

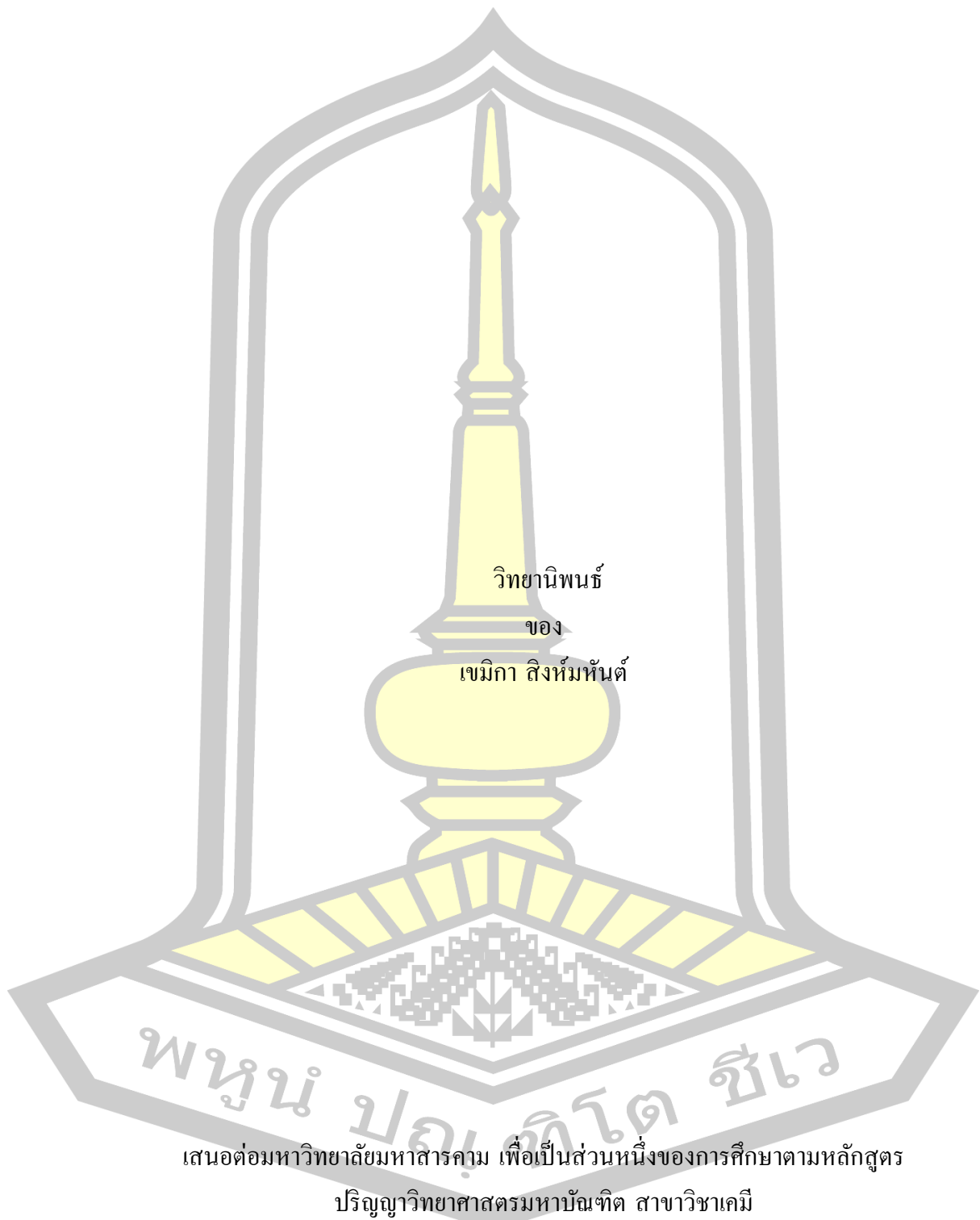
Chemical Constituents from Natural Fruiting Bodies of *Fulvifomes fastuosus* (Lév.)
Bondartseva & S. Herrera

Khemika Singmahan

A Thesis Submitted in Partial Fulfillment of Requirements for
degree of Master of Science in Chemistry
September 2025

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สารองค์ประกอบทางเคมีของดอกเห็ดธรรมชาติ *Fulvifomes fastuosus*



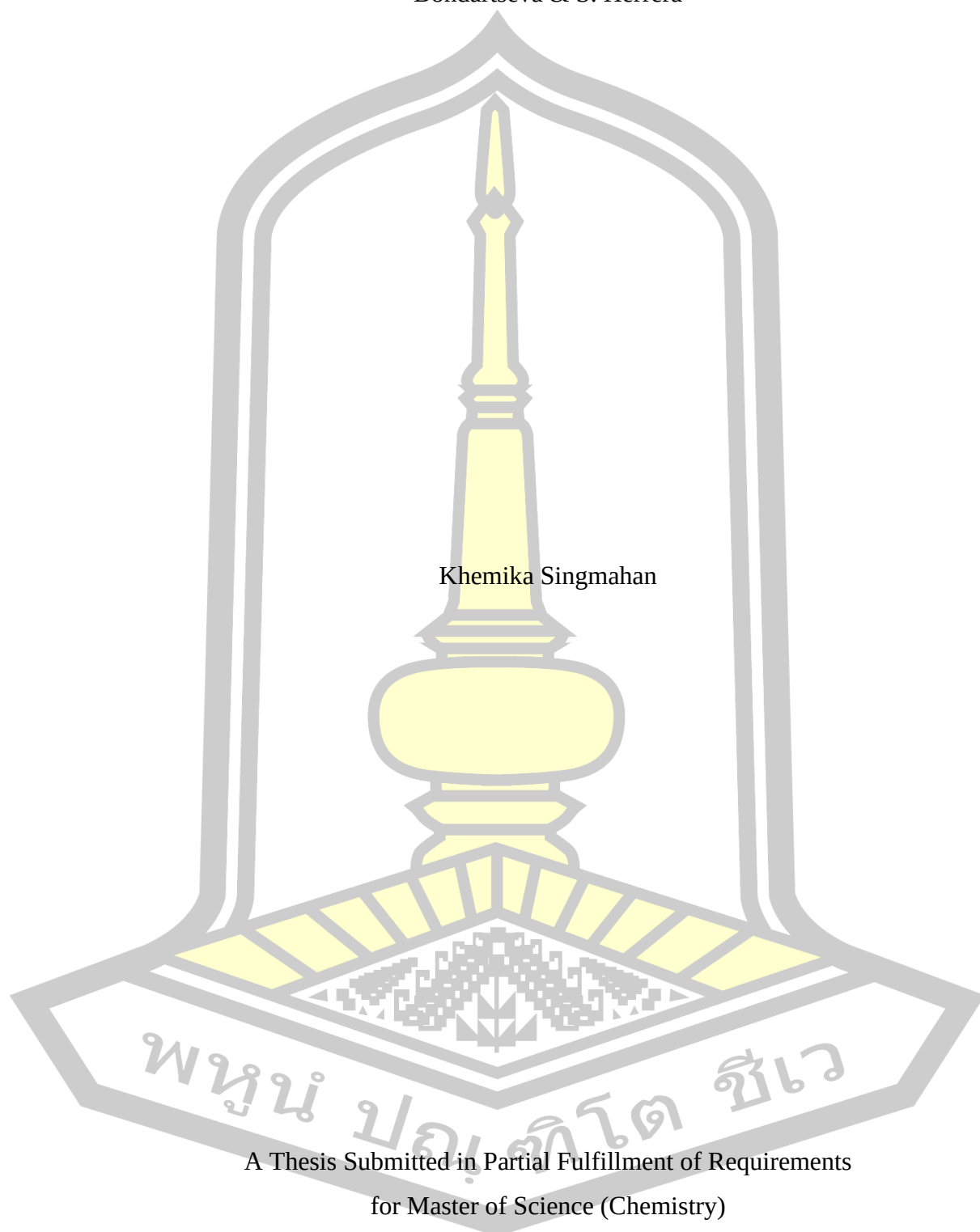
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for Master of Science (Chemistry)

September 2025

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The examining committee has unanimously approved this Thesis, submitted by Miss Khemika Singmahan , as a partial fulfillment of the requirements for the Master of Science Chemistry at Mahasarakham University

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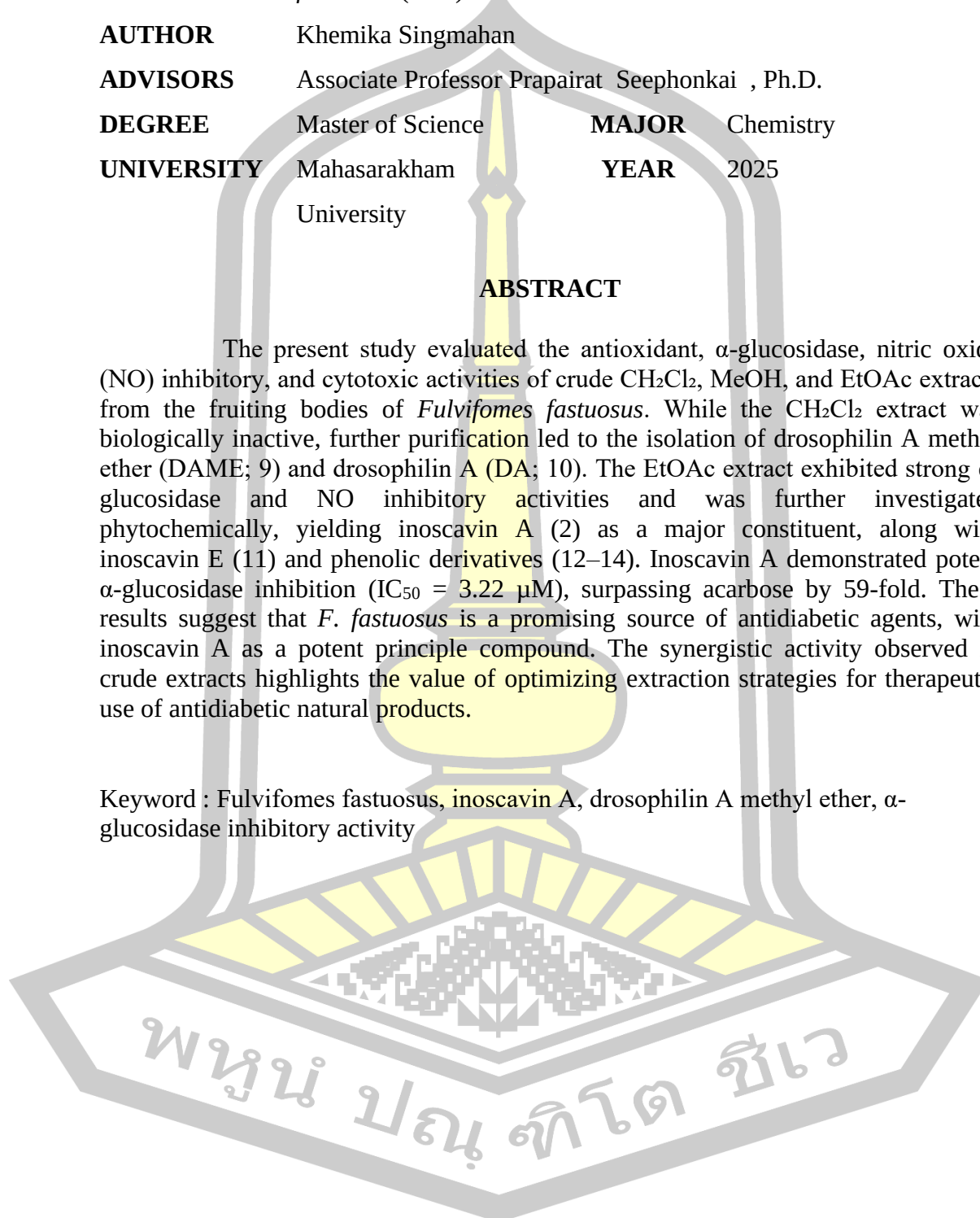
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ABSTRACT

The present study evaluated the antioxidant, α -glucosidase, nitric oxide (NO) inhibitory, and cytotoxic activities of crude CH_2Cl_2 , MeOH, and EtOAc extracts from the fruiting bodies of *Fulvifomes fastuosus*. While the CH_2Cl_2 extract was biologically inactive, further purification led to the isolation of drosophilin A methyl ether (DAME; 9) and drosophilin A (DA; 10). The EtOAc extract exhibited strong α -glucosidase and NO inhibitory activities and was further investigated phytochemically, yielding inoscavin A (2) as a major constituent, along with inoscavin E (11) and phenolic derivatives (12–14). Inoscavin A demonstrated potent α -glucosidase inhibition ($\text{IC}_{50} = 3.22 \mu\text{M}$), surpassing acarbose by 59-fold. These results suggest that *F. fastuosus* is a promising source of antidiabetic agents, with inoscavin A as a potent principle compound. The synergistic activity observed in crude extracts highlights the value of optimizing extraction strategies for therapeutic use of antidiabetic natural products.

Keyword : *Fulvifomes fastuosus*, inoscavin A, drosophilin A methyl ether, α -glucosidase inhibitory activity



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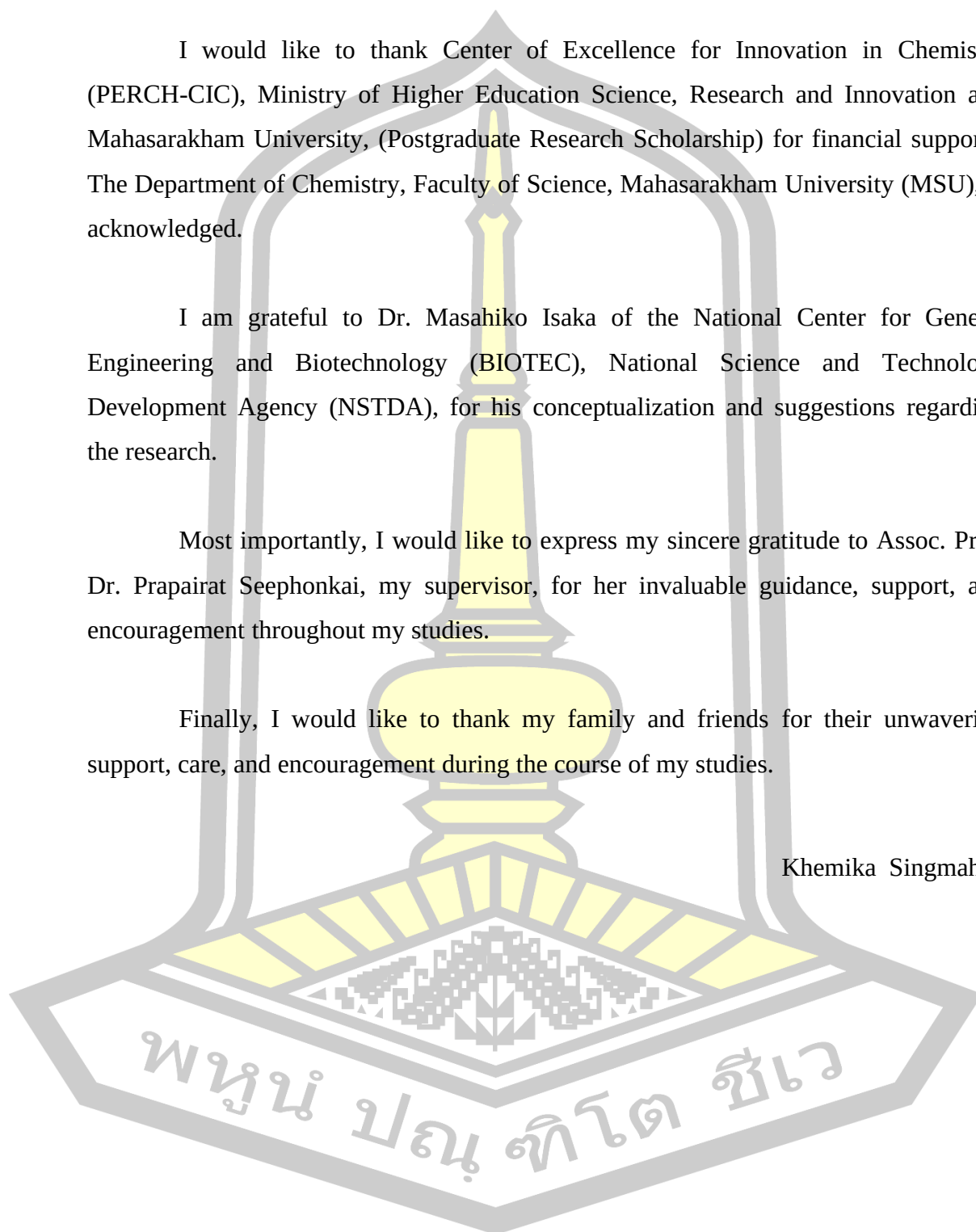
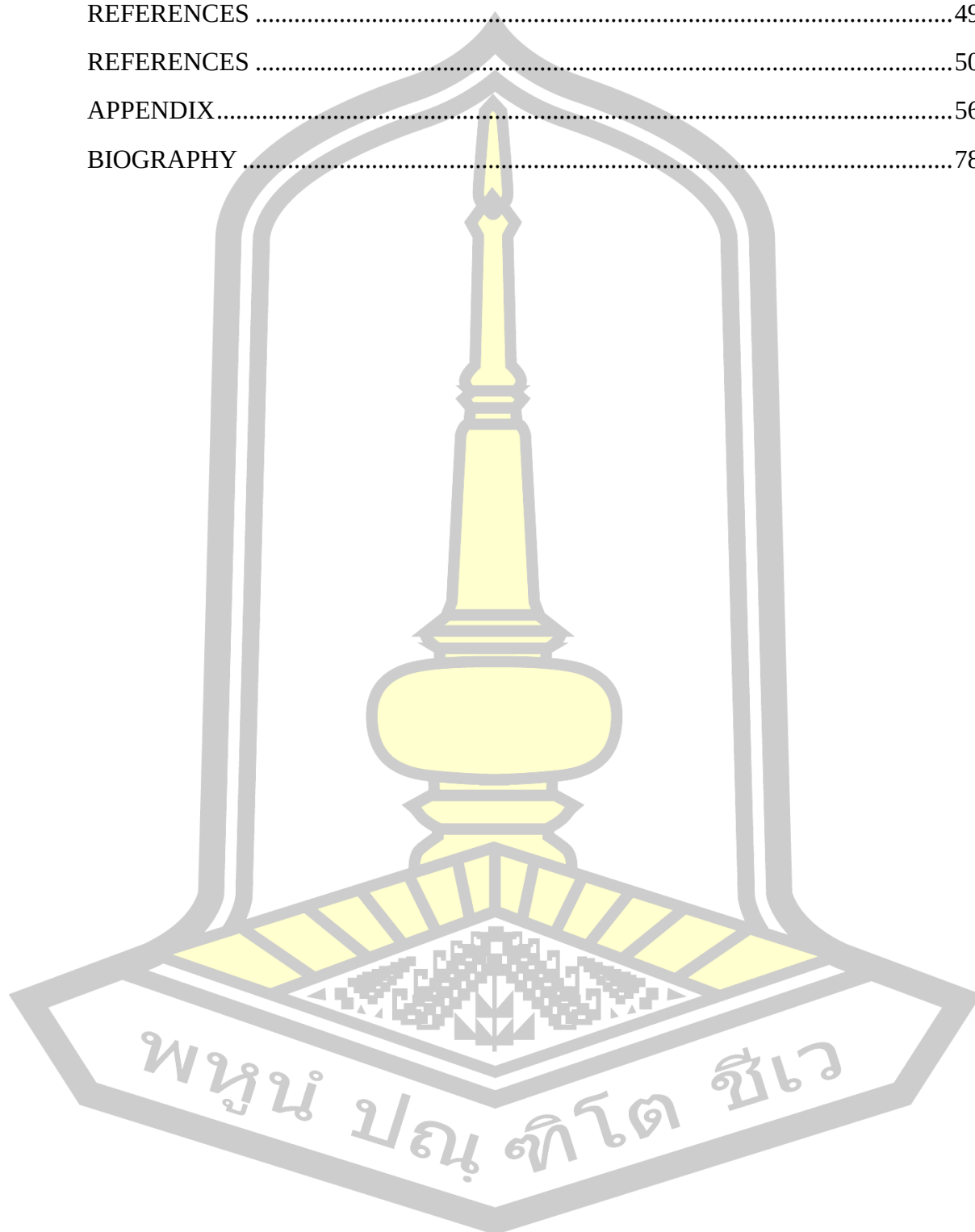


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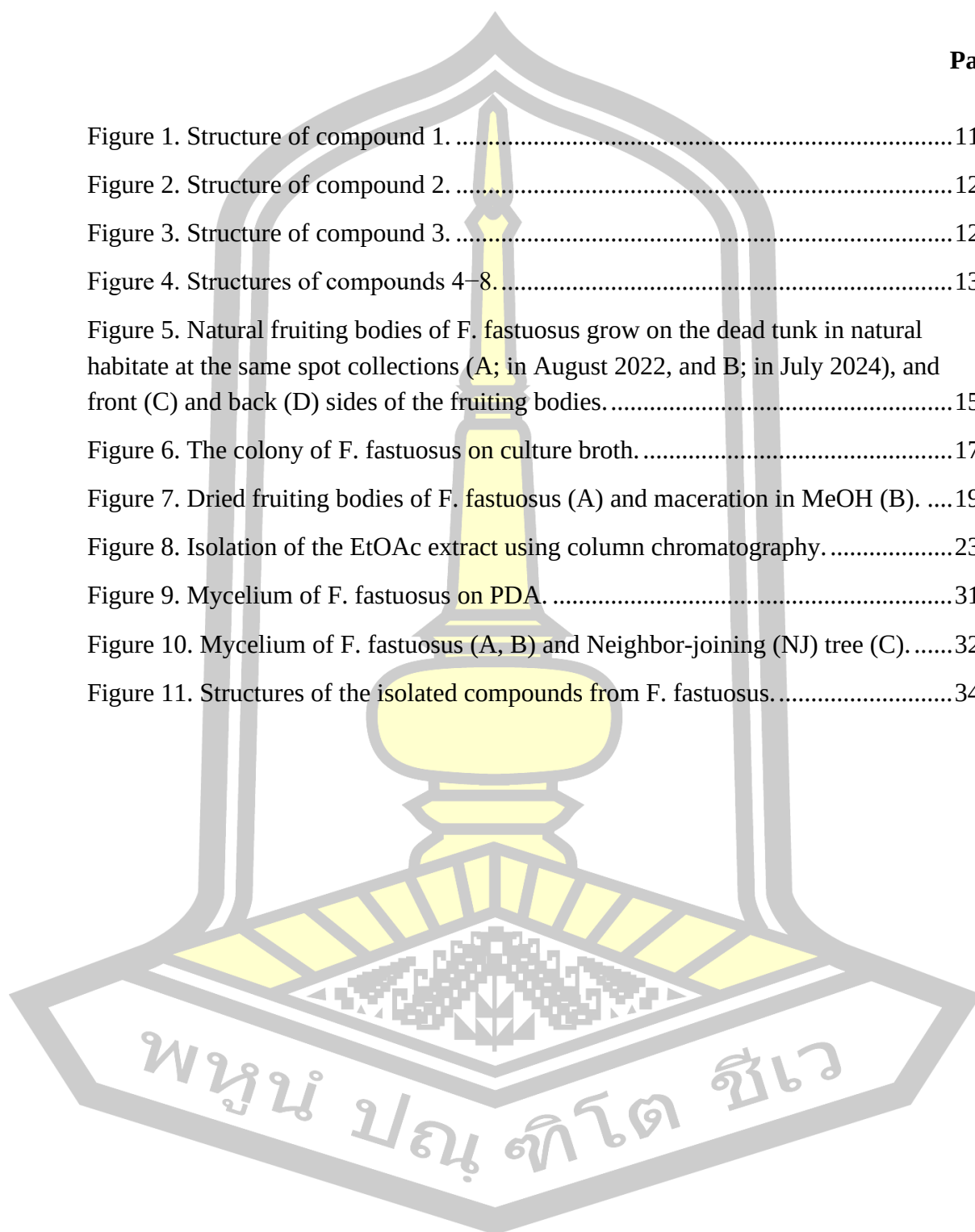


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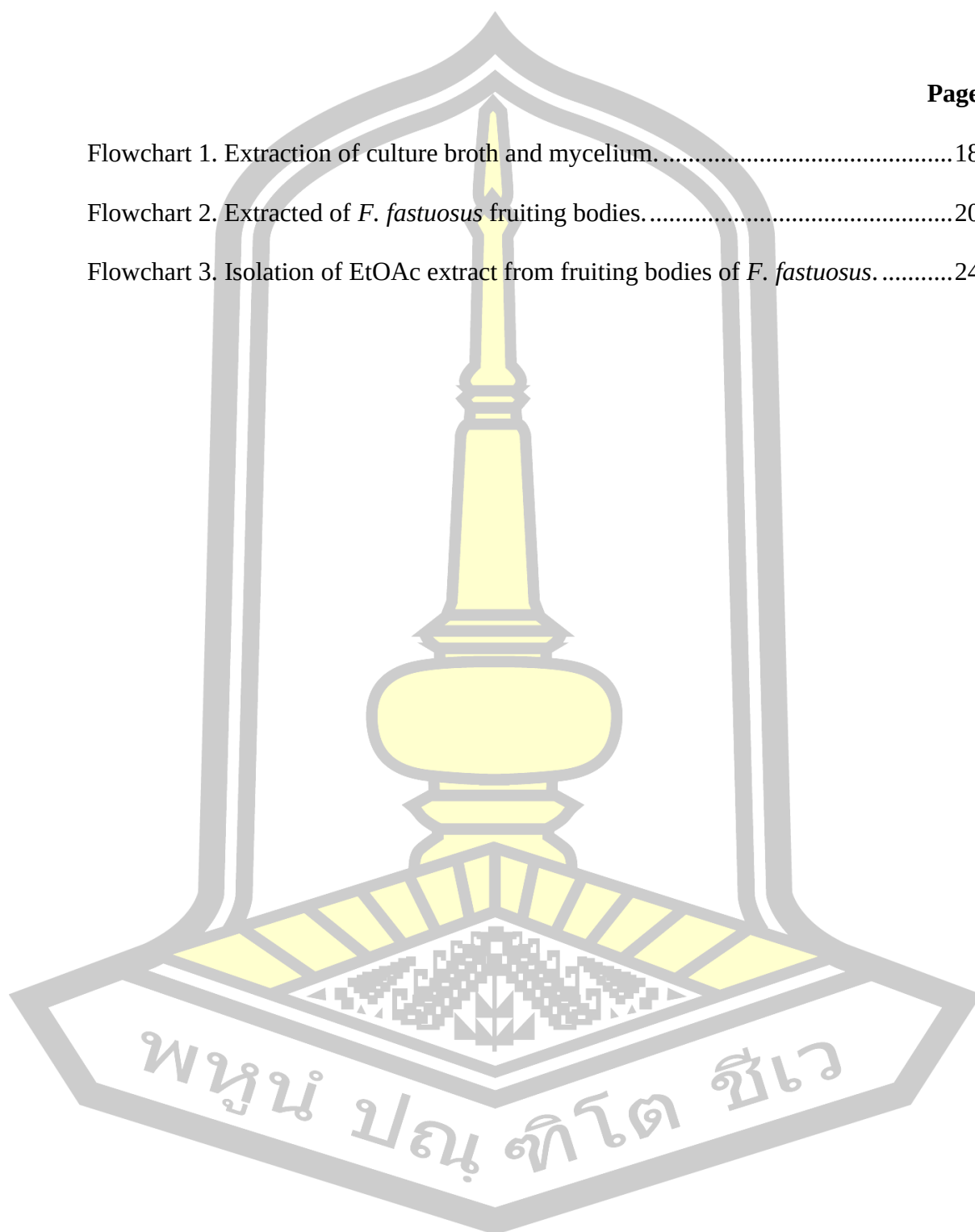
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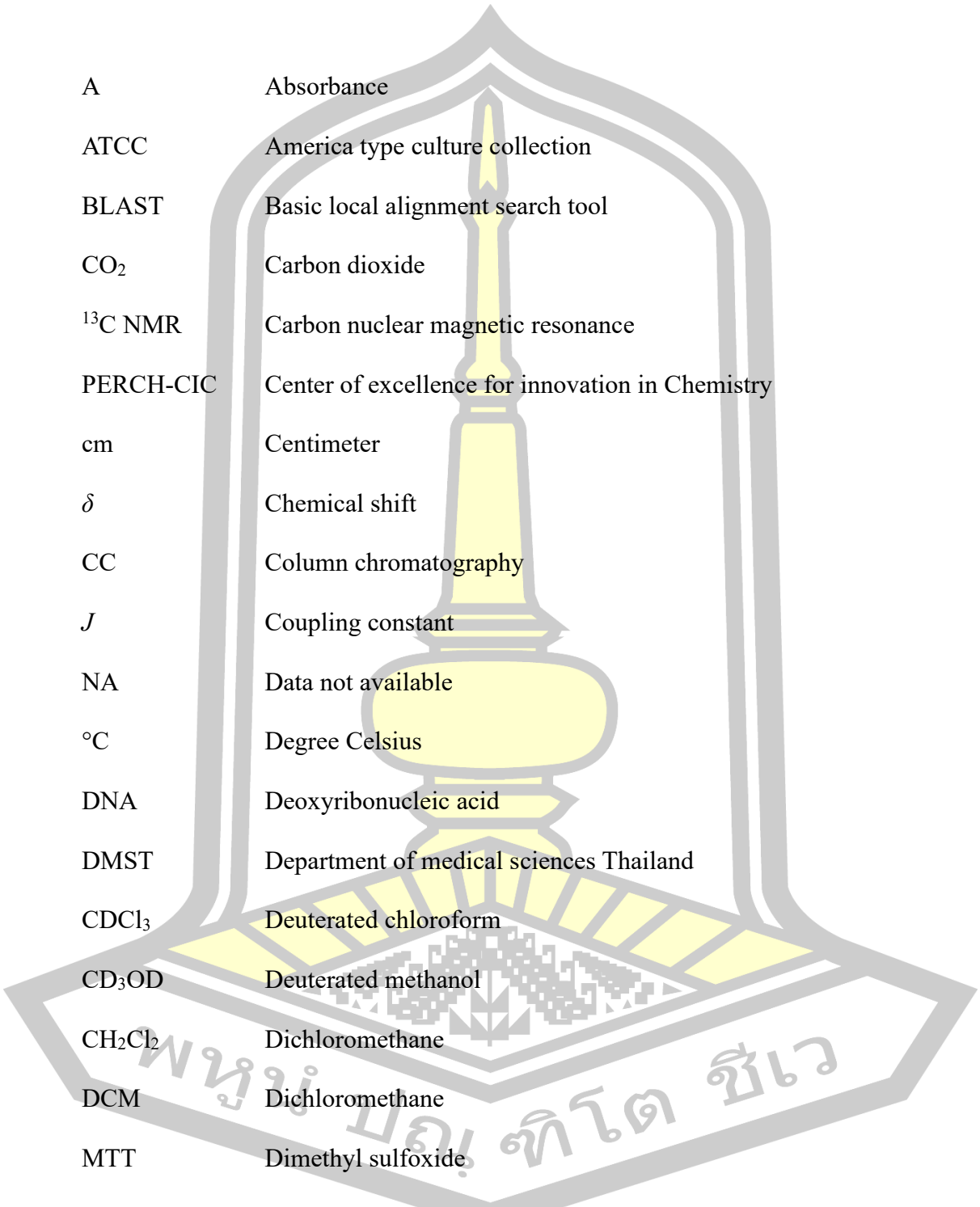


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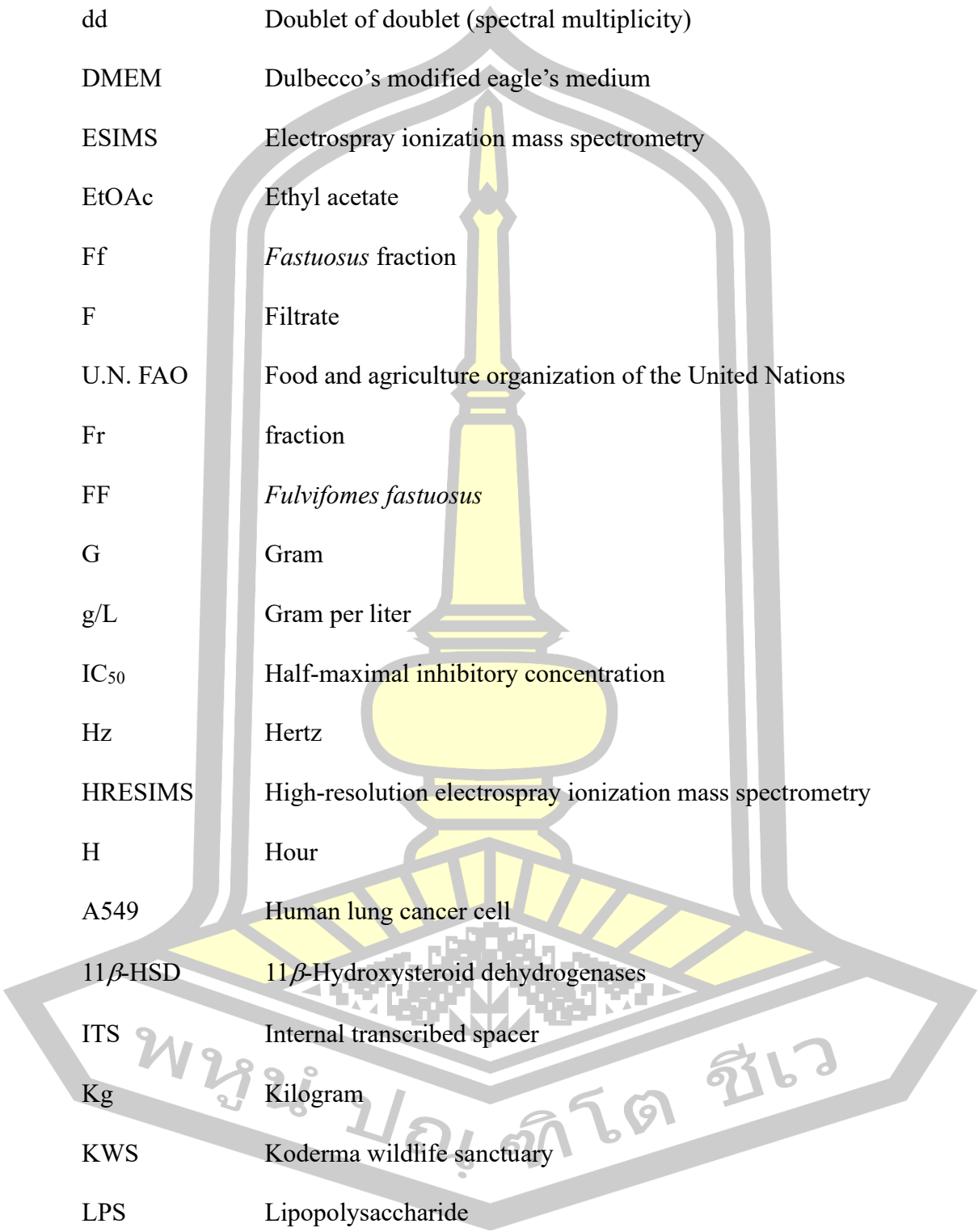
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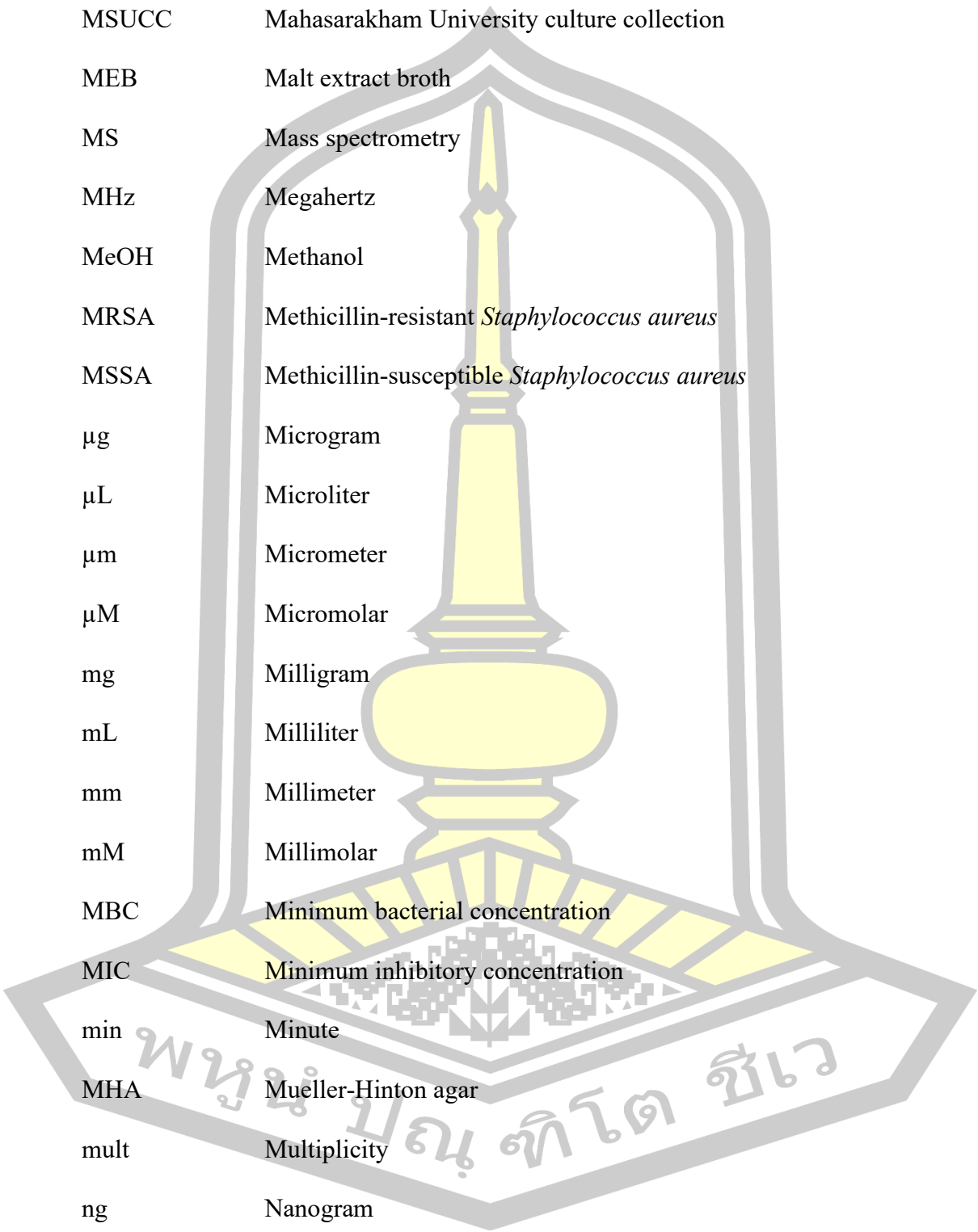
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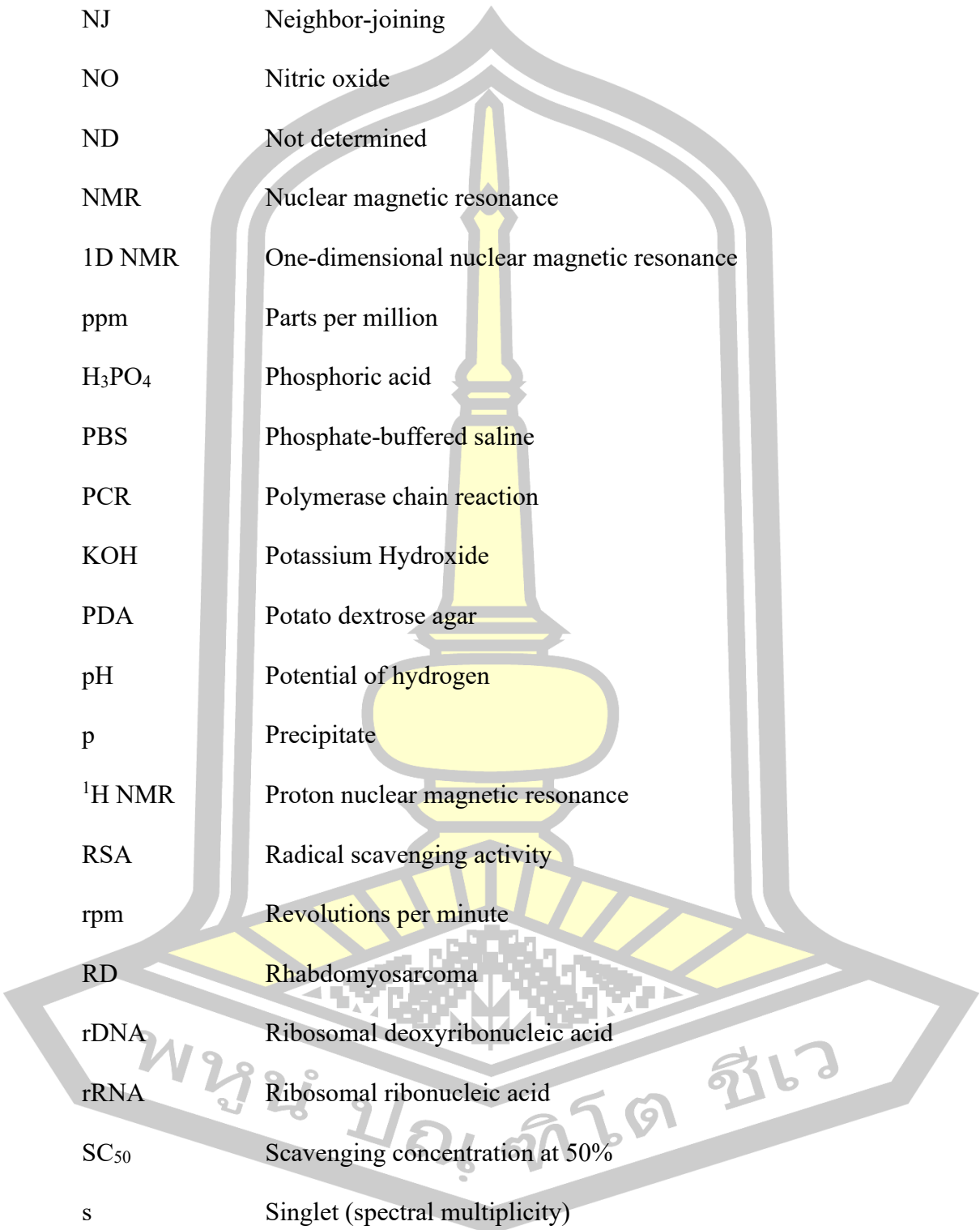
A	Absorbance
ATCC	America type culture collection
BLAST	Basic local alignment search tool
CO ₂	Carbon dioxide
¹³ C NMR	Carbon nuclear magnetic resonance
PERCH-CIC	Center of excellence for innovation in Chemistry
cm	Centimeter
δ	Chemical shift
CC	Column chromatography
<i>J</i>	Coupling constant
NA	Data not available
°C	Degree Celsius
DNA	Deoxyribonucleic acid
DMST	Department of medical sciences Thailand
CDCl ₃	Deuterated chloroform
CD ₃ OD	Deuterated methanol
CH ₂ Cl ₂	Dichloromethane
DCM	Dichloromethane
MTT	Dimethyl sulfoxide
DPPH	3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
DMSO	2,2-Diphenyl-1-picrylhydrazyl radical



d	Doublet (spectral multiplicity)
dd	Doublet of doublet (spectral multiplicity)
DMEM	Dulbecco's modified eagle's medium
ESIMS	Electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
Ff	<i>Fastuosus</i> fraction
F	Filtrate
U.N. FAO	Food and agriculture organization of the United Nations
Fr	fraction
FF	<i>Fulviformes fastuosus</i>
G	Gram
g/L	Gram per liter
IC ₅₀	Half-maximal inhibitory concentration
Hz	Hertz
HRESIMS	High-resolution electrospray ionization mass spectrometry
H	Hour
A549	Human lung cancer cell
11 β -HSD	11 β -Hydroxysteroid dehydrogenases
ITS	Internal transcribed spacer
Kg	Kilogram
KWS	Koderma wildlife sanctuary
LPS	Lipopolysaccharide
L	Liter

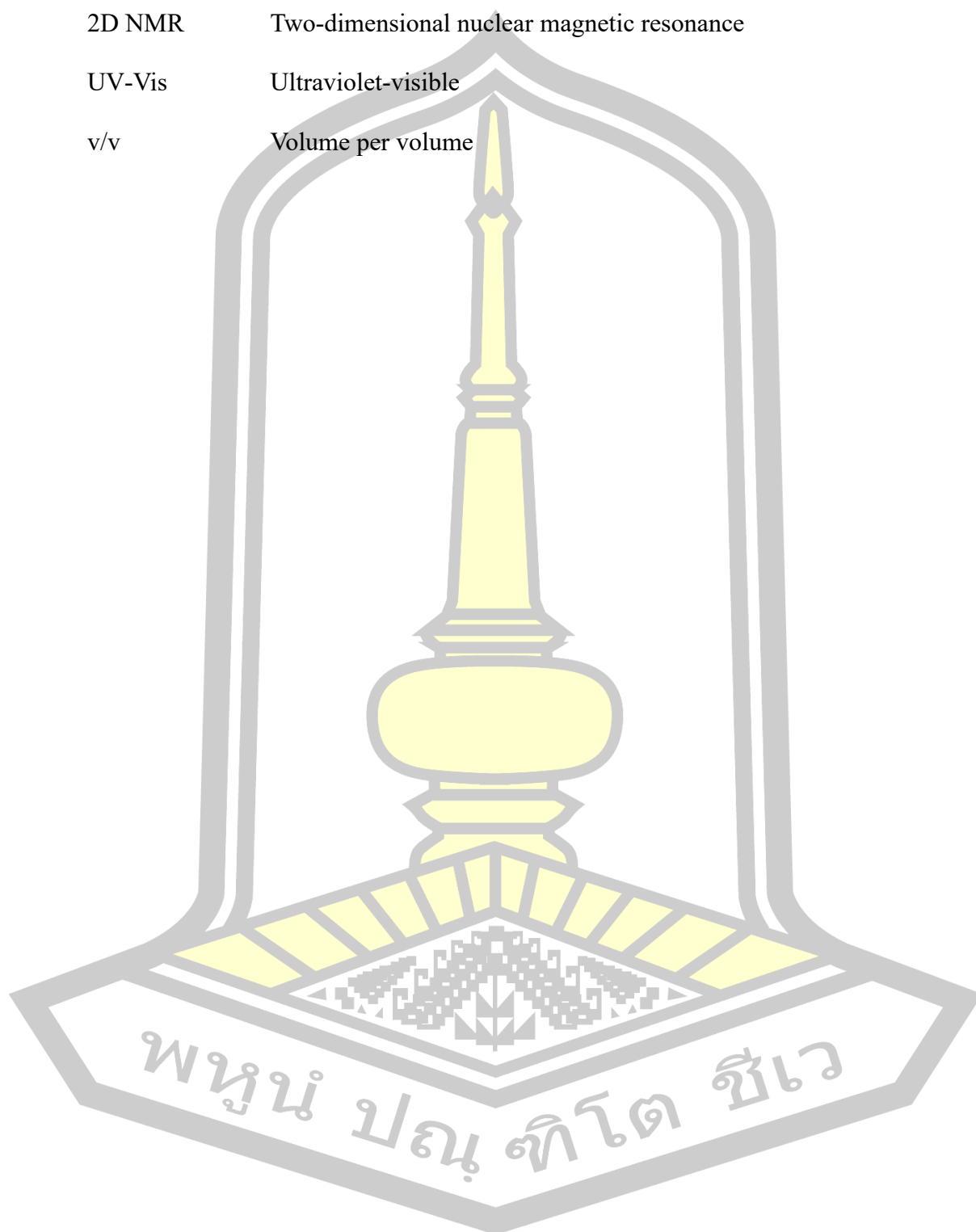


RAW 264.7	Macrophage-like cells
MSUCC	Maharakham University culture collection
MEB	Malt extract broth
MS	Mass spectrometry
MHz	Megahertz
MeOH	Methanol
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-susceptible <i>Staphylococcus aureus</i>
μg	Microgram
μL	Microliter
μm	Micrometer
μM	Micromolar
mg	Milligram
mL	Milliliter
mm	Millimeter
mM	Millimolar
MBC	Minimum bacterial concentration
MIC	Minimum inhibitory concentration
min	Minute
MHA	Mueller-Hinton agar
mult	Multiplicity
ng	Nanogram
nM	Nanometer



NCBI	National center for biotechnology information
NJ	Neighbor-joining
NO	Nitric oxide
ND	Not determined
NMR	Nuclear magnetic resonance
1D NMR	One-dimensional nuclear magnetic resonance
ppm	Parts per million
H ₃ PO ₄	Phosphoric acid
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
KOH	Potassium Hydroxide
PDA	Potato dextrose agar
pH	Potential of hydrogen
p	Precipitate
¹ H NMR	Proton nuclear magnetic resonance
RSA	Radical scavenging activity
rpm	Revolutions per minute
RD	Rhabdomyosarcoma
rDNA	Ribosomal deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
SC ₅₀	Scavenging concentration at 50%
s	Singlet (spectral multiplicity)
SD	Standard Deviation

TLC	Thin-layer chromatography
2D NMR	Two-dimensional nuclear magnetic resonance
UV-Vis	Ultraviolet-visible
v/v	Volume per volume



CHAPTER 1

INTRODUCTION

1.1. Background

Thailand is one of the most biodiverse countries in Southeast Asia and benefits greatly from its ecosystems, landscapes and habitats that support a rich variety of flora and fauna. According to the United Nations Food and Agriculture Organization (U.N. FAO), approximately 37% of Thailand is land area about 18,972,000 ha is covered by forested (Butler RA., 2000).

Kingdom Fungi, one of the most diverse and ancient branches of the tree of life, includes an estimated 2–5 million species that play vital roles in terrestrial and aquatic ecosystems (Heitman J et al., 2017). While fungi share some similarities with plants but are unable to do photosynthesis. Fungi are eukaryotic organisms; their cells contain membrane-bound organelles and clearly defined nuclei. Historically, fungi were included in the plant kingdom; however, because fungi lack chlorophyll and are distinguished by unique structural and physiological features, such as components of the cell wall and cell membrane, they have been separated from plants. In addition, fungi are clearly distinguished from all other living organisms (Wu F et al., 2014). There are two major forms of fungal growth: yeast-like cells and mycelium growth. Dimorphic fungi can switch between the yeast-like and mycelium form in response to the changes in environmental conditions (Wendland J., 2001). The Spores begin to germinate in a moist, nutrient-rich environment. A germ tubes are formed that extend into filamentous cells called hyphae. Hyphae are the major building block of fungi and can absorb and transport nutrients from the environment. The hyphae growing

and branching and forms a cross-linked structure called mycelium (Meyer V et al., 2020).

Mushrooms, particularly species from the genera *Inonotus* and *Phellinus*, are valued not only as functional foods but also as rich sources of bioactive compounds with therapeutic potential. These fungi produce diverse secondary metabolites, including yellow antioxidant pigments rich in hispidin and styrylpyranone derivatives, as well as polyphenols (Lee I et al., 2011 and He P et al., 2021).

Fulvifomes Murrill was established by Murrill in 1914 and typified with *F. robiniae* Murrill to accommodate species with perennial and sessile basidiocarps, sulcate surface, ungulate or applanate pilei and smooth, ferruginous or fulvous spores in the family Hymenochaetaceae (Murrill WA., 1914). *Fulvifomes* comprise 109 species according to Index Fungorum (Index Fungorum). *Fulvifomes* was considered as a synonym of *Phellinus* Quél. for several decades. (Ryvarden L and Johansen I., 1980; Gilbertson RL and Ryvarden L., 1987; Ryvarden L and Gilbertson RL., 1993; Núñez M and Ryvarden L., 2000). Until (Wagner T and Fischer M., 2002) provided evidence to confirm *Fulvifomes* as an independent generic rank within Hymenochaetaceae based on molecular phylogenetic analyses.

1.2. The objective of the research

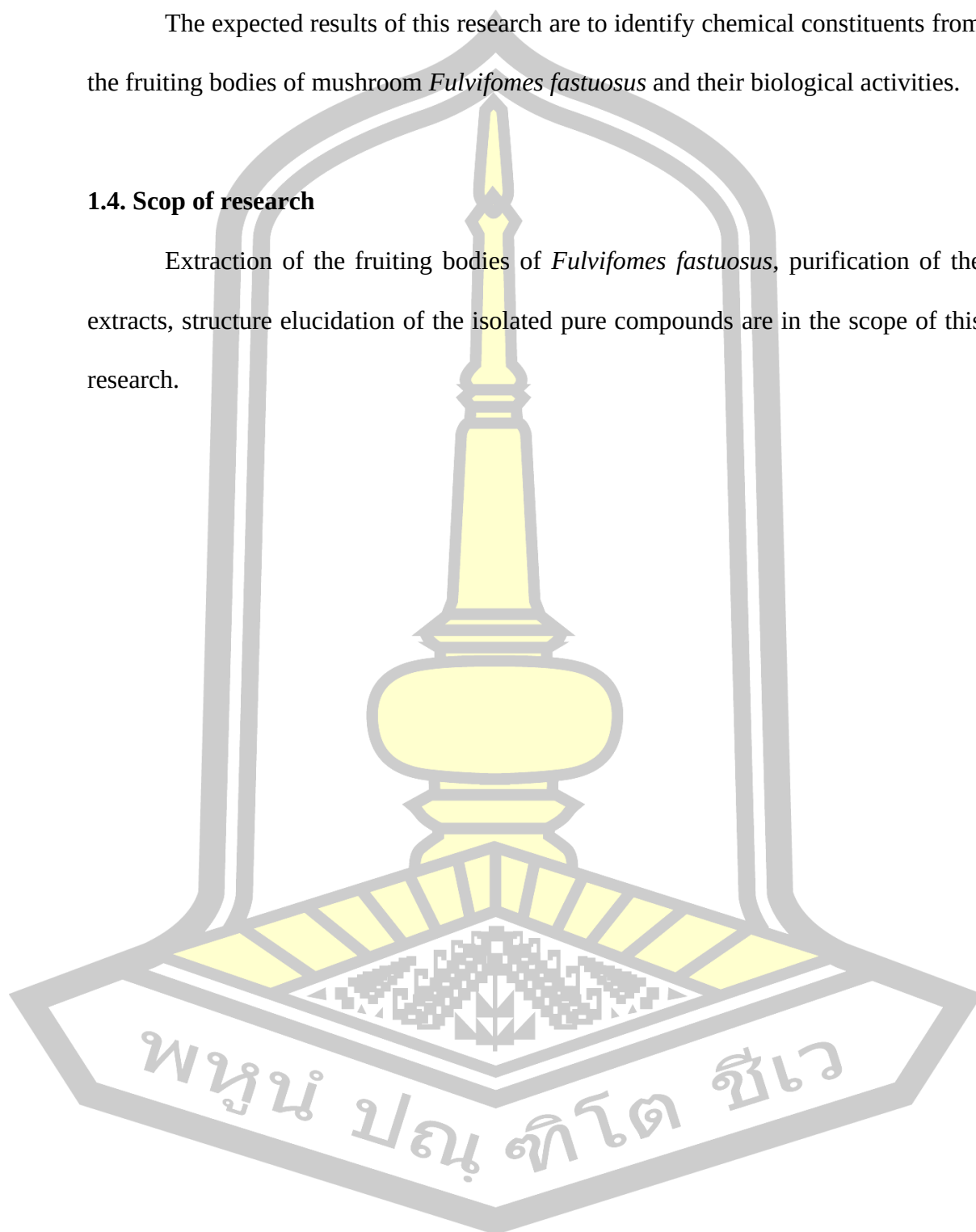
There have been only a few reports on the isolation of bioactive compounds from *Fulvifomes fastuosus*. Therefore, the objectives of this research are to isolate, structure elucidate and identify of compounds from the fruiting bodies of *Fulvifomes fastuosus* collected from northeastern Thailand.

1.3. Expected research

The expected results of this research are to identify chemical constituents from the fruiting bodies of mushroom *Fulvifomes fastuosus* and their biological activities.

1.4. Scop of research

Extraction of the fruiting bodies of *Fulvifomes fastuosus*, purification of the extracts, structure elucidation of the isolated pure compounds are in the scope of this research.



CHAPTER 2

LITERATURE REVIEW

For several decades, *Fulvifomes* was considered a synonym of *Phellinus* Quél. The species formerly known as *Phellinus fastuosus* was reclassified as *Fulvifomes fastuosus* (Ryvarden L and Johansen I., 1980; Gilbertson RL and Ryvarden L., 1987; Ryvarden L and Gilbertson RL., 1993; Núñez M and Ryvarden L., 2000). From literature survey, there are a few reports on the isolation of bioactive compounds from the genus *Fulvifomes*. Here is a summary of compounds isolated from *Fulvifomes* spp. found in sciencedirect and pubmed databases since the year 2000.

2.1. *Phellinus fastuosus* (*Fulvifomes fastuosus*)

Phellinus Quél is one of the largest genera of Hymenochaetaceae, which is comprised of about 220 species. Most *Phellinus* macro-fungi are perennial lignicolous mushrooms widely distributed on Earth (Kawashte G et al., 2021). Several species of *Phellinus* have pharmacologically important bioactive metabolites like polysaccharides, phenols, and flavonoids, which are traditionally used to treat different diseases such as anti-inflammatory (Ajith TA and Janardhanan KK., 2001), hepatoprotective (Chang HY et al., 2007), antidiabetic (Hwang HJ et al., 2005), anticancer (Sliva D et al., 2008 and Lu TL et al., 2009), antioxidant (Park BJ et al., 2009), immuno-stimulatory (Xue Q et al., 2010), cytoprotective (Park JM et al., 2015) and anti-microbial activities (Sonawane H et al., 2011). These mushrooms are a natural cocktail of a variety of secondary metabolites responsible for these activities (Bae JS et al., 2005).

2.2. Isolated compounds from *F. fastuosus*

In 2016, Fernando reported the isolation of ergone (**1**) (**Figure 1**) from the fruiting bodies of *Fulvifomes fastuosus* (Fernando D et al., 2016). This mushroom was collected from Sri Lanka, and molecular studies confirmed its identification. Isolated ergone exhibited cytotoxic activity against rhabdomyosarcoma (RD) cell line with 584.01 ± 2.74 ng/mL.

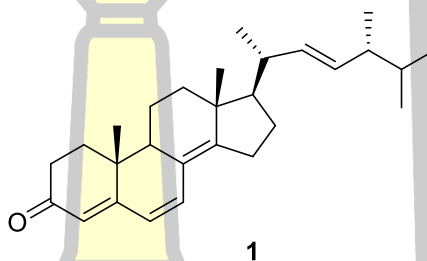


Figure 1. Structure of compound **1**.

2.3. Isolated compounds from *F. fastuosus*

In 2017, Fernando reported the invention relates to a novel method to isolate flavonoid, inoscavin A (**2**) (**Figure 2**), from the terrestrial basidiomycete *F. fastuosus* for the first time (Fernando D et al., 2017). In vitro cytotoxicity experiments demonstrated that the comprising inoscavin A strongly induced the apoptosis of human rhabdomyosarcoma (RD) cells with $IC_{50} = 27.99$ nM compared to the cytotoxicity produced on normal epithelial cells (CC-1) with SI = 6185.18.

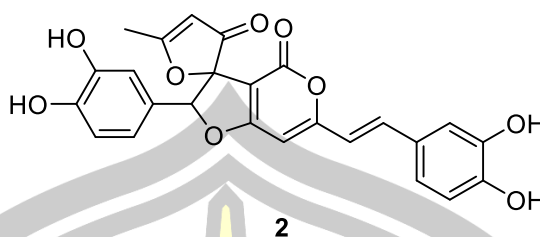


Figure 2. Structure of compound 2.

2.4. Isolated compounds from *F. kanehirae*

In 2018, Ding reported the isolation of a new guaiane sesquiterpene (**3**) (Figure 3) from the cultures of the fungus *F. kanehirae* (Ding J et al., 2019). This compound showed no significant activity against isozymes of 11 β -hydroxysteroid dehydrogenases (11 β -HSD) in the studied.

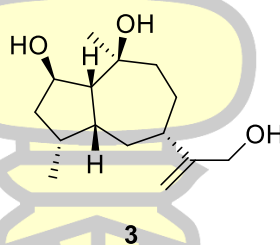


Figure 3. Structure of compound 3.

2.5. Isolated compounds from *F. xylocarpicola*

In 2021, Isaka reported the isolation of three undescribed limonoids, fulvifomins A–C (**4–6**), and two known compounds, 6-deoxydetigloyl-swietenine acetate (**7**) and methyl angolensate (**8**) (Figure 4) from natural fruiting body of *F. xylocarpicola* (Isaka M et al., 2021). The antimalarial and antitubercular activities of these compounds were evaluated. Fulvifomin B (**5**) exhibited moderate antimalarial

and antitubercular activities with half-maximal inhibitory concentration (IC_{50}) volume of 9.1 $\mu\text{g/mL}$ and minimum inhibitory concentration (MIC) volume of 12.5 $\mu\text{g/mL}$ respectively.

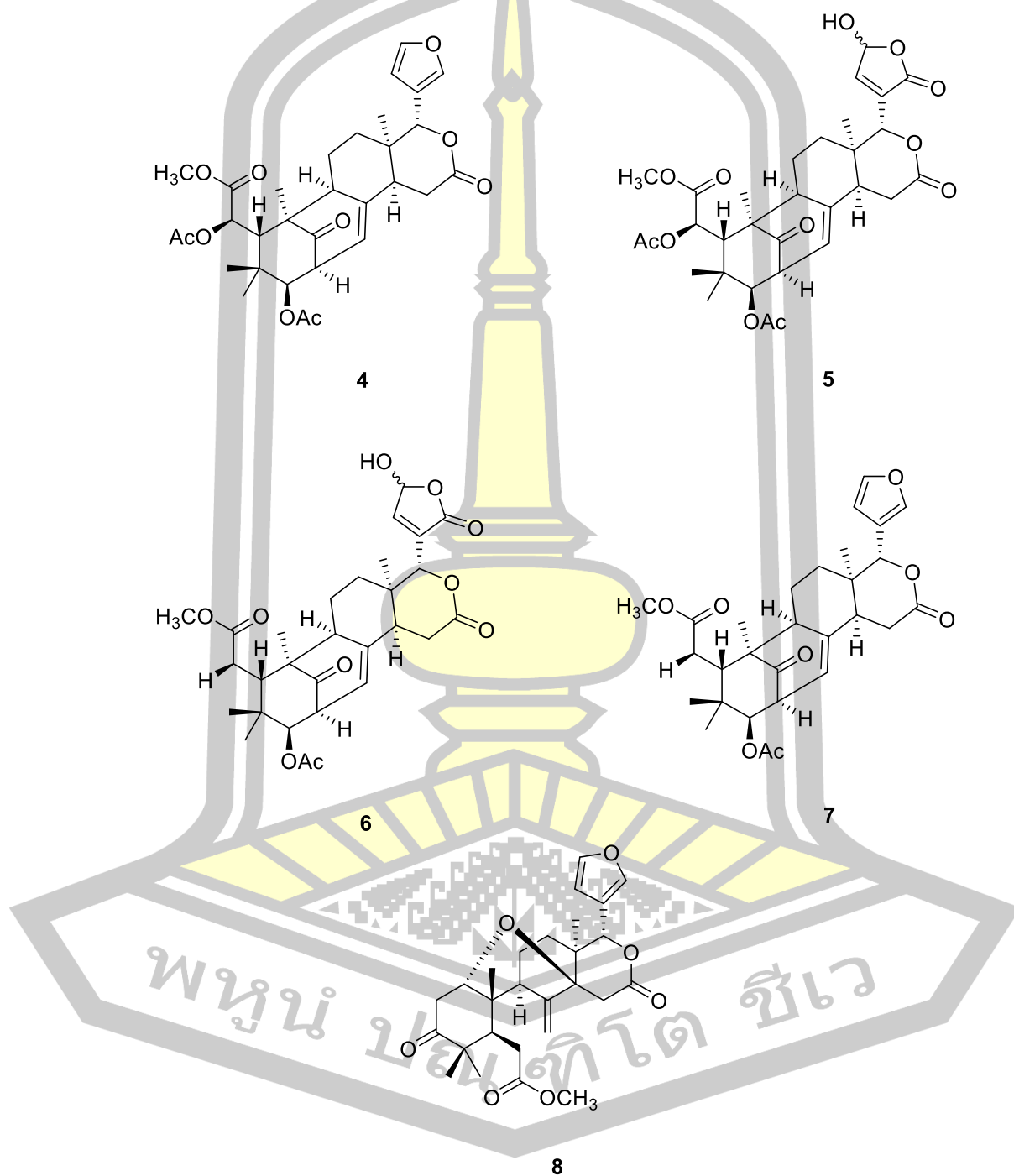


Figure 4. Structures of compounds 4–8.

CHAPTER 3

MATERIAL AND METHOD

3.1. General experimental procedures

NMR spectra were recorded on Bruker Ascend-400 (Bruker BioSpin, Rheinstetten, Germany) and on Bruker Avance III HD 400 and 500 MHz spectrometers. ESITOF mass spectra (HRMS) were measured using a Bruker micrOTOF mass spectrometer. Silica gel 60 (Merck, Darmstadt, Germany) and Sephadex LH-20 (Sigma Aldrich, St. Louis, MO, USA) were used for column chromatography. Pre-coated silica gel 60 F₂₅₄ on aluminium sheets (Merck, Darmstadt, Germany) was used for analytical thin-layer chromatography (TLC).

3.2. The fungus *Fulvifomes fastuosus*

3.2.1 Fungal material

Natural fruiting bodies of *Fulvifomes fastuosus* (*F. fastuosus*) (code MSU 043) (**Figure 5**) were collected from Ban Kham Yang Forest, Phasuk sub-District, Wang Sam Mo District, Udon Thani Province, Thailand, (GPS: 16.94973° N, 103.42831° E), on August, 2022 by Surachai Singmahan. This mushroom was deposited at the Natural Medicinal Mushroom Museum, Mahasarakham University.

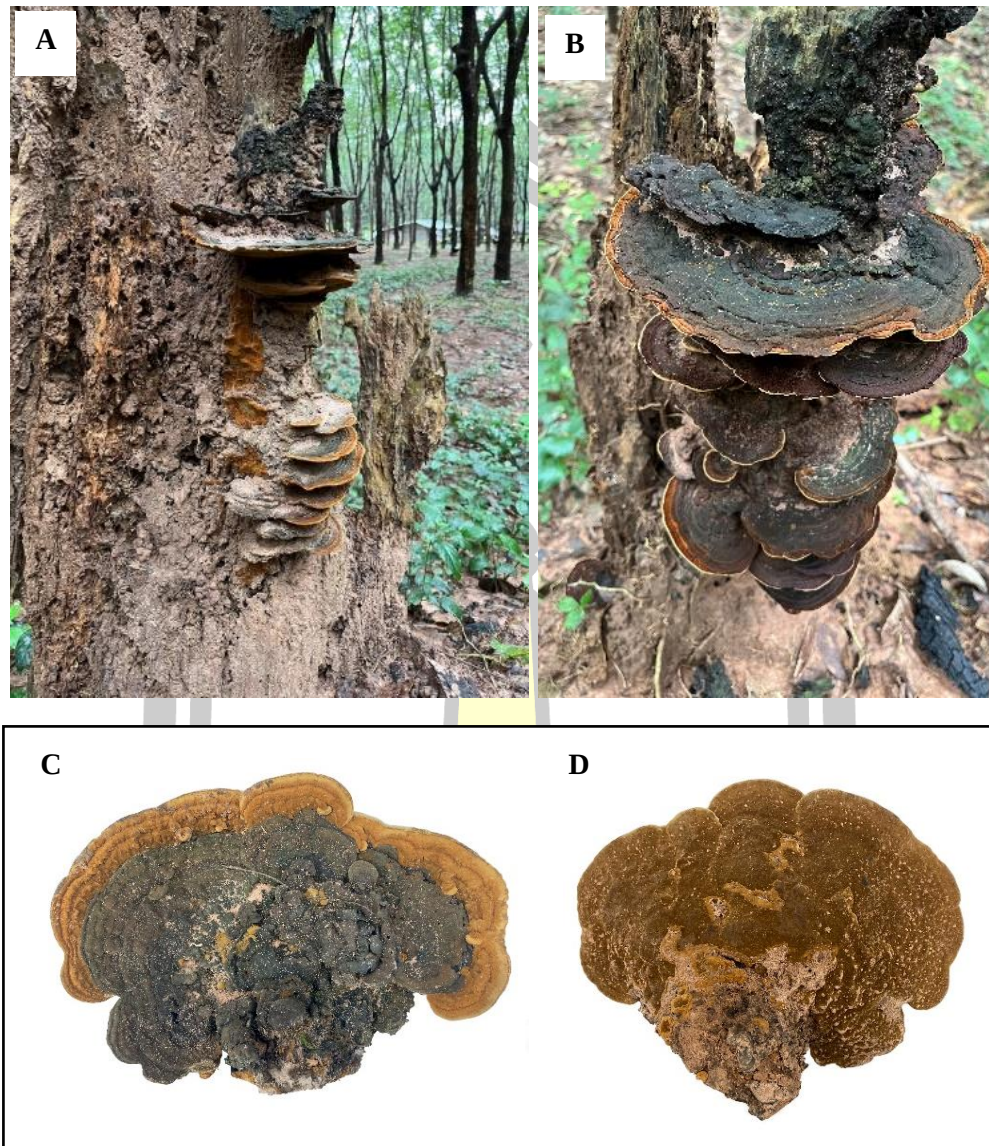


Figure 5. Natural fruiting bodies of *F. fastuosus* grow on the dead tunk in natural habitate at the same spot collections (**A**; in August 2022, and **B**; in July 2024), and front (**C**) and back (**D**) sides of the fruiting bodies.

3.2.2. Living isolation

The isolation of living culture of the natural mushroom specimen of *F. fastuosus* was examined. The inner tissue of *F. fastuosus* was cut, then soaked in

10% v/v clorox for 1 min and sterile water for 1 min, and then placed on petri dish containing potato dextrose agar (PDA) for cultivation. The fungal strain (MSUCC043) is preserved at the Department of Biology, Faculty of Science, Mahasarakham University.

3.2.3. DNA extraction

The pieces of fruiting bodies were homogenized in a cold mortar and pestle. The fruiting bodies powder was transferred into a microcentrifuge tube and genomic DNA was extracted using the plant DNA extraction kit handbook (Vivantis, Malaysia). DNA samples were checked on 1% agarose gel electrophoresis and stored at -20 °C for further experimental use.

3.2.4. Identification of the *F. fastuosus*

The partial small subunit rDNA was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White TJ et al., 1990). Polymerase chain reaction (PCR) was conducted for the ITS gene (Sangdee K et al., 2015). The resulting PCR products were purified using the Gel/PCR DNA Fragments Extraction Kit (Vivantis, Malaysia). Sequencing was carried out by Macrogen Inc. (Korea). The partial ITS sequence data were compared with those in the National Center for Biotechnology Information (NCBI) database using the nucleotide BLAST program (www.ncbi.nih.gov/blast). The novel partial sequence was deposited in the GenBank nucleotide sequence database. Reference sequences from related species were downloaded and aligned using ClustalW (www.genome.jp/tools/clustalw/). Phylogenetic analysis of the ITS region was

performed using MEGA program (Tamura K et al., 2013), and a neighbor-joining (NJ) tree was constructed with 1000 bootstrap replicates using the Kimura 2-parameter method for pairwise deletion at uniform rates.

3.2.5. Fermentation

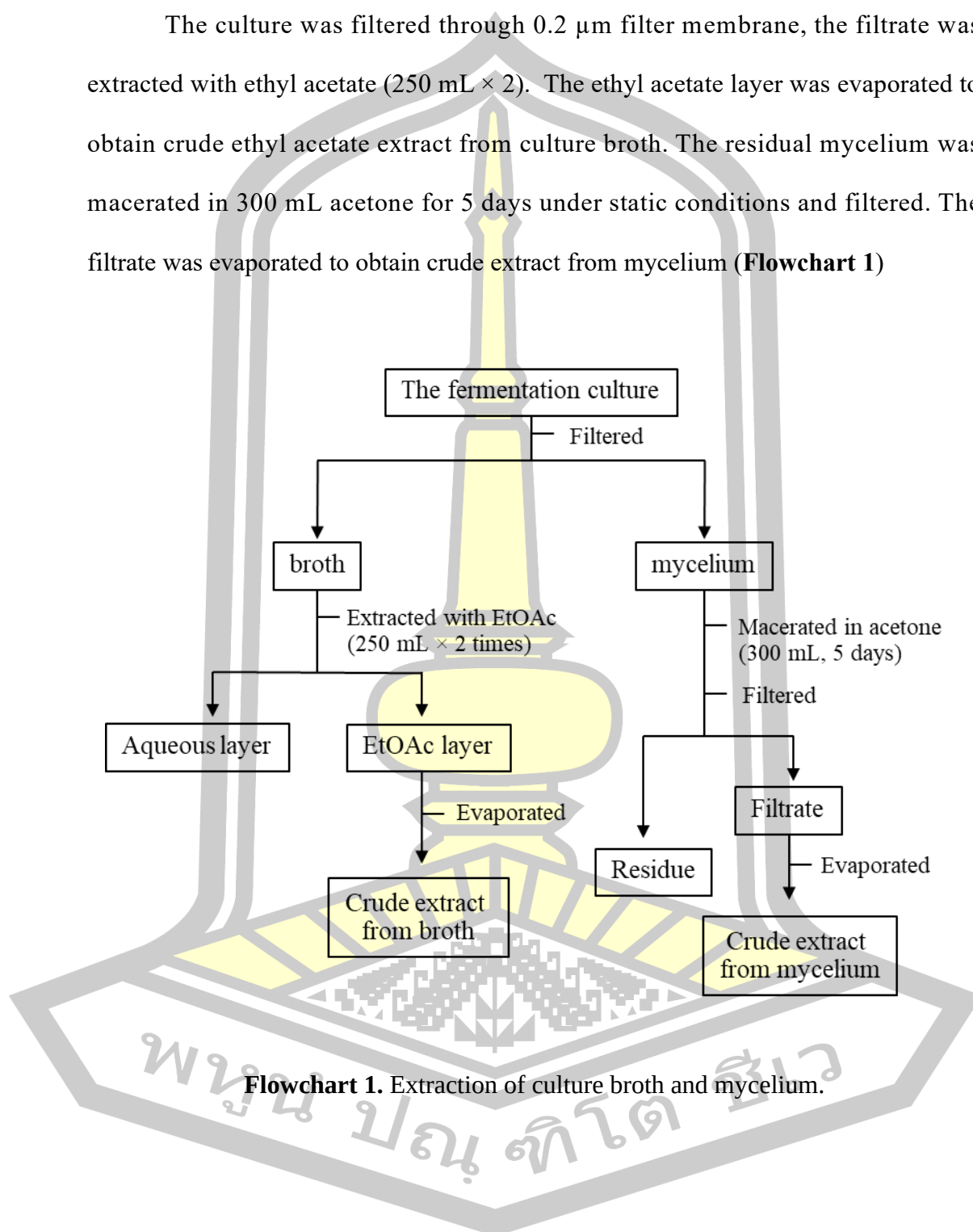
The culture of *F. fastuosus* was maintained on potato dextrose agar (PDA) at room temperature. The inoculum was prepared by growing initial fungus on PDA plate for 7 days at room temperature. Then, a 1 × 1 cm fungal colony was cut with a sterile inoculation needle and transferred to 25 mL of malt extract broth (MEB) (6 g/L of malt extract, 1.8 g/L of maltose, 6 g/L of dextrose, and 1.2 g/L of yeast extract in a 250 mL Erlenmeyer flask and incubated at room temperature on rotary shaker (125 rpm) for 7 days). After incubation, the culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (MEB) and carried out at room temperature for 20 days under static conditions (**Figure 6**).



Figure 6. The colony of *F. fastuosus* on culture broth.

3.2.6. Extraction of culture broth and mycelium

The culture was filtered through 0.2 μm filter membrane, the filtrate was extracted with ethyl acetate ($250\text{ mL} \times 2$). The ethyl acetate layer was evaporated to obtain crude ethyl acetate extract from culture broth. The residual mycelium was macerated in 300 mL acetone for 5 days under static conditions and filtered. The filtrate was evaporated to obtain crude extract from mycelium (**Flowchart 1**)

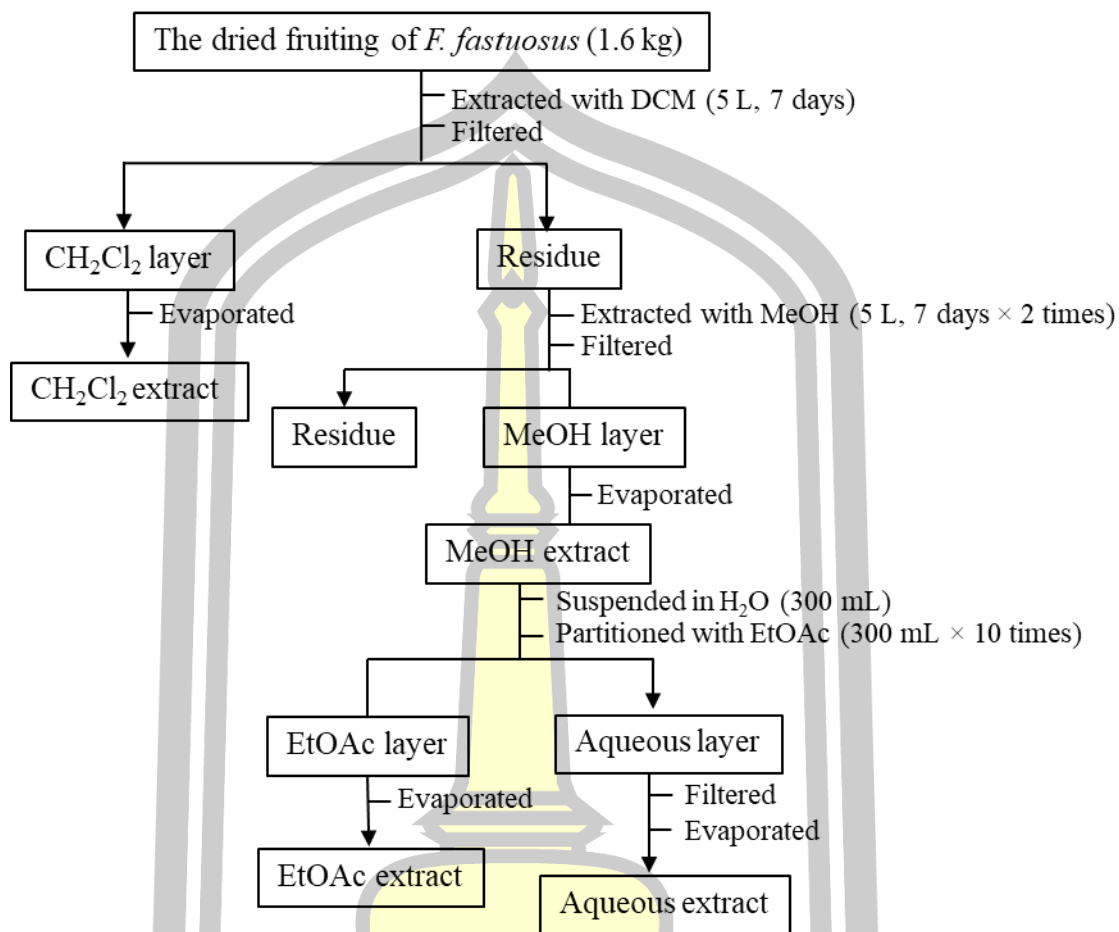


3.3. Extraction

The fruiting bodies of *F. fastuosus* was brush cleaned, broken into small pieces, and dried in the oven at 60 °C to a constant mass (1.6 kg). The sample was then macerated in dichloromethane (CH_2Cl_2) (5 L) at room temperature for 7 days. The mixture was filtered, and the filtrate was evaporated to obtain CH_2Cl_2 extract (1.78 g). The mushroom residue was then extracted with methanol (MeOH) (5 L, 7 days, 2 times) (**Figure 7**), and the MeOH filtrate was evaporated to obtain MeOH extract (35.64 g). The MeOH extract was suspended in water (300 mL) and successively partitioned with ethyl acetate (EtOAc) (300 mL, 10 times) to obtain EtOAc (22.0 g) extract. The aqueous residue from the partition was collected and filtered to remove dark solid precipitates. The filtrate was subjected to freeze dried to obtain crude aqueous extract (1.91 g). (**Flowchart 2**)



Figure 7. Dried fruiting bodies of *F. fastuosus* (A) and maceration in MeOH (B).



Flowchart 2. Extracted of *F. fastuosus* fruiting bodies.

3.4. Isolation

3.4.1. Isolation of the CH₂Cl₂ extract

The CH₂Cl₂ extract (1.77 g) was subjected to column chromatography (CC) on silica gel (eluted with Hexane–CH₂Cl₂ from 90:10 to 0:100) to yield **9** (colorless crystalline solid; 990 mg) and a trace amount of **10** (colorless crystalline solid; 4.2 mg).

3.4.2. Isolation of the EtOAc extract

The *F. fastuosus* EtOAc extract (FF extract; 22.0 g) was purified by silica gel CC (eluted with Hexane–EtOAc from 80:20 to 0:100, and then EtOAc–MeOH from 90:10 to 80:20) to obtain five fractions (**Ff.1–Ff.5**). **Ff.2** (386 mg) was applied over a silica gel column (eluted with Hexane–EtOAc from 80:20 to 0:100) to obtain a fraction which was further subjected to purify by Sephadex LH-20 CC (eluted with MeOH) to yield **12** (pale yellow powder; 5.0 mg). **Ff.3** (188 mg) was fractionated over silica gel column (eluted with Hexane–EtOAc from 90:10 to 0:100) to furnish **12** (8.3 mg).

Ff.4 (498 mg) was washed with MeOH (10 mL, stirred 20 min, $\times 3$) in a vial. The mixture was then left to separate to remove the filtrate (**Ff.4f**) from dark yellow precipitate (**Ff.4p**). **Ff.4p** (120 mg) was fractionated by Sephadex LH-20 CC (eluted with MeOH–H₂O from 50:50 to 90:10, and then CH₂Cl₂–MeOH 50:50), to obtain a sub-fraction **Fr.A** which was further purified by Sephadex LH-20 CC (eluted with CH₂Cl₂–MeOH 50:50), to yield **2** (yellow amorphous solid; 81.0 mg). **Ff.4f** (275 mg) was purified by Sephadex LH-20 CC (eluted with CH₂Cl₂–MeOH 50:50) to give three sub-fractions (**Fr.B–Fr.D**). **Fr.B** (22.1 mg) was applied over a Sephadex LH-20 column (eluted with MeOH) and the obtained fraction was further purified by silica gel CC (eluted with CH₂Cl₂–EtOAc from 80:20 to 0:100), to furnish **13** (pale yellow power; 1.6 mg). **Fr.D** (10.1 mg) was fractionated by silica gel CC (eluted with CH₂Cl₂–EtOAc from 80:20 to 0:100) and the obtained fraction was further purified by Sephadex LH-20 CC (eluted with MeOH) to yield **11** (yellow amorphous powder; 1.2 mg).

Ff.5 (3.89 g) was washed with MeOH (30 mL, stirred 30 min, $\times$ 3) in a small conical tube. The mixture was then left to separate to remove the filtrate (**Ff.5f**) from dark yellow precipitate of **2** (**Ff.5p**). A portion (110 mg) of **Ff.5p** (497 mg) was washed with MeOH (5 mL, stirred 20 min, $\times$ 3) in a vial and the yellow precipitate of **2** (102 mg) was obtained. A small amount of this sample was subjected to purify by analytical HPLC (Ultimate 300, Thermo Scientific) using a reverse phase column C18, 4.6 – 150 mm, 5 μ m; gradient elution with MeOH–H₂O from 30:70 to 100:0 over 30 min, flow rate 0.6 mL/min) to furnish **2** (10.4 mg, t_R 16.6 min). **Ff.5f** (3.39 g) was applied over a silica gel column (eluted with Hexane–EtOAc 60:40 to 0:100, and then EtOAc–MeOH from 90:10 to 0:100), to obtain five sub-fractions (**Fr.E–Fr.I**) and **14** (24.7 mg). **Fr.F** (59.0 mg) was fractioned by silica gel CC (eluted with Hexane–EtOAc from 60:40 to 0:100, and then EtOAc–MeOH from 90:10 to 0:100) to furnish **2** (2.8 mg). **Fr.G** (10.8 mg) was applied over a Sephadex LH-20 column (eluted with MeOH), to yield **2** (3.7 mg) while **Fr.H** (49.3 mg) was also purified by Sephadex LH-20 CC (eluted with MeOH), to yield **2** (7.4 mg). **Fr.C** (30.5 mg), **Fr.E** (10.1 mg) and **Fr.I** (243 mg) were subjected to HPLC purification; however, no compounds were successfully isolated. The isolation of the DCM and EtOAc extract from fruiting bodies of *F. fastuosus* is shown in **Flowchart 3**.

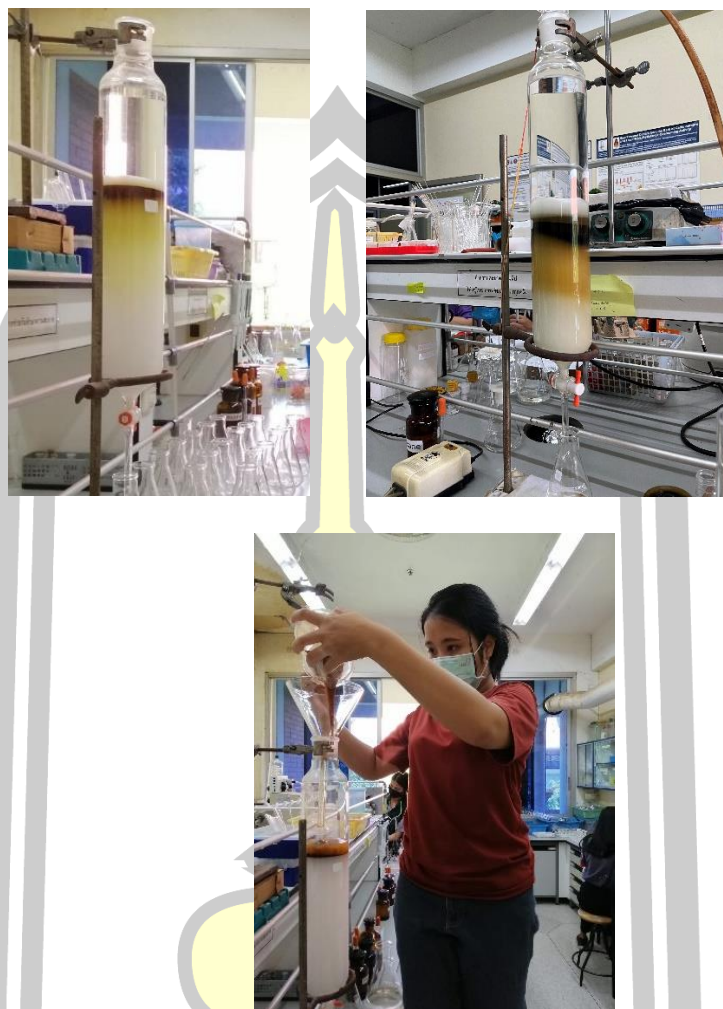
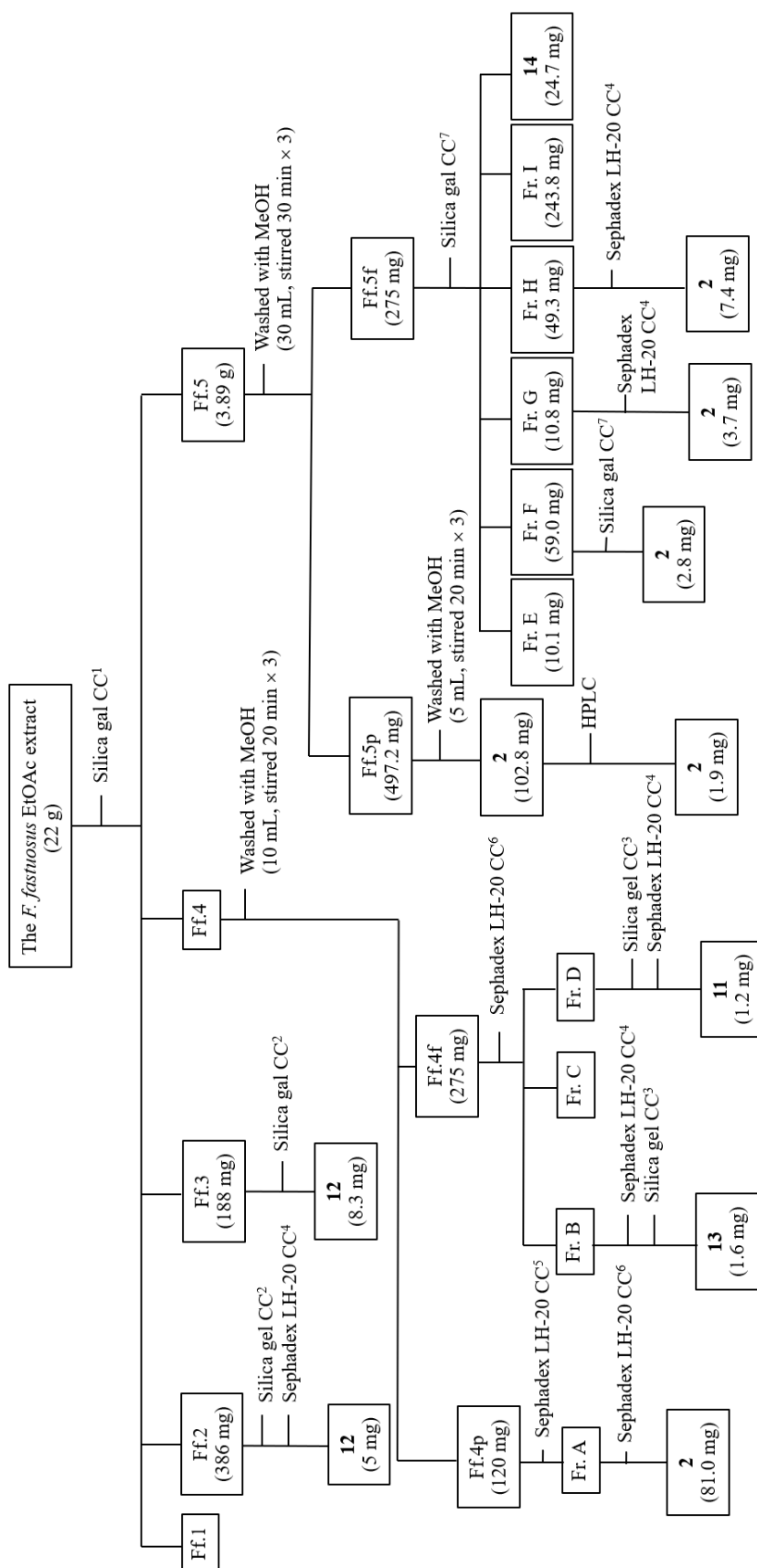


Figure 8. Isolation of the EtOAc extract using column chromatography.





Mobile phase system: ¹ Hexane-EtOAc from 80:20 to 0:100, and then EtOAc-MeOH from 90:10 to 80:20; ² Hexane-EtOAc from 80:20 to 0:100; ³ CH₂Cl₂-EtOAc from 80:20 to 0:100; ⁴ MeOH-H₂O from 50:50 to 90:10, and then CH₂Cl₂-MeOH 50:50; ⁵ CH₂Cl₂-MeOH 50:50; ⁶ Hexane-EtOAc 60:40 to 0:100, and then EtOAc-MeOH from 90:10 to 0:100; ⁷ Hexane-EtOAc 60:40 to 0:100, and then EtOAc-MeOH from 90:10 to 0:100.

Flowchart 3. Isolation of EtOAc extract from fruiting bodies of *F. fastuosus*.

3.5. Structural elucidation

The structures of pure compounds were analyzed by spectroscopic methods (1D and 2D Nuclear Magnetic Resonance (NMR) spectroscopy, Mass (MS) spectrometry, and Ultraviolet-Visible (UV-Vis) spectroscopy). The structures of the known compounds were confirmed by the comparison of their spectroscopic data to those reported in literatures.

3.6. HPLC analyses

The contents of resveratrol and oxyresveratrol were determined by HPLC (Promden W, Chanvorachote P, et al., 2024). The HPLC system (Agilent 1290 Infinity, Agilent Technologies, Santa Clara, CA, USA) consisted of a binary pump (G4220A), diode array detector (G4212A), and autosampler (G4226A). The separation was performed on a Zorbax[®] SB-C18 rapid resolution HD column (Agilent, 2.1 mm i.d. × 150 mm, 1.8 μm) coupled with a Zorbax[®] SB-C18 guard column (2.1 mm i.d. × 5 mm, 1.8 μm). The flow rate was 0.2 mL/min, and the injection volume was 5 μL. The detection wavelength was set at 320 nm, and the column temperature was maintained at 40 °C. The mobile phase comprised acetonitrile (A) and 0.5% acetic acid in water (B). The following gradient solvent system was applied: 0–10 min, 18–25% A; 10–25 min, 25–30% A; 25–27 min, 30–40% A; 27–30 min, 40–90% A. The data were collected and analyzed with 2D-LC software (version A.01.04).

3.7. Biological activity assay

The antibacterial activities, DPPH Radical Scavenging Activities, α -Glucosidase Inhibitory Activities, NO inhibitory Activities, and their Cytotoxicity of the extracts and isolated pure compounds will be tested.

3.7.1. Antibacterial activities assay

3.7.1.1. Bacterial strains and cultivation

The antibacterial activity was assayed against three Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20651, methicillin-susceptible *Staphylococcus aureus* (MSSA) DMST 2933, and *Bacillus cereus* ATCC 11778. These bacterial strains were cultured on Mueller-Hinton agar (MHA) at 37 °C for 16–18 h.

3.7.1.2. Agar well diffusion method

The agar well diffusion method was performed to determine antibacterial activity of the samples (Sangdee A et al., 2018). The sample solutions (50 mg/mL) were added to each well (0.1 mL per well) and incubated at 37 °C for 16–18 h. The zone of inhibition was measured after incubation of the plates. The negative controls of 10% (v/v) methanol and 10% dimethyl sulfoxide (DMSO) were used. A standard positive control was tetracycline (250 μ g/mL).

3.7.1.3. MIC and MBC determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were evaluated using the microdilution method (Sangdee A et al., 2018). MICs were determined by identifying the solutions that were clear after incubation compared with the control well. MBCs were determined by transferring bacterial suspensions from the MIC assay onto MHA plates. The MBC referred to the lowest concentration that showed no growth after incubation for 24 h. A reference standard antibiotic tetracycline (250 µg/mL) was used in the study.

3.7.2. DPPH Radical scavenging activity assay

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) (Sigma-Aldrich) radical scavenging activity assay was performed as previously described by Promden et al (Promden W et al., 2024). Briefly, 20 µL of each sample solution (5 µg/mL in dimethyl sulfoxide (DMSO)) was mixed with 180 µL of DPPH• solution (100 µM in MeOH) in a 96-well microplate. The mixture was incubated at 37 °C for 30 min in the dark. The absorbance (A) of the sample and control was measured at 515 nm using a FLUOstar® Omega plate reader (BMG LABTECH). The DPPH• radical scavenging activity (RSA) was calculated as of % RSA. For the active samples (%RSA >5%), the scavenging concentration at 50% (SC₅₀) was determined using a calibration curve within the linear range by plotting concentrations against % RSA. All experiments were performed in triplicate. Ascorbic acid was used as the standard compound.

3.7.3. α -Glucosidase inhibitory activity assay

The α -glucosidase inhibitory activity was evaluated as previously described (Promden W et al., 2024). Samples were dissolved in DMSO (final concentration: 50 μ g/mL in 2.5% DMSO buffer), and 10 μ L was added to each well of a 96-well microplate. Next, 130 μ L of 100 mM phosphate buffer (pH 6.8) and 20 μ L of α -glucosidase from *Saccharomyces cerevisiae* (0.5 U/mL, Sigma-Aldrich) were added. After incubation at 37 °C for 10 min, the reaction was initiated by adding 50 μ L of 2.5 mM *p*-nitrophenyl- α -D-glucopyranoside (*p*-PNPG, Sigma-Aldrich). Controls contained 10 μ L of 2.5% DMSO instead of sample. Absorbance was measured at 405 nm using a FLUOstar® Omega microplate reader (BMG LABTECH), and % inhibition was calculated. For samples showing >70% inhibition at 50 μ g/mL, IC₅₀ values were determined from concentration–response curves (3.125–100 μ g/mL). Acarbose (Sigma-Aldrich) served as the standard inhibitor.

3.7.4. NO inhibitory activity assay

Inhibition of nitric oxide (NO) production was assessed in lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophages (ATCC®, Lot: 70012232, Biomed, Thailand) as previously described with minor modifications (Makchuchit S et al., 2017). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco®, USA) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution (penicillin, streptomycin, amphotericin B), and maintained at 37 °C in 5% CO₂.

For the assay, RAW 264.7 cells (1×10^6 cells/well) were seeded in 96-well plates with 100 μ L of medium and incubated for 24 h. Medium was then replaced

with fresh DMEM containing 2 $\mu\text{g/mL}$ LPS and test samples (20 $\mu\text{g/mL}$). DMSO served as the solvent control. After 24 h, 100 μL of the supernatant was mixed with 100 μL of Griess reagent (1% sulfanilamide and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4) in a new 96-well plate. Absorbance was measured at 520 nm using a SPECTROstar Nano plate reader (BMG LABTECH), and % NO inhibition was calculated. For active compounds (>50% inhibition at 20 $\mu\text{g/mL}$), inhibitory concentration at 50% (IC_{50}) values were determined from dose–response curves (1–20 $\mu\text{g/mL}$ in 0.2% DMSO/DMEM). Diclofenac sodium (Thermo Scientific, China) was used as a positive control.

Cytotoxicity under assay conditions was evaluated via MTT assay. After removing the supernatant, 5 mg/mL MTT solution was added and incubated for 2 h at 37 °C. Formazan crystals were dissolved in 50 μL DMSO, and absorbance was read at 570 nm. Cell viability >70% compared to control was considered non-cytotoxic.

3.7.5. Cytotoxicity assay

Cytotoxic activity against human lung cancer (A549) cells (ATCC®, Lot: 70018877, Biomedica, Thailand) was evaluated as previously described with minor modifications (Alley MC et al., 1988). Cells were seeded in 96-well plates (100 μL /well) and incubated at 37 °C in a humidified 5% CO_2 atmosphere. After 24 h, 100 μL of medium containing test samples (final concentrations: 1, 10, 50, and 100 $\mu\text{g/mL}$) was added. DMSO (0.5% v/v) served as the solvent control. Plates were incubated for 72 h.

Following incubation, half of the medium was removed, and 10 μL of MTT solution (5 mg/mL in phosphate-buffered saline (PBS), Invitrogen®) was added. After

4 h at 37 °C, the medium was discarded and 50 μ L of DMSO was added to dissolve formazan crystals. Absorbance was measured at 570 nm using a SPECTROstar Nano plate reader (BMG LABTECH). Cell viability was calculated relative to the control. All assays were performed in triplicate, and results were expressed as % cytotoxicity.



CHAPTER 4

RESULTS AND DISCUSSION

4.1. The fungus *F. fastuosus*

4.1.1. Living isolation

The mycelium of *F. fastuosus* was grown on potato dextrose agar plate (PDA) and preserved at the Department of Biology, Faculty of Science, Mahasarakham University as MSUCC043 (Figure 9).



Figure 9. Mycelium of *F. fastuosus* on PDA.

4.1.2. Identification of the *F. fastuosus*

Phylogenetic relationships of *F. fastuosus* isolate MSUCC0043 and 17 related species were inferred based on partial internal transcribed spacer (ITS) gene sequences. A neighbor-joining (NJ) tree was constructed using MEGA version 10. Bootstrap values (expressed as percentages) are indicated above the branches and

represent the frequency with which each clade was recovered across 1,000 bootstrap replicates. Branches with less than 50% bootstrap support were collapsed (**Figure 10**).

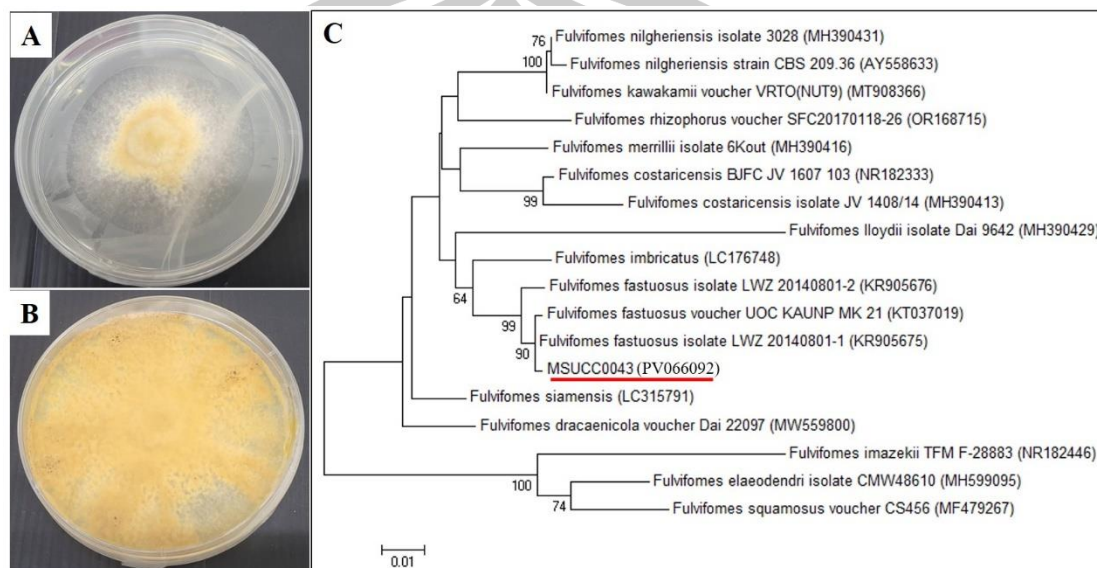


Figure 10. Mycelium of *F. fastuosus* (A, B) and Neighbor-joining (NJ) tree (C).

4.2. Fermentation

4.2.1. Extraction of culture broth and mycelium

The experimental result, the weight of the crude from the culture broth was 14.4 mg and the weight of the mycelium extract was 62.3 mg (**Table 1**).

Table 1. Fermentation and extraction yield of culture broth and mycelium of *F. fastuosus*.

25 mL of MEB		250 mL of MEB		Extract (mg)	
Started	Collected	Started	Collected	Broth	Mycelium
04/04/2023	11/04/2023	11/04/2023	03/05/2023	14.4	62.3

4.3. Extraction

The fruiting bodies of *F. fastuosus* was extracted with dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), and H₂O to obtain extracts. The dichloromethane extract was concentrated under reduced pressure to obtain a white crystals 1.8 g. The methanol solution was concentrated under reduced pressure to obtain a dark brown gum 35.6 g. The dark brown gum ethyl acetate extract was furnished from the partitioning of methanol extract to obtain EtOAc extract 22.0 g and H₂O extract 1.9 g (Table 2).

Table 2. The extract yields of natural fruiting bodies of *F. fastuosus*.

Extract	Weight (g)
DCM	1.8
MeOH	35.6
EtOAc	22.0
H ₂ O	1.9

4.4. ¹H NMR spectrum of the crude extracts

The broth and mycelium extracts (Figures A5–A6) from small scale fermentation, and the CH₂Cl₂, MeOH and EtOAc extracts (Figures A1–A6) from the fruiting bodies of *F. fastuosus* were subjected to ¹H NMR analyses.

4.5. Isolation

Purification of the CH₂Cl₂ extract (Figure A1) yielded chlorinated fungal metabolites, drosophilin A methyl ether (DAME; 9) (Song Y et al., 2008) (Figure 13),

as the major compound (56% yield), along with a trace amount of drosophilin A (DA; **10**) (**Figure 13**). The structure were elucidated based on its ^1H and ^{13}C NMR and MS data.

Fractionation of the crude EtOAc extract (**Figure A3**) led to the isolation of the major styrylpyranone inosvanin A (**2**) (Shou D et al., 2016 and Le AN et al., 2024), along with a trace amount of its derivative, inosvanin E (phellifuropyranone A) (**11**) (Lee I-K et al., 2007). Additional polyphenols including 3,4-dihydroxybenzalacetone (**12**) (Ohmura K et al., 1919), protocatechuic acid (**13**) (Keawsa-ard S et al., 2012), and caffeic acid methyl ester (**14**) (Pieters L et al., 1999) were also isolated. The structures of **2**, **11–14** (**Figure 11**) were elucidated based on their NMR spectroscopic data and confirmed by comparison with reported data.

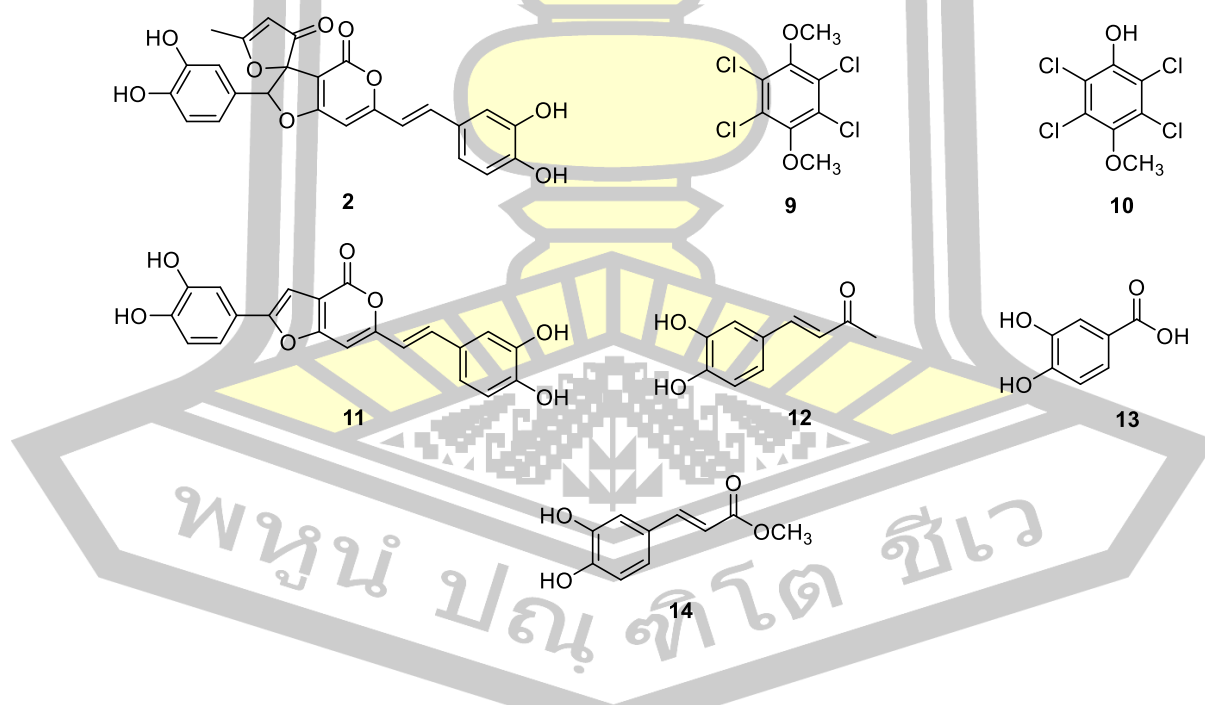


Figure 11. Structures of the isolated compounds from *F. fastuosus*.

4.6. Structure elucidation of the isolated compounds from *F. fastuosus*

Drosophilin A methyl ether (DAME; **9**) was isolated as a colorless crystalline solid. The molecular formula was determined to be $C_8H_8Cl_4O_2$ by HRESIMS. Although ^1H and ^{13}C NMR spectroscopic data for natural DAME had not been previously reported, the structure of the isolated compound **9** was elucidated based on its ^1H and ^{13}C NMR (**Figures A7** and **A8**) and confirmed by comparison with literature data for a synthetic analogue (**Table 3**) (Song Y et al., 2008). MS analysis further supported the identification, showing fragment ion at m/z 260.89 $[M - CH_3]^-$ and 245.96 $[M - 2CH_3]^-$ (**Figure A9**).

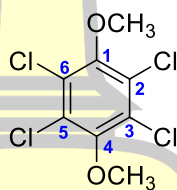


Table 3. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **9** in $CDCl_3$ compared with literature.

Position	9		Drosophilin A methyl ether ^a	
	δ_H , multi. (<i>J</i> in Hz)	δ_C	δ_H , multi. (<i>J</i> in Hz)	δ_C
1	-	150.5	-	150.7
2, 6	-	127.5	-	127.8
3, 5	-	127.5	-	127.8
4	-	150.5	-	150.7
1-OCH ₃	3.89, s	61.0	3.90, s	61.0
4-OCH ₃	3.89, s	61.0	3.90, s	61.0

Calibration of $CDCl_3$ δ_H 7.26 ppm. ^a Song Y et al., 2008

Drosophilin A (DA; **10**) was isolated as a colorless crystalline solid. The molecular formula was determined to be $C_7H_4Cl_4O_2$ by HRESIMS. The structure of **10** was similarly to **9**, as confirmed by NMR (**Table 4**) and MS data, including a fragment ion at m/z 260.89 $[M - H]^-$. (**Figure A12**)

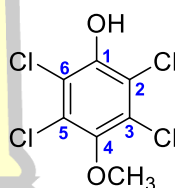


Table 4. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **10** (in $CDCl_3$).

Position	10	
	δ_H , multi. (J in Hz)	δ_C
1	5.99, br	146.1 ^a
2, 6	-	127.5 ^b
3, 5	-	118.8 ^b
4	-	147.3 ^a
4-OCH ₃	3.87, s	61.0

Calibration of $CDCl_3$ δ_H 7.26 ppm. ^{a,b} Chemical shifts interchangeable.

Inoscavin A (**2**) was isolated as a yellow amorphous solid. The molecular formula was determined to be $C_{25}H_{18}O_9$ by ESIMS. The structure of **2** were identified as inoscavin A based on spectroscopic data (**Table 5** and **Table 6**) compared with those reported in literature (Shou D et al., 2016 and Le AN et al., 2024). ^1H NMR spectrum of **2** in CD_3OD and acetone- d_6 was shown in **Figures A13–A16**.

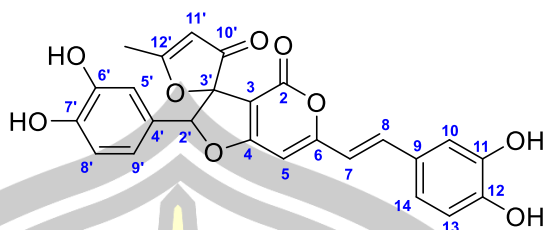


Table 5. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **2** in CD_3OD compared with literature.

Position	2 δ_{H} , multi. (J in Hz)	Inoscavin A ^a δ_{H} , multi. (J in Hz)
1	-	-
2	-	-
3	-	-
4	-	-
5	6.50, s	6.50, s
6	-	-
7	6.73, d (15.7)	6.72, d (15.7)
8	7.44, d (15.8)	7.43, d (15.8)
9	-	-
10	7.08, d (2.0)	7.07, d (1.7)
13	6.79, d (8.2)	6.79, d (8.2)
14	7.00, dd (8.3, 2.0)	6.99, dd (8.2, 1.7)
1'	-	-
2'	5.66, s	5.65, s
3'	-	-
4'	-	-
5'	6.71, d (2.1)	6.70, d (1.9)
8'	6.75, d (8.1)	6.75, d (8.1)
9'	6.59, dd (8.2, 2.1)	6.58, dd (8.2, 1.9)
10'	-	-
11'	5.58, s	5.57, s
12'	-	-
11-OH	-	-
12-OH	-	-
6'-OH	-	-
7'-OH	-	-
12'-CH ₃	1.97, s	1.97, s

Calibration of CD_3OD δ_{H} 3.31 ppm. ^a Shou D et al., 2016

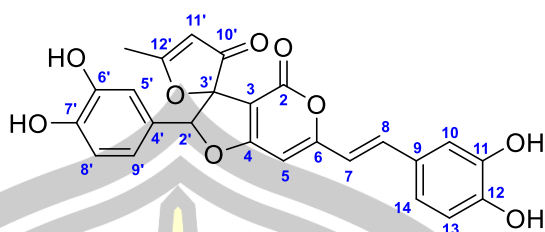


Table 6. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **2** in acetone- d_6 compared with literature.

Position	2	Inoscavin A ^a	
	δ_{H} , multi. (J in Hz)	δ_{H} , multi. (J in Hz)	δ_{C}
1	-	-	-
2	-	-	158.3
3	-	-	99.6
4	-	-	175.2
5	6.50, s	6.50, s	94.9
6	-	-	166.0
7	6.82, d (15.9)	6.82, d (15.5)	117.2
8	7.42, d (15.9)	7.42, d (16.0)	138.4
9	-	-	128.4
10	7.20, d (2.0)	7.20, d (2.0)	115.0
13	6.88, d (8.2)	6.89, d (8.3)	116.5
14	7.09, dd (8.2, 2.0)	7.09, dd (8.3, 2.0)	122.3
1'	-	-	-
2'	5.64, s	5.64, s	95.2
3'	-	-	93.6
4'	-	-	123.4
5'	6.81, d (2.0)	6.82, d (2.3)	115.4
8'	6.84, d (8.1)	6.84, d (8.4)	115.7
9'	6.66, dd (8.2, 2.0)	6.66, dd (8.4, 2.3)	120.2
10'	-	-	200.4
11'	5.52, s	5.52, s	104.6
12'	-	-	190.2
11-OH	-	-	146.4
12-OH	-	-	148.6
6'-OH	-	-	145.7
7'-OH	-	-	147.7
12'-CH ₃	1.93, s	1.93, s	16.5

Calibration of acetone- d_6 δ_{H} 2.05 ppm. ^a Le AN et al., 2024

Inoscavin E (**11**) was isolated as a pale yellow power. The molecular formula was determined to be $C_{21}H_{14}O_7$ by ESIMS. The structure of **11** were identified as inoscavin E based on spectroscopic data (**Table 7**) compared with those reported in literature (Lee I-K et al., 2007). ^1H NMR spectrum of **11** in CD_3OD **Figure A18**.

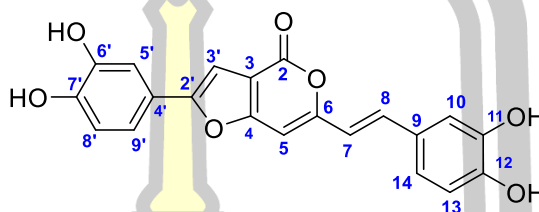


Table 7. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **11** in CD_3OD compared with literature.

Position	11	Inoscavin E ^a	
	δ_H , multi. (<i>J</i> in Hz)	δ_H , multi. (<i>J</i> in Hz)	δ_C
1	-	-	-
2	-	-	161.3
3	-	-	112.3
4	-	-	162.7
5	6.85, s	6.83, s	96.5
6	-	-	158.4
7	6.73, d (16.0)	6.70, d (16.0)	117.4
8	7.31, d (16.0)	7.30, d (16.0)	135.5
9	-	-	129.3
10	7.04, d (2.0)	7.04, d (2.0)	114.6
13	6.84, d (8.2)	6.77, d (8.0)	116.6
14	6.95, dd (9.6, 2.0)	6.95, dd (8.0, 2.0)	121.7
1'	-	-	-
2'	-	-	158.3
3'	6.97, s	6.96, s	100.3
4'	-	-	122.3
5'	7.21, d (2.0)	7.20, d (2.0)	112.6
8'	6.78, d (8.2)	6.83, d (8.4)	116.8
9'	7.17, dd (8.2, 2.0)	7.16, dd (8.4, 2.0)	117.9
11-OH	-	-	146.8
12-OH	-	-	148.3
6'-OH	-	-	146.9
7'-OH	-	-	148.0

Calibration of acetone- d_6 δ_H 2.05 ppm. ^a Lee I-K et al., 2007

3,4-dihydroxybenzalacetone (**12**) was isolated as a pale yellow powder, the molecular formula was determined to be $C_{10}H_{10}O_3$. The structure of **12** were identified as 3,4-dihydroxybenzalacetone based on spectroscopic data (**Table 8**) compared with those reported in literature (Ohmura K et al., 1919). ^1H NMR spectrum of **12** in $CDCl_3$ was shown in **Figure A21**.

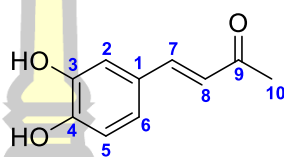


Table 8. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **12** in $CDCl_3$ compared with literature.

Position	12 δ_H , multi. (J in Hz)	3,4-Dihydroxybenzalacetone ^a δ_H , multi. (J in Hz)	δ_C
1	-	-	127.8
2	7.12, d (2.0)	7.07, d (2.0)	115.3
3	-	-	146.9
4	-	-	150.0
5	6.89, d (8.0)	6.78, d (8.0)	116.6
6	7.02, dd (8.0, 2.0)	7.01, dd (8.0, 2.0)	123.5
7	7.42, d (16.0)	7.51, d (16.5)	146.8
8	6.56, d (16.0)	6.51, d (16.5)	124.8
9	-	-	201.5
10	2.36, s	2.33, s	27.0

Calibration of $CDCl_3$ δ_H 7.26 ppm. ^a Ohmura K et al., 1919

Protocatechuic acid (**13**) was isolated as a pale yellow powder, the molecular formula was determined to be $C_7H_6O_4$. The structure of **13** were identified as protocatechuic acid based on spectroscopic data (**Table 9**) compared with those reported in literature (Keawsa-ard S et al., 2012). ^1H NMR spectrum of **13** in acetone- d_6 was shown in **Figure A23**.

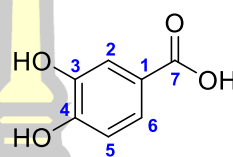


Table 9. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **13** in acetone- d_6 compared with literature.

Position	13	Protocatechuic acid ^a	
	δ_H , multi. (<i>J</i> in Hz)	δ_H , multi. (<i>J</i> in Hz)	δ_C
1	-	-	122.9
2	7.52, d (2.0)	7.53, d (2.0)	114.8
3	-	-	149.4
4	-	-	144.2
5	6.89, d (8.2)	6.91, d (8.0)	116.6
6	7.47, dd (8.2, 2.0)	7.46, dd (8.0, 2.0)	121.0
7-COOH	-	-	167.0

Calibration of acetone- d_6 δ_H 2.05 ppm. ^a Keawsa-ard S et al., 2012

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Caffeic acid methyl ester (**14**) was isolated as a brown powder. The molecular formula was determined to be $C_{10}H_{10}O_4$ by HRESIMS. The structure of **14** were identified as caffeic acid methyl ester based on spectroscopic data (**Table 10**) compared with those reported in literature (Pieters L et al., 1999). ^1H NMR spectrum of **14** in acetone- d_6 was shown in **Figure A25**.

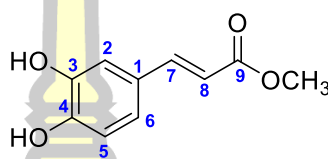


Table 10. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of **14** in acetone- d_6 compared with literature.

Position	14	Methyl caffeate ^a	δ_C
	δ_H , multi. (<i>J</i> in Hz)	δ_H , multi. (<i>J</i> in Hz)	
1	-	-	127.7
2	7.15, d (2.0)	7.15, d (2.1)	115.2
3	-	8.30, s	146.3
4	-	8.30, s	148.7
5	6.86, d (8.0)	6.86, d (8.0)	116.4
6	7.04, dd (8.0, 2.0)	7.03, dd (8.0, 2.1)	122.5
7	7.53, d (16.0)	7.53, d (15.8)	145.6
8	6.28, d (16.0)	6.27, d (16.0)	115.2
9	-	-	167.8
9-OCH ₃	3.71, s	3.70, s	51.4

Calibration of acetone- d_6 δ_H 2.05 ppm. ^a Pieters L et al., 1999

4.7. Biological activity of crude extracts and isolated fractions

The DPPH radical scavenging, α -glucosidase and nitric oxide (NO) inhibitory activities, as well as the cytotoxicity against A549 lung cancer cell lines of the crude CH_2Cl_2 , MeOH and EtOAc extracts from the fruiting bodies of *F. fastuosus* were evaluated (**Table 11**). The crude CH_2Cl_2 extract showed no activity in any of the assays. However, the presence of a colorless crystalline solid prompted further purification and structural investigation. In contrast, the crude EtOAc extract, obtained by partitioning the MeOH extract, exhibited notable α -glucosidase and NO inhibitory activities compared to the positive control (**Table 11**). Therefore, the crude EtOAc extract was selected for phytochemical investigation to identify its bioactive constituents. As mentioned earlier, the crude EtOAc extract exhibited potent α -glucosidase and NO inhibitory activities. Phytochemical investigation of this extract was undertaken to identify its bioactive constituents. During the purification process, selected fractions (Fr.A–I) of varying purity were analyzed by HPLC to determine their chemical profiles (**Figures A28–A29**) and subsequently evaluated for biological activities (**Table 11**). Based on the HPLC profiles, **3** (t_R 19 min) was detected in all fractions except Fr.D and E. In the DPPH radical scavenging assay, most fractions demonstrated strong antioxidant activity compared to ascorbic acid or the crude EtOAc extract, with the exception of Fr.D and F. Additionally, all fractions, Fr.A–I, exhibited significantly stronger α -glucosidase activity than acarbose, and better NO inhibitory activity than diclofenac. Fractions Fr.C, D and G showed cytotoxicity against A549 lung cancer cells comparable to cisplatin, the positive control, and better than that of the crude EtOAc extract (**Table 11**).

Table 11. Biological activities of the crude extracts of the fruiting bodies of *F. fastuosus*.

Sample	DPPH Radical scavenging activity (SC ₅₀ ; µg/mL)	α-Glucosidase inhibitory activity (IC ₅₀ ; µg/mL)	Nitric oxide inhibitory activity (IC ₅₀ ; µg/mL)	Cytotoxicity against A549 cells (IC ₅₀ ; µg/mL)
Crude extract				
CH ₂ Cl ₂	ND	ND	ND	ND
MeOH	25.60 ^d ± 1.52	1.54 ^a ± 0.01	53.92 ^c ± 4.19	49.89 ^d ± 0.18
EtOAc	18.30 ^c ± 2.35	1.38 ^a ± 0.03	42.62 ^d ± 2.47	39.52 ^{c,d} ± 2.51
fractions from EtOAc extract				
Fr.A	15.26 ^{b,c} ± 1.09	3.71 ^{a,b} ± 0.16	40.91 ^d ± 1.43	57.18 ^f ± 2.52
Fr.B	8.17 ^a ± 0.34	6.73 ^{b,c} ± 0.69	20.77 ^b ± 2.67	67.02 ^g ± 2.05
Fr.C	12.54 ^{a,b} ± 0.37	2.45 ^a ± 0.10	NA	9.64 ^a ± 0.09
Fr.D	30.34 ^d ± 5.10	1.32 ^a ± 0.04	NA	7.53 ^a ± 1.29
Fr.E	7.05 ^a ± 0.18	12.96 ^d ± 2.37	10.46 ^a ± 1.63	48.89 ^d ± 1.01
Fr.F	48.26 ^e ± 3.76	9.75 ^{c,d} ± 0.90	35.81 ^{c,d} ± 2.17	ND
Fr.G	9.32 ^{a,b} ± 0.57	1.55 ^a ± 0.08	NA	5.99 ^a ± 0.53
Fr.H	11.22 ^{a,b} ± 0.46	1.91 ^a ± 0.19	28.72 ^{b,c} ± 3.72	35.56 ^c ± 1.43
Fr.I	8.78 ^a ± 0.20	1.81 ^a ± 0.04	22.63 ^b ± 3.71	29.31 ^b ± 1.62
Control				
Ascorbic acid	9.77 ^{a,b} ± 2.64	-	-	-
Acarbose	-	123.07 ^c ± 3.06	-	-
Diclofenac	-	-	65.87 ^f ± 1.91	-
Cisplatin	-	-	-	6.83 ^a ± 0.76

Results are expressed as means ± SD of an experiment performed in triplicate. ND = Not determined. NA = Data not available due to the cytotoxicity of the samples on RAW 264.7 cells (cell viability <70%). Different lowercase superscript letters indicate significant differences between IC₅₀ values (p < 0.05, Tukey's HSD test) in each assay.

4.8. Biological activity of pure compounds

Compound **2**, isolated during purification, was evaluated for the DPPH radical scavenging, α -glucosidase and NO inhibitory activities, and cytotoxicity against A549 cells (**Table 12**). It exhibited potent α -glucosidase and NO inhibitory activities, surpassing the positive controls acarbose and diclofenac, respectively. Its α -glucosidase inhibitory activity (3.22–0.12 μ M; 1.48 μ g/mL) was 59-fold stronger than acarbose (190.60–4.74 μ M; 123.1 μ g/mL) (**Table 12**). However, this activity was slightly lower than that of the crude MeOH (1.54–0.01 μ g/mL) and EtOAc extracts (1.38–0.03 μ g/mL) (**Table 11**).

The DPPH, α -glucosidase, and NO inhibitory activities of the isolated DAME (**9**) were not assessed, as the crude CH_2Cl_2 extract was inactive in preliminary screening. However, due to the known bioactivity of chlorinated compounds, **9** was assayed for cytotoxicity against A549 cells but showed no activity. Furthermore, both compounds **9** and **2** were evaluated for antibacterial activity against *Staphylococcus aureus* (MSSA and MRSA) and *Bacillus cereus* at 1 mg/mL but were found to be inactive.

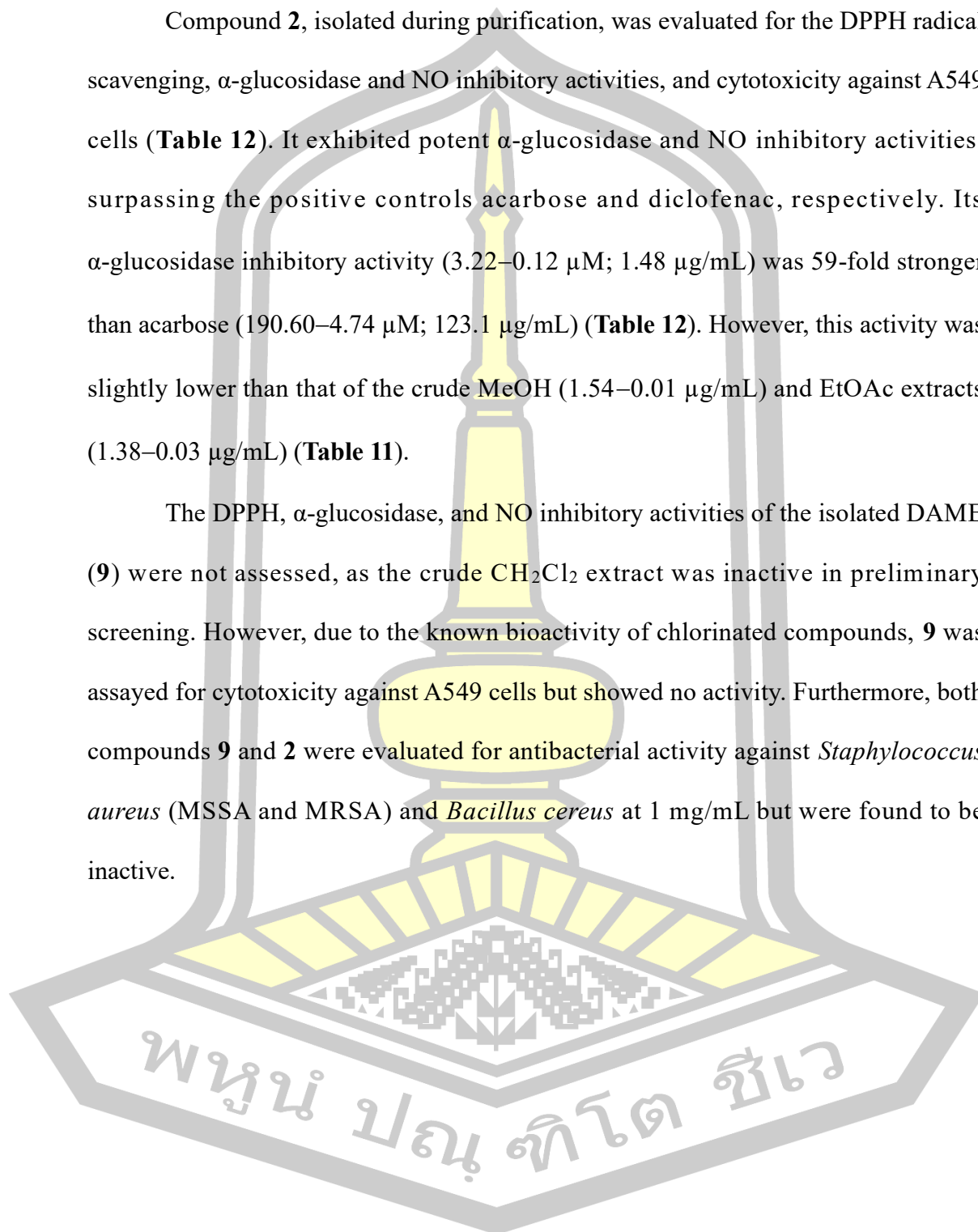
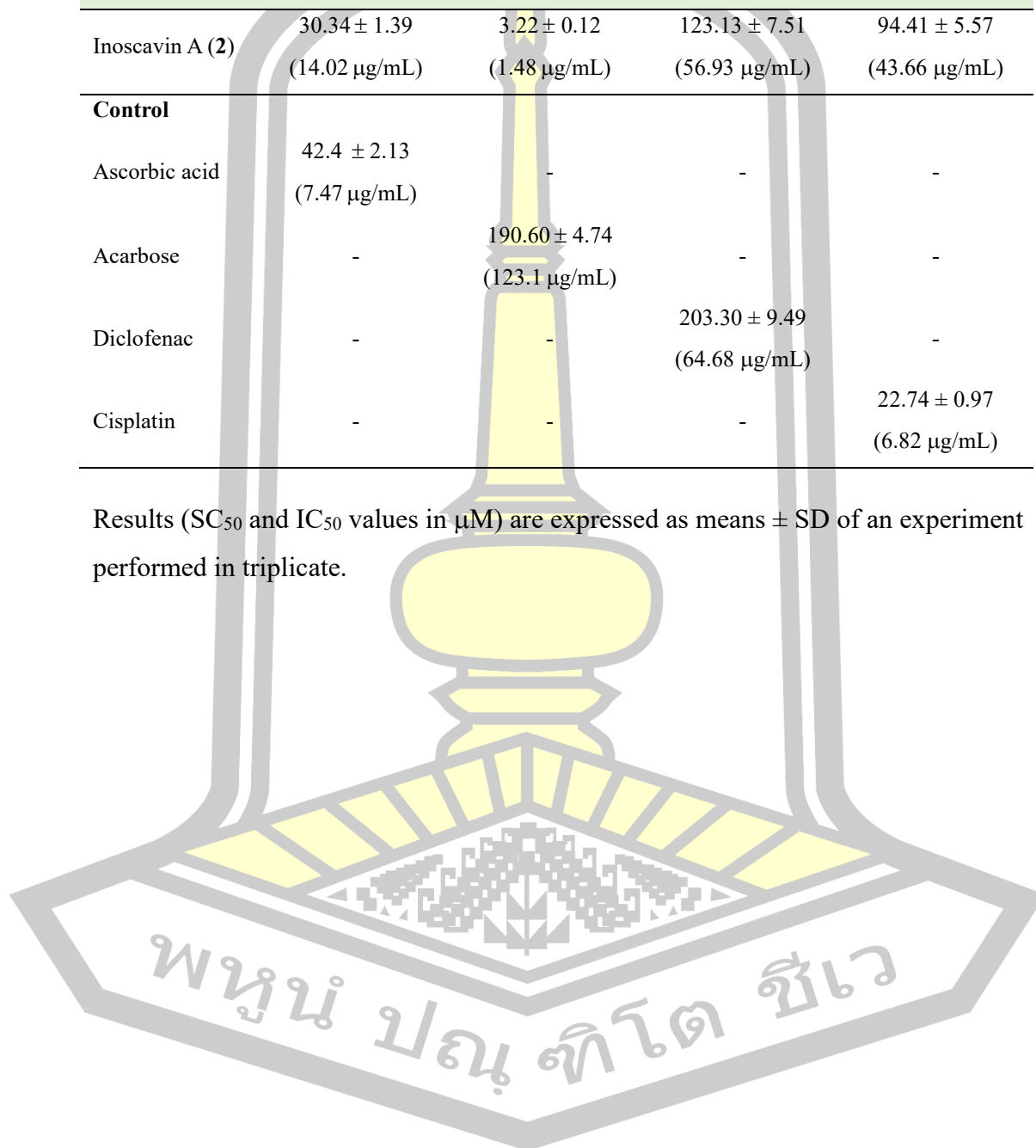


Table 12. Biological activities of inoscavin A (2).

Sample	DPPH Radical scavenging activity (SC ₅₀ ; μ M)	α -Glucosidase inhibitory activity (IC ₅₀ ; μ M)	Nitric oxide inhibitory activity (IC ₅₀ ; μ M)	Cytotoxicity against A549 cells (IC ₅₀ ; μ M)
Inoscavin A (2)	30.34 $\pm$ 1.39 (14.02 μ g/mL)	3.22 $\pm$ 0.12 (1.48 μ g/mL)	123.13 $\pm$ 7.51 (56.93 μ g/mL)	94.41 $\pm$ 5.57 (43.66 μ g/mL)
Control				
Ascorbic acid	42.4 $\pm$ 2.13 (7.47 μ g/mL)	-	-	-
Acarbose	-	190.60 $\pm$ 4.74 (123.1 μ g/mL)	-	-
Diclofenac	-	-	203.30 $\pm$ 9.49 (64.68 μ g/mL)	-
Cisplatin	-	-	-	22.74 $\pm$ 0.97 (6.82 μ g/mL)

Results (SC₅₀ and IC₅₀ values in μ M) are expressed as means $\pm$ SD of an experiment performed in triplicate.



CHAPTER 5

CONCLUSION

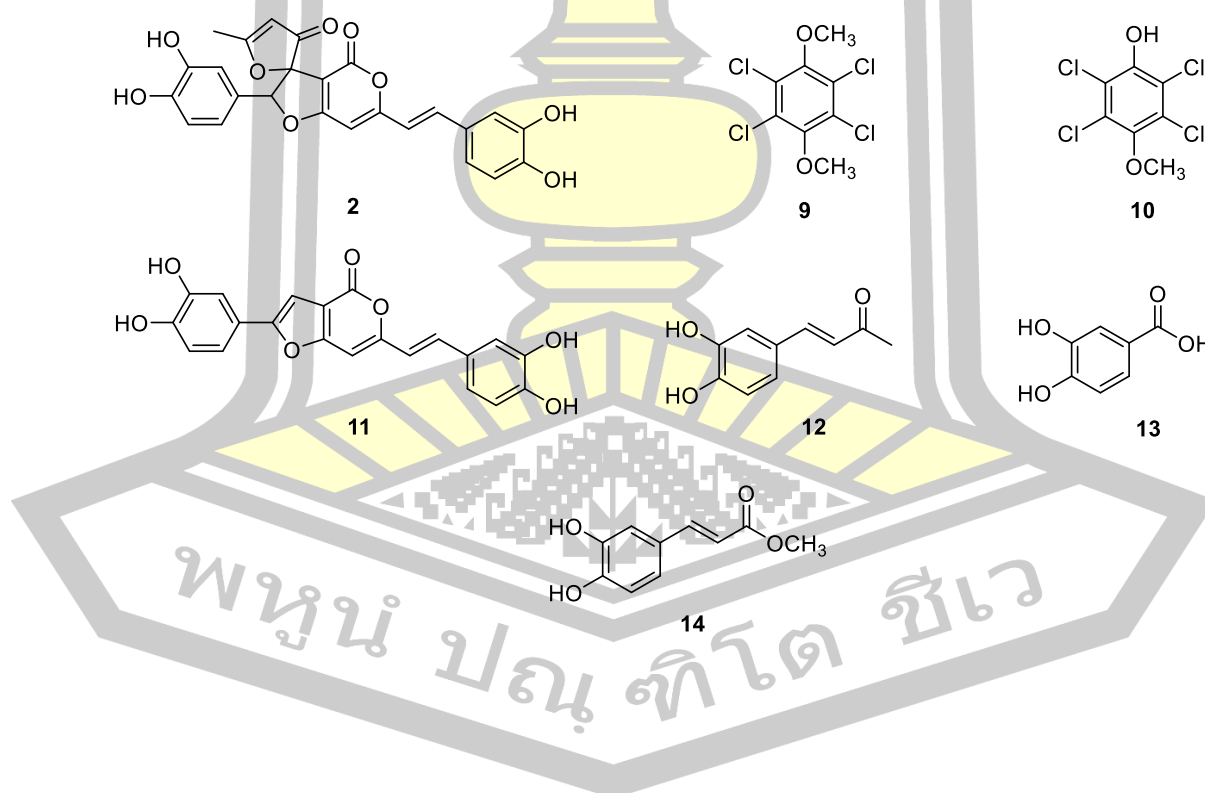
The natural fruiting bodies of *Fulvifomes fastuosus* (Lév.) Bondartseva & S. Herrera have been chemical investigated for its bioactive compounds. In this research, the natural fruiting bodies of *F. fastuosus* were collected from Udon Thani Province, Thailand. This fungus was identified as *F. fastuosus* based on ITS rDNA gene sequencing data. From literature survey, there are a few reports on the isolation of bioactive compounds from the genus *Fulvifomes*.

The DPPH radical scavenging, α -glucosidase and nitric oxide (NO) inhibitory activities, as well as the cytotoxicity against A549 lung cancer cell lines, of the crude CH_2Cl_2 , MeOH and EtOAc extracts from the fruiting bodies of *F. fastuosus* were evaluated. The crude CH_2Cl_2 extract showed no activity in any of the assays. However, the crude EtOAc extract, obtained by partitioning the MeOH extract, exhibited notable α -glucosidase ($1.38 \pm 0.03 \mu\text{g/mL}$) and NO ($42.62 \pm 2.47 \mu\text{g/mL}$) inhibitory activities compared to acarbose ($123.07 \pm 3.06 \mu\text{g/mL}$) and diclofenac ($65.87 \pm 1.91 \mu\text{g/mL}$), respectively.

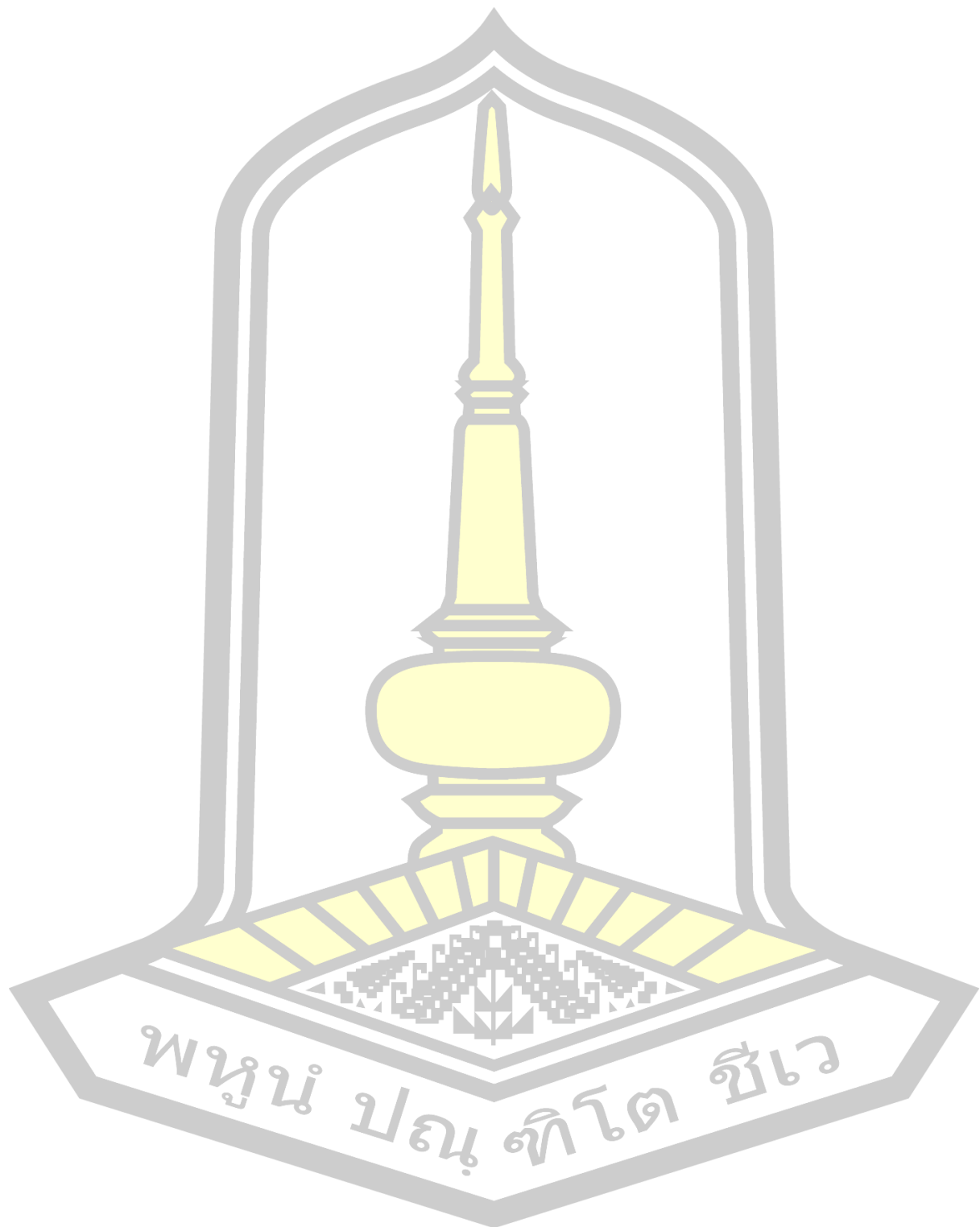
Chemical investigation of the CH_2Cl_2 extract led to the isolation of two chlorinated fungal metabolites; drosophilin A methyl ether (**9**) and drosophilin A (**10**). In contrast, inoscavin A (**2**), inoscavin E (**11**), 3,4-dihydroxybenzalacetone (**12**), protocatechuic acid (**13**), and caffeic acid methyl ester (**14**) were obtained from the EtOAc extract. Among these, inoscavin A (**2**) was identified as the major metabolite from the fruiting bodies of *F. fastuosus*.

Compound **2** exhibited potent α -glucosidase and NO inhibitory activities, with α -glucosidase inhibition ($3.22 \pm 0.12 \mu\text{M}$; $1.48 \mu\text{g/mL}$) and NO inhibition ($123.13 \pm 7.51 \mu\text{M}$; $56.93 \mu\text{g/mL}$), both stronger than those of acarbose ($190.60 \pm 4.74 \mu\text{M}$; $123.1 \mu\text{g/mL}$) and diclofenac ($203.30 \pm 9.49 \mu\text{M}$; $64.68 \mu\text{g/mL}$). This activity was comparable to that of the MeOH extract ($\text{IC}_{50} 1.54 \pm 0.01 \mu\text{g/mL}$) and the EtOAc extract ($\text{IC}_{50} 1.38 \pm 0.03 \mu\text{g/mL}$).

These results suggest that *F. fastuosus* is a promising source of antidiabetic agents, with inoscavin A as a potent lead compound. The synergistic activity observed in the crude extracts highlights the importance of optimizing extraction strategies for therapeutic application of antidiabetic natural products.



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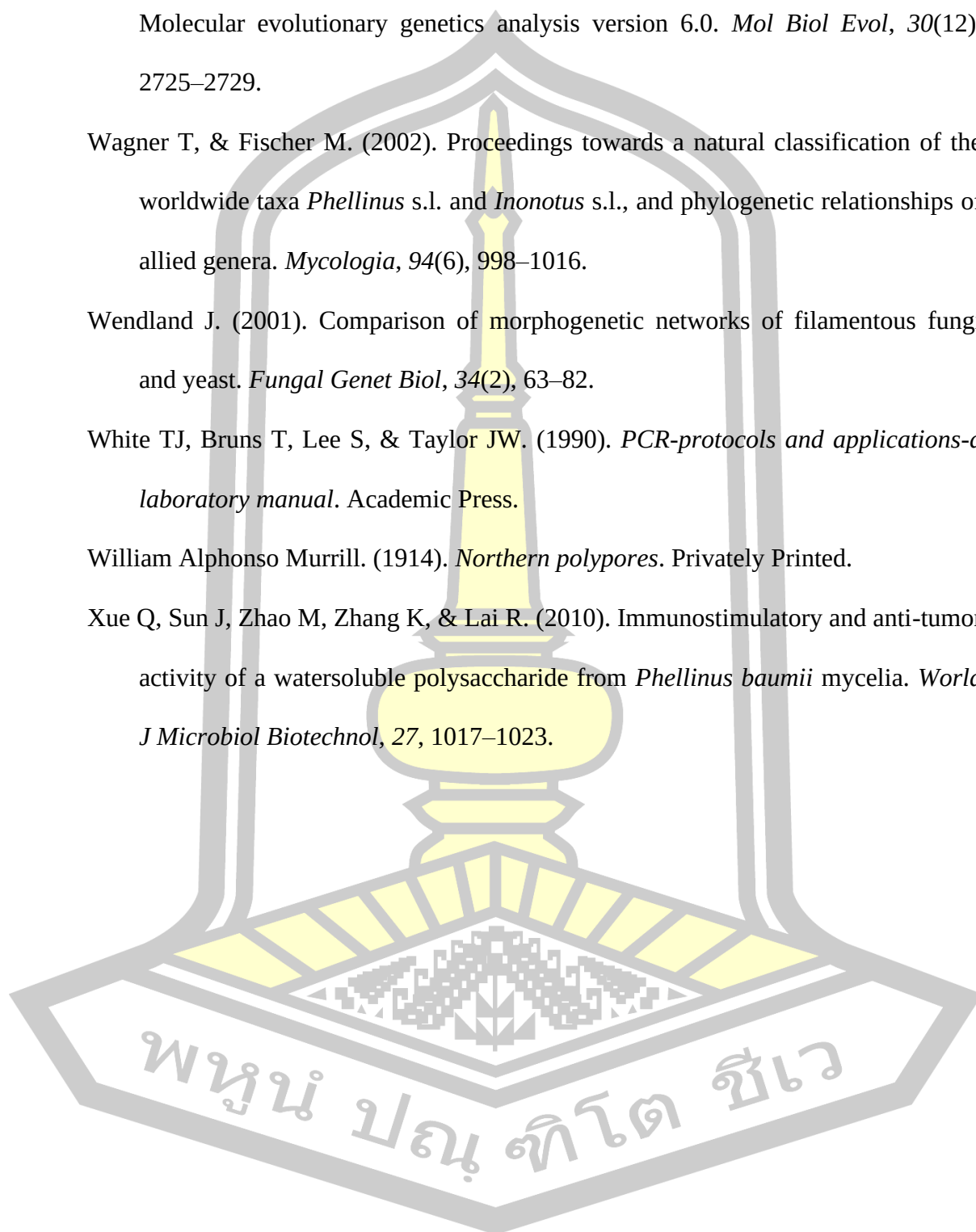
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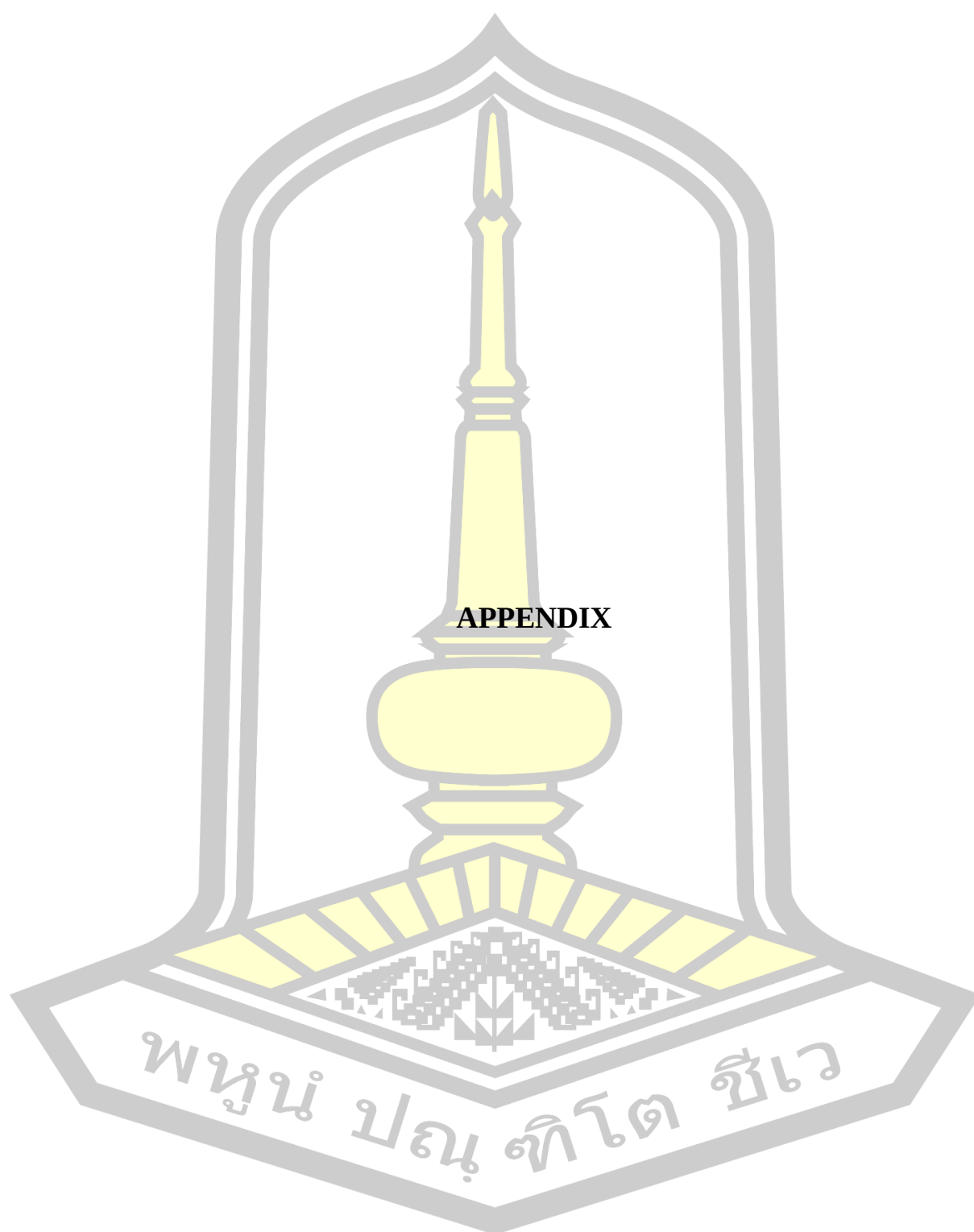
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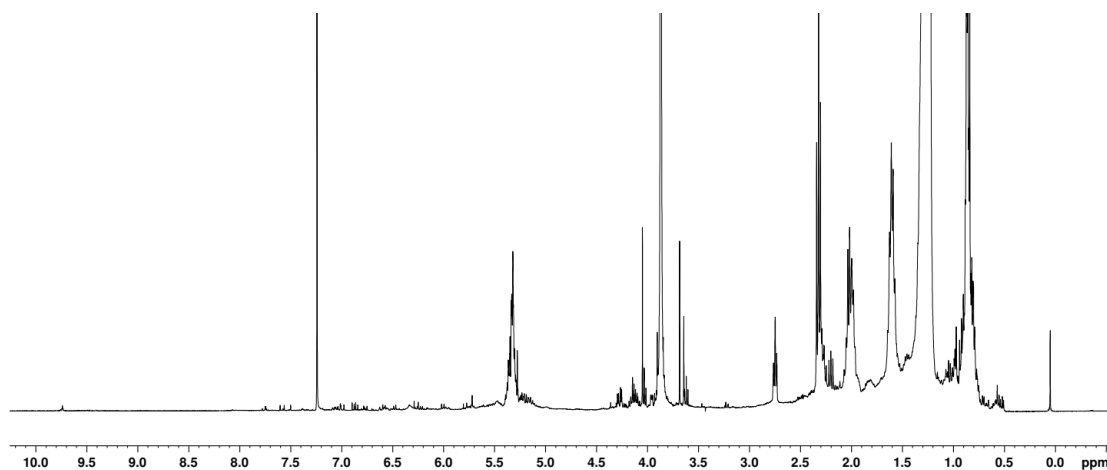


Figure A1. ^1H NMR spectrum (400 MHz) of the crude CH_2Cl_2 extract from the fruiting bodies of *F. fastuosus* in CDCl_3 .

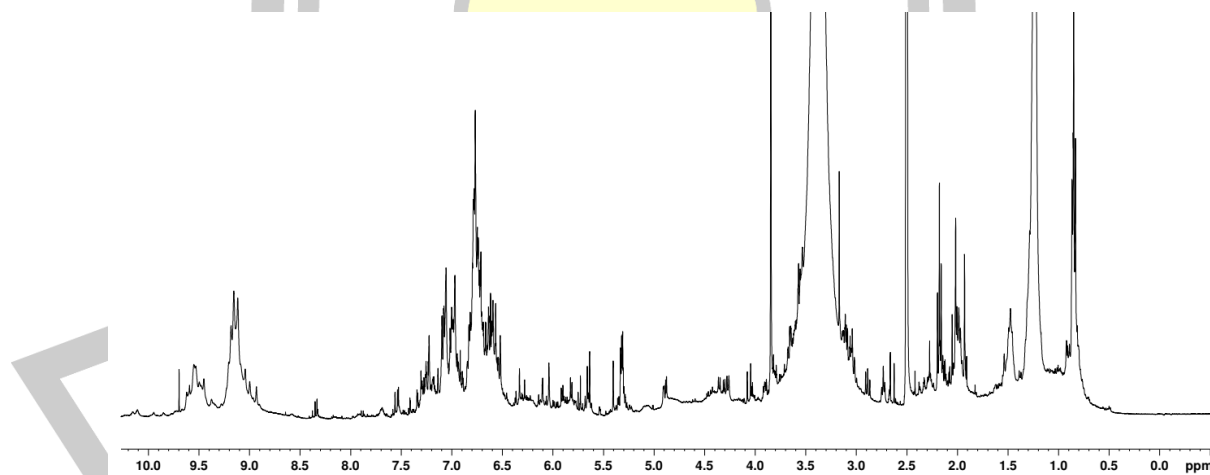


Figure A2. ^1H NMR spectrum (400 MHz) of the crude MeOH extract from the fruiting bodies of *F. fastuosus* in $\text{DMSO}-d_6$.

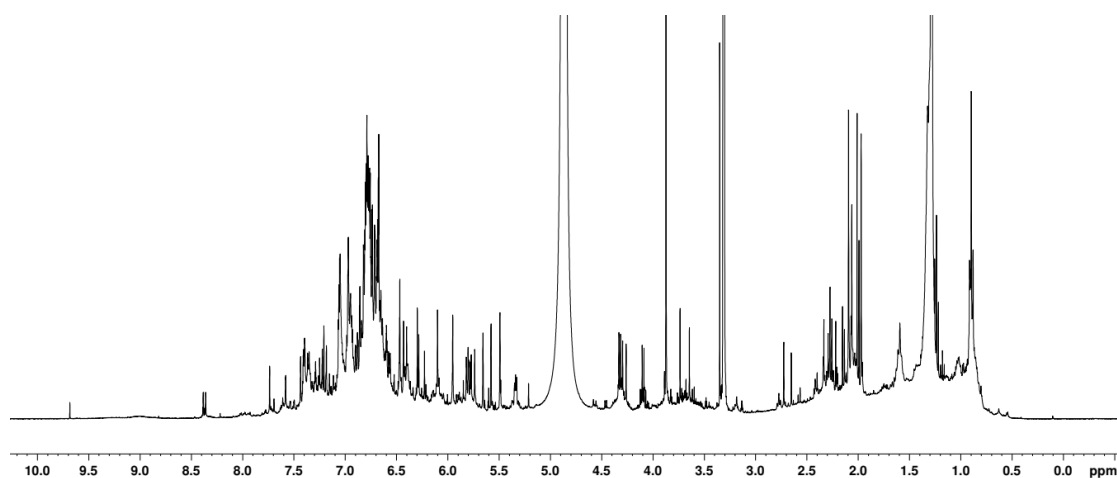


Figure A3. ¹H NMR spectrum (400 MHz) of the crude EtOAc extract from the fruiting bodies of *F. fastuosus* in CD₃OD.

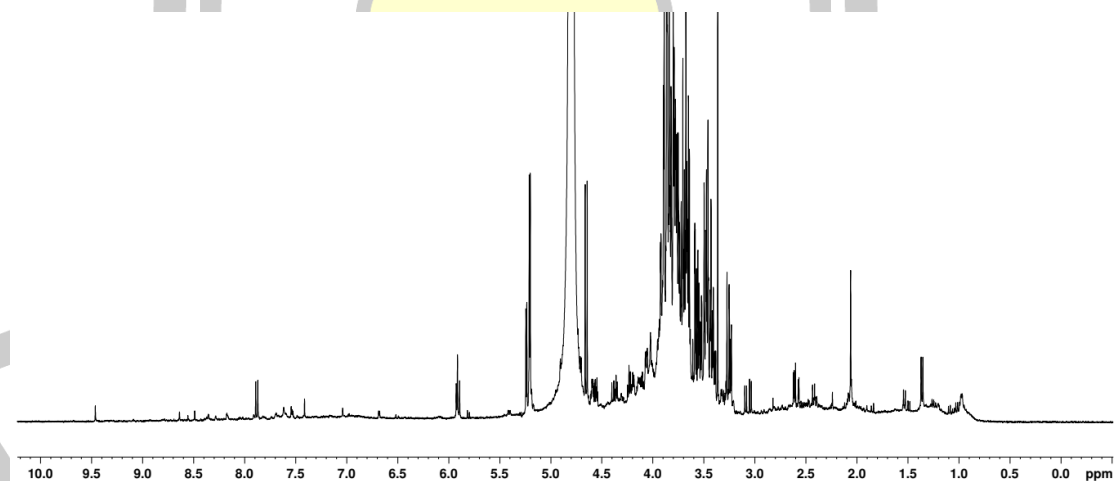


Figure A4. ¹H NMR spectrum (400 MHz) of the crude aqueous extract from the fruiting bodies of *F. fastuosus* in D₂O.

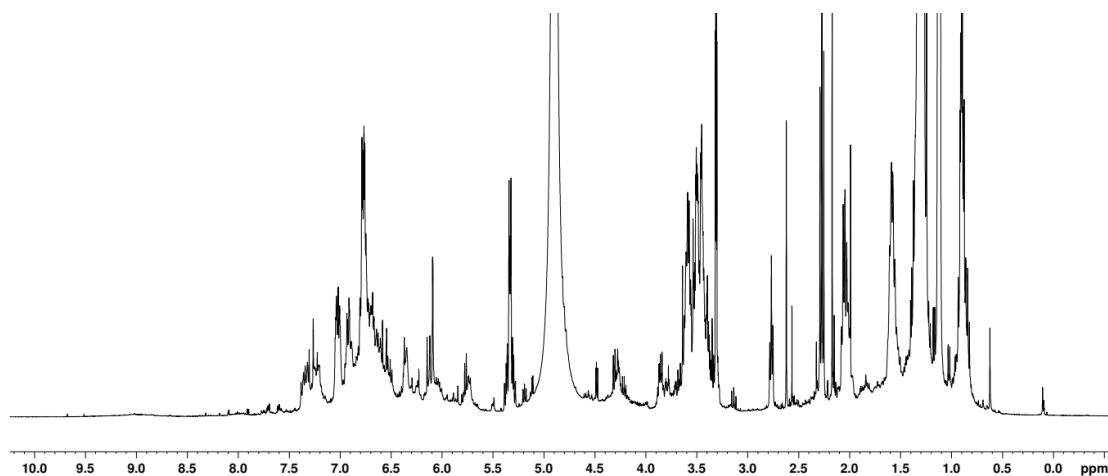


Figure A5. ^1H NMR spectrum (400 MHz) of the crude mycelial extract from the cultivated of *F. fastuosus* (MSUCC043) in CD_3OD .

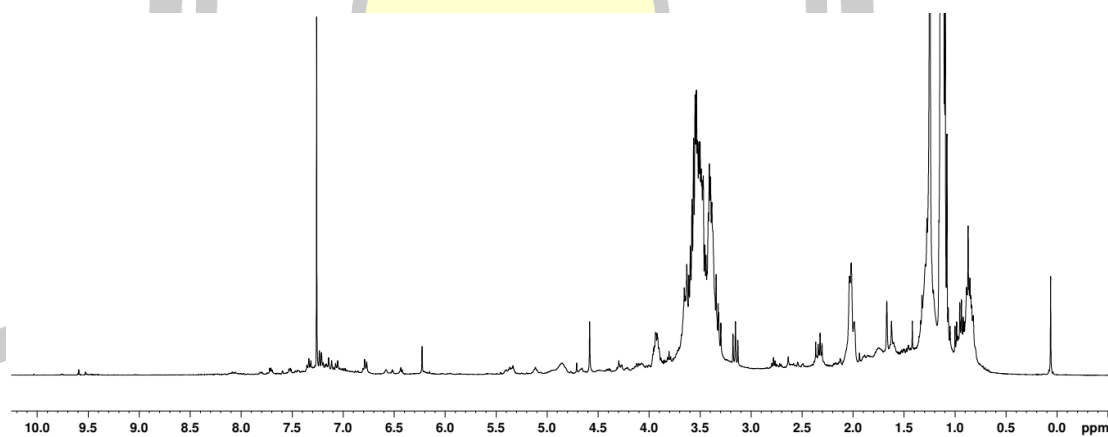


Figure A6. ^1H NMR spectrum (400 MHz) of the crude broth extract from the cultivated of *F. fastuosus* (MSUCC043) in CD_3OD .

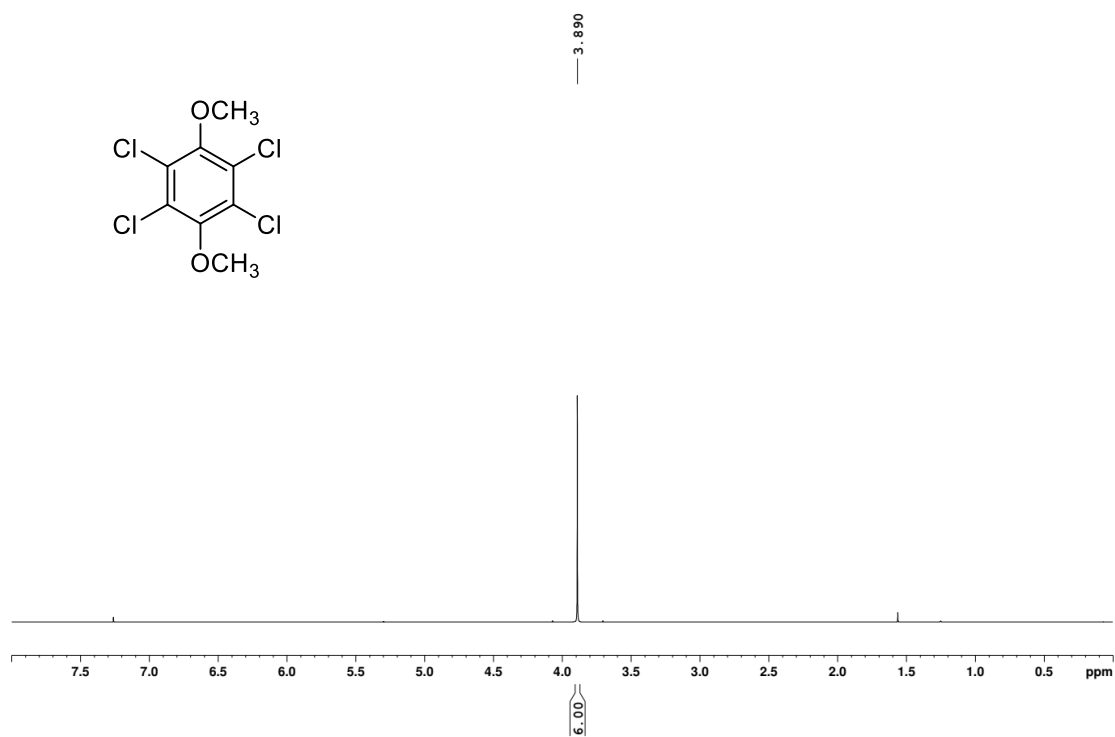


Figure A7. ^1H NMR spectrum (400 MHz) of **9** in CDCl_3 .

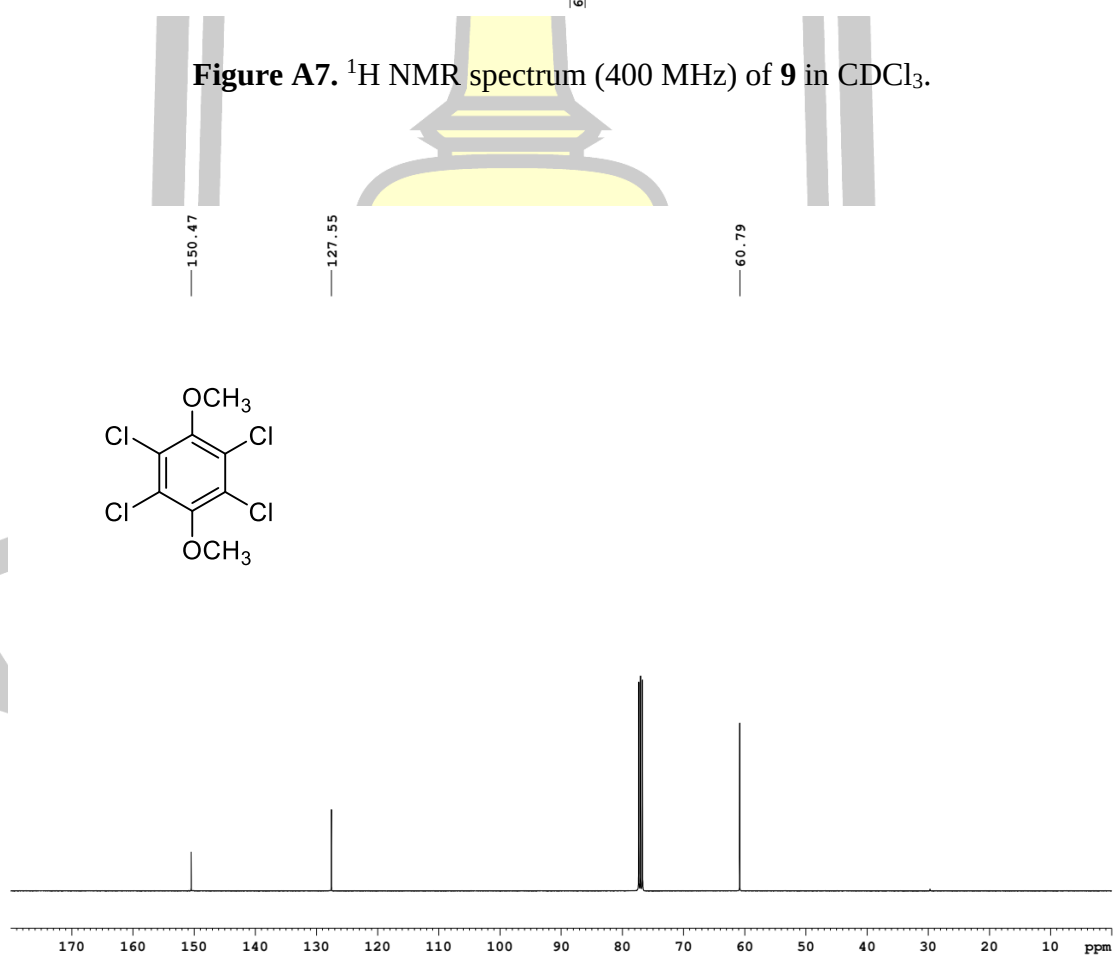


Figure A8. ^{13}C NMR spectrum (400 MHz) of **9** in CDCl_3 .

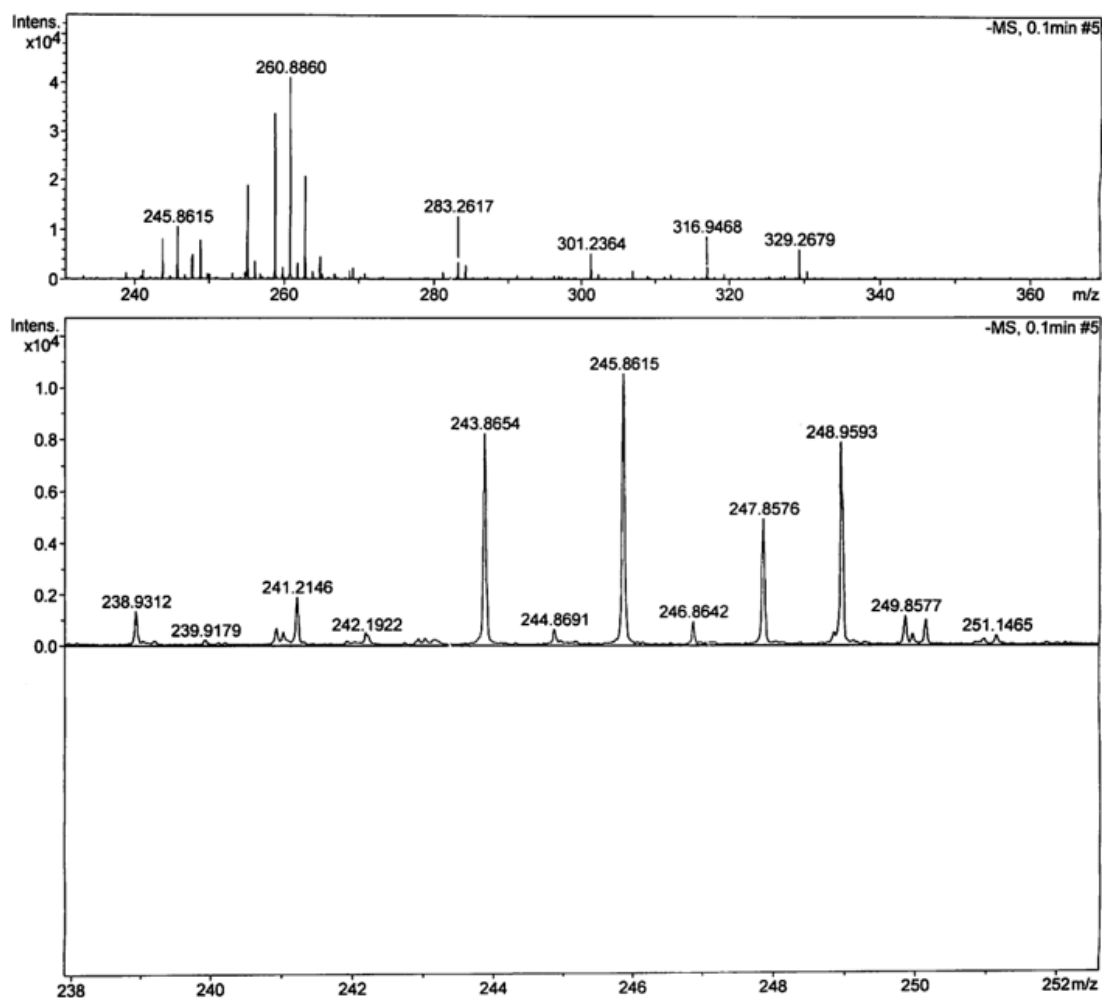
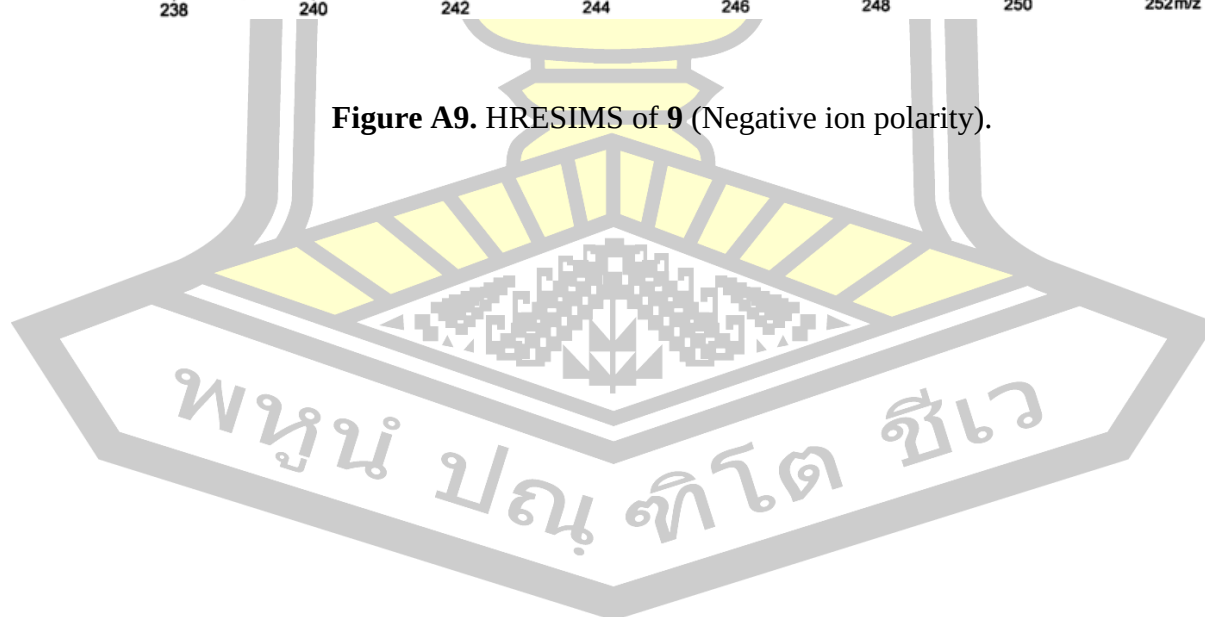


Figure A9. HRESIMS of **9** (Negative ion polarity).



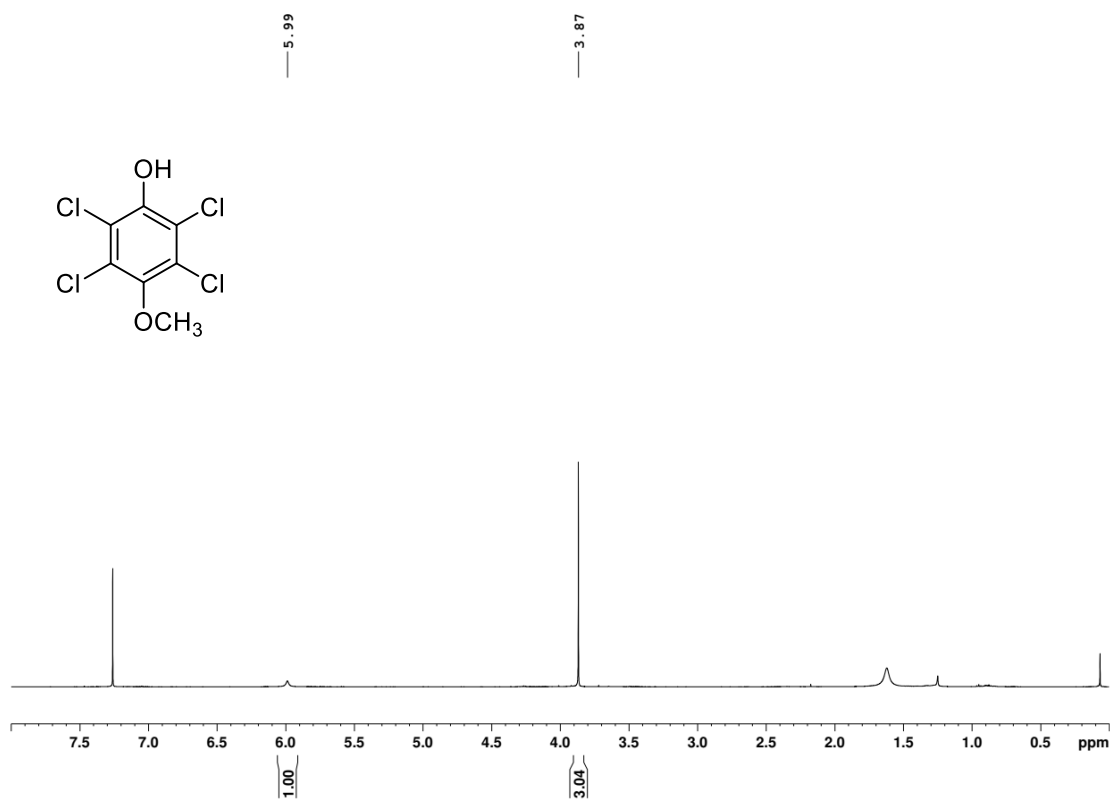


Figure A10. ^1H NMR spectrum (400 MHz) of **10** in CDCl_3 .

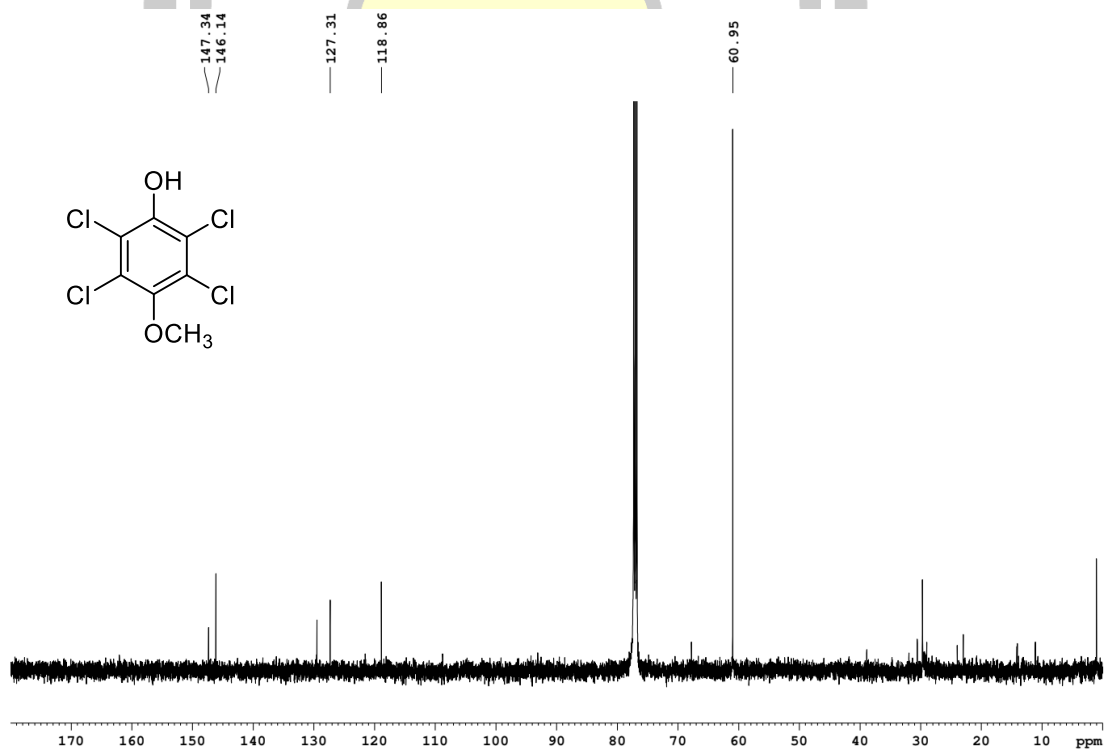


Figure A11. ^{13}C NMR spectrum (400 MHz) of **10** in CDCl_3 .

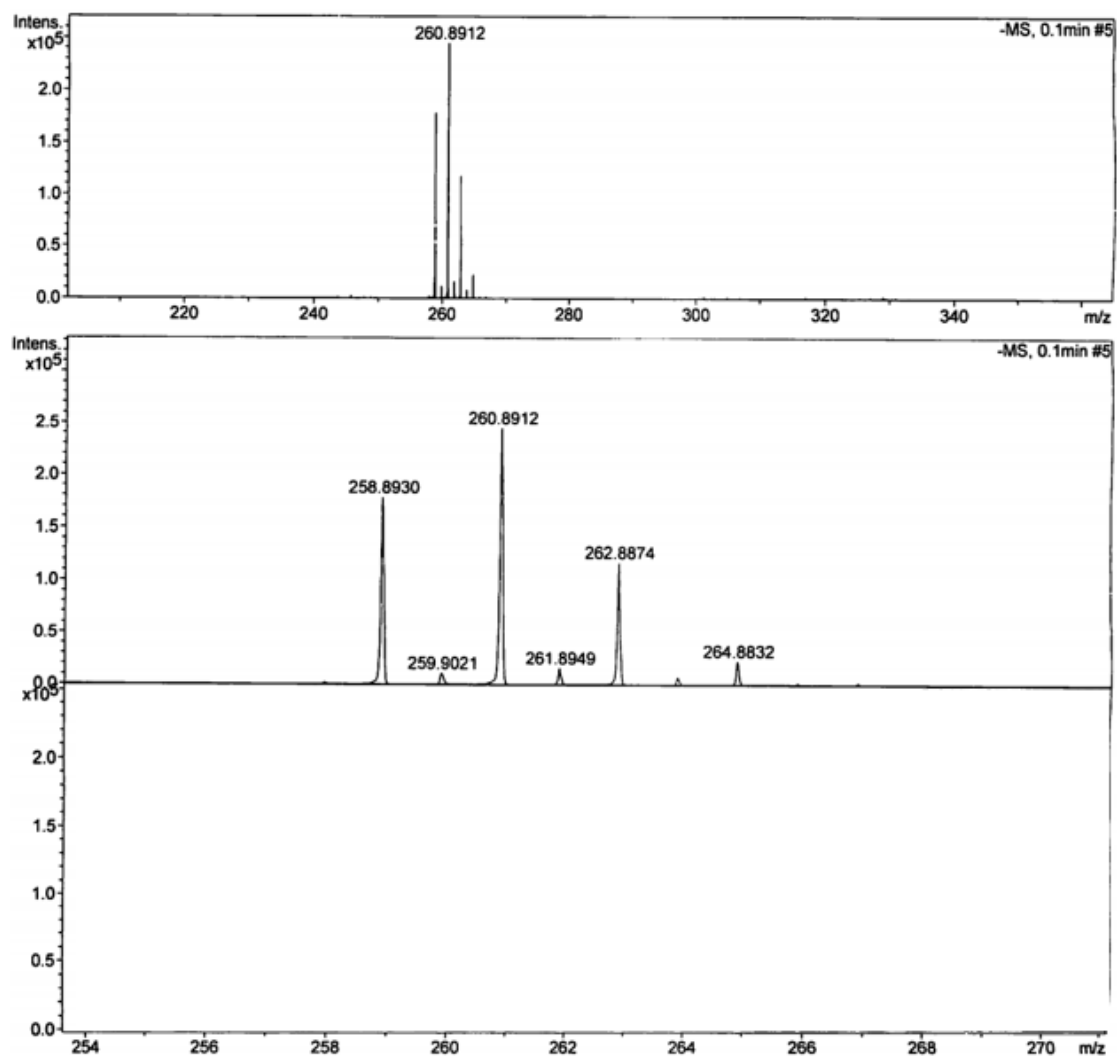


Figure A12. HRESIMS of **10** (Negative ion polarity).



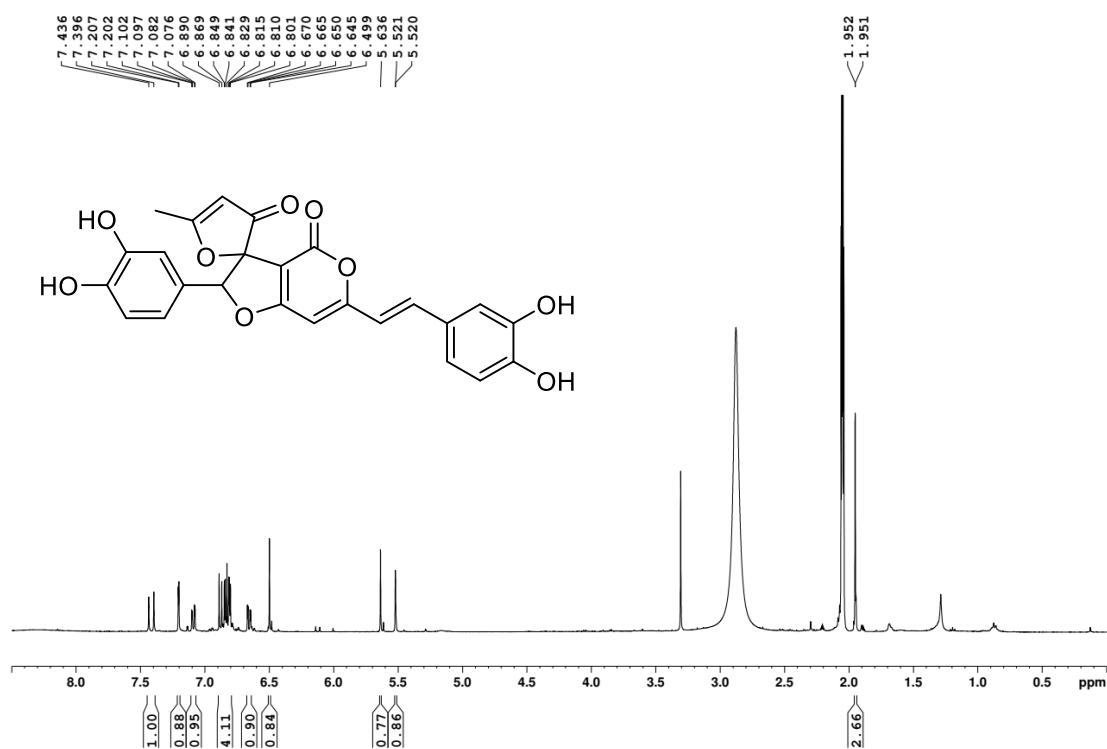


Figure A13. ^1H NMR spectrum (400 MHz) of **2** in acetone- d_6 .

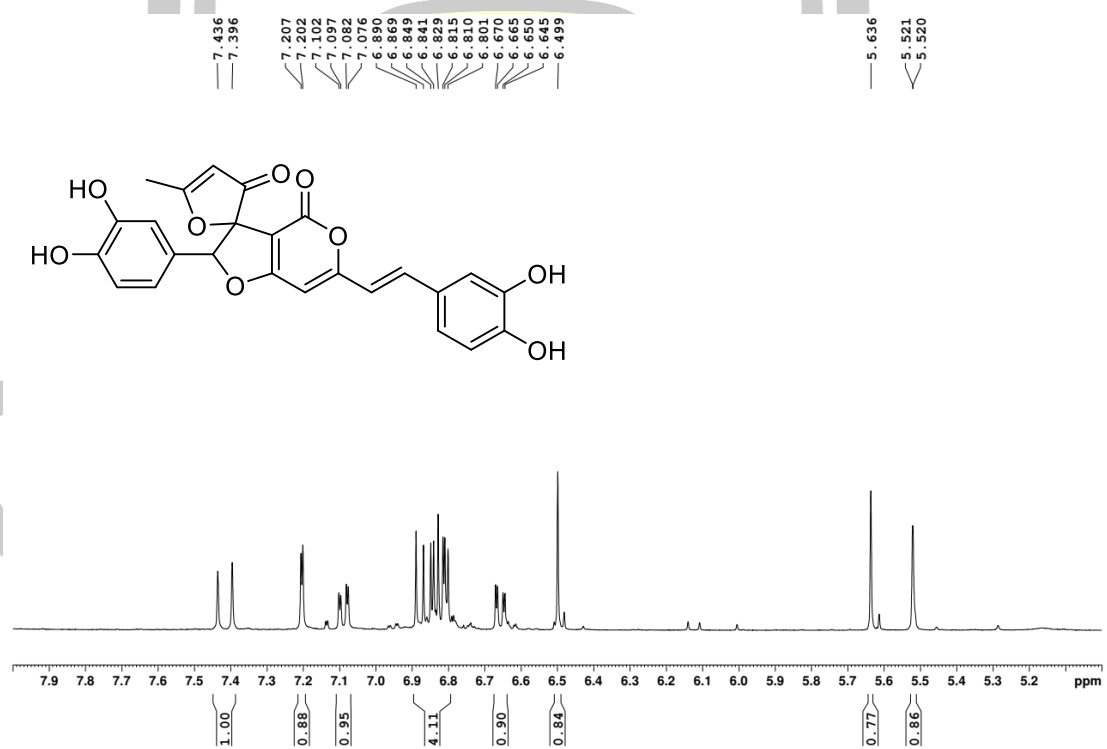


Figure A14. ^1H NMR spectrum (400 MHz) of **2** in acetone- d_6 .

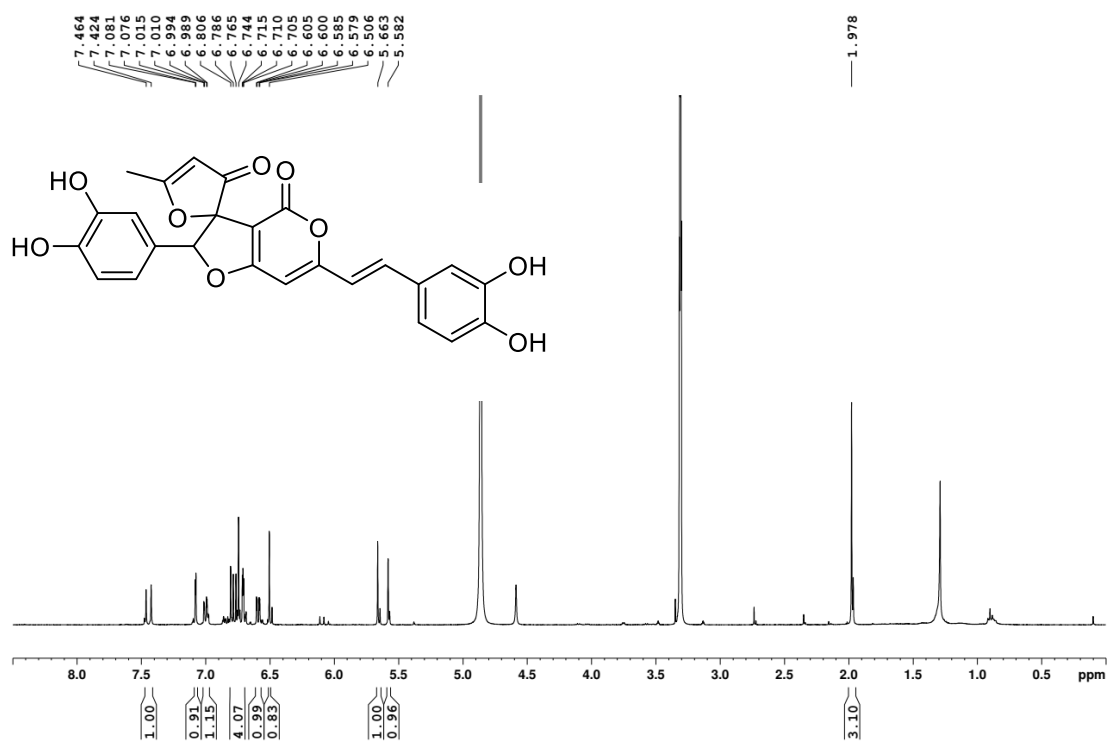


Figure A15. ^1H NMR spectrum (400 MHz) of 2 in CD_3OD .

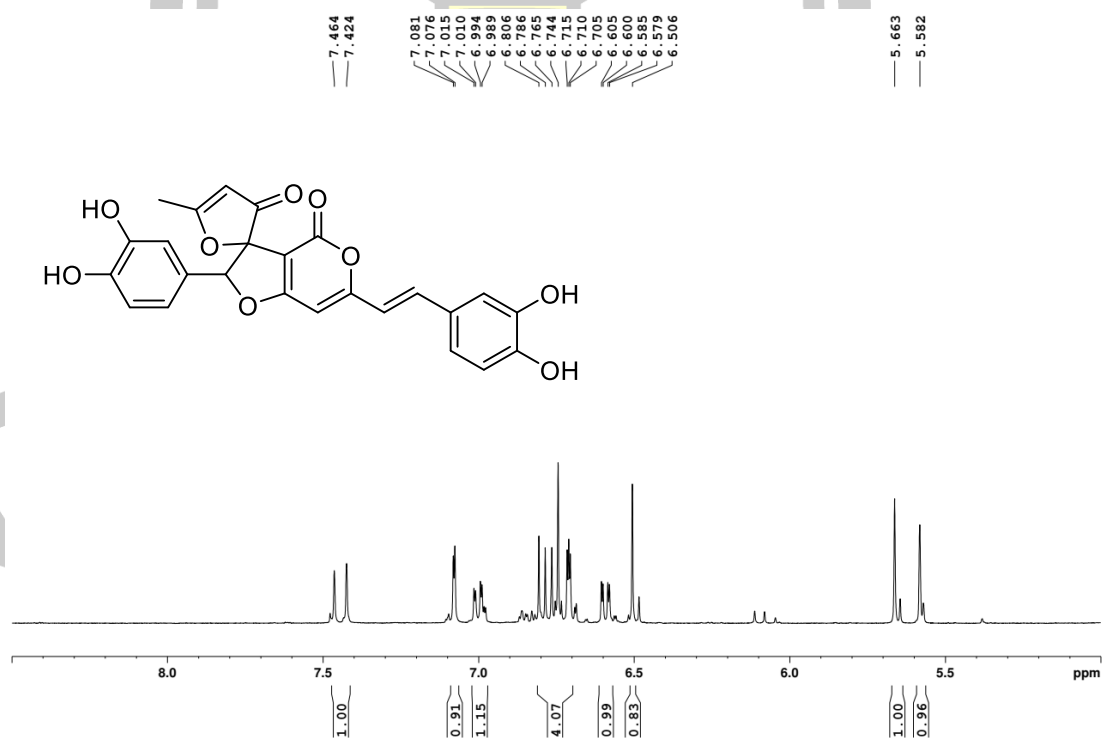


Figure A16. ^1H NMR spectrum (400 MHz) of 2 in CD_3OD .

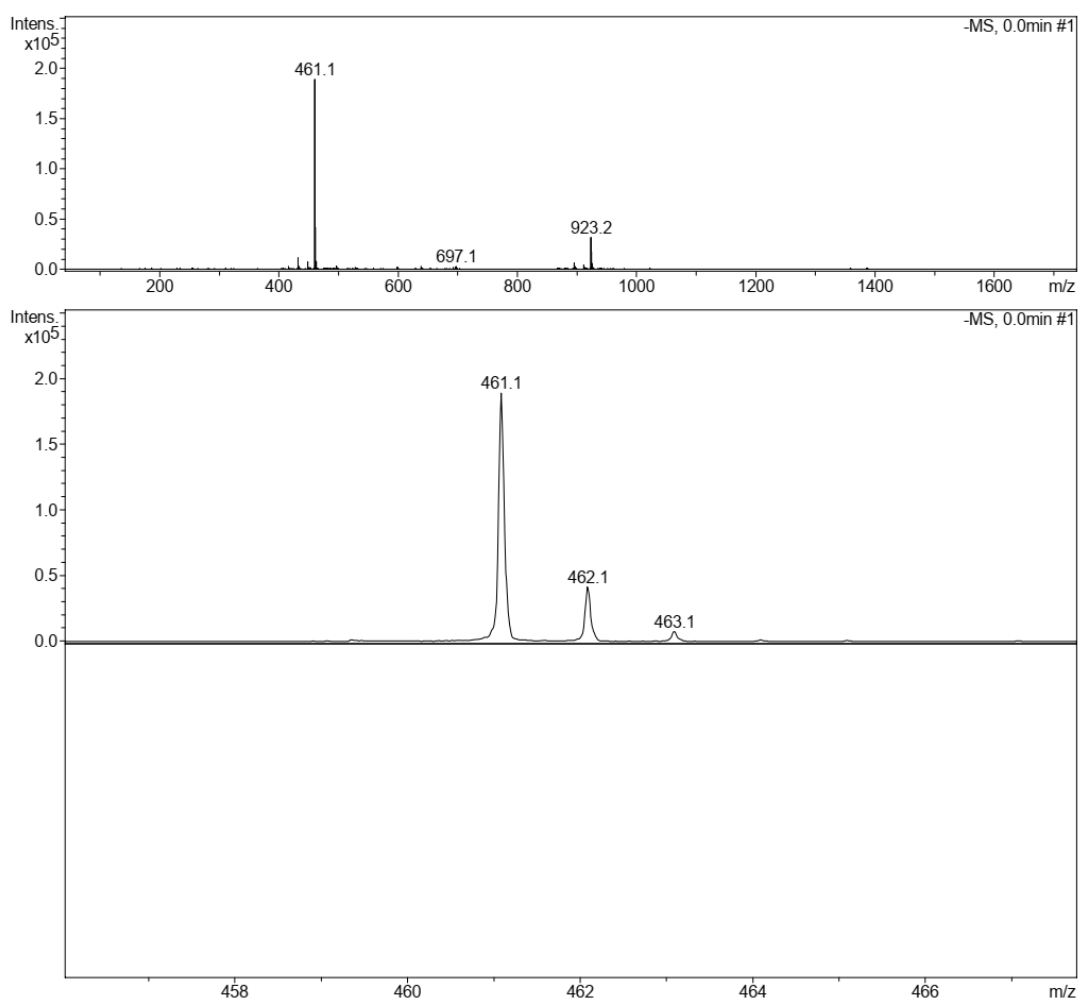
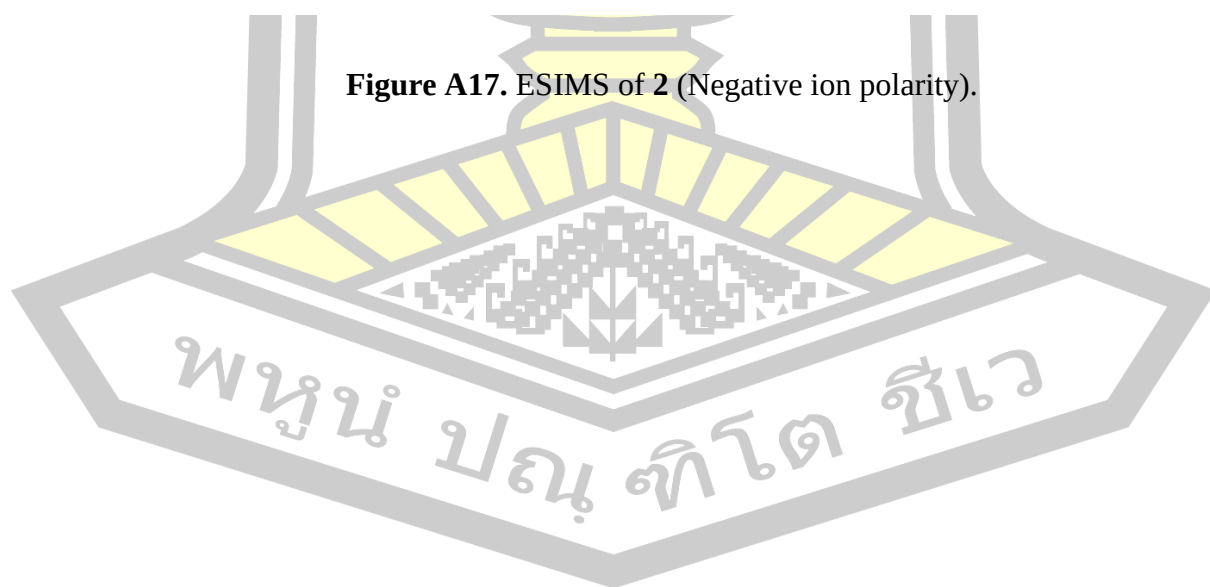


Figure A17. ESIMS of 2 (Negative ion polarity).



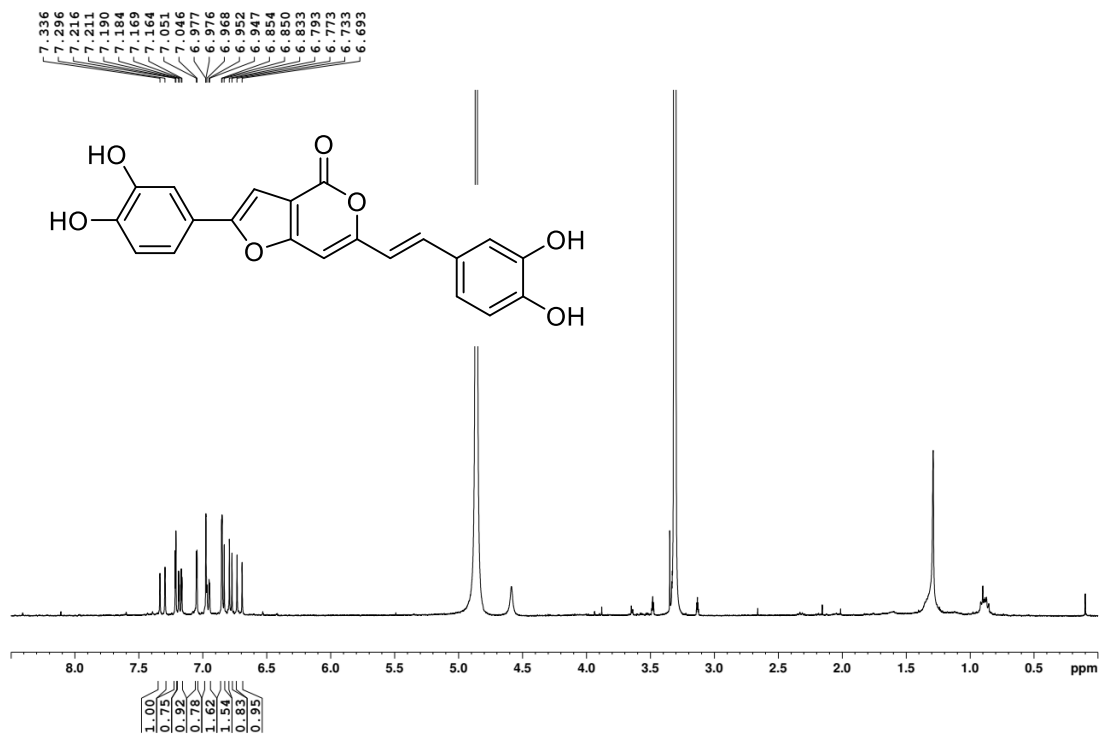


Figure A18. ¹H NMR spectrum (400 MHz) of **11** in CD₃OD.

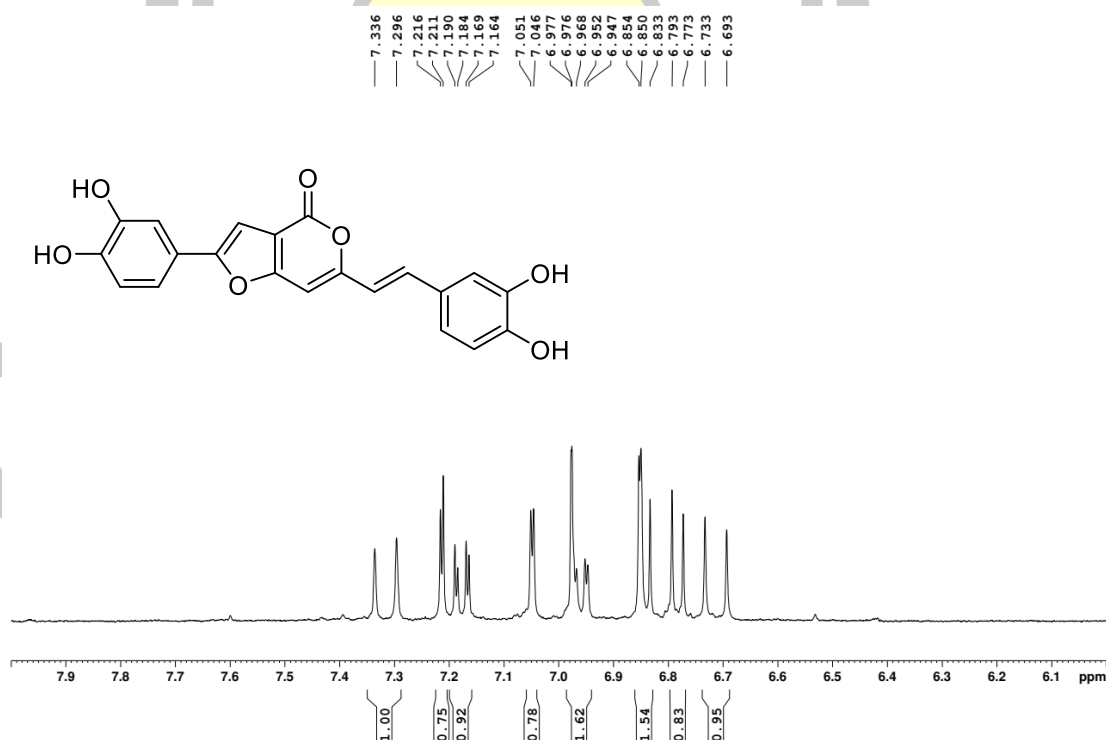


Figure A19. ¹H NMR spectrum (400 MHz) of **11** in CD₃OD.

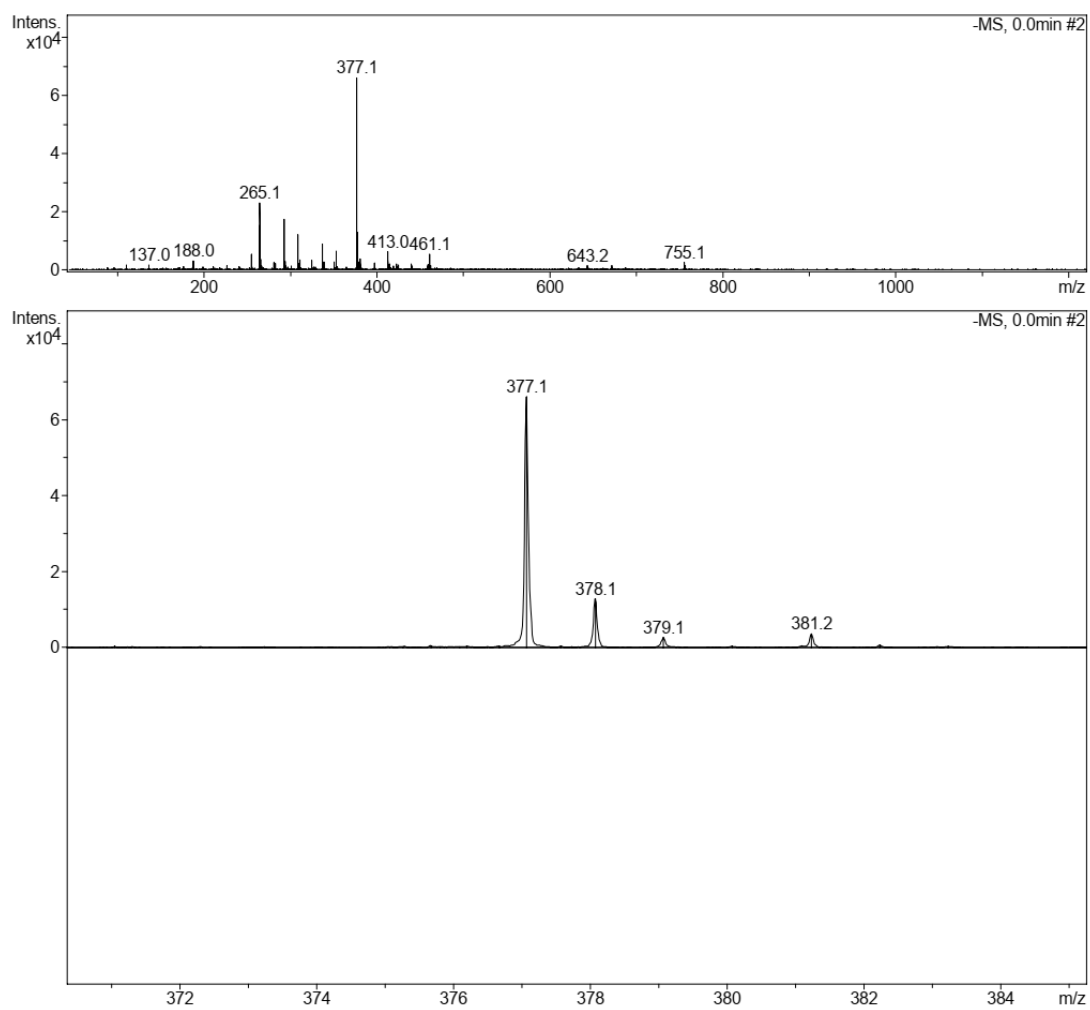
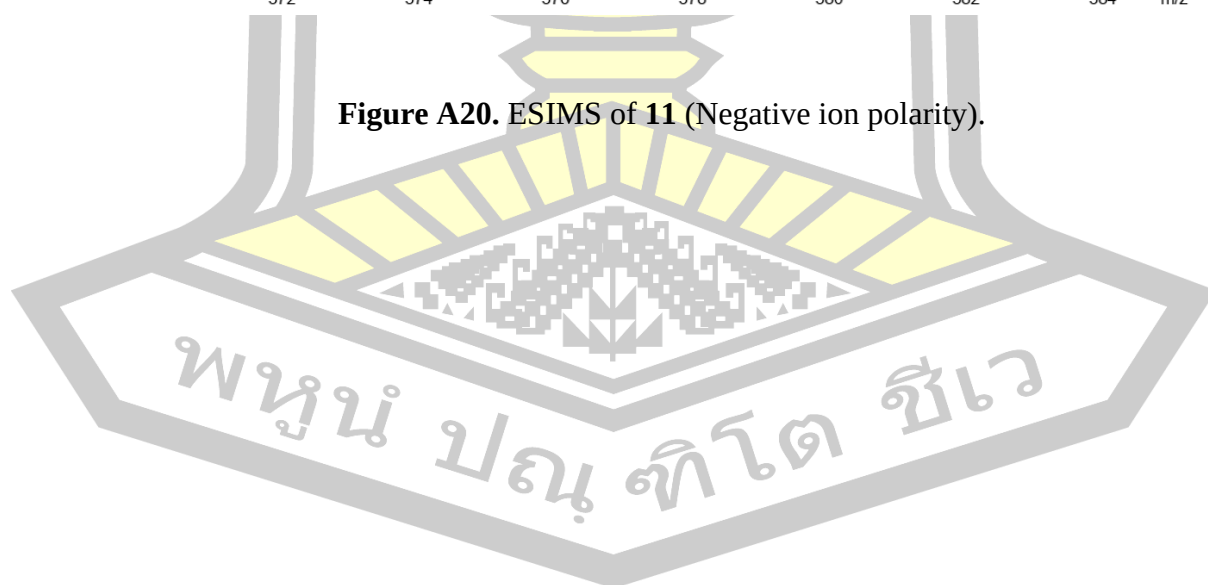


Figure A20. ESI-MS of **11** (Negative ion polarity).



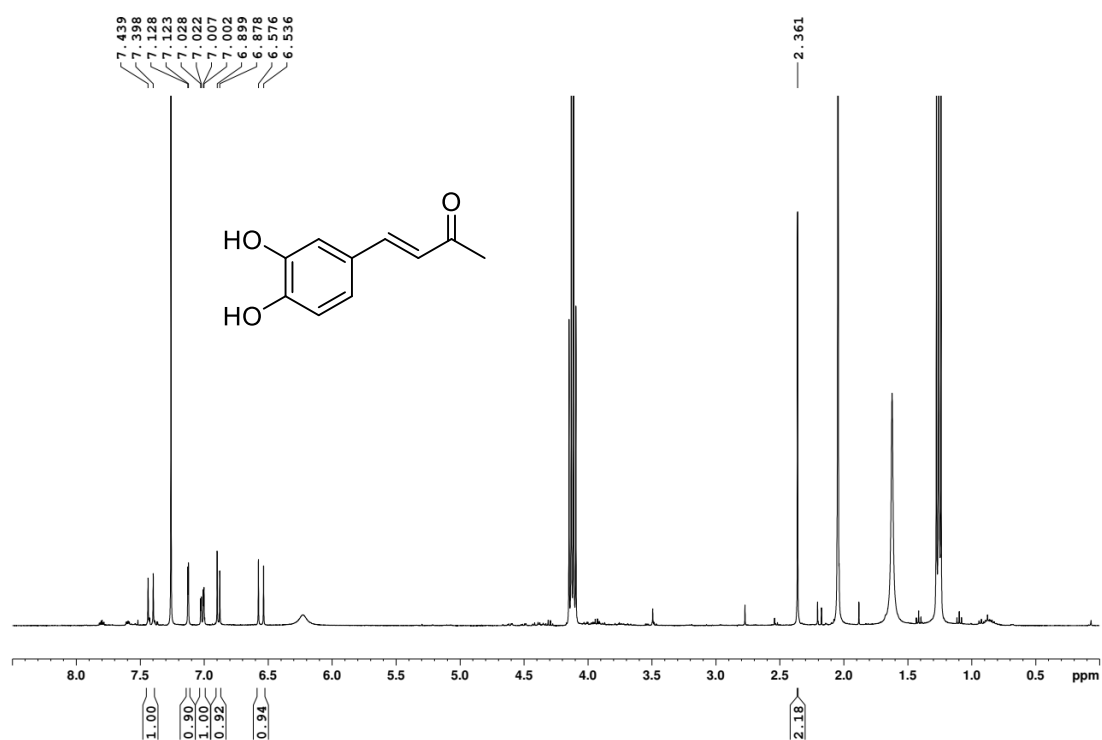


Figure A21. ¹H NMR spectrum (400 MHz) of **12** in CDCl₃.

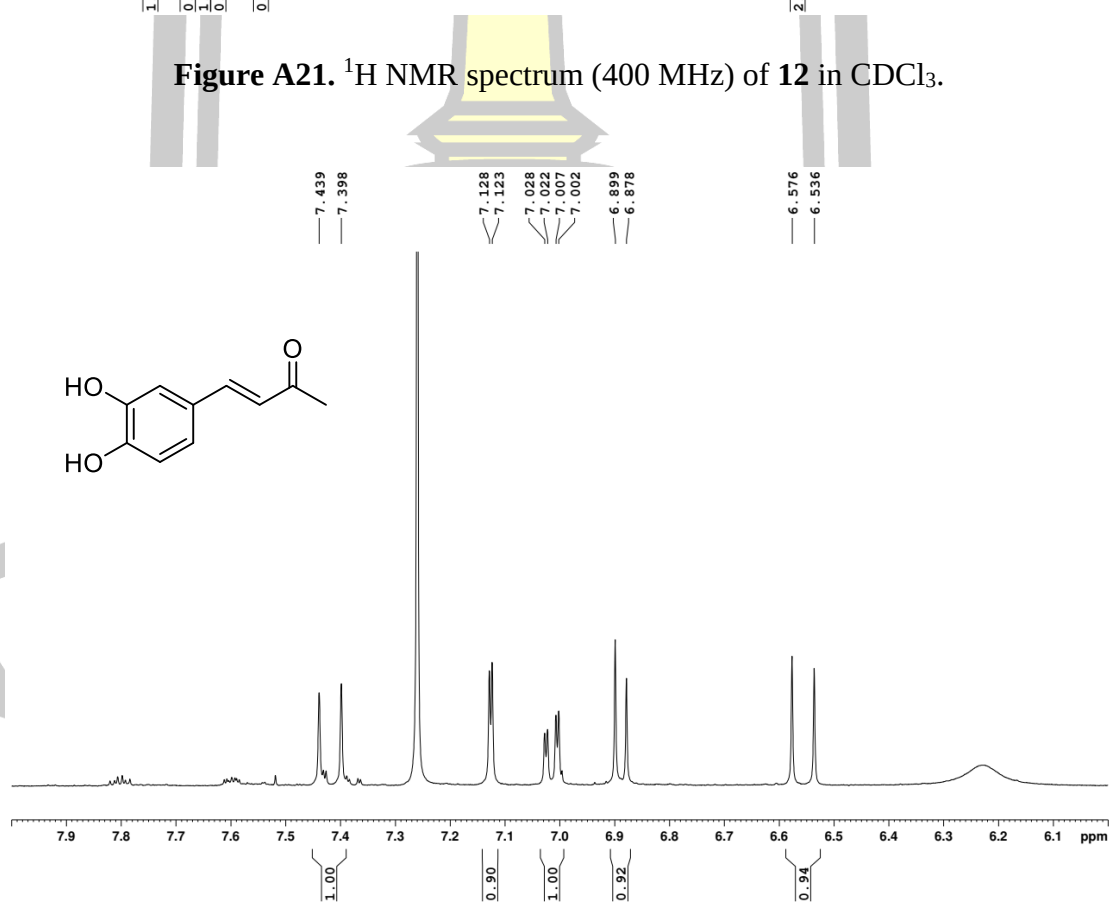


Figure A22. ¹H NMR spectrum (400 MHz) of **12** in CDCl₃.

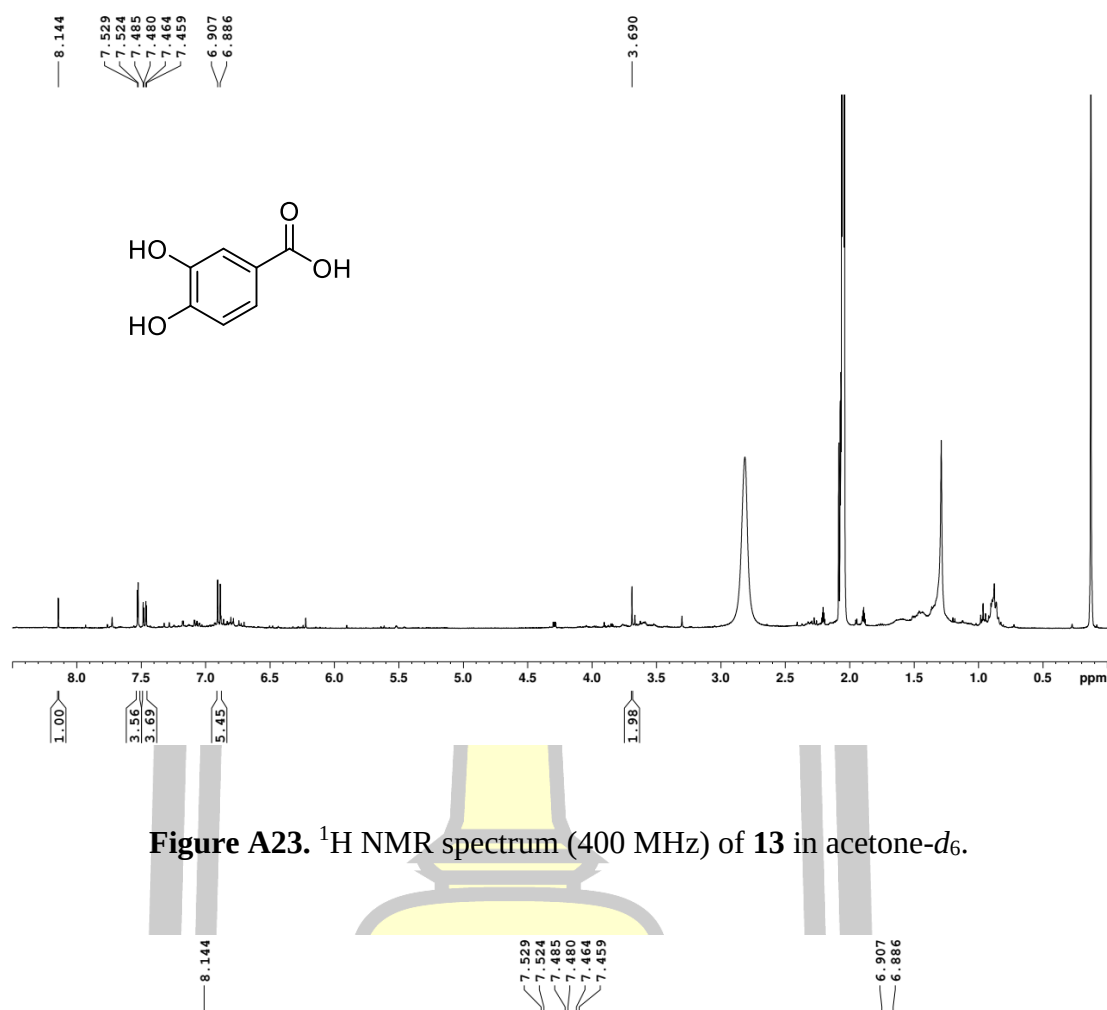


Figure A23. ¹H NMR spectrum (400 MHz) of **13** in acetone-*d*₆.

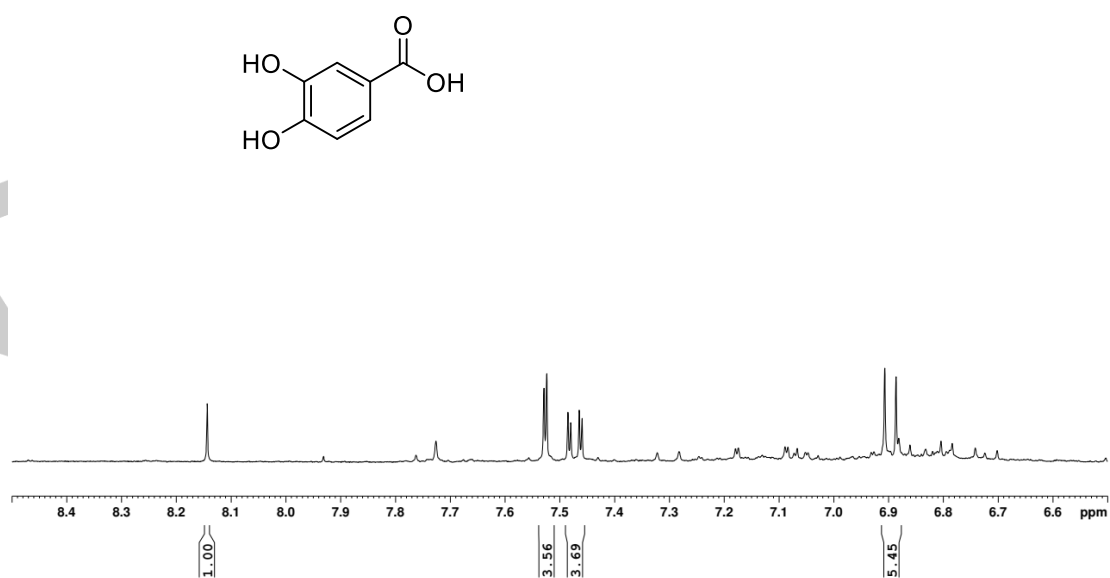


Figure A24. ¹H NMR spectrum (400 MHz) of **13** in acetone-*d*₆.

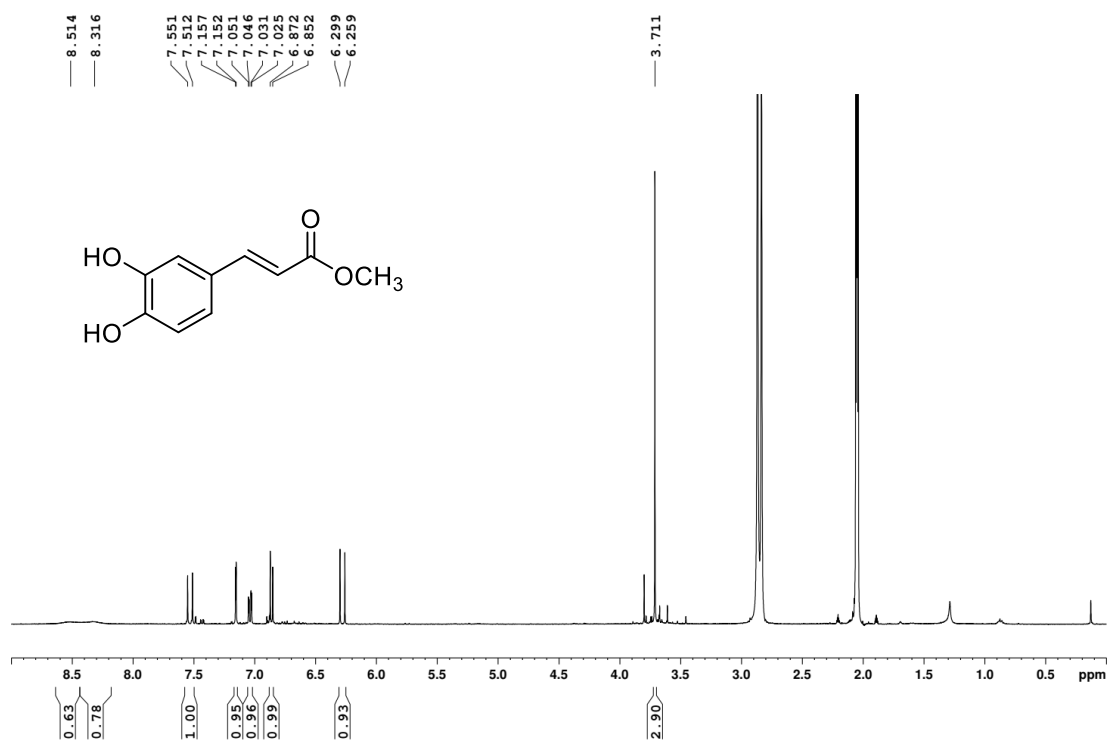


Figure A25. ¹H NMR spectrum (400 MHz) of **14** in acetone-*d*₆.

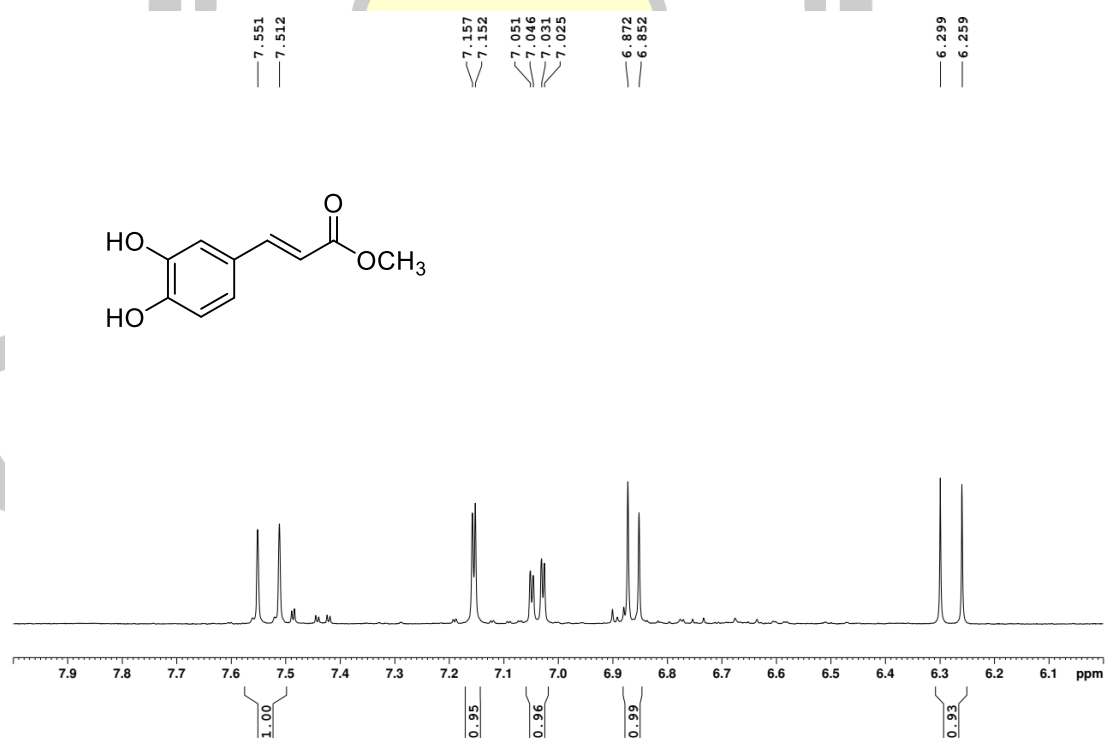


Figure A26. ¹H NMR spectrum (400 MHz) of **14** in acetone-*d*₆.

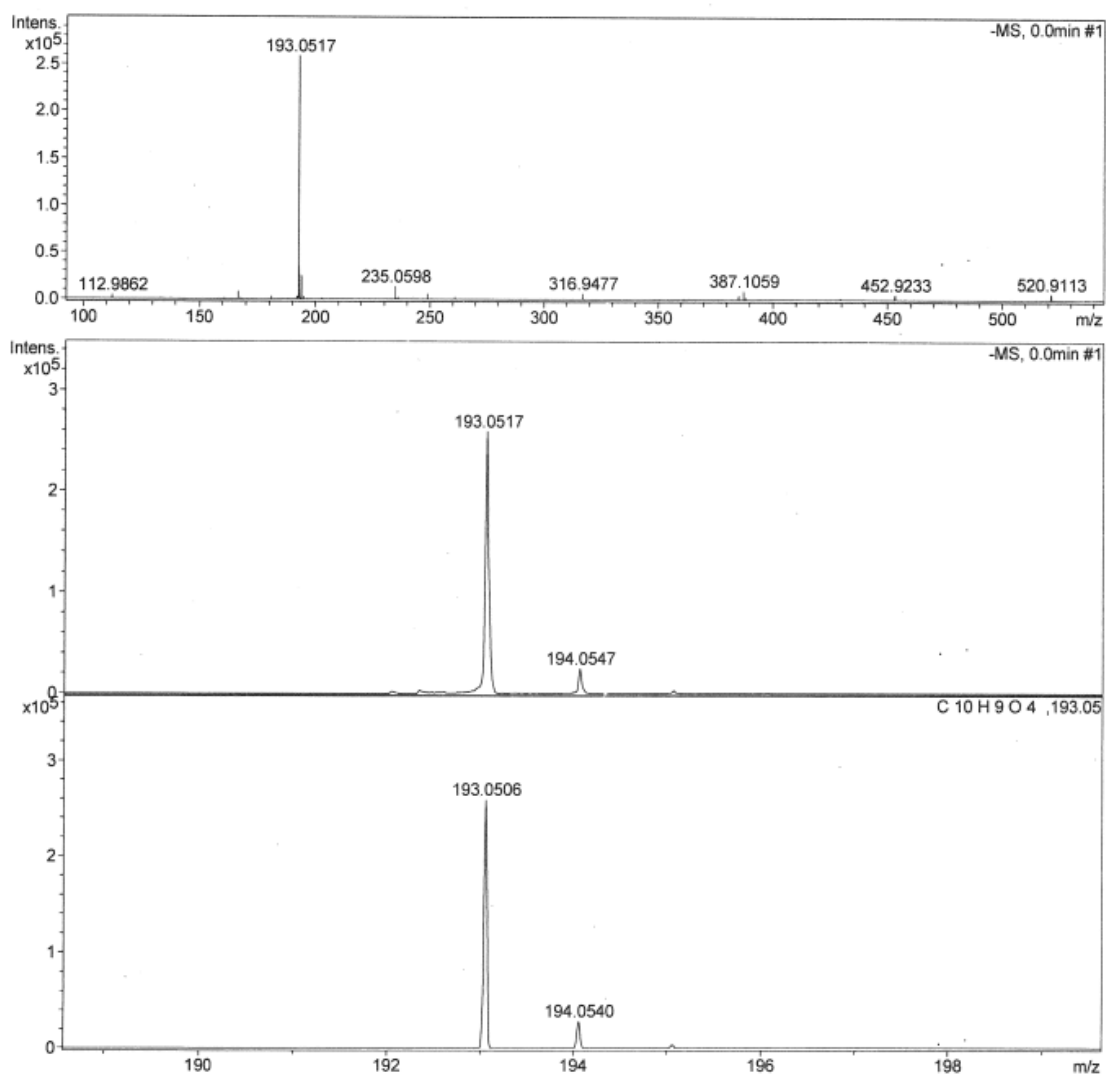
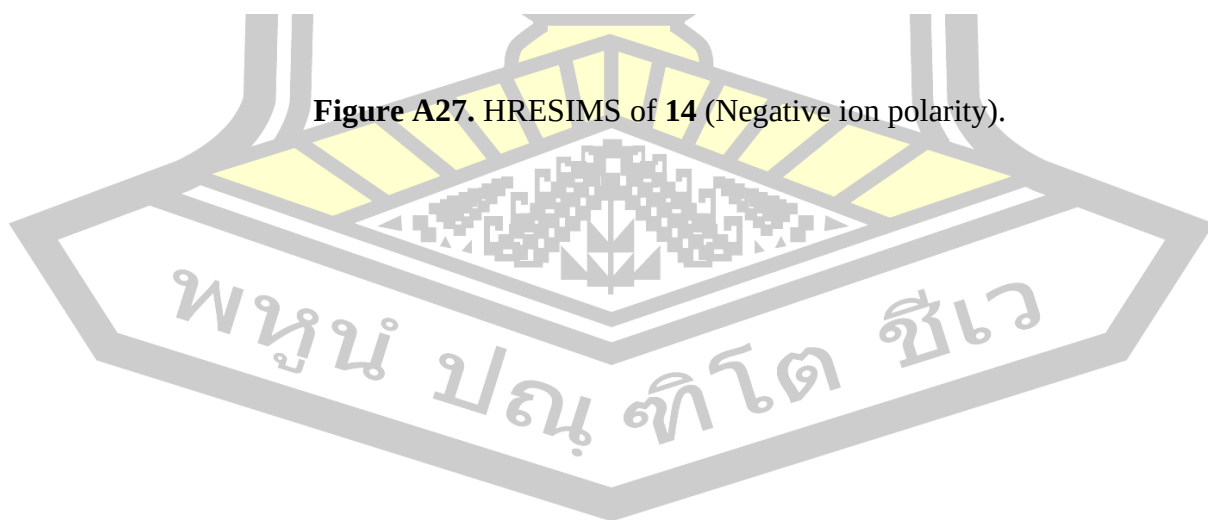


Figure A27. HRESIMS of 14 (Negative ion polarity).



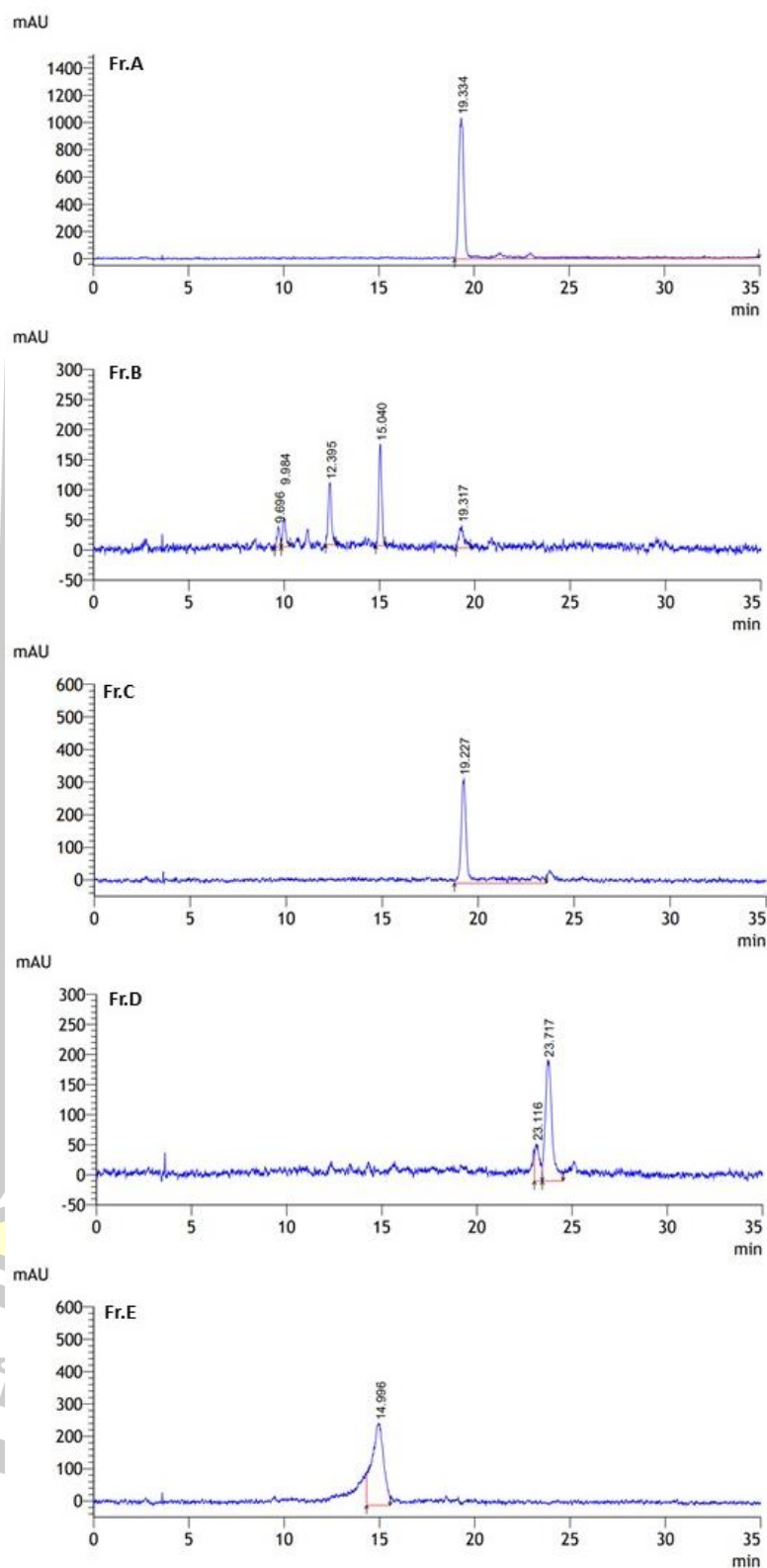


Figure A28. HPLC chromatograms of the isolated fractions from the crude EtOAc extract of *Fulvifomes fastuosus*, analyzed using an analytical C18 reversed-phase

column (4.6×150 mm, $5 \mu\text{m}$) with gradient elution (1% acetic acid–MeCN from 80:20 to 70:30 over 40 min) a flow rate of 1.0 mL/min. The sample concentration was 500 ppm, with a $20 \mu\text{L}$ injection volume, and detection was performed using a photodiode array at $\lambda = 400$ nm.

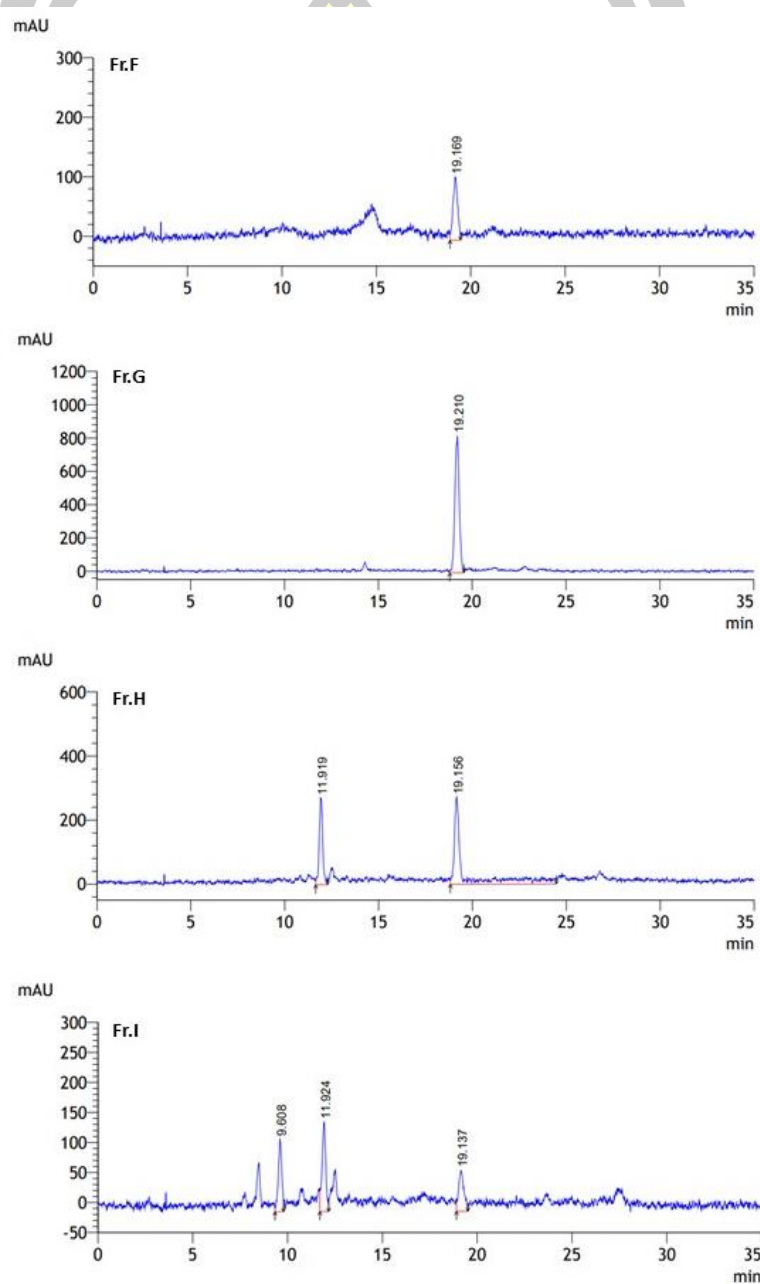


Figure A28. (cont.) HPLC chromatograms of the isolated fractions from the crude EtOAc extract of *Fulvifomes fastuosus*, analyzed using an analytical C18 reversed-phase column (4.6×150 mm, $5 \mu\text{m}$) with gradient elution (1% acetic acid–MeCN from 80:20 to 70:30 over 40 min) a flow rate of 1.0 mL/min. The sample concentration was 500 ppm, with a 20 μL injection volume, and detection was performed using a photodiode array at $\lambda = 400$ nm.



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