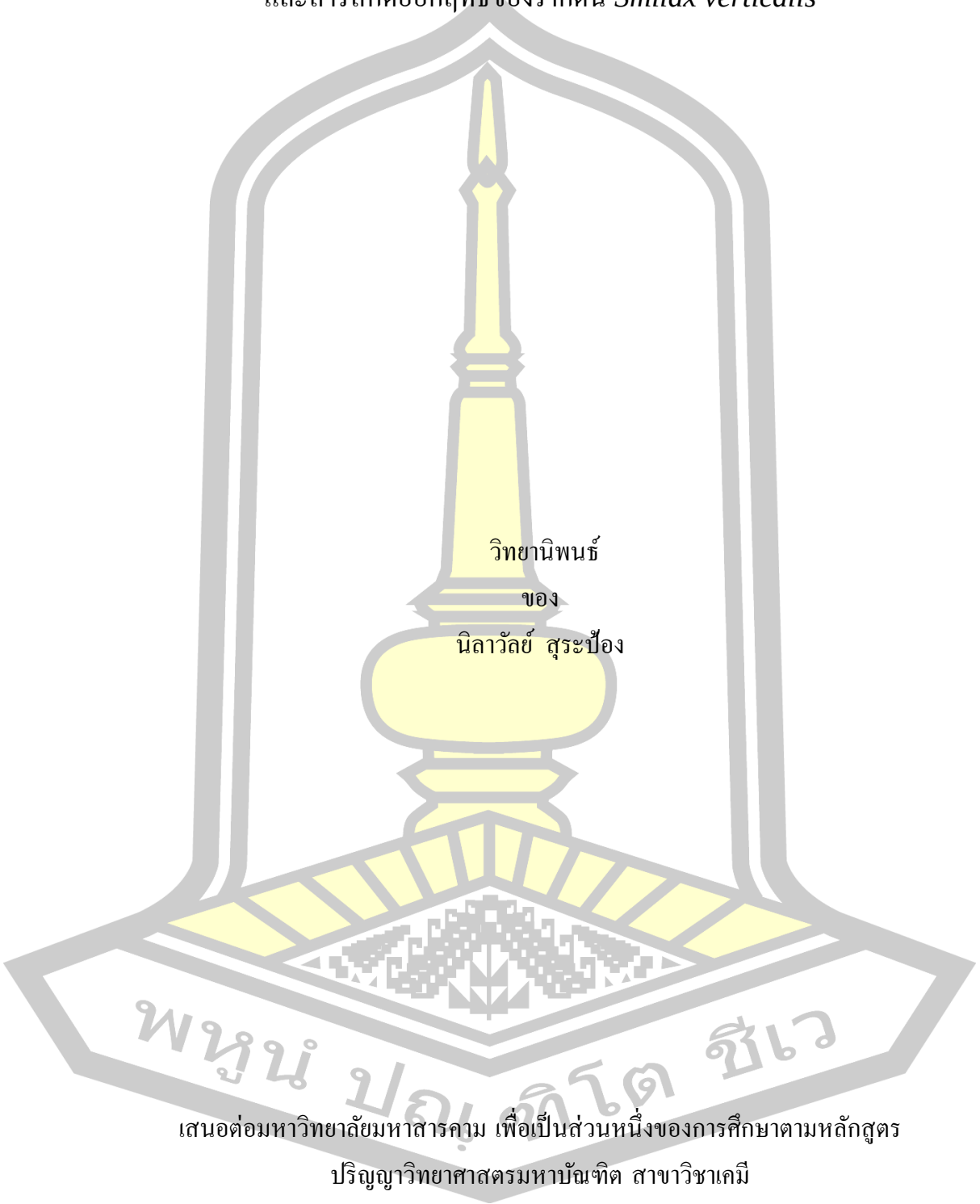
Bioactive Compounds from the Cultured Broth of the Fungus *Polycephalomyces nipponicus* and Active Extracts from the Root of *Smilax verticalis*

Nilawan Surapong

A Thesis Submitted in Partial Fulfillment of Requirements for
degree of Master of Science in Chemistry
November 2018

สารออกฤทธิ์ทางชีวภาพจากอาหารเหลวเลี้ยงเชื้อ *Polycephalomyces nipponicus* และสารสกัดออกฤทธิ์ของรากต้น *Smilax verticalis*



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TITLE Bioactive Compounds from the Cultured Broth of the Fungus *Polycephalomyces nipponicus* and Active Extracts from the Root of *Smilax verticalis*

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ABSTRACT

Cordytropolone (134) and (-)-leptosphaerone A (354), were isolated from the culture broth of the fungus *Polycephalomyces nipponicus*. The structures of these two compounds were elucidated by spectroscopic methods and from a comparison of the spectroscopic data with those reported previously. The structure of 134 was confirmed by X-ray crystallography for the first time while the leptosphaerone class, compound 354, was first isolated as its (+)-antipode from the fungus *Polycephalomyces* (*Cordyceps*). The fermentation process was monitored weekly by HPLC analysis for 12 weeks. The predominant compound 134 was produced at ~1 mg/mg of dry extract at weeks 11 and 12. Compound 134 exhibited modest antipathogenic fungi activity against *Collectrichum musae, C. capsici, C. gloeosporioides, Fusarium* spp. TFPK301, *F*. spp. FOC1708 and *Pestalotia* spp. with the percentage of mycelial growth inhibition (PGI) values of 3.74 ± 0.70, 12.86 ± 1.43, 0.91 ± 0.56, 5.46 ± 0.56, 7.93 ± 0.61, and 18.75 ± 5.24%, respectively, at 25 microgram/milliliter.

The root, stem and leaf of *S. verticalis* were small scale extracted in methanol. The methanol extracts from the root (SV-R), stem (SV-S) and leaf (SV-L) were tested for their antifungal, antioxidant, cytotoxic and antibacterial activities. The SV-R showed antifungal activity against six pathogenic fungal strains (*Pestalotia* spp., *Colletotrichum capsica, C. musae, C. gloeosporioides, Fusarium* spp. Foc 1708 and *F*. pp. TFPK301) with the percentage of mycelial growth inhibition (PGI) in the range of 3.61 ± 3.39-14.33 ± 3.84. The antioxidant activity of the SV-R (IC_{50} 35.76 ± 1.10 microgram/milliliter) was higher than the SV-S (IC_{50} 56.09 ± 1.33 microgram/milliliter) and SV-L (IC_{50} 90.68 ± 1.67 microgram/milliliter) by DPPH method. These three extracts had no cytotoxic (MCF-7, KB cancer cell lines and Vero cell lines) antiviral (HSV-1) and antibacterial activities.

Keyword : Polycephalomyces nipponicus, cordytropolone, (-)-leptosphaerone A, antipathogenic fungal activity, smilax verticalis

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Nilawan Surapong

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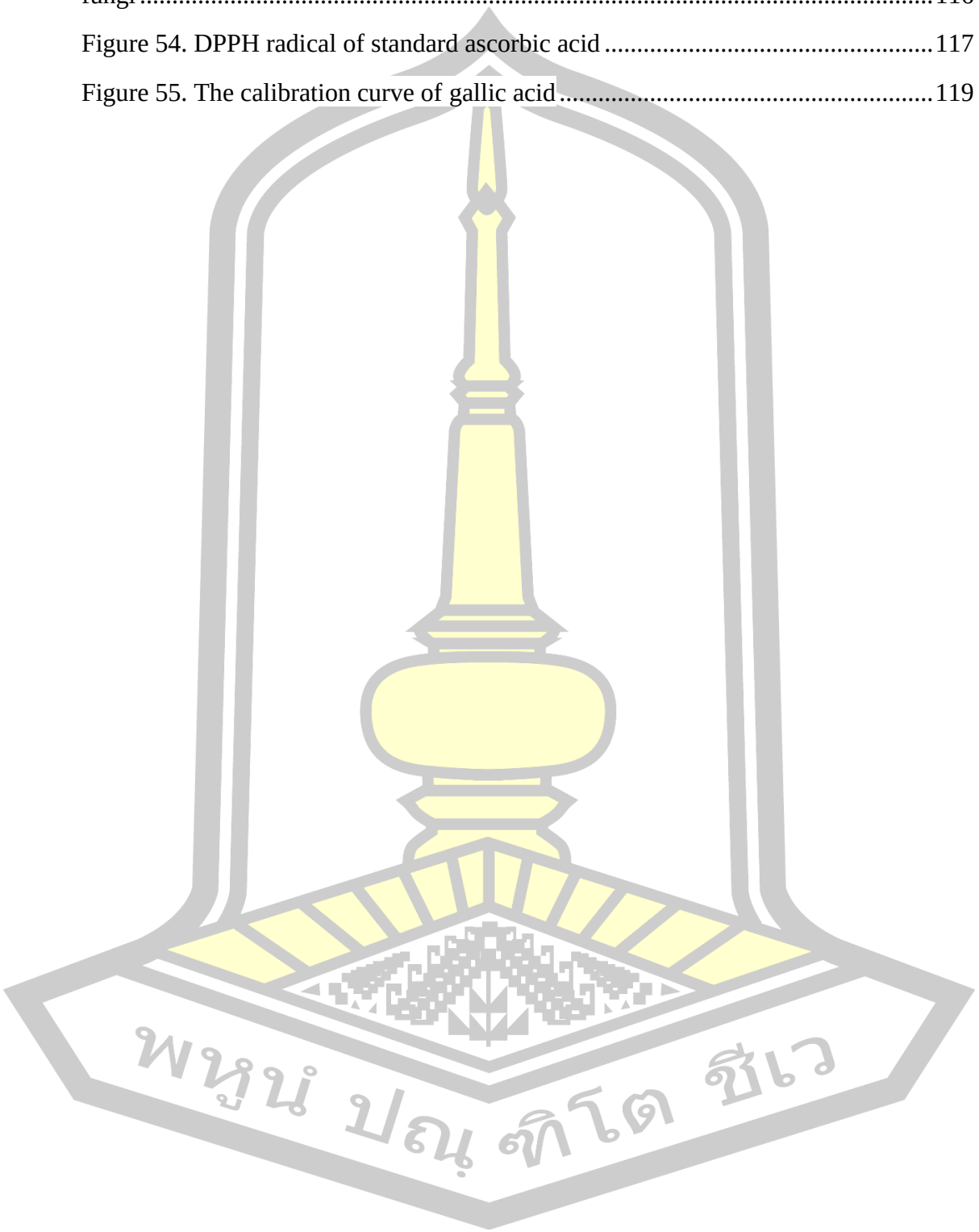

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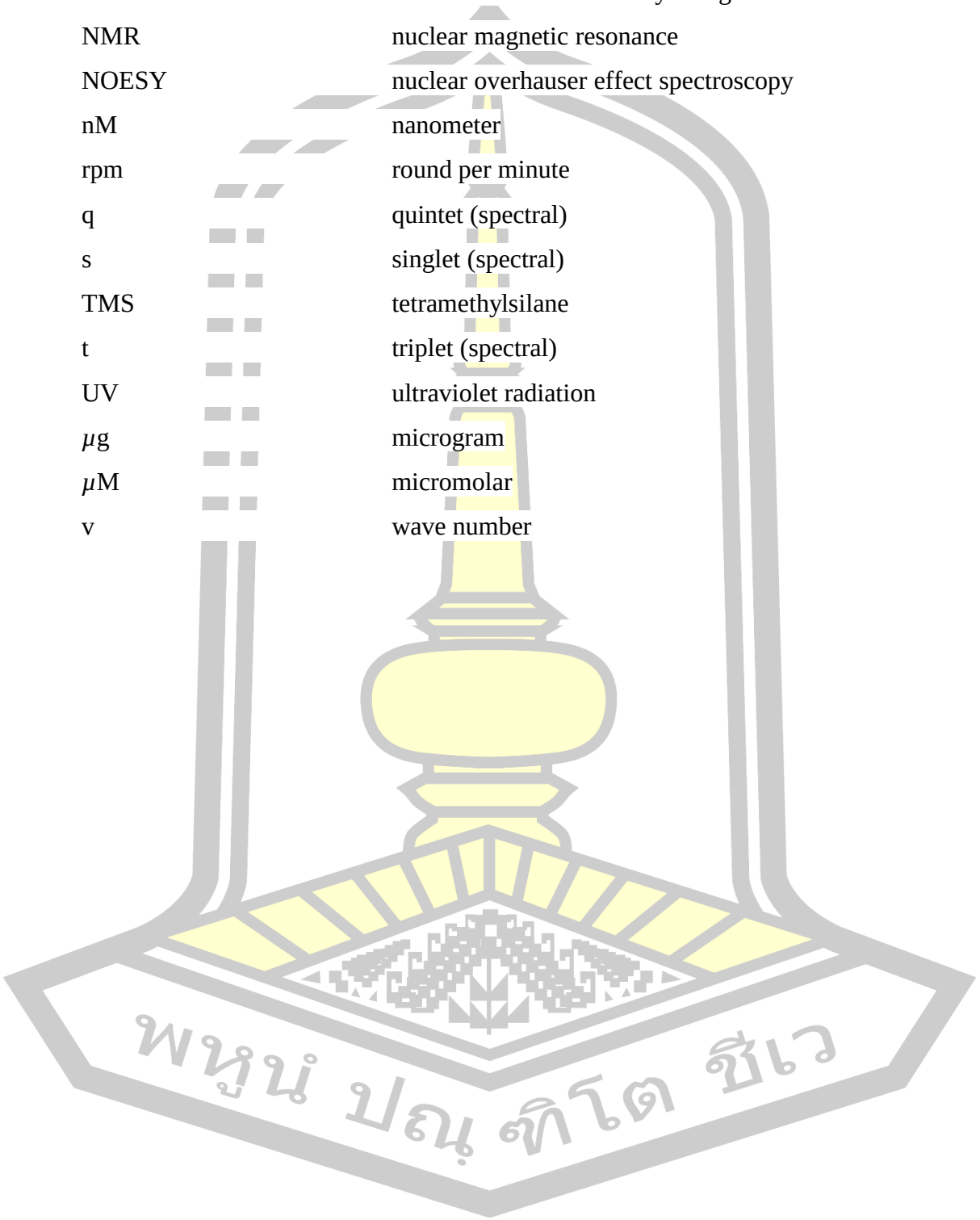
LIST OF ABBREVIATIONS

$^{\circ}C$	degree celsius
$CDCl_3$	deuterated chloroform
COSY	correlation spectroscopy
Ealcd.	calculated
cm^{-1}	wave number unit
DMSO-d_6	deuterated dimethylsulphoxide
d	doublet (spectral)
dd	doublet of doublet (spectral)
ddd	doublet of doublet of doublet (spectral)
dddd	doublet of doublet of doublet of doublet (spectral)
EtOAc	ethyl acetate
Eq	equatorial
g	gram
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	Hertz
IR	infrared radiation
IC_{50}	50% inhibitory concentration
J	coupling constant
MIC	minimum inhibitory concentration
MH_Z	megahertz
MS	mass spectroscopy
Me	methyl group
MeOH	methanol
MeOH-d_4	deuterated methanol
mL	millilitre
m	multiplet (spectral)
min	minute

mult	multiplicity
m/*z*	a value of mass divided by charge
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
nM	nanometer
rpm	round per minute
q	quintet (spectral)
s	singlet (spectral)
TMS	tetramethylsilane
t	triplet (spectral)
UV	ultraviolet radiation
μg	microgram
μM	micromolar
v	wave number



CHAPTER 1
INTRODUCTION

1.1 Rationale and background

Thailand has been considered a rich country in biodiversity, comprising approximately 6- 10% of total species known thus far. Plants, animals and microorganisms are diverse and live together in complex ecosystems. Natural sources of these living things have influenced the development of cultural diversity and traditional knowledge of people in the community. Therefore, a basic scientific knowledge of the natural resources is needed in order to facilitate conservation, utilization and management to better understand how to use biological resources in a sustainable manner to improve our quality of life.

1.2 Fungi

Fungi are organisms which are classified as kingdom " Fungi" and over 100,000 described species. Fungi do not photosynthesize and they are the principal decomposers in ecological systems. Some of the fungi live environmental friendly with other living. There are numbers of report on bioactive compounds from fungi during the past century. The most classical discovery was the discovery of penicillins from the fungi *Pennicilium notatum*. Penicillins show inhibition of protein in some of pathogenic bacteria cell wall, for example *Staphylococcus*, *Streptococcus*, *Neisseria gonorrhea* and *Corynebacterium*. This finding led to the development of antibiotic drug from fungal sources. However, some of the fungi are harmful to other organisms. Some examples of the fungi in this group are *Aspegillus flavus* and *Aspergillus paraciticus*. These fungi produce aflatoxins which are a toxin that sensitive to binding DNA, RNA and protein caused denaturation of DNA, RNA and protein. After the protein synthesis was disrupted will led to liver cancer, cirrhosis, encephalitis, lung disease and bronchiectasis. Some of the fungi are parasitic on insects called insect pathogenic fungi.

1.2.1 Insect pathogenic fungi

Some of fungi can be parasite on insects and kill the insects. This group of the fungi is classified as “insect pathogenic fungi”. The insects will be used as host for the groth of fungi by getting nutrient from insect. Once the fungi infected to the insect host, the fungi is going to develop as a yeast-like from, producing metabolites that inhibit the insect’s immune system and influence the insect’s behaviour [1], [2]. After the insect die, the fungi revert to a filamentous form and digests the remaining internal organs, leaving only the chitin or protein exoskeleton [3], [4]. The insect pathogenic fungi are widely distributed in tropical region. In Thailand, they can be found and collected from many locations. Most of the collected insect pathogenic fungi belong to the Ascomycota phylum that composite of three families as Cordycipitaceae, Opiocordycipitaceae and Clavipitaceae [5].

1.2.2 The genus *Cordyceps*

Cordyceps, an insect pathogenic fungus, is one of genus in Cordycipitaceae family. There are about 400 species of *Cordyceps* worldwide and about 200 species found in Thailand such as *Cordyceps militaris*, *Cordyceps sinensis*, *Cordyceps nipponica*, *Cordyceps pseudomilitaris*, *Cordyceps cylindrica*, *and Cordyceps unilateralis.* In traditional Chinese medicine, *Cordyceps* has long been used to prevent and cure human diseases for more than a millennium, especially the *C. sinensis*, the most well know species in this genus [6]. Till now, numerous bioactive constituents have been extracted and identified from the *Cordyceps*. Meanwhile, various pharmacological activities of the isolated compounds from the *Cordyceps* have also been reported. Research on bioactive compounds and biological activities of this genus is still progress.

1.3 Plants

Plants are classified as kingdom "Plantae" which contains about 300,000 species. This kingdom is very important for being sources of food and habitat for other living on earth. Plant is also an important source of secondary metabolites that has medicinal property. The compounds produced by plant for their defense mechanisms have been implicated in the therapeutic properties of most medicinal plants. There are numbers of report on bioactive compounds from plants. For example, the bark of *Cinchona* tree contains the alkaloid guanine. From the bioassay, this compound shows first effective treatment for malaria, appearing in therapeutic in the 17th the century [7]. Another example is the bioactive alkaloid solasolium from *Solanum indicum* which shows antimicrobial, antirheumatics, anticonvulsants, antiinflammatory, antioxidant and anticancer activities [8].

1.3.1 The genus *Smilax*

The genus *Smilax* (Liliaceae family) composes about 300 species which are mainly distributed in the tropical and warm areas throughout the world, especially in East Asia and North America [9]. The rhizomes of the *Smilax* species are most famous for their medical use. The rhizomes of *S. china* and *S. glabra*, called ''Jin Gang Teng'' and ''Tu Fu Lin'', respectively, in Pharmacopoeia of People's Republic of China, are used to treat chronic pelvic inflammatory disease and rheumatic arthritis [10]. The rhizomes of *S. riparia*, *S. nipponica*, *S. bockii*, *S. microphylla* and *S. discotis* were recorded in the Chinese Herbal Medicines to treat joint pain, edema, and rheumatoid arthritis [11]. There are numbers of report on bioactive compounds and phytochemical constituents of this genus. However, as far as our knowledge, the phytochemical study of the *S. verticalis* species has not been studied yet.

1.4 Purposes of the research

To search for bioactive natural products from the culture broth of the fungus *Polycephalomyces nipponicus* and the root of *Smilax verticalis*

1.5 Expected results obtained from the research

Identified natural product compounds from the fungus *P. nipponicus* and the root of *S. verticalis* and their biological activities are expected as basic scientific information from our research.

1.6 Scope of the research

The crude extracts from the culture broth of the fungus *P. nipponicus* and the root of *S. verticalis* will be obtained from the extraction. These extracts will be subjected to purify by column chromatography and the structures of isolated compounds will be elucidated by spectroscopic methods. Chemical compositions from the culture broth extract of *P. nipponicus* from small scale extraction will be analyzed by HPLC.

CHAPTER 2
LITERATURE REVIEW

2.1 Chemical constitutions of the fungus *Cordyceps*

During the past thirty years there are numbers of report of the chemical constituents and bioactive compounds from the insect pathogenic fungus in the genus *Cordyceps*. However, the chemical study of the fungus *Polycephalomyces nipponicus* (previously referred to *Cordyceps nipoponica*) has been reported once from literature survey. In this chapter, the bioactive compounds and chemical constituents isolated from the genus *Cordyceps* mostly from the cultivation in laboratory are reviewed. The culture broth and/or mycelium extracts were taken to purify to get pure compounds which were characterize for its chemical structures. Later, isolated compounds have been tested for their biological activities. In this review chemical constituents of eight species of the *Cordyceps* (*C. brunnearubra*, *C. cicadae*, *C. heteropoda*, *C. militaris*, *C. nipponica*, *C. pseudomilitaris*, *C. sinensis* and *C. unilateralis*) and seven unidentified species of *Cordyceps* sp. (BCC 1681, BCC 1788, BCC 16173, BCC 16176, BCC 1861, BCC 12671 and NBRC 106954), together with biological activities of isolated compounds published during the year 1997-2017 in data bases available to access have been summarized herein.

2.1.1 *C. brunnearubra*

In 2007, Isaka and coworkers reported the purification of the culture broth extract of the fungus C. *brunnearubra* (BCC 1395) collected from Sam Lan national park, Saraburi province, Thailand [12]. A new compound; cordyformamide (**1**), has been isolated (Figure 1). This compound showed antimalarial activity (*Plasmodium falciparum* K1 strain, drug-resistant) with an IC_{50} value of 18 μM. It also showed weak cytotoxicity against human breast cancer cell lines with an IC_{50} value of 39 μM, while its activity against oral human epidermoid carcinoma cell lines, human small cell lung cancer cell lines and noncancerous vero cell lines was inactive.

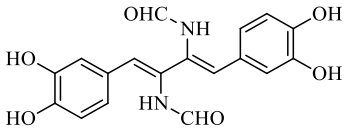


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Figure 1. Structure of a compound isolated from *C. brunnearubra*

2.1.2 *C. cicadae*

In 2014, Wang and coworkers reported the isolation of the ascocarps and insect-body portions extract of the fungus *C. cicadae* collected from Tongling city of Anhui province, China [13]. A new cyclodepsipeptide; cordycecin A (**2**), together with four known compounds; beauvericin E (**3**), beauvericin J (**4**), beauvericin (**5**) and beauvericin A (**6**), were isolated and identified (Figure 2). The pure compounds were evaluated for their inhibitory effect on HepG2 and HepG2/ADM cells. From the results, compounds **2-5** exhibited a significant inhibitory effect on HepG2 and HepG2/ADM cells with IC_{50} values ranging from 2.40 ± 0.37 to 14.48 ± 1.68 μM.

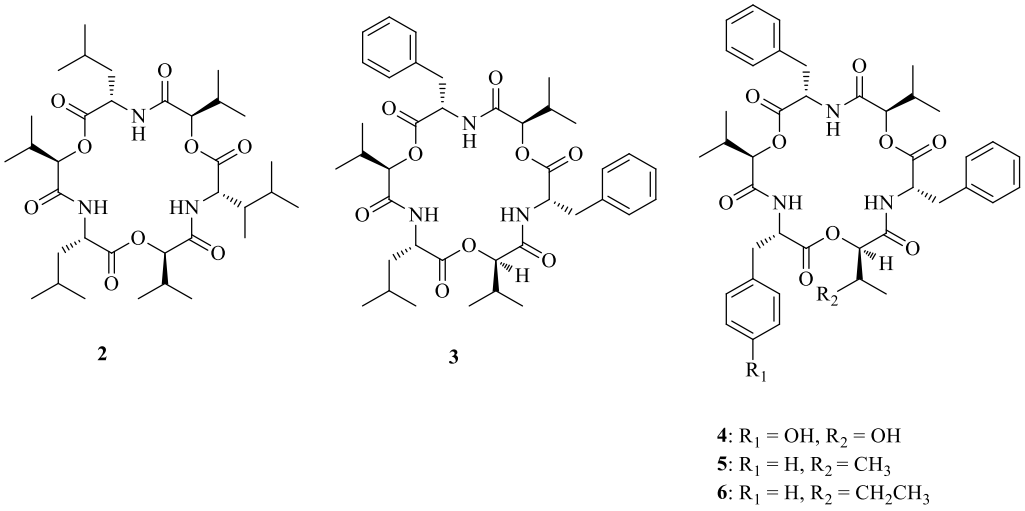


Figure 2. Structures of compounds isolated from *C. cicadae*

In 2017, Wang and coworkers investigated the chemical constituents from the mycelium and spores extracts of the fungus *C. cicadae* provided by Zhejiang

Bioasia Pharmaceutical Co., Ltd, China [14]. Nine known sterols; ergosterol (**7**), ergosterol peroxide (**8**), 9,11-dehydroergosterol peroxide (**9**), 3*β*,5*α*,9*α*-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**10**), 3*β*,5*α*,9*α*,14*α*-tetrahydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**11**), 5*α*,6*α*-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3*β*,7*α*-diol (**12**), 3*β*,5*α*,6*β*-(22*E*,24*R*)-ergosta-7,22-dien-3,5,6-triol (**13**), 3*β*,5*α*,6*α*-6-methoxyergosta-(22*E*,24*R*)-7,22-diene-3,5-diol (**14**), 4-hydroxy-17*R*-methylincisterol (**15**), together with a resorcinol derivative; 5-*n*-nonadecylresorcinol (**16**), a cyclodesipeptide; beauvericin (**5**) and a nucleoside; N^6-(2-hydroxyethyl) adenosine (**17**), were isolated and characterized (Figure 2). Compounds **8**-**15** were isolated from spores extract and compounds **7**, **8**, and **5**, **16**, **17** were isolated from mycelium extract. All of the isolated compounds were subjected to the biological testing against human lung cancer cell (A549) and human leukemia cell (HL-60), and only compound **5** was found to exhibit significant cytotoxicity with IC_{50} values of 5.995 and 5.800 μmol/L, respectively.

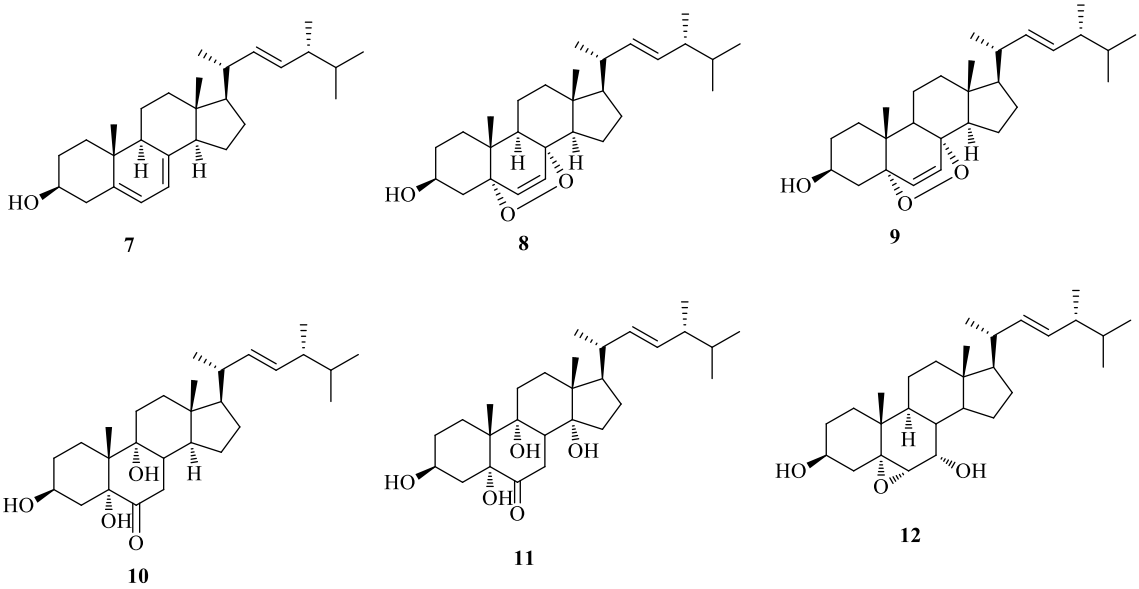


Figure 2. Structures of compounds isolated from *C. cicadae* (continued)

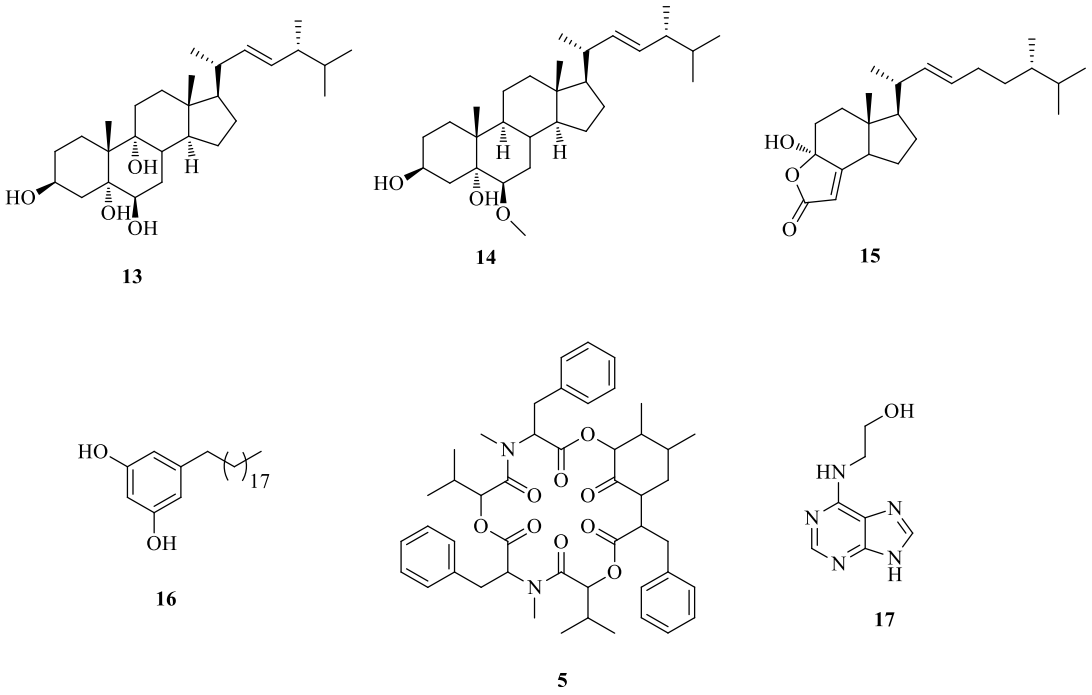
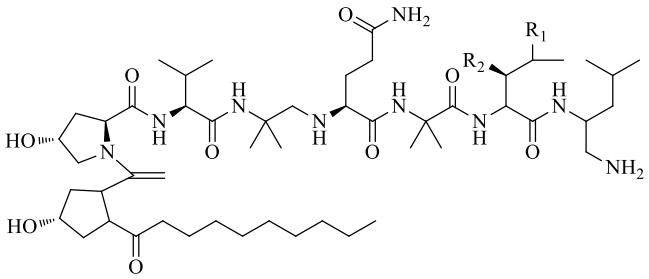


Figure 2. Structures of compounds isolated from *C. cicadae* (continued)

2.1.3 *C. heteropoda*

In 2004, kranoff and coworkers studied the culture broth extract of the fungus *C. heteropoda* which was isolated from an Australia cicada [15]. Two of peptides; cicadapeptin I (**18**) and cicadapeptin II (**19**), together with a known compound; myriocin (**20**), have been isolated (Figure 3). All of isolated compounds were tested against bacterial target strains. Compounds **18** and **19** produced clear kill zones against *Bacillus cereus* (13 and 12 mm, respectively), *Bacillus subtilis* (13 and 11 mm, respectively), and *Escherichia coli* (16 mm for both peptides). Compound **20** showed inactive activity against any of the bacterial targets, but it inhibited all the filamentous fungi tested, producing inhibition zones against *Botrytis cinerea*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides* and *Fusarium oxysporum*, of 28, 14, 8 and 17 mm, respectively.



18: $R_1 = CH_3$, $R_2 = H$
19: $R_1 = H$, $R_2 = CH_3$

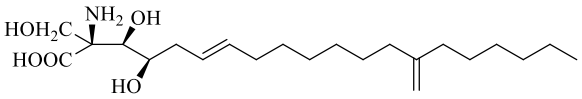


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Figure 3. Structures of compounds isolated from *C. heteropoda*

2.1.4 *C. militaris*

In 2004, Rukachaisirikul and coworkers reported the isolation and chemical elucidation of the culture broth extract of the fungus *C. militaris* (BCC 2816) provided by the BIOTEC [16]. Three new 10-membered macrolides (**21**-**23**), together with six known compounds; cepharosporolides C (**24**), cepharosporolides E (**25**), cepharosporolides F (**26**), 2-carboxymethyl-4-(3′-hydroxybutyl) furan (**27**), cordycepin (**28**) and pyridine-2,6-dicarboxylic acid (**29**) have been isolated (Figure 4). Compounds **21**-**24** and **28** were evaluated for their antimalarial activity (*P. falciparum* K1). Compound **28** exhibited antimalarial activity with an IC_{50} value of 4.5 μg/mL while other compounds were inactive on antimalarial testing.

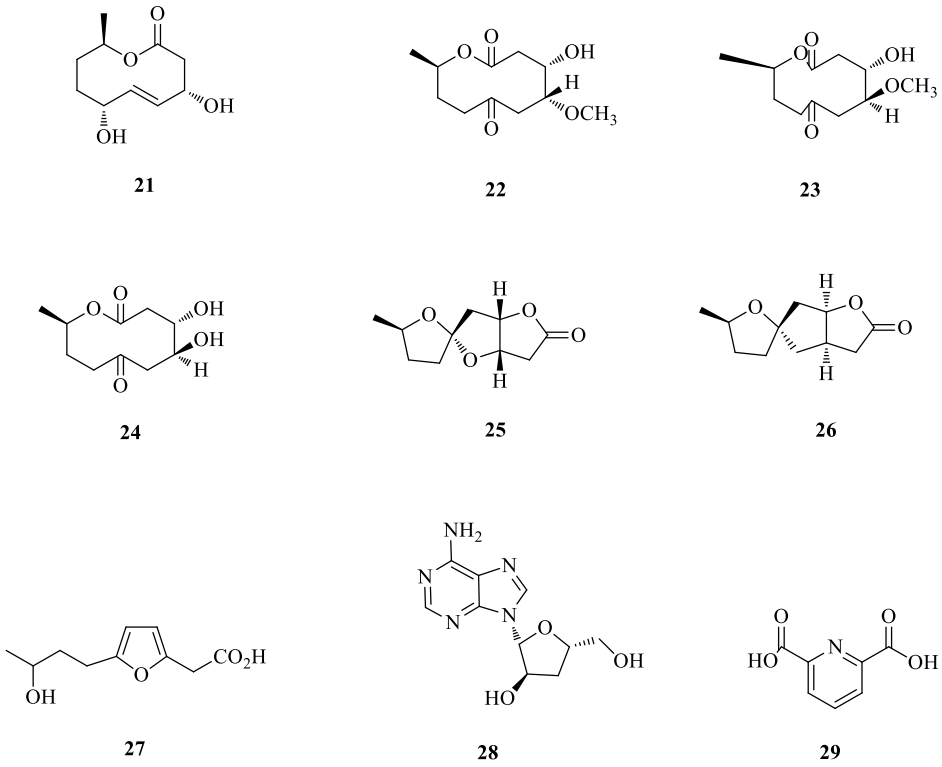


Figure 4. Structures of compounds isolated from *C. militaris*

In 2010, Rao and coworkers investigated the fruiting bodies extract of the fungus *C. militaris* obtained from Natural Products & Bioprocess Laboratory, Chaoyang University of Technology, Taiwan [17]. The investigation led to the isolation of ten pure compounds; ergosterol palmitate (**30**), palmitic acid (**31**), ergosterol (**7**), ergosterol peroxide (**8**), compound **32**, compound **33**, 3,4-*O*-isopropylidene-d-mannitol (**34**), cordycepin (**28**), d-mannitol (**35**) and d-glucose (**36**) (Figure 4). All of isolated compounds were examined for their growth inhibitory properties against nitricoxide (NO), tumor necrosisfactor (TNF)-α and interleukin (IL)12 enhanced production from LPS/ IFN-γ- stimulated macrophages. Additionally, the anti- proliferation effects of isolated compounds on human cancer cell lines, colon (colon 205), prostate (PC-3), and hepatoma (Hep G2) cells were also analyzed. Compound **28** displayed potent growth inhibition on NO, TNF-α and IL-12 production with IC_{50} values of 7.5, 6.3 and 7.6 μg/mL, respectively. A similar inhibitory trend on these inflammatory mediators was observed for compounds **7**, **34**, **35** and **36** with IC_{50} values ranging from 10.8 to 17.2 μg/mL. On the other hand, the compound **28** exhibited a strong growth inhibition

against the colon cancer cell line colon 205 with an IC_{50} of 32.6 μg/mL. The same result was observed by compound **7** in the prostate cancer cell line PC-3 with an IC_{50} value 35.6 μg/mL. The highest potency was observed for compounds **7** and **28** against the PC-3 and colon 205 cells, respectively. In Hep G2 cells only compound **7** showed moderate anti-proliferation activities with an IC_{50} value 61.5 μg/mL, while the other tested compounds were found to exhibit negligible effect with an IC_{50} value greater than 100 μg/mL.

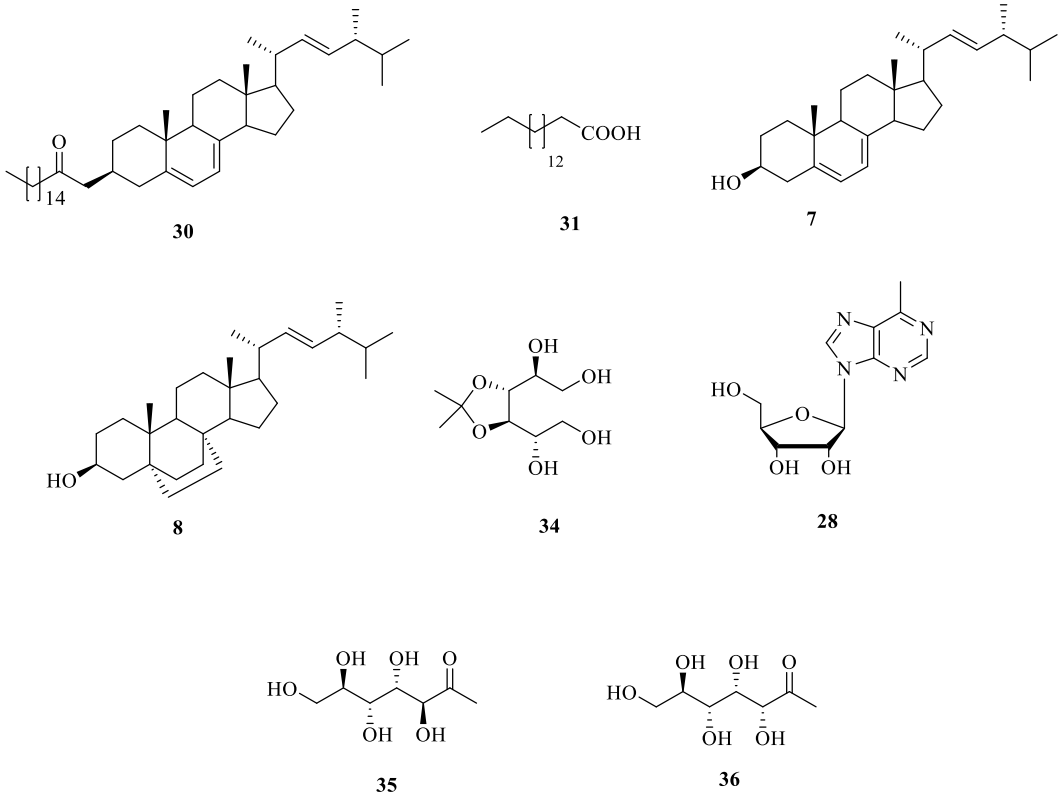


Figure 4. Structures of compounds isolated from *C. militaris* (continued)

In 2014, Kim and coworkers studied the characterization of active constituents from fruity bodies extract of the fungus C. *militaris* provided by Rural Development Administration, Korea [18]. The results of the isolation demonstrated two new compounds; cordyrroles A (**37**) and cordyrroles B (**42**), together with twelve known compounds; 5- (hydroxymethyl)- 1- (2- oxopiperidin- 3- yl)- 1*H*- pyrrole- 2- carbaldehyde (**38**) , dihydrouracil (**39**) , uracil (**40**), nicotinamide (**41**), N^6- (2-

hydroxyethyl) adenosine (**17**), cordycepin (**28**), adenosine (**43**), 2′-*O*-methyladenosine (**44**), xanthosine (**45**), 2′-deoxyuridine (**46**), uridine (**47**) and thymine (**48**) (Figure 4). Among the isolated compounds, compound **37** significantly inhibited adipocyte differentiation and pancreatic lipase activity, whereas compound **38** was more effective at inhibiting pancreatic lipase. Compound **28** decreased the rate of adipocyte differentiation.

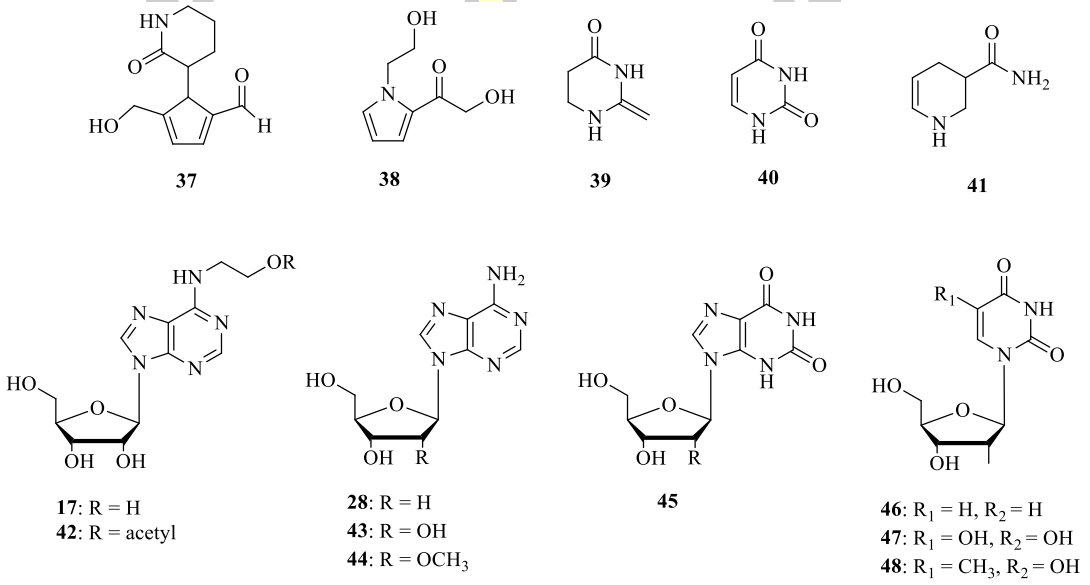


Figure 4. Structures of compounds isolated from *C. militaris* (continued)

In 2016, Chiu and coworkers purified the fruiting bodies extract of the fungus *C. militaris* provided by Chung-Shan Medical University, Taichung, Taiwan [19]. Eight compounds, including a new compound; cordycerebroside A (**49**), together with seven known compounds; soyacerebroside I (**50**), glucocerebroside (**51**), adenosine (**43**), cordycepin (**28**), ergosterol peroxide (**8**), cerevisterol (**52**) and ergosterol (**7**), have been isolated (Figure 4). Compounds **49-51** are cerebroside derivatives, **28** and **43** are nucleic acids and **7**, **8** and **52** are sterols. All of isolated compounds were tested biological activity against the production of NO, effect on iNOS and COX-2 protein expression. The resulted showed compounds **49-51** inhibited the accumulation of pro-inflammatory iNOS protein and reduced the expression of COX-2 protein in LPS-stimulated RAW264.7 macrophages.

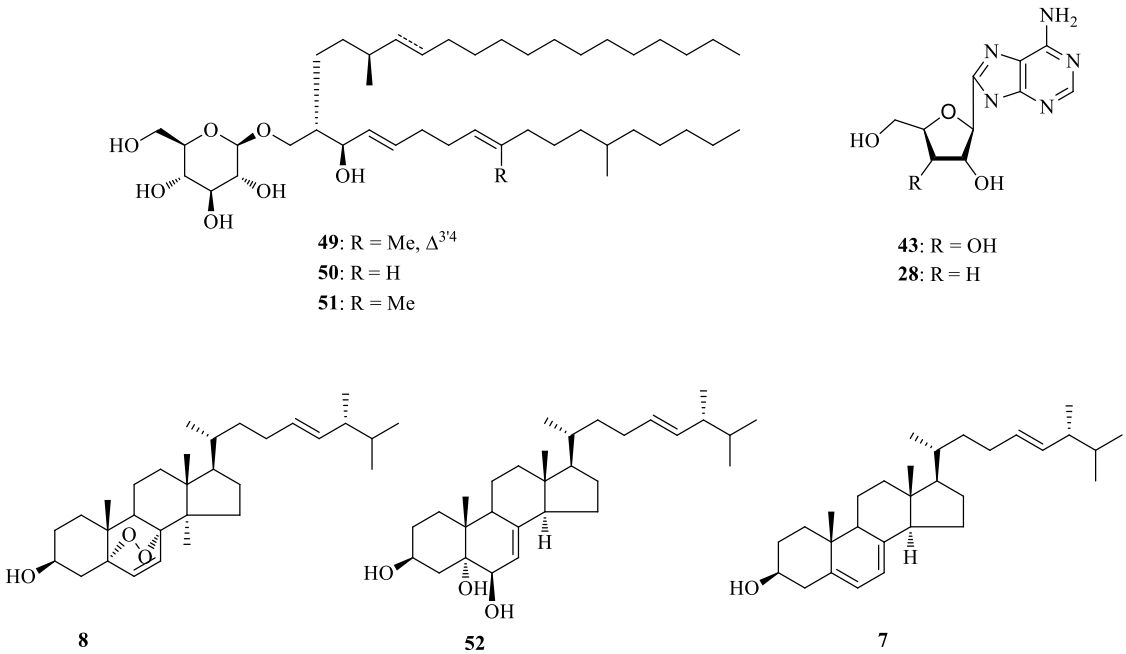


Figure 4. Structures of compounds isolated from *C. militaris* (continued)

In 2017, Sun and coworkers isolated the chemical constituents from the culture of the fungus *C. militaris* obtained from Yanbian Foresty Science Institute, Yanji, China [20]. Denosine (**53**) and fourteen known compounds; 3′-deoxyadenosine (**28**), N^6-(2-hydroxyethyl)adenosine (**17**), adenosine (**43**), 8-hydroxy-2,3-dihydro-4(1*H*)-quinolone (**54**), cholest-5-en-3*β*-ol (**55**), 3*β*-hydroxycholest-5-en-7-one (**56**), stigmasta-4,6,8(14), 22-tetraen-3-one (**57**), cholest-4-en-3-one (**58**), ergosterol peroxide (**8**), 3*β*,7*α*-dihydroxycholest-5-ene (**59**), 5*α*-cholest-3,6-dione (**60**), 22(*E*)-5,8-epidioxy-5*α*,8*α*-stigmata-6,9(11),22-(24*S*)-trie-3*β*-ol (**61**), ergosta-7,22-diene-3*β*,5*α*,6*β*-triol (**62**) and demethylincisterol A_4 (**63**), were isolated and chemical elucidated (Figure 4). The activities of the isolated compounds **8, 17, 43, 53-63** were tested by examining NF-*κ*B activation. The results showed that compound **28** showed significant inhibitory activity against TNF-*α*-induced NF-*κ*B reporter gene expression in HeLa cells from 3 to 100 *μ*M and even better than the positive control compound at 3 *μ*M.

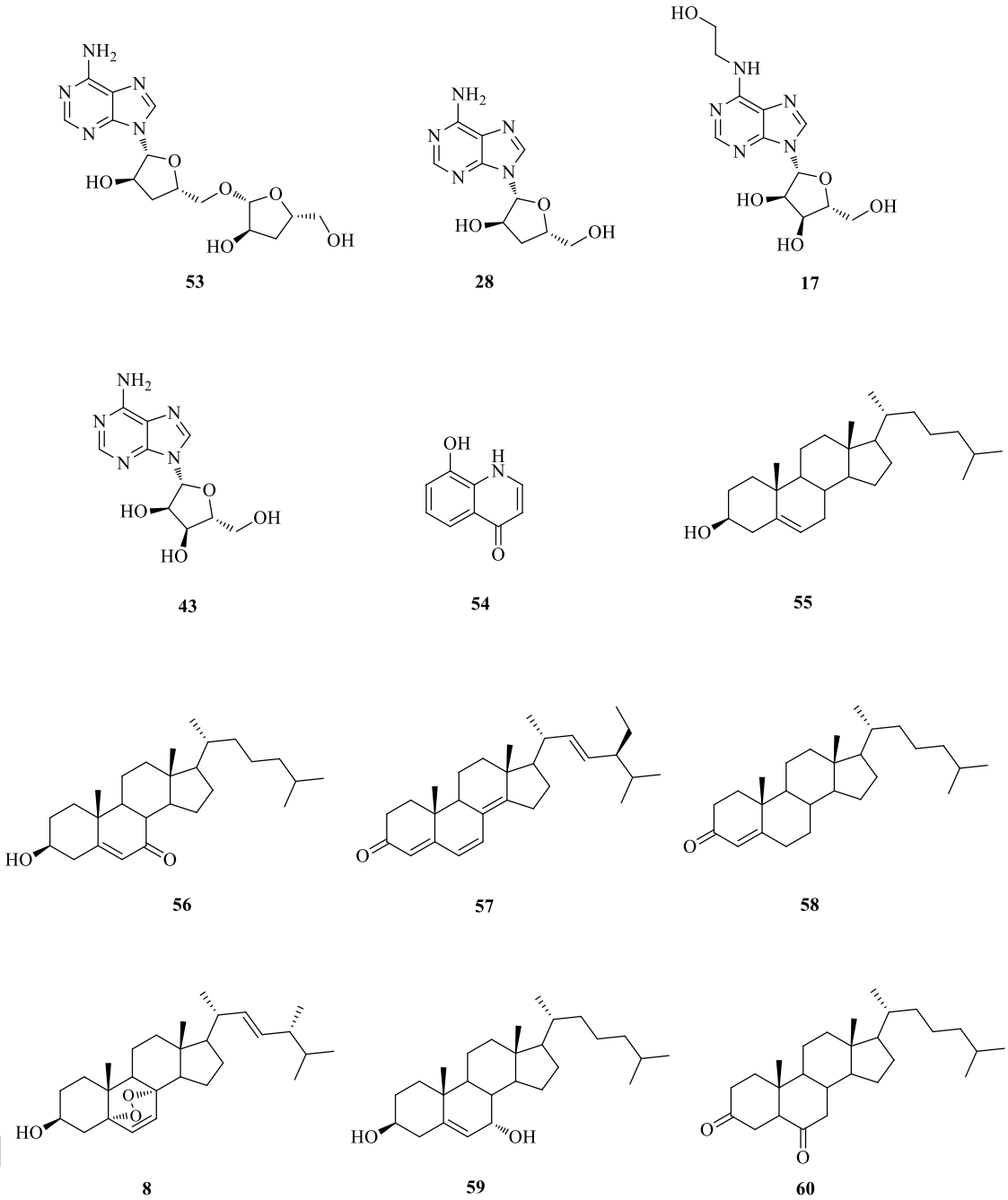
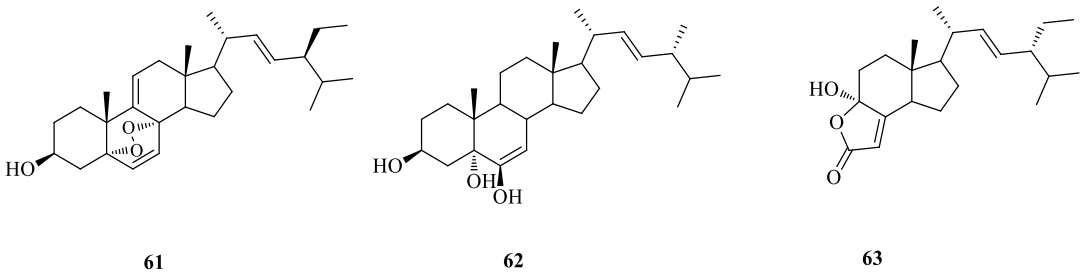


Figure 4. Structures of compounds isolated from *C. militaris* (continued)

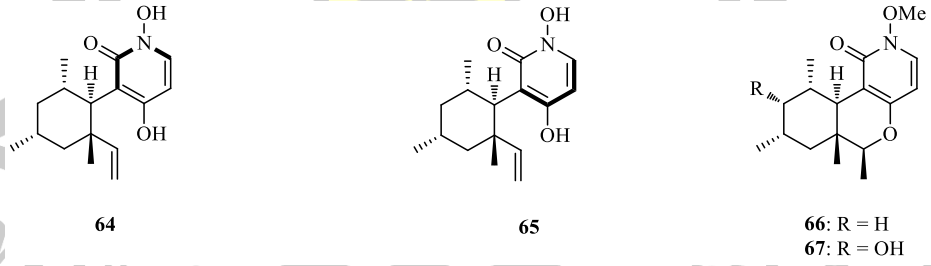


61 **62** **63**

Figure 4. Structures of compounds isolated from the *C. militaris* (continued)

2.1.5 *C. nipponica*

In 2001, Isaka and coworkers purified the culture broth extract of the fungus *C. nipponica* (BCC 1389) collected from Khao Yai national park, Thailand [21]. This investigation led to the isolation of *N*- hydroxy- and *N*- methoxy- 2- pyridones; cordypyridones A-D (**64**-**67**) (Figure 5). Cordypyridones A and cordypyridones C were previously isolated from an unidentified fungus OS-F61800 [22] and *Fusarium* sp. [23]. Cordypyridones A and B exhibited antimalarial activity (*P. falciparum* K1) with IC_{50} values of 0.066 and 0.037 μg/mL. Their cytotoxicity against three cell lines, breast cancer cell lines with IC_{50} values of 3.9 and 3.7 μg/mL, human epidermoid carcinoma in the mouth cell lines with IC_{50} values of 15.7 and 8.4 μg/mL and vero cell lines with IC_{50} values of 6.3 and 5.3 μg/mL, have been reported.

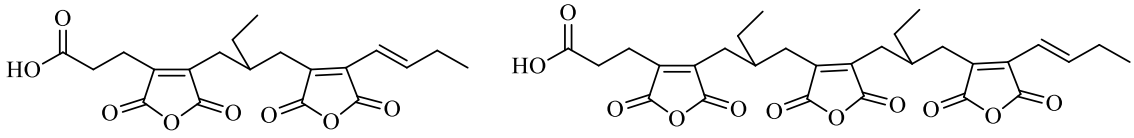


64 **65** **66**: R = H
67: R = OH

Figure 5. Structures of compounds isolated from *C. nipponica*

2.1.6 *C. pseudomilitaris*

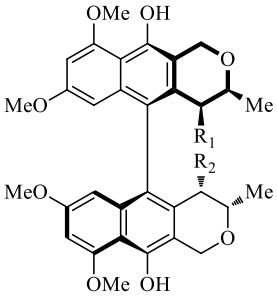
In 2000, Isaka and coworkers studied chemical constituents from the culture broth of the fungus *C. pseudomilitaris* (BCC 1620) collected from Sam Lan national park, Thailand [24]. Cordyanhydrides A (**68)** and cordyanhydrides B (**69**) were isolated and identified (Figure 6). Biological activity of these two compounds has not been reported in the year 2000.



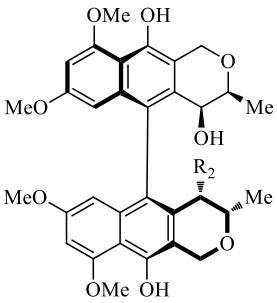
68 **69**

Figure 6. Structures of compounds isolated from *C. pseudomilitaris*

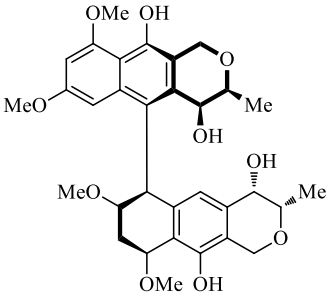
In 2001, Jaturapat and coworkers reported the isolation of the culture broth and mycelium extracts of the fungus *C. pseudomilitaris* (BCC 1620) collected from Sam Lan national park, Thailand [25]. Eleven bioxanthracenes (**70**- **80**) and two monomers (**81** and **82**) were isolated (Figure 6). Compounds **70**-**75** and **80** previously isolated from isolated from *Verticillium* sp. [26], [27] while compounds **76**-**89**, **81** and **82** were new compounds. Compounds **70**-**77**, **79**, **80** and **82** exhibited antimalarial activity (against *P. falciparum* K1) in the range of IC_{50} 1.1-6.4 μg/mL. The compounds were also screened for cytotoxicity against three cell lines, breast cancer cell lines, human epidermoid carcinoma in the mouth cell lines and vero cell lines. The bioxanthacenes showed no cytotoxic activity. Moreover, production of bioxanthracenes and cordyanhydrides in seven isolates of the fungus *C. pseudomilitaris*; BCC 188, 512, 1472, 1620, 1784, 1919 and 1979, were reported. All of fungus isolates of the fungus *C. pseudomilitaris* collected from Sam Lan National Park. The thin layer chromatography (TLC) results showed that the isolate BCC 188, 1472, 1620 and 1784, produced bioxanthracenes. Among the seven isolates tested, six isolates produced cordyanhydrides while isolate BCC 512 didn't produce cordyanhydrides.



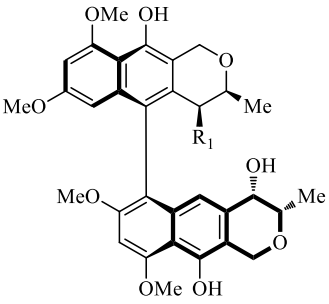
70: R_1 = OH, R_2 = OH
71: R_1 = OAc, R_2 = OH
72: R_1 = OAc, R_2 = OAc
73: R_1 = OH, R_2 = H
74: R_1 = OAc, R_2 = H
75: R_1 = H, R_2 = H



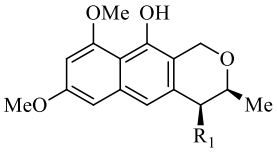
76: R_2 = OH
77: R_2 = H



78



79: R_1 = OH
80: R_1 = H

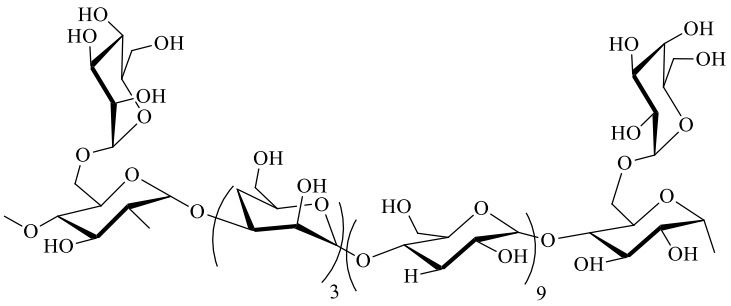


81: R_1 = OH
82: R_1 = H

Figure 6. Structures of compounds isolated from *C. pseudomilitaris* (continued)

2.1.7 *C. sinensis*

In 2010, Wang and coworkers investigated the chemical constituents from the culture broth extract of the fungus *C. sinensis* collected from Zhejiang, China [28]. The crude extract was purified to obtain a water-soluble polysaccharide; CPS-2 (**83**) (Figure 7). Its protective effect on the model of fulgerizing kidney-induced rats was tested. The results revealed that this polysaccharide had a significant protective effect of chronic renal failure at dosages of 40 mg/kg and 80 mg/kg.



83

Figure 7. Structures of a compound isolated from *C. sinensis*

In 2011, Yang and coworkers investigated the chemical constituents from the culture mycelium of the fungus *C. sinensis* provided by Taiwan Sugar Company, Taiwan [29]. The results led to the identification of fifty compounds, including five constituents; cordysinins A-E (**84**-**88**), which were reported from a natural source for the first time and forty five known compounds; ergosterol (**7**), (17*R*)-17-methylincisterol (**89**), ergosterol peroxide (**8**), ergosta-4,6,8(14),22-tetraen-3-one (**90**), fungisterol (**91**), mixture of *β*-sitosterol (**92**) and stigmasterol (**93**), mixture of *β*-sitosterol 3-*O*-acetate (**94**) and stigmasterol 3-*O*-acetate (**95**), 4,4-dimethyl-5*α*-ergosta-8,24(28)-dien-3*β*-ol (**96**), 3-*O*-ferulylcycloartenol (**97**), daidzein (**98**), *p*-hydroxybenzoic acid (**99**), vanillic acid (**100**), orobol (**101**), uracil (**40**), genistein (**102**), *d*-mannitol (**35**), *p*-methoxybenzoic acid (**103**), 3-hydroxy-2-methyl-4-pyrone (**104**), acetovanillone (**105**), *p*-hydroxyphenylacetic acid (**106**), cyclo(L-Pro-L-Val) (**107**), syringic acid (**108**), cyclo(L-Phe-L-Pro) (**109**), cyclo(L-Pro-L-Tyr) (**110**), 2-furancarboxylic acid (**111**), *p*-methoxyphenol (**112**), glycitein (**113**), salicylic acid (**114**), methyl-*p*-hydroxyphenylacetate (**115**), thymine (**48**), nicotinic acid (**116**), ergosteryl-3-*O*-*β*-D-glucopyranoside (**117**), flazin (**118**), 3′,4′,7-trihydroxyisoflavone (**119**), succinic acid (**120**), perlolyrine (**121**), 1-methylpyrimidine-2,4-dione (**122**), protocatechuic acid (**123**), 3,4-dihydroxyacetophenone (**124**), 4-hydroxyacetophenone (**125**), 2-deoxy-*d*-ribono-1,4-lactone (**126**), 1-acetyl-*β*-carboline (**127**) and adenosine (**43**), have been isolated and identified (Figure 7). All of these isolated compounds were tested for their anti-inflammatory activity. Compound **121** displayed the potent significant inhibition of superoxide anion generation and elastase release with IC_{50}

values of 0.45 ± 0.15 and 1.68 ± 0.32 μM, respectively. Among the tested compounds, only compound **119** displayed significant scavenging of DPPH free radicals with IC_{50} value of 31.97 μM.

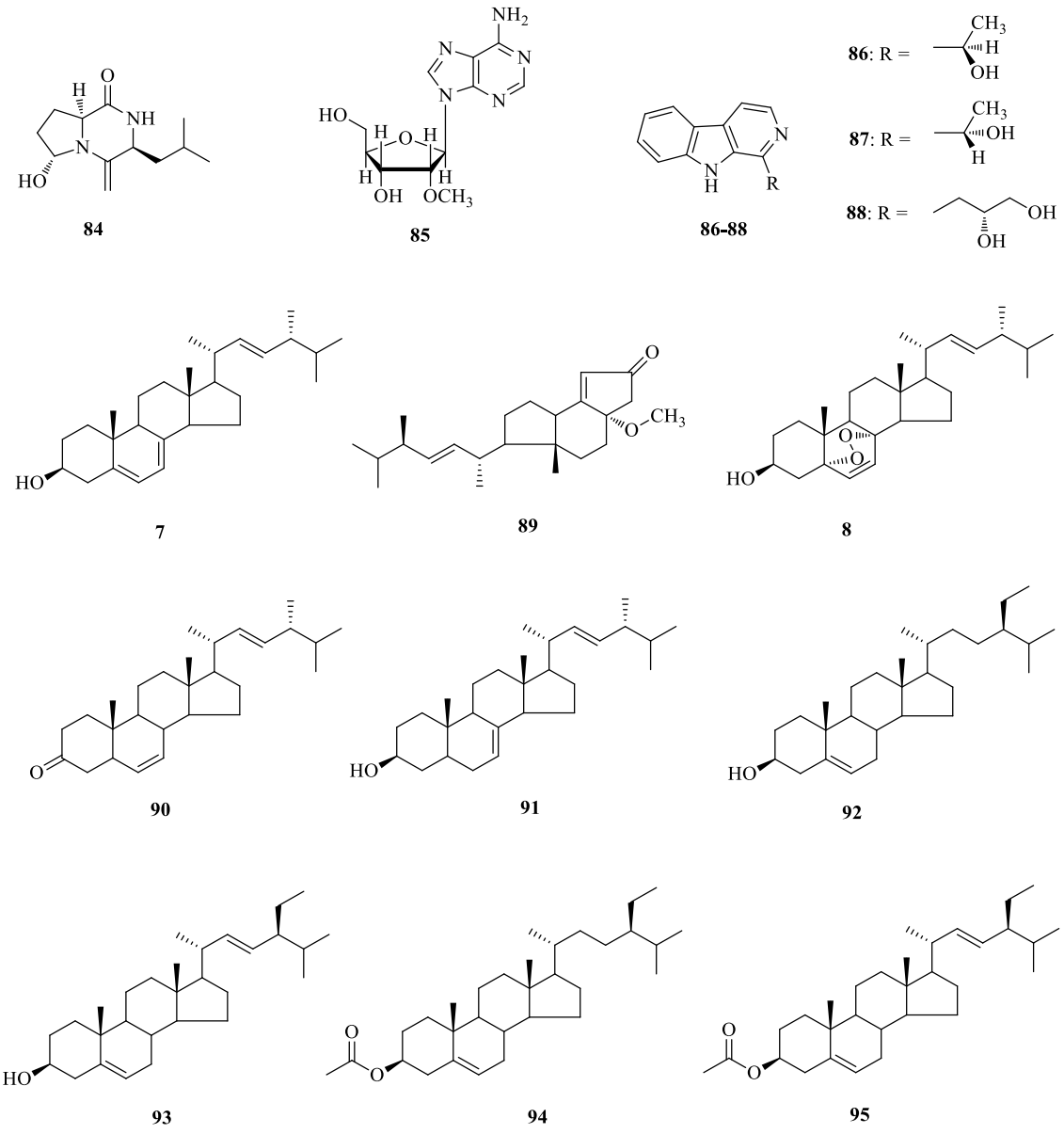


Figure 7. Structures of compounds isolated from *C. sinensis* (continued)

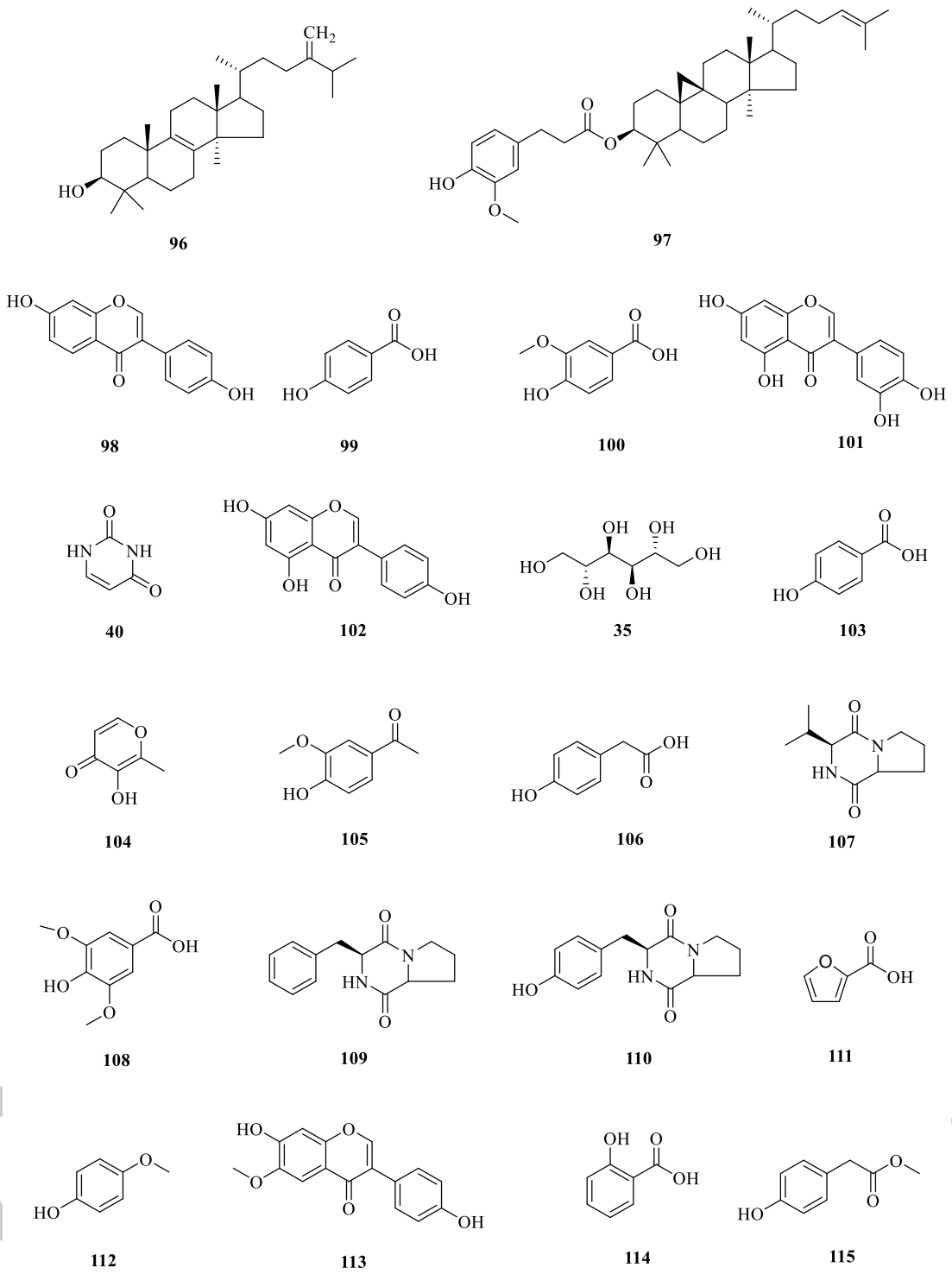


Figure 7. Structures of compounds isolated from *C. sinensis* (continued)

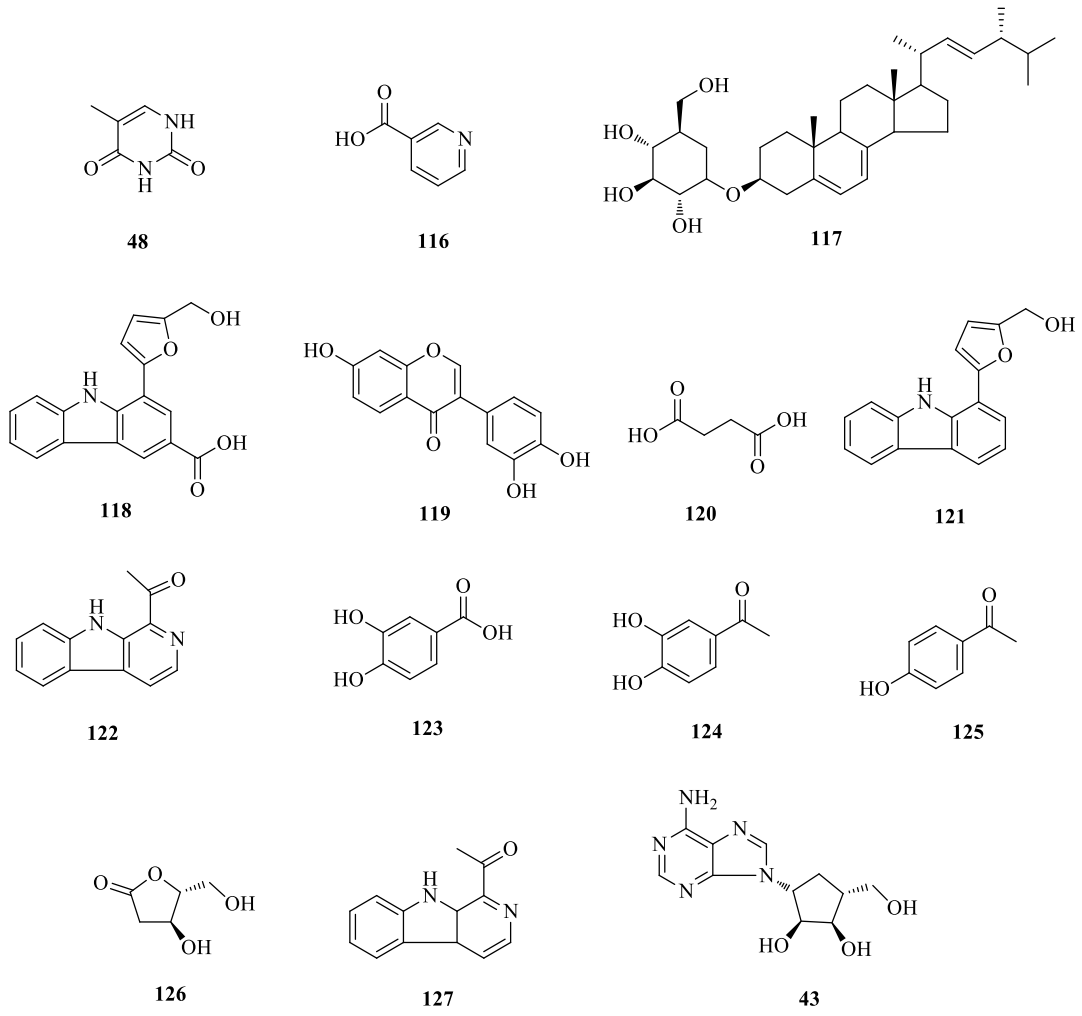
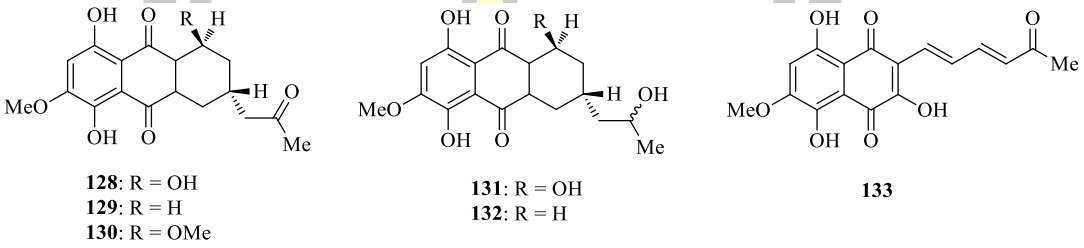


Figure 7. Structures of compounds isolated from *C. sinensis* (continued)

2.1.8 *C. unilateralis*

In 1999, Kittakoop and coworkers reported the purification of the culture broth extract of the fungus *C. unilateralis* (BCC1869) collected from the Khao Luang national park, Thailand [30]. Six bioactive naphthoquinone derivatives; erythrostominone (**128**), deoxyerythrostominone (**129**), 4-*O*-methyl erythrostominone (**130**), epierythrostominol (**131**), deoxyerythrostominol (**132**) and 3,5,8-trihydroxy-6-methoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone (**133**), were isolated (Figure 8). Compounds **128, 129, 131**, and **132** were previously reported to be antibacterial constituents in the fungus *Gnomonia erythrostoma*. Compounds **130** and **133** previously chemically synthesized from erythrostominone but never reported as a

natural product [31], [32], [33]. Compounds **128-133** showed antimalarial activity (*P. falciparum* K1) in the range of EC_{50} 2.5-10.1 μg/mL. Compounds **128-132** showed cytotoxicity against human breast cancer cell line in the range of EC_{50} 4.2-10 μg/mL, human epidermoid carcinoma in the mouth cell line in the range of EC_{50} 7.2-24.0 μg/mL and vero cell line in the range of EC_{50} 7.5-30.0 μg/mL. All of these naphthoquinone derivatives showed red color in acid condition and purple color in base condition. This is an alternative source for red pigment production [34].



128: R = OH
129: R = H
130: R = OMe

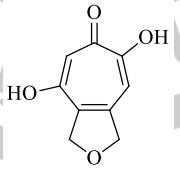
131: R = OH
132: R = H

133

Figure 8. Structures of compounds isolated from *C. unilateralis*

2.1.9 *Cordyceps* sp.

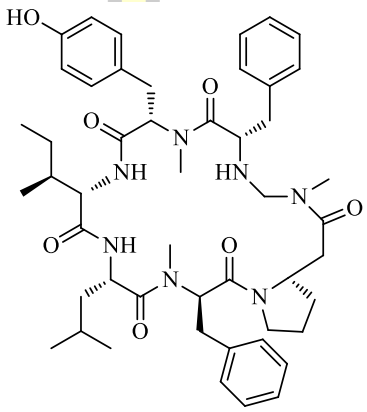
In 2001, Seephonkai and coworkers studied the chemical constituents of the culture broth extract of *Cordyceps sp.* (BCC 1681) collected from Khao Soi Dao wildlife sanctuary, Chantaburi province, Thailand [35]. A new cordytropolone (**134**) was isolated and characterized (Figure 9). Compound **134** exhibited antimalarial activity (*P. falciparum* Kl) with an IC_{50} value of IC_{50} 2.2 μg/mL. However, this compound also showed cytotoxicity against oral human epidermoid carcinoma, human breast cancer cell lines and vero cell lines with IC_{50} values of 17, 2.2 and 11 μg/mL, respectively.



134

Figure 9. Structure of a compound isolated from *Cordyceps* sp. (BCC 1681)

In 2006, Rukachaisirikul and coworkers reported the purification of the culture broth extract of the fungus *Cordyceps* sp. (BCC 1788) provided by BIOTEC [36]. A new cycloheptapeptide; cordyheptapeptide A (**135**), together with four known bioxanthracenes (**70, 72-74**), have been isolated (Figure 10). Compound **135** showed antimalarial activity (*P. falciparum* K1) with an IC_{50} value of 5.35 μM. This compound also showed cytotoxicity against vero cells with an IC_{50} value of more than 56.88 μM.



135

Figure 10. Structure of a compound isolated from *Cordyceps* sp. (BCC 1788)

In 2007, Isaka and coworkers reported the purification of the culture mycelium extract of the fungus *Cordyceps* sp. (BCC 16173) collected from Doi Innthanon national park, Chiang Mai province, Thailand [37]. Five new compounds (**136-140**) and nine known compounds (**70-74, 76** and **78-80**), were isolated and identified (Figure 11). Compounds **70, 73, 80** and **140** showed antimalarial activity (*P. falciparum* K1) with IC_{50} values of 8.1, 3.3, 12.0, and 3.3 μM, respectively.

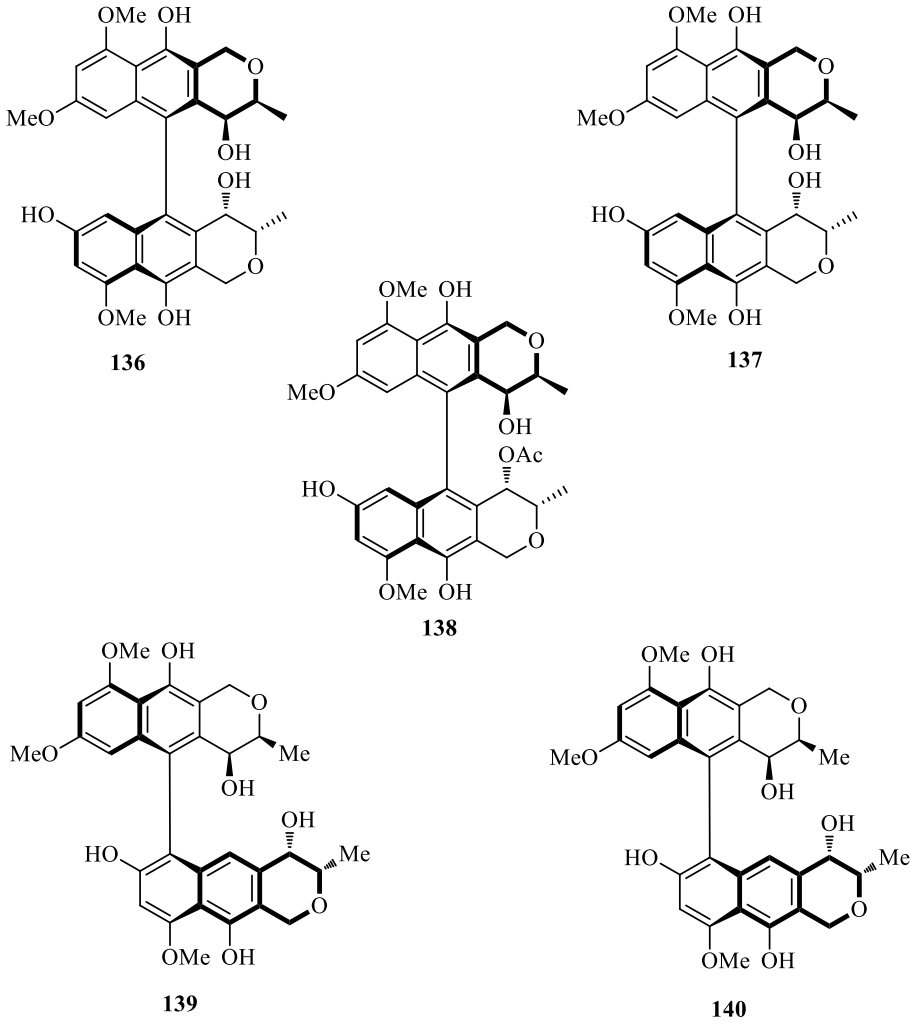
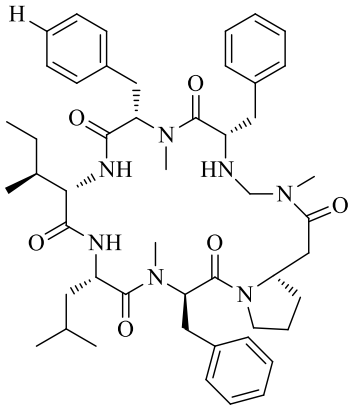


Figure 11. Structures of compounds isolated from *Cordyceps* sp. (BCC 16173)

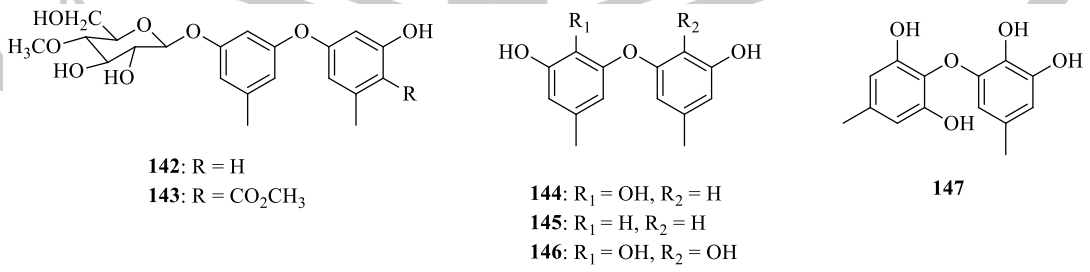
In 2007, Isaka and coworkers studied the chemical constituents of the culture mycelium extract of the fungus *Cordyceps* sp. (BCC 16176) collected from Doi Innthanon national park, Chiang Mai province, Thailand [37]. A new compound; cordyheptapeptide B (**141**) and a known compound; cordyheptapeptide A (**135**), were isolated (Figure 12). Compound **135** showed antimalarial activity (*P. falciparum* K1) with an IC_{50} value of 3.38 μM. It exhibited cytotoxicity against four cell lines, breast cancer cell line, human epidermoid carcinoma in the mouth cell lines, human small cell lung cancer cell lines and vero cell lines with IC_{50} values of 0.78, 0.28, 0.18 and 14.0 μM, respectively. In addition, Compound **141** also exhibited cytotoxicity against four cell lines with IC_{50} value of 2.0, 0.66, 3.1 and 1.6 μM, respectively.



141

Figure 12. Structure of a compound isolated from *Cordyceps* sp. (BCC 16176)

In 2007, Bunyapaiboosri and coworkers investigated the culture broth extract of the fungus *Cordyceps* sp. (BCC 1861) collected from Khao Laem national park, Kanchanaburi province, Thailand [38]. Two novel diphenyl ether glycosides; cordyol A (**142**) and cordyol B (**143**), a new diphenyl ether; cordyol C (**144**), together with three known compounds; diorcinol (**145**), violaceol-I (**146**) and violaceol-II (**147**), were isolated and identified (Figure 13). Biological activities of compound **142** and compound **144** were examined. Compound **142** displayed growth inhibitory activity against *Mycobacterium tuberculosis* ($H_{37}Ra$) with MIC value of 100 μg/mL. Compound **144** exhibited significant anti-herpes simplex virus type 1 activity with an IC_{50} value of 1.3 μg/mL. It also showed cytotoxic activity against breast cancer cells line and human small cell lung cancer cell lines with IC_{50} values of 8.65 and 3.72 μg/mL, respectively.



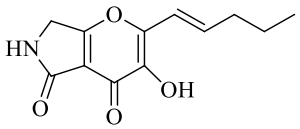
142: R = H
143: R = CO_2CH_3

144: R_1 = OH, R_2 = H
145: R_1 = H, R_2 = H
146: R_1 = OH, R_2 = OH

147

Figure 13. Structures of compounds isolated from *Cordyceps* sp. (BCC 1861)

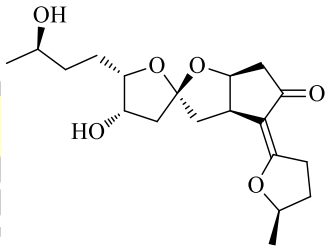
In 2013, Isaka and coworkers reported the isolation of the culture broth extract of the fungus *Cordyceps* sp. (BCC 12671) collected from Khao Yai national park, Nakhon Nayok province, Thailand [39]. A new alkaloid; cordylactam (**148**) was isolated (Figure 14). However, biological activity of this compound was not reported.



148

Figure 14. Structure of a compound isolated from *Cordyceps* sp. (BCC 12671)

In 2014, Grudniewska and coworkers reported the isolation of the culture broth extract of the fungus *Cordyceps* sp. (NBRC 106954) collected from Japan [40]. Opaliferin (**149**) was isolated and identified (Figure 15). Compound **149** was tested for its antitrypanosomal and antimalarial activities. Compound **149** showed no significant inhibitory activity against *Trypanosoma brucei* and *P. falciparum* and weak cytotoxicity against three tumor cell lines (HSC-2, HeLa, and RERF-LC-KJ).



149

Figure 15. Structure of a compound isolated from the *Cordyceps* sp. (NBRC 106954)

There are one hundred and forty nine compounds have been isolated and characterized from the fungus *Cordyceps* from our review. The chemical structures of the isolated compounds are divers and the biological activities of some active compounds are interesting. Table 1 shows the summary of chemical constituents of the

fungus *Cordyceps* published during the year 1997-2017.

Table 1. Chemical constituents of the fungus *Cordyceps* sp.

Cordyceps species	**Isolated compound**	**Reported year**	**Ref.**
C. brunnearubra	**1**	2007	[12]
C. cicadae	**2-6**	2014	[13]
	5 and **7-17**	2017	[14]
C. heteropoda	**18-20**	2004	[15]
C. militaris	**21-29**	2004	[16]
	7, **8**, **28**, **30-36**	2010	[17]
	17, **28** and **37-48**	2014	[18]
	7, **8**, **28**, **43** and **49-52**	2016	[19]
	8, **17**, **43** and **53-60**	2017	[20]
C. nipponica	**64-67**	2001	[21]
C. pseudomilitaris	**68-69**	2000	[24]
	70-82	2005	[25]
C. sinensis	**83**	2010	[28]
	7, **8**, **35**, **40**, **43**, **48**, **84-95**, **96-115** and **116-127**	2011	[29]
C. unilateralis	**128-133**	1999	[30]
Cordyceps sp.	**134**	2001	[35]
	70, **72-74** and **35**	2006	[36]
	70-74, **76**, **78-80** and **136-140**	2007	[37]
	135 and **141**	2007	[37]
	142-147	2007	[38]
	148	2013	[39]
	149	2014	[40]

2.2 Chemical constitutions of *Smilax*

During the past thirty years, there are numbers of report of the chemical constituents and bioactive compounds isolated from the genus *Smilax*. However, the phytochemical study of the *Smilax verticalis* has not been reported yet from literature survey. In this chapter the chemical constituents and bioactive compounds isolated from the genus *Smilax* are reviewed. The root, rhizome, tuber and/or leave extracts were taken to purify to get pure compounds which were characterized for its chemical structures. Later, isolated compounds have been tested for their biological activities. In this review, chemical constituents of eight species of the *Smilax* (*S. aspera, S. bockii, S. bracteate, S. china, S. corbularia, S. excelsa, S. fluminensis, S. macrophylla, S. riparia, S. scobinicaulis, S. sebeana* and *S. trinervula*) and the biological activities of isolated compounds published during the year 1995-2017 in data bases available to access have been summarized.

2.2.1 *S. aspera*

In 2008, Belhouchet and coworkers investigated the roots extract of the *S. aspera* subsp. *mauritanica*. collected in Mas de Jau from Case de Pènes, Roussillon, France [41]. Two new steroidal saponins (**150, 151**) together with the known compouds; curillin G (**152**), asparagoside E (**153**), asparoside A (**154**), asparoside B (**155**) and the phenolic compound; resveratrol (**156**), were isolated (Figure 16). Furthermore, their antifungal activity was tested against three human pathogenic yeasts (*Candida albicans*, *Candida glabrate* and *Candida tropicalis*). Compound **152** exhibited antifungal activity against *Candida albicans*, *Candida glabrata* and *Candida tropicalis* with MIC values of 25, 25 and 50 mg/ml, respectively whereas the other compounds were inactive.

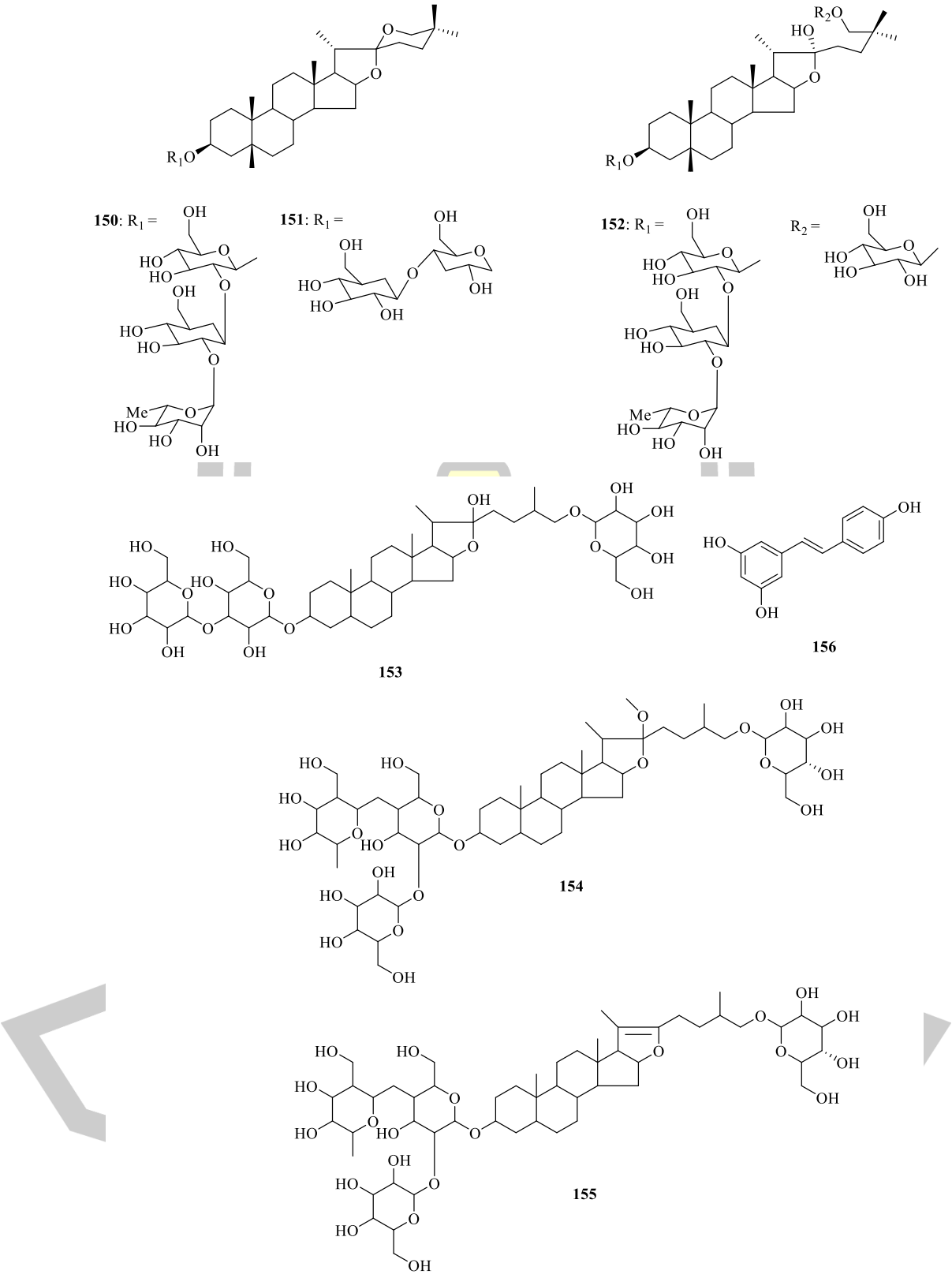
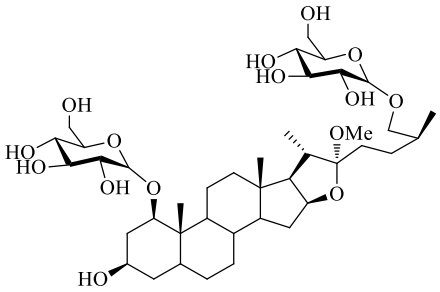
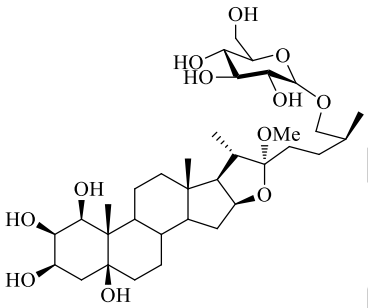


Figure 16. Structures of compounds isolated from *S. aspera*

In 2011, Ivanova and cowokers purified the chemical compositions of the *S. aspera* L. rhizomes extract collected near the Observatory of Nice, France [42]. Two new furostanol saponins; (25S)-26-*O*-*β*-D-glucopyranosyl-5*β*-furostan-1*β*,3*β*,22*α*,26-tetraol-1-*O*-*β*-D-glucopyranoside (**157**) and (25*S*)-26-*O*-*β*-D-glucopyranosyl-5*β*-furostan-1*β*,2*β*,3*β*,5*β*,22*α*,26-hexaol (**158**) and five known compounds; (25*S*)-26-*O*-*β*-D-glucopyranosyl-5*β*-furostan-3*β*,22*α*,26-triol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranoside (**159**), (25*S*)-26-*O*-*β*-D-glucopyranosyl-5*β*-furostan-3*β*,22*α*,26-triol-3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranoside (**160**), trans-resveratrol (**161**), (+) catechin (**162**) and (-) epicatechin (**163**), have been isolated (Figure 16). Compounds **157**-**160** were evaluated for cytotoxic activity against human normal amniotic (FL) and human lung carcinoma cell lines (A549). In vitro experiments compounds **157**-**160** showed significant cytotoxicity in a dose dependent manner with IC_{50} values of 32.98-94.53 μM.



157



158

Figure 16. Structures of compounds isolated from *S. aspera* (continued)

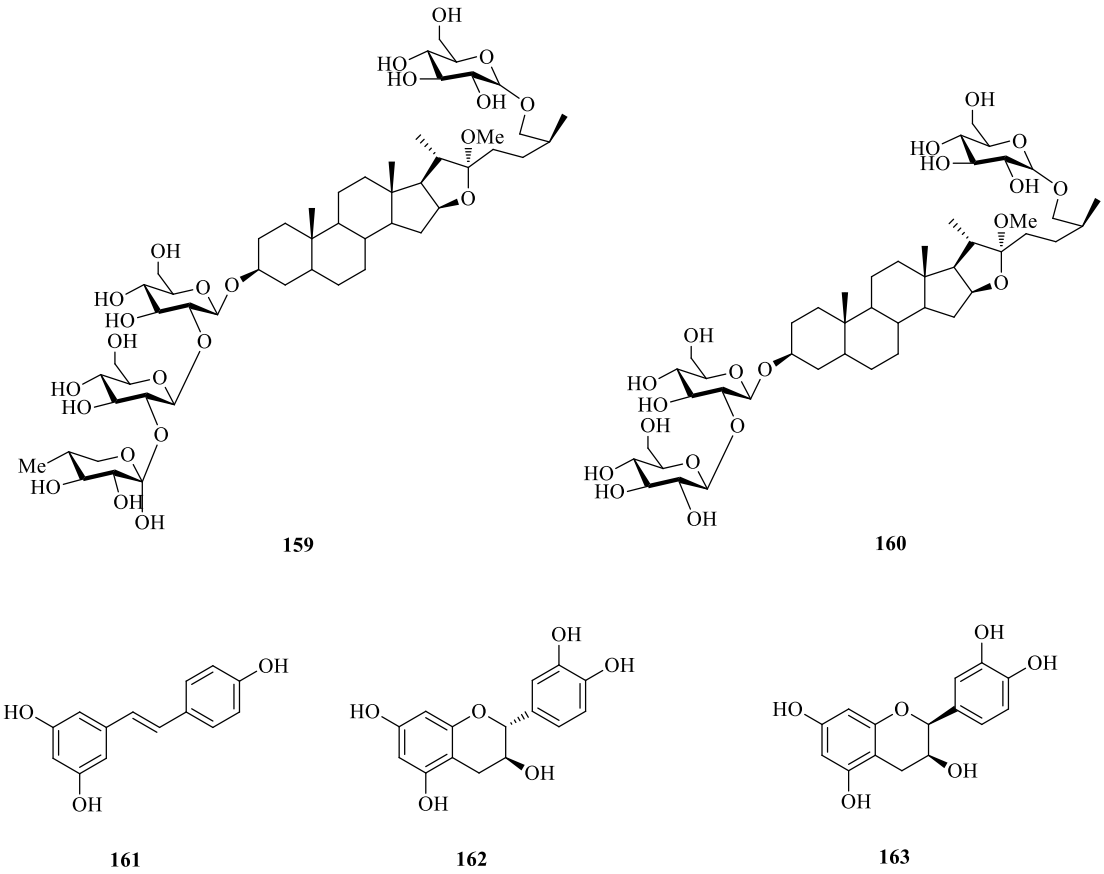


Figure 16. Structures of compounds isolated from *S. aspera* (continued)

2.2.2 *S. bockii*

In 2004, Guo and coworkers carried out a systematic phytochemical investigation of the tuber extract of the *S. bockii* collected from Sichuan Province, China [43]. This purification led to the isolation of twelve compounds, including two new maltol glucosides; bockioside A (**164**) and bockioside B (**165**), and ten known compounds; maltol 3-*O*-*β*-D-glucoside (**166**), hydroxymaltol 3-*O*-*β*-D-glucoside (**167**), isoinnovanoside (**168**), astilbin (**169**), engeletin (**170**), arthromerin B (**171**), rutin (**172**), 2-hydroxy-5-(2-hydroxyethyl)phenyl-*β*-D-glucopyranoside (**173**), pseudoproto-Pb (**174**) and pseudoprotodioscin (**175**) (Figure 17). However, biological activity of all isolated compounds was not reported.

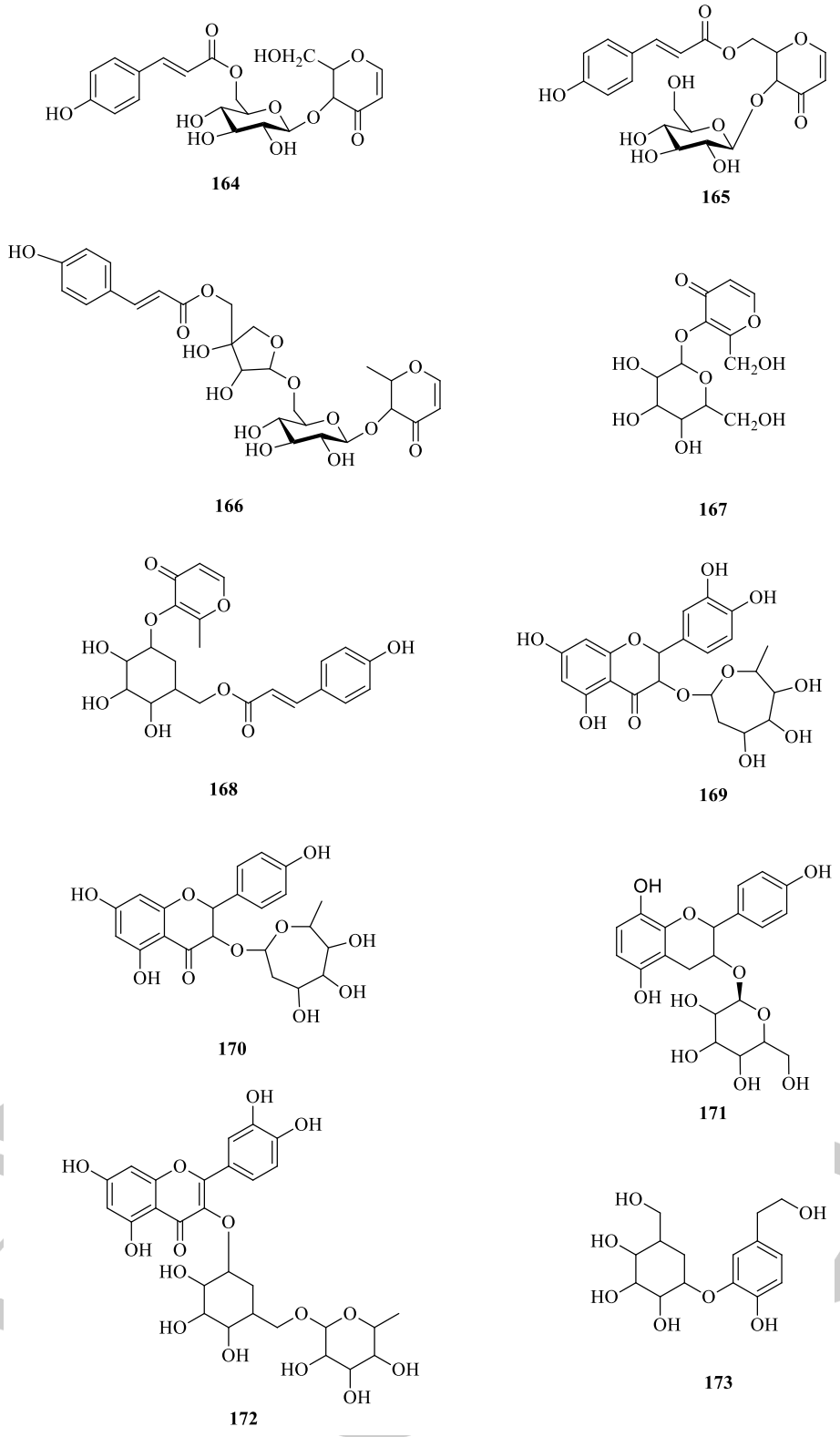


Figure 17. Structures of compounds isolated from *S. bockii*

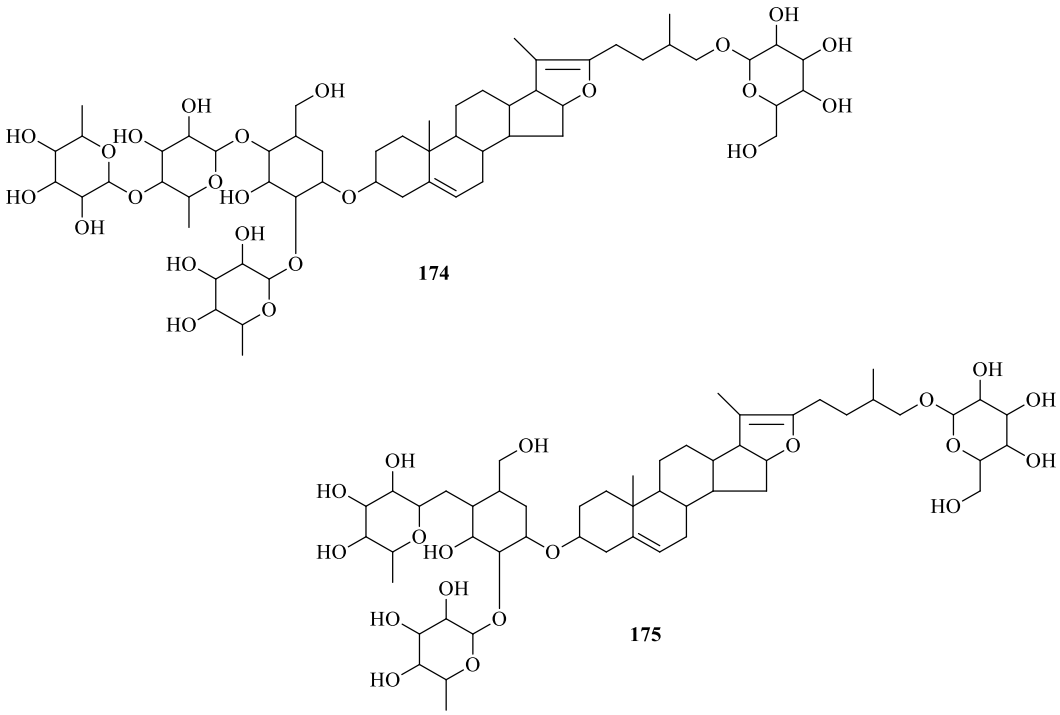
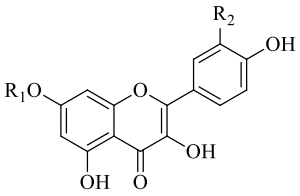
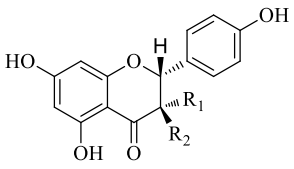


Figure 17. Structures of compounds isolated from *S. bockii* (continued)

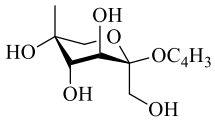
In 2005, Xu and coworkers investigated the chemical constituents from the roots extract of the collected from a county of Hunan province, China [44]. Nine compounds; kaempferol (**176**), kaempferol-7-*O*-*β*-D-glucopyranoside (**177**), quercetin (**178**), isorhamnetin (**179**), (+)-dihydrokaempferol (**180**), engeletin (**170**), isoengeletin (**181**), *n*-butyl-*β*-D-fructopyranoside (**182**) and caffeic acid *n*-butyl ester (**183**), were isolated and identified (Figure 17). The *in vitro* anti-inflammatory activity of the roots extract of the *S. bockii*, was founds to be moderate inhibited TNF-*α*-induced NF-KB activation with an IC_{50} value of 166.6 *μ*g/mL.



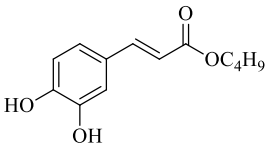
176: R_1= H, R_2 = H
177: R_1= Glc, R_2 = H
178: R_1= H, R_2 = OH
179: R_1= H, R_2 = OCH_3



180: R_1= H, R_2 = OH
170: R_1= H, R_2 = *O*-Rha
181: R_1= *O*-Rha, R_2 = H



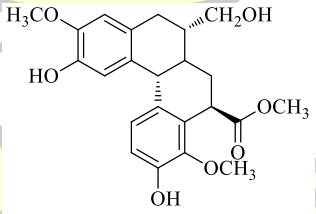
182



183

Figure 17. Structures of compounds isolated from *S. bockii* (continued)

In 2006, Xu and coworkers reported the chemical constituents from the the roots extract of the *S. bockii* collected from Hunan province, China [45]. New lignin; (-)-isolariciresinol-9″carboxylic acid methyl ester (**184**) was isolated (Figure 17).

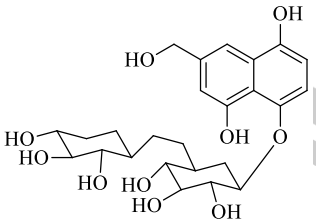


184

Figure 17. Structure of a compound isolated from *S. bockii* (continued)

In 2008, Li and coworkers studied phytochemical constituents of the roots extract of the *S. Bockii* collected from a county of Hunan province, China [46]. A new compound; 7- hydroxymethyl- 1,4,5- trihydroxynaphthalene- 4- *O*- *β*- D- xylopyranosyl (1→6)- *β*- D- glucopyranoside (**185**), was isolated and identified. The structure of the new compound was elucidated on the basis of spectroscopic methods (Figure 17).

However, biological activity of the isolated compound was not reported.



185

Figure 17. Structure of a compound isolated from *S. bockii* (continued)

2.2.3 *S. bracteata*

In 2008, Zhang and coworkers investigated the aerial part extract of the *S. bracteate* collected from the middle mountains of Taiwan [47]. Six phenylpropanoid glycosides; smilasides G-L (**186-191**), together with four known phenylpropanoid compounds; helonioside A (**192**), helonioside B (**193**), smilaside E (**194**) and (1-*p*-*O*-coumaroyl- 6- *O*- feruroyl) - *β*- D- fructofuranosyl- *α*- D- glucopyranoside (**195**) and fourteen known phenolic compounds; tricin (**196**), 5,7,4′-trihydroxy flavanone (**197**), 4,6,4′-trihydroxyaurone (**198**), vitexin (**199**), isovitexin (**200**), quercetin (**201**), 3-*O*-*α*-L-rhamnopyranosyl quercetin (**202**), 3,7-*O*-*α*-L-dirhamnopyranosyl quercetin (**203**), resveratrol (**156**), peceatannol (**204**), veraphenol (**205**), trans-scirpusin A (**206**), 2-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxy xanthone (**207**) and 5-*O*-caffeoylshikimic acid (**208**), were isolated (Figure 18). Moreover, compounds **186-191** exhibited moderate scavenging activities against DPPH radicals with EC_{50} values of 7.193, 7.935, 6.847, 2.667, 3.021 and 3.270 10^{-5} M, respectively.

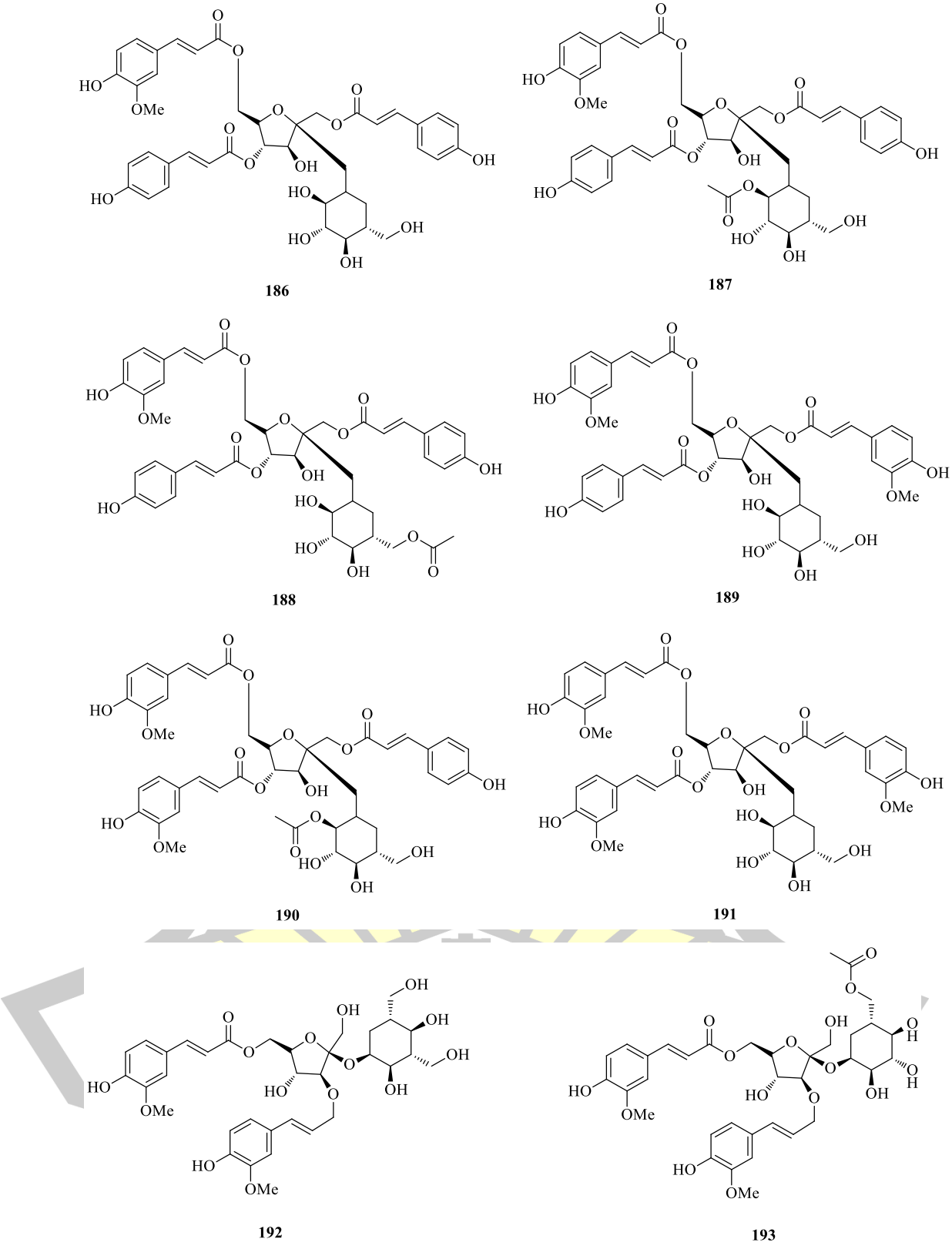


Figure 18. Structures of compounds isolated from *S. bracteata*

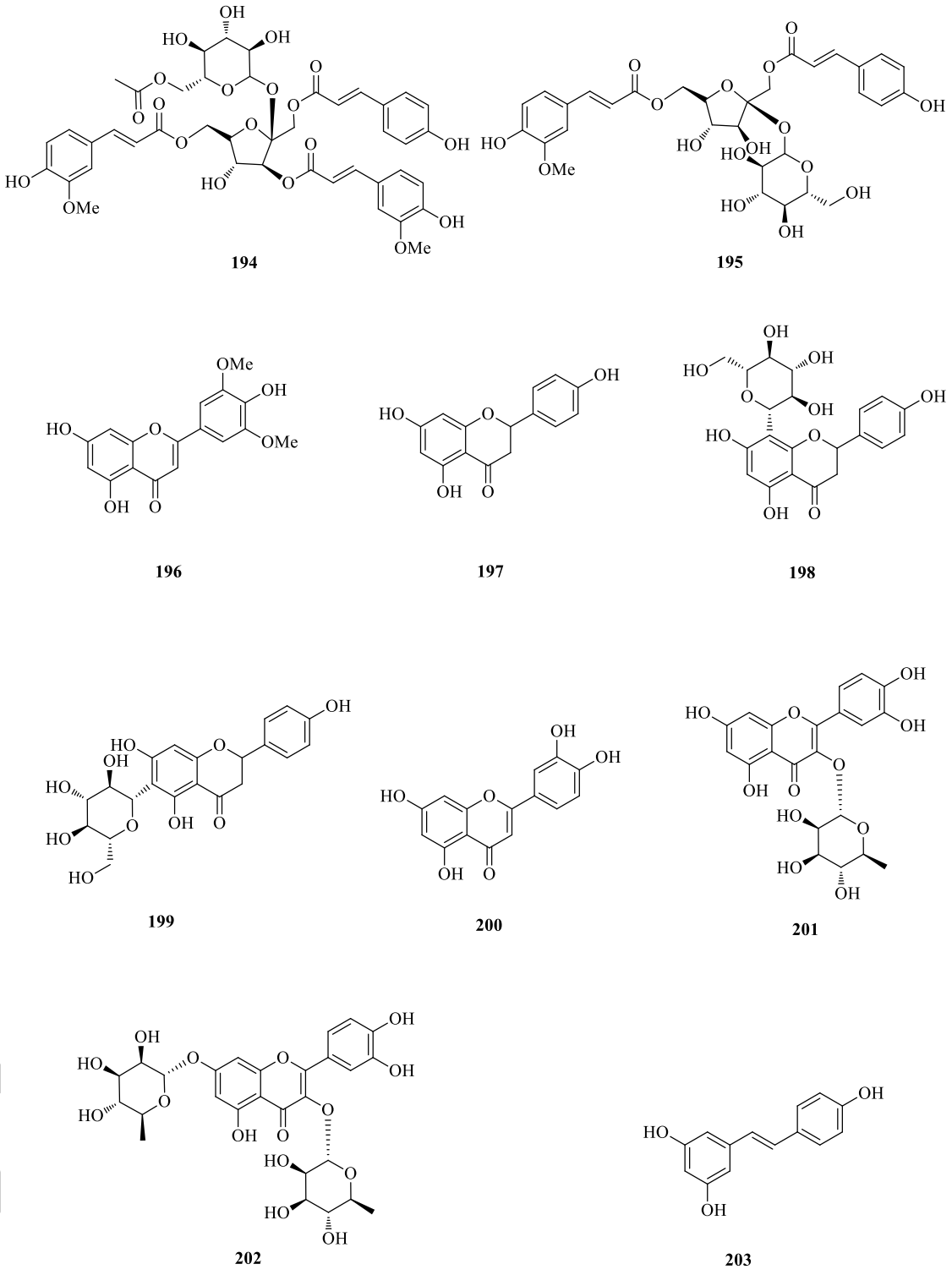


Figure 18. Structures of compounds isolated from *S. bracteata* (continued)

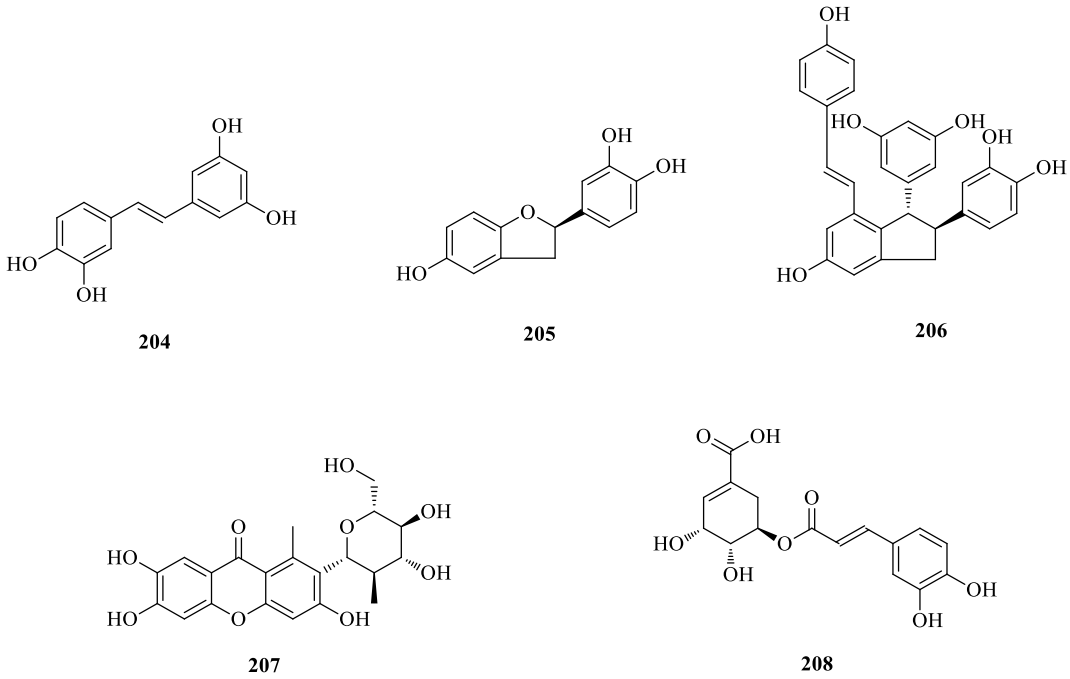
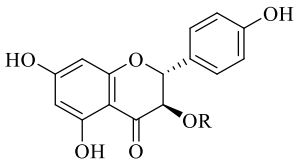


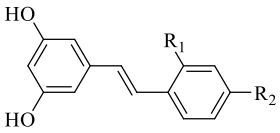
Figure 18. Structures of compounds isolated from *S. bracteata* (continued)

2.2.4 *S. china*

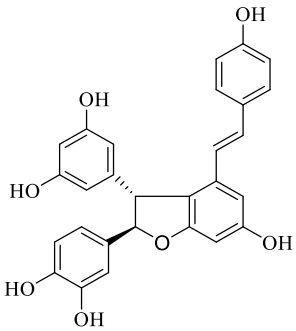
In 2010, Wu and coworkers purified the roots and tubers extract of the *S. china* L. purchased from Nanjing Pharmacy Ltd. Co., Nanjing, China [48]. Six polyphenols; dihydrokaempferol (**180**), resveratrol (**156**), oxyresveratrol (**209**), scirpusin A (**210**), kaempferol-7-*O*-*β*-D-glucoside (**211**) and dihydrokaempferol-3-*O*-*α*-L-rhamnoside (**212**), were obtained on the basis of a bioassay-guided isolation (Figure 19). Their breast tumor cytotoxic activities were tested. Compounds **156**, **180** and **209**-**212** showed anti-tumor activities against MCF-7 with IC_{50} values of 2.1-32.6 μg/mL and MDA-MB-231 with IC_{50} values of 2.9-38.9 μg/mL. All of isolated compounds can induce apoptosis for MCF-7 with apoptosis rates of 12.9-39.8% and MDA-MB-231 with apoptosis rates of 12.9-39.8%.



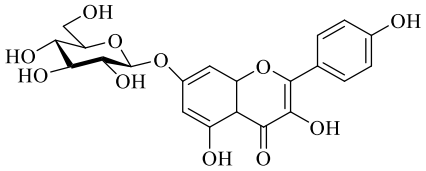
180: R = H
212: R = Rha



156: R_1 = H, R_2 = OH
209: R_1 = OH, R_2 = OH



210



211

Figure 19. Structures of compounds isolated from *S. china*

In 2012, Liang and coworkers chemical investigated the rhizomes extract of the *S. china*. purchased from Gangwon province, Korea [49]. Two known compounds; oxyresveratrol (**209**) and dioscin (**213**), were isolated by activity-guided column chromatography (Figure 19). Compound **213** showed little inhibition activity of tyrosinase, whereas compound **209**, a known tyrosinase inhibitor, showed a strong tyrosinase inhibitory activity. Interestingly, a mixture of compound **213** and compound **209** (1:1 ratio) showed higher inhibition on tyrosinase activities with L-tyrosine with the IC_{50} value of 5.1 μg/mL and L-DOPA with the IC_{50} value of 5.7 μg/mL as the substrate as compared to either compound **209** with the IC_{50} values of 7.8 and 10.9 μg/mL or compound **213** alone with the IC_{50} values >100 and 100 μg/mL.

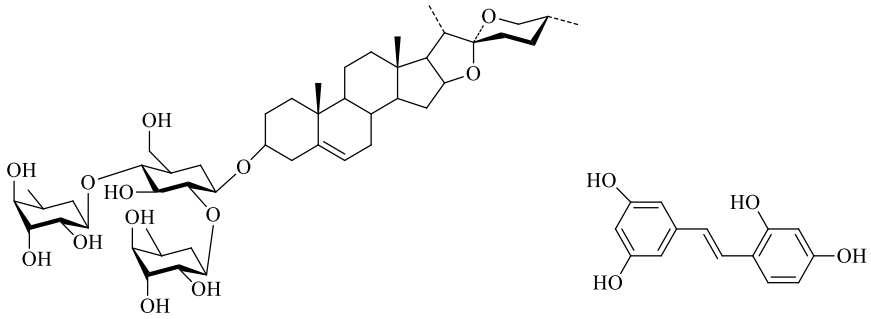
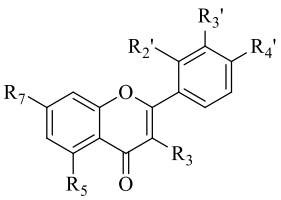
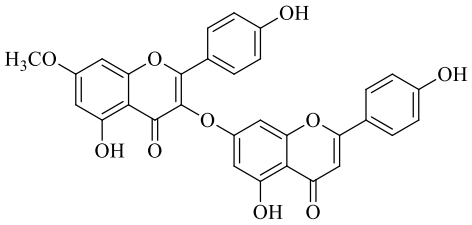


Figure 19. Structures of compounds isolated from *S. china* (continued)

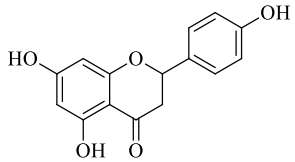
In 2016, Zhao and coworkers purified the leaves extract of the *S. china* L. collected from Cho- lye mountain in Daegu, Korea [50]. Two new flavonoids; bismilachinone (**214**) and smilachinin (**215**), were isolated together with fourteen known compounds; kaempferol (**176**), kaempferide (**216**), morin (**217**), kaempferol 7-*O*-*α*-L-rhamnoside (**218**), kaempferin (**219**), quercetin 4′-*O*-*β*-D-glucoside (**220**), vitexin (**199**), kaempferitrin (**221**), lepidoside (**222**), rutin (**172**), partensein (**223**), puerarin (**224**), naringenin (**225**) and 1,3,6-trithydroxyxanthone (**226**) (Figure 19). The PTP1B, *α*-glucosidase and DPP-IV inhibitory activities of all isolated compounds were evaluated at the molecular level. Compounds **218**, **199** and **172** showed moderate DPP-IV inhibitory activities with IC_{50} values of 20.81, 33.12 and 32.93 mM, respectively. Compounds **217**, **218**, **220**, **214**, **215** and **226** showed strong PTP1B inhibitory activities with respective IC_{50} values of 7.62, 10.80, 0.92, 2.68, 9.77 and 24.17 mM. Compounds **216**-**220**, **199**, **172**, **223**, **225** and **226** showed potent α-glucosidase inhibitory activities with respective IC_{50} values of 8.70, 81.66, 35.11, 35.92, 7.99, 26.28, 11.28, 62.68, 44.32 and 70.12 mM. In the kinetic study for the PTP1B enzyme, compounds **220**, **214** and **223** displayed competitive inhibition with K_i values of 3.20, 8.56 and 5.86 mM, respectively. Compounds **217**, **218** and **226** showed noncompetitive inhibition with K_i values of 18.75, 5.95 and 22.86 mM, respectively. Molecular docking study for the competitive inhibitors (**220**, **214** and **223**) radically corroborates the binding affinities and inhibition of PTP1B enzymes.





214

176: R_3, R_5, R_7, R_4' = OH, R_8, R_2', R_3' = H
216: R_3, R_5, R_7 = OH, R_8, R_2', R_3' = H, R_4' = OCH_3
217: R_3, R_5, R_7, R_2', R_4' = OH, R_8, R_3' = H
218: R_3, R_5, R_4' = OH, R_7 = O-Rham, R_8, R_2', R_3' = H
219: R_3 = O-Rham, R_5, R_7, R_4' = OH, R_8, R_2', R_3' = H
220: R_3, R_5, R_7, R_4' = OH, R_8, R_2' = H, R_4' = Glu
199: R_3, R_2', R_3' = H, R_5, R_7, R_4' = OH, R_8 = O-Glc
221: R_3, R_7 = O-Rham, R_5, R_4' = OH, R_8, R_2', R_3' = H
222: R_3 = O-Rham, R_5, R_4' = OH, R_7 = O-Xyl, R_8, R_2', R_3' = H
172: R_3 = O-rutinoside, R_5, R_7, R_3', R_4' = OH R_8, R_2' = H



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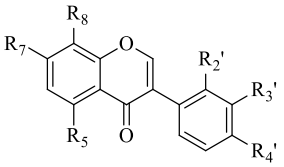
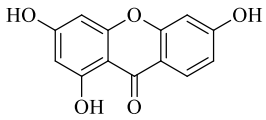


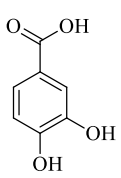


226

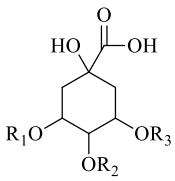
223: R_5, R_7, R_3' = OH, R_8, R_2' = H, R_4' = OCH_3
224: R_5, R_2', R_3' = H, R_7, R_4' = OH, R_8 = Glu
225: R_5, R_7 = OH, R_8, R_3' = H, R_2' = O-Glu, R_4' = OCH_3

Figure 19. Structures of compounds isolated from *S. china* (continued)

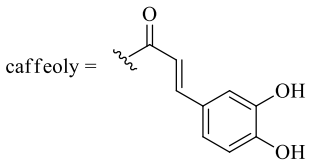
In 2017, Lee and coworkers chemical studied on the stems extract of the *S. china* L. collected from Yesan-gun, Chungcheongnam-do, Korea [51]. Ten compounds; protocatechuic acid (**227**), three chlorogenic acids; 5-*O*-caffeoylquinic acid (**228**), 3-*O*-caffeoylquinic acid (**229**) and 4-*O*-caffeoylquinic acid (**230**), four flavonoids; kaempferol 3-*O*-α-D-glucopyranosyl-7-*O*-β-L-rhamnopyranoside (**231**), quercitrin (**232**), afzelin (**233**) and *trans*-resveratrol (**161**), one stilbene; helonioside A (**192**) and one phenylpropanoid glycoside; isoscutellarein-8-*O*-rhamnoside (**234**), were isolated and identified (Figure 19). All isolated compounds were tested for their inhibitory effects against advanced glycation end products, as well as aldose reductase, α-gucosidase and lipase assays were also performed.



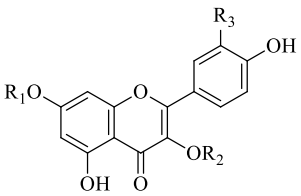
227



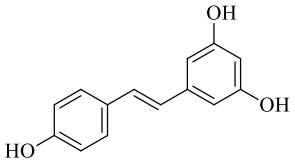
caffeoly =



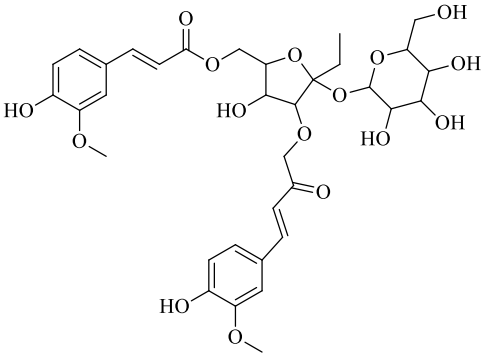
228: R_1 = caffeoly, R_2 = H, R_3 = H
229: R_1 = H, R_2 = H, R_3 = caffeoly
230: R_1 = H, R_2 = caffeoly, R_3 = H



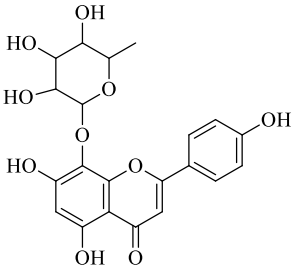
231: R_1 = Rhamnose, R_2 = Glcose, R_3 = H
232: R_1 = H, R_2 = Rhamnose, R_3 = OH
233: R_1 = H, R_2 = Rhamnose, R_3 = H



161



192



234

Figure 19. Structures of compounds isolated from *S. china* (continued)

In 2017, Zhong and coworkers reported the isolation of the rhizomes extract of the *S. china* L. purchased from Shenzhen Hongen Pharmaceutical Company, China [52]. A new triflavanoid; kandelin B-5 (**235**), together with six known phenylpropanoid substituted flavan-3-ols; cinchonain IIa (**236**), cinchonain IIb (**237**), cinchonain Ia (**238**), cinchonain Ib (**239**), catechin-[8,7-e]-4β-(3,4-dihydroxyphenyl)-dihyro-2(3*H*)-pyranone (**240**) and catechin-[8,7-e]-4α-(3,4-dihydroxyphenyl)-dihyro-2(3*H*)-pyranone (**241**), nine flavonoids; engeletin (**170**), astilbin (**169**), neoastilbin (**242**), isoastilbin (**243**), isoneoastilbin (**244**), quercetin-3-*O*-α-L-rhamnopyranoside (**245**),

luteolin-3-*O*-α-L-rhamnopyranoside (**246**), (-) epicatechin (**247**)and 5,7,4′- (**248**), two stilbenoids; scirpusin A (**210**) and resveratrol (**156**), and two other compounds; chlorogenic acid (**249**) and protocatechuic acid (**227**), were isolated and identified (Figure 19). Compounds **236**-**239**,**170**, **169**, **242**-**244**, **247**, **210** and **249** were evaluated for anti-inflammatory activity. Only compounds **242**, **247** and **210** showed slightly IL-1β expression inhibitory activities on LPS induced THP-1 cells, with inhibition rate of 15.8%, 37.3% and 35.8%, respectively.

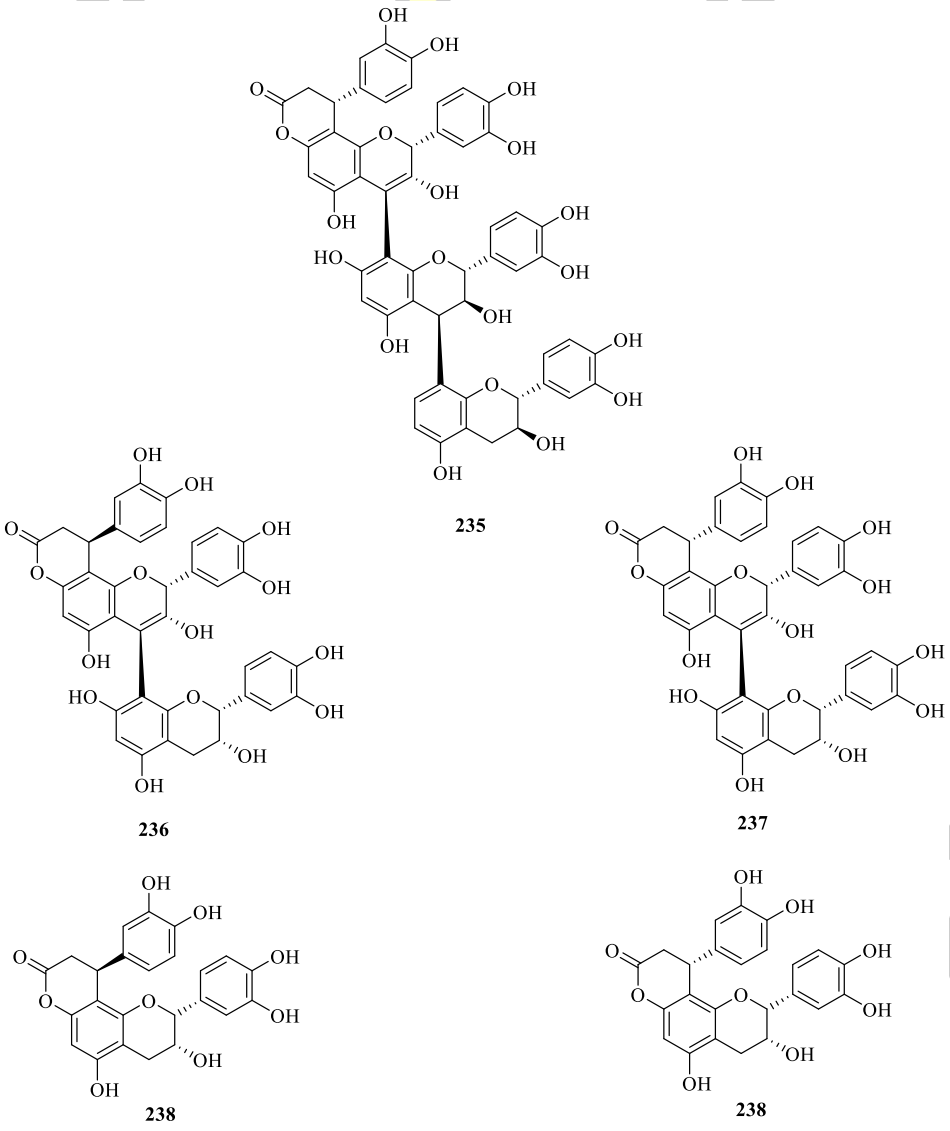


Figure 19. Structures of compounds isolated from *S. china* (continued)

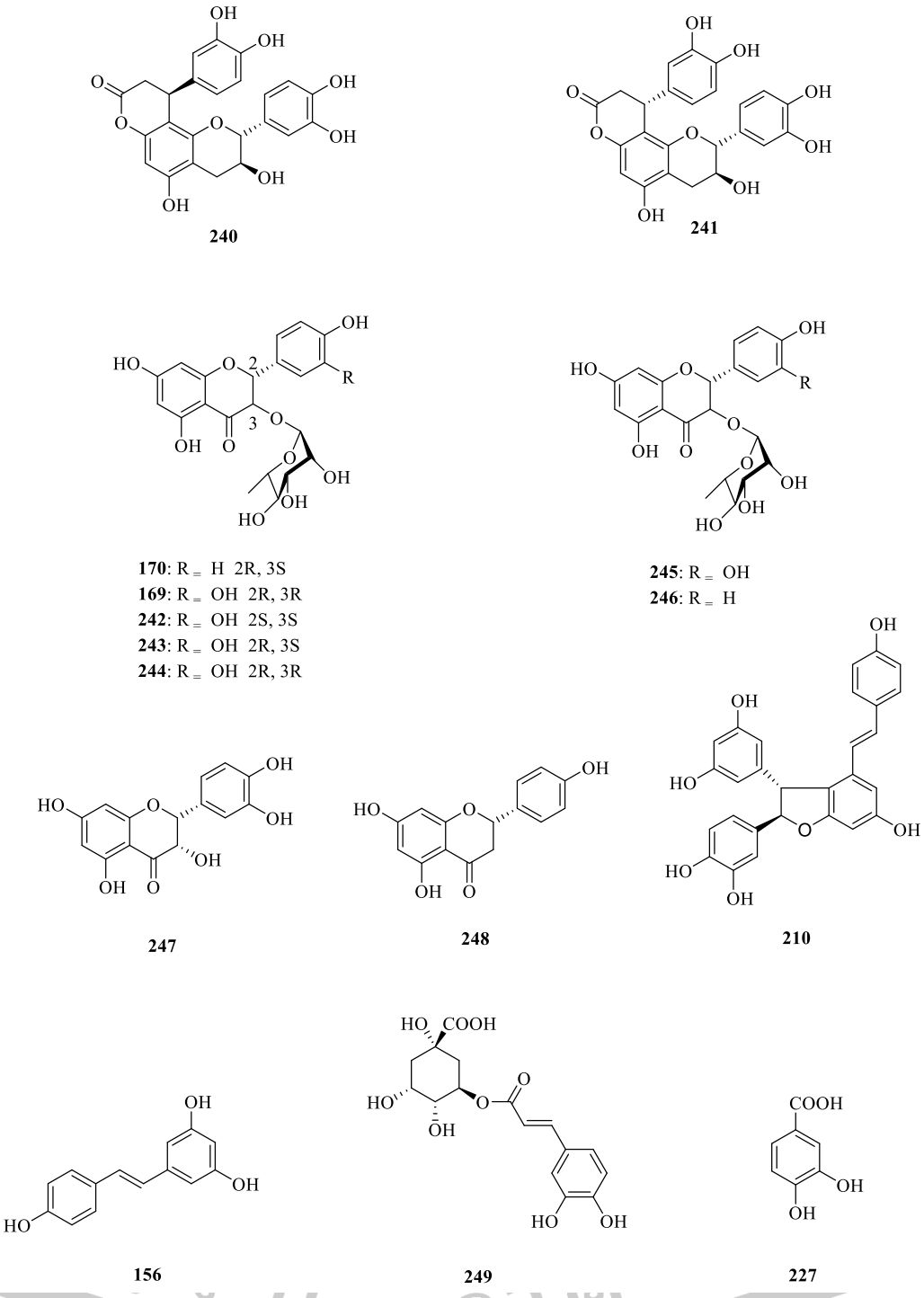


Figure 19. Structures of compounds isolated from *S. china* (continued)

2.2.5 *S. corbularia*

In 2011, Wungsintaweekul and coworkers reported the isolation of constituents from the rhizomes extract of the *S. corbularia* Kunth. purchased from a traditional Thai herb store in Nakhon Sri Thammaraj, Thailand [53]. Eleven compounds; (2*R*,3*R*)-2′-acetyl astilbin (**250**), (2*R*,3*R*)-3″-acetyl astilbin (**251**), (2*R*,3*R*)-4″-acetyl astilbin (**252**), (2*R*,3*R*)-3″-acetyl engeletin (**253**), (2*R*,3*S*)-4″-acetyl isoastilbin (**254**), 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*R*)-2*H*,8*H*-benzo[1,2-b:3,4-b′] dipyran-8-one (**255**), 2-(4-hydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-10-(3,4-dihydroxyphenyl)-(2*R*,3*R*,10*S*)-2*H*, 8*H*-benzo [1,2-b:3,4-b′] dipyran-8-one (**256**), 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxyphenyl)-5-[(1*E*)-2-(4-hydroxyphenyl) ethenyl]-2*H*-1-benzopyran-2-one (**257**), 3,4-dihydro-7-hydroxy-4-(3,4-dihydroxy-phenyl)-5-[(1*E*)-2-(3,4-dihydroxyphenyl) ethenyl]-2*H*-1-benzopyran-2-one (**258**), 3,4-dihydro-7-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-5-[(1*E*)-2-(4hydroxyphenyl)ethenyl-2*H*-1-benzopyran-2-one (**259**) and 5,7,3′, 4′-tetrahydroxy-3-phenylcoumarin (**260**), together with 34 known compounds; astilbin (**169**), neoastilbin (**242**), isoastilbin (**261**), neoisoastilbin (**262**), engeletin (**170**), isoengeletin (**181**), (+) taxifolin (**263**), (+) dihydrokaempferol (**180**), naringenin (**225**), eriodictyol (**264**), homoeriodictyol (**265**), quercetin (**201**), quercitrin (**232**), luteolin (**266**), (-) catechin (**267**), (-) epicatechin (**163**), cinchonain Ia (**238**), catechin-(7,8-b,c)-4b-(3,4-dihydroxyphenyl)-2(3*H*)-pyranone (**268**) cinchonain Ib (**239**) rhinchoin Ia (**269**), cinchonain Id (**270**), (4*S*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-b:3,4-b′] dipyran-2-one (**271**), cinchonain Ic (**272**), (4*R*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-b:3,4-b′] dipyran-2-one (**273**), phyllocoumarin (**274**), epiphyllocoumarin (**275**), trans-resveratrol (**161**), piceatannol (**276**), isorhapontigenin (**277**), eucryphin (**278**), (-) syringaresinol (**279**), 5-*O*-caffeoylshikimic acid (**208**), caffeic acid (**280**) and protocatechuic acid (**227**), were isolated and characterized (Figure 20). All isolated compounds had their estrogenic and anti-estrogenic activities determined using the estrogen-responsive human breast cancer cell lines (MCF-7 and T47D). The major constituents were recognized as compounds **250-254** by the suppressive effect on estradiol induced cell proliferation at a concentration of 1 μM. Meanwhile, flavanonol rhamnoside acetates

demonstrated estrogenic activity in both MCF-7 and T47D cells at a concentration of 100 μM, and they enhanced the effects of co-treated E2 on T47D cell proliferation at concentrations of more than 0.1 μM.

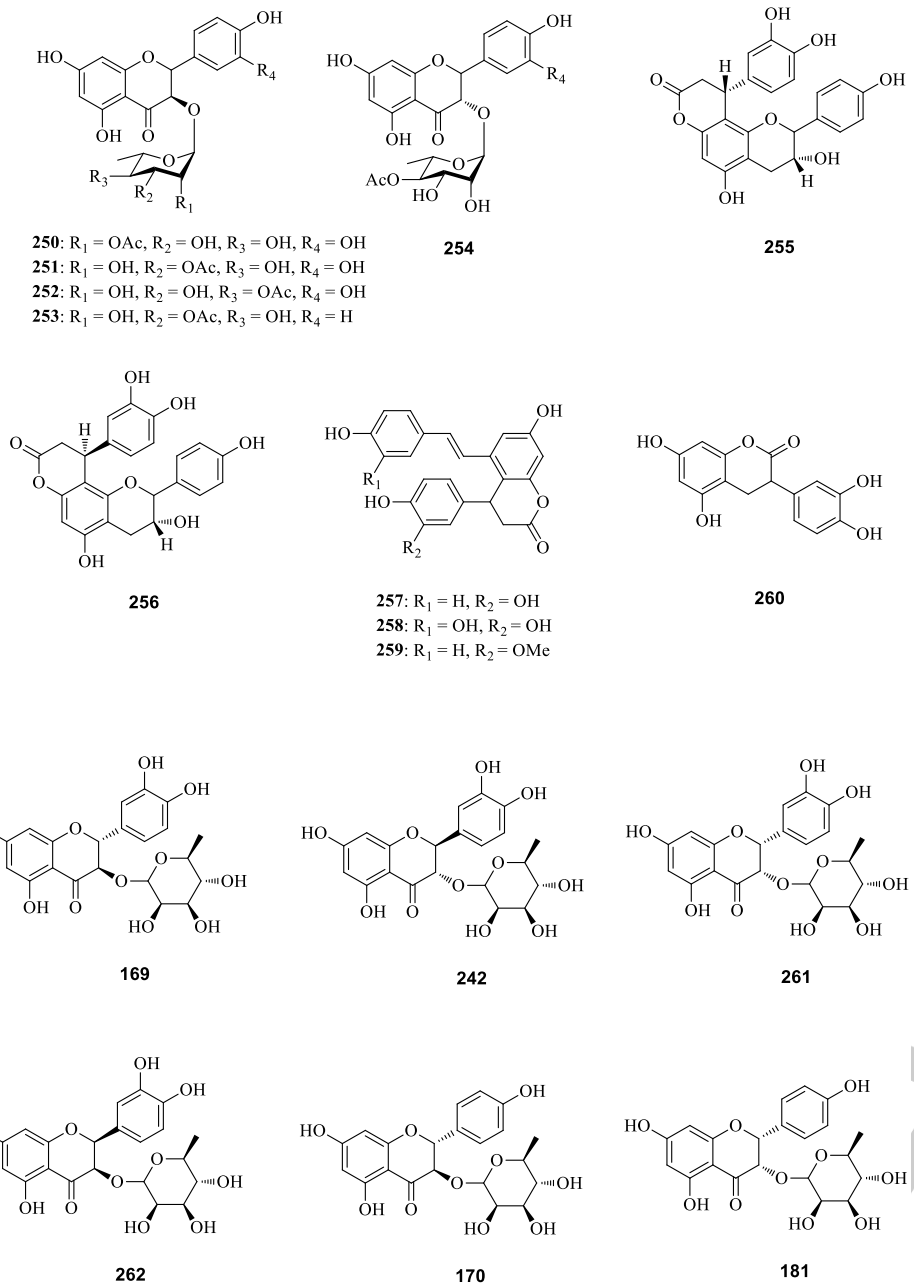


Figure 20. Structures of compounds isolated from *S. corbularia*

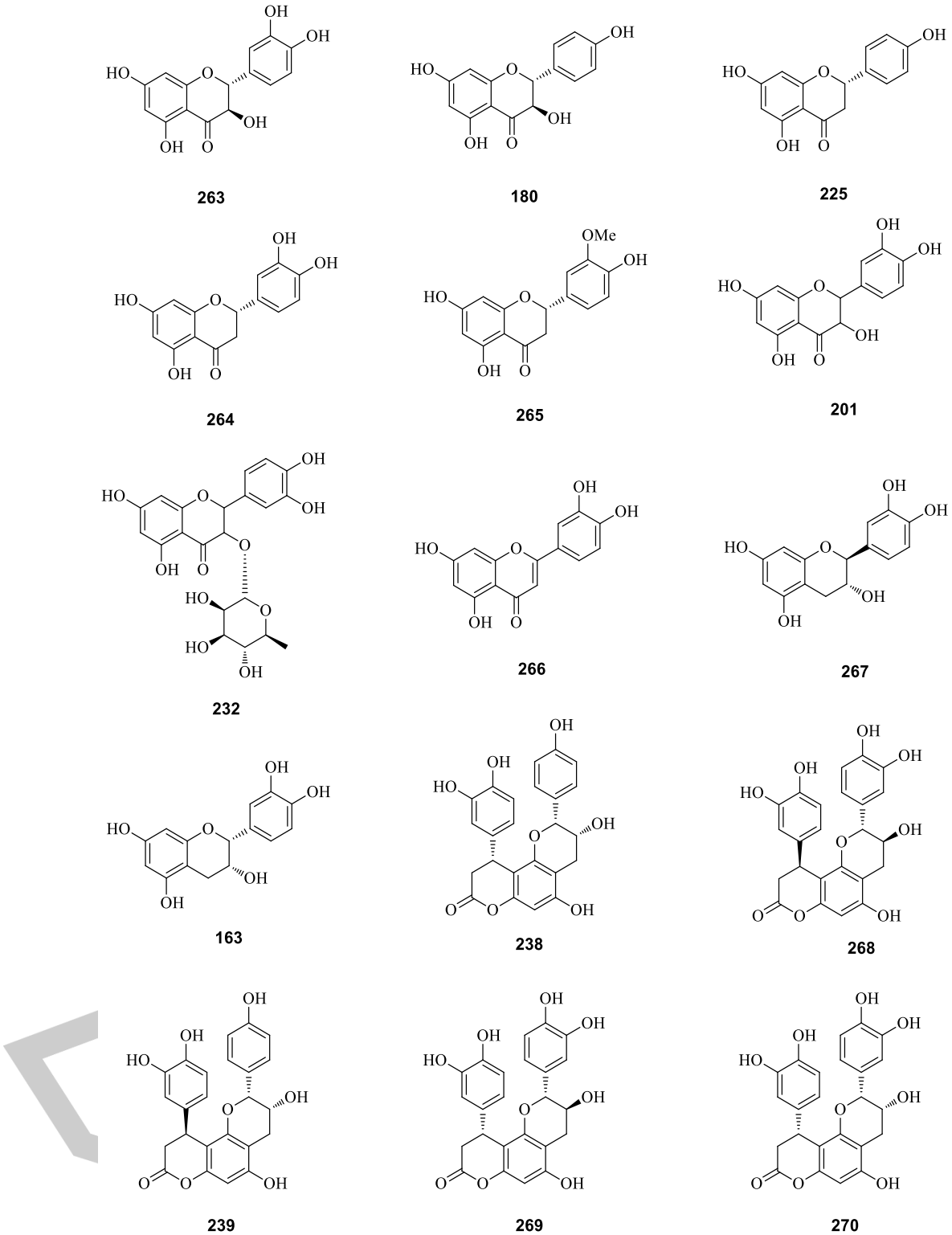


Figure 20. Structures of compounds isolated from *S. corbularia* (continued)

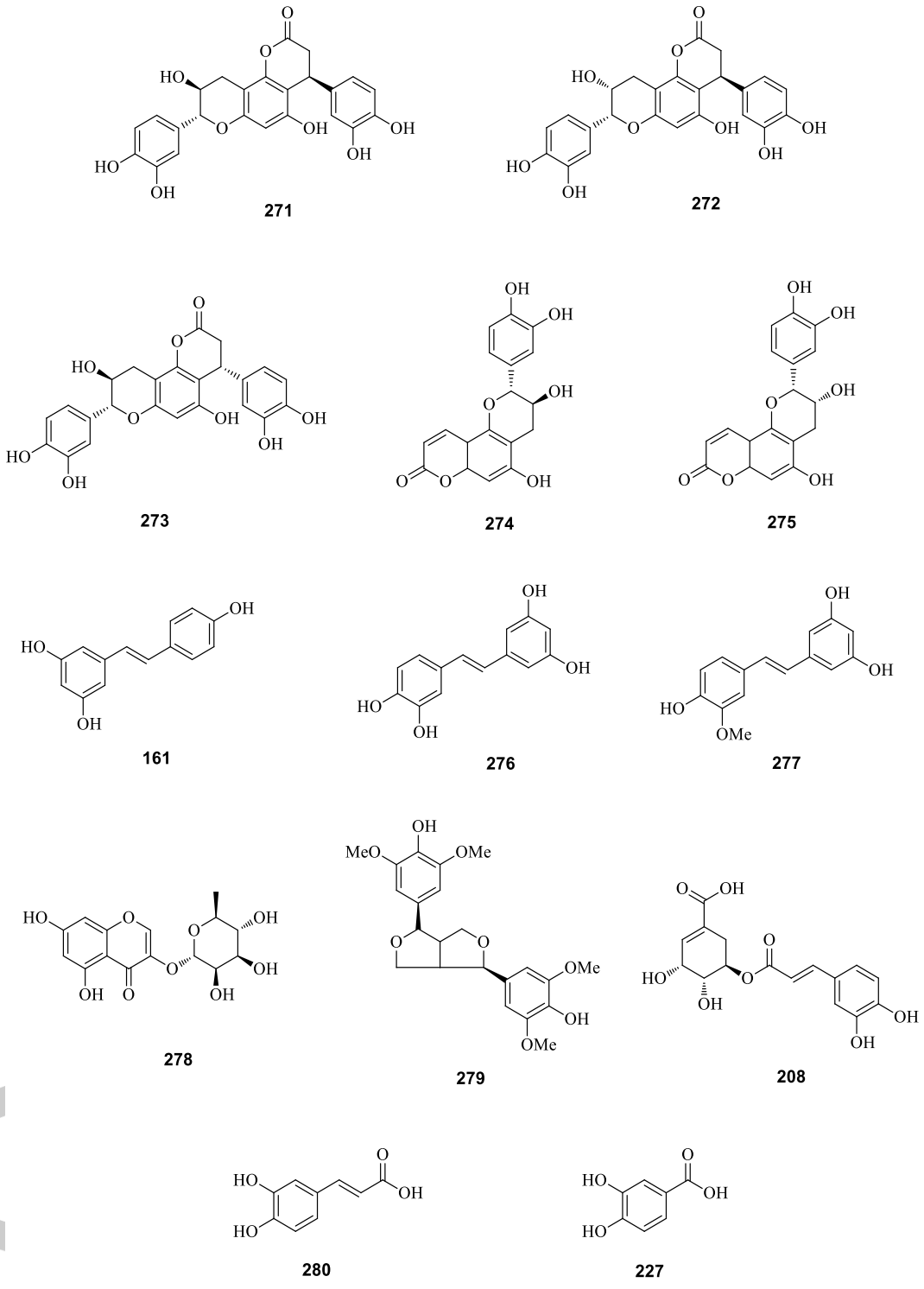


Figure 20. Structures of compounds isolated from *S. corbularia* (continued)

2.2.6 *S. excelsa*

In 2010, Ivanova and coworkers investigated phytochemical constituents of the rhizomes extract of the *S. excels* collected near Golden Sands, Varna, Bulgaria [54]. The results from the investigation led to the isolation and structural identification of *trans*-resveratrol (**161**), naringenin (**225**), 5-*O*-caffeoylshikimic acid (**208**), 1-*O*-*trans*-feruloylglycerol (**281**), 1-*O*-*trans*-*p*-coumaroylglycerol (**282**) and 1,2-*O*-di-*trans*-feruloylglycerol (**283**) (Figure 21). The antimicrobial and cytotoxic activities of the methanol, chloroform, *n*-butanol, and water-methanol extracts from the rhizomes extract were tested. The extracts showed no antimicrobial activity and cytotoxic activities with LC_{50} of 15.84, 10.08, 4.36, 32.62 and 0.45 μg/mL, respectively.

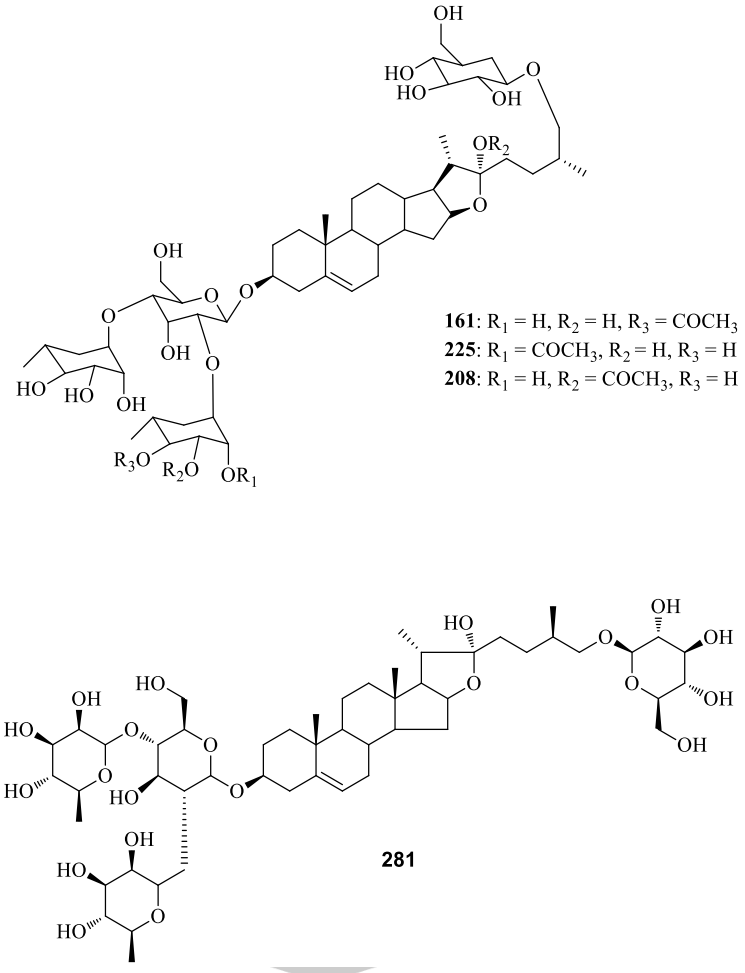


Figure 21. Structures of compounds isolated from *S. excelsa*

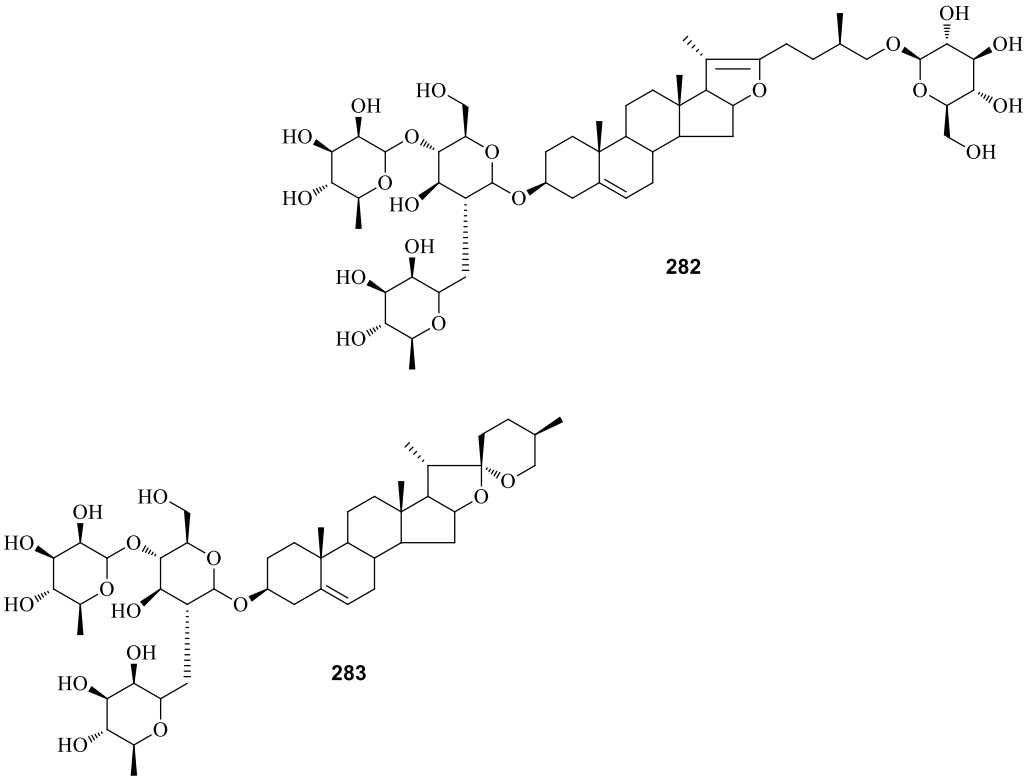


Figure 21. Structures of compounds isolated from *S. excelsa* (continued)

In 2016, Khaligh and coworkers carried out a phytochemical investigation on aerial parts extract of the *S. excels* collected from Baladza village, Sari, Iran [55]. This study led to isolation and structure elucidation of five compounds; solanesol (**284**), violasterol A (**285**), *trans*-resveratrol (**161**), 5-*O*-caffeoylshikimic acid (**208**) and 6-*O*-caffeoyl-*β*-D-fructofuranosyl-(2-1)-*α*-D-glucopyranoside (**286**) (Figure 21). The cytotoxicity and antibacterial activity of the isolated compounds were evaluated by MTT and MIC assays. Compounds **284** and **285** showed promising inhibition on MCF-7 cell line with IC_{50} of 161.6 and 190.0 μM, respectively. Compounds **285** and **161** also illustrated activity against *Staphylococcus aureus* with MIC values of 142.5 and 136.9 μM, respectively.

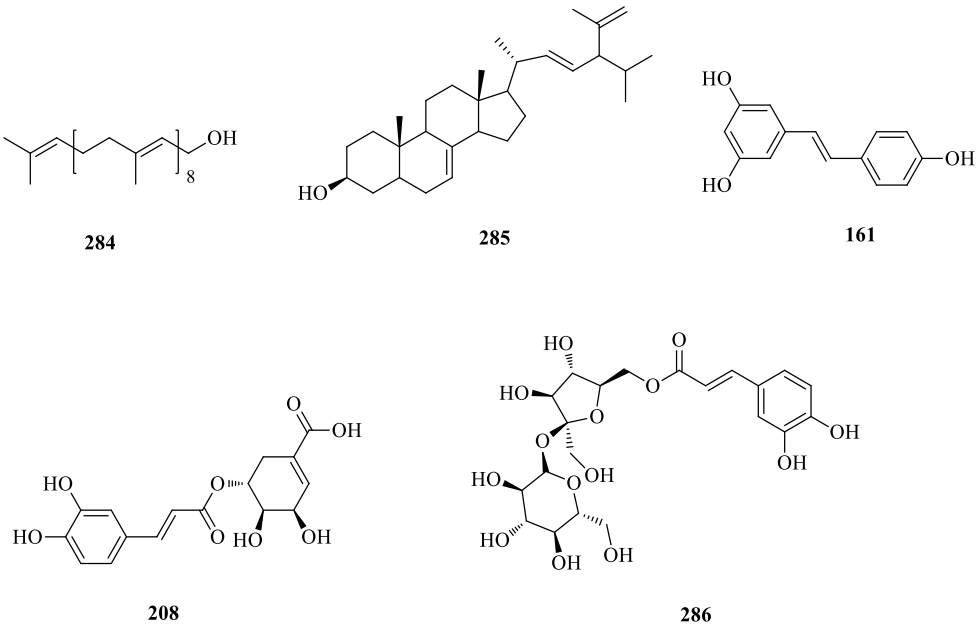


Figure 21. Structures of compounds isolated from *S. excelsa* (continued)

2.2.7 *S. fluminensis*

In 2014, Petrica and coworkers phytochemical studied the leaves extract of the *S. fluminensis* collected from Brazil [56]. The results led to the isolation and structure elucidation of two flavonoids; quercetin-3-*O*-*β*-L-rhamnopyranoside (1-6)-*O*-*β*-D-glucopyranoside (**287**) and quercetin-3-*O*-*β*-L-galactopyranoside (**288**) (Figure 22). Biological activity of these two compounds has not been reported.

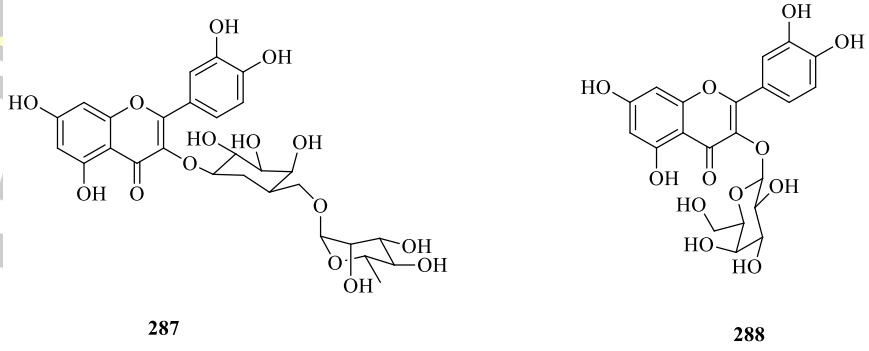
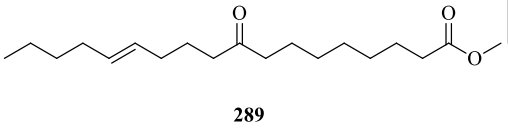


Figure 22. Structures of compounds isolated from *S. fluminensis*

2.2.8 *S. macrophylla*

In 1995, Dalutabad and cowokers reported the isolation of seed oil extract of the *S. macrophylla* collected from India [57]. A novel keto fatty acid; 9-keto-octadec-cis-13-enoic acid (**289**), was isolated and identified (Figure 23).

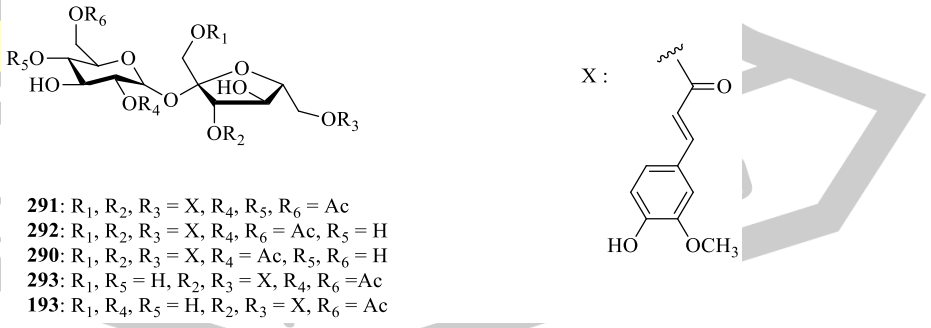


289

Figure 23. Structure of a compound isolated from *S. macrophylla*

2.2.9 *S. riparia*

In 2013, Wang and coworkers investigated the roots and rhizomes extract of the *S. riparia* perchared from Bozhou, Anhui, China [58]. New compound; smilaside P (**292**) and known compounds; smiglaside A (**290**), smiglaside B (**291**), 3,6-diferuloyl-2′,6′-diacetylsucrose (**293**) and helonioside B (**193**) have been isolated (Figure 24). Compound **290** was cytotoxic toward HL-60, SMMC-7721, A-549, MCF-7 and SW480 with IC_{50} values of 2.70, 3.80, 11.91, 3.79 and 3.93 μM, respectively. Moreover, compounds **290-292** showed moderate scavenging activities against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with IC_{50} values of 339.58, 330.66 and 314.49 μM, respectively.



291: R_1, R_2, R_3 = X, R_4, R_5, R_6 = Ac
292: R_1, R_2, R_3 = X, R_4, R_6 = Ac, R_5 = H
290: R_1, R_2, R_3 = X, R_4 = Ac, R_5, R_6 = H
293: R_1, R_5 = H, R_2, R_3 = X, R_4, R_6 =Ac
193: R_1, R_4, R_5 = H, R_2, R_3 = X, R_6 = Ac

Figure 24. Structures of compounds isolated from *S. riparia*

2.2.10 *S. scobinicaulis*

In 2012, Zhang and coworkers purified the rhizomes and roots extract of the *S. scobinicaulis* collected from Taibai mountain, Shaanxi province, China [59]. Two new spirostane- type steroidal saponins; smilscobinosides A (**294**) and smilscobinosides B (**295**), together with a known congener (**296**), have been isolated and reported (Figure 25).Compounds **294-296** were tested *in vitro* for their cytotoxicity against A549, Hela, and LAC human cancer cell lines. All of the tested compounds showed no cytotoxic activity (IC_{50} > 100 mM).

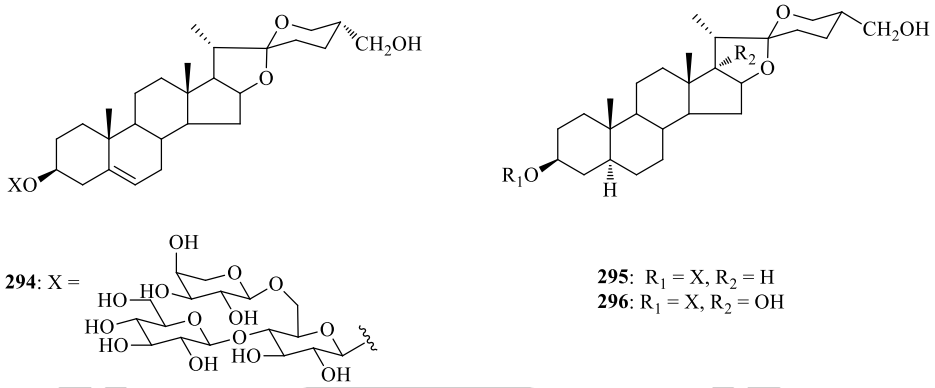


Figure 25. Structures of compounds isolated from *S. scobinicaulis*

In 2014, Xu and coworkers reported the isolation of the rhizomes and roots extract of the *S. scobinicaulis* collected from Taibai mountain, Shaanxi province, China [60]. Four new furostanol saponins; 26-*O*-*β*-D-glucopyranoside-3*β*,26-dihydroxy-(25*R*)-5*α*-furostan-22-methoxyl-6-one-3-*O*-*α*-L-arabinopyranosyl-(1→6-*β*-D-glucopyranoside (**297**), 26-*O*-*β*-D-glucopyranoside-3*β*,26-dihydroxy-(25*R*)-5*α*-furostan-22-methoxyl-6-one (**298**), 26-*O*-*β*-D-glucopyranoside-3*β*,26-dihydroxy-(25*R*)-5*α*-furostan-20(22)-en-6-one (**299**) and 26-*O*-*β*-D-glucopyranoside-3*β*,23,26-trihydroxy-(23*R*,25*R*)-5*α*-furostan-20(22)-en-6-one (**300**), together with two known furostanol saponins; 26-*O*-*β*-D-gluco pyranosyl-3*β*,22,26-trihydroxy-(25*R*)-5*α*-furostan-6-one-3-*O*-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (**301**) and 26-*O*-*β*-D-glucopyranosyl-3*β*,26-dihydroxy-(25*R*)-5*α*-furostan-20(22)-en-6-one-3-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (**302**) and a known spirostanol

saponin; sieboldogenin-3-*O*-α-L-arabino-pyranosyl-(1→6)-β-D-glucopyranoside (**303**), were isolated and characterize (Figure 25). The isolated saponins were evaluated for cytotoxic activity against two human cancer cell lines including Hela and SMMC-7221. The results revealed that compounds **298**-**302** were inactive (IC_{50} >100 μM), while compounds **297** and **303** displayed cytotoxicity against Hela carcinoma cell lines with IC_{50} values of 18.79 ± 1.12 μM and 9.73 ± 1.64 μM, respectively and against SMMC-7221 cancer cell lines with IC_{50} values of 28.57 ± 1.57 μM and 21.54 ± 1.64 μM, respectively.

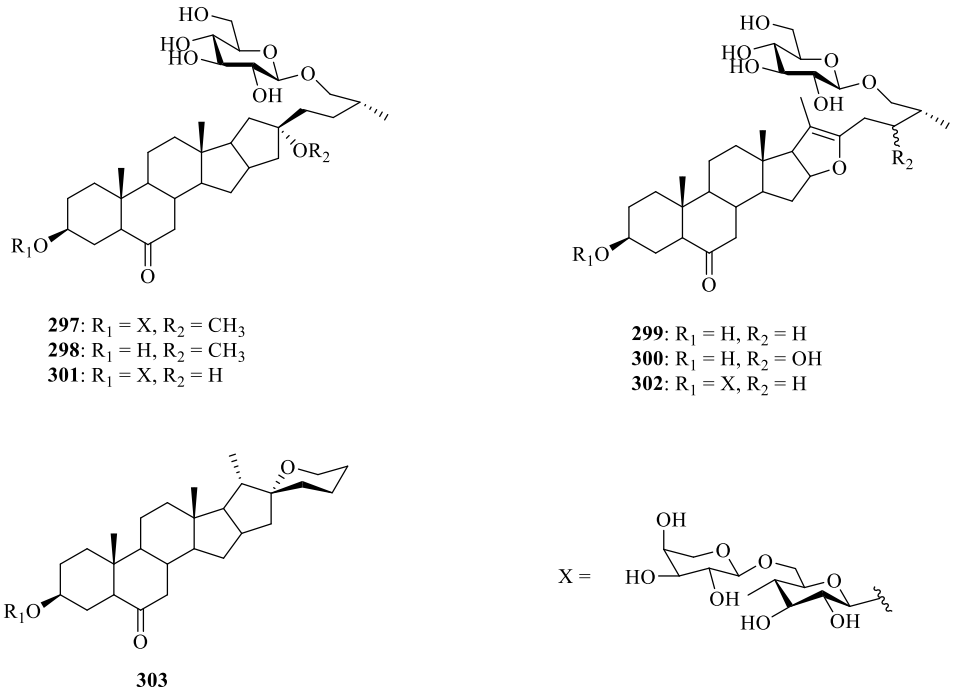


Figure 25. Structures of compounds isolated from *S. scobinicaulis* (continued)

In 2014, Zhang and coworkers phytochemical investigated of the rhizomes and roots extract of the *S. scobinicauli* collected from Taibai Mountain of Shaanxi Province, China [61]. The results led to the isolation of two new polymethoxylated flavones; 7,3′,5′-trihydroxy-5,6,4′-trimethoxyflavone (**304**) and 7-hydroxy-5,6,3′,4′,5′-pentamethoxyflavone (**305**), together with seventeen known compounds; 7,5-dihydroxy-5,6,3,4-tetramethoxyflavone (**306**), 5,8-dihydroxy-7-methoxyflavone (**307**), 5,7-dihydroxyflavanone (**308**), 7,4-dihydroxyisoflavone (**309**), methyl *p*-coumarate

(**310**), methyl 3,4-dihydroxybenzoate (**311**), 3,5-dimethoxybenzoic acid (**312**), 3-methoxybenzoic acid (**313**) 4-hydroxybenzaldehyde (**314**), 3,5-dimethoxy-4-hydroxybenzoic acid (**315**) 3-hydroxy-4-methoxybenzoic acid (**316**) 3-hydroxy-4-methoxycinnamic acid (**317**) 3,5-dihydroxybenzaldehyde (**318**), 4-hydroxycinamic acid (**319**) 5,6-dihydroxy-7-methoxyflavone (**320**), 5,7,4-trihydroxyflavone (**321**), and 5,7-dihydroxy-8-methoxyflavone (**322**) (Figure 25). The in *vitro* cytotoxicity evaluation of the new compounds demonstrated that compound **304** showed weak activity to the tested MCF-7 and H520 cancer cell lines with IC_{50} values of 65.1 and 82.0 μM, respectively, while compound **305** was found to be inactive to both cell lines.

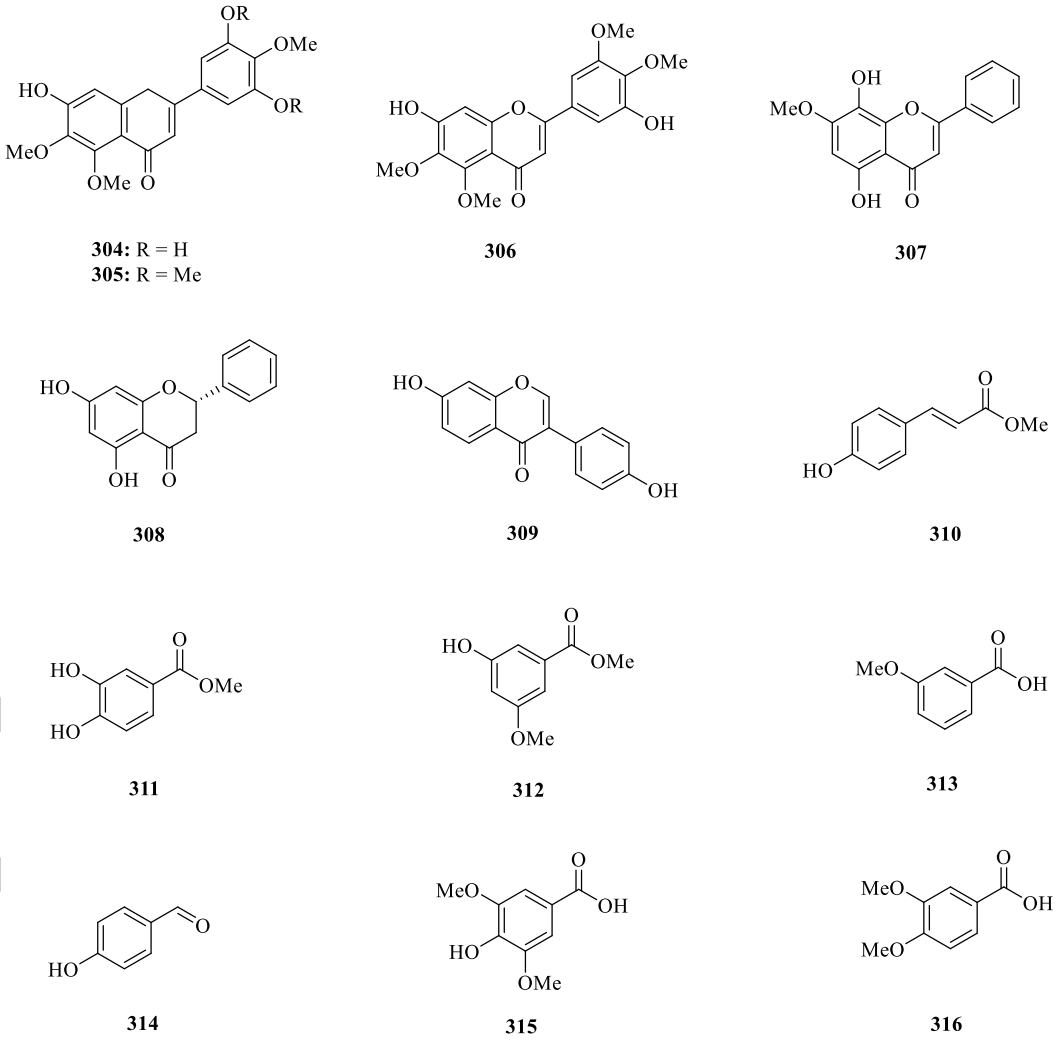


Figure 25. Structures of compounds isolated from *S. scobinicaulis* (continued)

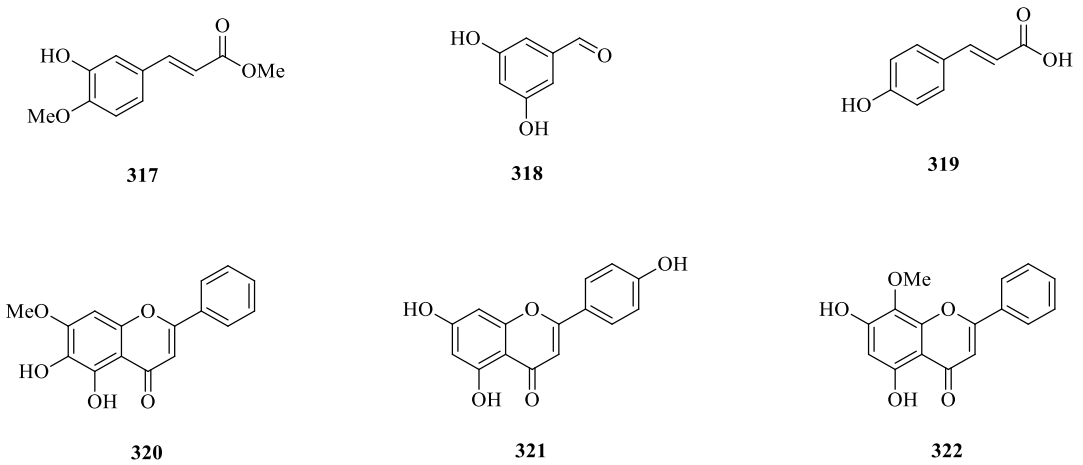
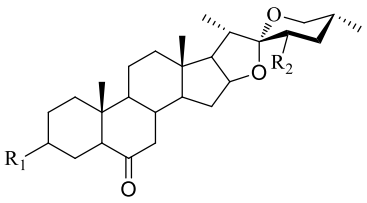
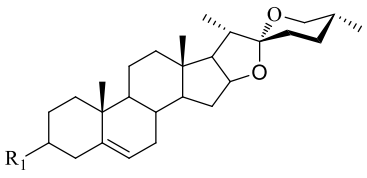


Figure 25. Structures of compounds isolated from *S. scobinicaulis* (continued)

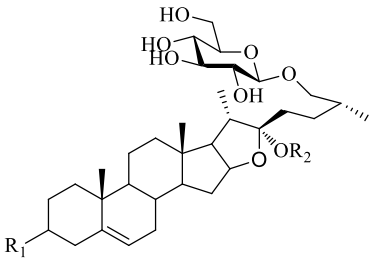
In 2017, Shu and coworkers reported the phytochemical investigations of the rhizomes extract of the *S. scobinicaulis* collected from Henan Province, China [62]. The investigation led to the isolation of seven steroidal saponins with new four compounds; smilscobinosides C- F (**323**, **325**, **326** and **327**) and three known compounds; (25*R*)-spirostan-3*β*-ol-6-one-3-*O*-[*α*-L-arabinopyranosyl(l-6)]-*β*-D-glucopyranoside (**324**), dioscin (**213**) and afromontoside (**328**) (Figure 25). The isolated compounds were evaluated for their cytotoxicity against four human tumor cell lines (SH-SY5Y, SGC-7901, HCT-116 and Lovo). Compounds **325** and **326** exhibited significant inhibition on HCT-116 with IC_{50} values of 10.5 and 7.8 μM, together with inhibition on SGC-7901 with IC_{50} values of 21.4 and 15.8 μM, respectively.



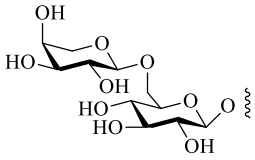
323: $R_1 = S_2$, $R_2 = OH$
324: $R_1 = S_1$, $R_2 = H$

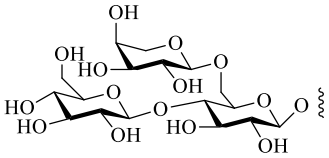


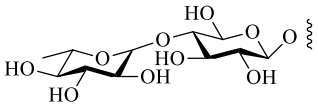
325: $R_1 = S_1$
326: $R_1 = S_2$
213: $R_1 = S_4$



327: $R_1 = S_1$
328: $R_1 = S_3$

$S_1 =$ 

$S_2 =$ 

$S_3 =$ 

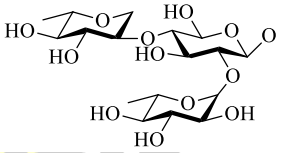
$S_4 =$ 

Figure 25. Structures of compounds isolated from *S. scobinicaulis* (continued)

2.2.11 *S. sebeana*

In 2011, Ao and coworkers reported the isolation and identification of bioactive compounds from the rhizomes and roots extract of the *S. sebeana* Miq. collected from the campus of University of the Ryukyus, Okinawa, Japan [63]. Six phenolic compounds; chlorogenic acid (**249**), 4-formylphenol (**329**), epicatechin (**330**), cinchonain IIa (**236**), cinchonain Ia (**328**) and cinchonain Ib (**329**), have been isolated and identified by spectroscopic analyses (Figure 26). The isolated compounds were

evaluated their potential antioxidant activities by DPPH and superoxide radical scavenging assays. Except compound **329**, other five compounds including **249**, **330**, **236**, **328** and **329** exhibited significant DPPH free radical scavenging capacities with EC_{50} values of 61.1, 11.3, 6.8, 10.9 and 12.7 mmol/L, respectively, and superoxide radical scavenging abilities with EC_{50} values of 65.8, 71.0, 26.5, 35.6 and 54.3 mmol/L, respectively.

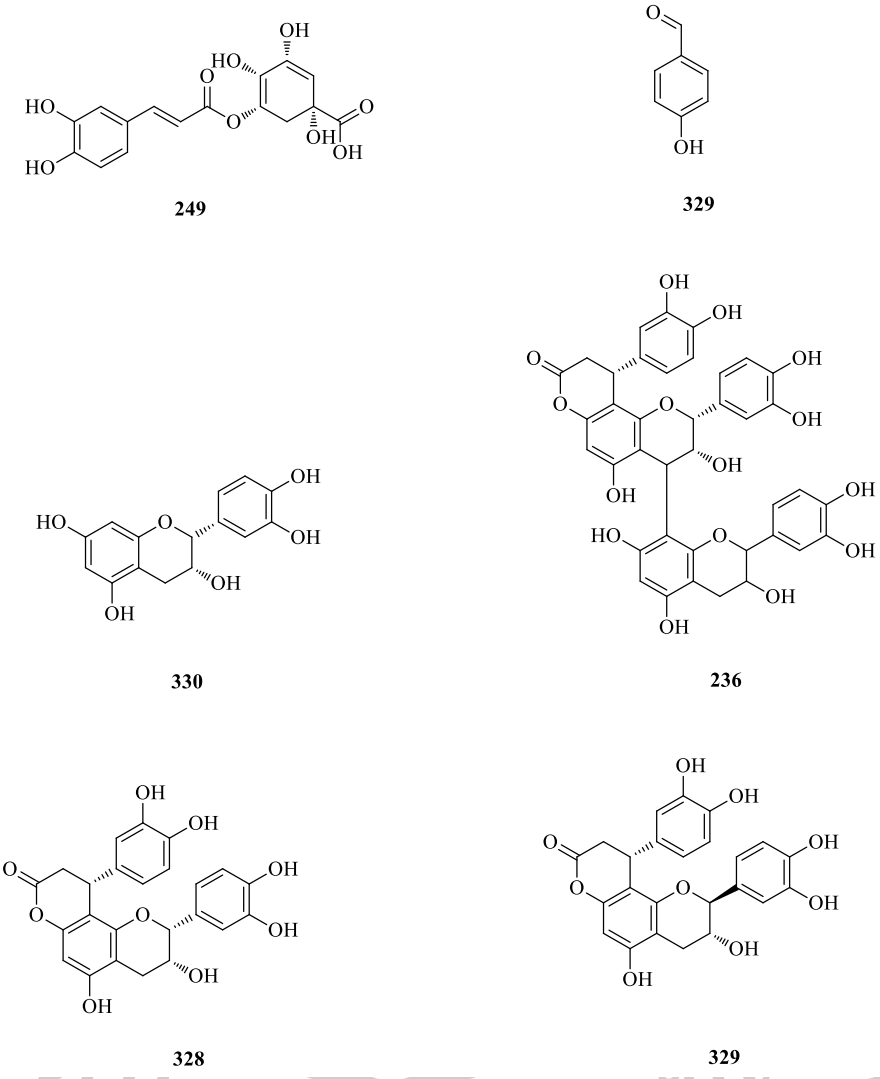


Figure 26. Structures of compounds isolated from *S. sebeana*

2.2.12 *S. trinervula*

In 2015, Shu and coworkers reported the isolation and identification of bioactive compounds from the rhizomes extract of the *S. trinervula* collected from

Yichun City, Jiangxi Province, China [64]. A new phenylpropanoid glucoside and two new neolignans; (1*S*, 2*R*)-1-(3,4,5-trimethoxyphenyl)-3-(*β*-D-glucopyranosyloxy)-1,2,3-propanetriol (**331**), (7*R*,8*R*)-4,7,9,9′-tetrahydroxy-3,5,3′,5′-tetramethoxy-8-4′-oxyneo lignan4-*O*-*β*-D-glucopyranoside (**332**) and 3′, 9, 9′-trihydroxy-3, 5-dimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside (**333**), together with a new natural product; (1*S*,2*R*) -1-(3,4,5-trimethoxyphenyl)-1,2,3-propanetriol (**334**) and four known compounds; (1*R*,2*R*)-1-(3,4,5-trimethoxyphenyl)-1,2,3-propanetriol (**335**), (7*S*,8*R*)-erythro-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside (**336**), 7*S*,8*R*-threo-4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (**337**) and 7*R*, 8*R*-threo-4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (**338**), were isolated and identified (Figure 27). Compounds **331**-**338** were tested in vitro for their cytotoxic activities against five human tumor cell lines (SH-SY5Y, SGC-7901, HCT-116, Lovo and Vero). Compounds **337** and **338** exhibited cytotoxic activity against Lovo, with IC_{50} values of 18.7 μM and 16.8 μM, respectively.

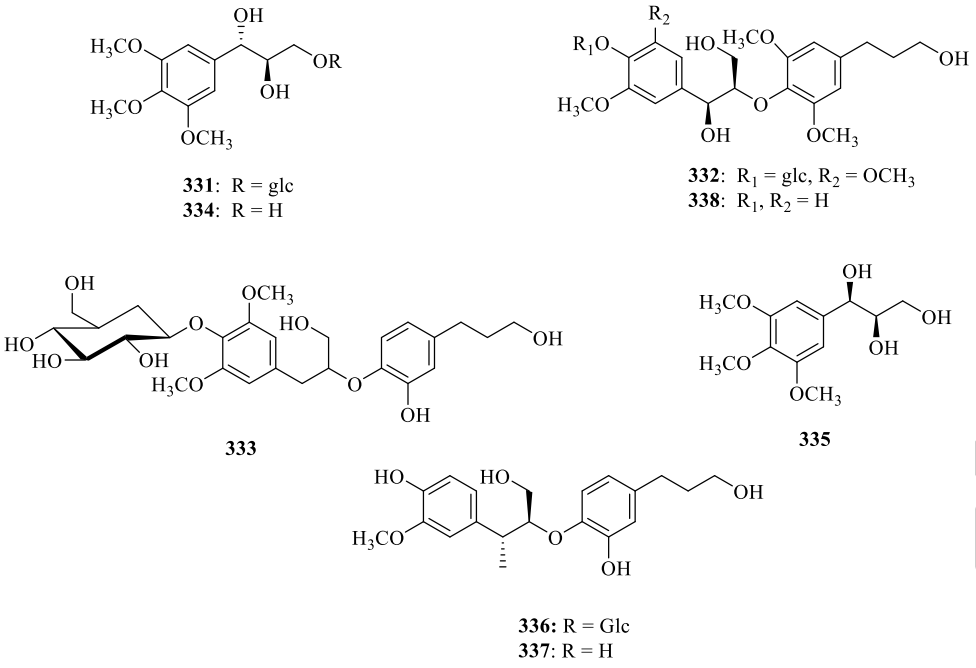


Figure 27. Structures of compounds isolated from *S. trinervula*

In 2016, Liang and coworkers purified the rhizomes and roots extract of the

S. trinervula collected from Yichun city, Jiangxi province, China [65]. Three new steroidal saponins; trinervulosides A- C (**339- 341**), together with four known compounds; dioscoreside E (**342**), smilaxchinoside A (**343**), pseudoprotodioscin (**175**) and anguiviosides XV (**344**), have been isolated (Figure 27). The cytotoxicities of compounds **175** and **339-344** were tested against SH-SY5Y, SGC-7901, HCT-116 and Lovo cell lines. The results showed that only compound **340** had activity against SGC-7901 with IC_{50} values of 8.1 mM and HCT-116 with an IC_{50} value of 5.5 mM. The other compounds were inactive (IC_{50} >100 mM).

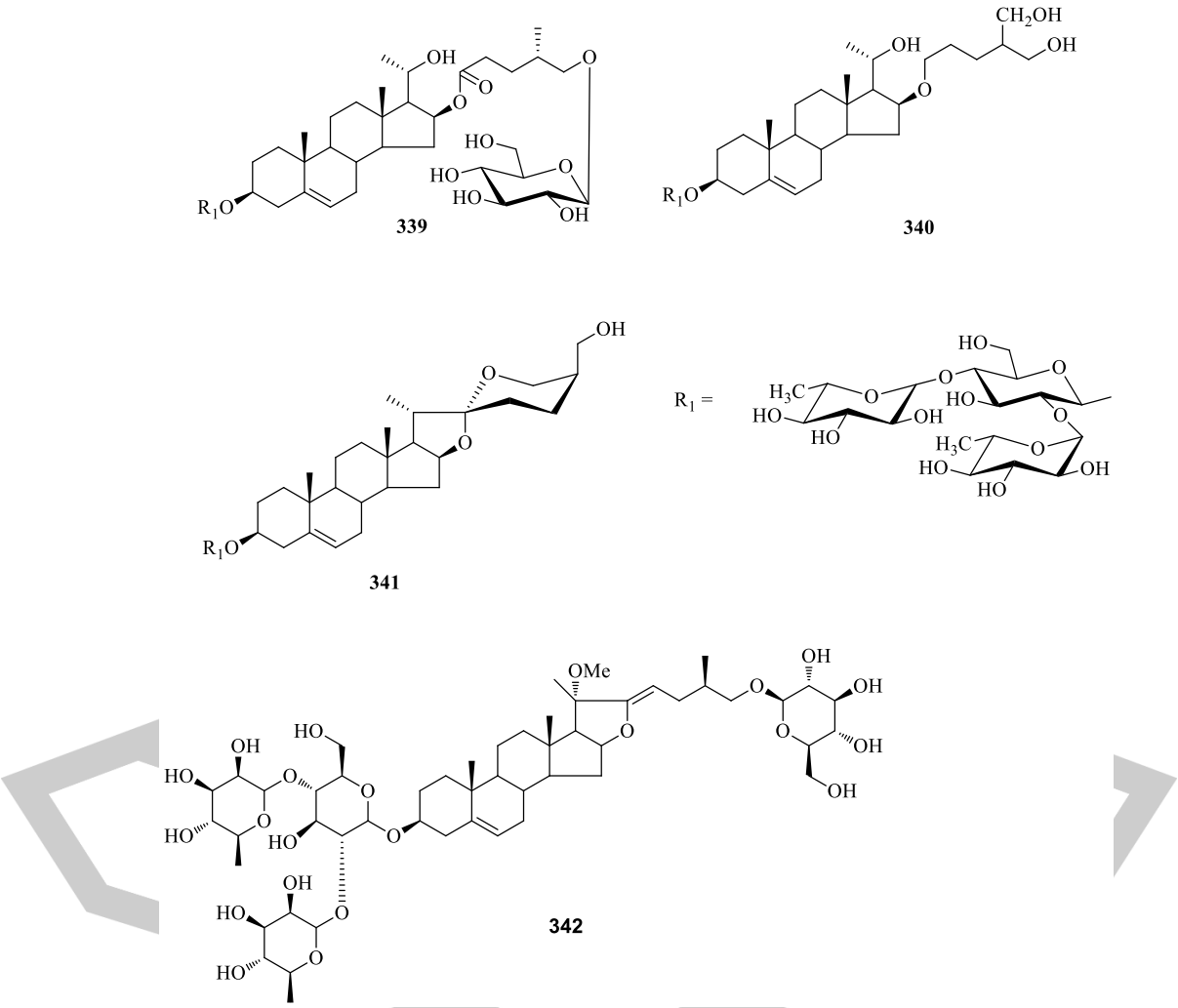
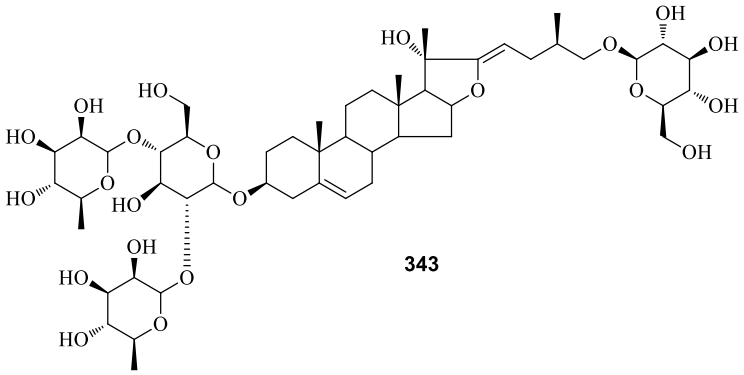
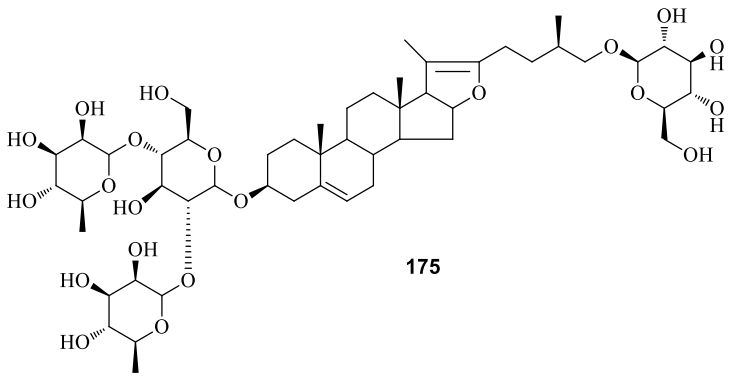


Figure 27. Structures of compounds isolated from *S. trinervula* (continued)



343



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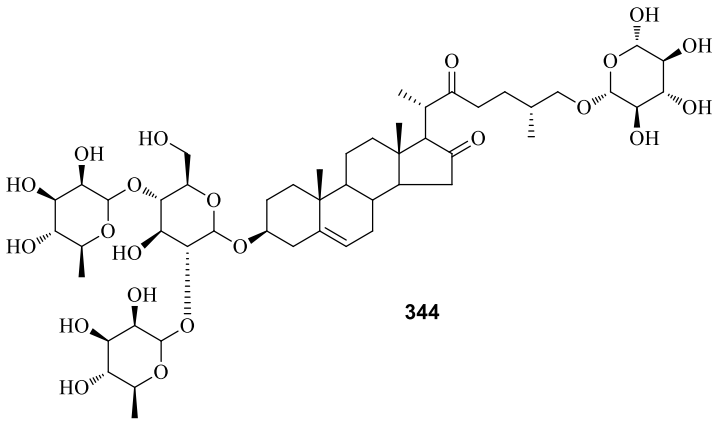


344

Figure 27. Structures of compounds isolated from *S. trinervula* (continued)

In 2017, Shu and coworkers reported the phytochemical investigation of the rhizomes extract of the *S. trinervala* collected from Yichun City, Jiangxi Province, China [66]. The investigation led to isolation and structure elucidation of eight lignan glycosides, including five new lignans; (7*S*,8*R*,8′*R*)-4,4′,9-trihydroxy-3,3′,5,5′-tetramethoxy-7,9′-epoxylignan-7′-one 4′-*O*-*β*-D-glucopyranoside (**345**), (7*S*,8*R*,8′*R*)-4,4′,9-trihydroxy-3,3′,5,5′-tetramethoxy-7,9′-epoxylignan-7′-one-4-*O*-*β*-D-glucopyranoside (**346**) (7*S*,8*R*)-4,9,9′-trihydroxy-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-

neolignan-9′-*O*-*β*-D-glucopyranoside (**347**), (7*R*,8*R*)-4,9,9′-trihydroxy-3,5-dimethoxy-7.O.4′, 8.O.3-neo lignan 9′-*O*-*β*-D-glucopyranoside (**348**) and (7S,8R)-4,9,9′-trihydroxy-3,3′,5-trimethoxy-8,4′-oxy-neolignan 4-*O*-*β*-D-glucopyranoside (**349**), together with three known compounds; (7*S*,8*R*)-4,9,9′-trihydroxy-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neo lignan 4-*O*-*β*-D-glucopyranoside (**350**), symplocosneolignan (**351**) and rourinoside (**352**) (Figure 27). Compounds **345**-**352** were tested *in vitro* for their cytotoxic activity against four human tumor cell lines (SH-SY5Y, SGC-7901, HCT-116, Lovo). Compounds **347** and **349** exhibited cytotoxic activity against Lovo cells with IC_{50} values of 10.4 mM and 8.5 mM, respectively.

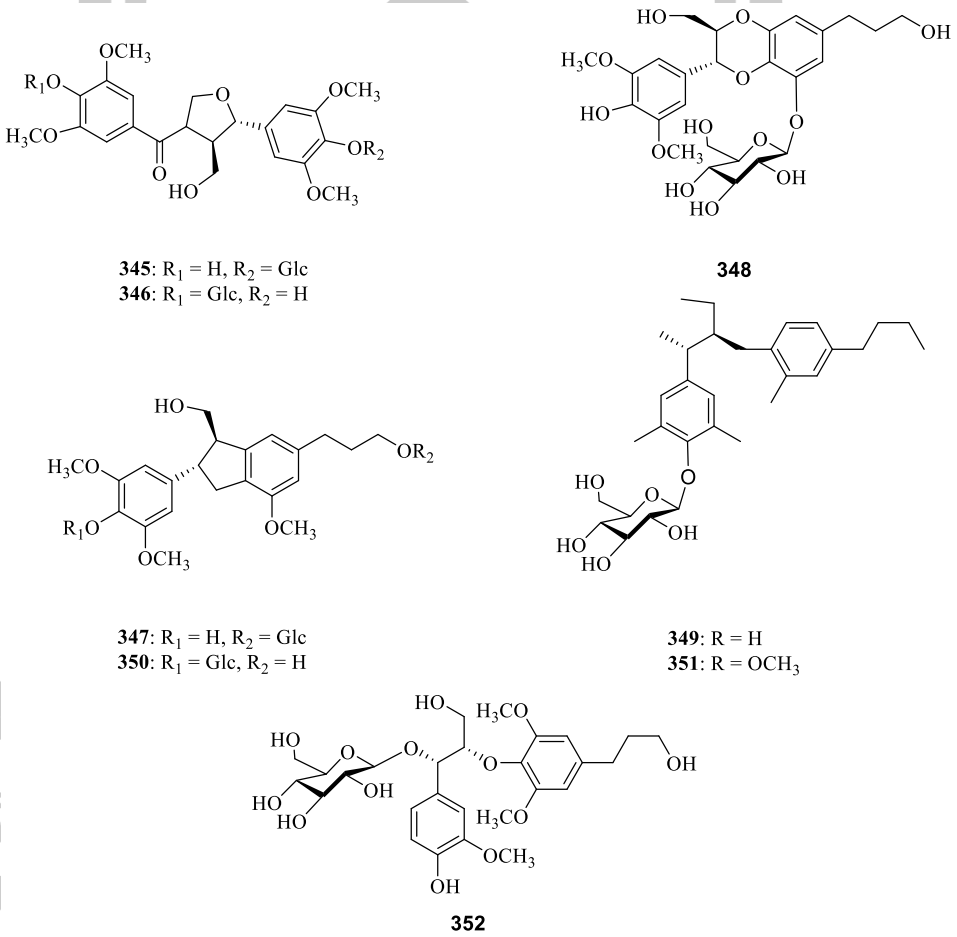


Figure 27. Structures of compounds isolated from *S. trinervula* (continued)

There are two hundred and three compounds have been isolated and characterized from the genus *Smilax* from our review. Most of the isolaed compounds

showed diversity of the chemical structures and the biological activities are interesting. Table 2 shows the summary of chemical constituents of the *Smilax* published during the year 1995-2017.

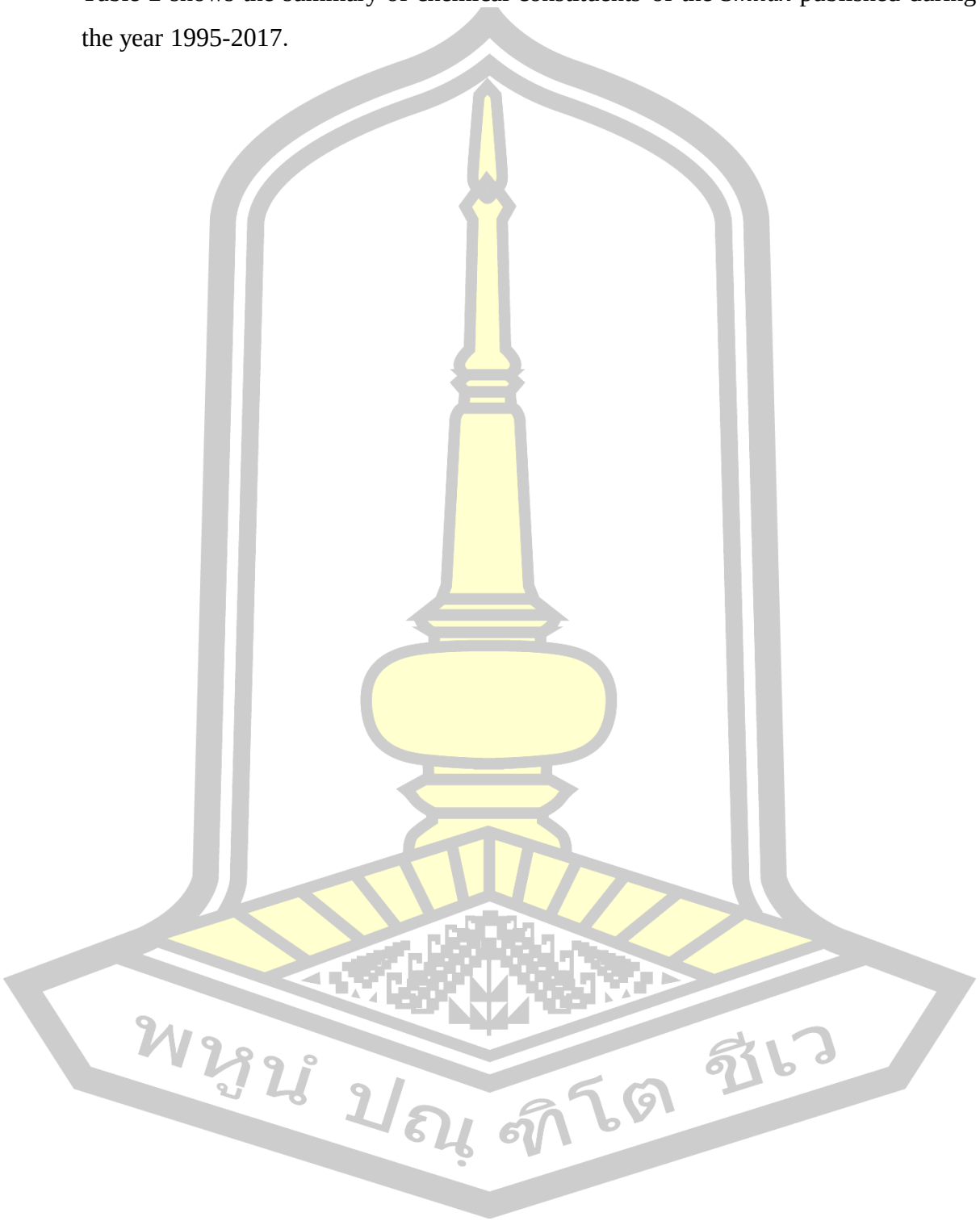


Table 2. Chemical constituents of the *Smilax* sp.

***Smilax* species**	**Isolated compounds**	**Reported year**	**Ref.**
S. aspera	**150-156**	2008	[41]
	157-163	2011	[42]
S. bockii	**164-175**	2004	[43]
	176-183	2005	[44]
	184	2006	[45]
	185	2008	[46]
S. bracteata	**156** and **186-208**	2008	[47]
S. china	**156**, **180** and **209-212**	2010	[48]
	209 and **213**	2012	[49]
	172, **176**, **199** and **218-226**	2016	[50]
	161, **192** and **227-234**	2017	[51]
	156, **169**, **170**, **210-227** and **235-249**	2017	[52]
S. corbularia	**161**, **163**, **169**, **181**, **201** and **250-280**	2011	[53]
S. excelsa	**161**, **208**, **225** and **281- 283**	2010	[54]
	161, **208** and **284- 286**	2016	[55]
S. fluminensis	**287** and **288**	2014	[56]
S. macrophylla	**289**	1995	[57]
S. riparia	**193** and **290- 293**	2013	[58]
S. scobinicaulis	**294-296**	2012	[59]
	297-303	2014	[60]
	304-322	2014	[61]
	213 and **323-328**	2017	[62]
S. sebeana	**236, 249** and **328-330**	2011	[63]
S. trinervula	**331-338**	2015	[64]
	175 and **339-344**	2016	[65]
	345-352	2017	[66]

CHAPTER 3
METHODOLOGY

3.1 General experimental procedures

The proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian Mercury Plus 400 MHz spectrometer. Complete assignment was performed using 2D experiments (COSY, HSQC, HMBC, and NOESY). The chemical shifts (δ) are given in ppm with respect to the deuterated solvents ($CDCl_3$, CD_3OD, D_2O and DMSO-d_6). Signals and coupling constants (J) are given in Hz. Optical rotations were determined by using JASCO DIP-1000 digital polarimeter. Infrared (IR) spectra were recorded by using a Bruker Tenser 27 spectrometer. High resolution mass spectra (HRMS) were obtained by using a Bruker micrOTOF mass spectrometer. BUCHI Rotary evaporator were used for solvent evaporation. Thin-layer chromatography (TLC) were performed with pre-coated MERCK silica gel 60 F254 as a stationary phase. The separated spots were visualized as black spots under 254 nm UV lamp. Column chromatography were carried out on MERCK silica gel 60 and Pharmacia Fine chemicals Sephadex G-75. High performance liquid chromatography (HPLC) were performed on Shimadzu system with SLC-10AD controller and detector using diode array detector (SPD-M20A; Shimadzu). C18 column (250 × 4.6 mm, 5-micron) were used in this analysis.

3.2 The fungus *P. nipponicus* (Cod-MK1201)

3.2.1 Fungal material

The insect pathogenic fungus *Polychaphalomyces nipponicus* (Cod-MK1201) was isolated from a dead cicada nymph and collected from Muang District, Maha Sarakham province, northeast Thailand. This fungus was identified by Associate Professor Aphidech Sangdee, Department of Biology, Mahasarakham University, Thailand (Figure 28).

Figure 28. The fungus *P. nipponicus* on cicada

3.2.2 Fermentation

3.2.2.1 Small scale fermentation

The culture used throughout the experiment was maintained on potato dextrose agar (PDA) slants at 28 ºC. For inoculum preparation, the fungus was initially grown at 25 ºC on a PDA plate for 14 days. The outer zone of the colony was punched with a sterile cutter and transferred to 25 mL of induced medium (35 g/L of sucrose, 5 g/L of peptone, 2.5 g/L of yeast extract, 0.5 g/L of $MgSO_4$, 1 g/L of KH_2PO_4 and 0.05 g/L of vitamin B1 and was adjusted to pH 5.2 in a 250 mL of flask and grown at 28 ºC [67]. Culture broth and mycelium were collected from each 5 flasks for 1-12 weeks.The mycelium on the surface of the induced medium was collected and dried at 50 °C for 2 days in an oven. The dried mycelium was powdered by using a pestle and mortar. The culture broth was filtrated through a 0.2 μm filter membrane before extraction.

3.2.2.2 Large scale fermentation

The culture used throughout the experiment was maintained on potato dextrose agar (PDA) slants at 28 ºC. For inoculum preparation, the fungus was initially grown at 25 ºC on a PDA plate for 14 days. The outer zone of the colony was punched with a sterile cutter and transferred to 25 mL of induced medium (35 g/L of sucrose, 5 g/L of peptone, 2.5 g/L of yeast extract, 0.5 g/L of $MgSO_4$, 1 g/L of KH_2PO_4 and 0.05 g/L of vitamin B1 and was adjusted to pH 5.2 in a 250 mL of 100 flasks and grown at 28 ºC for 20 days [67]. The mycelium on the surface of the induced medium was collected and dried at 50 °C for 2 days in an oven. The dried mycelium was powdered by using a pestle and mortar. The culture broth was filtrated through a 0.2 μm filter

membrane before extraction (Flow chart 1 and Figure 29).

Flow chart 1. Small and large scale fermentation of *P. nipponicus*

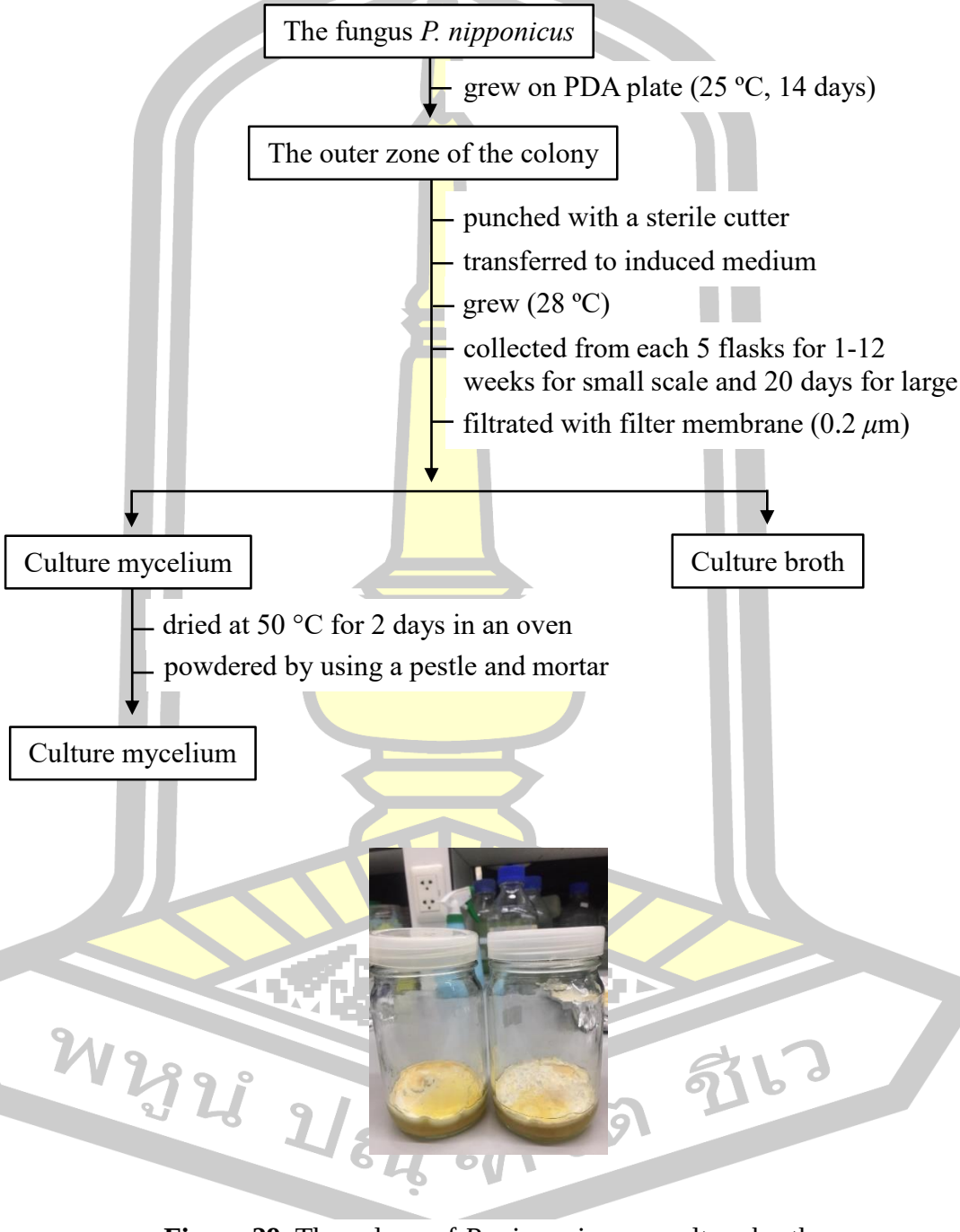


Figure 29. The colony of *P. nipponicus* on culture broth

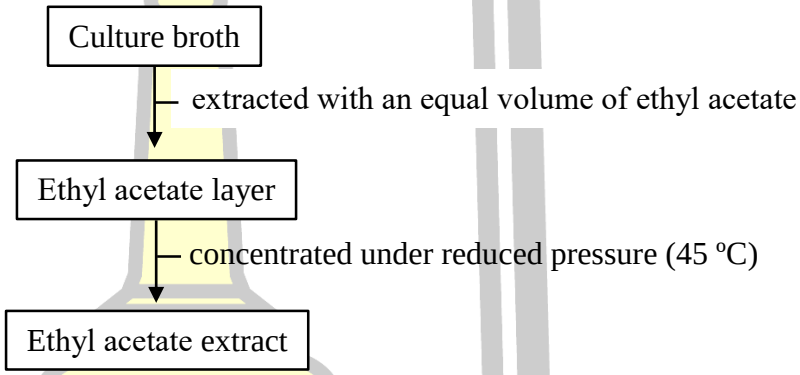
3.2.3 Extraction

3.2.3.1 Small scale extraction

3.2.3.1.1 Extraction of the culture broth

The culture broth of *P. nipponicus* was extracted with an equal volume of ethyl acetate (×2). The collected ethyl acetate layer was concentrated under reduced pressure at 45 ºC to obtain crude ethyl acetate layer extract from the culture broth (Flow chart 2).

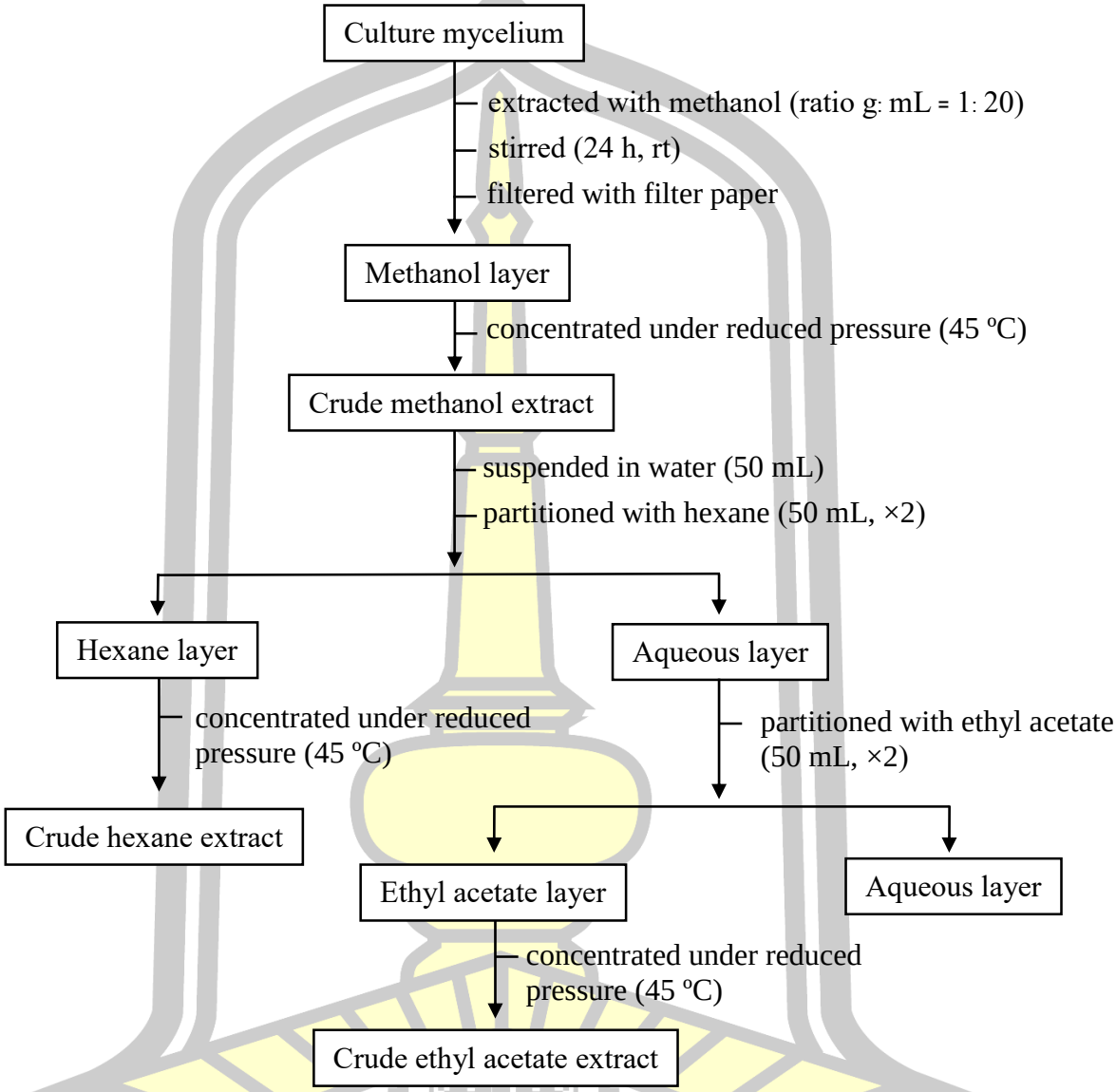
Flow chart 2. Small scale extraction of the culture broth



3.2.3.1.2 Extraction of mycelium

The dried and powdered mycelium of the fungus *P. nipponicus* was extracted with methanol (ratio g: mL = 1: 20) at room temperature and stirred for 24 h. The methanol layer was filtered with filter paper (Whatman No. 1) and concentrated under reduced pressure at 45 ºC to obtain methanol extract which was further suspended in water (50 mL) and partitioned with hexane (50 mL, ×2) and ethyl acetate (50 mL, ×2), respectively. The hexane and ethyl acetate layers were concentrated under reduced pressure at 45 ºC to obtain crude ethyl acetate and hexane extracts (Flow chart 3).

Flow chart 3. Small scale extraction of the culture mycelium

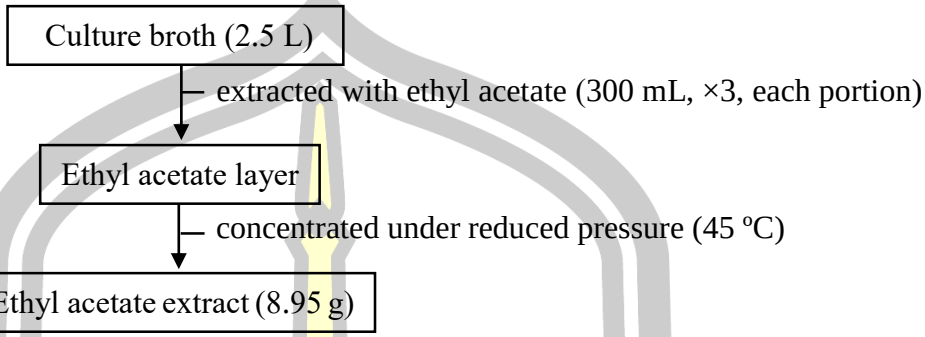


3.2.3.2 Large scale extraction

3.2.3.2.1 Extraction of the culture broth

The culture broth of the fungus *P. nipponicus* was extracted with ethyl acetate (300 mL, ×3, each portion). The collected ethyl acetate layer was combined and concentrated under reduced pressure at 45 ºC to obtain crude ethyl acetate extract from the culture broth (Flow chart 4).

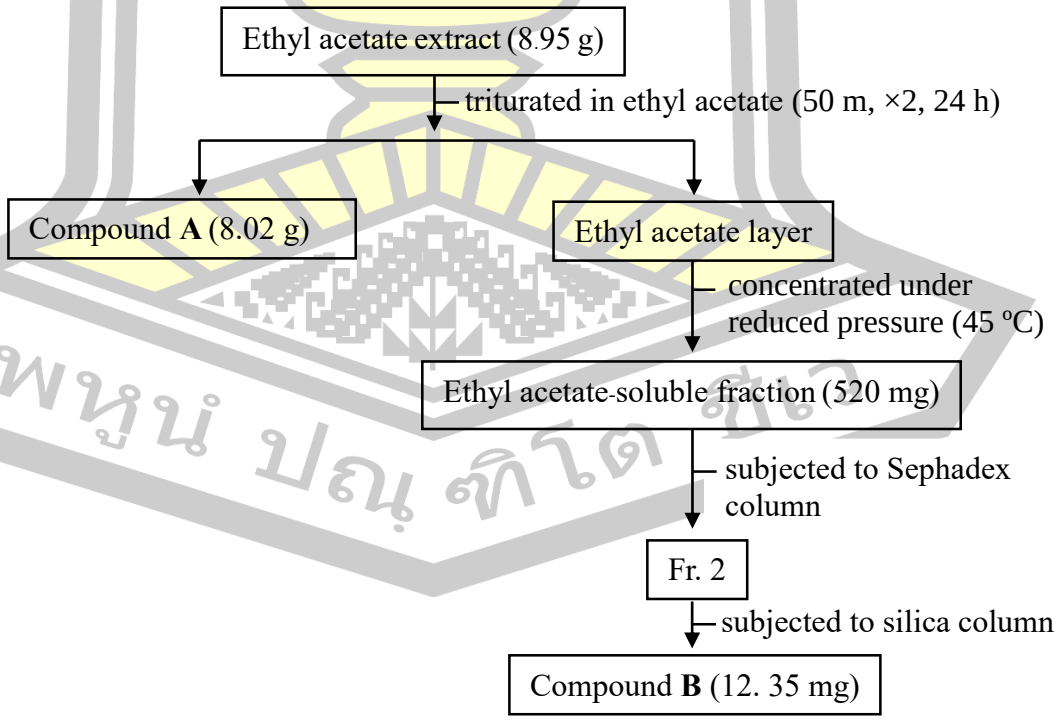
Flow chart 4. Large scale extraction of the *P. nipponicus* culture broth



3.2.4 Isolation of the crude extract

The ethyl acetate extract (8.95 g) was further triturated in ethyl acetate at room temperature and stirred for 24 h (50 mL, ×2) to yield compound **A** (8.02 g) and an ethyl acetate-soluble fraction (520 mg). The ethyl acetate-soluble fraction was subjected to purification over silica gel column chromatography to obtain six fractions (Fractions 1-6). Fraction 2 was furner purified by Sephadex G-75 column chromatography to give compound **B** (12.35 mg) as a pale brownish oil (Flow chart 5).

Flow chart 5. Isolation of compound **A** and **B** from the culture broth of *P. nipponicus*



3.2.5 Structural elucidation

Structures of compounds **A** and **B** were elucidated on the basis of spectroscopic data (^{1}H, ^{13}C NMR and 2D experimental data, MS, IR spectroscopy and X-ray crystallography).

3.2.5.1 Crystal data of compound **A**

Crystallization of compound **A** from methanol: water (~80:20) successfully provided small crystals. Single crystal of compound **A** was mounted to the end of a hollow glass fiber. X-ray diffraction data were collected using a Bruker D8 VENTURE and operating at T = 296(2) K. Data were measured using ω and ϕ scans and using Cu-Kα radiation (λ = 1.54056 Å). The total number of runs and images was based on the strategy calculation from the program APEX3 and unit cell indexing was refined using SAINT (V8.38A, Bruker, 2016). Data reduction and scaling were performed using SAINT (V8.38A) and SADABS-2016/2 was used for absorption correction (APEX3, SADABS and SAINT. Bruker AXS Inc., Madison, Wisconsin, USA, 2016).

3.2.6 HPLC analysis

The extract samples for HPLC were prepared at 2 mg/mL in methanol:water (1:1) while the standard compounds were prepared at 100 μg/mL in water. Each sample solution of 50 μL was injected to HPLC on a reversed phase C18 column. Methanol:milli-Q water (15:85 v/v) was used as a mobile phase with a constant flow rate of 1 mL/min. The column temperature was set at 30 ^{o}C and total run time was 30 min. All of samples were detected at 254 nm using a diode array detector.

3.2.7 Antifungal activity assay (Pore plate technique)

The antifungal activity of Compound A was performed by Associate Professor Aphidech Sangdee, Department of Biology, Mahasarakham University. Compound **A** (5 mL) as a solution in water at the concentration of 500 µg/mL, was mixed in 95 mL PDA medium before being plated to 90 mm Petri dishes. Seven-day-old mycelial discs of six plant pathogenic fungal pathogens (*Colletotrichum musae*, *C. capsici*, *C. gloeosporioides*, *Pestalotia* spp., *Fusarium* spp. TFPK301 and *Fusarium* spp. Foc 1708) were cut with a 7 mm sterilized cork borer under aseptic conditions and placed onto the 25 mL PDA plates containing 500 μg/mL of compound **A**. The plates were incubated at 28 ^{o}C and the mycelium growth was determined at day 7. The

percentage of mycelial growth inhibition (PGI) was calculated using the formula as shown below where R represents the fungal growth radius (mm) of the control culture and R1 represents the fungal growth radius distance (mm) in the treatment culture [68]. The experiment was done with five replications and the fungus grew on the PDA plate was used as a control plate.

$$PGI\ (\%) = R\text{-}R1/R \times 100$$

3.2.8 Antioxidant activity assay (DPPH scavenging assay)

The ability of compound **A** to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured by using slightly modified method of Anoosh [68]. The solution of 0.2 mM DPPH in methanol was prepared. The addition 1.0 mL of DPPH solution to 2.0 mL of sample solutions in methanol at different concentrations (10-100 μg/mL). The mixture was incubated at room temperature in dark for 30 min. The absorbance was measured at 517 nm using UV-VIS spectrophotometer. Ascorbic acid was used as reference standard compound. The percentage DPPH radical scavenging activity (%RSA) by the sample was calculated using the formula as shown below where $A_{control}$ was the absorbance of the control (blank, without sample) and A_{test} was the absorbance of the sample. All of the tests were run triplicate and the calculation was used with the mean values. The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percentage DPPH radical scavenging activity plotted against the respective concentration.

$$(\%RSA) = (A_{control} - A_{test})/A_{control} \times 100$$

3.2.9 Biological assay

Anti-herpes simplex virus type 1 (HSV-1) and cytotoxicity assays against human breast cancer (MCF-7), oral human epidermoid carcinoma (KB), and Vero (African green monkey kidney fibroblasts) cell lines of compound **B** were evaluated using a colorimetric method [69] at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. Antibacterial activity of compound **A** were evaluated using paper disc method. Antibacterial activity was performed by Associate

Professor Aphidech Sangdee, Department of Biology, Mahasarakham University.

3.3 The *Smilax verticalis*

3.3.1 Plant material

The roots of the *S. verticalis* was collected from the Muang district, Maha Sarakham province, northeast Thailand. It was identifided by Mr.Pornchai Kladwong, Ph. D. candidate at the department of Biology, Khonkaen University (Figure 30).

Figure 30. *S. verticalis*

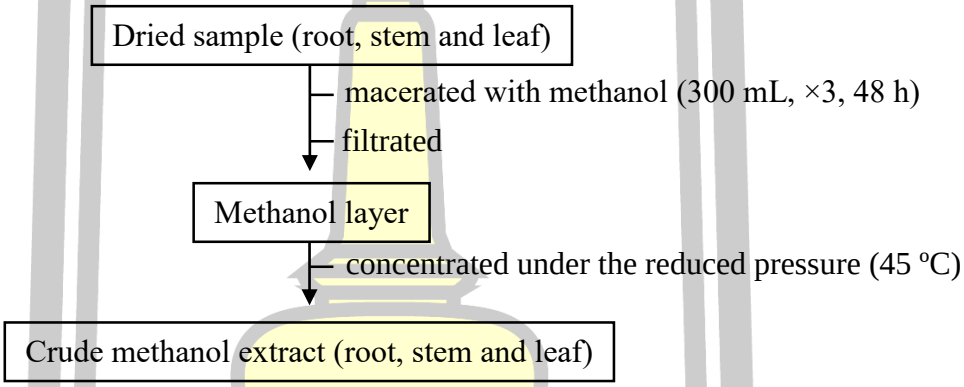
3.3.2 Extraction

3.3.2.1 Small scale extraction

The fresh sample (root, stem and leaf) of the *S. verticalis* (200 g) was cut into small pieces and then dried at room temperature. After that the dried sample (Figure 31) was macerated with methanol for 2 days (300 mL, ×3) and then filtrated. The filtrate was concentrated under the reduced pressure at 45 ºC to get crude methanol extract from the root (SV-R), stem (SV-S) and leaf (SV-L) of the *S. verticalis* (Flow chart 6).

Figure 31. Dried root, stem and leaf of *S. verticalis*

Flow chart 6. Small scale extraction of the root, stem and leaf of *S. verticalis*



3.3.2.2 Large scale extraction

The fresh roots of the *S. verticalis* was cut into small pieces and then dried at room temperature. After that the dried root (700 g) was macerated with methanol for 7 days (3 L, ×3) and then filtrated (Figure 32). The filtrate was concentrated under the reduced pressure at 45 ºC get crude methanol extract. The crude methanol extract was suspended in water and partitioned with ethyl acetate (350 mL, ×3) to get crude ethyl acetate extract from the root of the *S. verticalis* (10.419 g) (Flow chart 7).

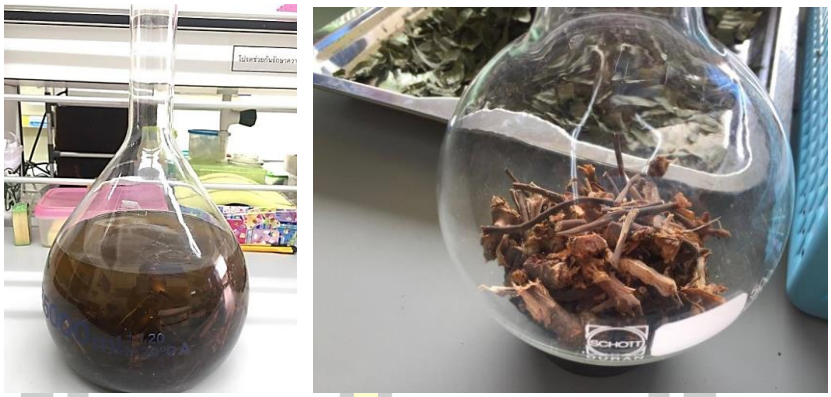
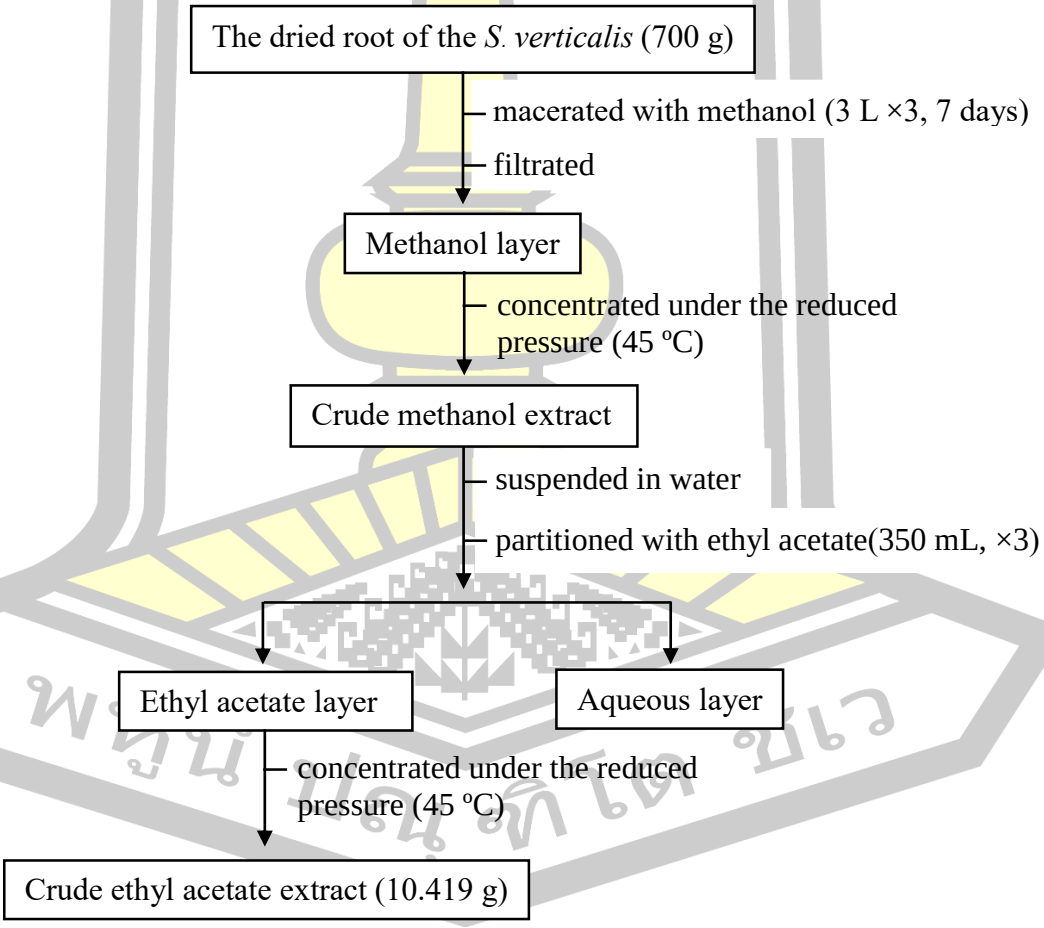


Figure 32. Large scale extraction of the root of *S. verticalis*

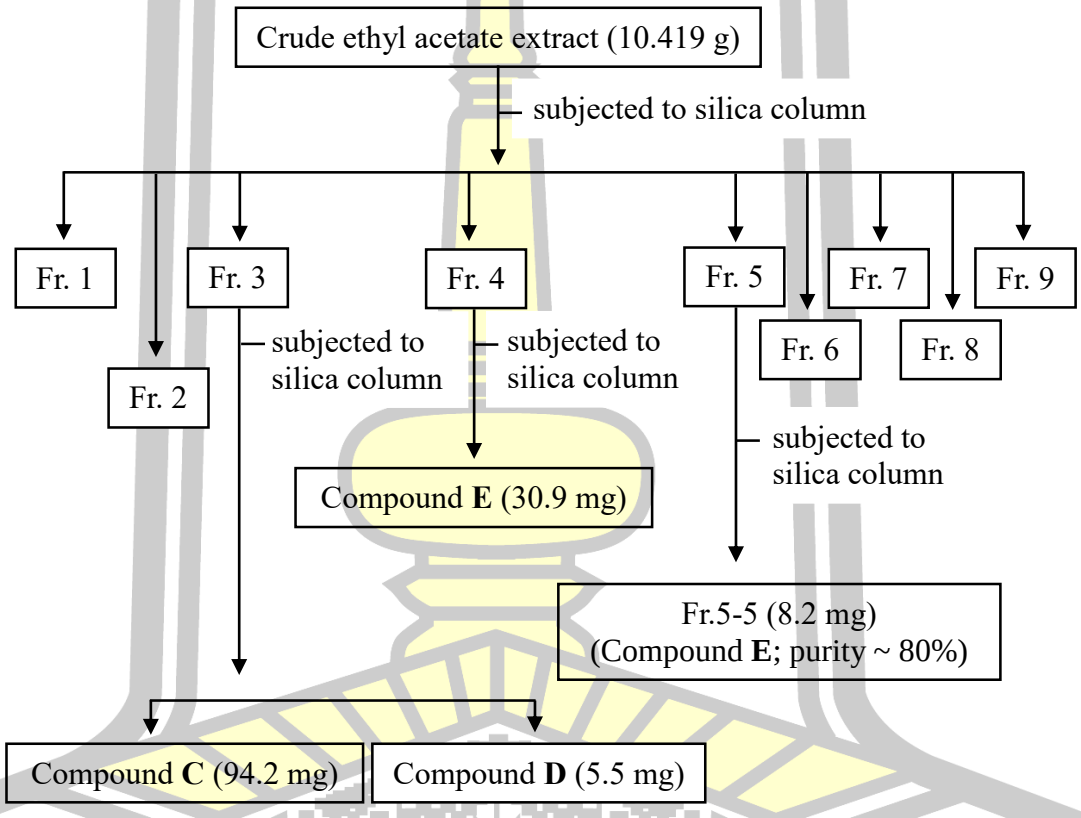
Flow chart 7. Extraction of the root of *S. verticalis*



3.3.3 Isolation of the crude extract

The ethyl acetate extract (10.419 g) was first chromatographed on a silica gel column chromatography to obtain nine fractions (fractions 1-9). Fraction 3 was further purified by a silica gel column chromatography to give compound **C** (94.2 mg) and compound **D** (5.5 mg). Fraction 4 was subjected to silica gel column chromatography to give compound **E** (31.9 mg) (Flow chart 8).

Flow chart 8. Isolation of compounds **C D** and **E** from the root of *S. verticalis*



3.3.4 Structural elucidation

Structures of compounds **C D** and **E** were elucidated on the basis of spectroscopic data (^{1}H, ^{13}C NMR and 2D experimental data, MS and IR spectroscopy).

3.3.5 Antifungal activity assay (Pore plate technique)

The antifungal activity of SV-R, SV-S and SV-L and pure compound **C** was performed by Associate Professor Aphidech Sangdee, Department of Biology,

Mahasarakham University. The antifungal activity assay was measured by using Pore plate technique following a slightly modified method of Kumer [68]. SV-R, SV-S and SV-L (5 mL) as solutions in water at the concentration of 500 μg/mL and compound **C** at the concentration of 5 μg/mL, were mixed in 95 mL PDA medium before being plated to 90 mm Petri dishes. Seven-day-old mycelial discs of six plant pathogenic fungal pathogens (*Colletotrichum musae*, *C. capsici*, *C. gloeosporioides*, *Pestalotia* spp., *Fusarium* spp. TFPK301 and *Fusarium* spp. Foc 1708) were cut with a 7 mm sterilized cork borer under aseptic conditions and placed onto the 25 mL PDA plates containing 500 μg/mL of SV-R, SV-S, SV-L and 5 μg/mL of compound **C** . The plates were incubated at 28 ^{o}C and the mycelium growth was determined at day 7. The percentage of mycelial growth inhibition (PGI) were calculated using the formula as shown below where R represents the fungal growth radius (mm) of the control culture and R_1 represents the fungal growth radius distance (mm) in the treatment culture. The experiment was done with five replications and the fungus grew on the PDA plate was used as a control plate.

$$PGI\ (\%) = R\text{-}R_1/R \times 100$$

3.3.6 Antioxidant activity assay (DPPH scavenging assay)

The ability of SV-R, SV-S and SV-L to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured by using slightly modified method of Anoosh [68]. The solution of 0.2 mM DPPH in methanol was prepared. The addition 1.0 mL of DPPH solution to 2.0 mL of sample solutions in methanol at different concentrations (10-100 μg/mL). The mixture was incubated at room temperature in dark for 30 min. The absorbance was measured at 517 nm using UV-VIS spectrophotometer. Ascorbic acid was used as reference standard compound. The percentage DPPH radical scavenging activity (%RSA) by the sample was calculated using the formula as shown below where $A_{control}$ was the absorbance of the control (blank, without sample) and A_{test} was the absorbance of the sample. All of the tests were run triplicate and the calculation was used with the mean values. The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percentage DPPH radical scavenging activity plotted against the respective

concentration.

$$\%RSA = (A_{control} - A_{test})/A_{control} \times 100$$

3.3.7 Total phenolic contant (Folin-Ciocalteu assay)

The total phenolic content of SV-R, SV-S and SV-L was determined by using Folin-Ciocalteu assay following a slightly modified method of Anoosh [70]. A volume of 1.0 mL of the sample solutions in methanol (50 μg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and 3 mL of sodium carbonate solution (7.5%, w/v). The mixture was incubated at room temperature in dark for 30 min. The absorbance was measured at 765 nm using UV-VIS spectrophotometer. Gallic acid (10, 30, 50, 100 and 150 μg/mL) was used as a reference standard for ploting calibration curve. The total phenolic content was determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/ 100 mg gallic acid equivalent (GAE) of dry extract. All of the tests were run triplicate and the calculation was used with the mean values.

3.3.8 Biological assay

Anti-herpes simplex virus type 1 (HSV-1) and cytotoxicity assays against human breast cancer (MCF-7), oral human epidermoid carcinoma (KB), and Vero (African green monkey kidney fibroblasts) cell lines of crude methanol extract from the root, leaf, stem and compound **C** were evaluated using a colorimetric method [69] at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. Antibacterial activity of crude methanol extract from the root, leaf, and stem was evaluated using paper disc method. Antibacterial activity was performed by Associate Professor Aphidech Sangdee, Department of Biology, Mahasarakham University.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 The fungus *P. nipponicus* (Cod-MK1201)

4.1.1 Fermentation

4.1.1.1 Small scale fermentation

The culture mycelium yield of the small scale fermentation (5 flasks fermentation each week) varied from 0.016-1.557 g. The dried mycelium was suddenly increased from week 1 to week 3 and slightly went up from week 4 to week 5. After that the yield was decrease from week 6 to week 12 (Figure 33).

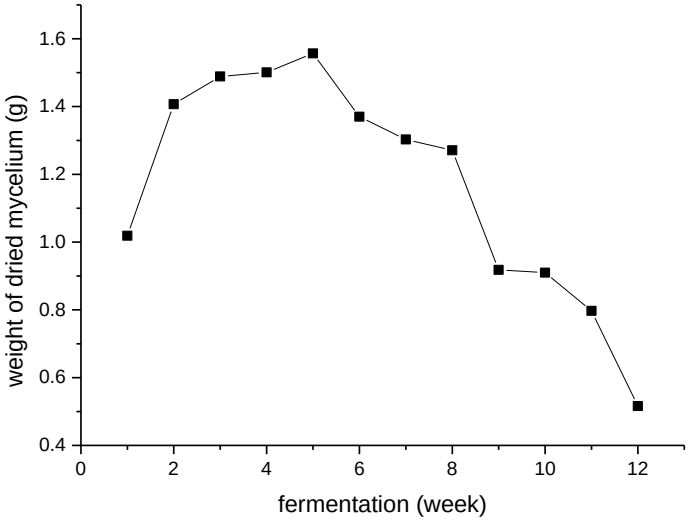


Figure 33. Weight of dried mycelium

4.1.1.2 Large scale fermentation

The culture broth (2.5 L) was collected for the large scale fermentation, 100 flasks of fermentation (Figure 34).

Figure 34. Culture broth of *P. nipponicus*

4.1.2 Extraction

4.1.2.1 Small scale extraction

From the results, the culture broth yield of the extraction (5 flasks fermentation each week) varied from 15.43-48.00 mg of dried extract/100 mL of broth and fluctuated without a regular pattern (Figure 35).

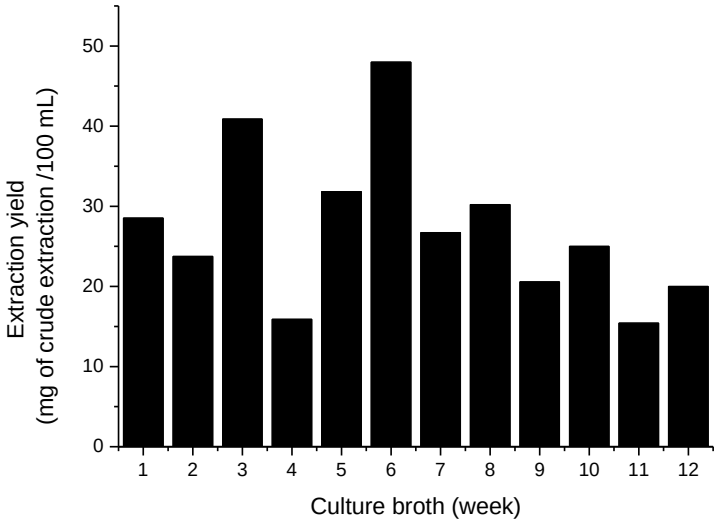


Figure 35. Extraction yield of culture broth

The culture mycelium yield of the extraction (5 flasks fermentation each week) varied from 2.70-17.05 mg of extract/g of dried mycelium and fluctuated without a regular pattern (Figure 36).

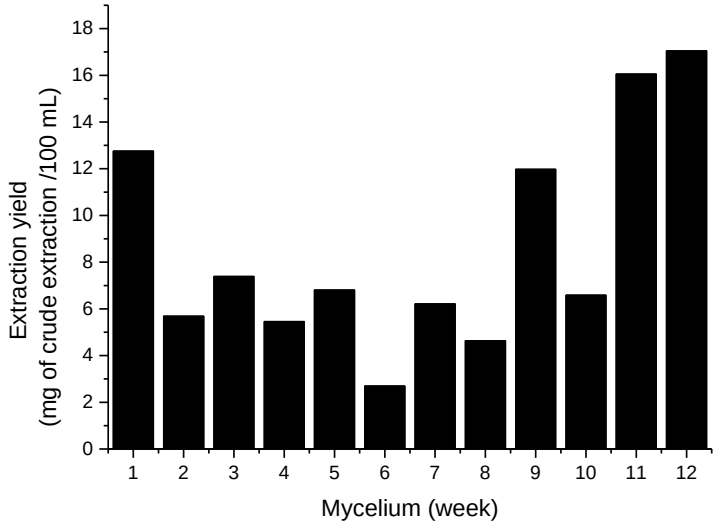


Figure 36. Extraction yield of culture mycelium

4.1.2.2 Large scale extraction

Lage scale extraction of *P. nipponicus* has been twice under the same amount of culture broth (2.5 L) and extraction condition. The crude extract from first bath large scale extraction was 8.95 g while second bath large scale extraction was 1.38 g. These results confirmed that the amount of extract from this fungus is not stable under the same fermentation condition.

4.1.3 Isolation of the crude extract

Compound **A** (8.02 g), a colorless amorphous powder, was obtained from 8.95 g of the dried broth extract from a large scale fermentation by trituration in ethyl acetate and compound **B** (12.35 mg), a pale brownish oil were isolated from the culture broth of the fungus *P. nipponicus*.

4.1.4 Structural elucidation

4.1.4.1 Structural elucidation of **A**

Compound **A** was identified as cordytropolone (**134**) ($C_9H_8O_4$). Its ^{1}H and ^{13}C-NMR spectroscopic data in DMSO-d_6 IR and MS spectrum were the same with those reported for cordytropolone from the fungus *Cordyceps* sp. BCC 1681 [35].

Crystallization of **134** from methanol: water successfully provided small crystals (Figure 37) and the structure of **134** was confirmed by X-ray crystallography for the first time (Figure 38). The structure was solved with the ShelXT structure solution program using combined Patterson and dual-space recycling methods [71]. The structure was refined by least squares using ShelXL [72]. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms of organic ligands were placed in calculated positions and refined using a riding model on attached atoms with isotropic thermal parameters 1.2 times those of their carrier atoms. The O−H hydrogen atoms were located in difference Fourier maps but refined with O−H = 0.82 ± 0.01 Å. The data have been deposited with the Cambridge Crystallographic Data Centre (CCDC) with CCDC number 1843739.

Figure 37. The single crystal of **134**

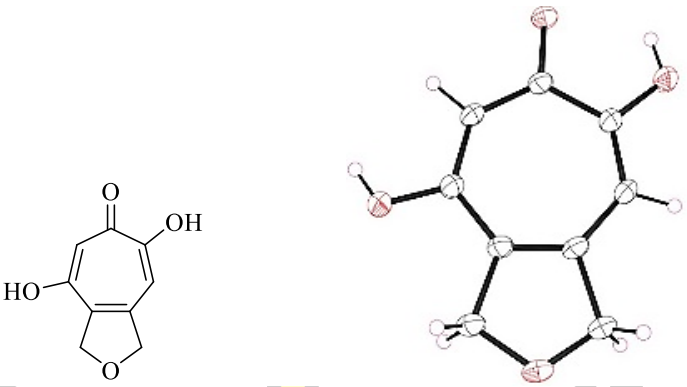


Figure 38. Structural compound and X-ray structure of **134**

4.1.4.2 Structural elucidation of **B**

Compound **B** has a molecular formula of $C_8H_{12}O_3$ (*m/z* 179.07 $[M+Na]^+$) as determined by ESIMS. The structure of **B** was elucidated on the basis of its NMR spectroscopic data in MeOH-d_4; ^{1}H NMR (500 MHz, CH_3OH-d_4) δ 5.85 (1H, s, H-6), 3.91 (1H, dd, *J* = 9.5, 5.5 Hz, H-3), 2.67 (1H, dd, *J* = 18.5, 5.5 Hz, H-4$_{eq.}$), 2.41 (1H, dd, *J* = 18.5, 5.5 Hz, H-4$_{ax.}$), 2.00 (3H, s, 5-CH_3), 1.22 (3H, s, 2-CH_3). ^{13}C NMR (125 MHz, CH_3OH-d_4) δ 203.7 (C, C-1), 162.5 (C, C-5), 125.0 (CH, C-6), 78.3 (C, C-2), 73.9 (CH, C-3), 39.4 (CH_2, C-4), 24.4 (CH_3, 5-*C*H_3), 18.1 (CH_3, 2-*C*H_3) (Table 3). IR (KBr) V_{max} cm^{-1}: 3381, 2978, 2924, 2846, 1662, 1631, 1435, 1382, 1262, 1162. From HSQC spectra indicated six HSQC correlation signals. The methyl protons at δ_H 1.22 (2-CH_3) showed the correlation signal to methyl carbon at δ_C 18.1 (2-*C*H_3) ppm. The methyl protons at δ_H 2.00 (5-CH_3) showed the correlation signal to methyl carbonat δ_C 24.4 (5-*C*H_3) while methylene proton at δ_H 2.41 (H-4$_{ax.}$) and 2.67 (H-4$_{eq.}$) showed the correlation signals to methylene carbon at δ_C 39.4 (C-4).The oxygenated methine proton at δ_H 3.91 (H-3) showed HSQC correlations to carbon at δ_C 73.9 (C-3) ppm. The methine proton at δ_H 5.85 (H-6) ppm exhibits the correlation signal to methine carbon δ_C 125.0 (C-6) ppm.

From HMBC spectra indicated nineteen HMBC correlation signals. The methyl proton at δ_H 1.22 (2-CH_3) showed three bond HMBC correlations to carbon at δ_C 73.9 (C-3), 78.3 (C-2) and 203.7 (C-1). The methyl proton at δ_H 2.00 (5-*C*H_3) showed the correlation signals to carbon at δ_C 39.4 (C-4), 125.0 (C-6) and 162.5 (C-5) while

methylene proton at δ_H 2.41 (H-4$_{ax}$.) showed the correlation signals to carbon at δ_C 73.9 (C-3), 125.0 (C-6) and 162.5 (C-5) ppm. The δ_H 2.67 (H-4$_{eq}$.) ppm showed the correlation signals to δ_C 24.4 (5-*C*H$_3$), 73.9 (C-3), 78.3 (C-2), 125.0 (C-6) and 162.5 (C-5) ppm. The oxygenated methine proton at δ_H 3.91 (H-3) ppm exhibits the correlation signals to methyl carbon at δ_C 18.1 (2-*C*H$_3$) and quaternary carbon at 78.3 (C-2) ppm. The correlatons of methine proton at δ_H 5.85 (H-6) with their attaching carbon at δ_C 24.4 (5-*C*H$_3$), 39.4 (C-4) and 78.3 (C-2) (Figure 39).

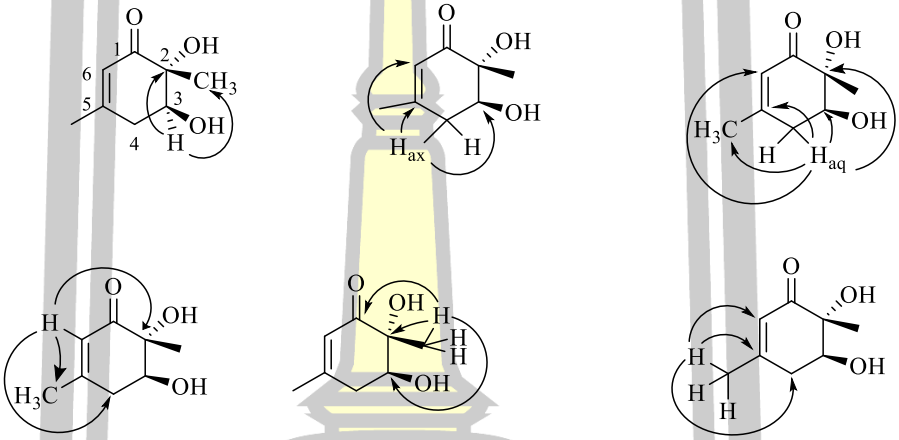


Figure 39. HMBC correlations of compound **B**

From COSY spectra indicated COSY correlation signals, methine proton at δ_H 5.85 (H-6) ppm showed COSYcorrelations to methylene protons at δ_H 2.67 (H-4$_{eq}$.) and 2.41 (H-4$_{ax}$.) ppm and methy proton at δ_H 2.00 (5-C*H*$_3$) ppm. The correlatons of methine proton at δ_H 3.91 with methylene protons at δ_H 2.67 (H-4$_{eq}$.) and 2.41 (H-4$_{ax}$.) ppm (Figure 40).

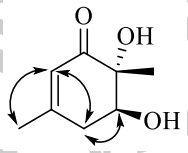


Figure 40. COSY correlations of compound **B**

Table 3. NMR spectral data of compound **B** in MeOH-d_4.

Position	**δ_H (multi., *J* in Hz)**	**δ_C**	**COSY**	**HMBC**
1	-	203.7	-	-
2	-	78.3	-	-
3	3.91 (dd, 9.5, 5.5)	73.9	H-4	C-2, 2-*C*H_3
$4_{ax.}$	2.41(dd, 18.5, 9.5)	39.4	H-$4_{eq.}$, H-3	C-3, C-5, C-6
$4_{eq.}$	2.67 (dd, 18.5, 5.5)		H-$4_{ax.}$, H-3	C-5, C-6
5	-	162.5		-
6	5.85 (s)	125.0	5-CH_3	C-2, C-4, 5-*C*H_3
2-CH_3	1.22 (s)	18.1		C-1, C-2, C-3
5-CH_3	2.00 (s)	24.4	H-6	C-4, C-5, C-6,

Based on spectroscopic data, the structure of **B** was elucidated as same structure of leptosphaerones A and B which have been isolated from the fungus *Leptosphaeria herpotrichoides* [73]. Comparison of the NMR spectroscopic data of **B** in $CDCl_3$ (Table 4) with those reported in the literature for leptosphaerone A (**353**) (Figure 41) in $CDCl_3$ were the same, except for their optical rotation values. Leptosphaerone A showed $[\alpha]_D$ +1.9 (*c* = 0.47, $CHCl_3$) while **B** displayed a specific rotation of the opposite sign $[\alpha]_D^{25}$ -1.7 (*c* = 0.49, $CHCl_3$). This information indicated that **B** was the enantiomer of leptosphaerone A (2*S*, 3*R*).

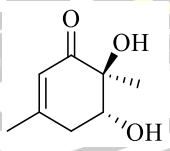


Figure 41. Structures of **353**

The lack of a NOESY correlation (in $CDCl_3$, Figure 42) from the methyl protons 2-CH_3, δ_H 1.26 (s), to the nearby oxygenated methine proton H-3, δ_H 4.00 (dd, *J* = 10.5, 6.0 Hz) supported the *trans* relationship between these protons. Hence, the stereogenic centers of **B** was confirmed to be 2*R*,3*S*. The name (-)-leptosphaerone A

(**354**) (Figure 43) was given for this new compound.

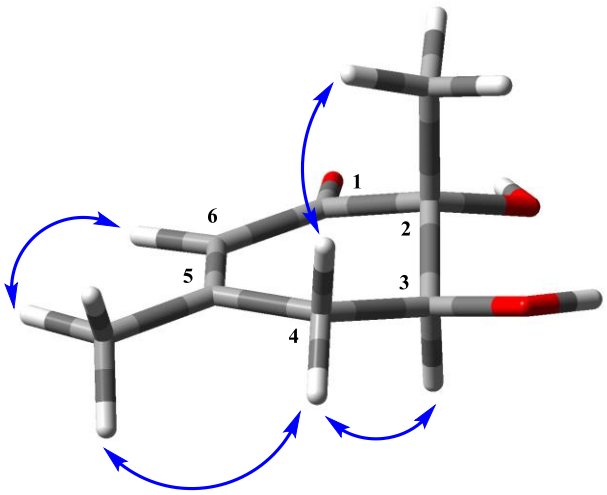


Figure 42. NOESY correlation of compound **B**

Table 4. NMR Data of **354** compared with **353** in $CDCl_3$

Position	**δ_H (multi., *J* in Hz)**		**δ_C**	
	354	**353**	**354**	**353**
1	-	-	201.8	201.6
2	-	-	77.2	77.3
3	4.00 (dd, 10.5, 6.0)	3.98 (dd, 10.0, 5.8)	72.9	72.8
$4_{ax.}$	2.40 (dd, 18.5, 10.5)	2.40 (dddq, 18.2, 10.5, 2.6,1.3)	37.7	37.6
$4_{eq.}$	2.60 (dd, 18.5, 6.0)	2.62 (dd, 18.5, 5.8)		
5	-	-	161.0	160.2
6	5.93 (s)	5.92 (dq, 2.6, 1.3)	123.5	123.4
2-CH_3	1.26 (s)	1.25 (s)	17.8	17.7
5-CH_3	2.02 (s)	2.00 (s)	24.5	24.4

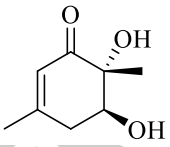


Figure 43. Structures of **354**

4.1.5 HPLC analysis

A time course of a small scale fermentation of *P. nipponicus* over 12 weeks was conducted in order to study the production of **134** as the predominant compound from the culture broth of this fungus. The production of **134** and **354** in the extracts was monitored by HPLC analysis using pure compounds **134** (t_R 2.52 min) and **354** (t_R 11.20 min) and also adenine (t_R 7.63 min) and adenosine (t_R 12.35 min) as reference standards. This study showed that compounds **134** and **354** were produced by *P. nipponicus* in every week of the fermentation as well as adenine and adenosine (Table 5). The quantity of **134** in the extracts was determined from its peak area calculated based on a standard linear equation of pure compound **134** (r^2 0.988) which was extremely high at weeks 11 and 12 (~1 mg/ mg of dried extract). Surprisingly, the production of **134** at week 3 (21 days) was very low when compared to most other weeks. These results were different from our large scale fermentation (20 days) results. Therefore, a second batch under large scale fermentation and the isolation of **134** under the same procedure was repeated. Only 756.20 mg (55% yield from 1.38 g of the dried extract) of **134** was obtained. These results confirmed that **134** is produced by this fungus but the quantity of **134** is not stable under the same fermentation conditions.

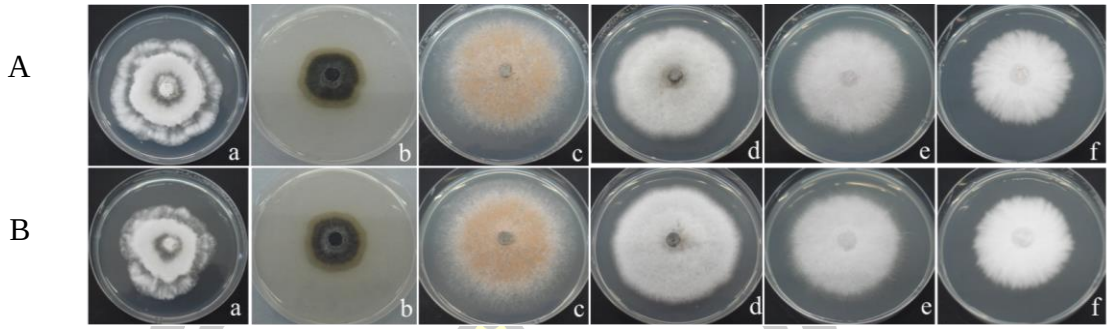
Table 5. Production of **134**, **354**, adenine and adenosine from the *P. nipponicus*

Fermentation (week)	Production of compound			
	134 (mg/mg of dried extract)	**354**	**adenine**	**adenosine**
1	0.08	+	+	+
2	0.12	+	+	+
3	0.07	+	+	+
4	0.03	+	+	+
5	0.35	+	+	+
6	0.22	+	+	+
7	0.10	+	+	+
8	0.48	+	+	+
9	0.65	+	+	+
10	0.31	+	+	+
11	1.00	+	+	+
12	0.99	+	+	+

+ indicates detectable in the broth extracts.

4.1.6 Antifungal activity

The antifungal activity of **134** at 25 μg/mL was tested against six plant pathogenic fungi. The results revealed that **134** had a slight inhibitory effect against fungal mycelial growth as shown in Figure 44 and Table 6. The antifungal activity of **134** against plant pathogenic fungi including *Colletotrichum capsici* and *C. gloeosporioides* has been reported recently [74]. However the mechanisms of action against fungal pathogens have not yet been described.



A = control, B = **134**, a = *Pestalotia* spp., b = *Colletotrichum capsica*, c = *Colletotrichum musae*, d = *Colletotrichum gloeosporioides*, e = *Fusarium* spp. Foc 1708, f = *Fusarium* spp. TFPK301

Figure 44. Mycelial growth inhibition of **134** against six plant pathogenic fungi

Table 6. The percentage of mycelial growth inhibition (PGI) of **134** against six plant pathogenic fungi.

Fungal strains	**Original host plant**	**The percentage (%) of mycelial growth inhibition**
Pestalotia spp.	Mango	18.75 ± 5.24
Colletotrichum capsici	Papaya	12.86 ± 1.43
Colletotrichum musae	Cultivated banana	3.74 ± 0.70
Colletotrichum gloeosporioides	Mango	0.91 ± 0.56
Fusarium spp. Foc 1708	Banana	7.93 ± 0.61
Fusarium spp. TFPK301	Tomato	5.46 ± 0.56

4.1.7 Antioxidant activity

The results of the antioxidant activity of **134**, toward DPPH radical was found to be inactive.

4.1.8 Biological activity

4.1.8.1 Cordytropolone

The antimalarial (*P. falciparum*, K1) and cytotoxic (KB and BC-1 cell lines) activities of cordytropolone have already been published [35]. The antibecteriral

activity of cordytropolone was found to be inactive.

4.1.8.2 (-)-Leptosphaerone A

(-)-Leptosphaerone A was tested for its cytotoxicity against human breast cancer (MCF-7), oral human epidermoid carcinoma cancer (KB) and Vero (African green monkey kidney fibroblasts) cell lines, and antivirus activity against Herpes simplex virus type-1 (HSV-1) at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. (-)-Leptosphaerone A was found to be inactive in all these tests. The cytotoxicity (A-549 cell lines) of leptosphaerone C (same identified structure with leptosphaerone A) isolated from the fungus *Penicillium* sp. have already been published (Table 7) [75].

Table 7. Biological activity of (-)-Leptosphaerone A

Test	**Activity**
cytotoxicity against human breast cancer (MCF-7 cell line)	inactive
cytotoxicity against oral human epidermoid carcinoma cancer (KB cell line)	inactive
cytotoxicity against Vero (African green monkey kidney fibroblasts cell line)	inactive
antivirus activity against Herpes simplex virus type-1 (HSV-1)	inactive

4.2 The *Smilax verticalis*

4.2.1 Extraction

4.2.1.1 Small scale extraction

From small scale extraction, the methanol extracts of root, stem and leaf were obtained 524.2 (0.26%), 356.7 (0.18%) and 720.4 mg (0.36%).

4.2.1.2 Large scale extraction

The ethyl acetate extract which was obtained from the methanol extract from the root of *S. verticalis* was 10.419 g (1.49%).

4.2.2 Isolation of the crude extract

Compound **C** (94.2 mg), a light brownish oil, a compound **D** (5.5 mg), a pale brownish solid, and a compound **E** (30.9 mg), a pale brownish solid were isolated from the ethyl acetate extract from the root of the *S. verticalis*.

4.2.3 Structural elucidation

4.2.3.1 Structural elucidation of **C**

The structure of **C** (Figure 45) was elucidated on the basis of its NMR spectroscopic data in MeOH-d_4; ^{1}H NMR (400 MHz, CH_3OH-d_4) δ 7.11 (2H, d, J = 8.8 Hz, H-3, H-7), 6.81 (2H, d, J = 8.8 Hz, H-4, H-6), 4.01-4.06 (1H, m, H-9), 3.89-4.00 (2H, m, H-8), 3.47-3.55 (2H, m, H-10), 3.37 (3H, s, H-11), 1.60 (3H, s, 1-CH_3), and ^{13}C NMR (100 MHz, CH_3OH-d_4) δ 156.7 (C, C-5), 143.2 (C, C-2), 127.4 (2C, C-3, C-7), 113.6 (C, C-4, C-6), 73.6 (C, C-10), 69.1 (C, C-8), 68.7 (C, C-9), 58.1 (C, C-11), 41.3 (C, C-1), 30.2 (C, 1-CH_3). NMR spectral data of compound **C** in MeOH-d_4 are shown in Table 8. All carbon signals were classified by DEPTs experiment which into five quaternary, ten methine, four methylene and four methyl carbons.

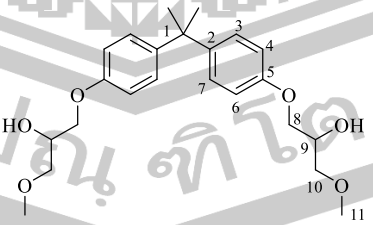


Figure 45. Structures of **C**

From HSQC spectra indicated seven HSQC correlation signals. The aromatic proton at δ_H 7.11 (H-3, H-7) showed the correlation signal to aromatic carbon at δ_C 127.4 (C-3, C-7). The aromatic proton at δ_H 6.81 (H-4, H-6) showed the correlation signal to aromatic carbon at δ_C 113.6 (C-4, C-6). Methylene protons at δ_H 3.89-4.00 (H-8) showed HSQC correlations to carbon at δ_C 69.1 (C-8). The methine proton at δ_H 4.01-4.06 (H-9) exhibited the correlation signal to methine carbon at δ_C 68.7 (C-9). The methylene protons at δ_H 3.47-3.55 (H-10) showed the correlation signals to methylene carbon at δ_C 73.6 (C-10) while methyl protons at δ_H 1.60 (1-C*H*$_3$) showed the correlation signal to methy carbon δ_C 30.2 (1-*C*H$_3$). The methoxyl protons at δ_H 3.37 (H-11) exhibited the correlation signal to methoxyl carbon at δ_C 58.1 (C-11).

From HMBC spectrum, aromatic proton at δ_H 6.81 (H-6) showed HMBC correlations to carbons δ_C 156.7 (C-5), 143.2 (C-2) and 113.6 (C-4). Aromatic protons at δ_H 7.11 (H-7) displayed the correlation signals to carbons δ_C 156.7 (C-5), 127.4 (C-3) 113.6 (C-6) and 41.3 (C-1) while methylene protons at δ_H 3.89-4.00 (H-8) showed the three bond HMBC correlations to their attaching carbon at δ_C 73.6 (C-10), 68.7 (C-9) and 156.7 (C-5). The methylene protons at δ_H 3.47-3.55 (H-10) showed the correlation signals to carbons at δ_C 69.1 (C-8), 68.7 (C-9), 58.1 (C-11) while methyl protons at δ_H 1.60 (1-C*H*$_3$) exhibited the correlation signals to aromatic carbon at δ_C 143.2 (C-2), quaternary carbon at δ_C 41.3 (C-1) and methyl carbon at δ_C 30.2 (1-*C*H$_3$). The correlatons of methoxyl protons at δ_H 3.37 (H-11) with methylene carbon at δ_C 73.6 (C-10) were observed (Figure 46).

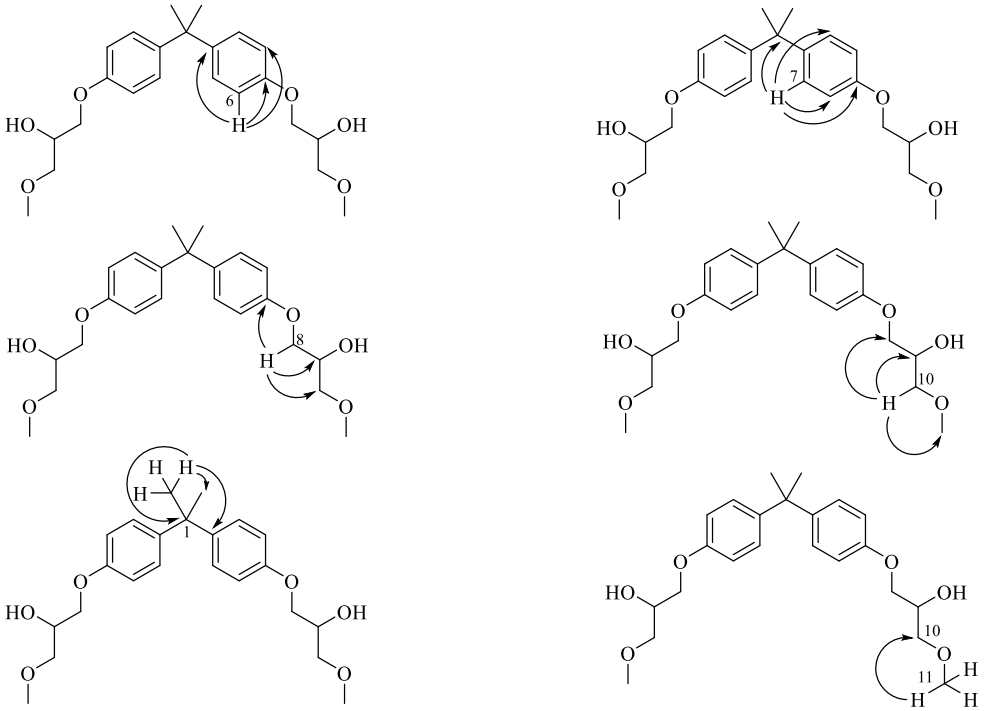


Figure 46. HMBC correlations of **C**

COSY spectra indicated three COSY correlation signals from methylene protons at δ_H 3.47-3.55 (H-10) to methine proton at δ_H 4.01-4.06 (H-9) and methoxyl protons at δ_H 3.37 (H-11) while methylene protons at δ_H 3.89-4.00 (H-8) showed COSY correlations to methine proton at δ_H 4.01-4.06 (H-9) (Figure 47).

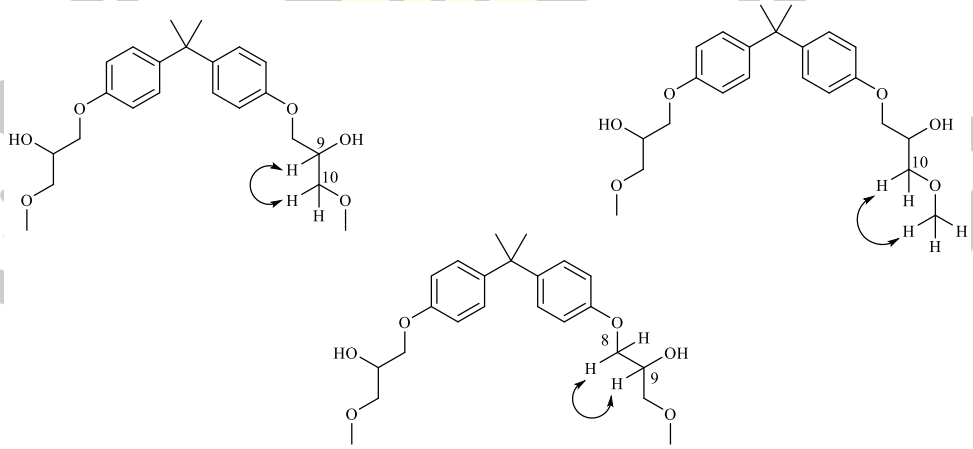


Figure 47. COSY correlations of **C**

Table 8. NMR spectral data of compound **C** in MeOH-d_4.

Position	δ_H (multi., *J* in Hz)	δ_C	COSY	HMBC
1	-	41.3	-	-
2	-	143.2	-	-
3	7.11 (d, 8.8)	127.4	H-4	-
4	6.81 (d, 8.8)	113.6	H-3	-
5	-	156.7	-	-
6	6.81 (d, 8.8)	113.6	H-7	C-2,C-4, C-5
7	7.11 (d, 8.8)	127.4	H-6	C-1, C-3, C-4, C-5
8	3.89-4.00 (m)	69.1	H-9	C-5, C-9, C-10
9	4.01-4.06 (m)	68.7	H-8, H-10	-
10	3.47-3.55 (m)	73.6	H-9, H-11	C-8, C-9, C-11
11	3.37 (s)	58.1	H-10	C-10
1-CH_3	1.60 (s)	30.2	-	C-1, 1-*C*H_3, C-2

NOESY spectra of compound **C** showed correlation between aromatic proton at δ_H 7.11 (H-7, H-3) to methy proton at δ_H 1.60 (1-CH_3) and aromatic proton at δ_H 6.81 (H-6, H-4). Aromatic proton at δ_H 6.81 (H-6, H-4) exibitied NOESY correlations to proton at δ_H 3.89-4.00 (H-8) while proton at δ_H 4.01-4.06 (H-9) showed correlation signals to methylene proton at δ_H 3.89-4.00 (H-8) and δ_H 3.47-3.55 (H-10). Methylene proton at δ_H 3.47-3.55 (H-10) exhibitied NOESY correlations to methine proton at δ_H 4.01-4.06 (H-9) while methoxyl protons at δ_H 3.37 (H-11) showed correlation signal to δ_H 3.47-3.55 (H-10) (Figure 48).

On the basis of these spectroscopic data, the structure of compound **C** was elucidated as 3,3'-(4,4'-(Propane-2,2-diyl)bis(4,1-phenylene))bis(oxy)bis (1-methoxypropan-2-ol) (**355**). The compound is a synthetic plastic agent and might contaminated as a plasticizer in the methanol sovent.

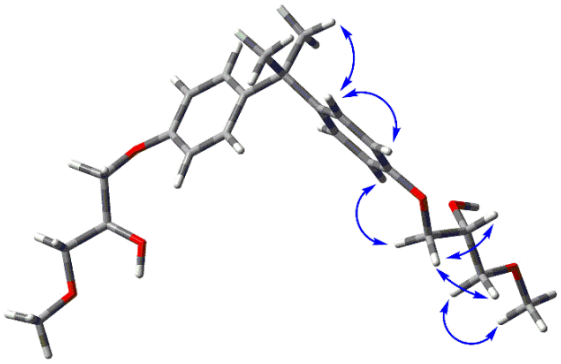


Figure 48. NOESY correlations of **C**

The synthesis of compound **355** was reported [76], [77]. The synthesized method was started with a solution of racemic isphenol A diglycidyl ether (BADGE) (32 mg, 0.094 mmol. 1 equiv) in methanol (0.3 mL) and added solid erbium (III) trifluoromethanesulfonate (58 mg, 0.094 mmol. 1 equiv) in portions over an hour, the mixture was stirred at room temperature for 6 h. The organic solvent was evaporated under a stream of nitrogen, and the residue was purified by flash column chromatography on silica gel Sep pak (10 g) (eluent: 5% methanol in dichloromethane) to provide 28 (31 mg, 82%) as a colourless solid (Figure 49) [77].

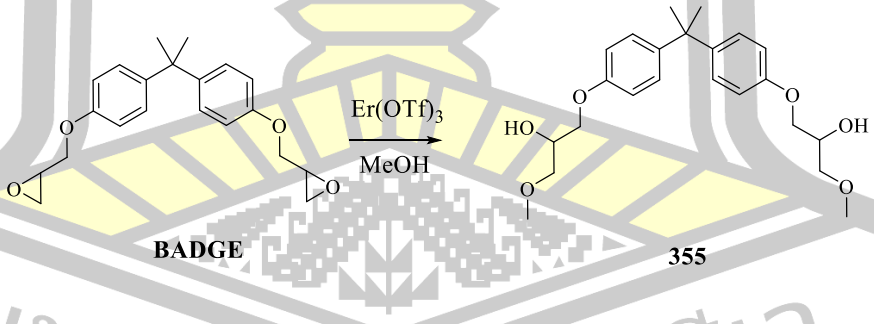


Figure 49. The synthesis scheme of 355

4.2.3.2 Structural elucidation of **D**

Compound **D** gave similar ^{1}H NMR and ^{13}C NMR spectra to compound **C**. This compound is a derivative of compound **D** and should be also a plasticizer. Its NMR spectroscopic data was recorded in MeOH-d_4; ^{1}H NMR (400 MHz, CH_3OH-d_4) δ 7.11 (2H, d, J = 8.4 Hz, H-3, H-7), 6.83 (2H, t, J = 8.4 Hz, H-4, H-6), 4.01-4.10 (1H,

m, H-9), 3.90-4.00 (2H, m, H-8), 3.47-3.56 (2H, m, H-10), 3.37 (2H, s, H-11), 1.60 (3H, s, 1-CH_3), and ^{13}C NMR (100 MHz, CH_3OH-d_4) δ 1567.0 (C, C-5), 143.5 (C, C-2), 127.5 (2C, C-3, C-7), 113.6 (C, C-4, C-6), 73.6 (C, C-10), 69.0 (C, C-8), 68.7 (C, C-9), 57.9 (C, C-11), 41.1 (C, C-1), 30.0 (C, C-1-CH_3). ^{1}H and ^{13}C NMR spectral data of compound **D** in MeOH-d_4 are shown in Table 9. On the basis of ^{1}H and ^{13}C NMR spectral data and comparison of ^{1}H and ^{13}C with NMR spectral data with **355**, the structure of compound **D** was propored (Figure 50).

Table 9. NMR spectral data of compound **356** in MeOH-d_4.

Position	**δ_H (multi., *J* in Hz)**	**δ_C**
1	-	41.1
2	-	143.5
3	7.11 (d, 8.4)	127.5
4	6.83 (d, 8.4)	113.6
5	-	157.0
6	6.83 (d, 8.4)	113.6
7	7.11 (d, 8.4)	127.4
8	3.90-4.00 (m)	69.0
9	4.01-4.10 (m)	68.7
10	3.47-3.56 (m)	73.6
11	3.37 (s)	57.9
1-CH_3	1.60 (s)	30.0

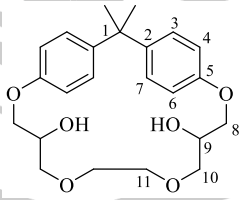


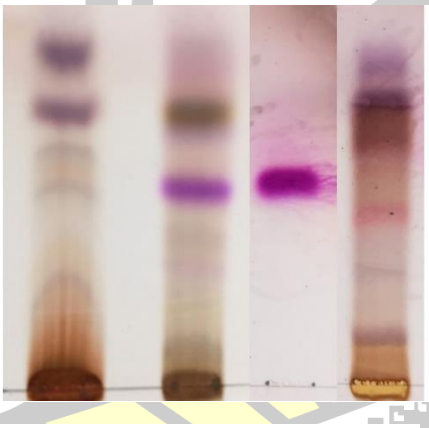
Figure 50. Structures of **356**

4.2.3.3 Spectoscopic data of **E**

Compound **E** showed similar ^{1}H NMR spectra to compound **C**. Its NMR spectroscopic data was recorded in MeOH-d_4; ^{1}H NMR (400 MHz, CH_3OH-d_4) δ 7.10 (2H, d, J = 8.7 Hz, H-3, H-7), 6.81 (2H, d, J = 8.7, H-4, H-6), 4.00-4.05 (1H, m, H-9), 3.80-3.99 (2H, m, H-8), 3.47-3.69 (2H, m, H-10), 3.37 (1H, s, H-11), 1.59 (3H, s, 1-CH_3). The structure of E has not been proposed yet.

4.2.4 Proof of contamination by thin layer chromatography (TLC)

To prove that compound **C** (**355**) is a plasticizer that contaminated in the ethyl acetate extract, 50 g of the root of *S. verticalis* was extracted once again in ethanol and the ethanol extract was analyzed by TLC compared with ethyl acetate extract, methanol extract and pure compound **C** (Figure 51). From the result, a band belong to **355** only occured in the ethyl acetate extract from large scale extraction. This information confirmed that **355** is not a metabolite produced by *S. verticalis*. It should be a contaminant from organic solvents during extraction process.



A: Methanol extract
B: Ethy acetate extract
C: Compound **355**
D: Ethanol extract
Mobile phase: 100% Ethy acetate
Spray reagent: *p*-Anisaldehye

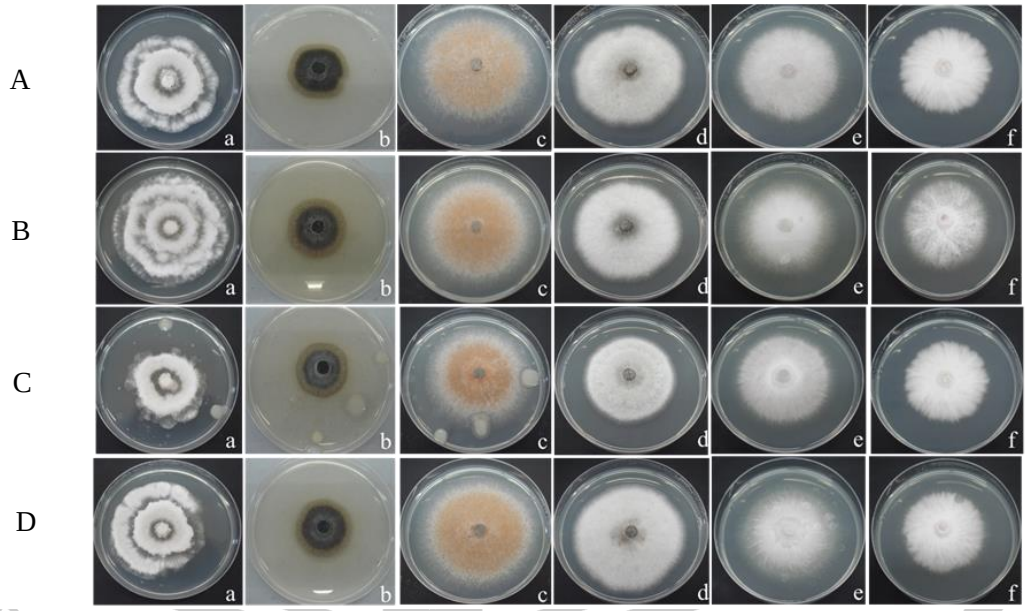
Figure 51. TLC profile of compound **355** compared with extracts.

4.2.5 Antifungal activity

The antifungal activity of SV-R, SV-S and SV-L at 25 μg/mL and compound **355** at 5μg/mL were tested against six plant pathogenic fungi. The results revealed that SV-R, SV-S, SV-L (Figure 52 and Table 10) and **355** (Figure 53 and Table 11) had weak inhibitory effect against fungal mycelial growth.

Table 10. The percentage of mycelial growth inhibition (PGI) of crude methanol extractfrom the root, stem and leaf against six plant pathogenic fungi

Fungal strains	**Original host plant**	**The percentage (%) of mycelial growth inhibition**		
		SV-R	**SV-S**	**SV-L**
Pestalotia spp.	Mango	14.33 ± 3.84	14.83 ± 13.82	0.00 ± 0.00
C. capsici	Papaya	12.57 ± 2.55	0.00 ± 0.00	0.00 ± 0.00
C. musae	Cultivated banana	8.59 ± 1.95	0.00 ± 0.00	0.00 ± 0.00
C. gloeosporioides	Mango	11.24 ± 1.82	0.00 ± 0.00	0.00 ± 0.00
F. spp. Foc 1708	Banana	4.88 ± 2.77	1.82 ± 2.05	0.91 ± 0.00
F. spp. TFPK301	Tomato	3.61 ± 3.39	1.18 ± 0.99	0.00 ± 0.00



A = control, B =SV-L, C = SV-R, D = SV-S, a = *Pestalotia* spp., b = *Colletotrichum capsica*, c = *Colletotrichum musae*, d = *Colletotrichum gloeosporioides*, e = *Fusarium* spp. Foc 1708, f = *Fusarium* spp. TFPK301

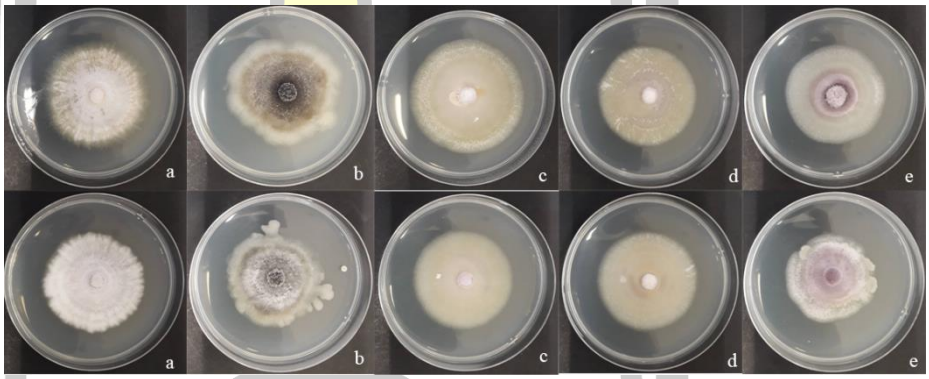
Figure 52. Mycelial growth inhibition of crude methanol extract from the root, stem and leaf against six plant pathogenic fungi

Table 11. The percentage of mycelial growth inhibition (PGI) of compound **355** against six plant pathogenic fungi

Fungal strains	**Original host plant**	**The percentage (%) of mycelial growth inhibition**
		355
Pestalotia spp.	Mango	4.71 ± 2.29
C. capsici	Papaya	10.86 ± 2.35
C. musae	Cultivated banana	11.47 ± 3.14
Fusarium spp. Foc 1708	Banana	0.00 ± 0.00
Fusarium spp. TFPK301	Tomato	17.74 ± 1.16

A

B



A = control, B = compound **355**, a = *Pestalotia* spp., b = *Colletotrichum capsica*, c = *Colletotrichum musae*, d = *Fusarium* spp. Foc 1708, e = *Fusarium* spp. TFPK301

Figure 53. Mycelial growth inhibition of compound **355** against six plant pathogenic fungi

4.2.6 Antioxidant activity

The scavenging activity of toward DPPH radical of standard ascorbic acid is shown in Figure 54. Based on the calculation from %RSA to IC_{50}, ascorbic acid showed scavenging activity of toward DPPH radical at IC_{50} value of 4.81 ± 0.22. The DPPH scavenging activity of the crude methanol extract from the root, leaf and stem are shown in Table 12. Crude methanol extract from the root exhibited the lowest IC_{50} value (35.76 ± 1.1 μg/mL) as compared to crude methanol extract from the stem (56.09

± 1.33 μg/mL) and leaf (90.68 ± 1.67 μg/mL). The IC_{50} values of all crude methanol extract were found to be significant ($P < 0.05$) as compared with ascorbic acid.

The results were analyzed using the Statistical Package for Social Sciences (SPSS). All the data were expressed as mean ± SD and analyzed by One-way ANOVA with the post-hoc Tukey's test and values were considered significant at $P < 0.05$.

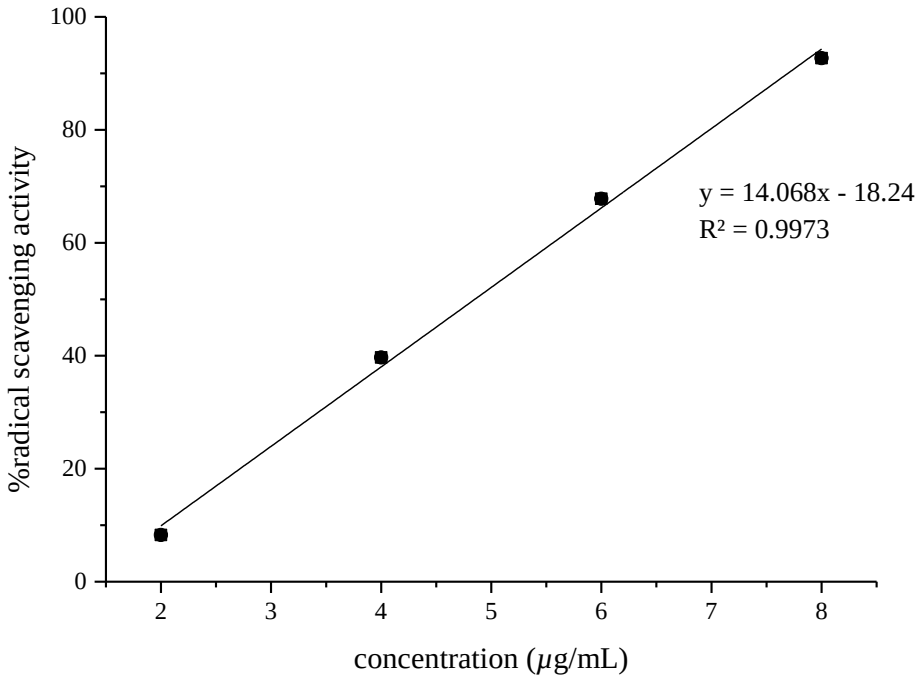


Figure 54. DPPH radical of standard ascorbic acid

Table 12. The antioxidant activity of crude methanol extract from the root, stem and leaf

Sample	**Antioxidant activity (IC_{50}, μg/mL)**
SV-R	35.76 ± 1.10^{b}
SV-S	56.09 ± 1.33^{c}
SV-L	90.68 ± 1.67^{d}
Ascorbic acid	4.81 ± 0.22^{a}

*Different lower case letters denote significant differences ($P < 0.05$) between IC_{50} values.

4.2.7 Total phenolic content

The total phenolic content was calculated using the following linear equation based on the calibration curve of gallic acid; $Y = 0.0142X + 0.2149$, $R^2 = 0.9957$, where Y is absorbance and X is concentration of gallic acid in μg/mL (Figure 55). The content of total phenolic compounds expressed as mg/100 mg gallic acid equivalent (GAE) of dry extract. The content of total phenolic compounds of crude methanol extract from the root was detected at 7.71± 0.57 mg/100 mg GAE while the content of total phenolic compounds of crude methanol extract from the leaf and stem were not detected with this assay at the concentration of 50 μg/mL (Table 9).

Table 13. The total phenolic contents of SV-R, SV-S and SV-L

Sample	**The total phenolic contents (mg/ 100 mg GAE)**
SV-R	7.71 ± 0.57
SV-S	not detected*
SV-L	not detected*

* not detected with this assay at the concentration of 50 μg/mL with linear equation based on the calibration curve of gallic acid in the range of 10- 150 μg/mL

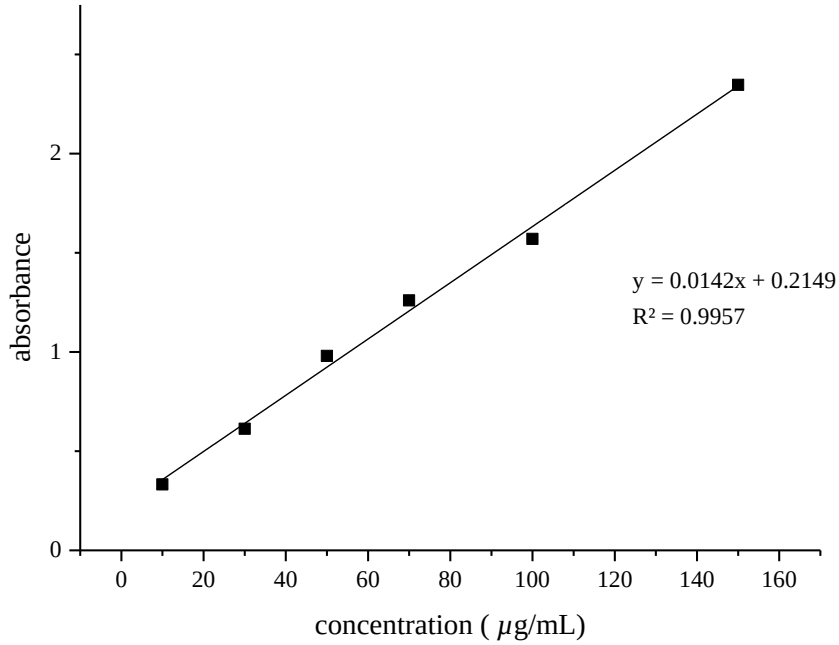


Figure 55. The calibration curve of gallic acid

4.2.8 Biological activity

Crude methanol extract from the root, stem and leaf were tested for theirs cytotoxicity against human breast cancer (MCF-7), oral human epidermoid carcinoma cancer (KB) and Vero (African green monkey kidney fibroblasts) cell lines, antivirus activity against Herpes simplex virus type-1 (HSV-1) and antibecteriral activity. Crude methanol extract from the root, stem and leaf were inactive in all these tests. Compound **355** was tested for its cytotoxicity against human breast cancer (MCF-7) and Vero (African green monkey kidney fibroblasts) cell lines. It exhibited cytotoxicity against Vero cell line with %cytotoxicity values of 62.54% and non- cytotoxicity against MCF-7 and NCI-H187-Small cell lung cancer cell line (Table 14).

Table 14. Biological activity of SV-R, SV-S, SV-L and **355**

Sample	**Test**	**Activity**
SV-R, SV-S and SV-L	cytotoxicity against human breast cancer (MCF-7 cell line)	inactive
	oral human epidermoid carcinoma cancer (KB cell line)	inactive
	cytotoxicity against Vero (African green monkey kidney fibroblasts cell line)	inactive
	antivirus activity against Herpes simplex virus type-1 (HSV-1)	inactive
	antibecteriral activity	inactive
355	cytotoxicity against human breast cancer (MCF-7 cell line)	inactive
	cytotoxicity against Vero (African green monkey kidney fibroblasts cell line)	active
	cytotoxicity against NCI-H187-Small cell lung cancer cell line	inactive

CHAPTER 5
CONCLUSION

5.1 The fungus *P. nipponicus* (Cod-MK1201)

Two biologically active compounds, cordytropolone (**134**; 8.02 g) and and a new compound (-)-leptosphaerone A (**354**; 12.35 mg), have been isolated from the culture broth (2.5 L) of the insect pathogenic fungus *Polycephalomyces nipponicus* (formerly known as *Cordyceps nipponica*) which was collected from Maha Sarakham province, northeast Thailand. The structures of these two compounds were elucidated by spectroscopic methods and compared with spectral data those reported previously in the literatures. In this research, the structure of **134** was confirmed by X-ray crystallographic technique for the first time while the leptosphaerone class, compound **354**, was first isolated as its (+)-antipode from the fungus *Polycephalomyces* (*Cordyceps*). The production of 134, a predominant compound, from the culture broth of *P. nipponicus* in between 1-12 weeks small scale cultivation was determined by using High Performance Liquid Chromatography (HPLC) compared with adenine and adenosine metabolites and the antifungal activity of **134** were studied. From the results, compounds **134**, **354**, adenine and adenosine were detected in every week from the culture broth extracts. The highest production of **134** was found in week 11 from the small scale cultivation. Compound **134** exhibited weak antifungal activity against six tested fungal species; *Collectrichum capsica*, *Collectrichum gloeosporioides*, *Collectrichum musae*, *Fusarium* spp. FOC1708, *Fusarium* spp. TFPK301 and *Pestalotia* sp. with the PGI values of 12.86 ± 2.86, 0.91 ± 1.25, 3.74 ± 1.39, 7.93 ± 1.36, 5.46 ± 1.26 and 18.75 ± 10.48 respectively, at 25 μg/mL.

5.2 The *Smilax verticalis*

The root, stem and leaf of *S. verticalis* were small scale extracted in methanol. The methanol extracts from the root (SV-R), stem (SV-S) and leaf (SV-L) were tested for their antifungal, antioxidant, cytotoxic and antibacterial activities. The SV-R

showed antifungal activity against six pathogenic fungal strains (*Pestalotia* spp., *Colletotrichum capsica*, *Colletotrichum musae*, *Colletotrichum gloeosporioides*, *Fusarium* spp. Foc 1708 and *Fusarium* spp. TFPK301) with the percentage of mycelial growth inhibition (PGI) in the range of 3.61 ± 3.39-14.33 ± 3.84. The antioxidant activity of the SV-R (IC_{50} 35.76 ± 1.10 μg/mL) was higher than the SV-S (IC_{50} 56.09 ± 1.33 μg/mL) and SV-L (IC_{50} 90.68 ± 1.67 μg/mL) by DPPH method. These three extracts had no cytotoxic (MCF-7, KB cancer cell lines and Vero cell lines) antiviral (HSV-1) and antibacterial activities. Purification of the ethyl acetate extract from large scale extraction of the root of *S. verticalis* led to the isolation of compound **355** (compound **C**) and its two derivative, compounds **356** (compound **D**) compound **E**. Based on spectroscopic data the structure of **355** was elucidated as 3,3'-(4,4'-(Propane-2,2-diyl)bis(4,1-phenylene))bis(oxy)bis(1-methoxypropan-2-ol) and the structure of **356** was proposed. Compound **355** is a commercial plastic substance commonly used in many types of plastic packaging. The TLC chromatogram of **355** compared with the methanol, ethyl acetate and ethanol extracts from the root of *S. verticalis* suggested that **355** is a plasticizer contaminated in the ethyl acetate extract. Compound **D** showed ^{1}H and ^{13}C NMR spectroscopic data similar to **355** while compound **E** showed ^{1}H NMR spectroscopic data similar to **355**. These two compounds are derivatives of **355** and should also be plasticizers contaminated in the ethyl acetate extract.

REFERENCES

REFERENCES

[1] Samson RA, Evans HC, Latge JP. Atlas of entomopathogenic fungi. Heidelberg, Germany: Springer-Verlag, Berlin; 1988.

[2] Hajek AE, Leger RJS. Interactions between fungal pathogens and insect hosts. Annu Rev Entomol. 1994; 39(1): 293-322.

[3] Griesch J, Vilcinskas A. Proteases released by entomopathogenic fungi impair phagocytic activity, attachment and spreading of plasmatocytes isolated from haemolymph of the greater wax moth *Galleria mellonella*. Biocontrol Sci Technol. 1998; 8(4): 517-531.

[4] Vey A, Matha V, Dumas C. Effects of the peptide mycotoxin destruxin E on insect haemocytes and on dynamics and efficiency of the multicellular immune reaction. J Invertebr Pathol. 2002; 80(3): 177-187.

[5] Mongkolsamrit S, Luangsa-ard JJ, Tasanatai K, Sivichai S. Invertebrate-Pathogenic Fungi of Thailand. Pathum Thaina, Thailand: National Center for Genetic Engineering and Biotechnology (BIOTECH); 2010.

[6] Russell R, Paterson M. *Cordyceps* - A traditional Chinese medicine and another fungal therapeutic biofactory? Phytochemistry. 2008; 69(7): 1469-1495.

[7] Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, Baliraine PJ, FN, Alessandro UD. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malar J. 2011; 10(144): 1-12.

[8] Jayakumar K, Murugan K. *Solanum* alkaloids and their pharmaceutical roles: a review. J Anal Pharm Res. 2016; 3(6): 1-14.

[9] Editorial Committee of the flora of the Chinese Aacdemy of Sciences, Flora reipublicae popularis sinicae. Beijing House of Science Press, 1978.

[10] Chinese Pharmacopoeia Commission. Chinese Pharmacopoeiatle. In: China Medical Science Press. Beijing; 2015.

[11] Compilation Group of Chinese Hebral Medicine. The Assembly of Chinese Herbal Medicine. In: People's Medical Publishing House. Beijing; 1975.

[12] Isaka M, Boonkhao B, Rachtawee P, Auncharoen P. A xanthocillin-like alkaloid from the insect pathogenic fungus *Cordyceps brunnearubra* BCC 1395. J Nat Prod. 2007; 70(4): 656-658.

[13] Wang J, Zhang DM, Jia JF, Peng QL, Tian HY, Wang L, Ye WC. Cyclodepsipeptides from the ascocarps and insect-body portions of fungus *Cordyceps cicadae*. Fitoterapia. 2014; 97: 23-27

[14] Wang JH, Zhang ZL, Wang YQ, Yang M, Wang CH, Li XW, Guo TW. Chemical Constituents from Mycelia and Spores of Fungus *Cordyceps cicadae*. Chinese Herb Med. 2017; 9(2): 188-192.

[15] Krasnoff SB, Reátegui RF, Wagenaar MM, Gloer JB, Gibson DM. Cicadapeptins I and II: new aib-containing peptides from the entomopathogenic fungus *Cordyceps heteropoda*. J Nat Prod. 2005; 68(1): 50-55.

[16] Rukachaisirikul V, Pramjit S, Pakawatchai C, Isaka M, Supothina S. 10-Membered macrolides from the insect pathogenic fungus *Cordyceps militaris* BCC 2816. J Nat Prod. 2004; 67(11): 1953-1955.

[17] Rao YK, Fang SH, Wu WS, Tzeng YM. Constituents isolated from *Cordyceps militaris* suppress enhanced inflammatory mediator's production and human cancer cell proliferation. J Ethnopharmacol. 2010; 131(2): 363-367.

[18] Kim SB, Ahn B, Kim M, Ji HJ, Shin SK, Hong IP, Kim CY, Hwang BY, Lee MK. Effect of *Cordyceps militaris* extract and active constituents on metabolic parameters of obesity induced by high-fat diet in C58BL/6J mice. J Ethnopharmacol. 2014; 151(1): 478-484.

[19] Chiu CP, Liu SC, Tang CH, Chan Y, El-Shazly M, Lee CL, Du YC, Wu TY, Chang FR, Wu YC. Anti-inflammatory cerebrosides from cultivated *Cordyceps militaris*. J Agric Food Chem. 2016; 64(7): 1540-1548.

[20] Sun J, Jin M, Zhou W, Diao S, Zhou Y, Li S, Wang X, Pan S, Jin X, Li G. A new ribonucleotide from *Cordyceps militaris*. Nat Prod Res. 2017; 31(21): 2537-2543.

[21] Isaka M, Tanticharoen M, Kongsaeree P, Thebtaranonth Y. Structures of cordypyridones A-D, Antimalarial *N*-hydroxy- and *N*-methoxy-2-pyridones from the insect pathogenic fungus *Cordyceps nipponica*. J Org Chem. 2001; 66(14): 4803-4808.

[22] Cai P, Smith D, Cunningham B, Brown SS, Katz B, Pearce C, Venables D, Houck D. 8-Methyl-pyridoxatin: a novel *N*-hydroxy pyridone from fungus OS-F61800 that induces erythropoietin in human cells. J Nat Prod. 1999; 62(2): 397-399.

[23] McBrien KD, Gao Q, Huang S, Klohr SE, Wang RR, Pirnik DM, Neddermann KM, Bursuker I, Kadow KF, Leet JE. Fusaricide, a new cytotoxic *N*-hydroxypyridone from *Fusarium* sp. J Nat Prod. 1996; 59(12): 1151-1153.

[24] Isaka M, Tanticharoen M, Thebtaranonth Y. Cordyanhydrides A and B. two unique anhydrides from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. Tetrahedron Lett. 2000; 41(10): 1657-1660.

[25] Isaka M, Kongsaeree P, Thebtaranonth Y. Bioxanthracenes from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. II. structure elucidation. J Antibiot (Tokyo). 2001; 54(1): 36-43.

[26] Ando K, Kawamoto I, Yoshida M, Matsuda Y. ES-242-2,-3,-4,-5, -6,-7, and -8, novel bioxantracenes produced by *Verticillium* sp.,which act on the *N*-methy-D-aspartate receptor. J Antibiot. 1992; 45(7): 1047-1054.

[27] Ando K, Yoshida M, Kawamoto I, Matsuda Y. a novel compound from *Verticillium* sp., binds to a site on *N*-methyl-D-aspartate receptor that is coupled to the channel domain. J Antibiot. 1992; 45(1): 88-93.

[28] Wang Y, Yin H, Lv X, Wang Y, Gao H, Wang M. Protection of chronic renal failure by a polysaccharide from *Cordyceps sinensis*. Fitoterapia. 2010; 81(5): 397-402.

[29] Yang ML, Kuo PC, Hwang TL, Wu TS. Anti-inflammatory principles from *Cordyceps sinensis*. J Nat Prod. 2011; 74(9): 1996-2000.

[30] Kittakoop P, Punya J, Kongsaeree P, Lertwerawat Y, Jintasirikul A, Tanticharoen M, Thebtaranonth Y. Bioactive naphthoquinones from *Cordyceps unilateralis*. Phytochemistry. 1999; 52(3): 453-457.

[31] Cross BE, Edinberry MN, Turner WB. Three pyranonaphthazarin pigments from *Gnomonia erythrostoma*. J Chem Soc. 1970; (4): 209-210.

[32] Cross BE, Edinberry MN, Turner WE. Pigments of *Gnomonia erythrostoma*. Part I.The structures of erythrostominone, deoxyerythrostominone, and deoxyerythrostominol. J Chem Soc, Perkin Trans 1. 1972; (3): 380-390.

[33] Cross, BE, Zammitt LJ. Pigments of *Gnomonia erythrostoma*. Part II. Epierythrostominol and epideoxyerythrostominol. J Chem Soc, Perkin Trans 1. 1973; (23): 2975-2576.

[34] Unagul P, Wongsa P, Kittakoop P, Intamas S, Srikitikulchai P, Tanticharoen M. Production of red pigments by the insect pathogenic fungus *Cordyceps unilateralis* BCC 1869. J Ind Microbiol Biotechnol. 2005; 32(4): 135-140.

[35] Seephonkai P, Isaka M, Kittakoop P, Trakulnaleamsai S, Rattanajak R, Tanticharoen M, Thebtaranonth Y. A new tropolone from the insect pathogenic fungus *Cordyceps* sp. BCC 1681. J Antibiot (Tokyo). 2001; 54(9): 751-752.

[36] Rukachaisirikul V, Chantaruk S, Tansakul C, Saithong S, Chaicharernwimonkoon L, Pakawatchai C, Isaka M, Intereya K. A cyclopeptide from the insect pathogenic fungus *Cordyceps* sp. BCC 1788. J Nat Prod. 2006; 69(2): 305-307.

[37] Isaka M, Srisanoh U, Lartpornmatulee N, Boonruangprapa T. ES-242 derivatives and cycloheptapeptides from *Cordyceps* sp. strains BCC 16173 and BCC 16176. J Nat Prod. 2007; 70(10): 1601-1604.

[38] Bunyapaiboonsri T, Yoiprommarat S, Intereya K, Kocharin K. New diphenyl ethers from the insect pathogenic fungus *Cordyceps* sp. BCC 1861. Chem Pharm Bull. 2007; 55(2): 304-307.

[39] Isaka M, Chinthanom P, Rachtawee P, Somyong W, Luangsa-Ard JJ, Hywel-Jones NL. Cordylactam, a new alkaloid from the spider pathogenic fungus *Cordyceps* sp. BCC 12671. Phytochem Lett. 2013; 6(2): 162-164.

[40] Grudniewska A, Hayashi S, Shimizu M, Kato M, Suenaga M, Imagawa H, Ito T, Asakawa Y, Ban S, Kumada T, Hashimoto T, Umeyama A. Opaliferin, a new polyketide from cultures of entomopathogenic fungus *Cordyceps* sp. NBRC 106954. Org Lett. 2014; 16(18): 4695-4697.

[41] Belhouchet Z, Sautour M, Miyamoto T, Lacaille-Dubois MA. Steroidal saponins from the roots of *Smilax aspera* subsp. *mauritanica*. Chem Pharm Bull (Tokyo). 2008; 56(9): 1324-1327.

[42] Ivanova A, Mikhova B, Batsalova T, Dzhambazov B, Kostova I. New furostanol saponins from *Smilax aspera* L. and their *in vitro* cytotoxicity. Fitoterapia. 2011; 82(2): 282-287.

[43] Guo H, Koike K, Li W, Guo D, Nikaido T. Maltol glucosides from the tuber of *Smilax bockii*. Phytochemistry. 2004; 65(4): 481-484.

[44] Xu J, Li X, Zhang P, Li ZL, Wang Y. Antiinflammatory constituents from the roots of *Smilax bockii* warb. Arch Pharm Res. 2005; 28(4): 395-399.

[45] Xu J, Li X, Zhang P, Li N, Meng DL. A new lignan from *Smilax bockii* warb. Pharmazie. 2006; 61(9): 812.

[46] Li X, Xu J, Zhang P, Li N, Meng DL. A new naphthalene glycoside from the roots of *Smilax bockii*. Fitoterapia. 2008; 79(6): 479-480.

[47] Zhang L, Liao CC, Huang HC, Shen YC, Yang LM, Kuo YH. Antioxidant phenylpropanoid glycosides from *Smilax bracteata*. Phytochemistry. 2008; 69(6): 1398-1404.

[48] Wu LS, Wang XJ, Wang H, Yang HW, Jia AQ, Ding Q. Cytotoxic polyphenols against breast tumor cell in *Smilax china* L. J Ethnopharmacol. 2010; 130(3): 460-464.

[49] Liang C, Lim JH, Kim SH, Kim DS. Dioscin: A synergistic tyrosinase inhibitor from the roots of *Smilax china*. Food Chem. 2012; 134(2): 1146-1148.

[50] Zhao BT, Le DD, Nguyen PH, Ali MY, Choi JS, Min BS, Shin HM, Rhee HI, Woo MH. PTP1B, α-glucosidase, and DPP-IV inhibitory effects for chromene derivatives from the leaves of *Smilax china* L. Chem Biol Interact. 2016; 253: 27-37.

[51] Lee HE, Kim JA, Whang WK. Chemical constituents of *Smilax china* l. stems and their inhibitory activities against glycation, aldose reductase, α-glucosidase, and lipase. Molecules. 2017; 451(22): 1-18.

[52] Zhong C, Hu D, Hou LB, Song LY, Zhang YJ, Xie Y, Tian LW. Phenolic compounds from the rhizomes of *Smilax china* L. and their anti-inflammatory activity. Molecules. 2017; 22(4): 8-12.

[53] Wungsintaweekul B, Umehara K, Miyase T, Noguchi H. Estrogenic and anti-estrogenic compounds from the Thai medicinal plant, *Smilax corbularia* (Smilacaceae). Phytochemistry. 2011; 72(6): 495-502.

[54] Ivanova A, Mikhova B, Kostova I, Evstatieva L. Bioactive chemical constituents from *Smilax excelsa*. Chem Nat Compd. 2010; 46(2): 295-297.

[55] Khaligh P, Salehi P, Farimani MM, Ali AS, Esmaeili MA, Nejad ES. Bioactive compounds from *Smilax excelsa* L. J Iran Chem Soc. 2016; 13(6): 1055-1059.

[56] Petrica EEA, Sinhorin AP, Sinhorin VDG, Júnior GMV. First phytochemical studies of japecanga (*Smilax fluminensis*) leaves: flavonoids analysis. Rev Bras Farm. 2014; 24: 443-445.

[57] Datllatabad CD, Bhat GG, Jamkhand AM. A keto fatty acid from *Smilax macrophylla* seed oil. 1996; 42(3): 889-890.

[58] Wang WX, Li TX, Ma H, Zhang JF, Jia AQ. Tumoral cytotoxic and antioxidative phenylpropanoid glycosides in *Smilax riparia* A. DC. J Ethnopharmacol. 2013; 149(2): 527-532.

[59] Zhang CL, Gao JM, Zhu W. Steroidal saponins from the rhizomes and roots of *Smilax scobinicaulis*. Phytochem Lett. 2012; 5(1): 49-52.

[60] Xu J, Feng S, Wang Q, Cao Y, Sun M, Zhang C. Four new furostanol saponins from the rhizomes and roots of *Smilax scobinicaulis* and their cytotoxicity. Molecules. 2014; 19(12): 20975-20987.

[61] Zhang CL, Feng SX, Wang Q, Wang P, Xu J, Chen T. Flavonoids and phenolic compounds from *Smilax scobinicaulis*. Chem Nat Compd. 2014; 50(2): 254-255.

[62] Shu J, Zhu G, Huang G, Huang H, Liang Y, Liu X, Yu J, Zhou M, Li L, Deng J. New steroidal saponins with l-arabinose moiety from the rhizomes of *Smilax scobinicaulis*. Phytochem Lett. 2017; 21: 194-199.

[63] Ao C, Higa T, Khanh TD, Upadhyay A, Tawata S. Antioxidant phenolic compounds from *Smilax sebeana* Miq. LWT-Food Sci Technol. 2011; 44(7): 1681-1686.

[64] Shu J, Liang F, Liang J, Liang Y, Li F, Shao F, Liu R, Huang H. Phenylpropanoids and neolignans from *Smilax trinervula*. Fitoterapia. 2015; 104: 64-68.

[65] Liang F, He JW, Zhu GH, Liu RH, Shu JC, Shao F, Huang HL. New steroidal saponins with cytotoxic activities from *Smilax trinervula*. Phytochem Lett. 2016; 16: 294-298.

[66] Shu J, Liang F, Zhu G, Liu X, Yu J, Huang H. Lignan glycosides from the rhizomes of *Smilax trinervula* and their biological activities. Phytochem Lett. 2017; 20: 1-8.

[67] Huang L, Li QZ, Chen YY, Wang XF, Zhou XW. Determination and analysis of cordycepin and adenosine in the products of *Cordyceps* spp. African J Microbiol Res. 2009; 3(12): 957-961.

[68] Kumar S, Reddy NPE, Reddy KH, Devi MC. Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in Agri Export Zone of Andhra Pradesh, India. Plant Pathol Bull. 2007; 16: 157-160.

[69] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anti-cancer drug screening. J Natl Cancer Inst. 1990; 82(13): 1107-1112.

[70] Eghdami A, Fateh V, Asli DE, Houshmandfar A. Antioxidant activity, total phenolic and flavonoids contents in methanolic and aqueous extract of *Achillea millefolium* L. Adv Environ Biol. 2011; 5(5): 929-932.

[71] Sheldrick GM. SHELXT - Integrated space-group and crystal-structure determination. Acta Crystallogr Sect A Found Crystallogr. 2015; 71(1): 3-8.

[72] Sheldrick GM. Crystal structure refinement with SHELXL. Acta Crystallogr Sect C Struct Chem. 2015; 71: 3-8.

[73] Ayer WA, Gökdemir T, Miao S, Trifonov LS. Leptosphaerones A and B, new cyclohexenones from *Leptosphaeria herpotric*hoides. J Nat Prod. 1993; 56, (9): 1647-1650.

[74] Jaihan P, Sangdee K, Sangdee A. Disease suppressive activity of extracts from entomopathogenic fungus *Ophiocordyceps sobolifera* against chili anthracnose fungi *Colletotrichum* spp. in a pot experiment. J Gen Plant Pathol. 2018; 84(3): 237-342.

[75] Lin Z, Zhu T, Fang Y, Gu Q, Zhu W. Polyketides from Penicillium sp. JP-1, an endophytic fungus associated with the mangrove plant Aegiceras corniculatum. Phytochemistry. 2008; 69(5): 1273-1278.

[76] Jose FY, Santiago GB, Francisco JDA, Justo GG. Chemically resistant polyester resins. Patronato de Investigacion Cientifica y Tecnica “Juan de la Cierva.” Patent. 1975; 7.

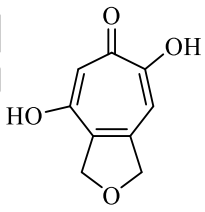
[77] John RN, Garcia FJ, Dorothy SM, Nasrin M, Adriana BC, Wang J. Preparation of bisphenol derivatives as androgen receptor modulators. Patent. 2012; 158

APPENDIX

APPENDIX I

THE FUNGUS *POLYCEPHALOMYCES NIPPONICUS*

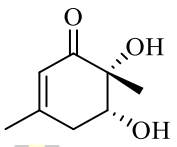
Compound 134



134

Common name	Cordytropolone
Physical appearance	Colorless amorphous powder
Molecular formula	$C_9H_8O_4$
MS m/z	179.0341 $[M-H]^-$
IR (KBr) V_{max}	3387, 1656, 1526, 2846, 1662, 1440, 1121, 891, 617 cm^{-1}

Compound 354



Common name	(-)-Leptrospaerone A
Isolated fungus	*Polycephalomyces nipponicus*
$[\alpha]_D^{25}$	-1.7 (*c* = 0.49, $CHCl_3$)
Physical appearance	a pale brownish oil
Molecular formula	$C_8H_{12}O_3$
MS (ESIMS) *m/z*	179.07 $[M+Na]^+$
IR V_{max}	3381, 2978, 2924, 2846, 1662, 1631, 1435, 1382, 1262, 1162 cm^{-1}

^{1}H NMR (500 MHz, CH_3OH-d_4)

δ 5.88 (1H, s, H-6), 3.91 (1H, dd, *J* = 9.5, 5.5 Hz, H-3), 2.67 (1H, dd, *J* = 18.5, 5.5 Hz, H-4eq.), 2.41 (1H, dd, *J* = 18.5, 5.5 Hz, H-4ax.), 2.00 (3H, s, 5-C*H*$_3$), 1.22 (3H, s, 2-C*H*$_3$)

^{13}C NMR (125 MHz, CH_3OH-d_4)

δ 203.7 (C, C-1), 162.5 (C, C-5), 125.0 (CH, C-6), 78.3 (C, C-2), 73.9 (CH-C-3), 39.4 (CH_2, C-4), 24.4 (CH_3, 5-*C*H_3), 18.1 (CH_3, 2-*C*H_3)

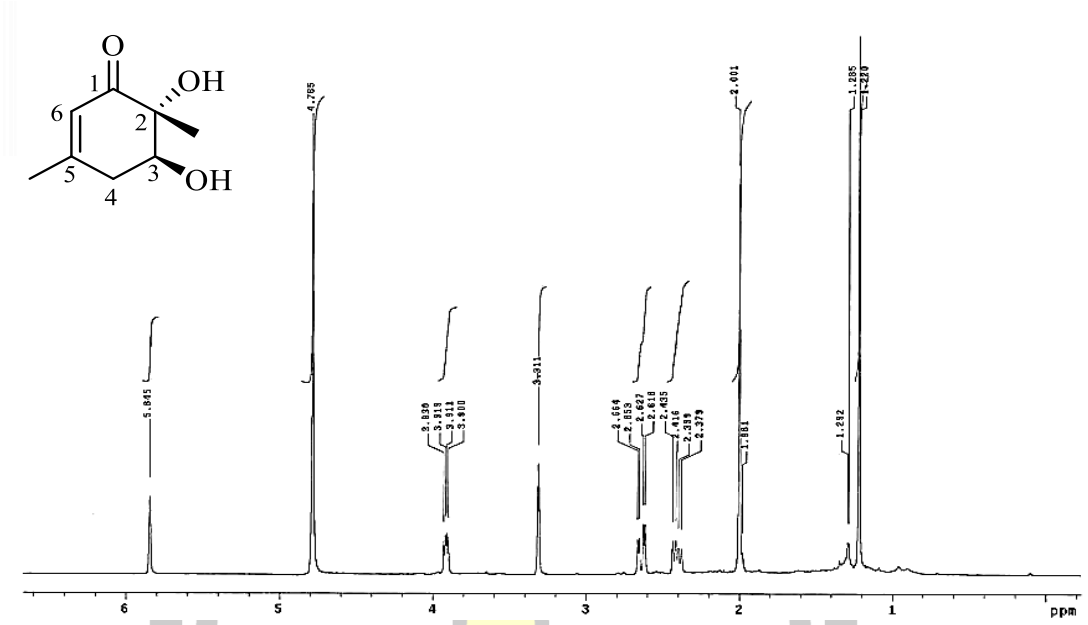


Figure 1A. [1]H-NMR spectrum (500 MHz) in MeOH-d_4 of compound **354**

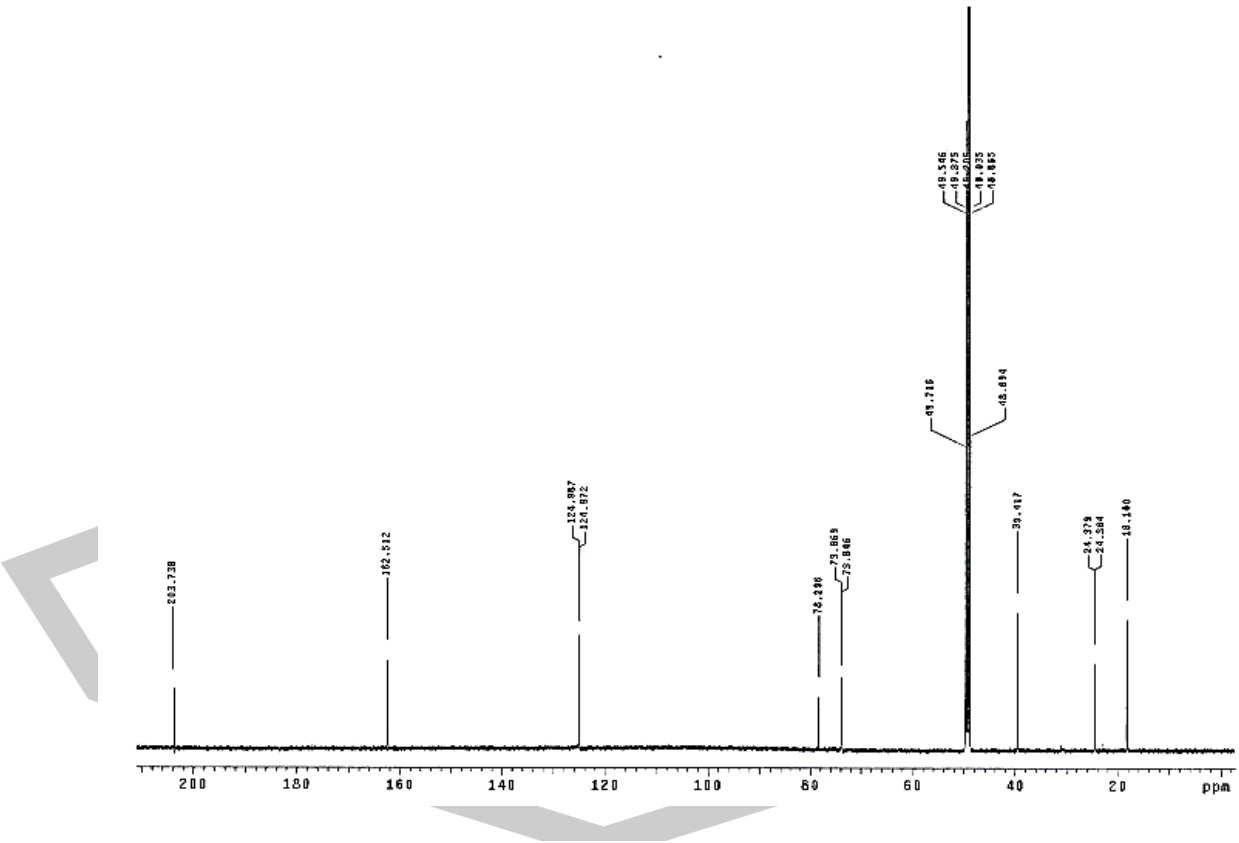


Figure 2A.[13]C-NMR spectrum (125 MHz) in MeOH-d_4 of compound **354**

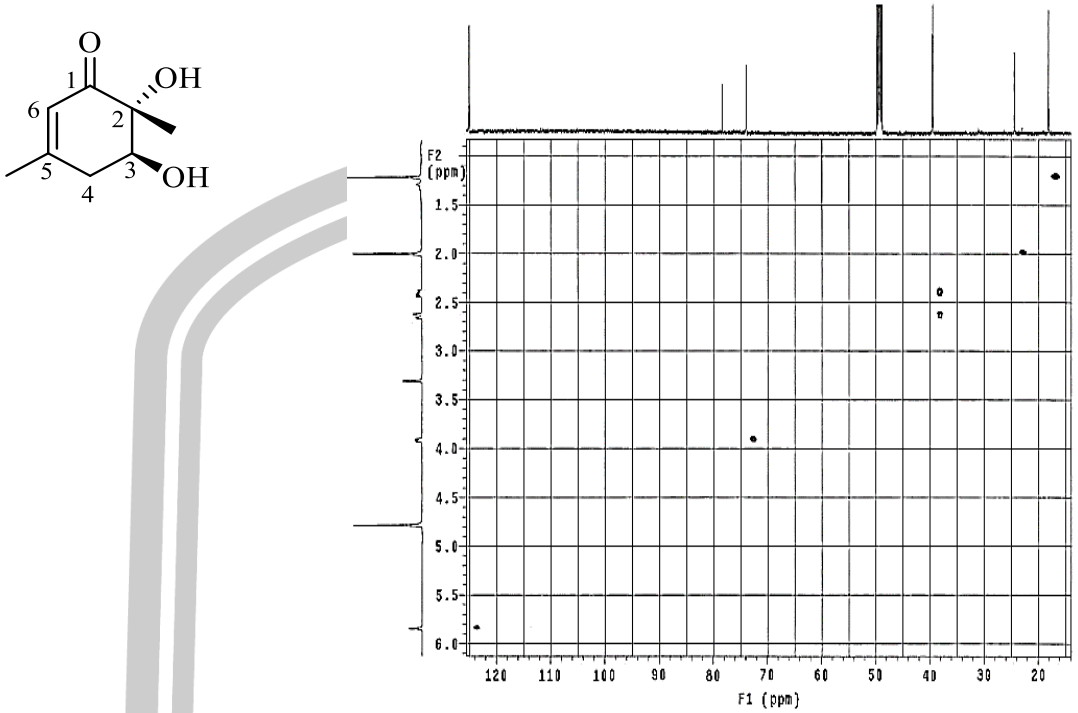


Figure 3A. HSQC spectrum (500 MHz) in MeOH-d_4 of compound **354**

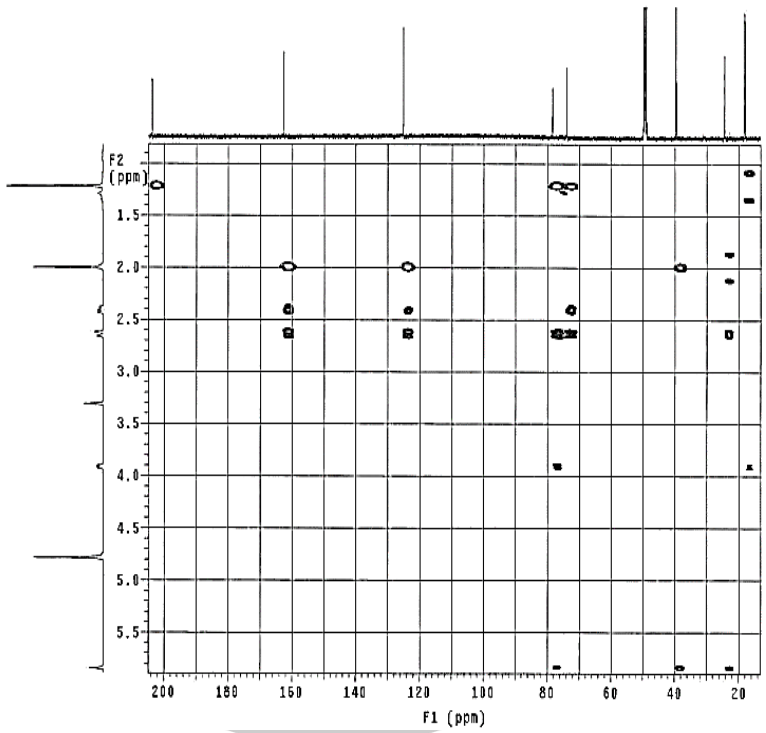


Figure 4A. HMBC spectrum (500 MHz) in MeOH-d_4 of compound **354**

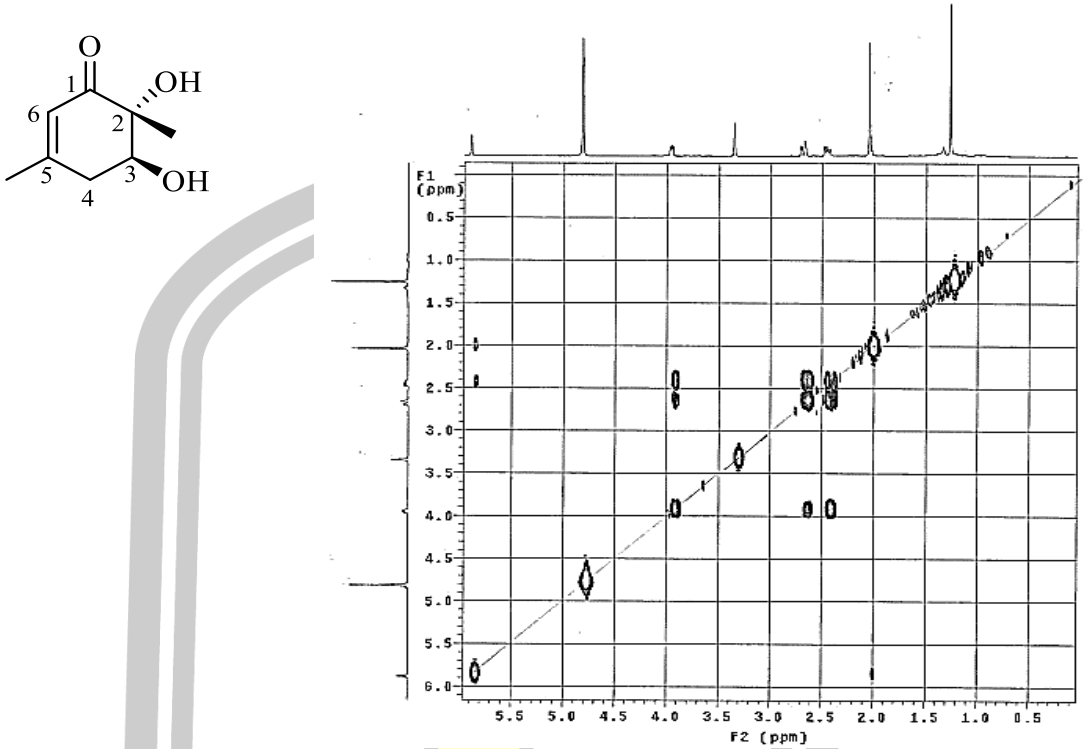


Figure 5A. COSY spectrum (500 MHz) in MeOH-d_4 of compound **354**

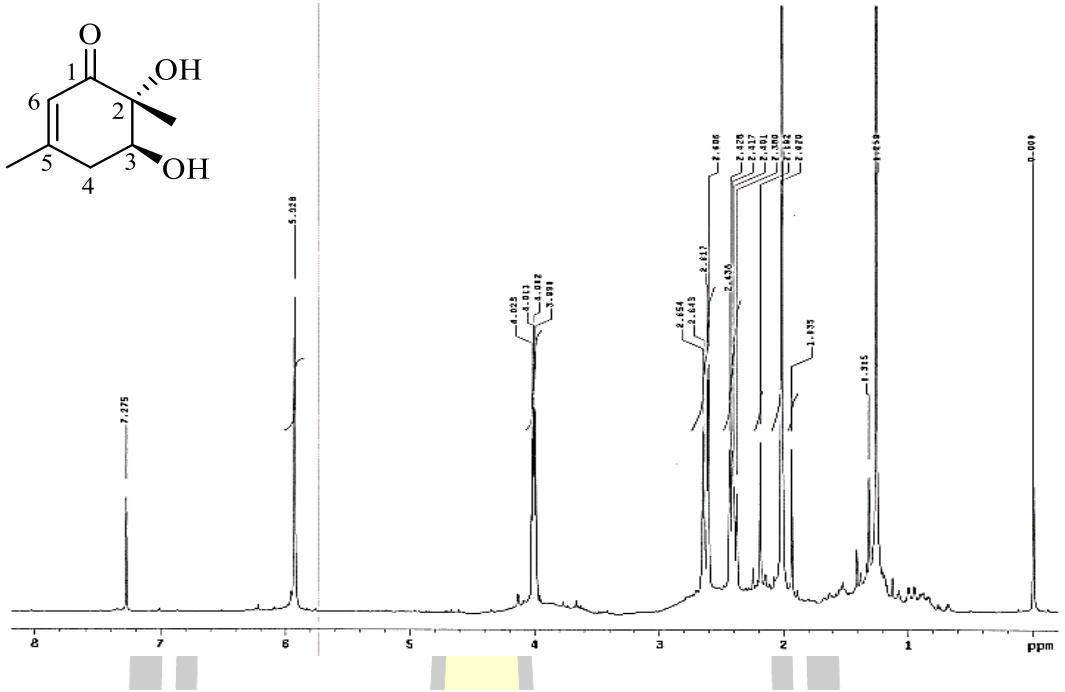


Figure 6A. ^{1}H-NMR spectrum (500 MHz) in $CDCl_3$ of compound **354**

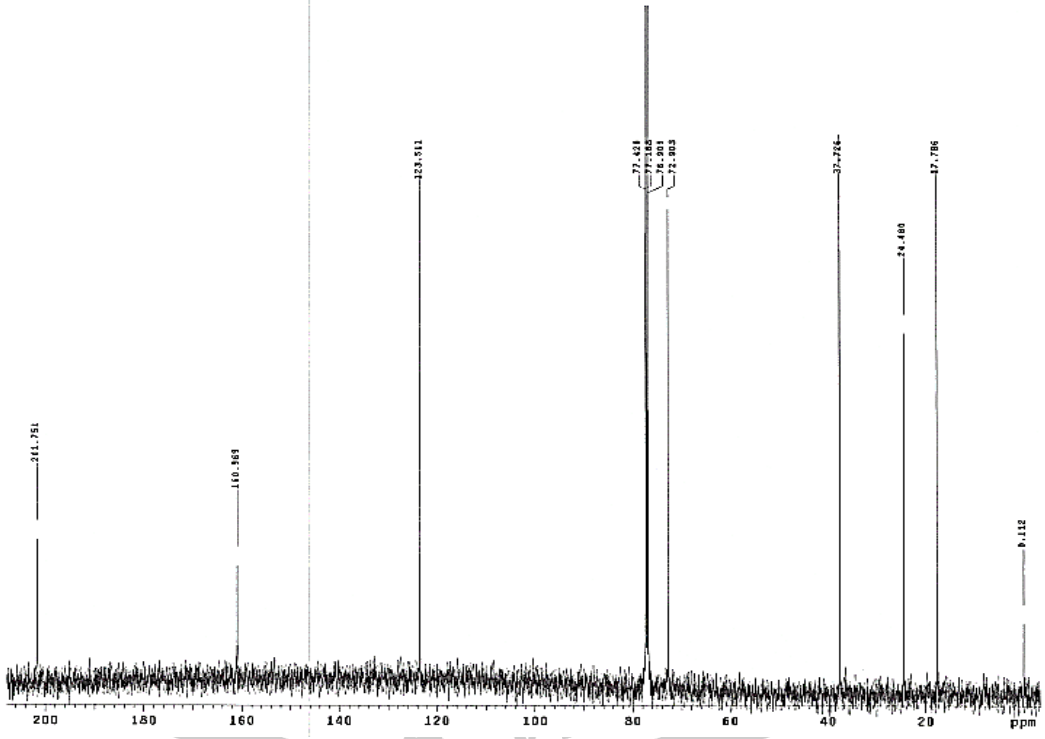


Figure 7A. ^{13}C-NMR spectrum (500 MHz) in $CDCl_3$ of compound **354**

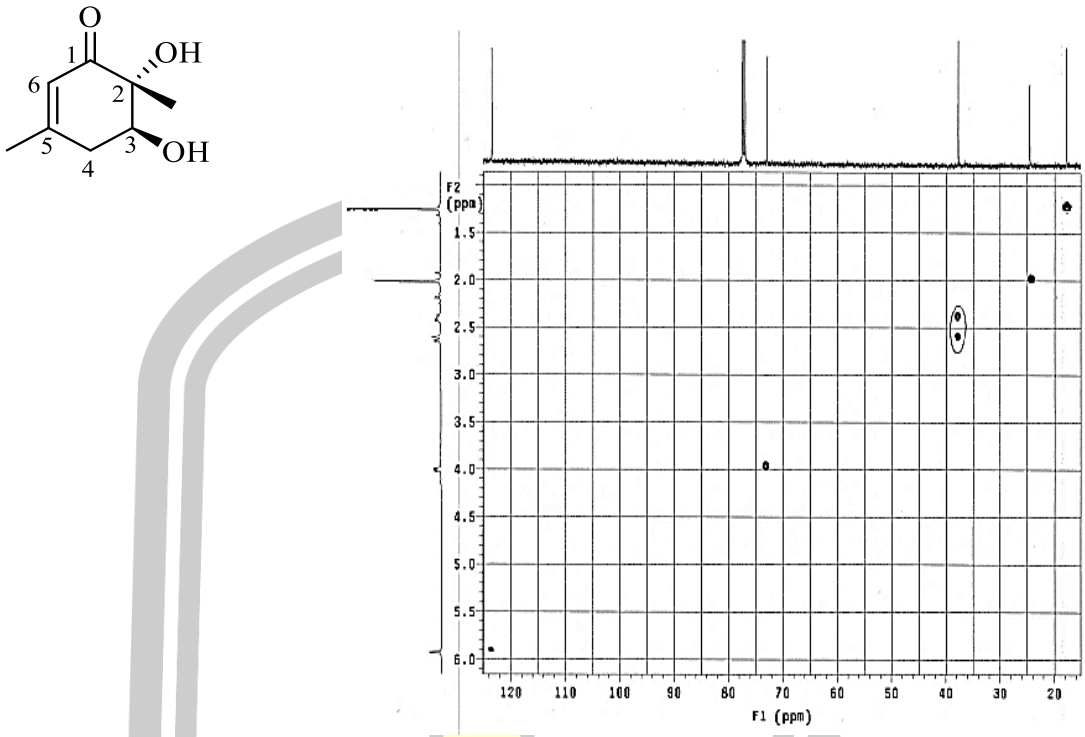


Figure 8A. HSQC spectrum (500 MHz) in $CDCl_3$ of compound **354**

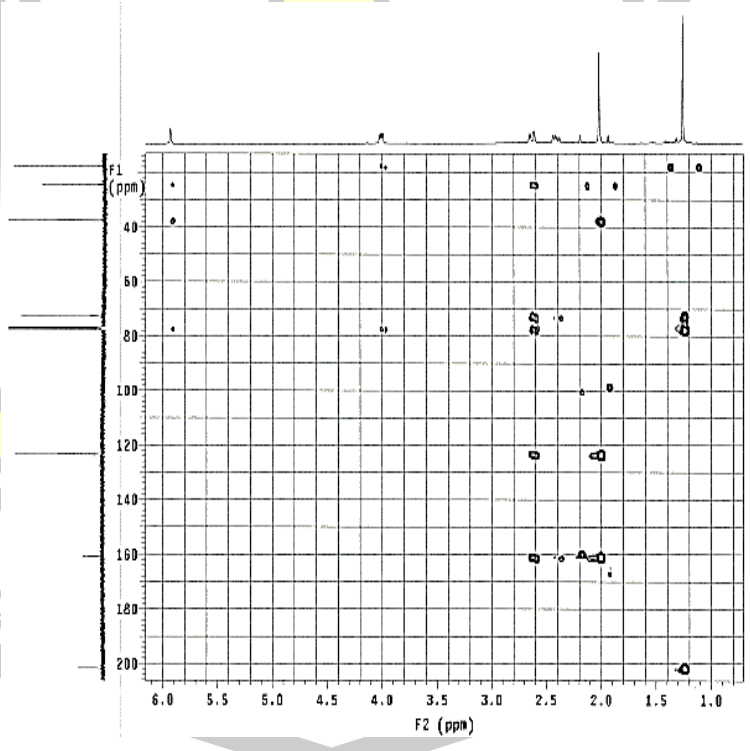


Figure 9A. HMBC spectrum (500 MHz) in $CDCl_3$ of compound **354**

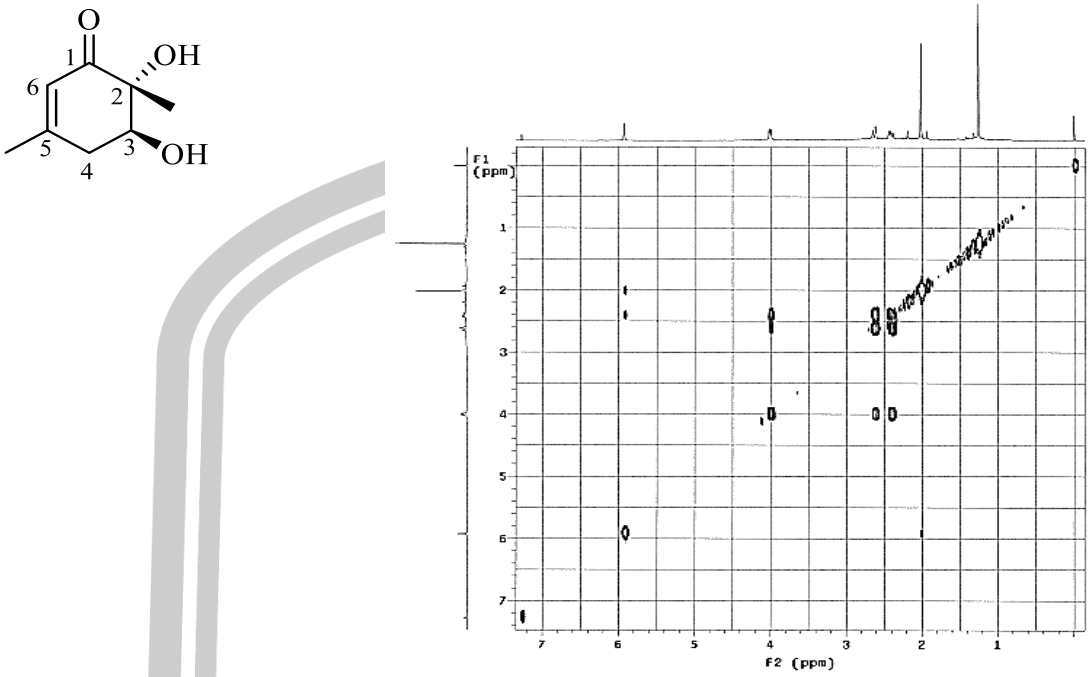


Figure 10A. COSY spectrum (500 MHz) in $CDCl_3$ of compound **354**

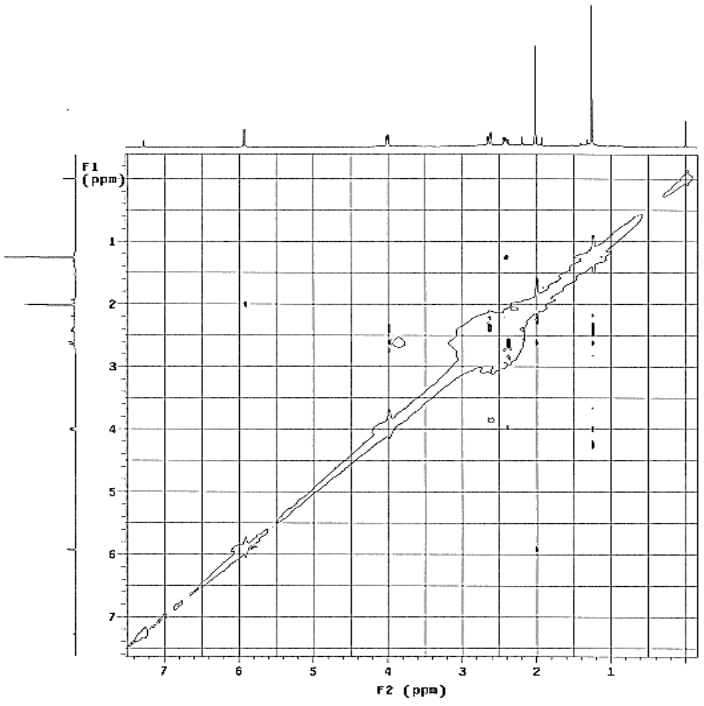


Figure 11A. NOESY spectrum (500 MHz) in $CDCl_3$ of compound **354**

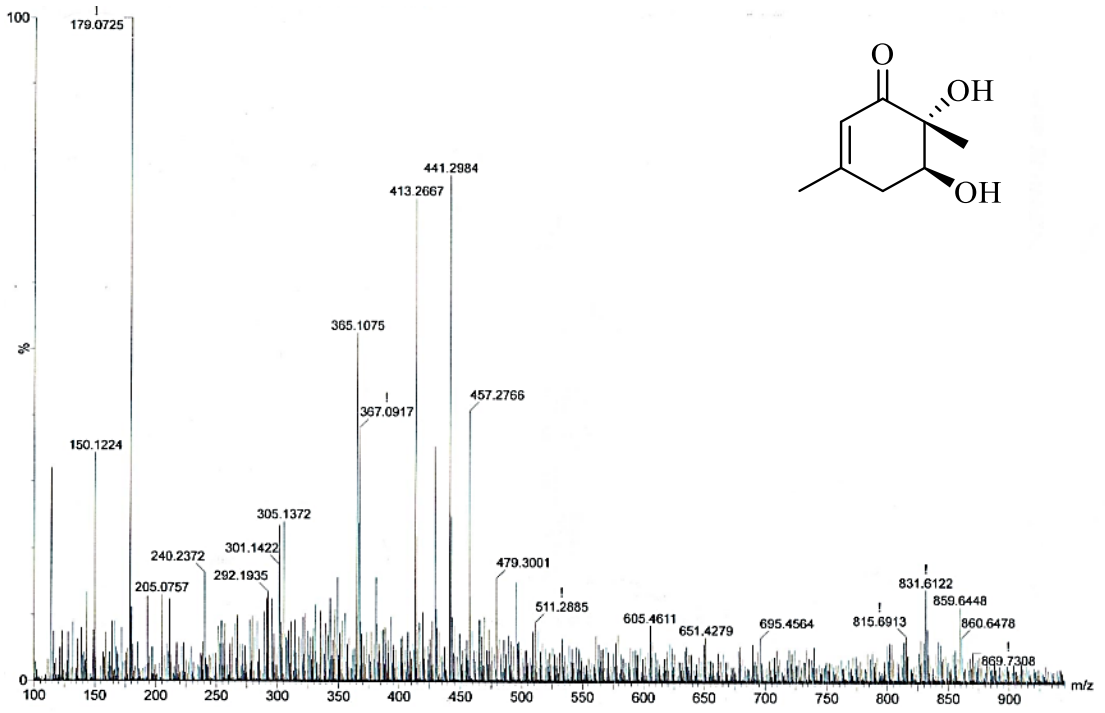


Figure 12A. HRESIMS spectrum of compound **354**

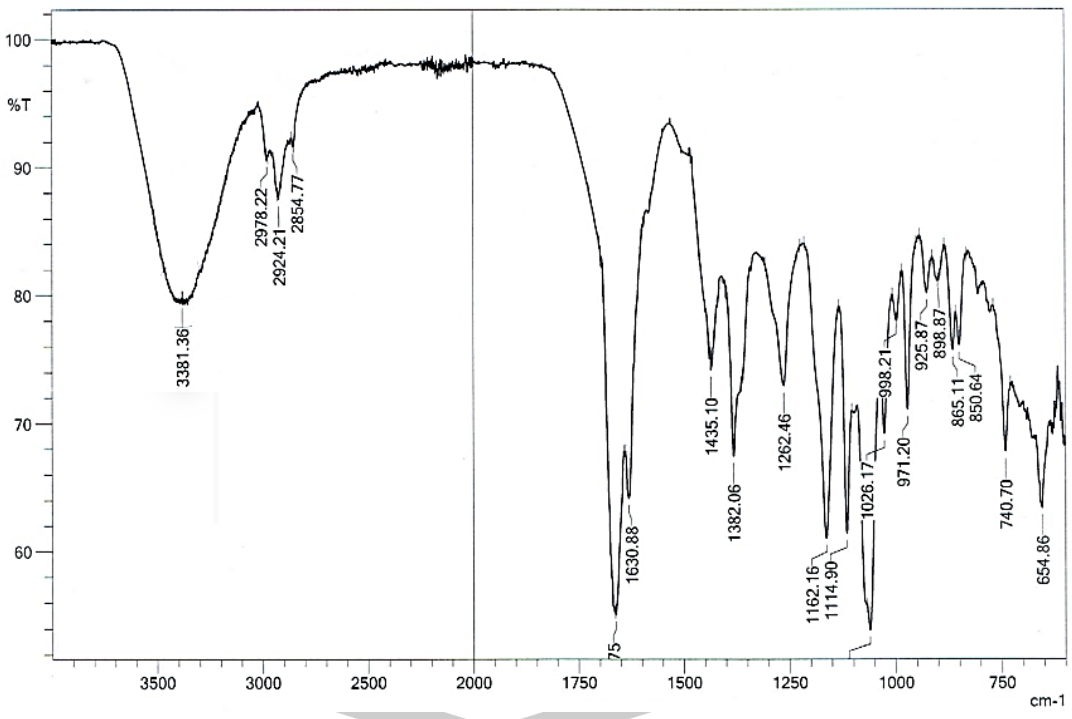


Figure 13A. IR spectrum of compound **354**

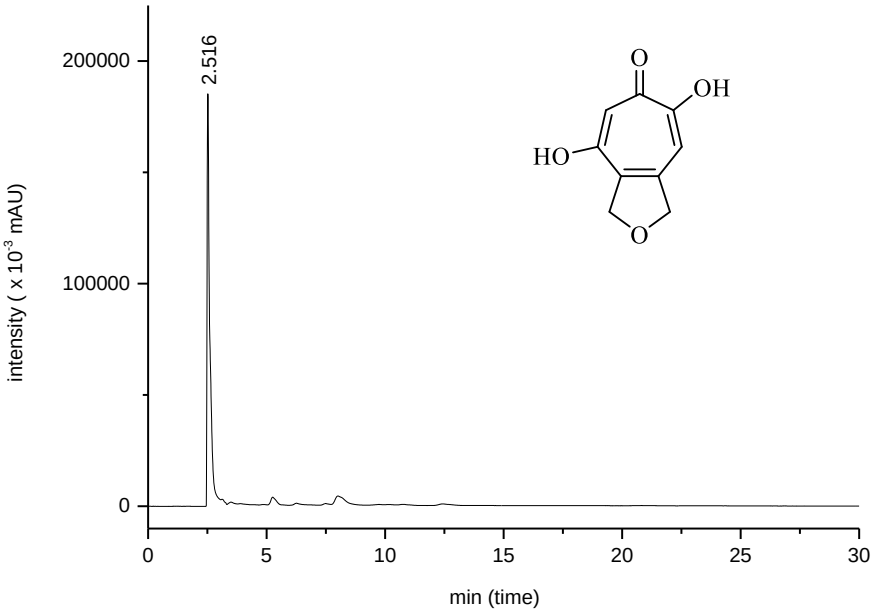


Figure 14A. HPLC chromatogram of compound **134**

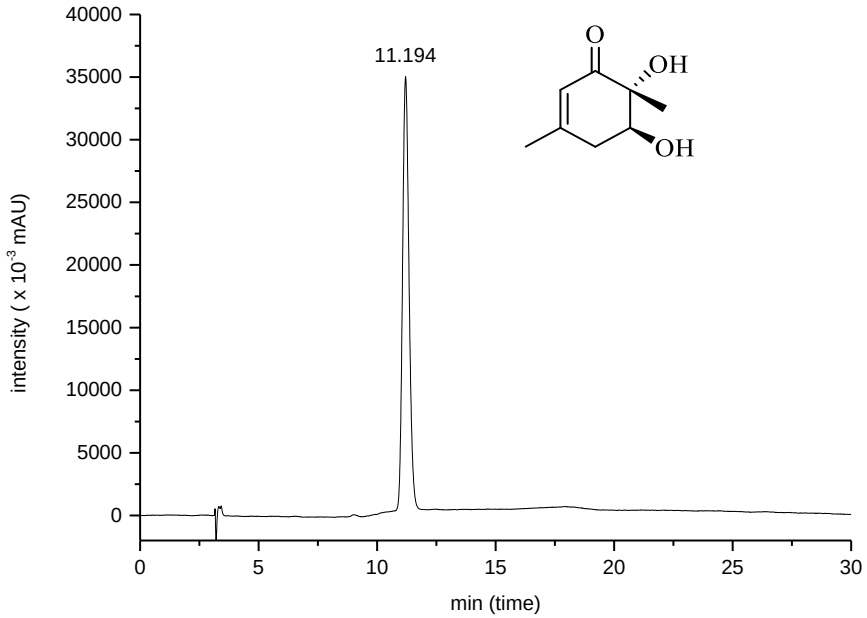


Figure 15A. HPLC chromatogram of compound 354

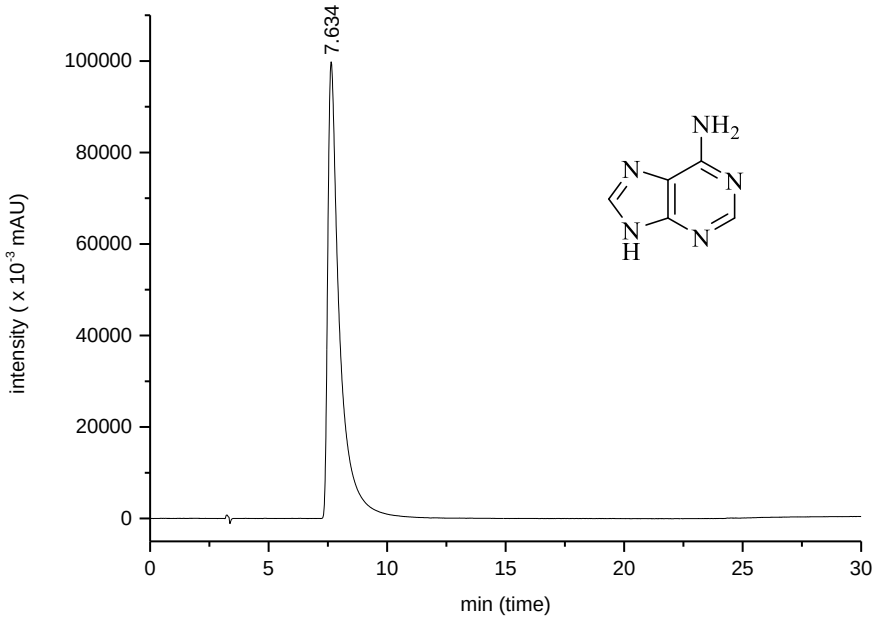


Figure 16A. HPLC chromatogram of adenine

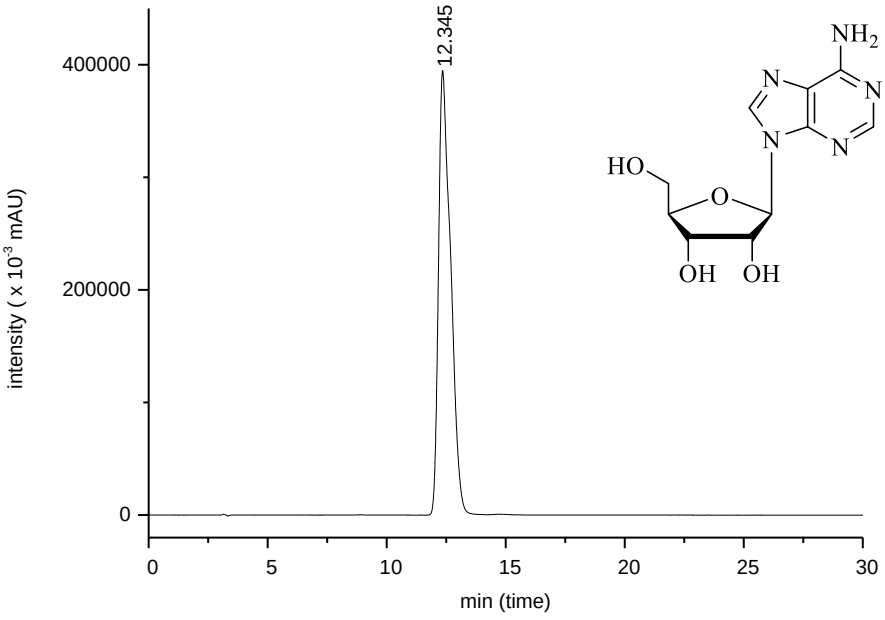


Figure 17A. HPLC chromatogram of adenosine

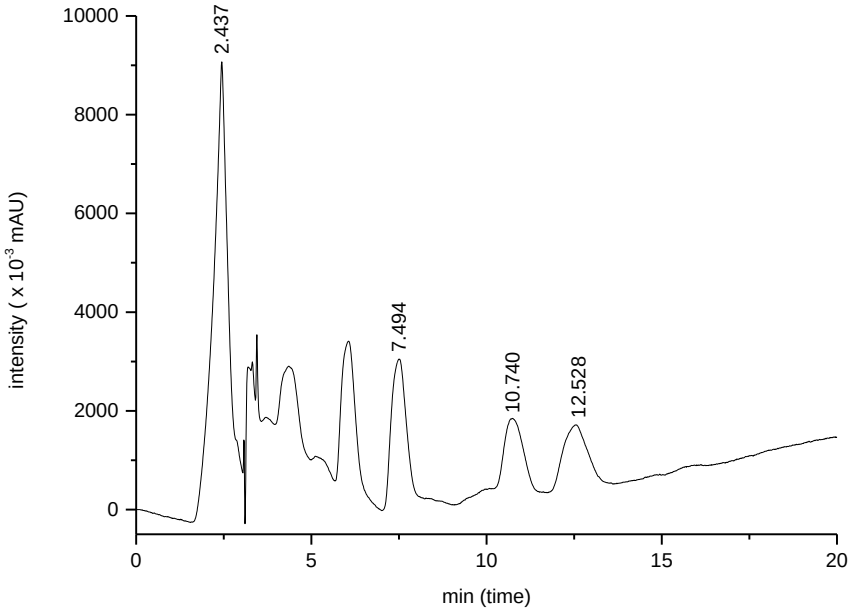


Figure 18A. HPLC chromatogram of culture broth week 1

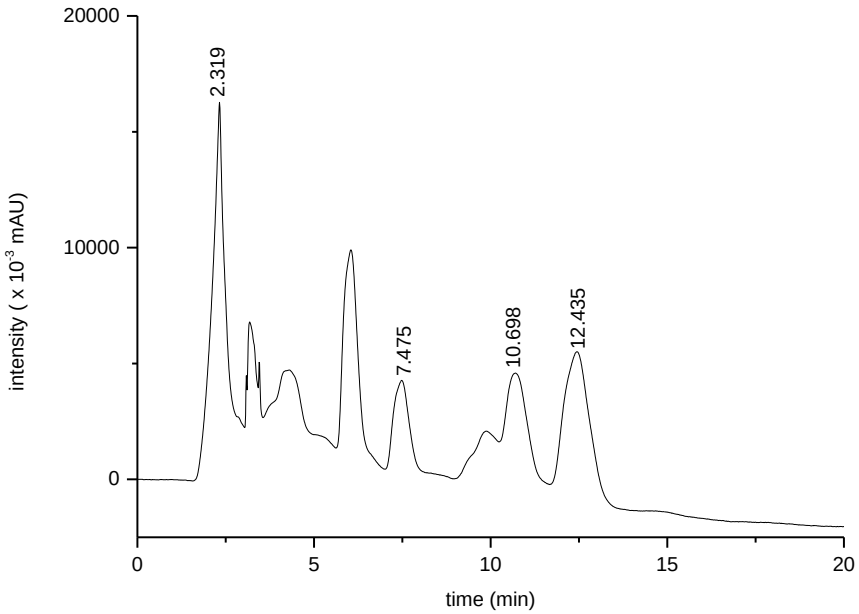


Figure 19A. HPLC chromatogram of culture broth week 2

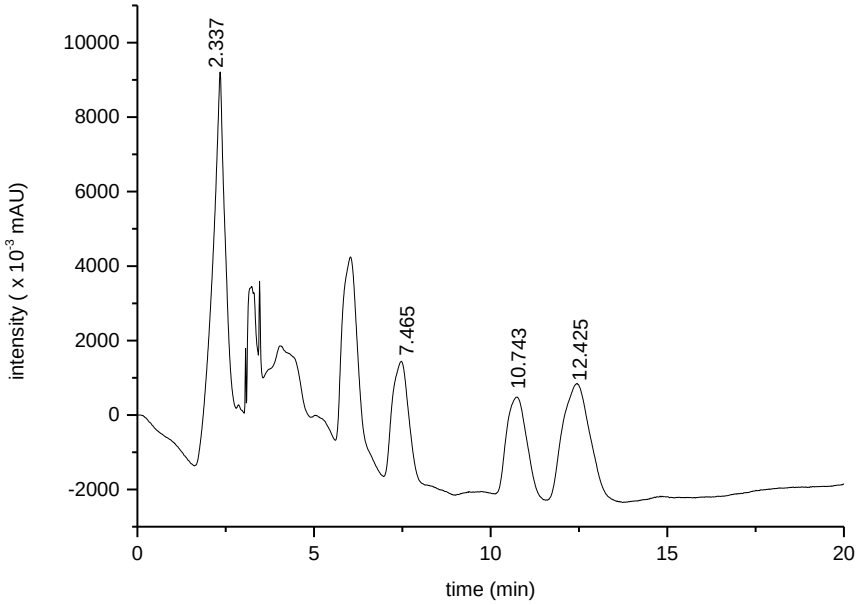


Figure 20A. HPLC chromatogram of culture broth week 3

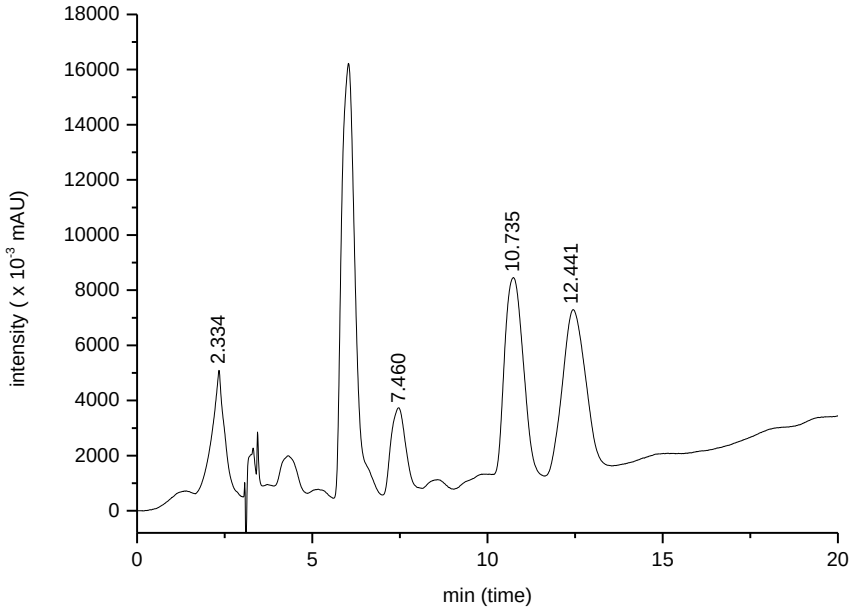


Figure 21A. HPLC chromatogram of culture broth week 4

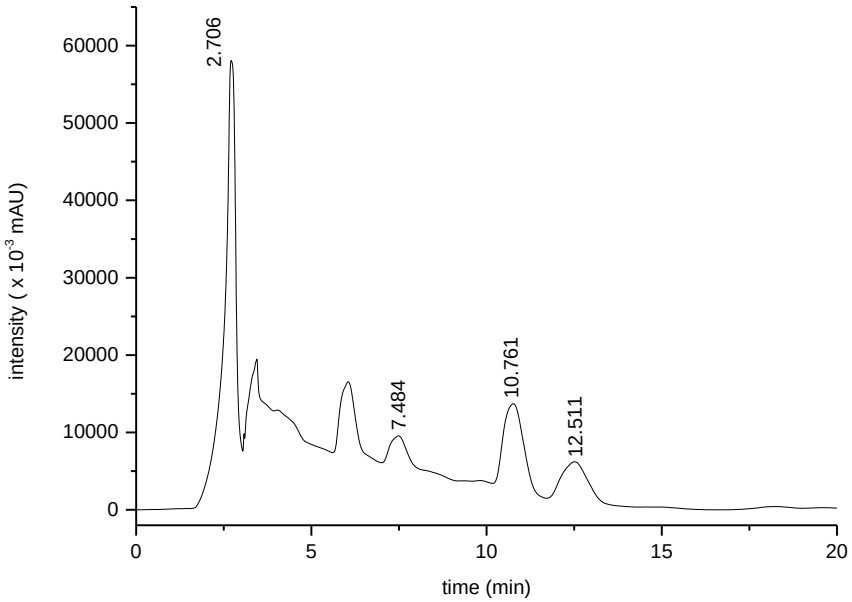


Figure 22A. HPLC chromatogram of culture broth week 5

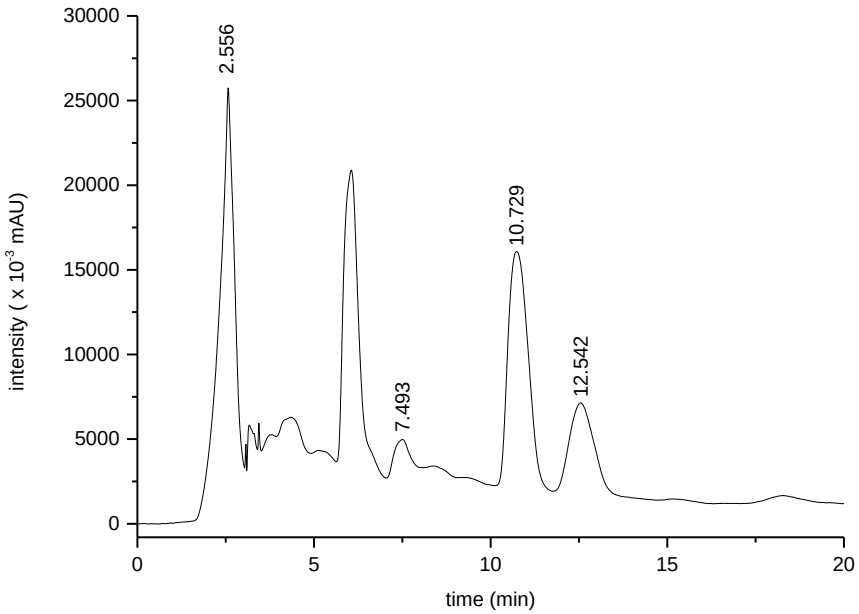


Figure 23A. HPLC chromatogram of culture broth week 6

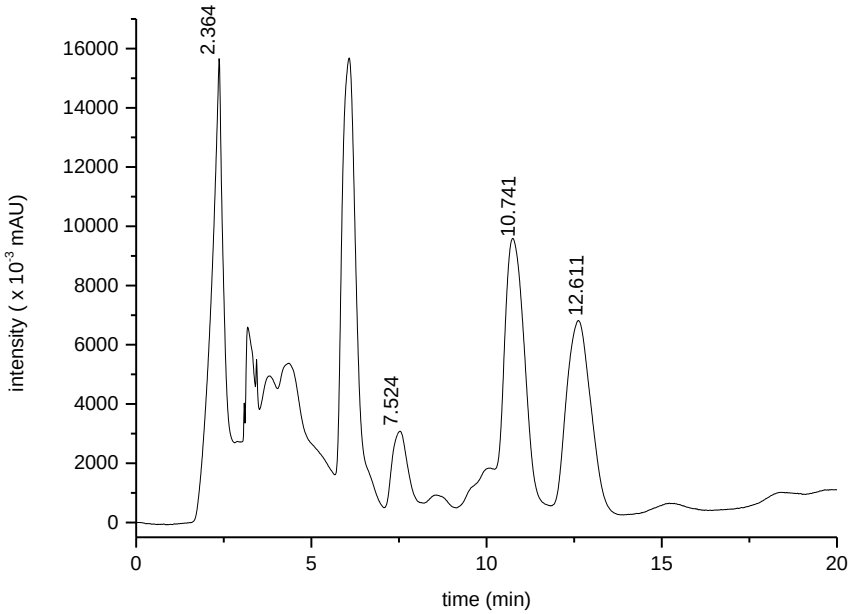


Figure 24A. HPLC chromatogram of culture broth week 7

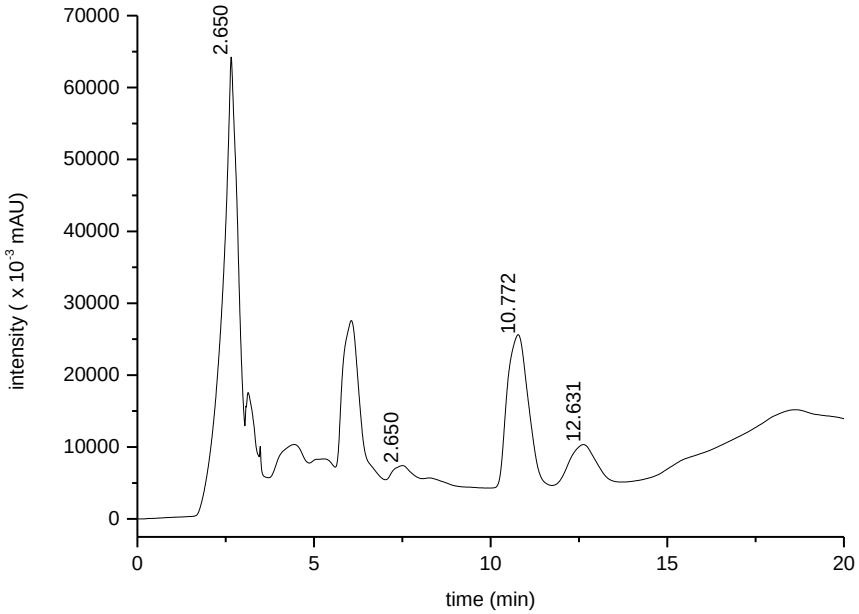


Figure 25A. HPLC chromatogram of culture broth week 8

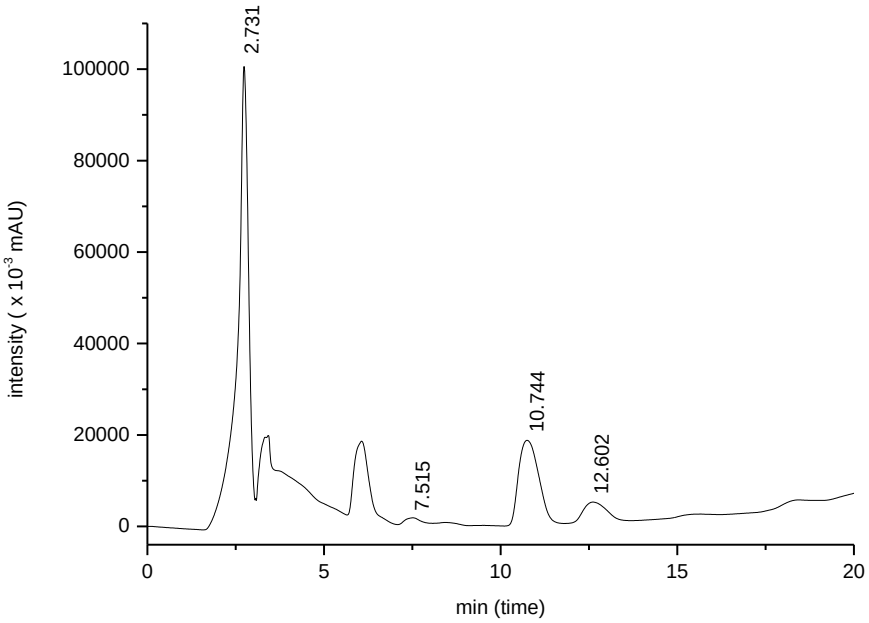


Figure 26A. HPLC chromatogram of culture broth week 9

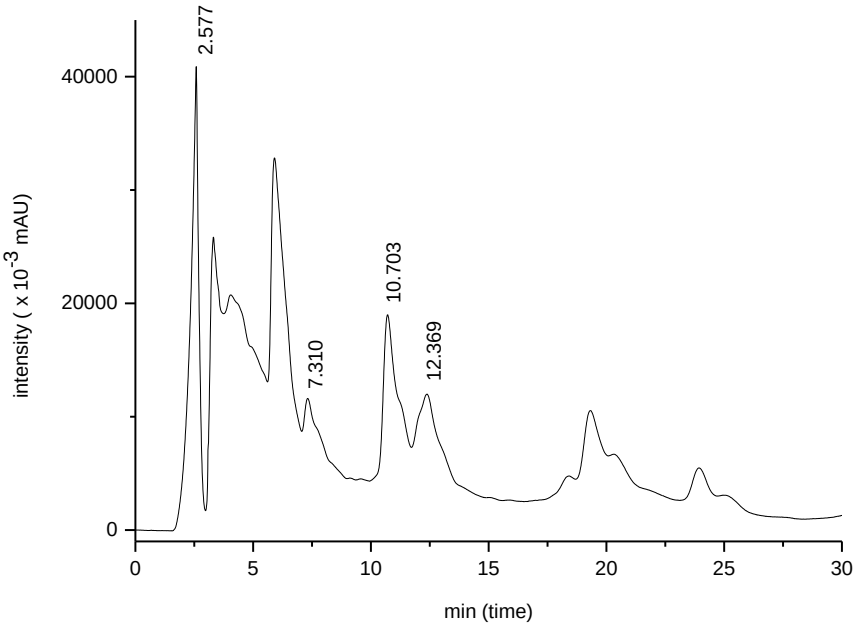


Figure 27A. HPLC chromatogram of culture broth week 10

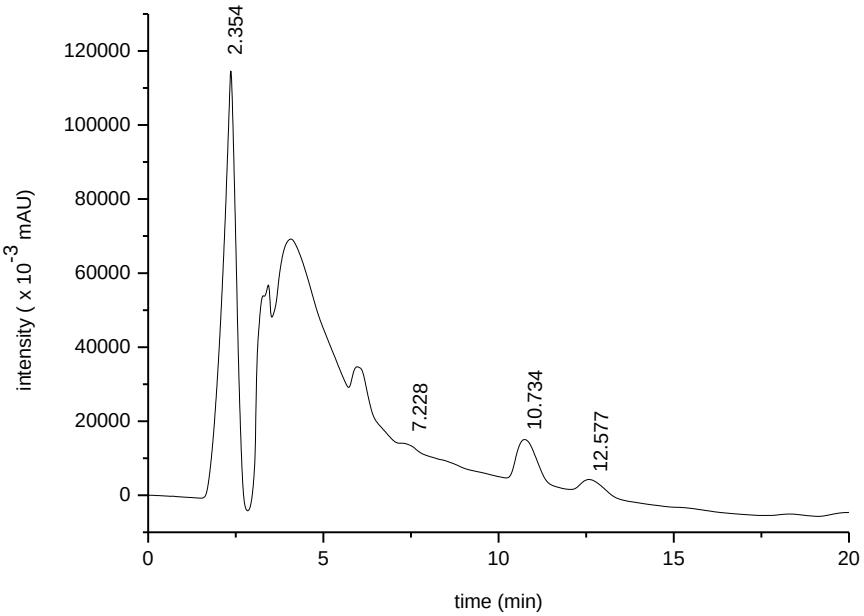


Figure 28A. HPLC chromatogram of culture broth week 11

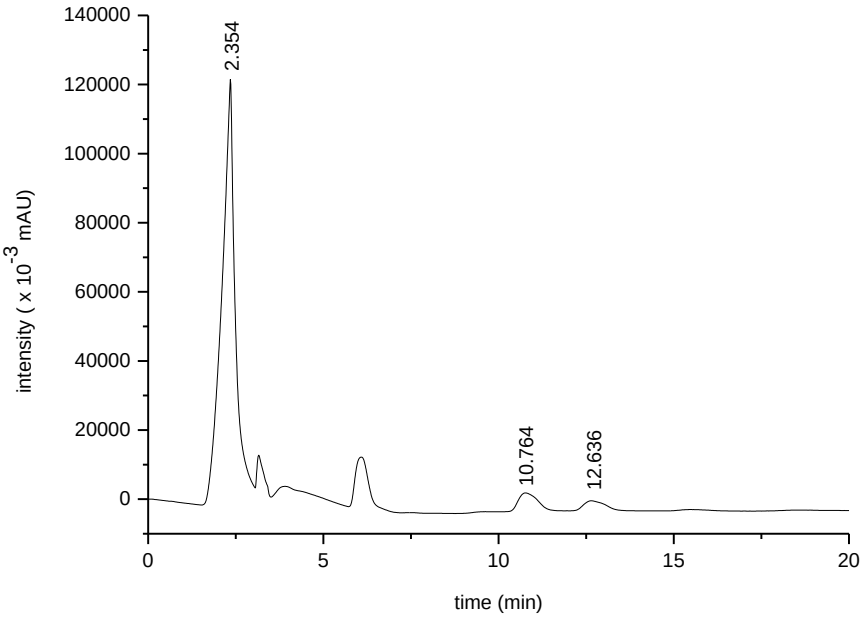
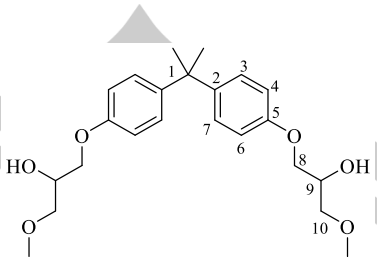


Figure 29A. HPLC chromatogram of culture broth week 12

APPENDIX II

THE *SMILAX VERTICALIS*

Compound 355



Physical appearance a light brownish oil

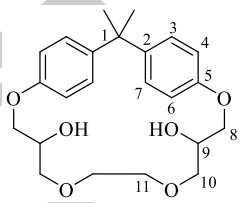
^{1}H NMR (400 MHz, CH_3OH-d_4)

δ 7.11 (2H, d, *J* = 8.8 Hz, H-3, H-7), 6.81 (2H, d, *J* = 8.8 Hz, H-4, H-6), 4.01-4.06 (1H, m, H-9), 3.89-4.00 (2H, m, H-8), 3.47-3.55 (2H, m, H-10), 3.37 (3H, s, H-11), 1.60 (3H, s, 1-CH_3)

^{13}C NMR (100 MHz, CH_3OH-d_4)

δ 156.7 (C, C-5), 143.2 (C, C-2), 127.4 (2C, C-3, C-7), 113.6 (C, C-4, C-6), 73.6 (C, C-10), 69.1 (C, C-8), 68.7 (C, C-9), 58.1 (C, C-11), 41.3 (C, C-1), 30.2 (C, 1-CH_3)

Compound 356



Physical appearance pale brownish solid

^{1}H NMR (400 MHz, CH_3OH-d_4)

δ 7.11 (2H, d, *J* = 8.4 Hz, H-3, H-7), 6.83 (2H, t, *J* = 8.4 Hz, H-4, H-6), 4.01-4.10 (1H, m, H-9), 3.90-4.00 (2H, m, H-8), 3.47-3.56 (2H, m, H-10), 3.37 (2H, s, H-11), 1.60 (3H, s, 1-CH_3)

^{13}C NMR (100 MHz, CH_3OH-d_4)

δ 1567.0 (C, C-5), 143.5 (C, C-2), 127.5 (2C, C-3, C-7), 113.6 (C, C-4, C-6), 73.6 (C, C-10), 69.0 (C, C-8), 68.7 (C, C-9), 57.9 (C, C-11), 41.1 (C, C-1), 30.0 (C, C-1-CH_3).

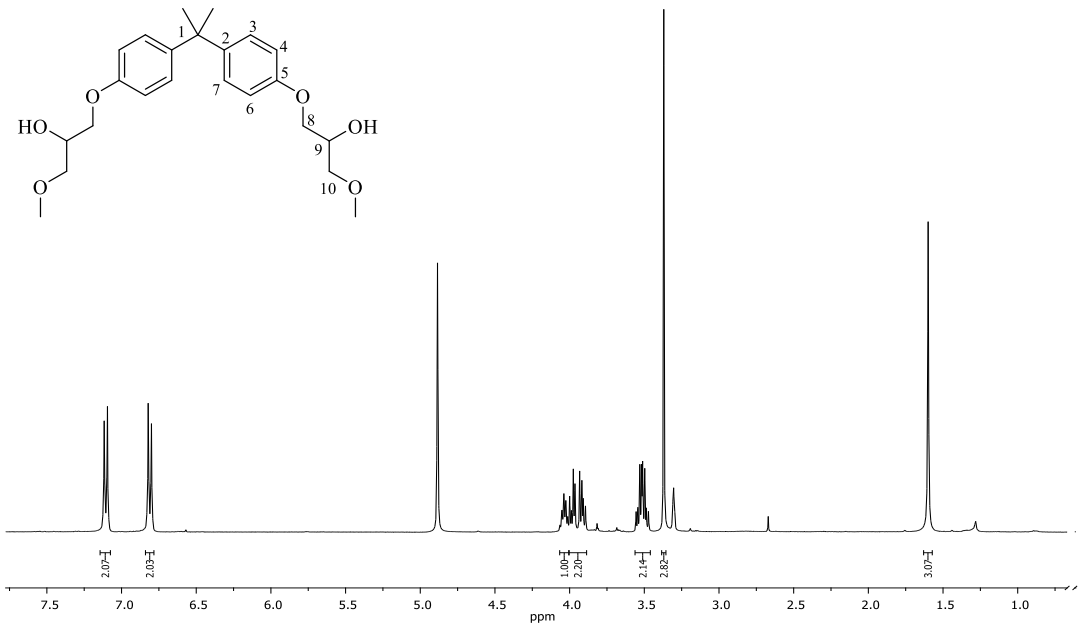


Figure 30A. ^{1}H-NMR spectrum (400 MHz) in MeOH-d_4 of compound **355**

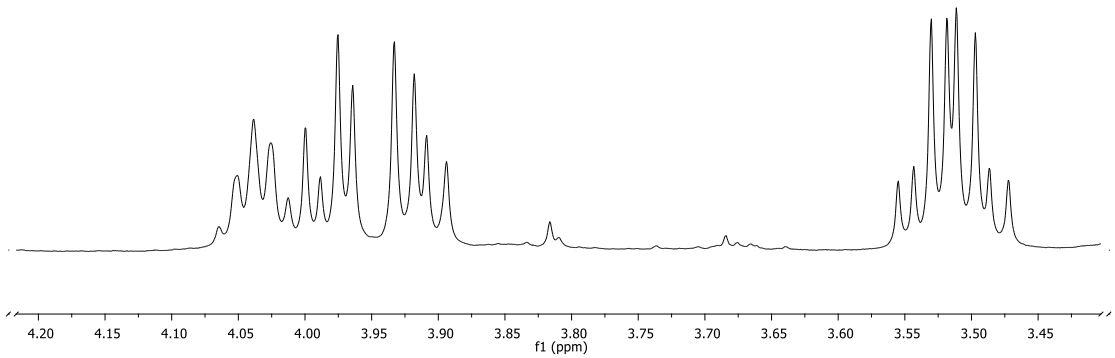


Figure 32A. Expansion of ^{1}H-NMR spectrum (400 MHz) in MeOH-d_4 of compound **355**

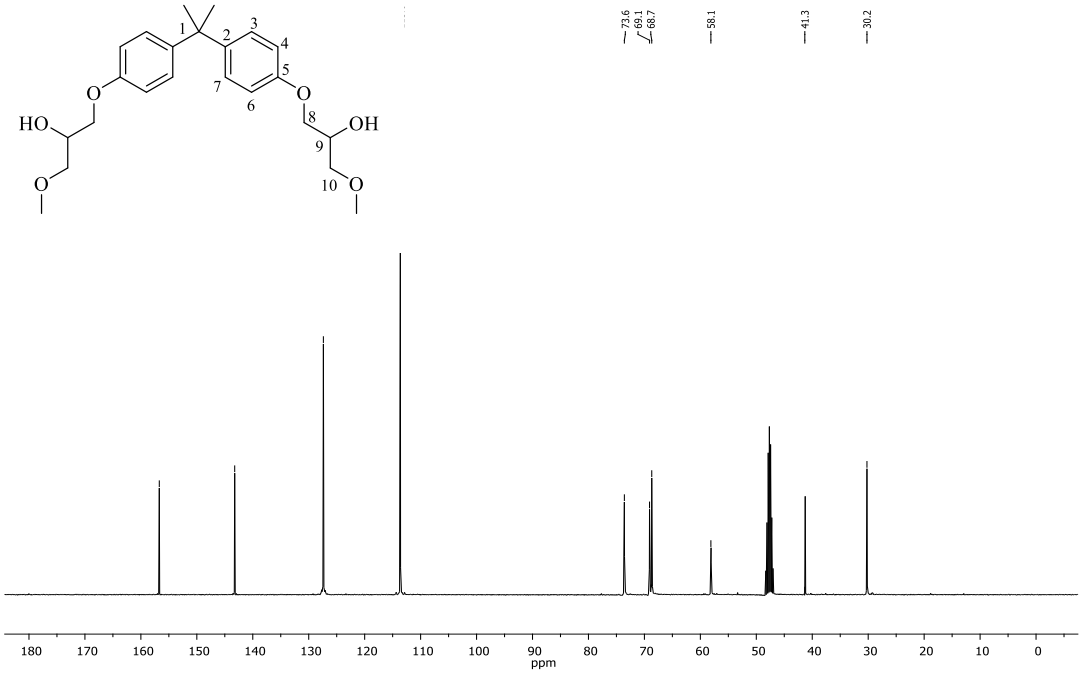


Figure 33A. ^{13}C-NMR spectrum (100 MHz) in $CDCl_3$ of compound **355**

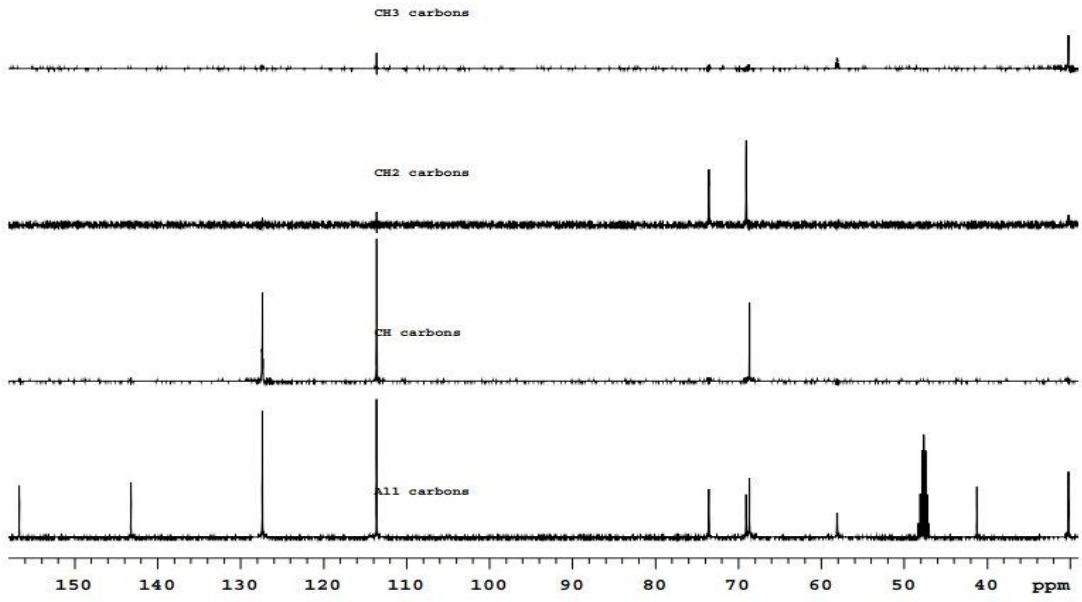


Figure 34A. DEPT spectrum (400 MHz) in MeOH-d_4 of compound **355**

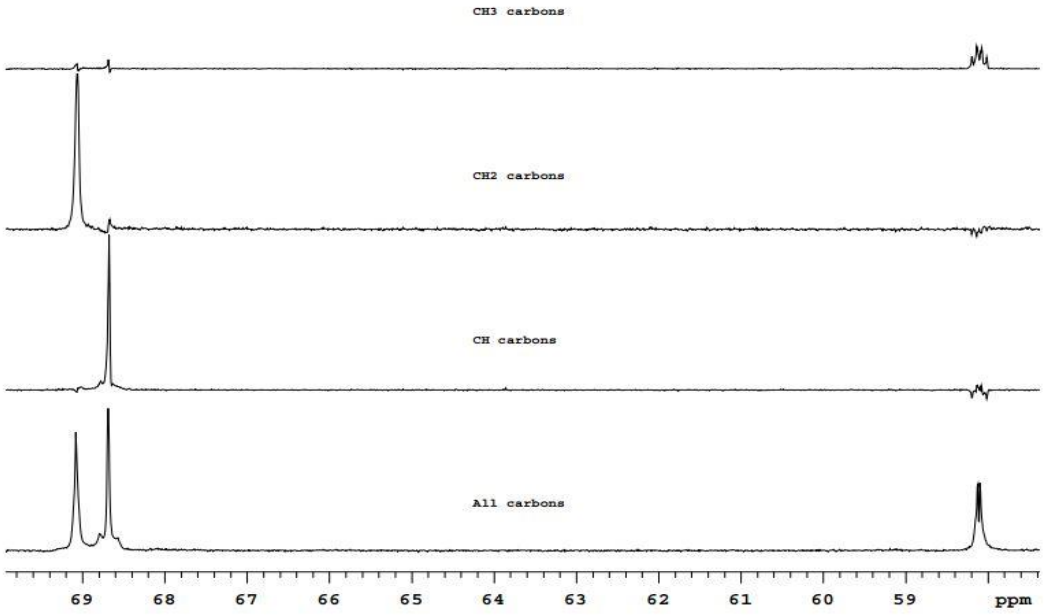


Figure 35A. Expansion of DEPT spectrum (400 MHz) in MeOH-d_4 of compound **355**

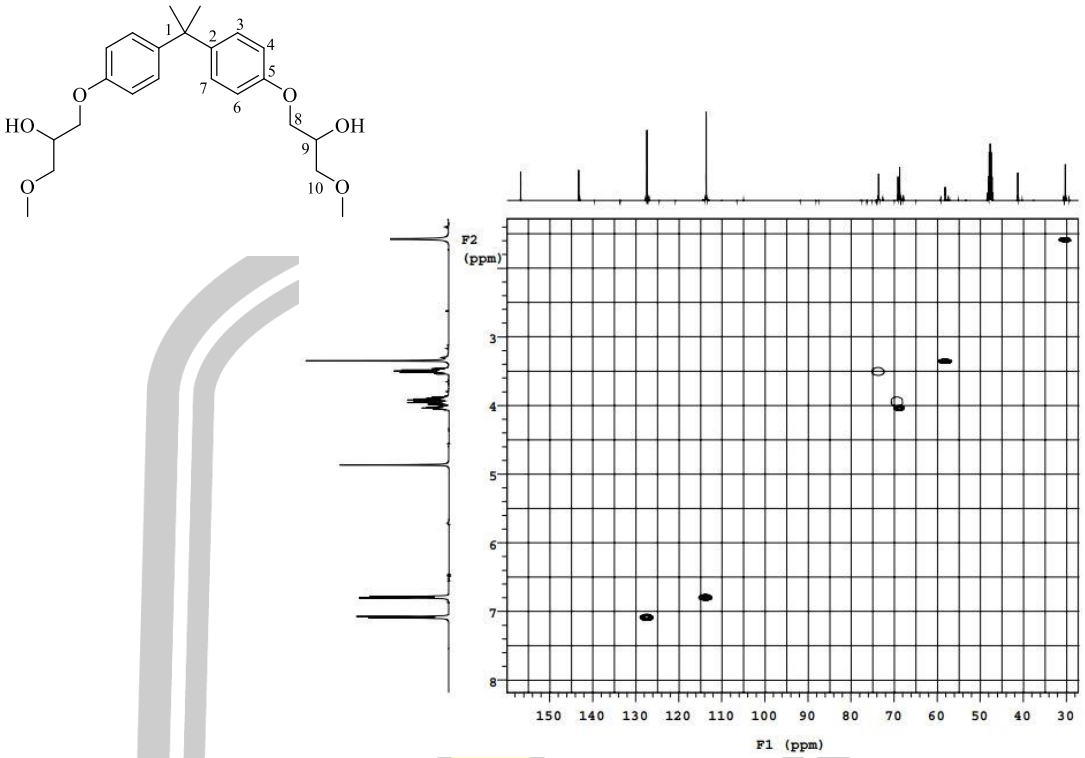


Figure 36A. HSQC spectrum (400 MHz) in MeOH-d_4 of compound **355**

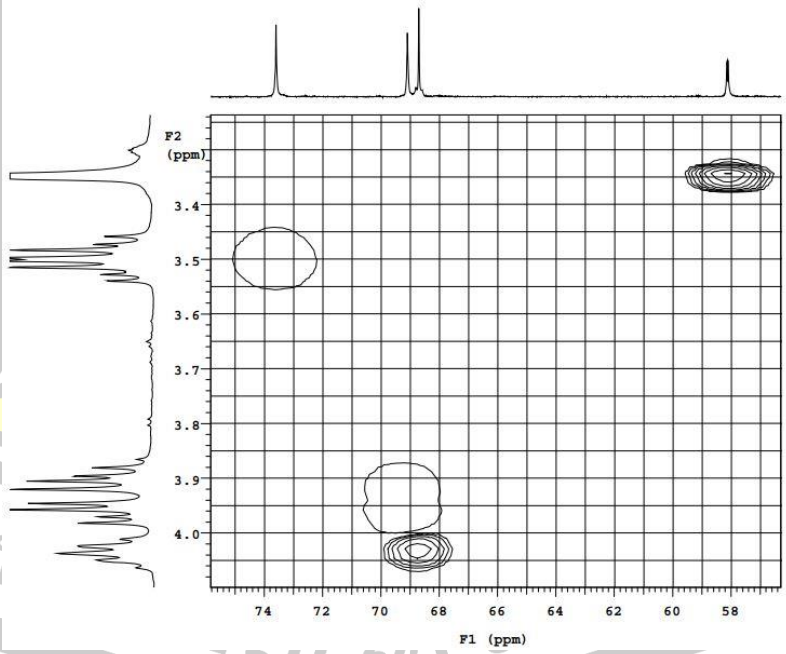


Figure 37A. Expansion of HSQC spectrum (400 MHz) in MeOH-d_4 of compound **355**

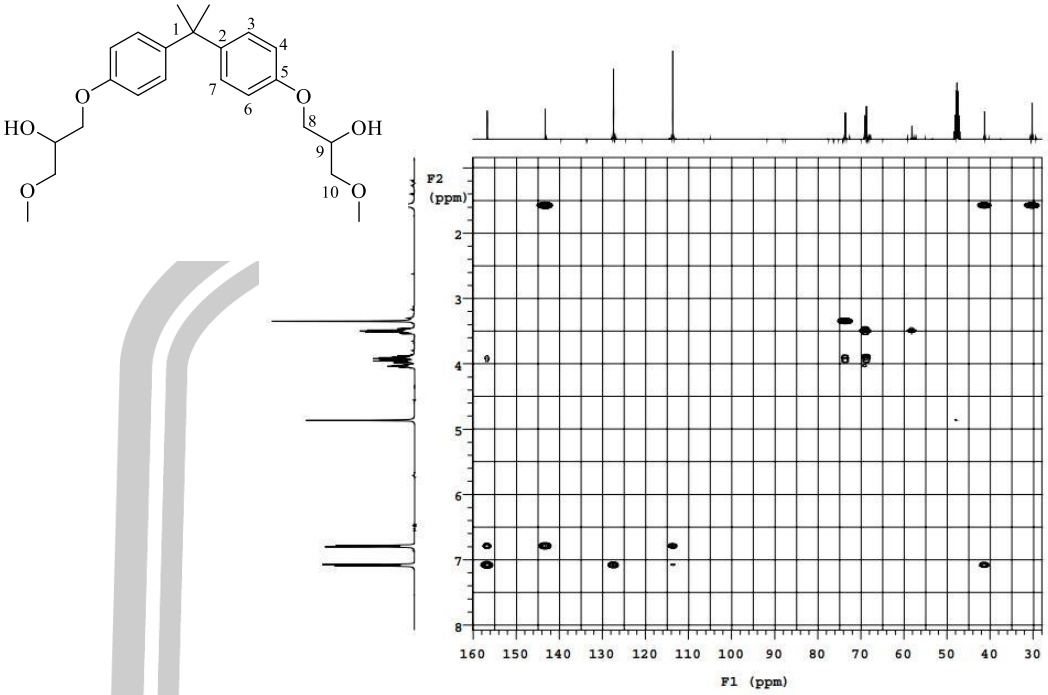


Figure 38A. HMBC spectrum (400 MHz) in MeOH-d_4 of compound **355**

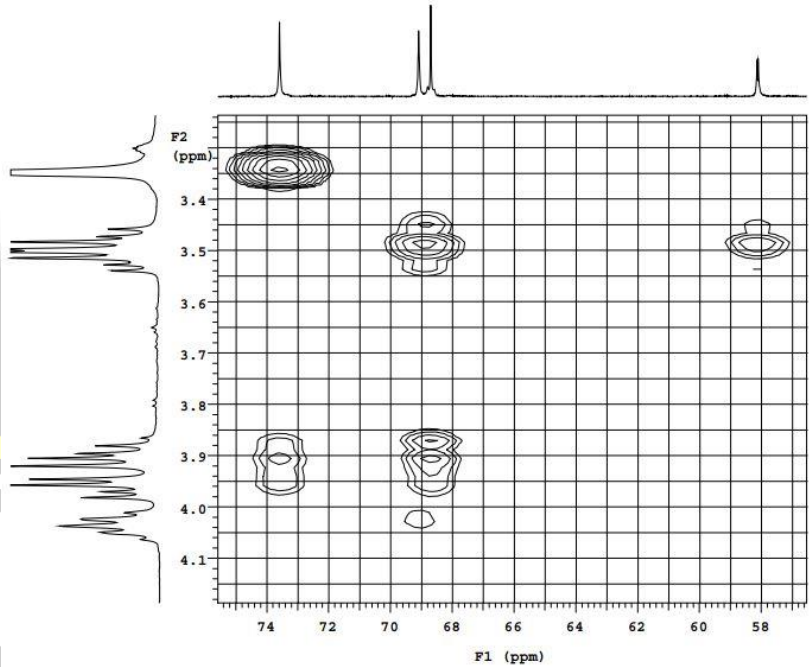


Figure 39A. Expansion of HMBC spectrum (400 MHz) in MeOH-d_4 of compound **355**

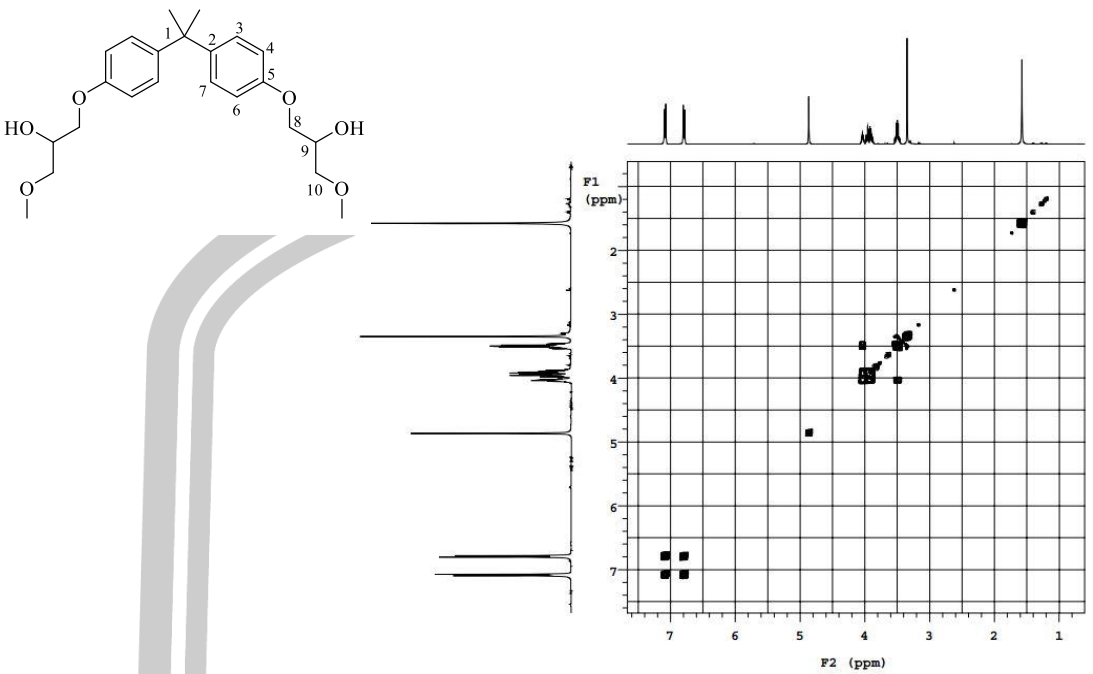


Figure 40A. COSY spectrum (400 MHz) in MeOH-d_4 of compound **355**

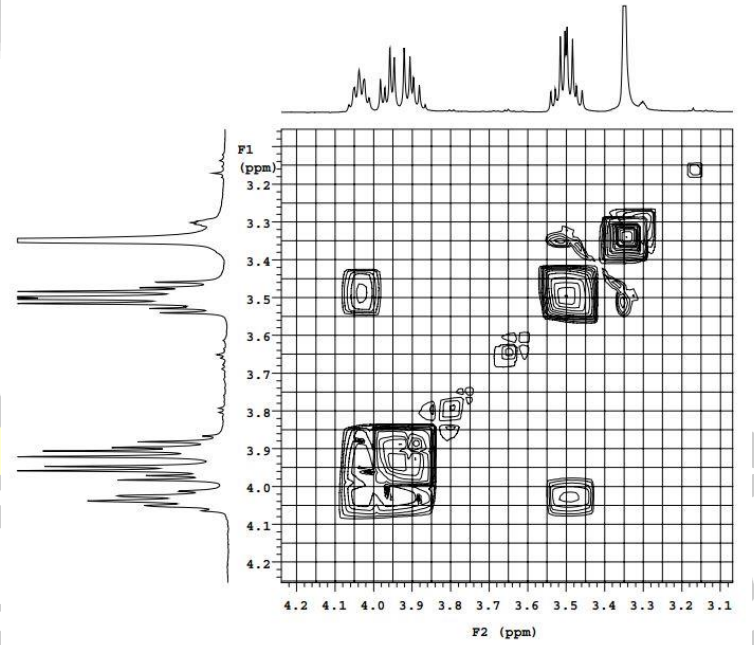


Figure 41A. Expansion of COSY spectrum (400 MHz) in MeOH-d_4 of compound **355**

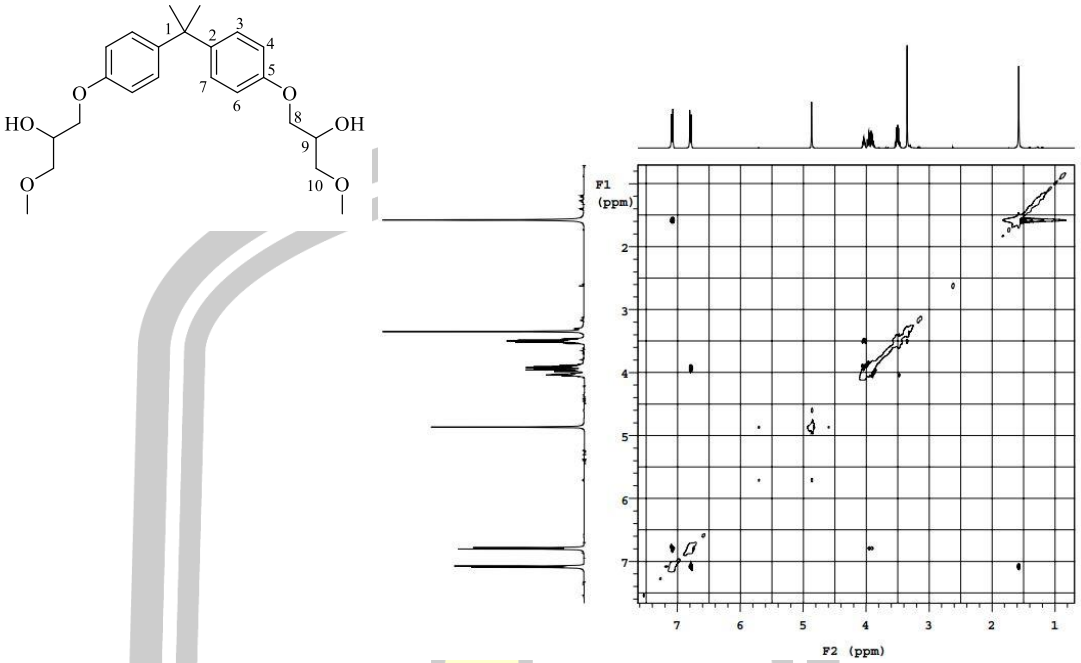


Figure 42A. NOESY spectrum (400 MHz) in MeOH-d_4 of compound **355**

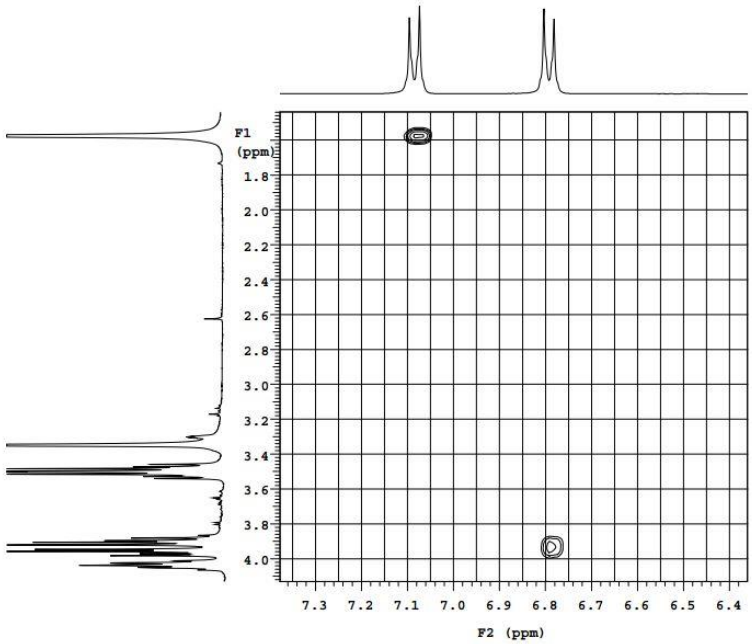


Figure 43A. Expansion A of NOESY spectrum (400 MHz) in MeOH-d_4 of compound **355**

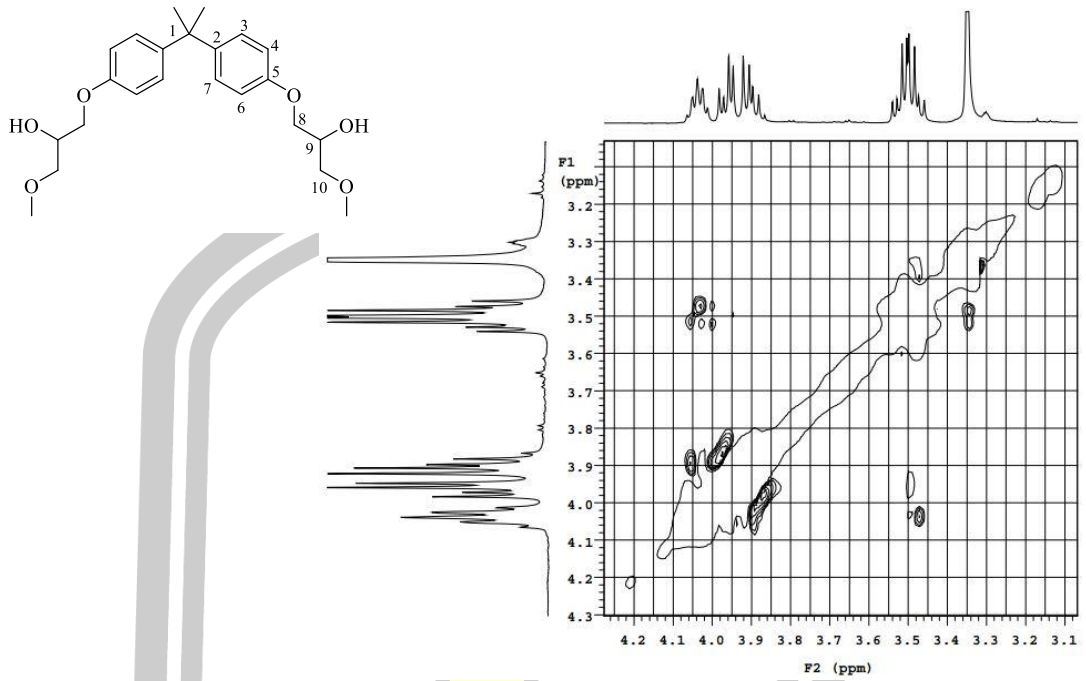


Figure 44A. Expansion B of NOESY spectrum (400 MHz) in MeOH-d_4 of compound **355**

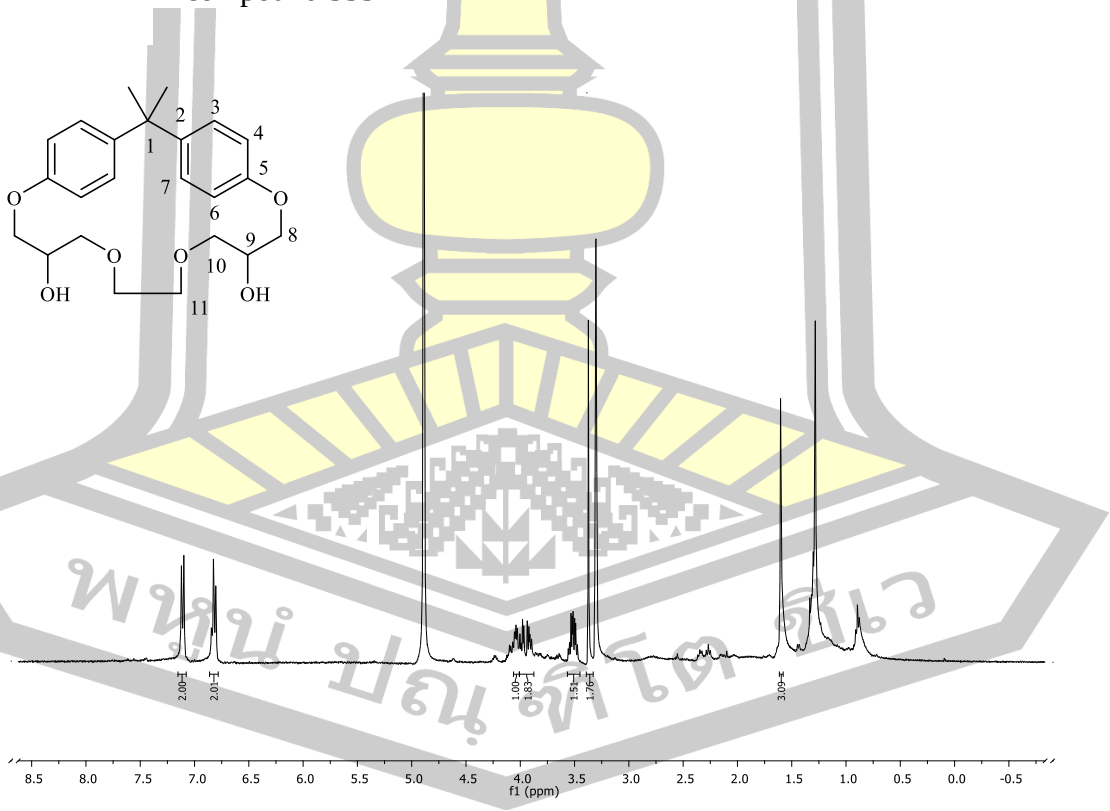
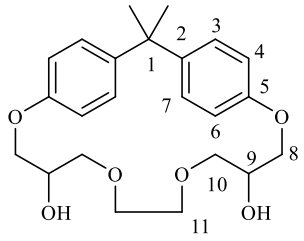


Figure 45A. ^{1}H-NMR spectrum (400 MHz) in MeOH-d_4 of compound **356**



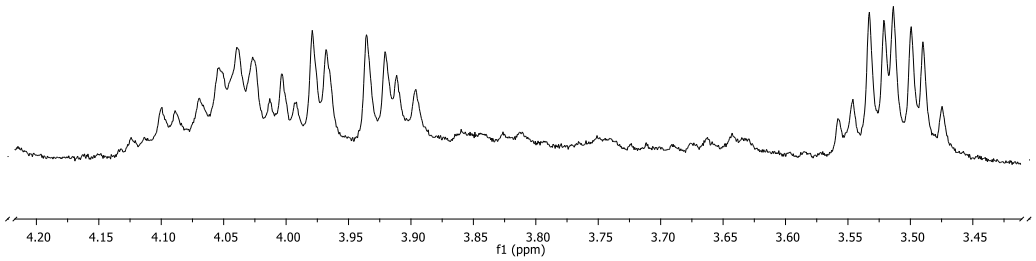


Figure 46A. Expansion of ^{1}H-NMR spectrum (400 MHz) in MeOH-d_4 of compound **356**

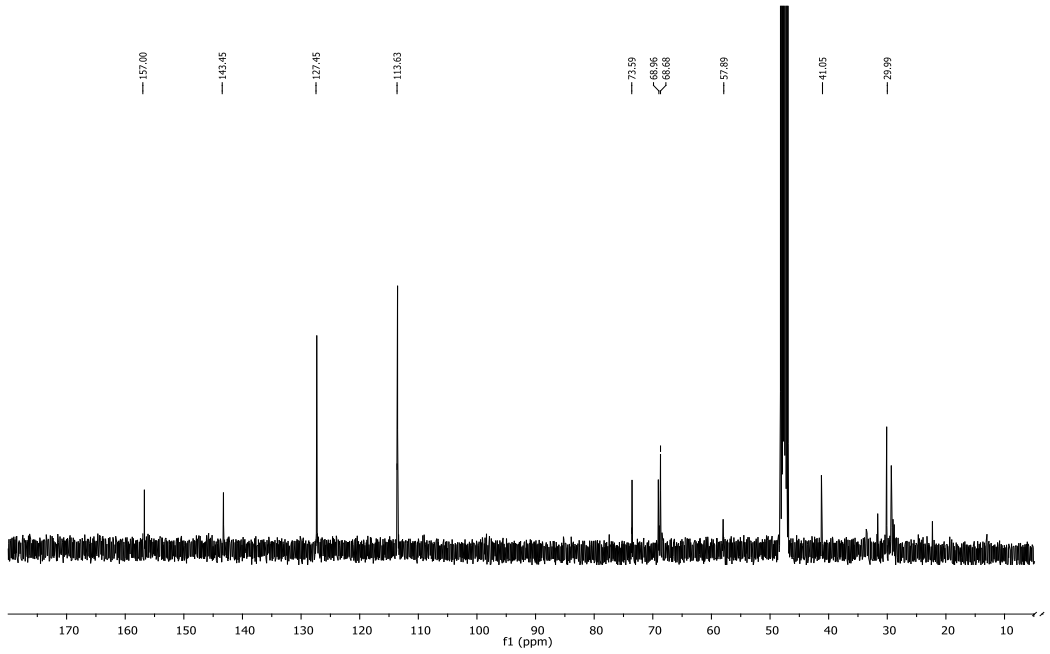


Figure 47A. ^{13}C-NMR spectrum (100 MHz) in $CDCl_3$ of compound **356**

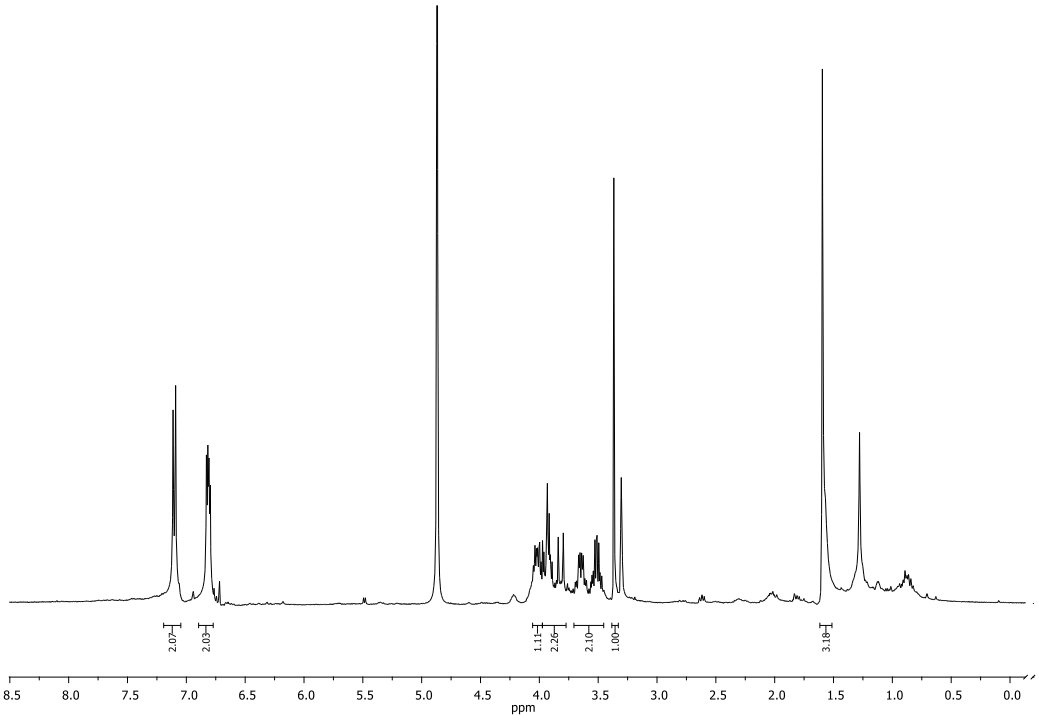


Figure 48A. ^{1}H-NMR spectrum (400 MHz) in MeOH-d_4 of compound **357**

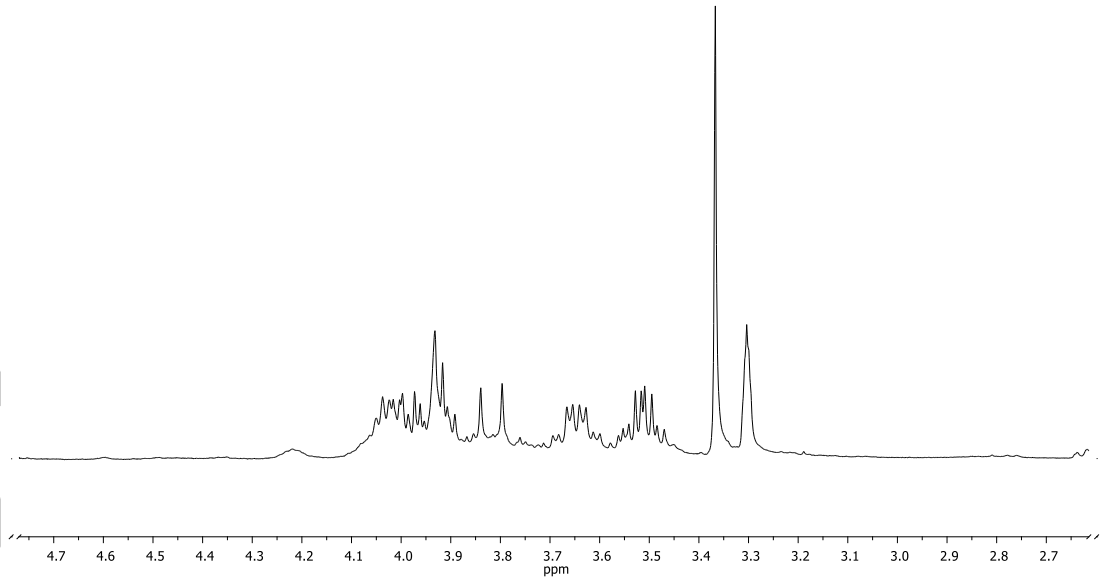


Figure 49A. Expansion of ^{1}H-NMR spectrum (400 MHz) in MeOH-d_4 of compound **357**

BIOGRAPHY

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Research grants & awards	Science Achievement Scholarship of Thailand (SAST)
Research output	1) Sangdee K, Seephonkai P, Buranrat B, Surapong N, Sangdee A. Effects of ethyl acetate extracts from the Polycephalomyces nipponicus isolate cod-MK1201 (Ascomycetes) against human pathogenic bacteria and a breast cancer cell line. Int J Med Mushrooms. 2016; 18(8): 733-743. 2) Surapong N and Seephonkai P, Bioactive compounds from Cordyceps fungi collected in Thailand. KKU Sci. J. 2018; 46(2): 186-200.