and repeated dose toxicity studies after oral administration of TBE up to the dose level of 2000 mg/kg.	Chauhan *et al*. (2018)
		Fruits	The cytotoxicity of ethanolic extract of *T. bellirica* fruits using Vero, L-6 and 3T3 cell lines. The results showed that the extracts did not confer any cytotoxicity with an LC_{50} value greater than 1000 µg/mL.	Das and Devi (2015)
		Fruits	The cytotoxic activity was exhibited by water extracts on Vero cells with IC_{50} values of 238.70 ± 8.45 µg/mL.	Pinmai *et.al.* (2010)
Terminalia chebula Retz.	Antipyretic	Fruits	The 95% ethanol extract at 400 and 600 mg/kg, p.o. dose showed significant antipyretic activity by reduced pyrexia on brewer's yeast induced fever in albino rats.	Lahon *et al.* (2012)
	Analgesic	Fruits	The ethanolic extract of *T. chebula* fruits was evaluated for their analgesic activity using the tail immersion model in mice. The ethanolic extract of the plant exhibited analgesic response at 200,400 and 800mg/kg body weight in acute pain and in chronic pain studied for 15 days with maximum analgesic response on 14^{th} day.	Kaur and Jaggi (2010)
		Fruits	The ethanolic extract of fruit at doses of 250 mg/kg and 500 mg/kg, i.p. in Wister albino rats and Swiss Albino mice showed a significant increase in the mean reaction time to heat stimuli in hot plate method at both 250 mg/kg and 500 mg/kg BW doses throughout the observation period in 30 minutes and 60 minutes after treatment, which was comparable to the standard ketorolac and control group.	Jami *et al.* (2014)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia chebula Retz.	Analgesic	Fruits	The methanolic extract of fruit at various doses (300, 500 and 1000 mg/kg p.o.) were evaluated for their analgesic activity using the tail immersion technique and acetic acid induced writhing test in mice. *T. chebula* fruit extract possessed varying degree of analgesic activity significant at 300 mg/kg and highly significant at 500 and 1000 mg/kg in comparison to control. The results were almost similar to standard drug. In acetic acid induced writhing test, maximum inhibition of writhing was observed at 1000 mg/kg where the number of writhes decreased from 14.1 to 5.2 indicating 63.1% inhibition.	Ahmed *et al.* (2015)
	Anti-inflammatory	Chebulagic acid	Chebulagic acid were isolated form fruit. The result found this compound showed potent COX–LOX dual inhibition activity with IC_{50} values of 15 ± 0.288, 0.92 ± 0.011 and 2.1 ± 0.057 µM for COX-1, COX-2 and 5-LOX respectively.	Reddy *et al*. (2009)
		Fruits	The 70% ethanol extract at 250 mg/kg, p.o. dose caused 69.96% reduction in carrageenin-induced rat paw edema and demonstrated 96.72% protective effect on human RBC membrane stability. Besides, *T. chebula* fruit extract significantly reduced the *in vivo* formation of TBARS in carrageenin-induced rat liver with IC_{50} 94.96 mg/kg, p.o.	Bag *et al*. (2013)
		Gallotannins and Triterpenoids	Two gallotannins [chebulinic acid (1) and 2,3,6-tri-*O*-galloyl-ß-D-glucose (2)] and two triterpenoids [arjunic acid (3) and arjunolic acid (4)] efficiently reduced nitric oxide (NO) production with IC_{50} values of 53.4, 55.2, 48.8, and 38.0 µM, respectively. The protein expressions of iNOS and COX-2 were decreased in macrophages by treatment with compounds 1-4 (54-69% and 33-37%, respectively) at 50 µM.	Yang *et al*. (2014)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia chebula Retz.	Anti-inflammatory	Fruits	The ethanolic extract at 300 mg/kg, p.o. dose in carrageenin-induced rat paw edema. The ethanolic extract of *T. chebula* produced dose-dependent and significant inhibition of carrageenan-induced paw edema. The inhibition was significant at the dose of 300 mg/kg (52.625%) to that of the standard drug, Diclofenac sodium (58.985%).	Jami *et al*. (2013)
		Tannins	Tannin rich fraction (TRF) isolated from hydroalcoholic extract of *T. chebula* including gallic acid, methyl gallate, corilagin, chebulagic acid, and chebulinic acid. TRF at 250 µg/mL dose exhibited 84.48, 84.33, 81.98, and 82.3% inhibition of protein denaturation, membrane lysis, proteinase, and hyaluronidase enzyme activities, respectively.	Priya *et al*. (2018)
		Fruits	The ethanolic extract of *T. chebula* treatment on RAW 264.7 cells revealed the anti-inflammatory properties by regulating nitrite and TNF-α production; iNOS, COX-2 levels, and translocation of NF-κB protein.	Shendge *et.al.* (2020)
	Immunomodulatory	Fruits	The aqueous extract of *T. chebula* produced an increase in humoral antibody (HA) titer and delayed-type hypersensitivity (DTH) in mice.	Shivaprasad *et al*. (2006)
		Fruits	The 70% ethanol extract of *T. chebula* increased spleen lymphocyte proliferation. Based on RT-PCR analysis, the expression of cytokines, viz, IL-2, IL-10 and TNF-, was more in *T. chebula*-treated than in vehicle- and cyclophosphamide- treated groups.	Aher and Wahi (2011)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia chebula Retz.	Immunomodulatory	Fruits	The alcoholic extract of *T. chebula* at 100 mg/kg, p.o. dose was found to increase the neutrophils and lymphocytes as compared to vehicle and cyclophosphamide treated groups. *T. chebula* alcoholic extract showed linear time dependent significant phagocytic activity as compared to SRBC sensitized and cyclophosphamide treated group. In zinc sulphate turbidity test *T. chebula* treated rats serum showed more turbidity (cloudy) which indicate the increase in the immunoglobulin level as compared to vehicle, SRBC sensitized and cyclophosphamide treated group.	Aher *et al.* (2018)
	Antioxidant	Fruits	The 6 extracts of *T. chebula* showed potent anti-lipid peroxidation, anti-superoxide radical formation and free radical scavenging activities. IC_{50} of MeOH, $CHCl_3$, EtOAc, *n*-Butanol, Organic aqueous and Water extracts against lipid peroxidation ranging from 4.05 to 8.97 µg/mL, against superoxide radical ranging from 0.48 to 2.42 µg/mL, whereas against free radical ranging from 0.004 to 0.021 µg/mL, respectively.	Cheng *et.al.* (2003)
		Casuarinin, Chebulanin, Chebulinic acid and 1,6-Di-*O*-galloyl-β-*D*-glucose	Casuarinin, Chebulanin, Chebulinic acid and 1,6-Di-*O*-galloyl-β-*D*-glucose compounds were isolated form *T. chebula* fruits showed potent anti-lipid peroxidation, anti-superoxide radical formation, and free radical scavenging activities. IC_{50} of Casuarinin, Chebulanin, Chebulinic acid and 1,6-Di-*O*-galloyl-β-*D*-glucose compounds against lipid peroxidation were 29.67, 3.96, 7.27 and ND µg/mL, against superoxide radical were 1.06, 0.04, 1.17 and 1.30 µg/mL, whereas against free radical radical were 0.006, 0.031, 0.002 and 0.161 µg/mL, respectively.	Cheng *et.al.* (2003)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia chebula Retz.	Antioxidant	Fruits	The polyphenolic extract of *T. chebula* was evaluated for antioxidant activity by determiningthe reducing power, total antioxidant capacity, DPPH radical concentration (IC_{50} = 14 µg/mL), nitric oxide radical concentration (IC_{50} = 30.51 µg/mL) and hydrogen peroxide scavenging activity (IC_{50} = 265.53 µg/mL) under *in vitro* conditions.	Saha and Verma (2016)
	Antimalarials	Fruits	The water extract of *T. chebula* fruits showed antimalarials effect by against *Plasmodium falciparum* (K1 strain) *in vitro* by assessing their ability to inhibit the uptake of [^{3}H] hypoxanthine with IC_{50} values of 15.41 ± 0.61 µg/mL.	Pinmai *et.al*. (2010)
	Antibacterial	Fruits	The ethanolic extract of *T. chebula* showed antibacterial activity using the disc diffusion method and the minimum inhibitory concentration (MIC). The extract was highly effective against *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* ATCC 27853. The MIC was determined as 1 mg/mL for *S. typhi*.	Kannan *et.al.* (2009)
	Antivirals	Fruits	The 50% ethanolic extract and chebulagic acid and chebulinic acid both purified from *T. chebula* fruits showed against Herpes simplex viruses-2 using MTT assay with an IC_{50} value were 0.01 ± 0.0002, 1.41 ± 0.51 and 0.06 ± 0.002 µg/mL, respectively.	Kesharwani *et.al.* (2017)
		Seeds	The water extract of *T. chebula* reduced the titre of influenza A virus A/Teal/Tunka/7/2010 (H3N8) by approximately five-fold. The plaque reduction neutralisation tests revealed that none of the extract tested was able to inhibit formation of plaques by 90%. However, *T. chebula* was able to inhibit formation of plaques by more than 50% at low dilutions from 1:3 to 1:14. The *T. chebula* extract had a concentration-dependent inhibitory effect.	Oyuntsetseg *et.al*. (2014)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia chebula Retz.	Anticancer	Fruits	The ethanolic extract of *T. chebula* were active against human lung cancer (A549) and breast cancer (MCF-7) cell lines. The ethanolic extract showed cytotoxicity toward A549 (IC_{50} = 359.06 ± 20.04 µg/mL), and MCF-7 (IC_{50} = 61.02 ± 5.55 µg/mL) cells.	Shendge *et.al.* (2020)
	Laxative (Stool softeners or wetting agents)	Fruits	Intestinal transit time was studied in two dosage forms of *T. chebula* fruits, powdered (Churna) and tablet (Vati), at a dose of 550 mg/kg and evaluation on intestinal transit time was carried out by adopting kaolin expulsion test in Swiss albino mice. The results show that powdered and tablet dosage forms significantly shortened intestinal transit time with kaolin pellet expulsion time of 193.60±4.82 min (37.94%) and 200.10±3.54 min (35.85), respectively. The mechanism of observed effect may be due to interference with local stimulant effect on motility or acceleration of gastric emptying.	Jirankalgikar *et al.* (2012)
	Stimulatory and inhibitory effects on gastrointestinal	Fruits	The crude extract of Tri-sa-maw recipe (including *Terminalia chebula*, *Terminalia* sp. and *Terminalia bellirica*) showed both stimulatory (two *in vivo* models were used gastric emptying and gastrointestinal transit in male Sprague-Dawley rats) and inhibitory effects (*in vitro* isolated guinea pig ileum experiment) on the gastrointestinal function. Not only did the extract at the dose of 1,000 mg/kg inhibit the gastric emptying time, but also stimulate the movement of the digestive tract by increasing the mobility of charcoal. In the isolated guinea pig ileum experiment, the extract at low concentration (0.1 ng/mL) induced the contraction of isolated guinea pig ileum. However, the stimulation effect on contractions of isolated guinea pig ileum was very much decreased at the high concentration (0.2-1 ng/mL) of the extract.	Wannasiri *et al.* (2015)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia chebula Retz.	Laxative (Stool softeners or wetting agents)	Fruits	Crude aqueous extract of *T.chebula* fruits at doses 100 and 200 mg/kg respectively was investigated for laxative activity in albino rats and compared with standard drug Bisacodyl (8mg/kg, p.o.) in gum acacia. The rats were fasted for 12 hours before the experiment. After 8 hours of drug administration the faeces were collected and weighed. The extract was found to produce significant laxative activity in dose dependant manner.	Suresh *et al.* (2013)
	Spasmogenic	Seeds	The aqueous extract of *T. chebula* seeds showed spasmogenic activity in rat small intestine. The extract increased the frequency of ileum motility and tension of contraction dose-dependently ($P < 0.05$). Responses induced by extract were inhibited by pre-treatment of the tissue with verapamil. The extract activities were not affected by atropine, hexamethonium, and indomethacin. The faecal number and faecal water content were increased dose-dependently by extract ($P < 0.05$).	Mard *et al.* (2011)
	Toxicity	Fruits	The acute oral toxicity 50% ethanolic extract of *T. chebula* in mice at dose 10 g/kg (1,000-fold the size of the treatment in humans). The results showed not toxicity in mice.	Department of Medical Sciences (2003)
		Fruits	The aqueous extract of *T. chebula* fruits was further evaluated using the rat primary hepatocyte system, and its cytotoxicity was assessed by incubating the cells with doses of the extract of up to a dose of 1000 mg/mL. At the maximum dose of extract, no cytotoxicity was found, since cell viability remained above the level of the control after 30 min of incubation.	Lee *et al.* (2005)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Terminalia chebula Retz.	Toxicity	Fruits	Acute oral toxicity of T. chebula fruit extract (up to a dosage of 500 mg/kg body weight/day) for 30 days produced no effect on the general behaviour or appearance of the animals and all the rats survived the test period. There were no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions, coma. Assay of pathophysiological enzymes such as ALP, AST and ALT in plasma revealed the nontoxic nature of fruit extract.	Kumar *et al.* (2006)
		Fruits	The cytotoxic activity was exhibited by water extracts on Vero cells with IC_{50} values of 257.47 ± 12.53µg/mL.	Pinmai *et.al*. (2010)
		Fruits	The acute oral toxicity of aqueous extract of the *T.chebula* fruits at the doses of 1000, 2000 and 4000 mg/kg, p.o.in BALB/c mice. The extract did not produce any mortality up to a dose level of 4000 mg/kg. Hence, $1/20^{th}$ (200 mg/kg), $1/10^{th}$ (400 mg/kg) and $1/5^{th}$ (800 mg/kg) of this dose were used for further investigations.	Sheng *et al.* (2016)
Tiliacora triandra (Colebr.) Diels	Antipyretic	Roots	Powder of *T. triandra* at 40 mg/kg, p.o. significantly reduced rectal temperature from the first hour after brewer's yeast induced pyxeria in rats.	Konsue *et al*. (2008)
		Roots	The ethanolic extract of *T. triandra* at various doses (25-400 mg/kg) significantly ($p < 0.05$) reduced body temperature on lipopolysaccaride (LPS) in rats.	Jongchanapong *et al*. (2010)
	Analgesic	Roots	The ethanolic extract of *T. triandra* at various doses (25-400 mg/kg p.o.) were evaluated for their analgesic activity using the hot-plate, tail-flick and acetic acid-induced writhing models in mice. The ethanolic extract produced a significant analgesic response compared to vehicle controls.	Jongchanapong *et al*. (2010)
	Anti-inflammatory	Roots	The 95% ethanol extract of *T. triandra* showed activity against the over-expression of NO in LPS-stimulated RAW 264.7 with IC_{50} values of 54.65 ± 5.34 µg/mL.	Juckmeta and Itharat (2012); Nutmakul *et al.* (2016)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Tiliacora triandra (Colebr.) Diels	Antimalarials	Alkaloids	The alkaloids including tiliacorine, tiliacorinine, nor-tiliacorinine A, and two uncharracterized alkaloids G and H were isolated form *T. triandra* root. Alkaloid G was found to be the most active in vitro schizontocide (ID_{50}, 344 ng/mL) followed by nor-tiliacorinine A and tiliacorine (ID_{50} of 558 and 675 ng/mL, respectively).	Pavanand *et al*. (1989)
		Roots	The dichloromethane, methanol and water extracts of *T. triandra* inhibited *Plasmodium falciparum* (Pf3D7) with IC_{50} values were 1.22 ± 0.07, 1.95 ± 0.56 and 9.61 ± 2.87 µg/mL, respectively. Moreover, dichloromethane and methanol extracts of *T. triandra* inhibited *Plasmodium falciparum* (PfW2) with IC_{50} values were 3.99 ± 0.67 and 5.73 ± 1.15 µg/mL, respectively.	Nutmakul *et al*. (2016)
	Antioxidant	Roots	The ethanolic extract of *T. triandra* showed antioxidant activity, with EC_{50} values of 15.38 µg/mL in DPPH radical.	Juckmeta and Itharat (2012)
		Roots	The ethanolic extract of *T. triandra* showed antioxidant activity, with IC_{50} values of 17.77 ± 0.22 mg/mL in DPPH radical and 21.16 ± 1.06 mg/mL in ABTS radical and a reducing power of 30.58 ± 1.13 mM Fe^{2+}/g in FRAP assay.	Arjin *et al*. (2020)
	Antibacterial	Alkaloids	Bisbenzylisoquinoline alkaloids, tiliacorinine, 2′-nortiliacorinine, tiliacorine, and 13′-bromo-tiliacorinine, isolated from *T. triandra* root showed MIC values ranging from 0.7 to 6.2 µg/mL, but they exhibited the MIC value at 3.1 µg/mL against most multidrug-resistant *Mycobacterium tuberculosis* (MDR-MTB) isolates.	Sureram *et al*. (2012)
		Roots	The ethanolic extract of *T. triandra* inhibited *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and methicillin resistant *Staphylococcus aureus* (MRSA) with MIC of 7.81, 15.63, 7.81 mg/mL and MBC of 125, 125, 125 mg/mL, respectively.	Janta and Thaharn (2018)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Tiliacora triandra (Colebr.) Diels	Antivirals	Roots	The ethanolic extract of *T. triandra* at a concentration of 1250 µg/mL significantly inhibited porcine reproductive and respiratory syndrome virus (PRRSV) infectivity in MARC-145 cells [virus titer 3.5 median tissue culture infective dose ($TCID_{50}$)/mL (log10)] at 24 h post-infection.	Arjin *et al.* (2020)
	Anticancer	Tiliacorinine	Tiliacorinine was isolated form root and stem of *T. triandra* significantly inhibited proliferation of human CCA cell lines mice of bile duct cancer with IC_{50} 4.5-7 µM by inducing apoptosis through caspase activation, upregulation of BAX, and down-regulation of BclxL and XIAP.	Janeklang *et al.* (2014)
		Roots	The ethanolic extract of *T. triandra* showed cytotoxic activity against all cancer cell lines of the respiratory tract with IC_{50} values in the range of 10.1 to 45.2 µg/mL.	Juckmeta *et al.* (2019)
	Toxicity	Alkaloids	The 50% ethanolic extract of *T. triandra* root showed not antipyretic properties but was toxic in rats. Two types of alkaloids were water-insoluble alkaloids and water-soluble quarternary bases that were isolated. The water-soluble quarternary base is toxic to rats and has curare-like effects.	Dechatiwongse *et al.* (1974)
		Roots	A single oral administration of the water extract at a dose of 5,000 mg/kg body weight (5 males, 5 females) did not produce signs of toxicity, behavioral changes, mortality, changes on gross appearance or histopathological changes of internal organs. The subchronic toxicity was determined by oral feeding both male and female rats (10 males, 10 females) daily with the test substance at the doses of 300, 600 and 1,200 mg/kg body weight continuously for 90 days. The examinations of signs, animal behavior and health monitoring showed no abnormalities in the test groups as compared to the controls.	Sireeratawong *et al.* (2008)
		Roots	The ethanolic and water extracts of *T. triandra* showed LC_{50} of 44 and 200 µg/mL on *Artemia salina* L. (Brine shrimp larva) eggs, respectively.	Singharachai *et al.* (2011)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Tiliacora triandra (Colebr.) Diels	Toxicity	Roots	The ethanolic extract of *T. triandra* showed cytotoxicity effect with CD_{50} 1.483 ± 0.512 mg/mL on the Vero cell by MTT assay.	Janta and Thaharn (2018)
Tinospora crispa (L.) Hook. f. & Thomson	Antipyretic	Stems	The methanol extract (METC) and various subfractions (pet ether, PEFTC; *n*-hexane, NHFTC; and chloroform, CFTC) significantly ($p < .05$) reduced pyrexia in a dose-dependent manner on brewer's yeast induced fever in rats.	Rakib *et al.* (2019)
	Analgesic	Stems	At a dose of 400 mg/kg body weight, the crude methanol extract and its other fractions of stem significantly ($p<0.05$) produced inhibition of acetic acid-induced writhing compared to the standard (Diclofenac Sodium) in Swiss albino mice.	Islam *et al.* (2014)
	Anti-inflammatory	Stems	The hot-water extracts, at all concentrations (50, 100 and 150 mg/kg), significantly inhibited swelling of the paw edema induced by carrageenin in rats.	Hipol *et al.* (2012)
		Tinocrisposide	Tinocrisposide (1, 5, 25, 50, and 100 µM) was isolated form stem. Showed activity against the over-expression of NO in LPS-stimulated RAW 264.7 with % inhibition of 22.67 – 73%.	Adnan *et al.* (2018)
	Immunomodulatory	Stems / Syringin	The 80% ethanolic extract increased the chemotaxis and phagocytic activity of macrophages and significantly enhanced the production of ROS, NO and pro-inflammatory cytokines (IL-1ß, TNF-α, IL6, PGE_2 and MCP-1). In contrast, syringin potently reduced the chemotaxis, phagocytic activity, ROS and NO productions.	Ahmad *et al.* (2018)
		Stems	The crude extract and its isolated fraction significantly stimulate RAW264.7 cell viability ($P \leq 0.05$) and intracellular INF-γ, IL-6, and IL-8 expressions.	Abood *et al.* (2014)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Tinospora crispa (L.) Hook. f. & Thomson	Antioxidant	*N-cis*-feruloyltyramine, *N-trans*-feruloyltyramine and secoisolariciresinol	*N-cis*-feruloyltyramine, *N-trans*-feruloyltyramine and secoisolariciresinol were isolated form *T. crispa* stem. The result found these compounds exhibited proved to be more active than the synthetic antioxidant BHT.	Cavin *et al*. (1998)
		Stems	The DPPH assay showed that the methanol extract had highest scavenging activity in a dose-dependent manner where the IC_{50} value was 12 µg/mL.	Ibahim *et al.* (2011)
		Stems	The ethanol extract has higher antioxidant potential than ascorbic acid. The FRAP value of *T. crispa* extract is 11011.11 ± 1145.42 µmol Fe^{+2}/g, and its DPPH inhibition percentage is 55.79 ± 7.9, with 22 µg/mL IC_{50}.	Abood *et al*. (2014)
	Antimalarials	Stems	Mice were inoculated with Plasmodium yoelii then treated with the crude extract of Tinospora crispa at doses of 20, 40 and 80 mg/kg. Mice receiving the dose of 20 mg/kg died on average on Day 8. Mice remained alive longer when treated of the dose of 40 mg/kg or even longer under the treatment of the dose of 80 mg/kg.	Rungruang and Boonmars (2009)
		Stems	The methanolic extract of *T. crispa* has antimalarial activity in dose-dependent manner, especially at doses of 100 and 200 mg/kg with percent inhibition of 35% and 50%, respectively. However, the extract at dose of 20 mg/kg had no antimalarial effect.	Niljan *et al*. (2014)
	Antibacterial	Stems	The methanol extract at concentration 0.4 mg/ disc showed a little, but insignificant inhibition zone of extract against *S. aureus* (7.9 mm), *B. cereus* (4.6 mm), *B. megaterium* (3.6 mm), *B. subtilis* (5.2 mm), *S. lutea* (3.4 mm), *E. coli* (2.8 mm), *P. aeruginosa* (3.5 mm), *S. paratyphi* (5.0 mm), *S. typhi* (4.8 mm), *S. boydii* (3.6 mm), *S. dysenteriae* (5.2 mm), *V. mimicus* (4.0 mm) and *V. parahemolyticus* (5.5 mm).	Islam *et al.* (2014)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Tinospora crispa (L.) Hook. f. & Thomson	Antivirals	Stems	The ethanolic extract of stem showed direct virucidal activity by against Oncorhynchus masou virus (OMV) using plaque method with plaque reduction rate of 90%.	Direkbusarakom *et al.* (1996)
	Anticancer	Stems	The methanolic extract of stem, on its own, moderately decreased the cell viability by MTT assay in Triple Negative Breast Cancer cells with an IC_{50} of 66±3µg/mL and 60±4µg/mL in MDA-MB-231 and HCC1806 cells respectively.	Al-Rashidi *et al.* (2016)
	Toxicity	Stems	The acute toxicity study revealed that the ethanol extract of *T. crispa* stem did not cause any signs of toxicity or animal death at a dose of 4.0 g/kg of body weight (g/kg BW). However, the chronic toxicity test for 6 months exhibited that administration of the ethanol extract at a dose of 9.26 g/kg BW/day to rats caused hepatic and renal toxicities.	Chavalittumrong *et al.* (1997)
		Stems	A human hepatotoxicity case was reported due to chronic overuse of herbal preparation of *T. crispa* stem as a prophylactic agent against malaria.	Denis *et al.* (2007)
		Stems	The chloroform, petroleum ether and methanol extracts of *T. crispa* exhibited lethality effect to *Artemia salina* L. with the LC_{50} of 11.5, 12.6 and 12.0 µg/mL, respectively.	Aminul *et al.* (2011)
		Stems	The oral administration of the ethanol extract of *T. crispa* at doses of 100 and 200 mg/kg for 8 weeks potentiated the thioacetamide induced hepatotoxicity in rats. Moreover, they reported that the ethanol extract of *T. crispa* contained certain hepatotoxins which may be responsible for this effect.	Kadir *et al.* (2011)
		Stems	Recently, an incidence of toxic hepatitis linked with chronic use of high doses of *T. crispa*. They observed that a patient who received 10 pellets per day of T. crispa had problem of dark urine and pale stools, linked with asthenia and right hypochondrial pain which lead to jaundice. The histopathological results also confirmed a toxic reaction.	Langrand *et al.* (2014)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Tinospora crispa (L.) Hook. f. & Thomson	Toxicity	Stems	*T. crispa* causes acute hepatitis by liver injury induced, suggesting its *cis*-Clerodane-type furano-diterpenoids might be an important factor of inducing hepatotoxicity.	Cachet *et al.* (2018), Huang *et al.* (2019)
Vetiveria zizanioides (L.) Nash	Antipyretic	Roots	The hexane and methanol extracts at 75, 150 and 300 mg/kg dose significantly ($p < 0.05$) reduced pyrexia on brewer's yeast induced fever in albino rats.	Narkhede *et al.* (2012a)
	Analgesic	Roots	Four fractions (n-hexane, chloroform, ethyl acetate and butanol) of *V. zizanioides* at a dose 200 mg/kg, p.o. were tested. In carrageenan induced paw oedema and cotton pellet induced granuloma in rats, the ethyl acetate and chloroform fraction were found to be more significant ($p< 0.01$).	Rahul *et al.* (2013)
	Anti-inflammatory	Roots	The methanol extract of *V. zizanioides* at 300 mg/kg and 600 mg/kg exhibited significant anti-inflammatory effect. Maximum inhibition (66.17 %) was noted at the dose of 300 mg/kg after 6 hr of drug treatment in carrageenan induced paw edema. In cotton pellet induced granuloma model the methanol extract (600 mg/kg) and standard drug (Indomethacin, 10 mg/kg) showed decreased formation of granuloma tissue by 53.69 % and 56.70 % ($p < 0.001$) respectively.	Narkhede *et al.* (2012b)
		Roots	Four fractions (n-hexane, chloroform, ethyl acetate and butanol) of *V. zizanioides* at a dose 200 mg/kg, p.o. were tested. In acetic acid induced writhing reaction in mice, the ethyl acetate and chloroform fraction were found to be more significant ($p< 0.01$). The n-hexane fraction along with ethyl acetate and chloroform fraction showed significant ($p< 0.01$) increase in reaction time in tail immersion method.	Rahul *et al.* (2013)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

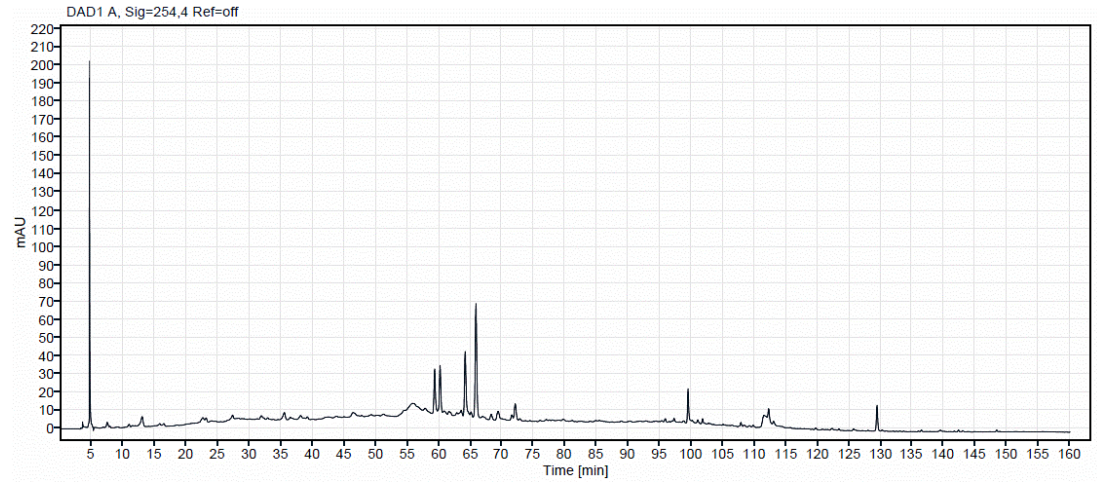
Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Vetiveria zizanioides (L.) Nash	Antioxidant	Roots	The ethanolic extract of *V. zizanioides* showed reducing power increased significantly ($P<0.01$) with increasing concentration of the extract was lower than the standard BHT. The extract showed significant ($P<0.001$) superoxide inhibiting activity at a concentration ranging from 25- 400 µg/mL. The IC_{50} of the extract was found to be 130.36±6.13 µg/mL whereas the IC_{50} of the standard ascorbic acid is 100.33±2.61 µg/mL. The degradation of deoxyribose by Fe^{3+}-ascorbate-EDTA-H_2O_2 system was markedly decreased by extract indicating the significant ($P<0.001$) hydroxyl radical scavenging activity. The IC_{50} of the extract was found to be 39.5± 2.5 µg/mL whereas the IC_{50} of the standard quercetin was 15.5 ±3.42 µg/mL.	Subhadradevi *et al*. (2010)
		Essential oil	Essential oil from *V. zizanioides* had antioxidant activity by DPPH assay with IC_{50} = 0.635±0.036 mg/mL (90.18±0.84%) and showed inhibit lipid peroxidation by TBARs assay with 23.90±5.68%.	Veerapan and Khunkitti (2011)
	Anticonvulsant	Roots	Administration of ethanolic extract of *V. zizanioides* in mice at dose 100, 200 and 400 mg/kg p.o. was determined by maximal electroshock stimulation (MES) and pentylenetetrazole (PTZ) in mice for 8 d experimental protocol. LD_{50} value of extract in mice was found at a dose of 600 mg/kg body weight. Extract at a dose of 400 mg/kg significantly (p50.001) reduced flexion (l5.98 to 3.73 s), extension (13.73 to 0.96 s), clonus (14.07 to 4.93 s), stupor (6.29 to 1.22 s) in the MES model. Further, it increases onset of clonic (88.25 to 708.32 s/30 min) and tonic (139.52 to 1126.39 s/30 min) in the PTZ model. In the PTZ model, 33% normal control and 83% extract (100 mg/kg) animals were alive, while 100% protection was achieved in standard drug phenobarbital (20 mg/kg), extract (200 mg/kg) and extract (400 mg/kg) animals.	Gupta *et al.* (2013)

Biological activities of plant components in Mo-Ha-Rak remedy (Continued)

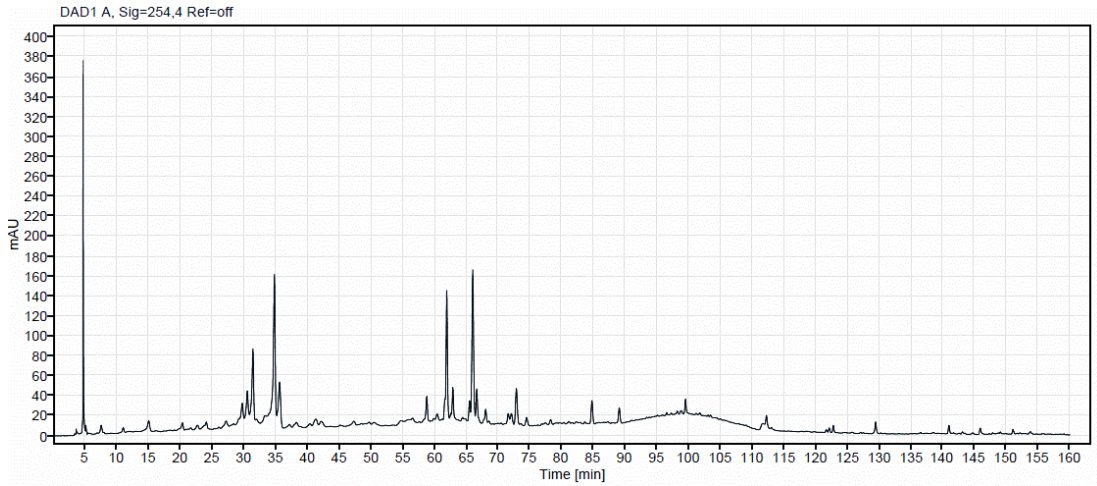
Botanical Names	Activities	Part used / Compounds	Biological activities	Reference
Vetiveria zizanioides (L.) Nash	Antibacterial	Roots	Ethanolic extract of *V. zizanioides* at concentration 150-750 µg inhibited *S. aureus*, *P. aeurogenesa* and *E. coli* with inhibition zone ranging from 11 to 25 mm were less potent than ciprofloxacin (zone of inhibition ranging from 30 to 36 mm).	Devi *et al*. (2010)
	Toxicity	Roots	The acute oral toxicity 50% ethanolic extract of *V. zizanioides* in mice at dose 10 g/kg (7,143-fold the size of the treatment in humans). The results showed not toxicity in mice.	Department of Medical Sciences (2003)
		Roots	The acute oral toxicity ethanolic extract of *V. zizanioides* in mice at dose 100, 200, 300, 400, 500 and 600 mg/kg. The mice treated with ethanolic extract did not show any mortality and observable neurobehavioral effects up to the 400 mg/kg dose. Its LD_{50} value was found at a dose of 600 mg/kg.	Gupta *et al.* (2013)

Appendix B

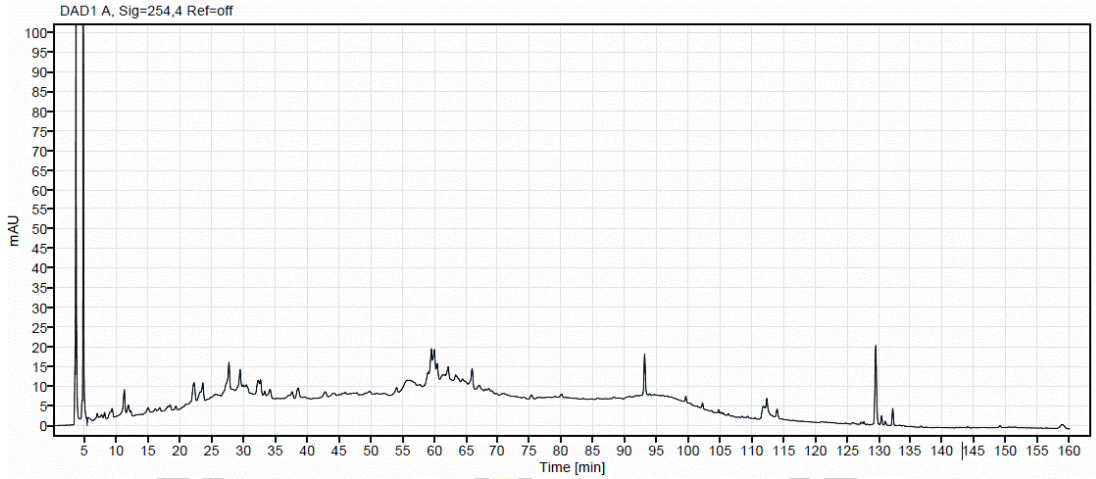
HPLC chromatograms of the Mo-Ha-Rak component ethanolic extract



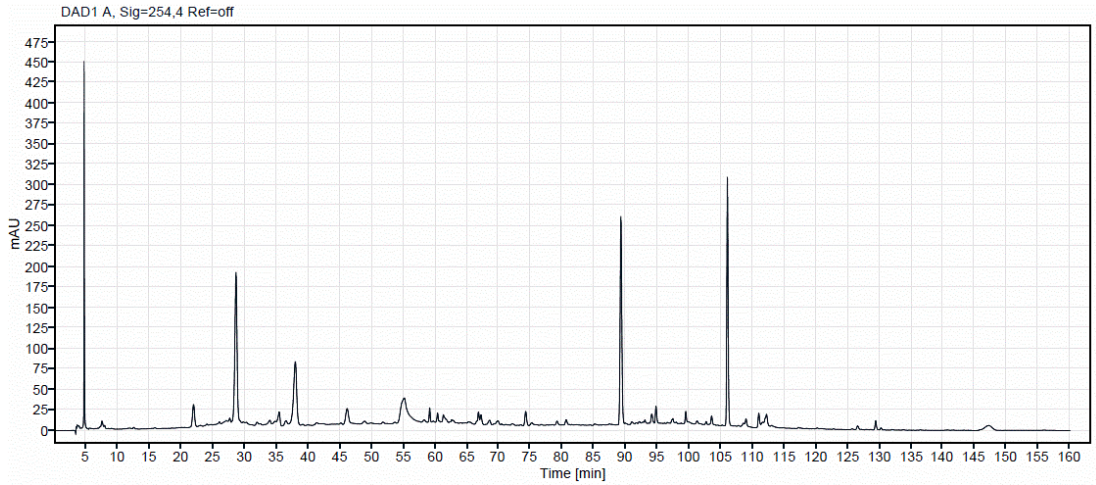
HPLC chromatograms of ethanolic extract of *Azadirachta indica* (สะเดา) at wavelength 254 nm



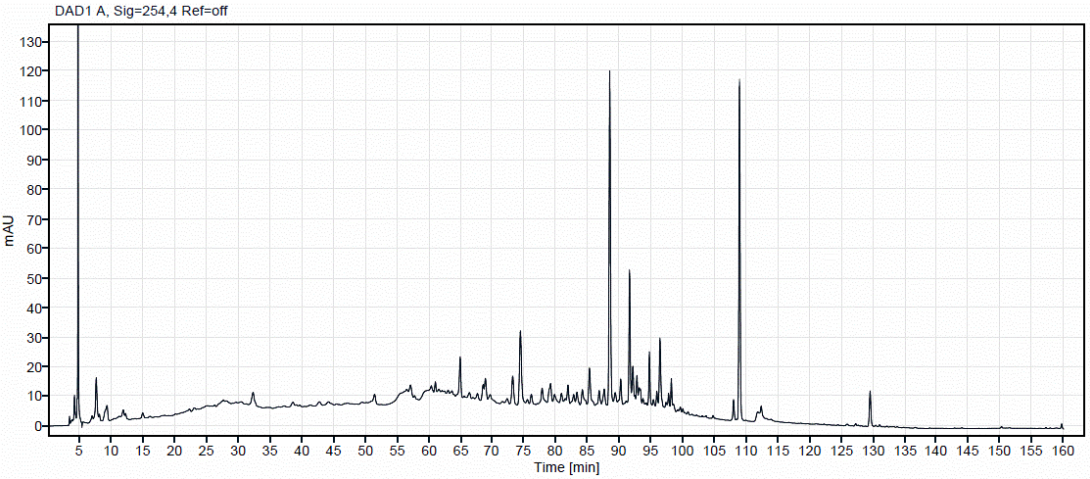
HPLC chromatograms of ethanolic extract of *Bridelia ovata* (มะกา) at wavelength 254 nm



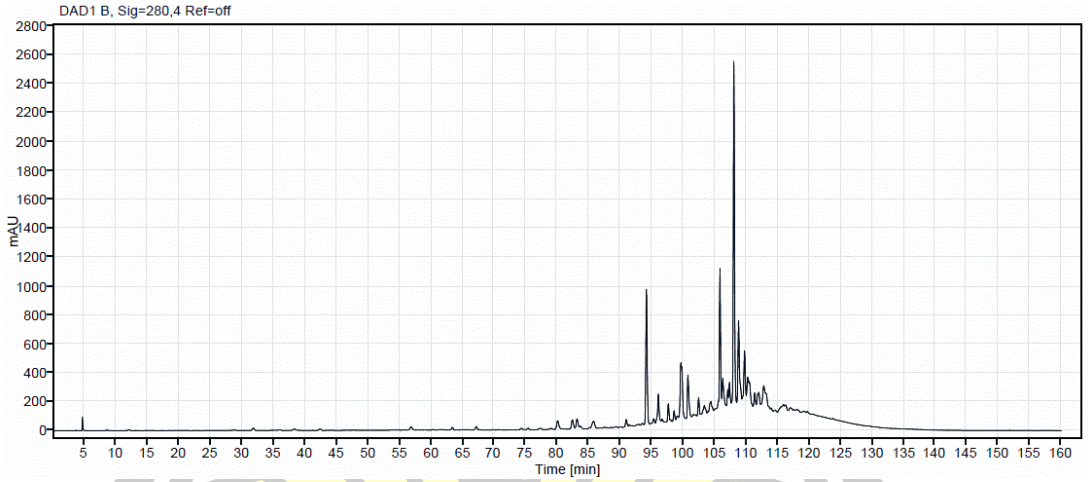
HPLC chromatograms of ethanolic extract of *Capparis micracantha* (ชิงชี่) at wavelength 254 nm



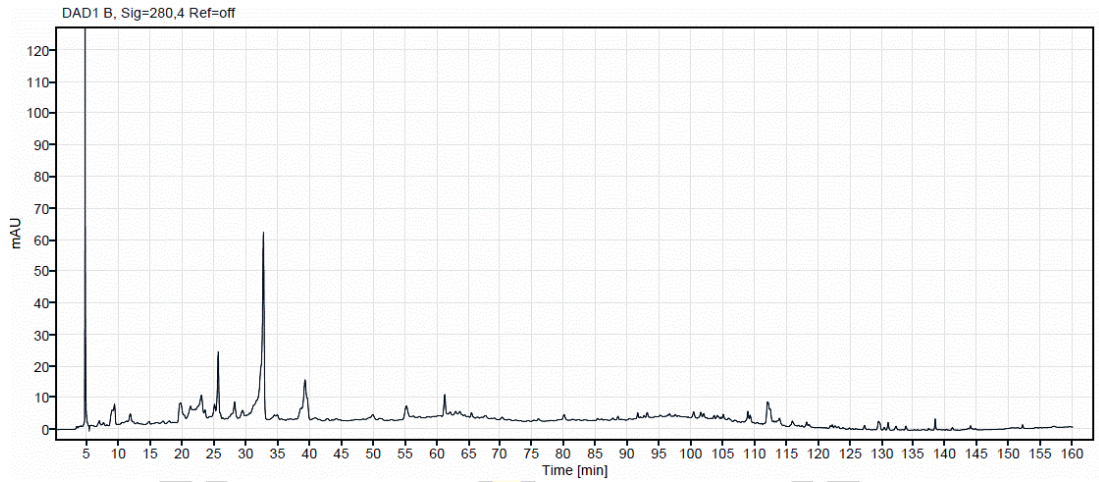
HPLC chromatograms of ethanolic extract of *Cassia fistula* (คูน) at wavelength 254 nm



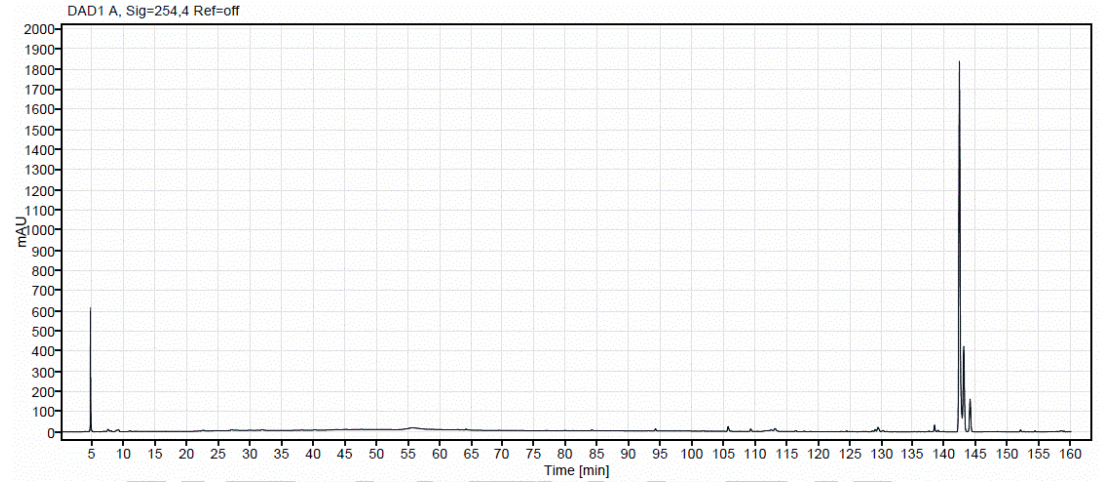
HPLC chromatograms of ethanolic extract of *Clerodendrum indicum* (เท้ายายม่อม) at wavelength 254 nm



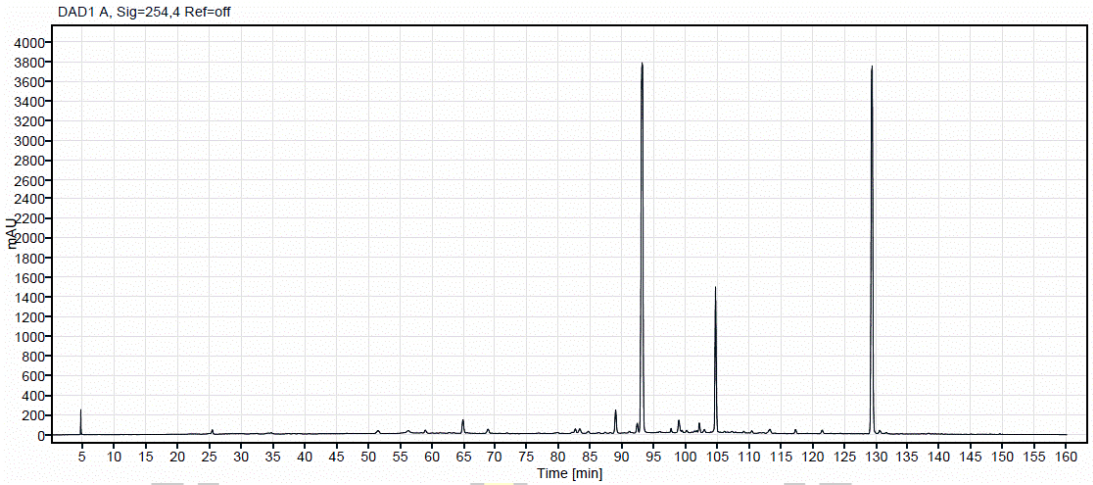
HPLC chromatograms of ethanolic extract of *Dracaena cochinchinensis* (จันทน์แดง) at wavelength 280 nm



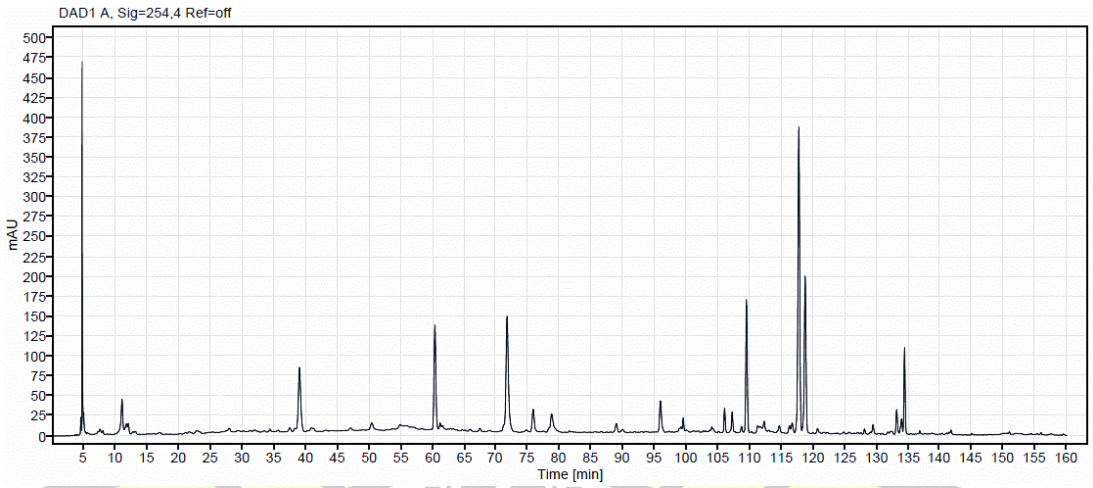
HPLC chromatograms of ethanolic extract of *Ficus racemosa* (มะเดื่อชุมพร) at wavelength 280 nm



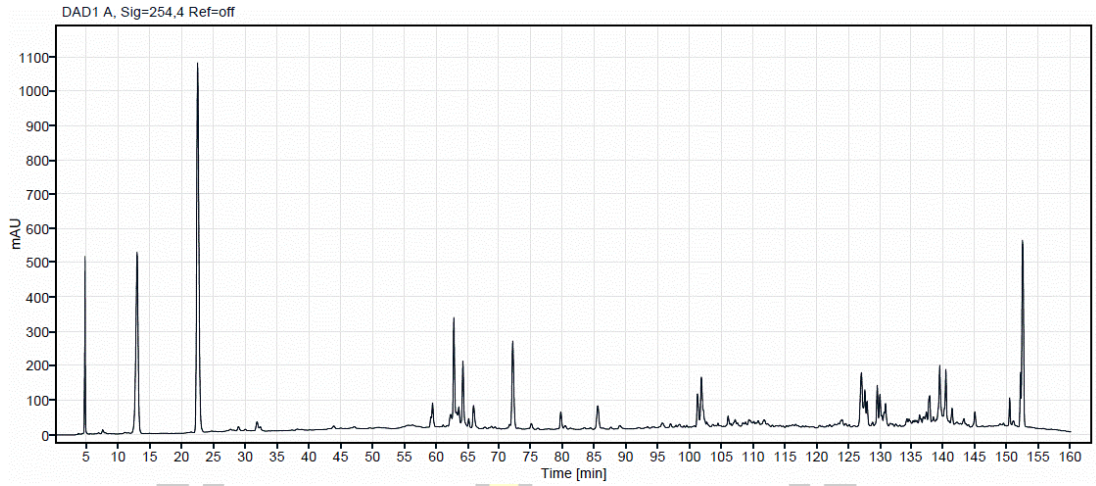
HPLC chromatograms of ethanolic extract of *Gymnopetalum chinense* (กระดอม) at wavelength 254 nm



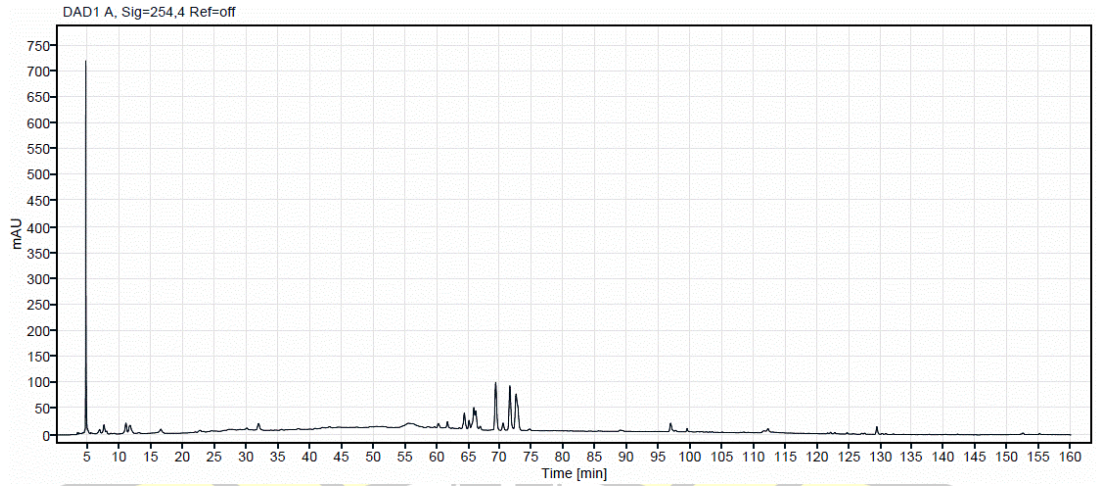
HPLC chromatograms of ethanolic extract of *Harrisonia perforata* (คนทา) at wavelength 254 nm



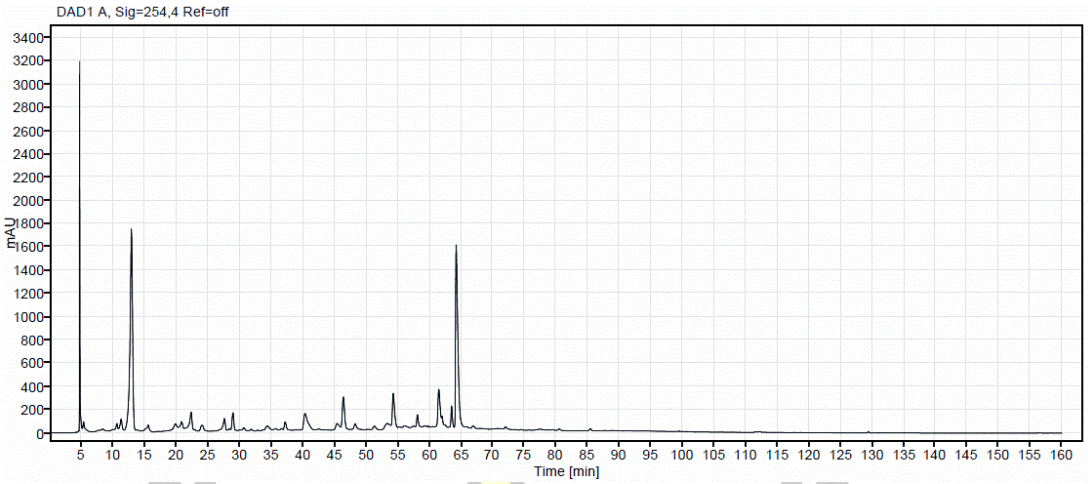
HPLC chromatograms of ethanolic extract of *Ligusticum sinense* (โกฐหัวบัว)at wavelength 254 nm



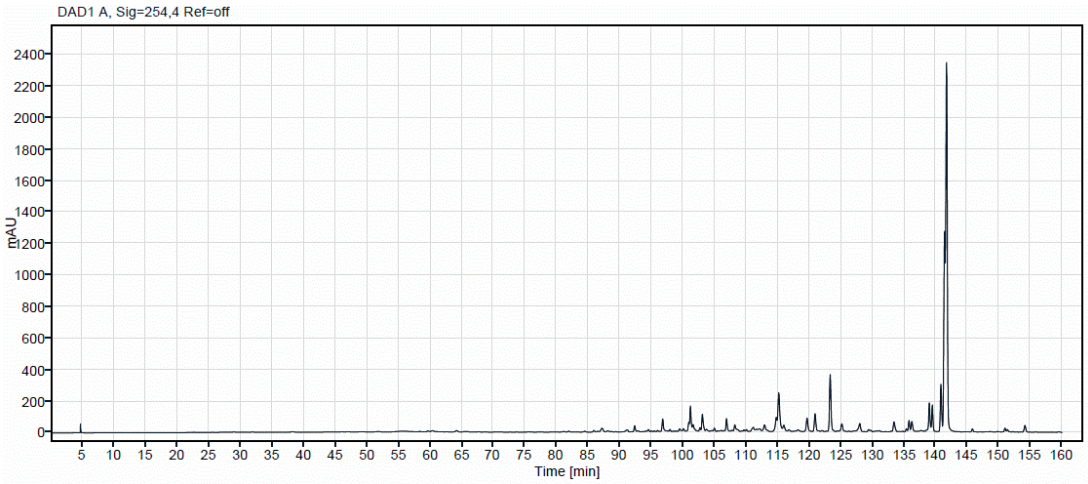
HPLC chromatograms of ethanolic extract of *Mesua ferrea* (บุนนาค) at wavelength 254 nm



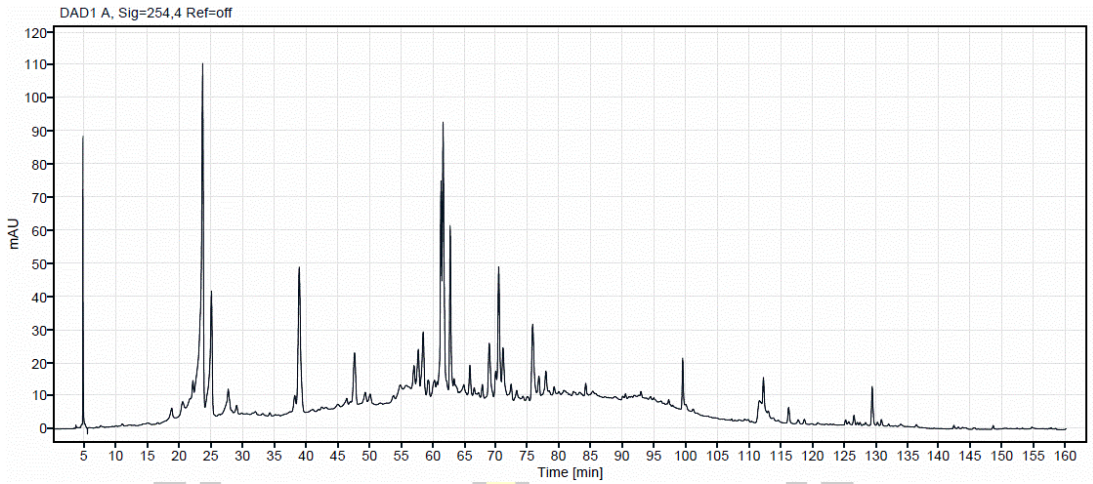
HPLC chromatograms of ethanolic extract of *Nelumbo nucifera* (บัวหลวง) at wavelength 254 nm



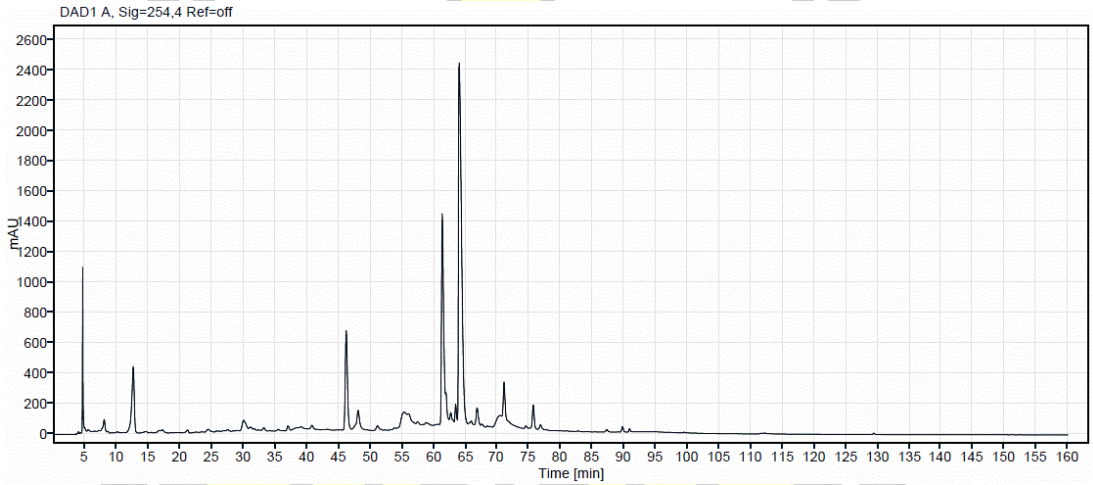
HPLC chromatograms of ethanolic extract of *Phyllanthus emblica* (มะขามป้อม) at wavelength 254 nm



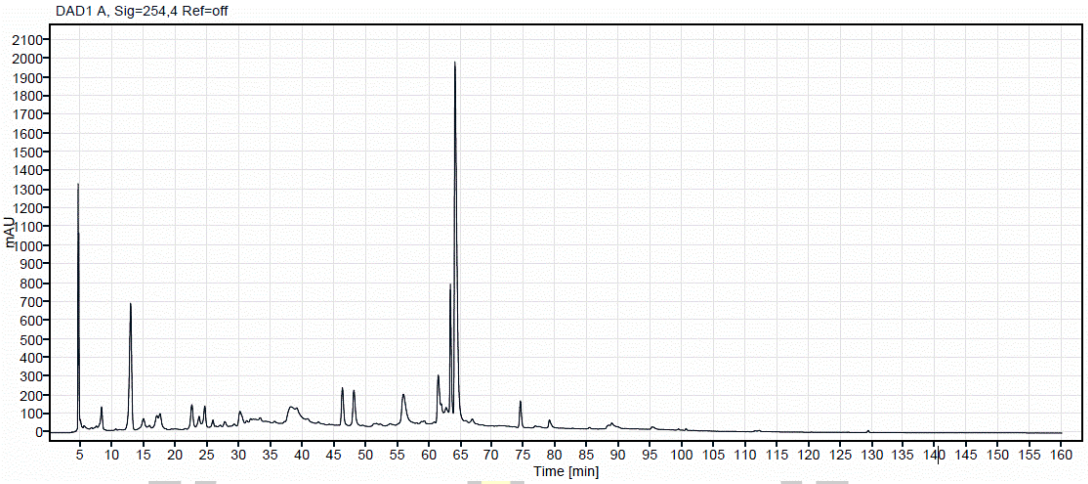
HPLC chromatograms of ethanolic extract of *Pinus kesiya* (สน) at wavelength 254 nm



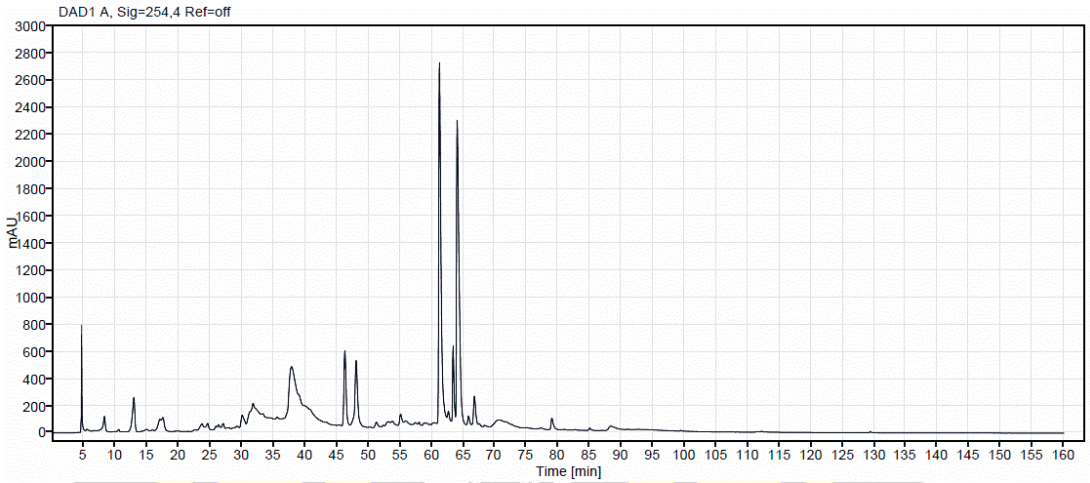
HPLC chromatograms of ethanolic extract of *Tarenna hoaensis* (จันทน์ขาว) at wavelength 254 nm



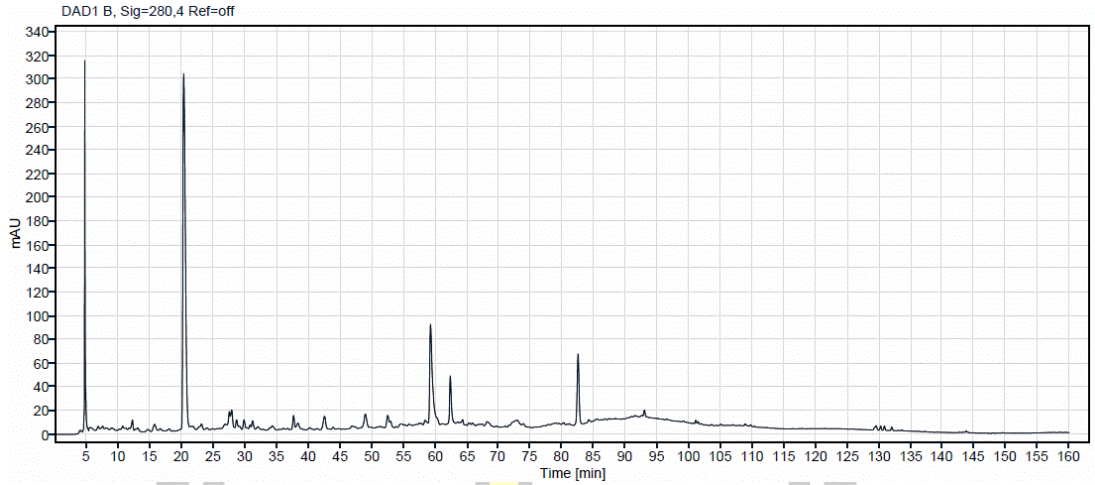
HPLC chromatograms of ethanolic extract of *Terminalia bellirica* (สมอพิเภก) at wavelength 254 nm



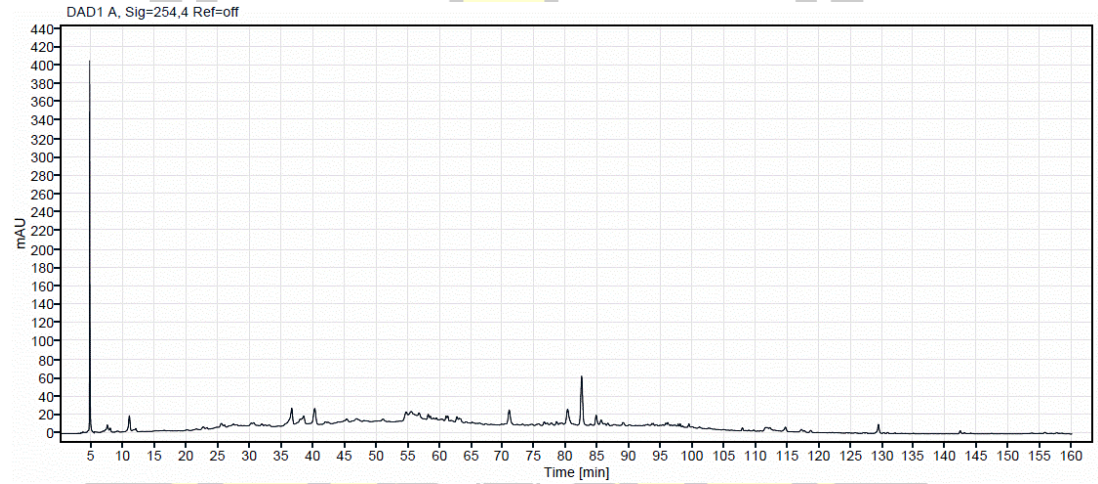
HPLC chromatograms of ethanolic extract of *Terminalia chebula* (สมอไทย) at wavelength 254 nm



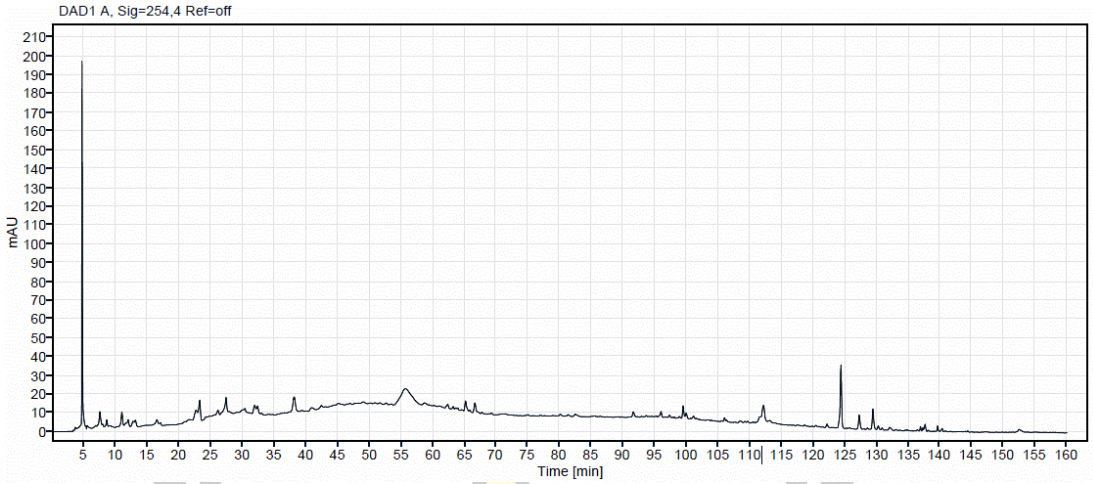
HPLC chromatograms of ethanolic extract of *Terminalia* sp. “Samo Thet” (สมอเทศ) at wavelength 254 nm



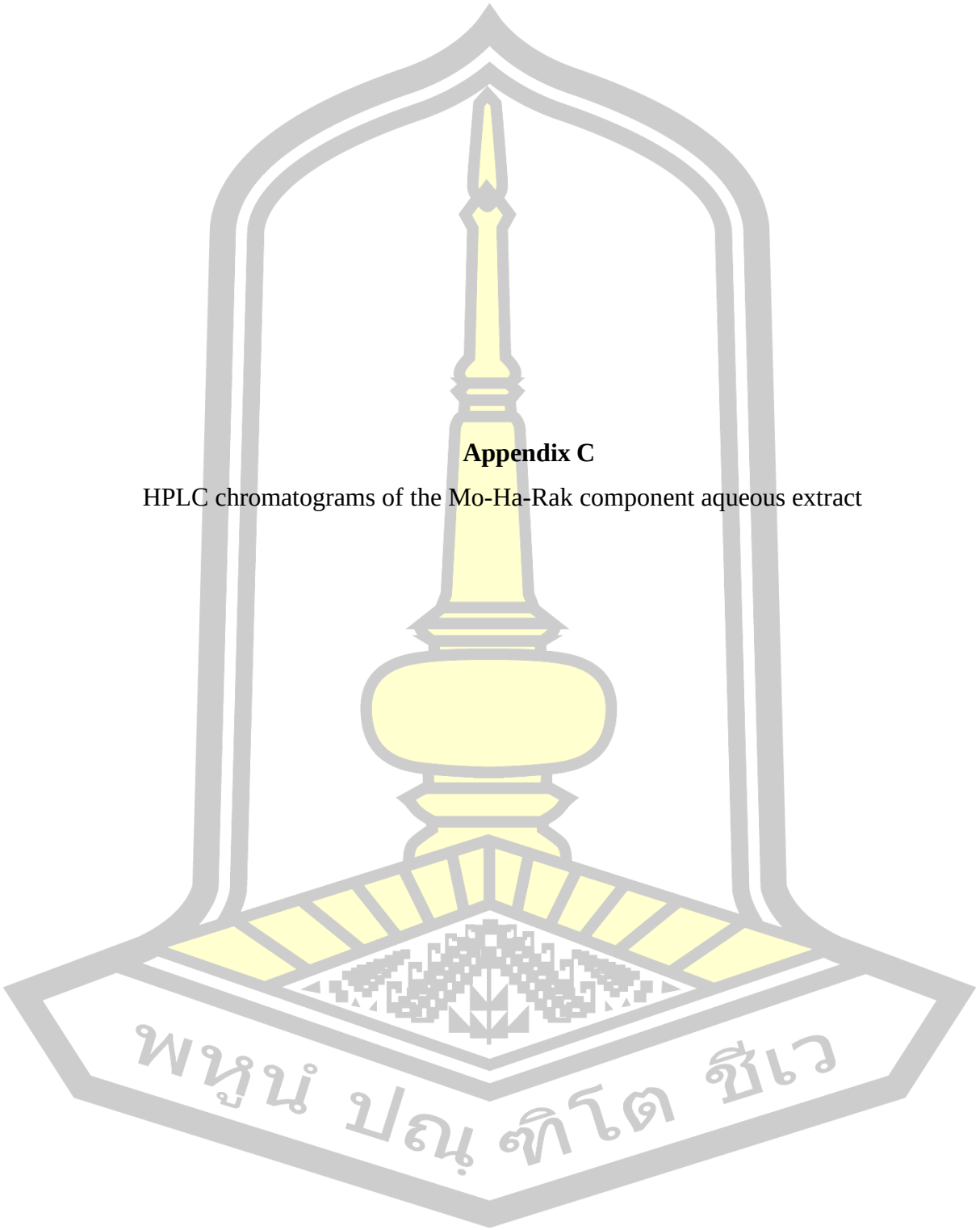
HPLC chromatograms of ethanolic extract of *Tiliacora triandra* (ย่านาง) at wavelength 280 nm



HPLC chromatograms of ethanolic extract of *Tinospora crispa* (บอระเพ็ด) at wavelength 254 nm

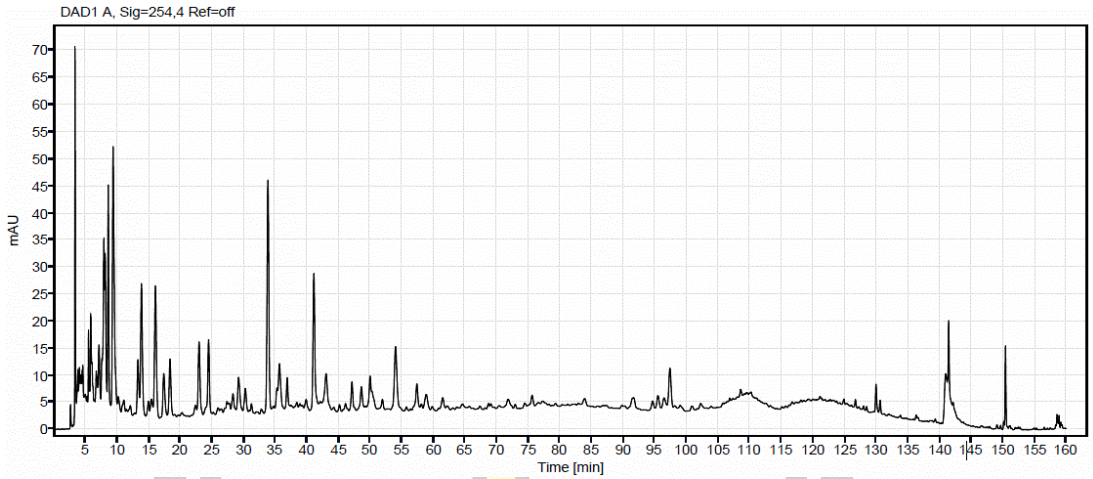


HPLC chromatograms of ethanolic extract of *Vetiveria zizanioides* (แฝกหอม) at wavelength 254 nm

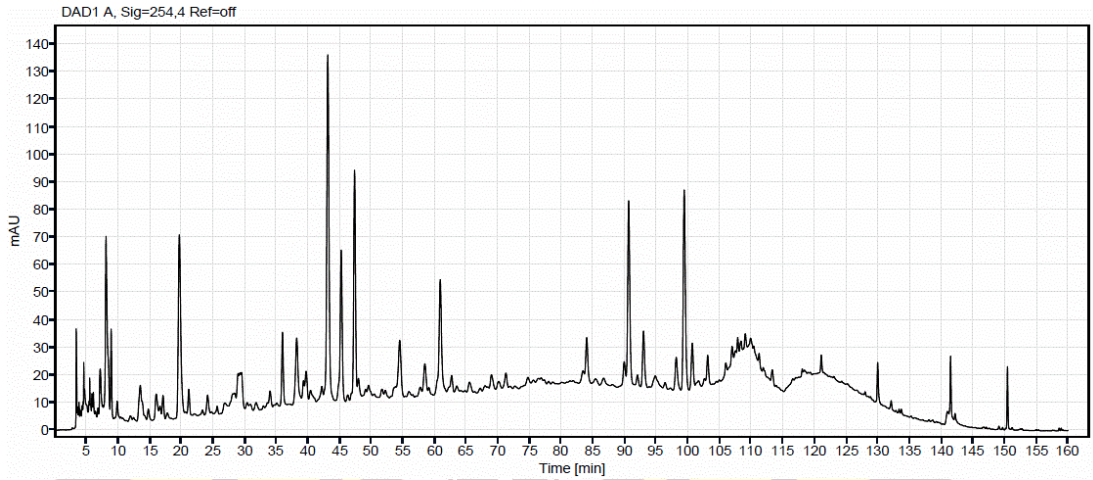


Appendix C

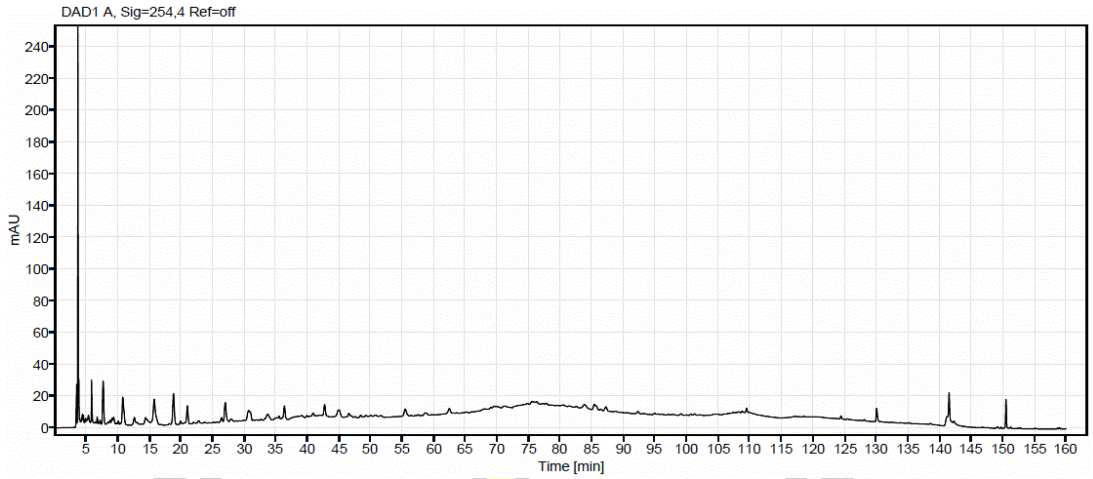
HPLC chromatograms of the Mo-Ha-Rak component aqueous extract



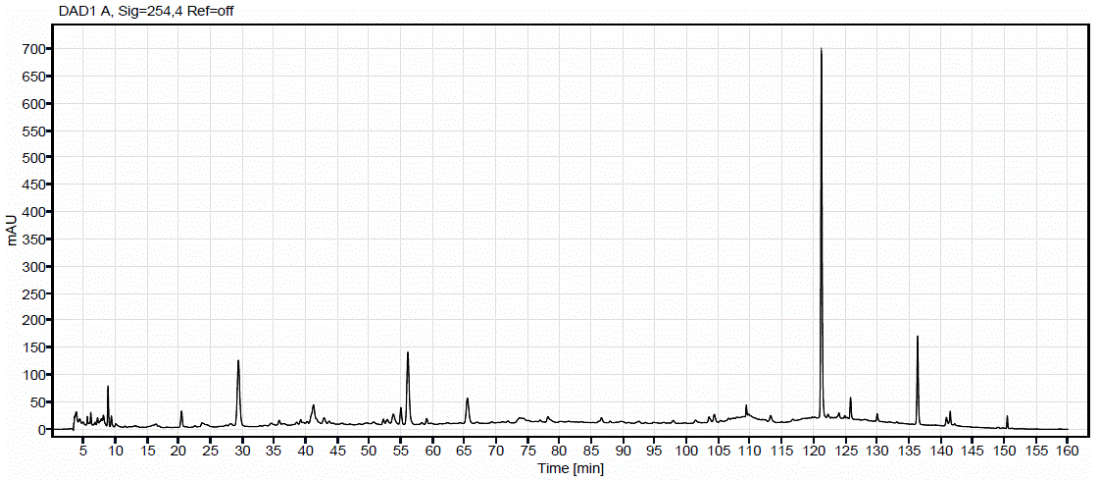
HPLC chromatograms of aqueous extract of *Azadirachta indica* (สะเดา) at wavelength 254 nm



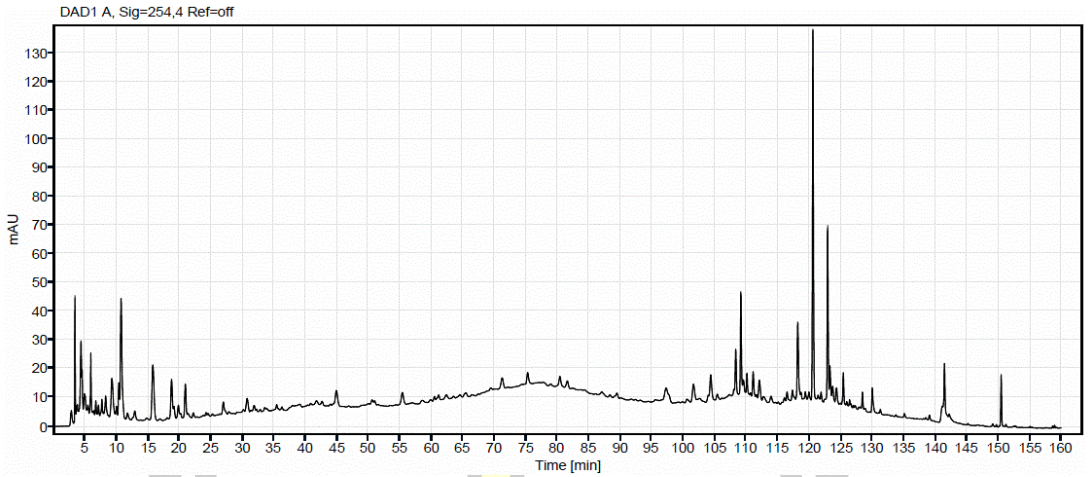
HPLC chromatograms of aqueous extract of *Bridelia ovata* (มะกา) at wavelength 254 nm



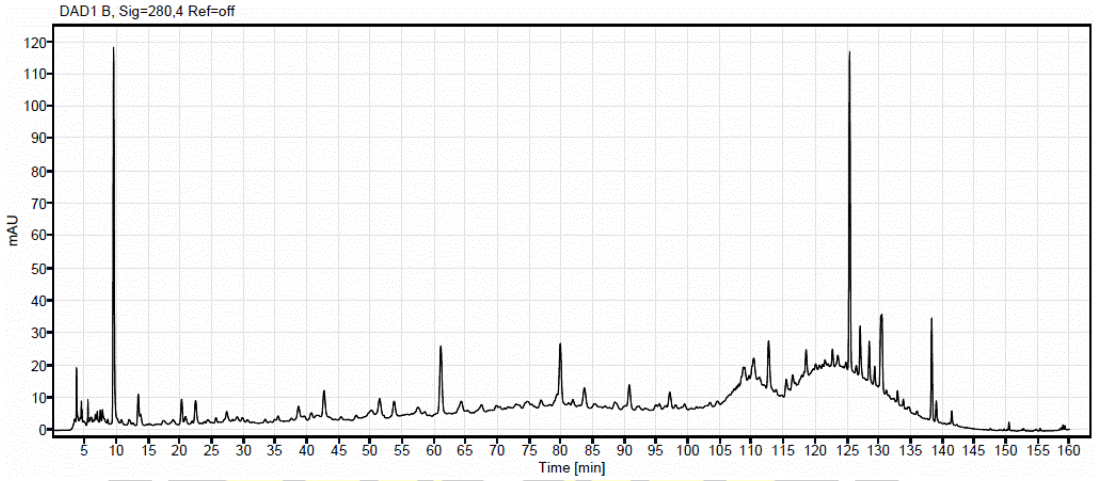
HPLC chromatograms of aqueous extract of *Capparis micracantha* (ชิงชี่) at wavelength 254 nm



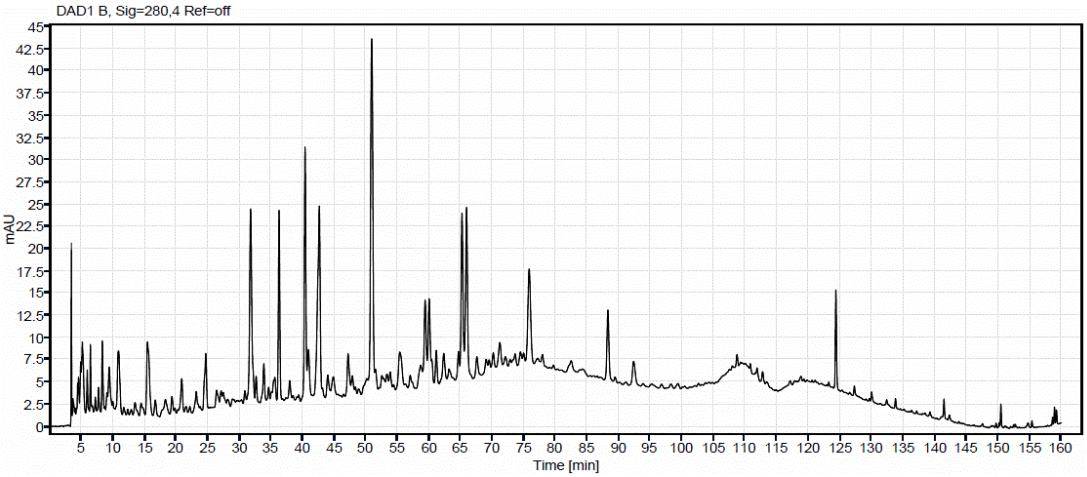
HPLC chromatograms of aqueous extract of *Cassia fistula* (ลูน) at wavelength 254 nm



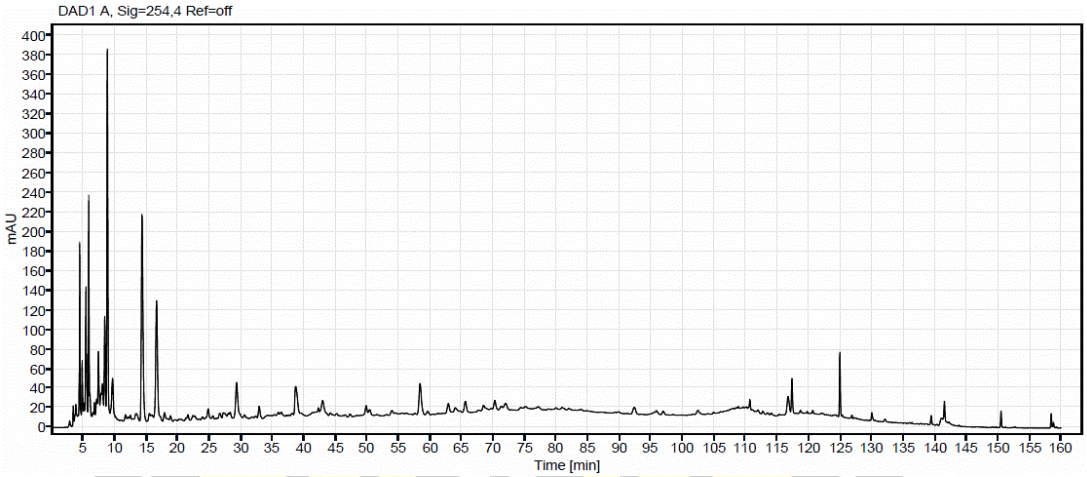
HPLC chromatograms of aqueous extract of *Clerodendrum indicum* (เท้ายายม่อม) at wavelength 254 nm



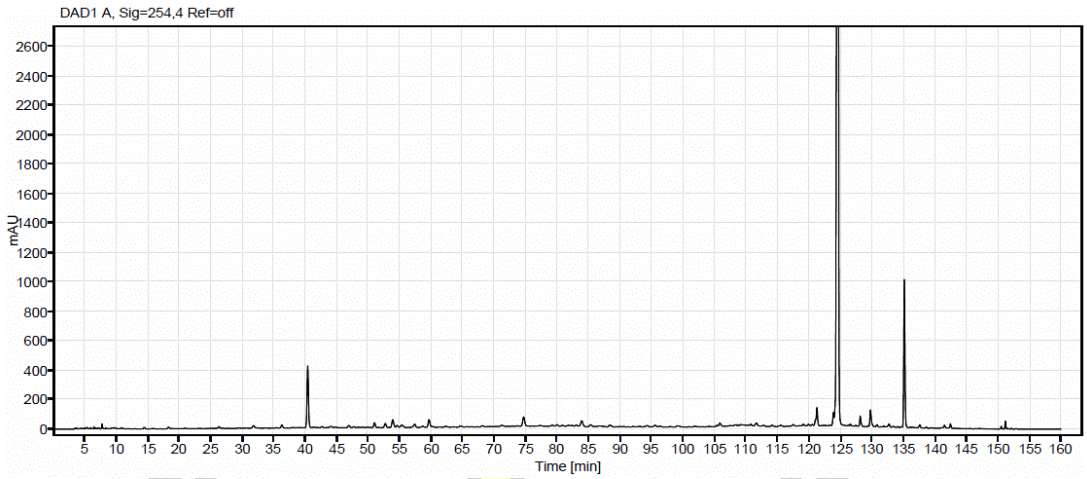
HPLC chromatograms of aqueous extract of *Dracaena cochinchinensis* (จันทน์แดง) at wavelength 280 nm



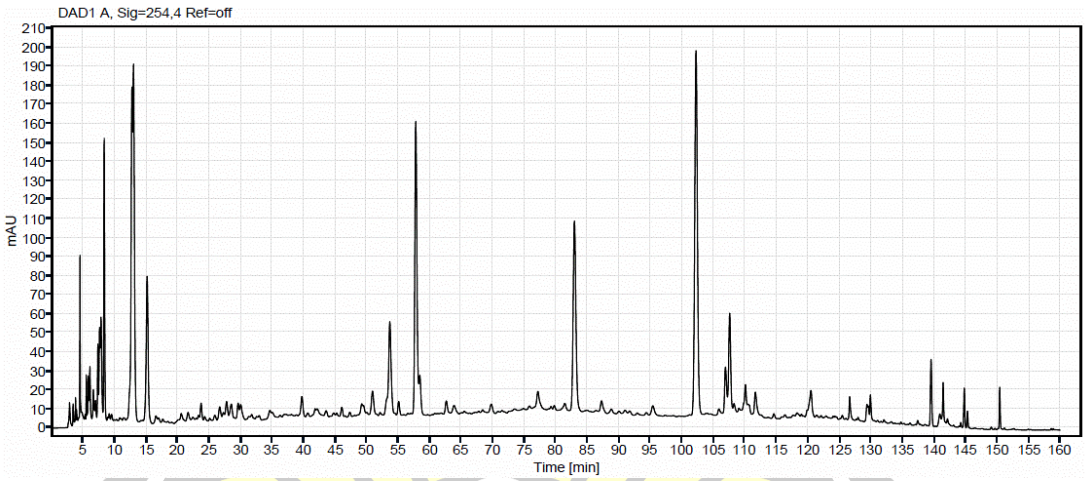
HPLC chromatograms of aqueous extract of *Ficus racemosa* (มะเดื่อชุมพร) at wavelength 280 nm



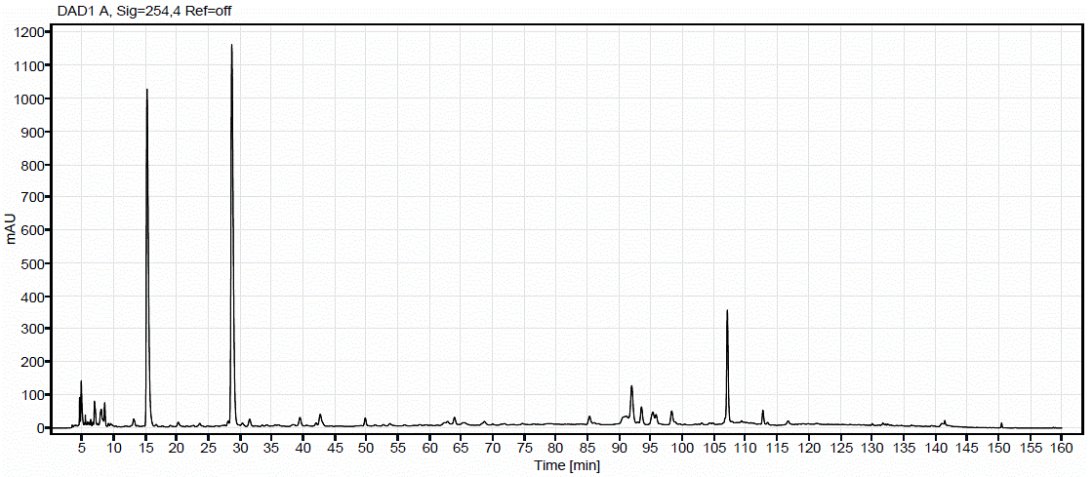
HPLC chromatograms of aqueous extract of *Gymnopetalum chinense* (กระดอม) at wavelength 254 nm



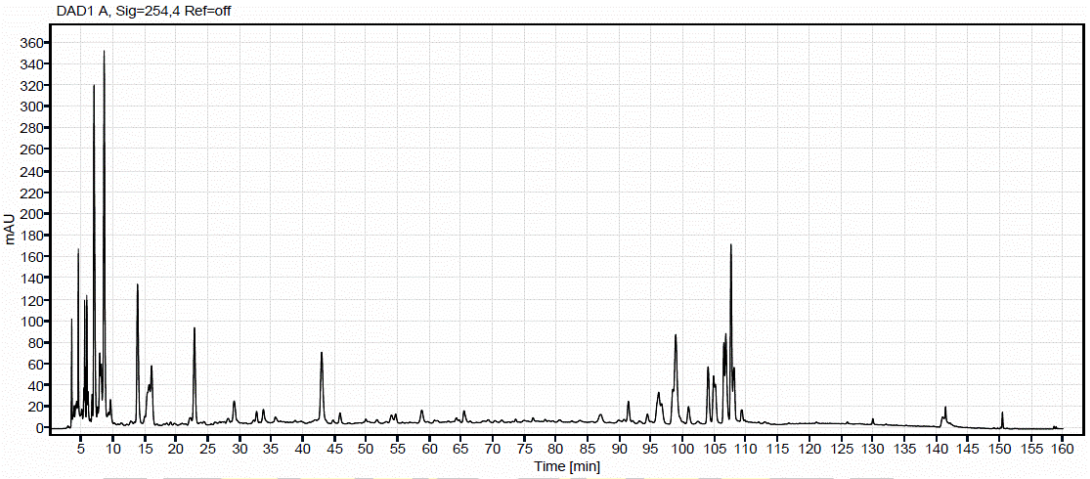
HPLC chromatograms of aqueous extract of *Harrisonia perforate* (คนทา) at wavelength 254 nm



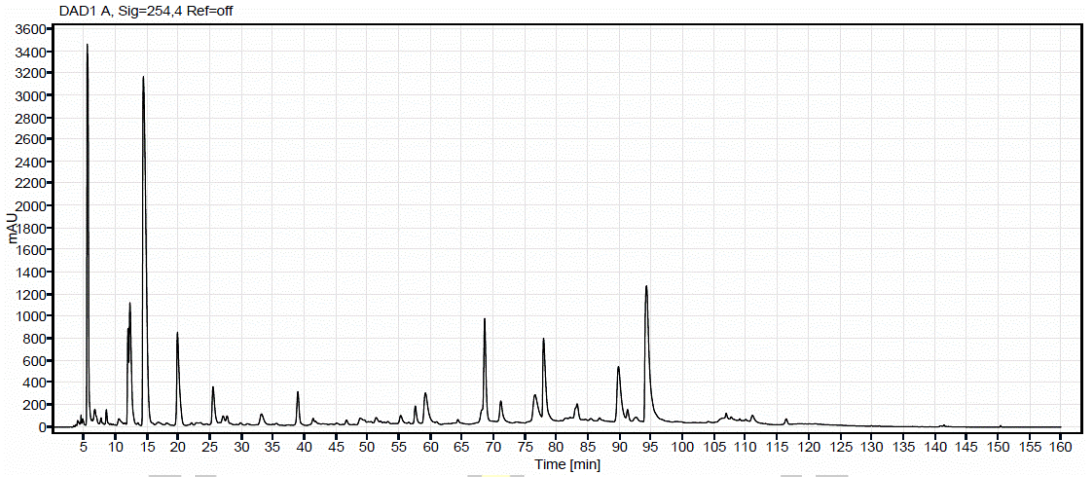
HPLC chromatograms of aqueous extract of *Ligusticum sinense* (โกฐหัวบัว) at wavelength 254 nm



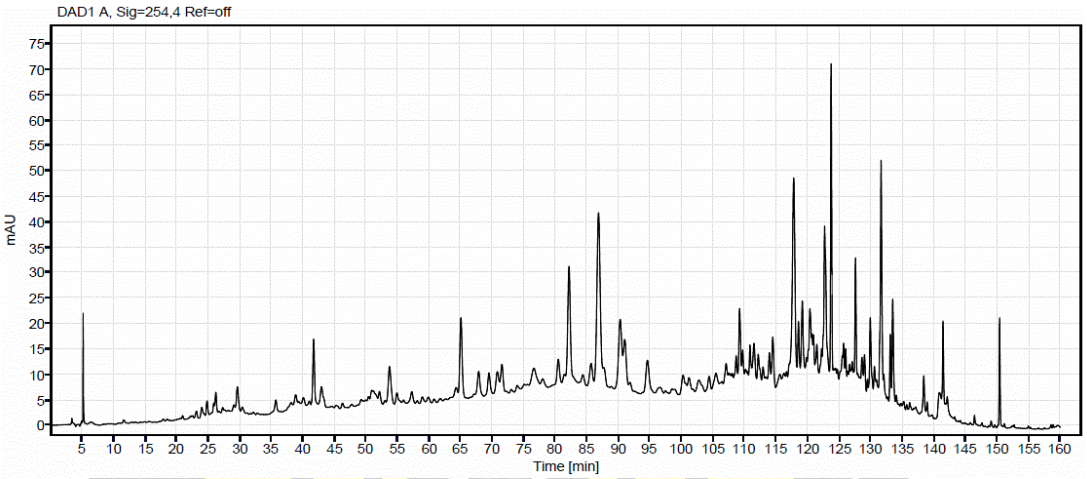
HPLC chromatograms of aqueous extract of *Mesua ferrea* (บุนนาค) at wavelength 254 nm



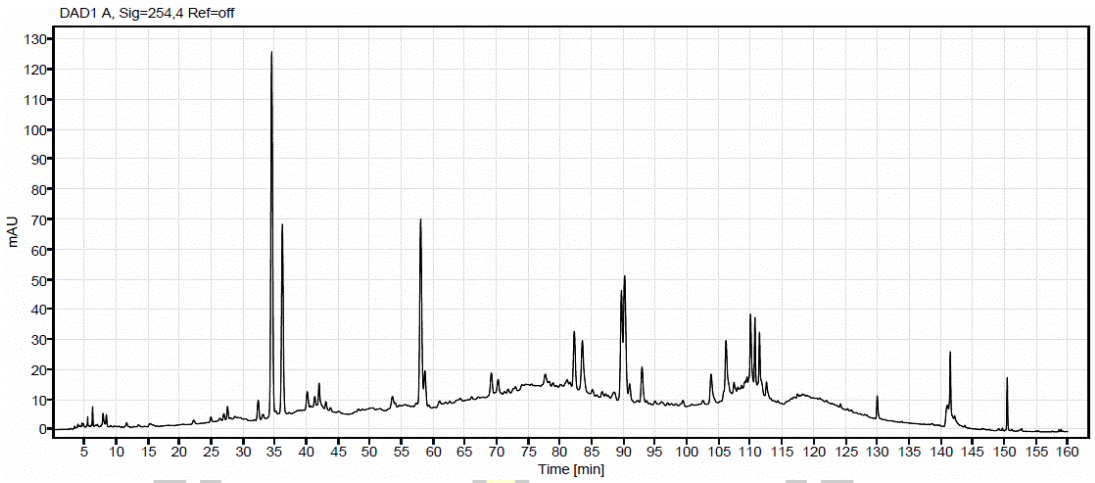
HPLC chromatograms of aqueous extract of *Nelumbo nucifera* (บัวหลวง) at wavelength 254 nm



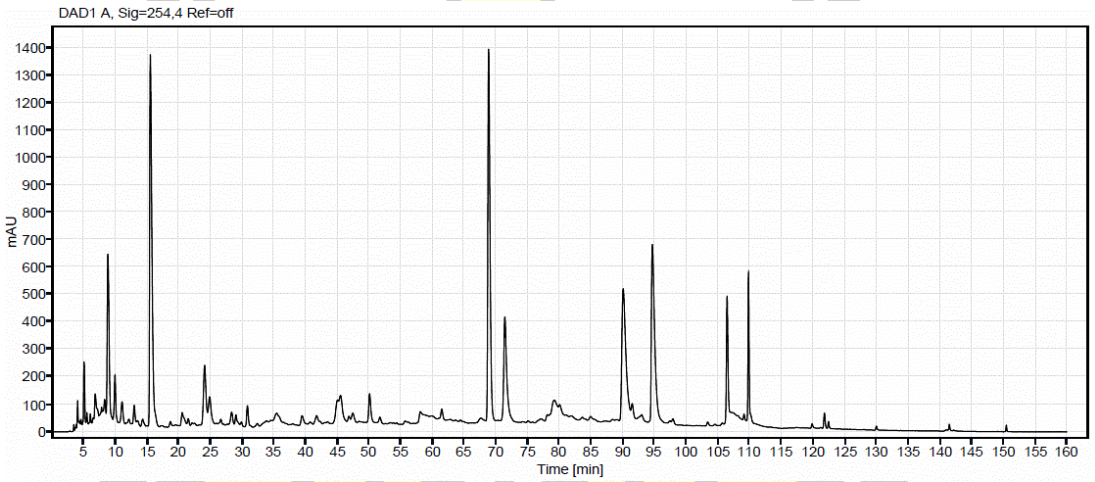
HPLC chromatograms of aqueous extract of *Phyllanthus emblica* (มะขามป้อม) at wavelength 254 nm



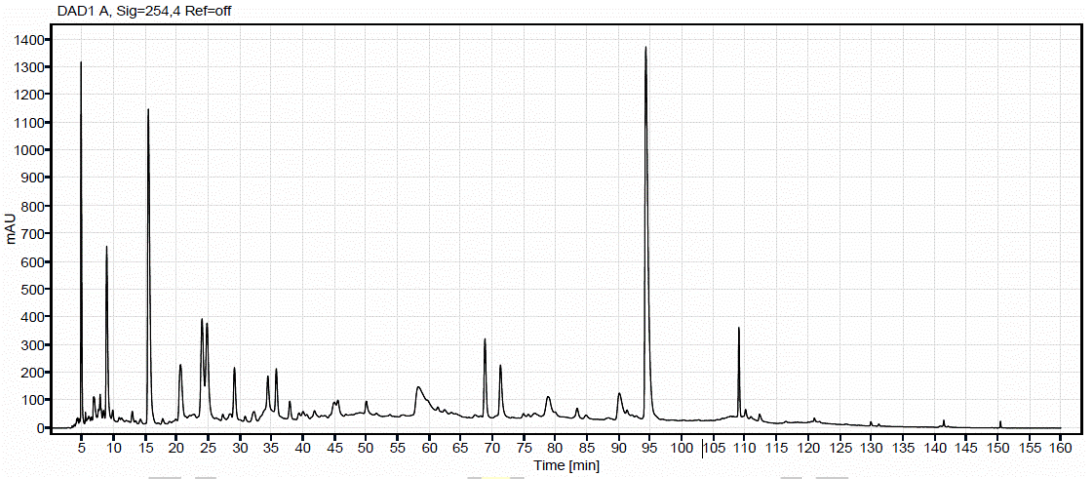
HPLC chromatograms of aqueous extract of *Pinus kesiya* (สน) at wavelength 254 nm



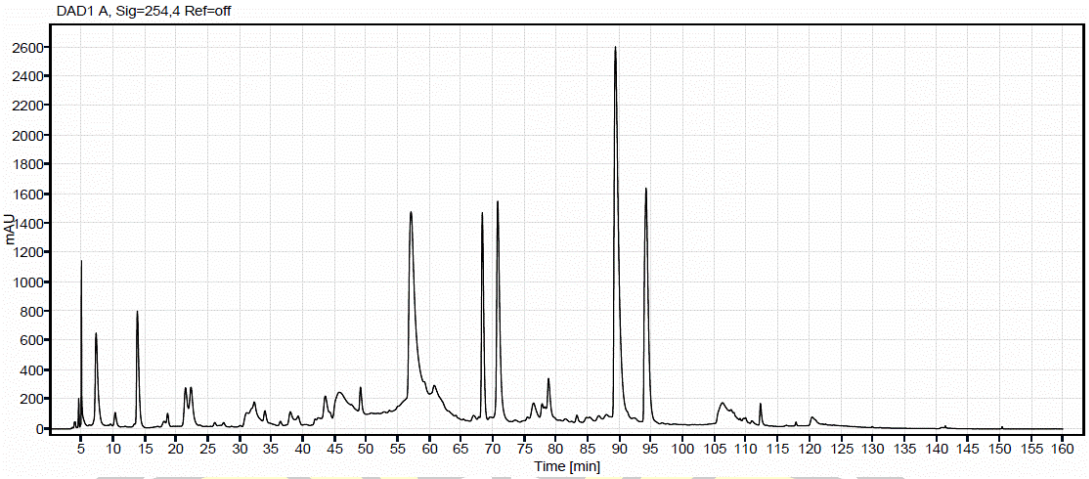
HPLC chromatograms of aqueous extract of *Tarenna hoaensis* (จันทน์ขาว) at wavelength 254 nm



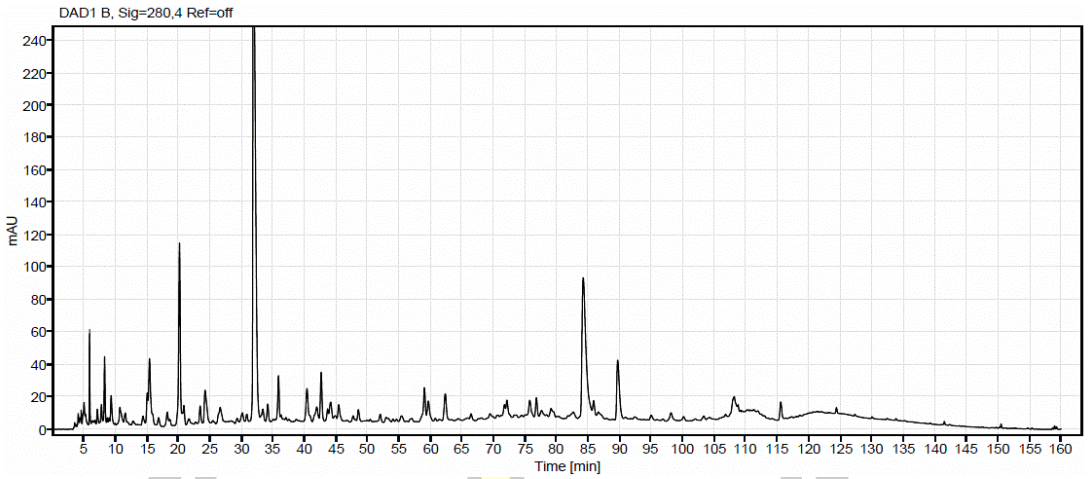
HPLC chromatograms of aqueous extract of *Terminalia bellirica* (สมอพิเภก) at wavelength 254 nm



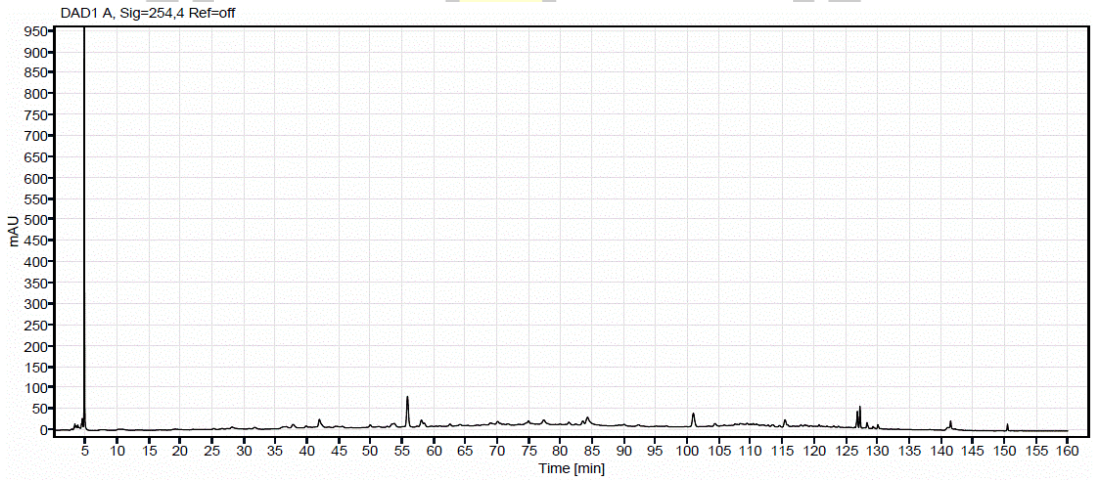
HPLC chromatograms of aqueous extract of *Terminalia chebula* (สมอไทย) at wavelength 254 nm



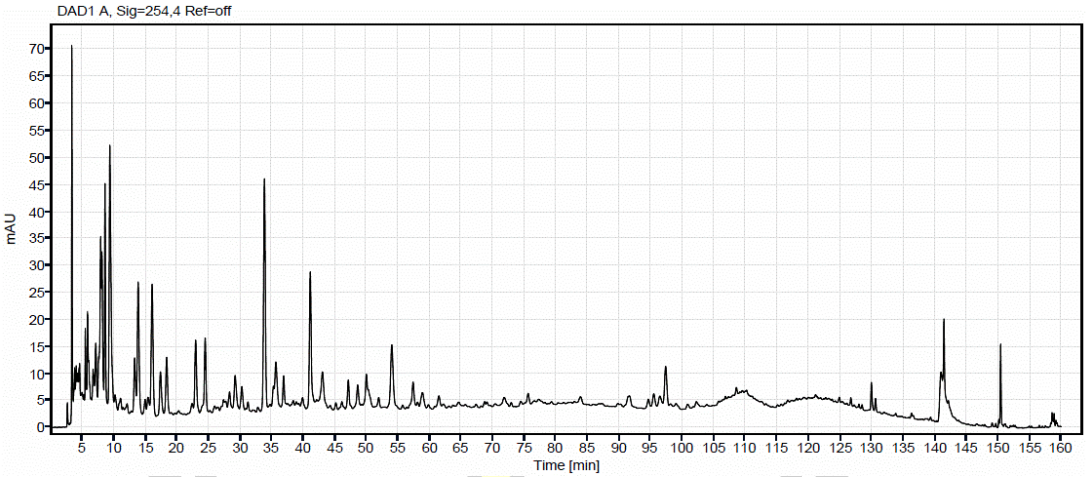
HPLC chromatograms of aqueous extract of *Terminalia* sp. “Samo Thet” (สมอแทศ) at wavelength 254 nm



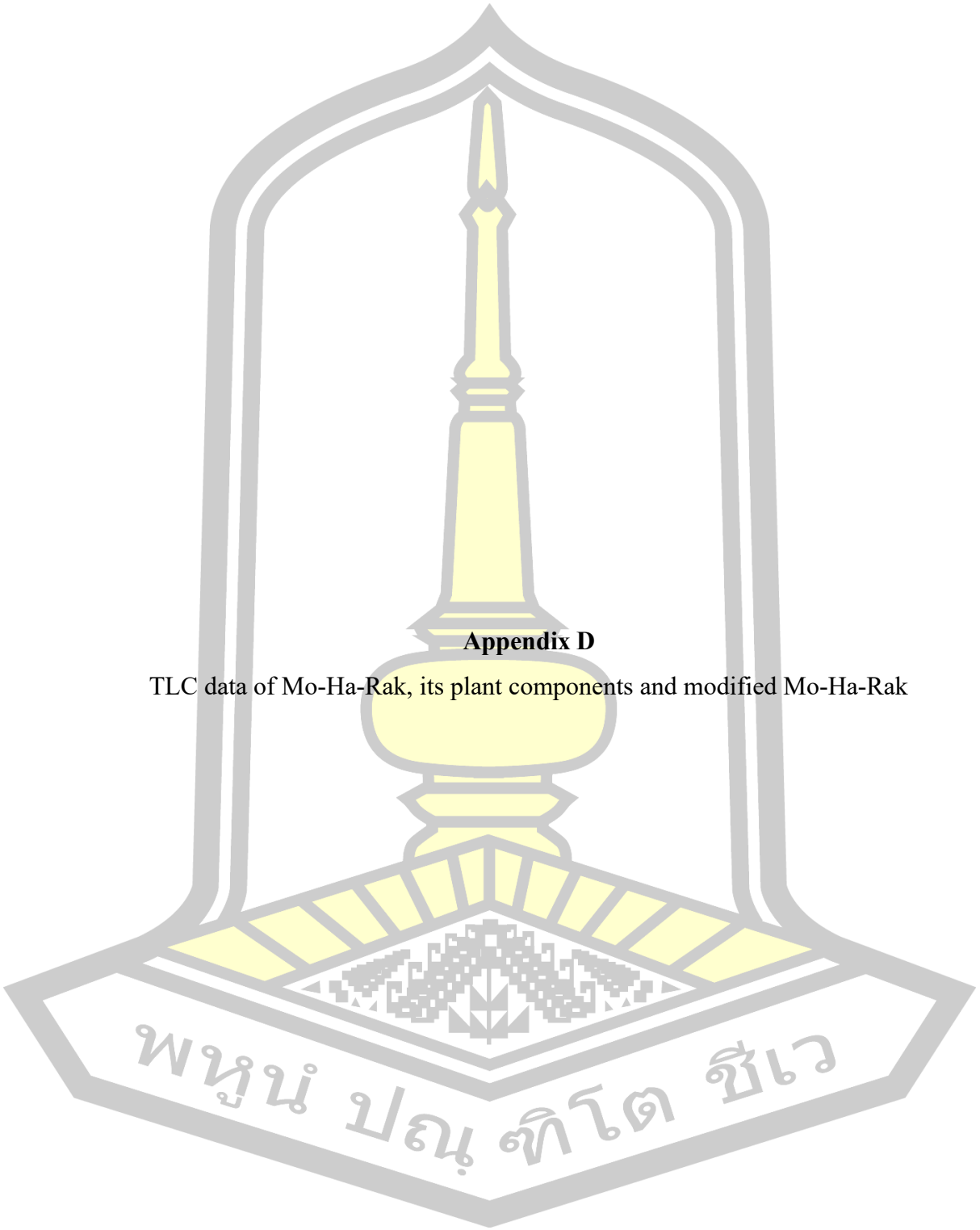
HPLC chromatograms of aqueous extract of *Tiliacora triandra* (ย่านาง) at wavelength 280 nm



HPLC chromatograms of aqueous extract of *Tinospora crispa* (บอระเพ็ด) at wavelength 254 nm



HPLC chromatograms of aqueous extract of *Vetiveria zizanioides* (แฝกหอม) at wavelength 254 nm



Appendix D

TLC data of Mo-Ha-Rak, its plant components and modified Mo-Ha-Rak

Table 22 Rf value (System I) of MHR compared with herbal components and standard chemicals, detected under UV 254 nm.

Rf	Samples (detection with UV 254 nm)																		
	1	S1	S2	S3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.16-0.20	quenching																		✓
0.26-0.28	quenching							✓											
0.30-0.32	quenching							✓							✓				
0.35-0.36	quenching							✓										✓	
0.40-0.41	quenching							✓			✓		✓			✓	✓		
0.45-0.46	quenching					✓		✓					✓		✓	✓		✓	
0.58-0.60	quenching					✓		✓					✓						
0.72-0.74	quenching														✓	✓			
0.77-0.80	quenching							✓											
0.90-0.93	quenching															✓			

Samples: (1) Mo-Ha-Rak, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (ลูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 23 Rf value (System I) of MHR compared with herbal components, modified Mo-Ha-Rak and standard chemicals, detected under UV 254 nm.

Rf	Samples (detection with UV 254 nm)																			
	1	S1	S2	S3	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0.19-0.21	quenching				✓	✓	✓	✓							✓	✓		✓	✓	✓
0.30-0.32	quenching										✓	✓	✓	✓	✓		✓	✓	✓	
0.33-0.34	quenching				✓			✓												✓
0.35-0.37	quenching					✓	✓	✓			✓	✓			✓	✓		✓		✓
0.46-0.48	quenching							✓			✓	✓	✓	✓	✓		✓	✓	✓	
0.50-0.51	quenching										✓	✓	✓	✓	✓					
0.59-0.61	quenching							✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.72-0.73	quenching							✓												✓
0.74-0.78	quenching										✓	✓	✓	✓	✓		✓	✓	✓	
0.89-0.91	quenching										✓	✓								

Samples: (1) Mo-Ha-Rak, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 24 Rf value (System I) of MHR compared with herbal components and standard chemicals, detected under UV 366 nm.

Rf	Samples (detection with UV 366 nm)																		
	1	S1	S2	S3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.04-0.06	blue				✓	✓												✓	
0.10-0.13	blue					✓													✓
0.15-0.18	quenching																		✓
0.20-0.24	green																		✓
0.27-0.28	blue												✓						
0.29-0.31	quenching							✓											
0.32-0.33	blue					✓		✓	✓			✓							
0.35-0.38	light blue				✓	✓		✓	✓				✓						
0.40-0.42	blue							✓	✓	✓			✓	✓	✓				
0.45-0.46	blue							✓					✓		✓				
0.47-0.48	red									✓		✓					✓		
0.50-0.51	blue														✓				
0.55-0.57	blue							✓					✓			✓			
0.59-0.60	yellow																	✓	
0.64-0.66	blue							✓							✓				
0.71-0.74	red																✓		
0.76-0.77	blue				✓	✓	✓		✓		✓		✓		✓	✓			
0.79-0.82	blue							✓			✓	✓	✓	✓	✓	✓	✓		
0.89-0.92	blue						✓	✓								✓			

Samples: (1) Mo-Ha-Rak, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (ลูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 25 Rf value (System I) of MHR compared with herbal components, modified MHR and standard chemicals, detected under UV 366 nm.

Rf	Samples (detection with UV 366 nm)																			
	1	S1	S2	S3	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0.04-0.06	blue				✓	✓		✓								✓				
0.10-0.13	blue							✓							✓	✓		✓		
0.15-0.18	quenching				✓	✓	✓	✓							✓	✓		✓	✓	✓
0.20-0.24	green														✓	✓	✓			
0.27-0.28	blue							✓			✓	✓	✓	✓	✓		✓	✓	✓	
0.29-0.31	quenching										✓	✓	✓	✓	✓		✓	✓	✓	
0.32-0.33	blue										✓	✓	✓	✓	✓		✓	✓	✓	
0.35-0.38	light blue										✓	✓			✓		✓	✓	✓	
0.40-0.42	blue										✓	✓	✓	✓	✓	✓	✓	✓	✓	
0.45-0.46	blue									✓	✓	✓	✓	✓	✓		✓	✓	✓	
0.47-0.48	red										✓	✓			✓	✓	✓			
0.50-0.51	blue										✓									
0.55-0.57	blue										✓	✓		✓			✓			
0.59-0.60	yellow														✓	✓	✓			
0.64-0.66	blue										✓			✓			✓	✓	✓	
0.71-0.74	red										✓	✓			✓	✓	✓			
0.76-0.77	blue							✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓
0.79-0.82	blue				✓		✓		✓	✓	✓	✓		✓	✓		✓	✓	✓	✓
0.89-0.92	blue								✓		✓		✓	✓	✓			✓	✓	✓

Samples: (1) Mo-Ha-Rak, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 26 Rf value (System I) of MHR compared with herbal components and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm.

Rf	Samples (detection with anisaldehyde-sulphuric acid under UV 366 nm)																		
	1	S1	S2	S3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.04-0.06	blue				✓	✓												✓	
0.10-0.13	blue					✓													✓
0.15-0.18	dark blue																		✓
0.20-0.24	green				✓	✓	✓	✓		✓	✓	✓		✓	✓		✓	✓	✓
0.27-0.28	blue							✓					✓						
0.29-0.31	quenching							✓											
0.32-0.33	blue					✓		✓	✓			✓							
0.35-0.38	light blue												✓						
0.40-0.42	blue					✓		✓		✓			✓	✓	✓			✓	
0.45-0.46	blue							✓		✓	✓	✓	✓		✓				
0.47-0.48	red																✓		
0.50-0.51	blue										✓	✓	✓		✓				
0.55-0.57	blue					✓		✓									✓		
0.59-0.60	yellow																	✓	
0.64-0.66	light purple	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓
0.69-0.70	purple				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
0.75-0.78	light purple		✓							✓		✓	✓		✓		✓		
0.79-0.82	blue					✓		✓			✓	✓		✓	✓				
0.89-0.92	blue						✓		✓		✓		✓			✓	✓		

Samples: (1) Mo-Ha-Rak, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (ลูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 27 Rf value (System I) of MHR compared with herbal components, modified Mo-Ha-Rak and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm.

Rf	Samples (detection with anisaldehyde-sulphuric acid under UV 366 nm)																			
	1	**S1**	**S2**	**S3**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
0.04-0.06	blue				✓	✓		✓								✓				
0.10-0.13	blue							✓							✓	✓		✓		
0.15-0.18	dark blue				✓	✓	✓	✓							✓	✓		✓	✓	✓
0.20-0.24	green				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.27-0.28	blue				✓	✓		✓			✓	✓		✓	✓	✓	✓	✓	✓	
0.29-0.31	quenching										✓	✓	✓	✓	✓		✓	✓	✓	
0.32-0.33	blue										✓	✓	✓	✓	✓		✓	✓	✓	
0.35-0.38	light blue										✓	✓			✓		✓	✓	✓	
0.40-0.42	blue				✓			✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	
0.45-0.46	blue									✓	✓	✓	✓	✓	✓		✓	✓	✓	
0.47-0.48	red										✓	✓			✓	✓	✓			
0.50-0.51	blue										✓	✓	✓	✓	✓					
0.55-0.57	blue				✓			✓			✓	✓		✓			✓	✓	✓	✓
0.59-0.60	yellow														✓	✓	✓			
0.64-0.66	light purple	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.69-0.70	purple				✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.75-0.78	light purple		✓						✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.79-0.82	blue				✓		✓		✓	✓	✓	✓		✓	✓		✓	✓	✓	✓
0.89-0.92	blue								✓		✓		✓	✓	✓			✓	✓	✓

Samples: (1) Mo-Ha-Rak, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 28 Rf value (System II) of MHR compared with herbal components and standard chemicals, detected under UV 254 nm.

Rf	Samples (detection with UV 254 nm)																		
	1	S1	S2	S3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.04-0.05	quenching						✓	✓	✓			✓		✓	✓				✓
0.08-0.12	quenching					✓			✓								✓		✓
0.16-0.18	quenching					✓		✓	✓			✓	✓	✓	✓		✓	✓	✓
0.22-0.23	quenching							✓	✓			✓		✓				✓	✓
0.28-0.30	quenching							✓	✓	✓				✓			✓		✓
0.39-0.41	quenching																✓		
0.46-0.48	quenching																		✓
0.54-0.56	quenching																		✓
0.56-0.59	quenching							✓										✓	
0.68-0.70	quenching							✓	✓			✓	✓					✓	
0.73-0.74	quenching				✓	✓	✓	✓	✓	✓	✓		✓		✓		✓	✓	
0.78-0.80	quenching					✓					✓		✓			✓		✓	
0.87-0.90	quenching							✓											
0.97-0.99	quenching															✓	✓		

Samples: (1) Mo-Ha-Rak, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (ดูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

***T*able 29** Rf value (System II) of MHR compared with herbal components, modified MHR and standard chemicals, detected under UV 254 nm.

Rf	Samples (detection with UV 254 nm)																			
	1	S1	S2	S3	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0.04-0.05	quenching				✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.08-0.12	quenching				✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.15-0.16	quenching				✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.22-0.23	quenching				✓			✓	✓						✓	✓		✓		
0.26-0.27	quenching				✓	✓									✓	✓		✓		
0.35-0.36	quenching						✓				✓	✓			✓	✓		✓		✓
0.46-0.48	quenching				✓	✓	✓	✓							✓	✓		✓	✓	✓
0.50-0.52	quenching				✓	✓	✓	✓							✓	✓		✓	✓	✓
0.57-0.59	quenching						✓				✓	✓	✓	✓	✓		✓	✓	✓	
0.65-0.66	quenching					✓	✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.68-0.69	quenching				✓		✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.76-0.77	quenching										✓	✓		✓			✓	✓	✓	
0.88-0.90	quenching				✓						✓	✓		✓	✓		✓	✓	✓	
0.97-0.99	quenching										✓	✓						✓	✓	

Samples: (1) Mo-Ha-Rak, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 30 Rf value (System II) of MHR compared with herbal components and standard chemicals, detected under UV 366 nm.

Rf	Samples (detection with UV 366 nm)																		
	1	S1	S2	S3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.04-0.05	blue						✓	✓	✓			✓		✓	✓		✓	✓	✓
0.08-0.13	blue					✓			✓								✓		✓
0.13-0.15	dark blue																		✓
0.18-0.20	blue				✓	✓		✓						✓	✓		✓		✓
0.25-0.27	blue							✓		✓				✓	✓		✓	✓	
0.28-0.30	blue					✓								✓				✓	✓
0.38-0.41	blue					✓									✓				
0.46-0.48	blue					✓													✓
0.48-0.51	green																	✓	
0.52-0.54	blue							✓											
0.55-0.57	quenching																		✓
0.58-0.60	quenching							✓											
0.63-0.65	blue					✓						✓	✓						
0.66-0.68	blue					✓							✓		✓				
0.69-0.71	blue					✓		✓		✓			✓	✓	✓		✓	✓	
0.73-0.74	red									✓		✓					✓	✓	✓
0.74-0.75	blue							✓							✓	✓			
0.76-0.77	red									✓		✓					✓		✓
0.78-0.80	blue							✓							✓	✓			
0.84-0.85	red																✓		
0.88-0.89	blue				✓	✓	✓		✓		✓	✓	✓	✓	✓	✓			
0.90-0.91	red									✓							✓		
0.96-0.98	blue						✓									✓			

Samples: (1) Mo-Ha-Rak, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (ลูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 31 Rf value (System II) of MHR compared with herbal components, modified MHR and standard chemicals, detected under UV 366 nm.

Rf	Samples (detection with UV 366 nm)																			
	1	S1	S2	S3	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0.04-0.05	blue				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.08-0.13	blue				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.13-0.15	dark blue				✓	✓	✓								✓	✓		✓		
0.18-0.20	blue				✓		✓	✓			✓	✓	✓	✓					✓	✓
0.22-0.24	blue					✓									✓	✓	✓			
0.24-0.26	dark blue				✓	✓														
0.28-0.30	blue				✓		✓	✓			✓	✓	✓	✓		✓	✓	✓	✓	✓
0.38-0.40	blue				✓			✓												
0.42-0.43	green										✓	✓			✓				✓	
0.45-0.46	blue														✓	✓		✓		
0.47-0.50	dark blue				✓	✓	✓	✓							✓	✓		✓		✓
0.52-0.54	blue									✓										
0.56-0.58	green										✓	✓	✓	✓	✓	✓	✓	✓	✓	
0.58-0.59	quenching										✓	✓	✓	✓	✓		✓	✓	✓	
0.63-0.65	light blue							✓			✓	✓			✓		✓	✓	✓	
0.66-0.67	light blue							✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
0.68-0.69	red														✓	✓	✓			
0.69-0.70	red										✓	✓			✓	✓	✓			
0.71-0.72	yellow														✓	✓	✓			
0.79-0.80	red															✓	✓			
0.87-0.88	red										✓	✓			✓	✓	✓			
0.90-0.92	blue				✓		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.96-0.98	blue								✓				✓	✓	✓				✓	✓

Samples: (1) Mo-Ha-Rak, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 32 Rf value (System II) of MHR compared with herbal components and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm.

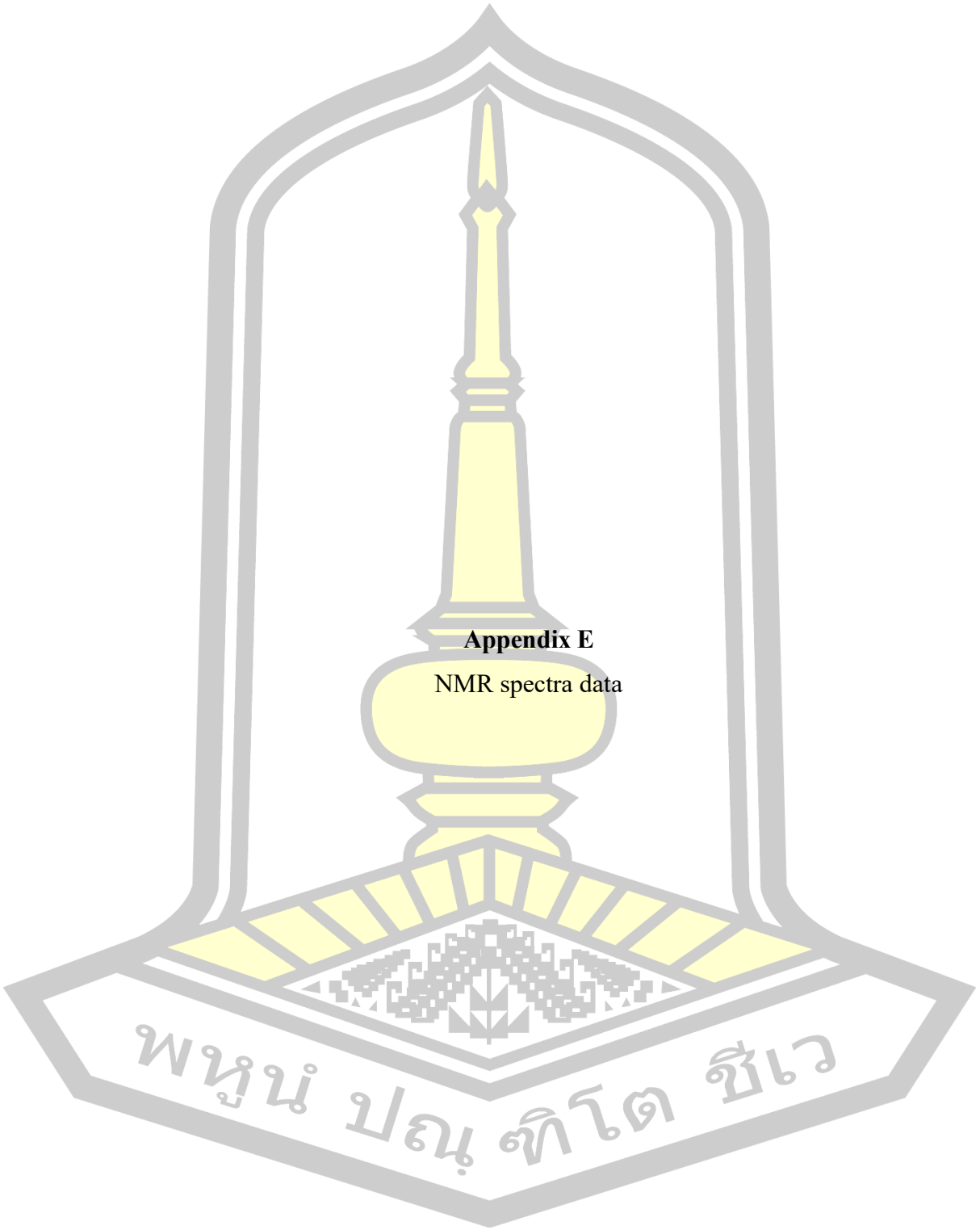
Rf	Samples (detection with anisaldehyde-sulphuric acid under UV 366 nm)																		
	1	S1	S2	S3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.04-0.05	blue				✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓
0.08-0.13	orange					✓	✓	✓	✓	✓	✓	✓			✓		✓	✓	✓
0.13-0.15	dark blue																		✓
0.20-0.22	blue					✓	✓		✓						✓				
0.25-0.27	blue							✓		✓				✓	✓		✓	✓	
0.28-0.31	orange												✓	✓					
0.35-0.37	blue					✓									✓				✓
0.43-0.45	blue					✓													✓
0.55-0.57	dark blue																		✓
0.58-0.60	orange							✓											
0.61-0.62	green				✓		✓			✓	✓			✓	✓		✓	✓	✓
0.63-0.65	blue					✓						✓	✓	✓				✓	
0.66-0.67	red												✓						
0.68-0.69	red												✓						
0.69-0.71	blue								✓			✓	✓	✓	✓		✓	✓	
0.73-0.74	red									✓	✓	✓				✓	✓		
0.76-0.77	red							✓			✓					✓			
0.81-0.83	light purple	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓
0.81-0.83	light purple		✓							✓		✓	✓		✓		✓		
0.89-0.91	blue					✓		✓			✓	✓		✓	✓				
0.96-0.98	blue						✓		✓		✓		✓			✓	✓		

Samples: (1) Mo-Ha-Rak, (2) *C. micracantha* (ชิงชี่), (3) *C. indicum* (เท้ายายม่อม), (4) *F. racemosa* (มะเดื่อชุมพร), (5) *H. perforata* (คนทา), (6) *T. triandra* (ย่านาง), (7) *A. indica* (สะเดา), (8) *G. chinense* (กระดอม), (9) *T. crispa* (บอระเพ็ด), (10) *D. cochinchinensis* (จันทน์แดง), (11) *T. hoaensis* (จันทน์ขาว), (12) *L. sinense* (โกฐหัวบัว), (13) *P. kesiya* (สน), (14) *B. ovata* (มะกา), (15) *C. fistula* (คูน), (16) *T. bellirica* (สมอพิเภก), (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

Table 33 Rf value (System II) of MHR compared with herbal components, modified MHR and standard chemicals, detected with anisaldehyde-sulphuric acid under UV 366 nm

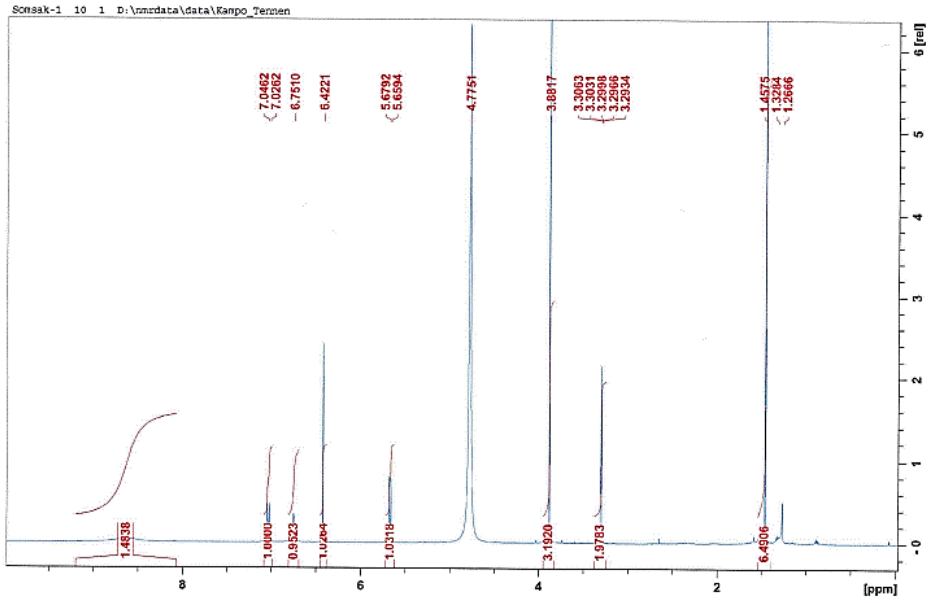
Rf	Samples (detection with anisaldehyde-sulphuric acid under UV 366 nm)																			
	1	S1	S2	S3	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0.04-0.05	blue				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.08-0.13	orange				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.13-0.15	dark blue				✓	✓	✓								✓	✓		✓		
0.18-0.20	blue				✓		✓	✓			✓	✓	✓	✓					✓	✓
0.22-0.24	blue					✓									✓	✓	✓			
0.24-0.26	dark blue				✓	✓														
0.28-0.30	blue				✓		✓	✓			✓	✓	✓	✓		✓	✓	✓	✓	✓
0.38-0.40	blue				✓			✓												
0.42-0.43	green										✓	✓			✓				✓	
0.45-0.46	blue														✓	✓		✓		
0.47-0.50	dark blue				✓	✓	✓	✓							✓	✓		✓		✓
0.52-0.54	blue									✓										
0.56-0.58	green										✓	✓	✓	✓	✓	✓	✓	✓	✓	
0.58-0.59	quenching										✓	✓	✓	✓	✓		✓	✓	✓	
0.63-0.65	light blue							✓			✓	✓			✓		✓	✓	✓	
0.66-0.67	light blue							✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
0.68-0.69	red										✓	✓			✓	✓	✓			
0.69-0.70	red										✓	✓			✓	✓	✓			
0.71-0.72	yellow										✓	✓			✓	✓	✓			
0.78-0.80	light purple	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.82-0.84	purple				✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.85-0.87	light purple		✓					✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0.90-0.92	blue				✓		✓		✓	✓	✓	✓		✓	✓		✓	✓	✓	✓
0.96-0.98	blue								✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Samples: (1) Mo-Ha-Rak, (17) *T. chebula* (สมอไทย), (18) *Terminalia* sp. (สมอเทศ), (19) *P. emblica* (มะขามป้อม), (20) *M. ferrea* (บุนนาค), (21) *N. nucifera* (บัวหลวง), (22) *V. zizanioides* (แฝกหอม), (23) 02E, (24) 31E, (25) 32E, (26) 33E, (27) 03E, (28) 91E, (29) 12E, (30) 14E, (31) 10E, (32) 92E, (S1) β-sitosterol, (S2) lupeol, (S3) stigmasterol.

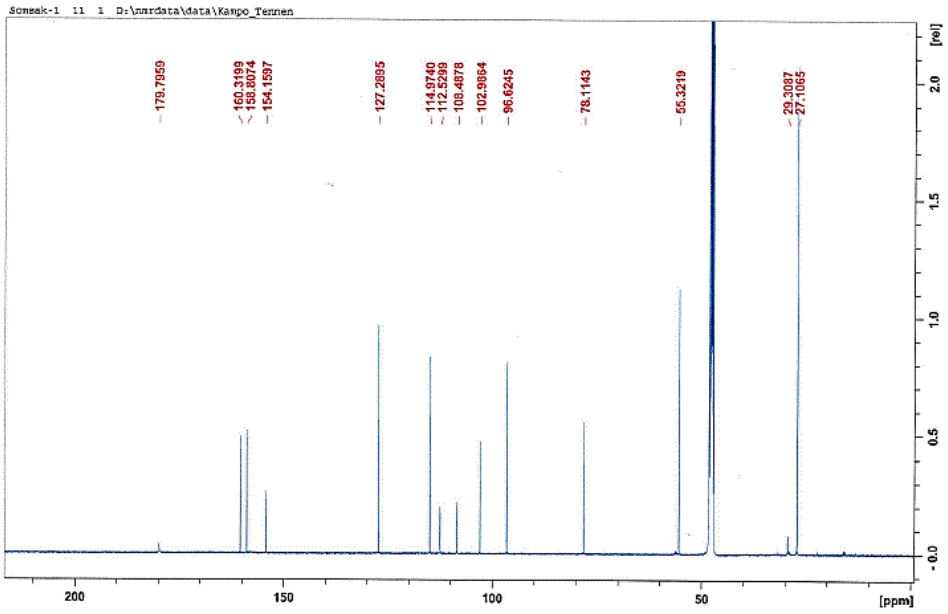


Appendix E

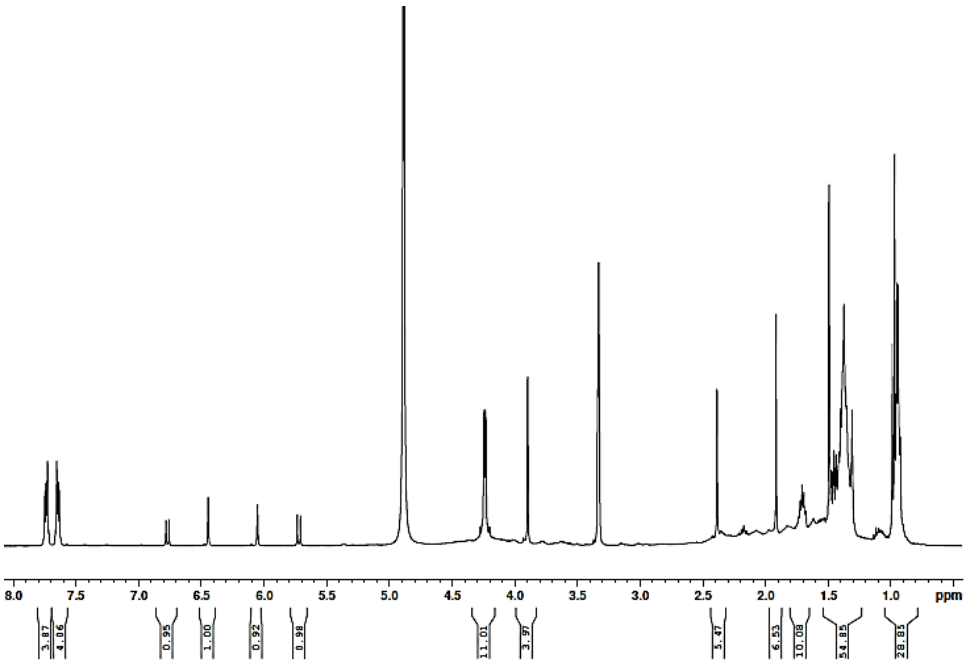
NMR spectra data



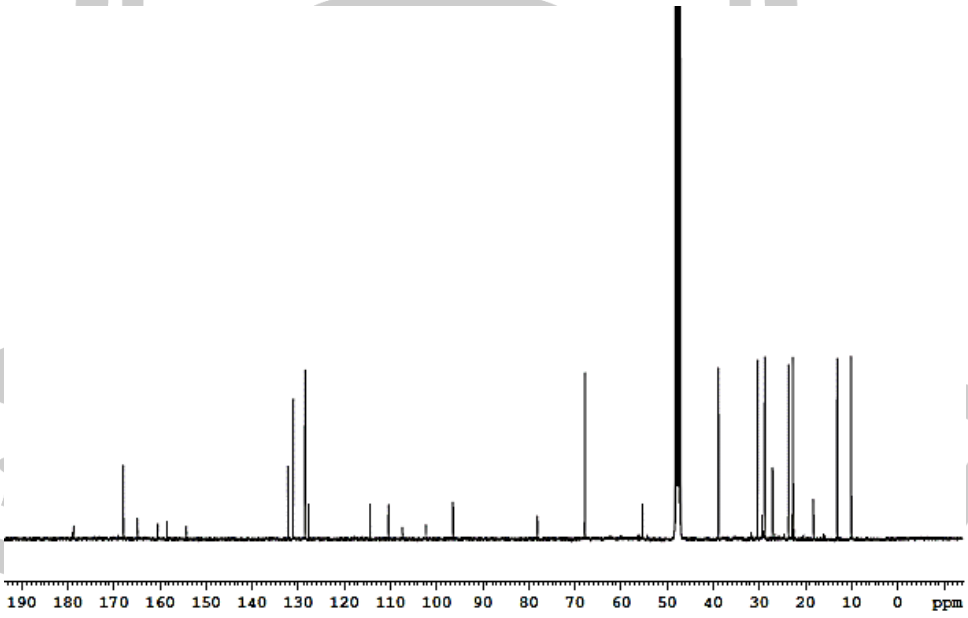
^{1}H NMR (400 MHz) spectrum of perforatic acid (CD_3OD).



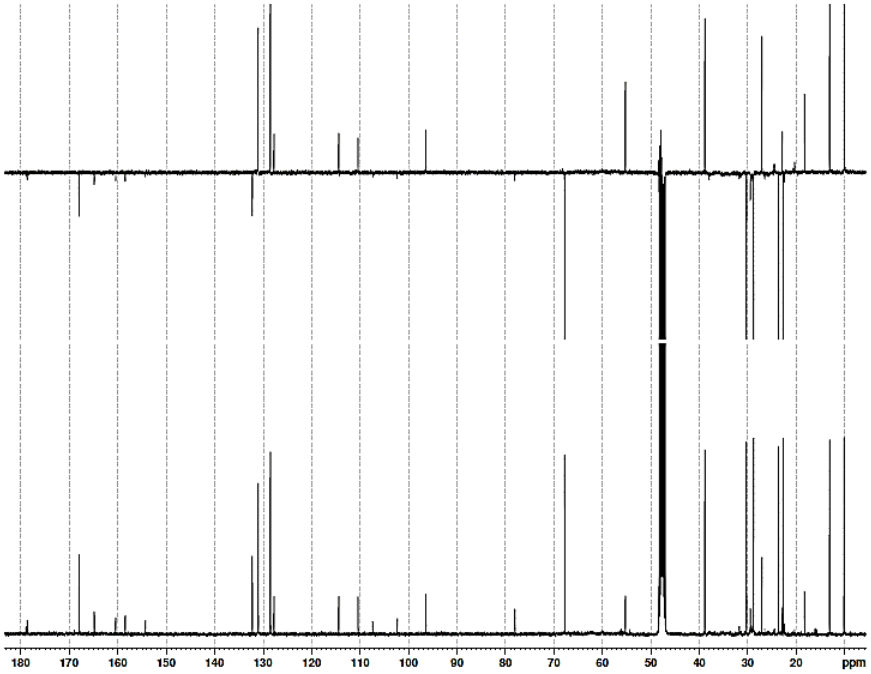
^{13}C NMR (400 MHz) spectrum of perforatic acid (CD_3OD).



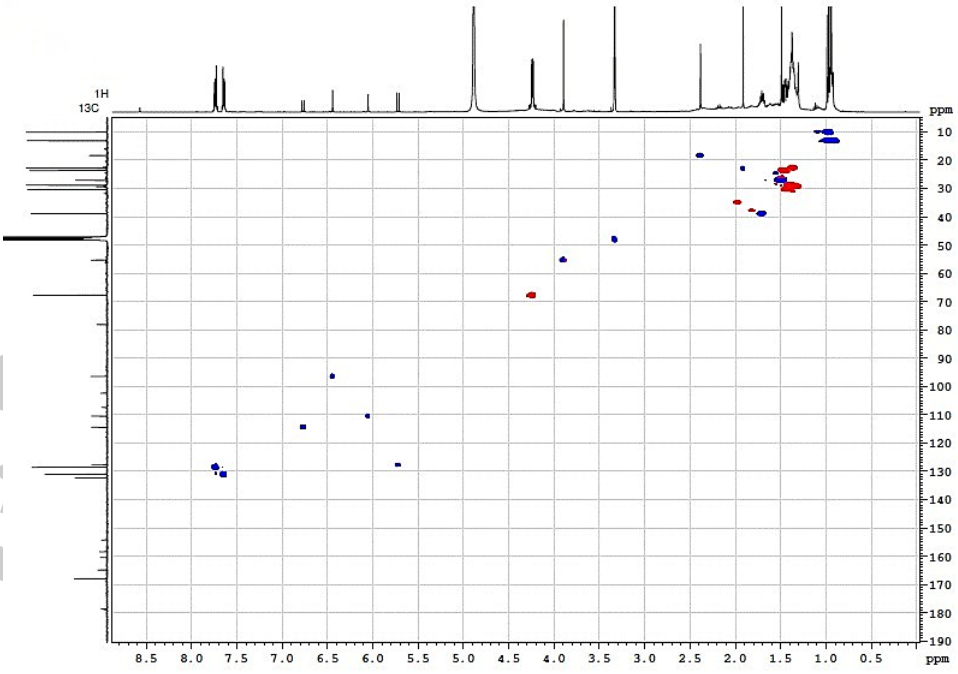
^{1}H NMR (400 MHz) spectrum of *O*-methyllaloptaeroxyrin (CD_3OD).



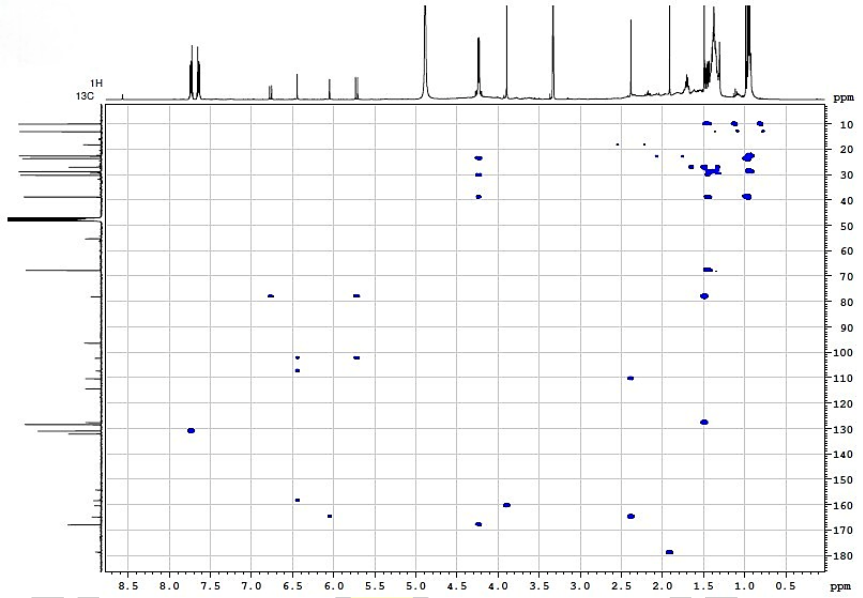
^{13}C NMR (100 MHz) spectrum of *O*-methyllaloptaeroxyrin (CD_3OD).



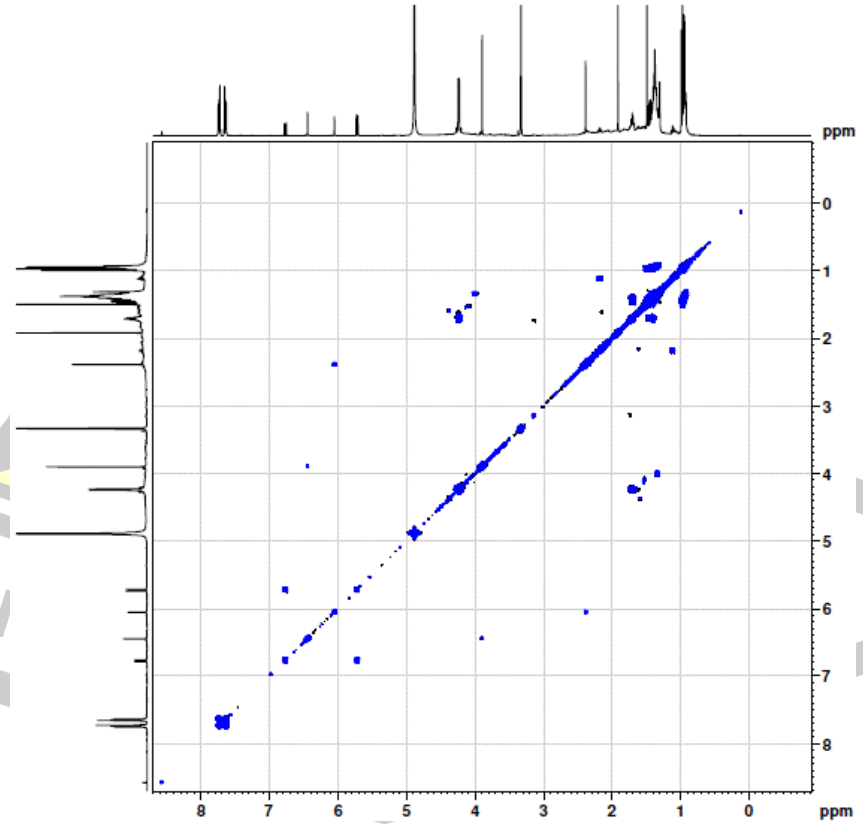
DEPTQ-135 (100 MHz) spectrum of *O*-methyllaloptaeroxyrin (CD_3OD).



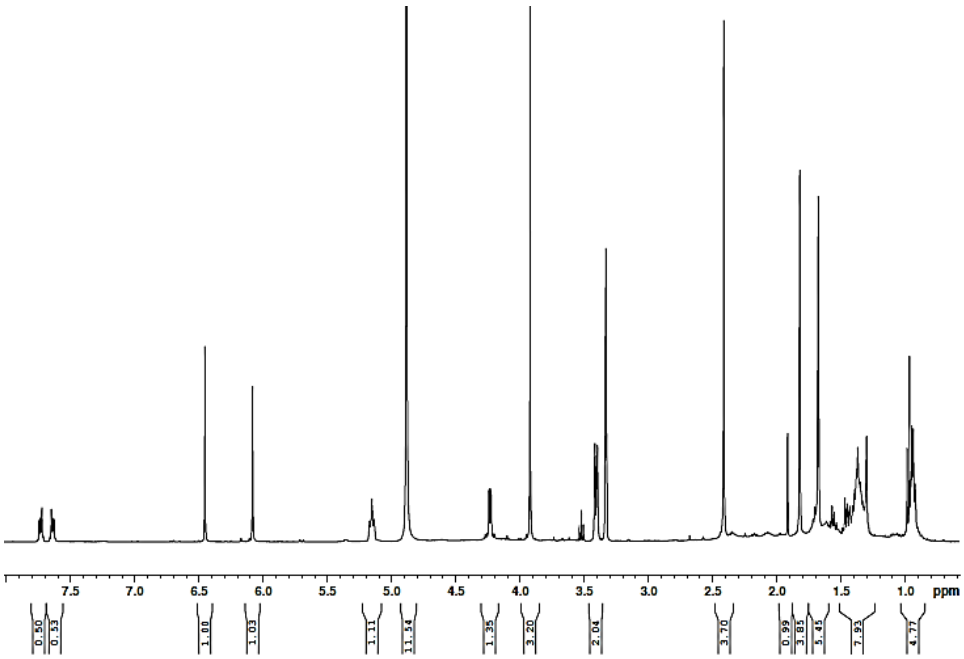
HSQC spectrum of *O*-methyllaloptaeroxyrin (CD_3OD).



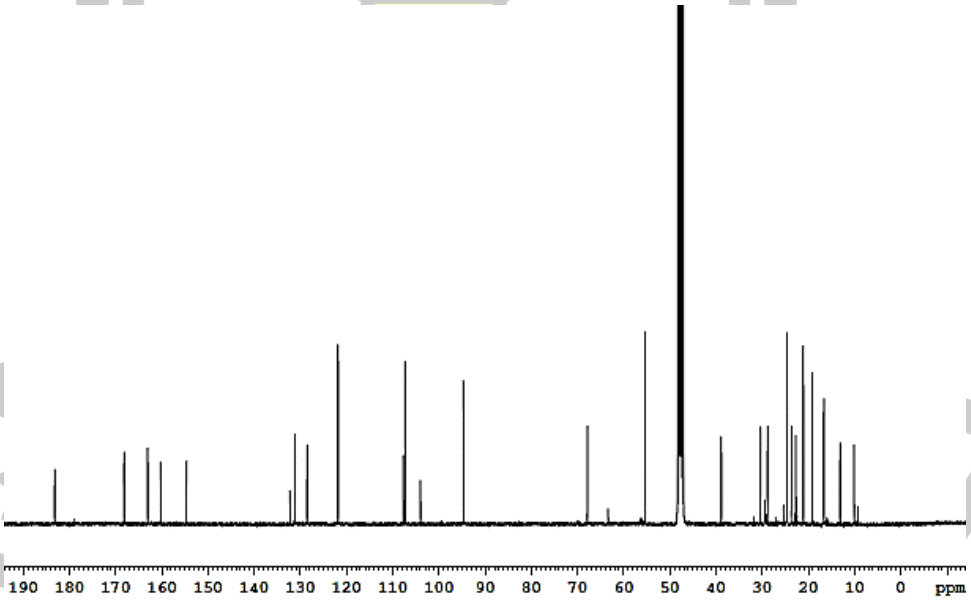
HMBC spectrum of *O*-methyllaloptaeroxyrin (CD_3OD).



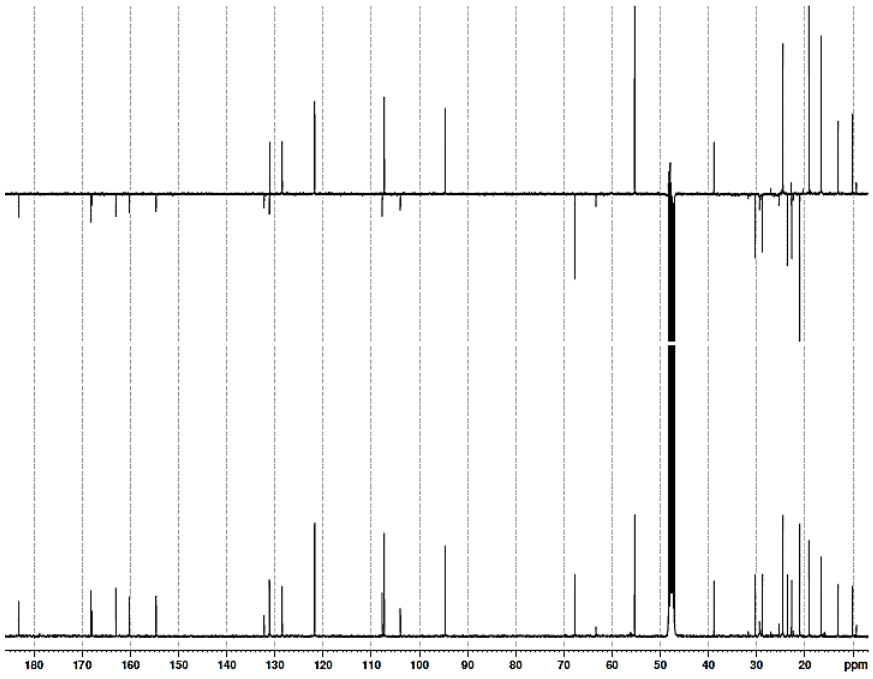
COSY (400 MHz) spectrum of *O*-methyllaloptaeroxyrin (CD_3OD).



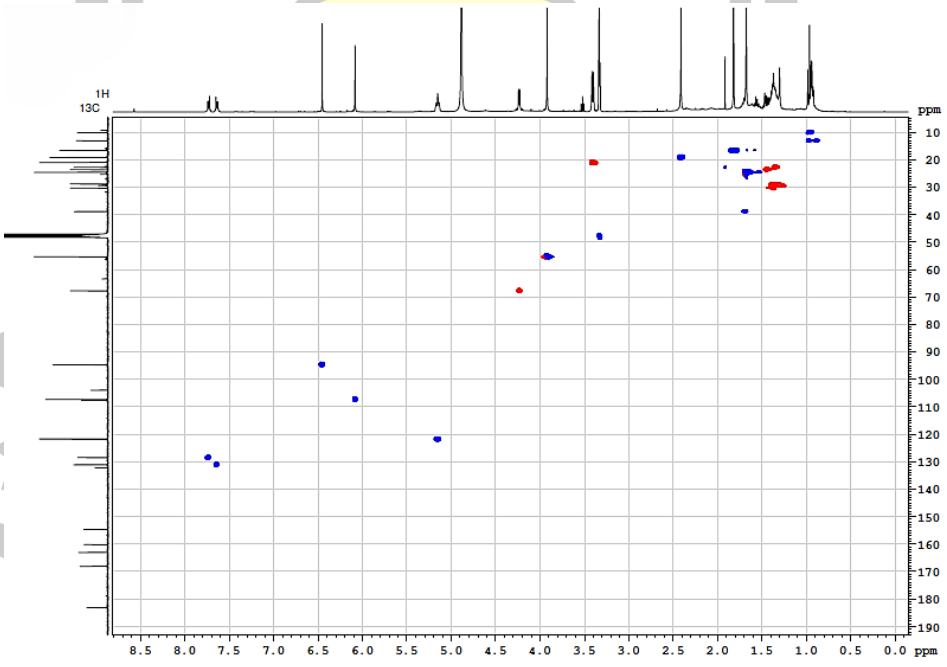
^{1}H NMR (400 MHz) spectrum of peucenin-7-methyl ether (CD_3OD).



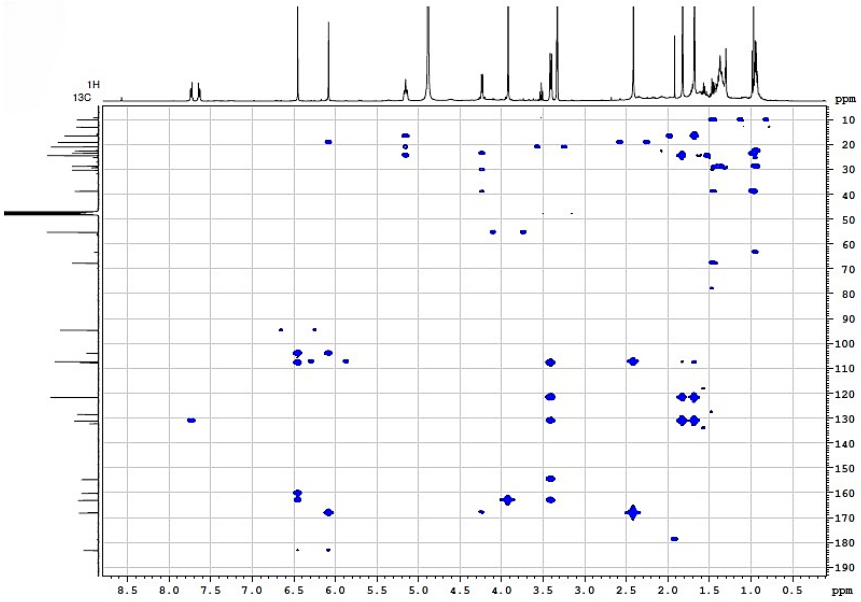
^{13}C NMR (100 MHz) spectrum of peucenin-7-methyl ether (CD_3OD).



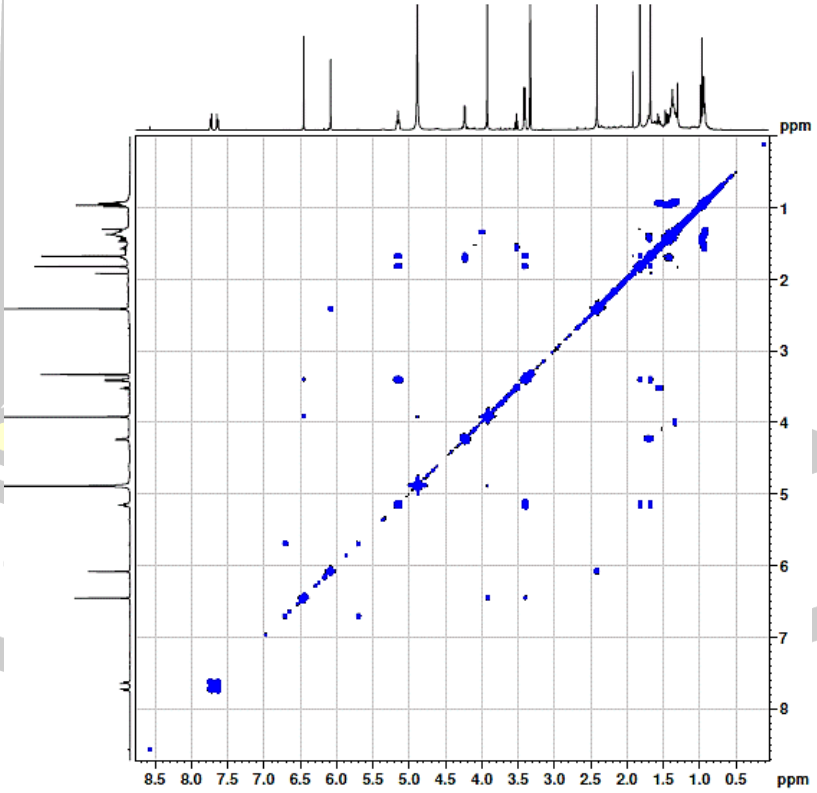
DEPTQ-135 (100 MHz) spectrum of peucenin-7-methyl ether (CD_3OD).



HSQC spectrum of peucenin-7-methyl ether (CD_3OD).



HMBC spectrum of peucenin-7-methyl ether (CD_3OD).



COSY (400 MHz) spectrum of peucenin-7-methyl ether (CD_3OD).

BIOGRAPHY

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Research output	Research publication Chaloemram C. (2020). Principles of using medicinal plants in Withikutdharok scripture. Journal of Thai Traditional and Alternative Medicine, 18(1), 147-165.

Chaloemram C, Sedlak S. (2020). Folk medicine recorded in Plam leaf manuscripts of Isan: A case study of skin disorders. Journal of Science and Technology Mahasarakham University, 39(4), 457-471.

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Nammatra R, Chaloemram C, Chanhan P. (2021). Phytochemical contents and antioxidant activities of Thai herbal tea from leaves of Morus alba and Citrus hystrix. Journal of Science and Agricultural Technology, 2(1), 21-27.

Nammatra R, Srihawong T, Chaloemram C. (2021). Evaluation of phytochemical constituents and antioxidant activities of different formula of heart tonic herbal teas. Journal of Sustainability Science and Management, 16(2), 94-104.

Oral presentation

Chinnaphat Chaloemram, Ruchilak Rattarom, Somsak Nualkaew. Efficacy of green banana-supplemented diet for management of persistent diarrhea in children: a systematic review and meta-analysis of randomized controlled trials. The Third Graduate Student Conference in Health Science Research, Mahasarakham University, 5 April 2022, Maha Sarakham, Thailand.

Chinnaphat Chaloemram, Ruchilak Rattarom, Somsak Nualkaew. Phytochemistry and in-vitro evaluation of α-glucosidase inhibitory, anti-inflammatory and antioxidant activities of Gymnopetalum chinensis fruits. 7th International Symposium on Phytochemicals in Medicine and Food, 2-7 August 2023, Beijing, China.

Chinnaphat Chaloemram, Ruchilak Rattarom, Somsak Nualkaew. Phytochemistry and in-vitro evaluation of α-glucosidase inhibitory, anti-inflammatory and antioxidant activities of Gymnopetalum chinensis fruits. The Fourth Graduate Student Conference in Health Science Research, Mahasarakham University, 24 August 2023, Maha Sarakham, Thailand.

Chinnaphat Chaloemram, Ruchilak Rattarom, Somsak Nualkaew. Anti-inflammatory activity and toxicity of Thai traditional polyherbal medicine, Mor-Ha-Rak and its group components. International conference on medical plants and natural drug research, 5-7 June 2024, Al-Farabi Kazakh National University, Almaty, Kazakhstan.