博　　士　　論　　文

STUDIES ON BIOLOGICALLY ACTIVE SECONDARY METABOLITES FROM SOME MEDICINAL PLANTS IN VIET NAM

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Contents

Abbreviations

TLC	Thin layer chromatography
CC	Column chromatography
RP-18	Reversed phase C_{18} column
GC	Gas chromatography
HPLC	High performance liquid chromatography
MPLC	Medium performance liquid chromatography
CD	Circular dichroism
MS/HR-MS	Mass spectrum/High resolution-mass spectroscopy
NMR	Nuclear magnetic resonance
1D/2D	One dimension/two dimension
$^{1}H/^{13}C$-NMR	Proton/Carbon-NMR
DEPT	Distortionless enhancement by polarization transfer
COSY	Correlated spectroscopy
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum coherence
NOESY	Nuclear Overhauser enhancement spectroscopy
δ/*J*	Chemical shift/coupling constant
fr.s	Fractions
MIC	Minimum inhibitory concentration
mg/μg/mL	Milligram/microgam/millilitre
MHz	Megahertz
m/z	Mass to charge ratio

Abstract

Historical records have evidently accumulated in showing that the use of traditional antibiotics, which are derived from synthetic substances, is always accompanied by a long duration of treatment, high costs, and drug resistances. Therefore, calls for new antibiotic drugs from natural sources are warranted. For thousands of years, Vietnamese traditional herbal medicine, also known as Southern Medicine, is considered as a useful tool to quickly drive various diseases. From generation to generation, the recording medical documents exposured that either of Vietnamese people or other parts of the world treated diseases with available resources, utilizing orally herbs and plants which were gathered or cultivated. The search for bioactive molecules and novel drugs have always drawn much more attentions to scientists, especially chemists. In the recent days, modern chromatography techniques have been applied to isolate numerous number of secondary metabolites. It, therefore, would lay a good platform to get further extensive steps in discovery of new drugs and preparations.

As my present work for Ph.D thesis, I would like to point out that my career would be taken good advances for the dissertation entitled "**Studies on Biologically Active Secondary Metabolites from Some Medicinal Plants in Viet Nam**", which emphasizes on phytochemical, spectroscopic, and biological studies to enhance my responsibilities and skillful development of future professionals. This study deals with two Vietnamese medicinal plants *Dalbergia tonkinensis* Prain and *Cratoxylum formosum* (Jack) Dyer in Hook. The timber tree *D. tonkinensis* is endemic to Viet Nam, which is valuable for wood craft, workship and ornamentation. *D. tonkinensis* is on The IUCN Red List of Threatened Species and Vietnam Government Decree No, 32/2016/ND-CP, prohibiting exploitation, shipping, and storage. Furniture made from *D. tonkinensis* heartwood is particularly prized in Vietnam, while commercial sales of this plant are

quietly banned. Of pharmacological aspect, *D. tonkinensis* species is recognized to be potential agent in treating diabetes. *C. formosum* species grows up as shrubs or small to medium-sized trees. It has pink flowers and can be up to 20 meters tall. The leaf of this herbal plant has traditionally been employed for tea, heat detoxification, digestive benefits, and anti-aging. As the chemical components of this plant are rich in tannins and flavonoids, the pharmacological reports of *C. formosum* species are associated with the significant antioxidant capacity. Despite the fact that these two plants have been playing an important role in Vietnam, but to date, specific phytochemical investigations on these are quietly limited. In order to explore further scientific aspects on these plants, my thesis has aimed at discovering the plant extracts/fractions and natural compounds with new kinds of biological activities from several parts of these two plants. The results are as follows:

Chapter 1 An overview of studies on *Dalbergia* genus:

It is necessary to demonstrate a board spectrum of the previous studies relating to the plants from *Dalbergia* species. As a consesquence, this chapter deals with an overview of the botanical description, traditional uses, especially phytochemical investigations, and pharmacological results. Most of *Dalbergia* species have claimed for protection due to over-exploitation. Adding that, the perennial plants from *Dalbergia* genus are well-known to be rich in flavonoids and their glycosides. Thus, pharmacological outcomes arising from *in vitro* and *in vivo* examimnations give a general insight in employing *Dalbegria* plants as good candidates against micro-bacterial, free radicals, diabetes, cancer, cardiac heart failure, and so on.

Chapter 2 Chemical constituents from *Dalbergia tonkinensis* Prain:

In this chapter, the experimental performance mainly focused on the phytochemical investigations on heartwood, leaf, and root of Vietnamese *D. tonkinensis* plant. Briefly, the first stage involved in the process to produce the crude extracts obtained by using methanol or ethanol

solvents. These crude extracts were gone on to partition with an increase in solvents using such as *n*-hexane, chloroform, dichloromethane, and ethyl acetate. Utilizing silica gel, Sephadex, RP-18 and HPLC columns in multi-chromatographical actions, total 47 compounds were isolated. Among them, two new rare flavanone derivatives (2*S*)-6,8-dicarboxyethylpinocembrin (**20**), and (2*S*)-8-carboxyethylnaringenin (**21**) and one new sesquiterpene *rel*-(3*R*,6*S*,7*S*)-6α-hydroxycyclonerolidol (**25**) were isolated from heartwood, while one new unique alkaloid 3-(1*H*-indol-3-yl)-2-methoxypropanamide (**31**) and one new isoflavone glycoside isocaviunin 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**33**) were identified presenting in the leaf. In addition, other new sesquiterpene 2,5'-dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47**) was found in the root. The result also suggested that flavonoids and flavonoid glycosides predominated over in *D. tonkinensis* plant. Of particular interests, among several secondary metabolites, sesquisterpenes nerolidol derivatives were only found in Vietnamese *D. tonkinensis* and Chinese *D. odorifera*, thereby making a close affinity between them.

Chapter 3 Chemical constituents from *Cratoxylum formosum*:

Phytochemical study on *C. formosum* led to the isolation of 9 compounds from the methanol extract of leaf. Isolated compounds were categorized as phytosterols, xanthone glycosides, flavonoids, and flavonoid glycosides.

Chapter 4 Biological activities:

With regards to biological assays, antimicrobacterial, enzyme active and inhibitory, and antioxidant assays were carried out. In the present study, several kinds of the extracts, fractions, and isolated compounds from the plants of *Dalbergia* genus were submitted to bioassays. *D. tonkinensis* methanol extracts of leaf, stem bark and root, choroform fraction of heartwood methanol extract exhibited inhibitory percentage of more than 60% against *Bacillus cereus*,

Escherichia coli and *Streptococcus pneumoniae*. Among the isolated compounds, flavanone pinocembrin and isoflavone biochanin A suppressed 80% of the growth of the two bacterial strains *B cereus* and *S. pneumonia* compared with the positive control. Likewise, the strongest percentage of 80% against bacterium *S. pneumonia* was assigned to the isoflavanone sativanone and chalcone isoliquiritigenin.

The products from *D. tonkinensis* (extracts, fractions, and isolated compounds) were accessed by serine protease active and inhibitive assays. The results suggest that at concentrations of 25-250 ng/mL, the methanol extracts of leaf and root, the root ethanol extract and its dichloromethane fraction, and heartwood water decoction extract can serve as useful sources to stimulate trypsin enzyme activity. In addition, water decoction extracts of leaf and stem bark may explain unknown ethno-pharmacology due to the high inhibitory percentages in trypsin-chymotrypsin-elastase experiments. Among isolated compounds, quercetin inhibited the activities of trypsin and chymotrypsin with the IC_{50} 9.7 μM.

Finally, for antioxidant evaluation, DPPH radical scavenging assay was used and the result suggested that isoflavan 3*R*-vestitol (from *D. tonkinensis*) and isoquercetin (from *C. formosum*) possessed the inhibitory capacity as comparable as standard compound catechin (IC_{50} 42.98 μg/mL).

In this thesis, the aforemenioned data and results are discussed in detail and settled in the experimental section.

Chapter 1 An overview of studies on *Dalbergia* genus

1.1. Botany

Dalbergia, belonging to the pea family Fabaceae, has a wide distribution, native to all over the world, especially in the tropical and subtropical regions [1]. According to database of The Plant List (www.theplantlist.org, 2019), the following acceptable names of 274 of 647 *Dalbergia* species were listed at a level of high confidence [2]. The members of the genus are various considerably in shape but generally displayed as small to medium size trees, shrubs and lianas with leathery, alternate, and compound leaves. Leaflets are alternate, rarely, some subopposite and glabrous. Corolla small, rarely fragrant, white to cream, sometimes flushed purplish. Fruits are oblong-lanceolate, indehiscent, usually flattened, and seeded. Seeds are kidney shaped, thin and flat, light brown in color. Root nodulation is very common [1].

1.2. Traditional uses

Numerous species of *Dalbegia* genus have a long history of traditional medicinal use in many countries. The heartwood of medicinal plant *D. odorifera* was found in China with brand name “Jiangxiang”, “Kangjinhyang” in Korean, and “Koshinko” in Japanese drugs for cardiovascular diseases, cancer, diabetes, blood disorder, ischemia, swelling, necrosis, rheumatic pains, and so forth [3-5]. In the employments of *D. sissoo* in Jharkhand-India, the decoction of its leaf possessed acute stage of gonorrhea, removing pus in urine, as alleviates profuse menstruation [6]. Generally, *Dalbegia* species have been employing either as single drugs or in combination with other materials. Representative Table 1 reveals the most striking features in ethnomedical uses of some *Dalbergia* species.

Table 1. Traditional and medicinal uses of some *Dalbergia* species

Name	Parts	Pharmacological uses
D. cochinchinensis	Heartwood	Anti-tumor and blood stasis [7]
D. lanceolaria	Bark	A decoction of the bark is used in dyspepsia [1]
D. latifolia	Whole plant	Whole plant is used in diarrhea, dyspepsia, leprosy and obesity [1]
D. melanoxylon	Stem Root bark	In Senegal, stem and root bark are used in the treatment of diarrhea with baobab or tamarind fruits [1]
D. nitidula	Leaf	Chewed leaf is applied to treat snakebites and leaves are rubbed on abscesses [1]
D. obovata	Root	A root infusion is used in the treatment of stomach-ache and toothache
D. odorifera	Heartwood	Cardiovascular diseases, cancer, blood disorder, ischemia, swelling, necrosis, rheumatic pains [3-5]
D. retusa	Heartwood	The heartwood is extremely resistant to attack by marine boring organisms [8]
D. sissoo	Leaf	Decoction of leaf possesses acute stage of gonorrhea, removing pus in urine, and alleviates profuse menstruation [6].
D. volubilis	Leaf	Decoction of leaf is used as anti-inflammatory and antiarthritic, applied in aphthae, and also used in sore-throat as gargle [1]

Beside the important role in traditional ethnopharmacology and pharmaceutical applications, many species of *Dalbergia* are important timber trees, valued for their decoratives and often fragrant woods, rich in aromatic oils. For instances, *D. sissoo* wood is suitable for quality furniture, plywoods, bridge piles, sport goods, railway sleepers due to strong and tough properties, and *D. melanoxylon* is an intensely black wood in demand for making woodwind musical instruments [9]. It should be noted that many wild plant *Dalbergia* species have been threatening by habit loss and over exploitation for timber usage. It is not for commercial sales, therefore, the protection and growth of this genus is an urgent task.

1.3. Phytochemical studies

Nowadays, the procedures of isolation, purification, and structural elucidation of the interesting secondary metabolites are facilitated by continual development of chromatographic

techniques such as thin layer chromatography (TLC), column chromatography (CC), gas chromatography (GC), and high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), and spectroscopic analyses, for instance, nuclear magnetic resonance (NMR), and mass spectrum (MS). Components of *Dalbergia* species are classified into a wide range of compounds, comprising of the predominant amounts of flavonoids and phenolic compounds, as well as sesquiterpenes, aryl benzofurans, quinones, fatty acids, ketones, and ester derivatives. As shown in Fig. 1, flavonoids in *Dalbergia* species have been found in several categories, such as flavones, isoflavones, flavanones, isoflavanones, isoflavans, isoflavens, neoflavones, pterocarpans, chalcones, and bisflavonoids. While glucopyranosyl, apiosyl-glucopyranosyl, and glucopyranosyl-glucopyranosyl units are the most common sugar of the flavonoid glycosides. Particularly, isoflavone 7-*O*-β-apiofuranosyl-(1→6)-β-glucopyranoside is to be a crucial chemotaxonomic marker for this genus.

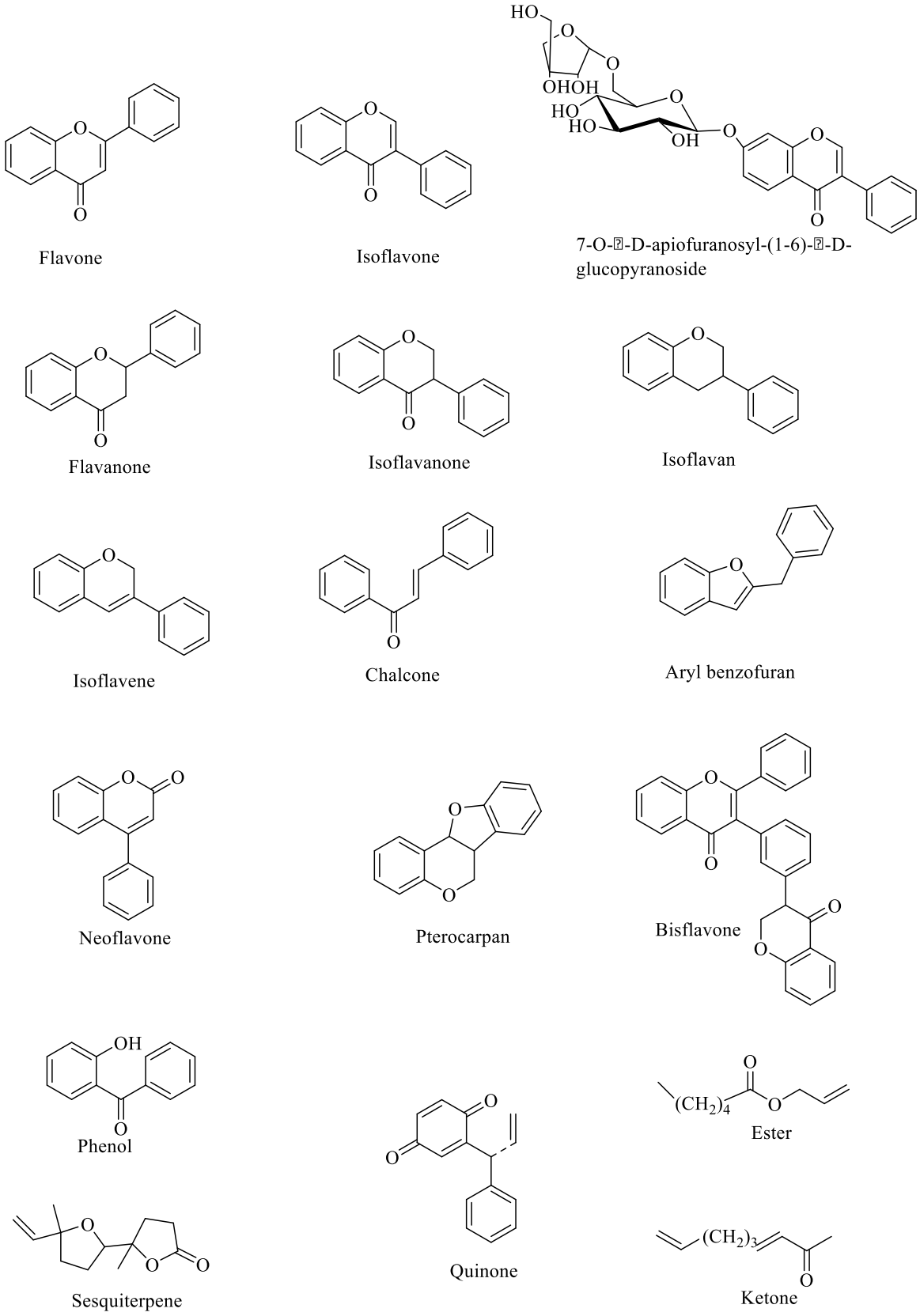


Figure 1. Main skeletons found in isolated compounds of *Dalbergia* species

1.4. Biological investigations

The *Dalbergia* genus is shown to consist of main classes of flavonoid, phenol, and sesquiterpene derivatives, as well as several arylbenzofurans, quinones, and fatty acids. On the other hand, biological studies on extracts, and isolated compounds from species of *Dalbergia* genus involve in cytotoxic, antibacterial, antioxidative, antiinflammatory, antithrombotic, antiplatelet, antiosteosarcoma, antiosteoporosis, antiangiogenesis, vasorelaxant activities, α-glucosidase inhibitory activities, and many other valuable effects. Interestingly, as the valuable resources for natural new drug developments, *Dalbergia* species is widely used as medicinal drugs in many countries for treatments, such as cardiovascular diseases, cancer, blood disorder, ischemia, swelling, necrosis, or rheumatic pains.

Regarding to the cytotoxic activity of chemical constituents of *Dalbergia* species, Choi *et al.* reported the inhibitory effects of pterocarpan medicarpin and a phenolic compound hydroxyobtustyrene on the proliferation of four human tumor cell lines, human uterine carcinoma cell line (MES-SA), multidrug resistant subline of MES-SA (MES-SA/DX5), human colorectal adenocarcinoma cell line (HCT-15), and multidrug resistant subline of HCT15/CL02 [10], in which medicarpin and hydroxyobtustyrene established the significant ED_{50} values of 5.7-7.3 and 5.1-6.8 μM, respectively.

A poorly aqueous soluble chalcone, namely butein, was precipitated out of methanol extract of *D. odorifera* species heartwood [11], which chelated with metal ions Fe^{2+} (UV λ_{max}: 286 and 422 nm) and Cu^{2+} (286 and 454 nm), and showed as potential antioxidant agent with iron-induced lipid peroxidation inhibition in rat brain homogenate

in a concentration dependent manner with the IC_{50} value of 3.3 ± 0.4 μM, the IC_{50} value of 9.2 ± 1.8 μM in DPPH reducing experiment (which was more potential than reference compounds α-tocopherol 11.9 ± 0.2 μM, and BHT 14.5 ± 2.5 μM), the IC_{50} value of 5.9 ± 0.3 μM in xanthine oxidase-induced uric acid formation inhibitory activity [12].

Notes on several antiinflammatory studies. Isoliquiritigenin did not show cytotoxicity for RAW 264.7 macrophages event at the concentration of 20 μM, but, at the concentration of 10 μM, displayed the results in inhibitory percentage of 86 and 79% for NO and IL-1β production when LPS (200 ng/mL) stimulated RAW 264.7 macrophages respectively, and absolutely inhibited iNOS mRNA and protein, TNF-α mRNA expression [13]. A neoflavone 9-hydroxy-6,7-dimethoxydalbergiquinol and two arylbenzofuran derivatives, (2*R*,3*R*)-obtusafuran and isoparvifuran were isolated from the heartwood of *D. parviflora*, *D. odorifera* and *D. louvelii* species, respectively. The viability of cells incubated with the various concentrations of 2,4,5-trimethoxydalbergiquinol (5-50 μM), two arylbenzofurans (1-20 μM) did not affect significantly to BV2 microglia, whereas both 9-hydroxy-6,7-dimethoxydalbergiquinol and (2*R*,3*R*)-obtusafuran inhibited the levels of proinflammatory mediators NO, PGE_2, TNF-α, IL-1β, and decreased iNOS and COX-2 appearance when BV2 microglia was stimulated by LPS at the doses of 500 ng/mL and 1 μg/mL, respectively. But isoparvifuran did not show any decrease levels of above proinflammatory agents and iNOS and COX-2 expressions event at the concentration of 20 μM [14-15].

The screening results for the motility and the viability of phytopathogenic *Aphanomyces cochlioides* zoospores showed that three flavonoid derivatives daidzein,

dalbergin, and (6a*R*, 11a*R*)-variabiin from heartwood of *D. stevensonii*, *D. odorifera* and bark of *D. frutescans*, and medicarpin showed repellent activity at 150 μg/ml, whereas claussequinone and formononetin showed stimulating and attracting activity at 100 and 50 μg/ml, respectively. A mixture of daidzein, dalbergin, and (6a*R*, 11a*R*)-variabiin (1:1:1, w/w/w) had advantages in repellent activity at 50 μg/ml [16].

As part of ongoing search for natural products with anti-cancers effects, 4-methoxydalbergione was found to suppress growth and induce apoptosis in human osteosarcoma cells *in vitro* and *in vivo* xenograft model through down-regulation of the JAK2/STAT3 pathway [17].

The latent *vasorelaxant* relaxation of the butein on rat aortic rings precontracted with various vasoconstrictors phenylephrine (3 μM), KCl (60 mM), U-46619 (1 μM), endothelin-1 (1 nM), and angiotensin II (3 μM) had the EC_{50} values of 7.4 ± 1.6, 10.5 ± 2.3, 14.3 ± 3.3, 11.8 ± 2.0, 13.6 ± 3.7 μM, respectively [18]. Meantime, isoliquiritigenin from the heartwood of *D. cochinchinensis*, *D. louvelii*, and *D. odorifera* species was found to relax rat aorta contracted with phenylephrine, KCl, U-46619, endothelin and 5-hydroxytryptamine, with EC_{50}s of 7.4 ± 1.6, 10.5 ± 2.3, 14.3 ± 3.3, 11.8 ± 2.0 and 13.6 ± 3.7 μM, respectively [19].

Taking a look at pharmacological screening of the extracts from genus *Dalbergia*, the methanol extract (60%) of the Chinese medicinal plant *D. odorifera* heartwood showed potent repellent activity against *A. cochlioides* zoospores at the dose 200 μg/mL [16]. Taking the analgesic activtity into consideration, the methanol extract of *D. spinosa* species was found to showe potential analgesic activity in the acetic acid-induced

writhing model with an inhibition of 40% at a dose of 500 mg/kg body weight as compared with the control [1].

2. Studies on *Dalbergia* species in Vietnam

According to "An illustrated Flora of Vietnam" (2002), 48 plant species of *Dalbergia* genus were recorded in Vietnam, and distributed in different areas of the country, particularly in the North [15]. However, up to now, the phytochemical and biological studies are quite limited. To the best of our knowledge, the phytochemical investigation of methanol extracts of *D. oliveri*, *D. vietnamensis*, and *D. tonkinensis* led to the isolation of total twenty two compounds, including daidzein, formononetin, pinocembrin, 2′,4′,5′-trimethoxy-7-hydroxyisoflavanone, liquiritigenin, violanone isoliquiritigenin, (3*R*)-5'-methoxyvestitol, sativanone, isoduratin, naringenin, 3′-hydroxy-2,4,5-trimethoxydalbergiquinol, butein, caviunin, pratensein, maackiain, (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan, 3-hydroxy-9-methoxypterocarpan, lanceolarin, genistein-6-C-glucoside, daltonkins A and B, dalspinosin 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, caviunin 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, caviunin 7-*O*-(5-*O*-*trans*-p-coumaroyl)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, and 9,10-*threo*-3-[7-(3,10-dihydroxy-9-hydroxymethyl-2,5-dimethoxy)-9,10-dihydrophenanthrenyl]-propenal [20-22].

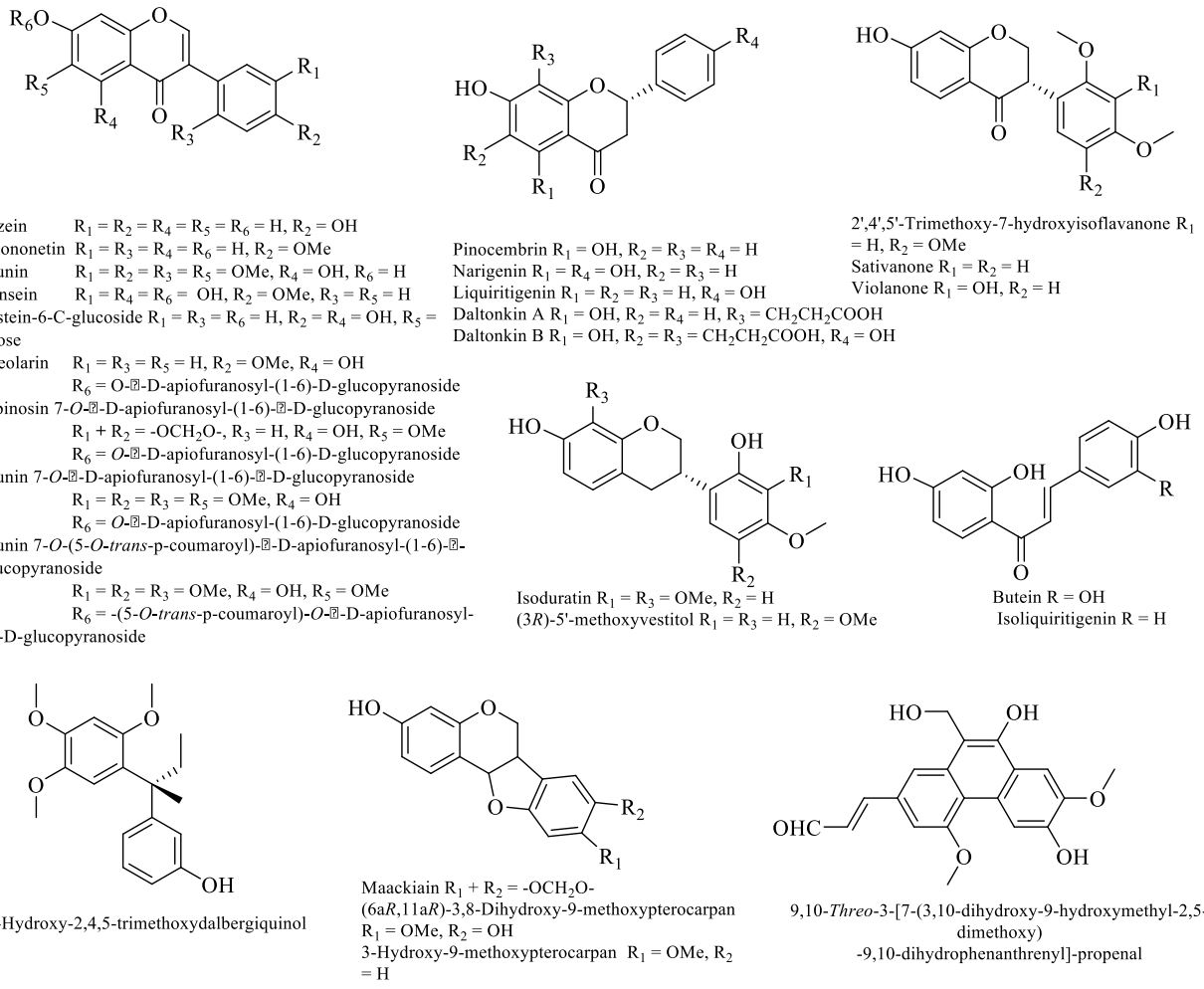


Figure 2. Chemical constituents from the Vietnamese *Dalbergia* species

In the cytotoxic experiment, two pterocarpans (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan and 3-hydroxy-9 methoxyterocarpan from *D. oliveri* heartwood were reported to inhibit four cell lines KB, LU-1, Hep G2, and MCF7 with the strong IC_{50} values of 3.76-7.09 μg/ml, whereas with regards to the antimicrobial assays, these compounds as well as liquiritigenin, genistein 6-C-glucoside, maackiain, formononetin, pratensein, violanone isoliquiritigenin, and (3*R*)-5'-methoxyvestiol were inactive against all tested bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus fermentum*, *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albican* [21].

Among *Dalbergia* species, the precious tree *D. tonkinensis* has established in the vulnerable level in the International Union for Conservation of Nature (IUCN) Red List of

Threatened Species by excessive exploitation for commercial purpose. International publications involved in *D. tonkinensis* which mostly focused on taxonomic differents between it and its other related species principally based on using DNA identifications and chemical comparison with GC-MS techniques [23,24]. Recently, a survey conducted by Nguyen et al suggested that the methanol extract of the trunk-bark, the heartwood, and the leaf of this plant had demonstrated potential α-glucosidase inhibition [25].

Chapter 2 Chemical constituents from *Dalbergia tonkinensis*

2.1. Introduction

Scientific name: *Dalbergia tonkinensis* Prain and local name: Sua do, Trac thoi;

Family: Fabaceae; genus: *Dalbergia*;

Morphology: The perennial trees, 5-13 m tall, leaves 9-20 cm, rachis glabrous, stipules caducous, small, tawny, puberulent, leaflets (7) 9-11, petiolule glabrous, blades ovate, 4-9×(1) 3-5 cm, subleathery, sparsely puberulent when young, soon glabrous, base rounded, apex shortly acuminate. Panicles corymbose, axillary, ca. 5×3.8 cm. Flowers white, fragrant. Legume distinctly stipitate, ovoid or oblong, 5–7.5×ca. 2 cm, reticulate opposite seeds, base cuneate, apex subacute. Seeds reniform, compressed, ca. 9×5 mm [26].

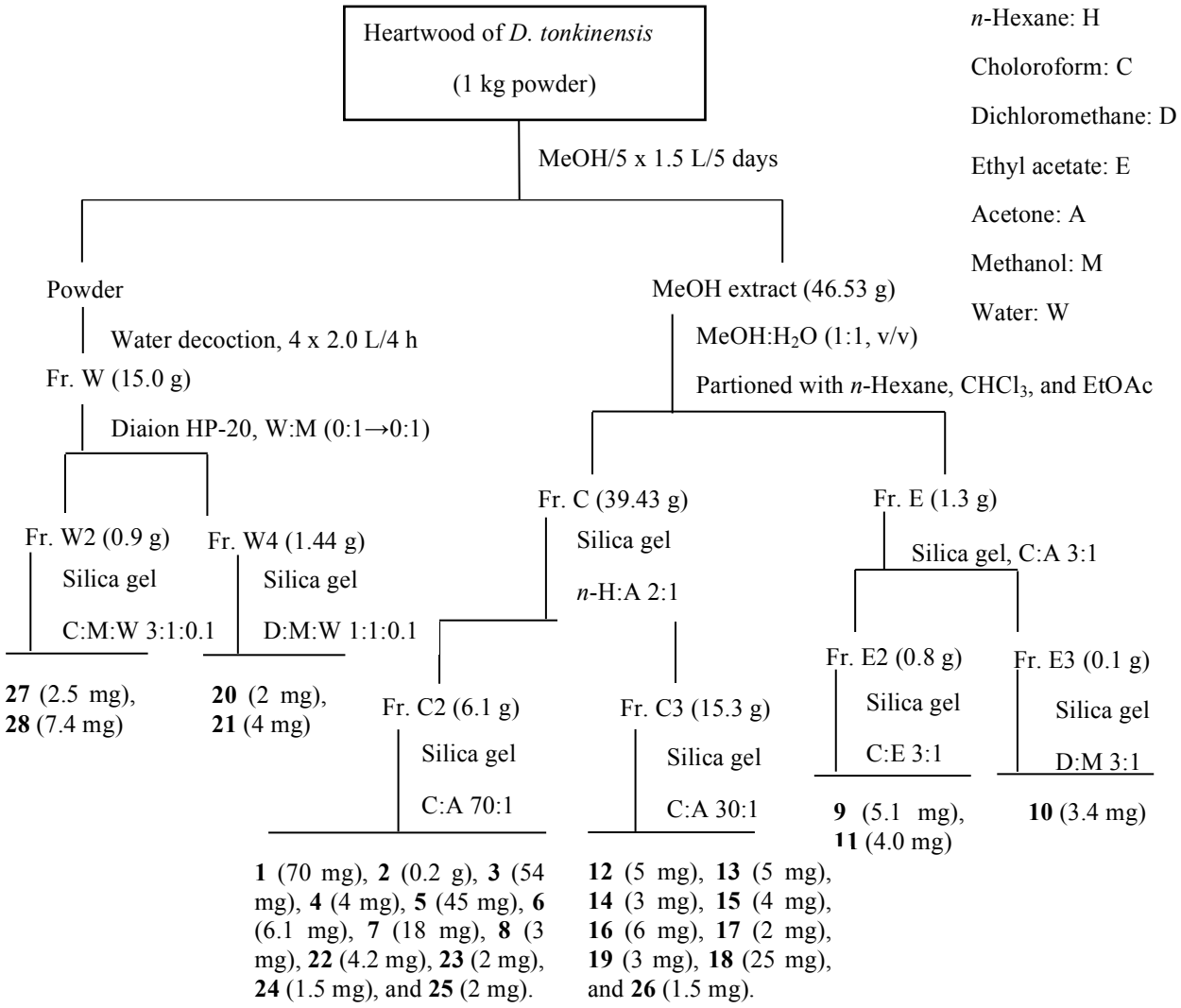
Distribution: Ha Noi, Vinh Phu, Ha Nam Ninh, Ha Son Binh, Dong Nai [15].

Conservation status: Vulnerable level.

Figure 3. Heartwood, root, and leaf of *D. tonkinensis*

2.2. Phytochemical investigation of heartwood extract and structural elucidation

2.2.1. Isolated procedure



From the chloroform ($CHCl_3$), ethyl acetate (EtOAc) fr.s of the methanol, and aqueous decoction extracts of *D. tonkinensis* heartwood, three new compounds **20**, **21** and **25**, along with twenty five known compounds **1-19**, **22-24**, and **26-28** were isolated. By comparison of their ^{1}H, and ^{13}C-NMR spectroscopic data with the literature data, the chemical structures of the known compounds were identified as formononetin (**1**), sativanone (**2**), naringenin (**3**), liquiritigenin (**4**), pinocembrin (**5**), medicarpin (**6**), 3'-hyrdoxy-2,4,5-trimethoxydalbergiquinol (**7**), buteaspermanol

(**8**), butin (**9**), 3'-hyrdoxymelanettin (**10**), biochanin A (**11**), calycosin (**12**), eriodictoyl (**13**), isoliquiritigenin (**14**), 3'-*O*-methylviolanone (**15**), luteolin (**16**), quercetin (**17**), apigenin (**18**), sulfuretin (**19**), dalbergin (**22**), homoferreirin (**23**), neroplomacrol (**24**), kaemfperol (**26**), formyl phenol (**27**), and naringin (**28**) [9, 27-33].

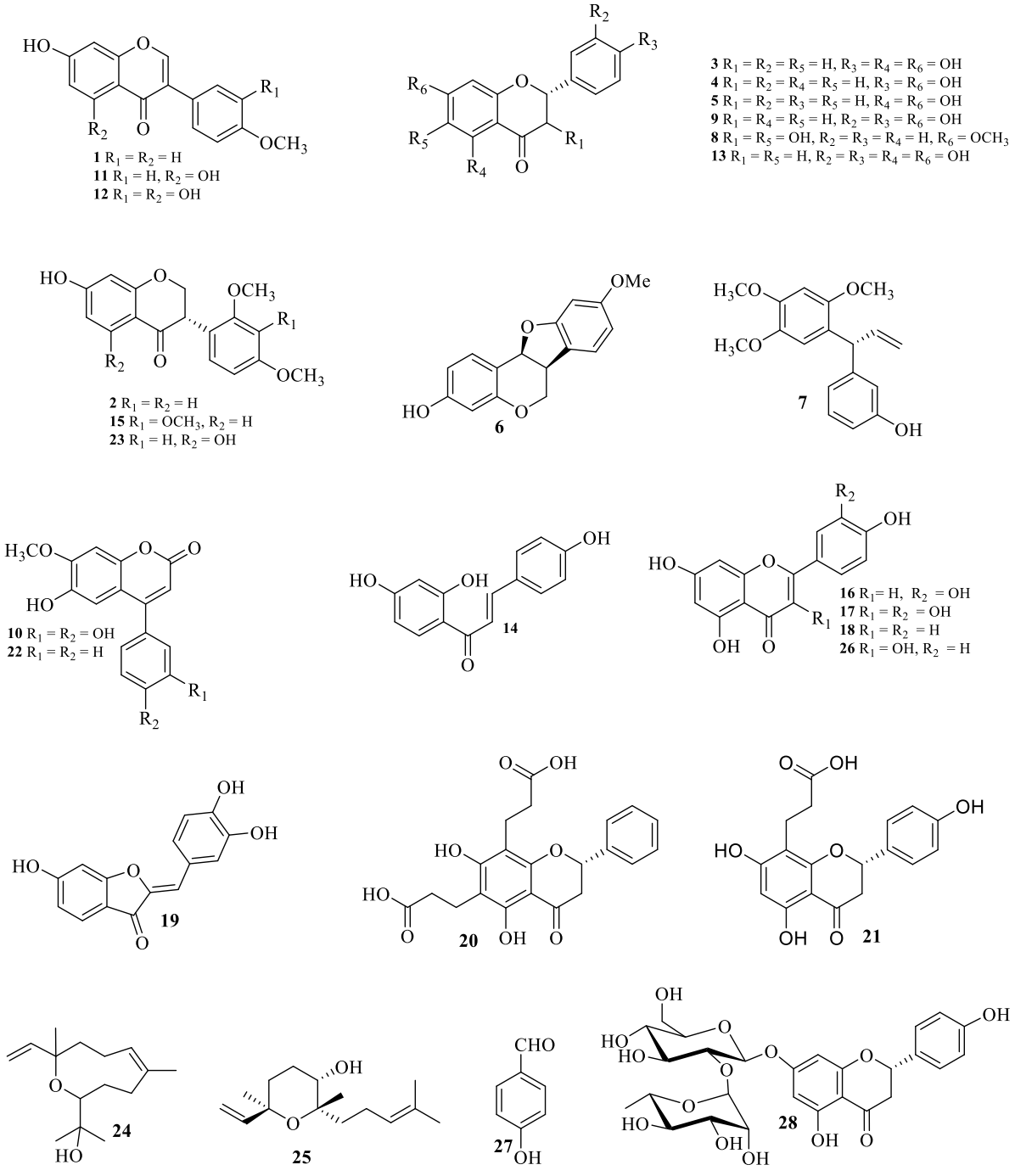


Figure 4. Compounds isolated from *D. tonkinensis* heartwood

2.2.2. Structural elucidation of new compounds 20, 21 and 25

Compound **20**: (2*S*)-6,8-Dicarboxyethylpinocembrin

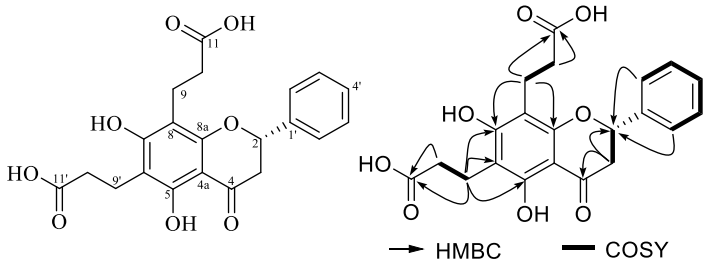


Figure 5. Structure, and COSY and HMBC key correlations of **20**

Table 2. ^{1}H- and ^{13}C-NMR data of compounds **20** and **21** in CD_3OD

Position	20		21	
	δ_H (*J* in Hz) [a]	δ_C [b]	δ_H (*J* in Hz) [a]	δ_C [b]
2	5.50 (dd, 13.0, 3.0)	80.2	5.34 (dd, 13.0, 2.5)	80.5
3	2.84 (dd, 17.0, 3.0)	44.2	2.72 (dd, 17.0, 2.5)	44.1
	3.11 (dd, 17.0, 13.0)		3.14 (dd, 17.0, 13.0)	
4		198.1		198.0
4a		103.5		103.2
5		159.9		162.9
6		109.1	5.97 (s)	95.7
7		163.9		166.3
8		108.3		108.6
8a		161.1		162.7
9	2.88 (m)	19.5	2.84 (t, 8.0)	18.8
10	2.54 (m)	34.7	2.48 (t, 8.0)	34.6
11		178.7		177.9
9'	2.88 (m)	18.8		
10'	2.54 (m)	34.3		
11'		178.5		
1'		140.6		131.2

2',6'	7.54 (d, 7.5)	127.2	7.33 (d, 8.5)	129.0
4'	7.39 (t, 7.5)	129.5		159.0
3',5'	7.44 (d, 7.5)	129.7	6.84 (d, 8.5)	116.3

[a] 500 MHz, [b] 125 MHz.

Compound **20** was isolated as a white amorphous solid. The molecular formula of **20** was consistent with $C_{21}H_{20}O_8$ deduced from the quasi-molecular ion peak at *m/z* 399.1080 $[M-H]^-$ in the negative HR-ESI-MS. Its IR spectrum displayed the absorption bands at 3370 and 1721 cm^{-1}, due to hydroxyl and carbonyl groups, respectively. The ^1H and ^{13}C-NMR spectroscopic data of **20** were similar to those of (2*S*)-pinocembrin [34], except for the absence of two aromatic protons H-6 and H-8 in (2*S*)- pinocembrin which were replaced by two carboxyethyl groups [H-9' (δ_H 2.88)/C-9' (δ_C 18.8), H-10' (δ_H 2.54)/C-10' (δ_C 34.3), and C-11' (δ_C 178.5)] and [δ_H 2.88 (H-9)/δ_C 19.5 (C-9), δ_H 2.54 (H-10)/δ_C 34.7 (C-10), and δ_C 178.7 (C-11)] in **20** (Table 2). Analysis of ^1H-^1H correlation spectroscopy (COSY) and HMBC spectra enabled the linkage of one carboxyethyl side chain to be on the carbon C-6 by COSY correlation of H-9' and H-10' and HMBC correlations of H-9' and H-10'/C-11', and H-9'/C-5, C-6 and C-7 (Fig. 5). Similarly, COSY cross peak between H-9 and H-10, as well as HMBC correlations between H-9 and H-10/C-11, and H-9/C-7 and C-8a confirmed the connectivity of the other carboxyethyl side chain on the carbon C-8. The CD spectrum of **20** revealed positive Cotton effect at $\Delta\varepsilon_{339}$ (nm) +0.83 ($n-\pi^*$ transition) and negative Cotton effects at $\Delta\varepsilon_{290}$ (nm) −8.21 ($\pi-\pi^*$ transition), thereby indicating the 2*S*-configuration [35]. Consequently, the chemical structure of **20** was characterized as (2*S*)-6,8-dicarboxyethylpinocembrin.

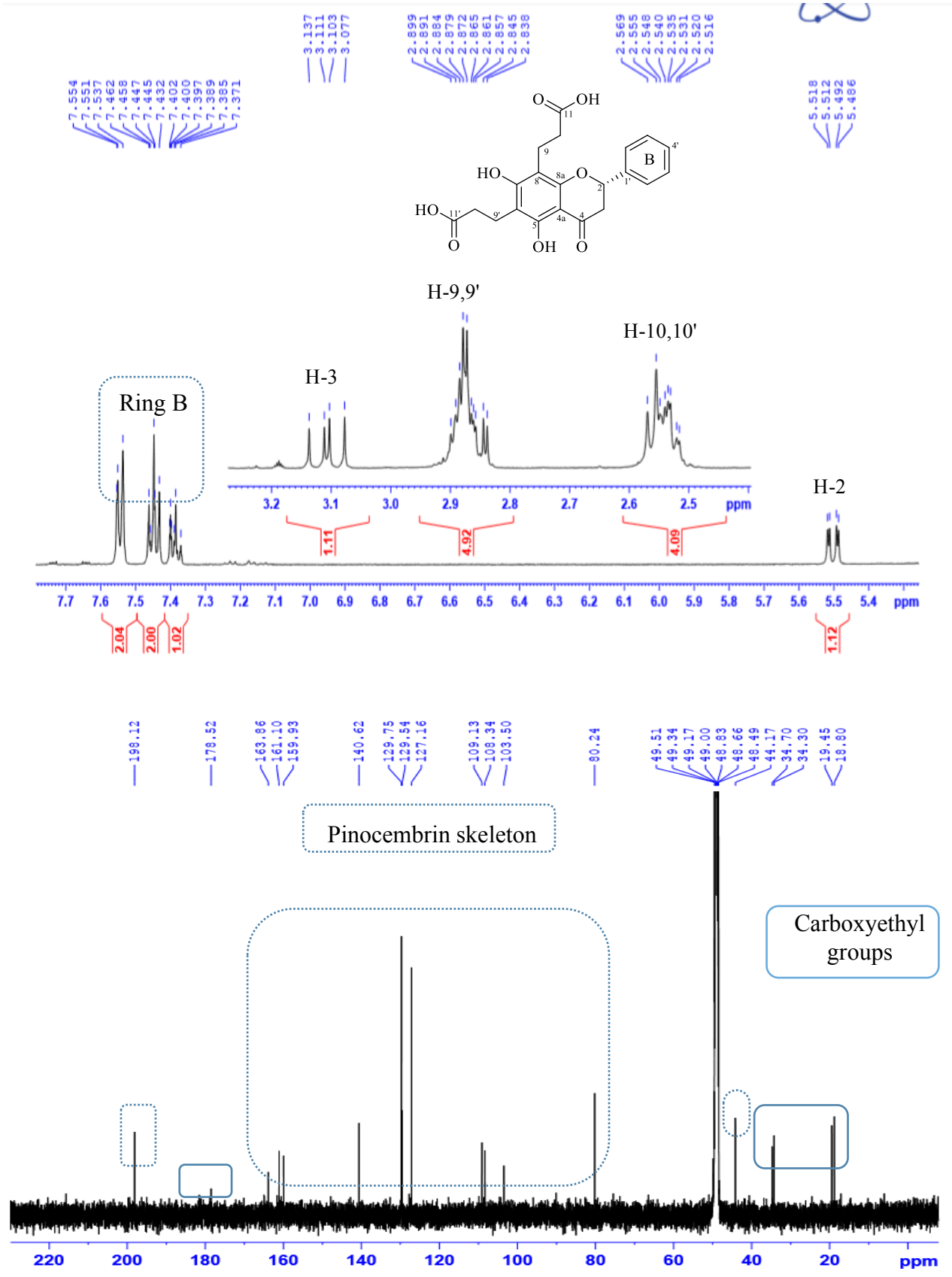
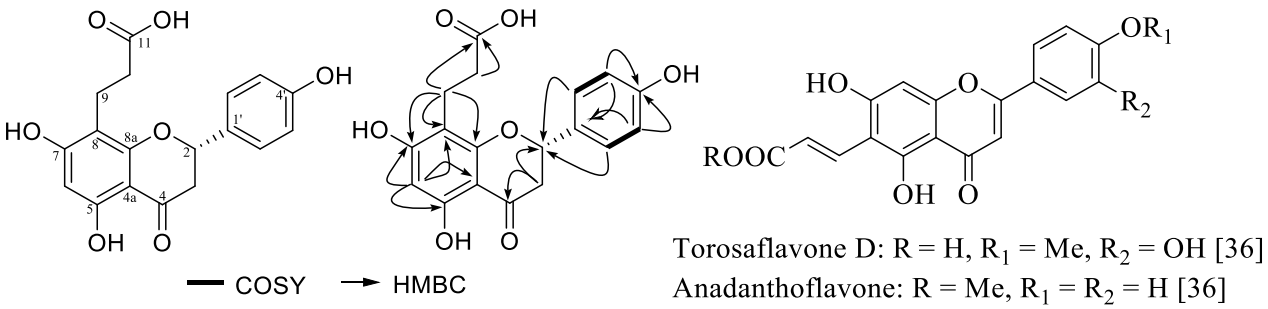


Figure 6. ^{1}H-NMR/^{13}C-NMR spectra of compound **20** in CD_3OD

Compound **21**: (2*S*)-8-Carboxyethylnaringenin



Torosaflavone D: R = H, R_1 = Me, R_2 = OH [36]
Anadanthoflavone: R = Me, R_1 = R_2 = H [36]

Figure 7. Structure, COSY and HMBC key correlations of **21**

Compound **21** was obtained as a white amorphous solid, and its molecular formula $C_{18}H_{16}O_7$ was deduced from the quasi-molecular ion peak at *m/z* 343.0815 $[M-H]^-$ in the negative high resolution-electrospray ionization mass spectroscopy (HR-ESI-MS). The IR spectrum of **21** showed absorption bands at 3356 and 1737 cm^{-1}, attributing to hydroxyl and carbonyl groups, respectively. The ^1H and ^{13}C-NMR spectroscopic data of **21** were similar to those of (2*S*)-naringenin [34], except for the absence of the aromatic proton H-8 in (2*S*)-naringenin which was replaced by a carboxyethyl group [δ_H 2.84 (H-9)/δ_C 18.8 (C-9), δ_H 2.48 (H-10)/δ_C 34.6 (C-10), and δ_C 177.9 (C-11)] existing in **21** (Table 2). This was further substantiated by heteronuclear HMBC correlations between H-6 and C-5/C-7/C-8 (Fig. 7). In addition, the HMBC correlations of H-9 to C-7/C-8/C-8a suggested that the carboxyethyl group was located at C-8 (Fig. 7). The CD spectrum of **21** showed positive Cotton effect at $\Delta\varepsilon_{337}$ (nm) +0.19 ($n-\pi^*$ transition) and negative Cotton effect at $\Delta\varepsilon_{293}$ (nm) −0.71 ($\pi-\pi^*$ transition), which was consistent with the 2*S*-configuration [35]. According to the analysis of spectroscopic data, the chemical structure of compound **21** was elucidated as (2*S*)-8-carboxyethylnaringenin. Two rare isolated compounds **20** and **21** indicated the same manner with the previous natural product torosaflavone D and its derivative ananthoflavone [36].

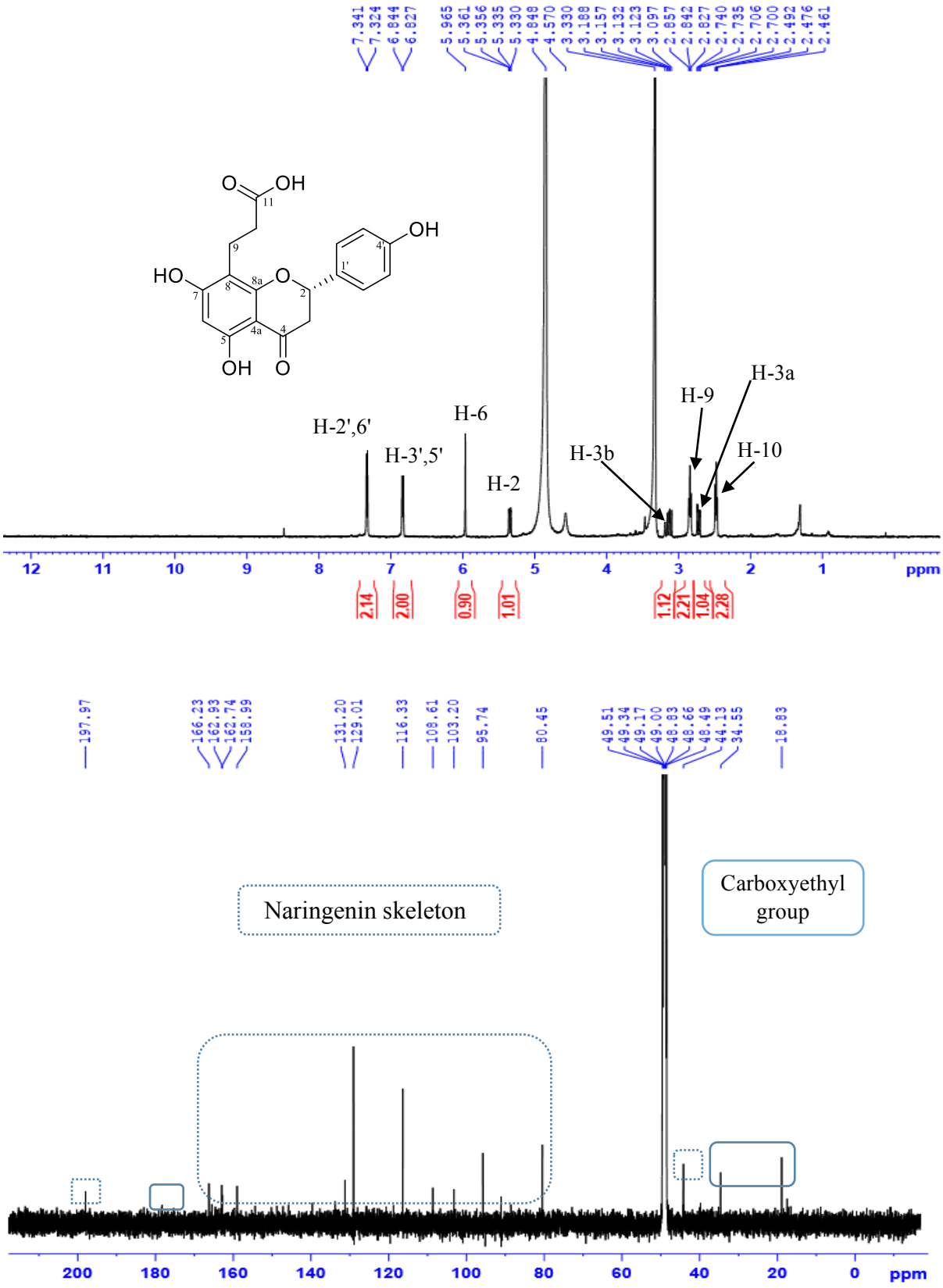


Figure 8. ^{1}H-NMR/^{13}C-NMR spectra of compound **21** in CD_3OD

Compound **25**: *Rel*-(3*R*,6*S*,7*S*)-6α-hydroxycyclonerolidol

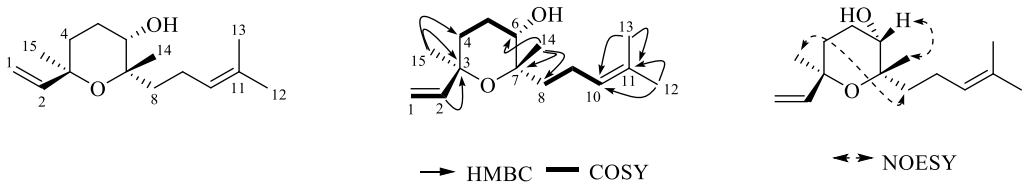


Figure 9. Structure, and COSY, HMBC and NOESY key correlations of **25**

Table 3. ^{1}H- and ^{13}C-NMR data of compound **25** in CD_3OD

Position	δ_H (*J* in Hz) [a)]	δ_C [b)]	**Position**	δ_H (*J* in Hz) [a)]	δ_C [b)]
1	5.22 (dd, 17.5, 1.5) 5.04 (dd, 10.5, 1.5)	112.0	9	2.08 (m)	23.0
2	5.93 (dd, 17.5, 10.5)	146.6	10	5.14 (br t, 7.5)	126.1
3		73.9	11		131.2
4	1.52 (m) 1.87 (m)	40.9	12	1.69 (s)	25.9
5	1.40 (m) 1.73 (m)	26.6	13	1.64 (s)	17.7
6	3.27 (br d, 10.5)	79.3	14	1.12 (s)	22.1
7		75.5	15	1.28 (s)	27.9
8	1.46 (m) 1.55 (m)	39.4			

[a)] 500 MHz, [b)] 125 MHz.

Compound **25** was obtained as a white amorphous solid. The molecular formula of **25** was determined to be $C_{15}H_{26}O_2$ based on the quasi-molecular ion peak observed at *m/z* 239.2011 $[M+H]^+$ in the positive HR-ESI-MS spectrum. The IR spectrum showed absorption bands at 3362 and 1642 cm^{-1}, which were attributed to hydroxyl and double bond groups, respectively. The ^{1}H NMR spectrum of **25** showed signals similar to those of 6α-hydroxycyclonerolidol and *rel*-(3*R*,6*R*,7*S*)-3,7,11-trimethyl-3,7-epoxy-1,10-dodecadien-6-ol [37, 38], as follows: olefinic proton signals of H_2-1 *exo*-olefinic methylene at δ_H 5.22 (dd, *J* = 17.5, 1.5 Hz, H-1a) and 5.04 (dd, *J* = 10.5, 1.5 Hz, H-1b), H-2 at δ_H 5.93 (dd, *J* = 17.5, 10.5 Hz) and H-10 at δ_H 5.14 (br t, *J* = 7.5 Hz), oxygenated methine proton H-6 at δ_H 3.27 (br d, *J* = 10.5 Hz), two pairs of vicinal-multiplet methylene protons H_2-4 at δ_H 1.52 (m, H-4a) and 1.87 (m, H-4b), H_2-5 at δ_H 1.40 (m,

H-5a) and 1.73 (m, H-5b), H_2-8 at δ_H 1.46 (H-8a) and 1.55 (H-8b), H_2-9 at δ_H 2.08, and four methyl groups [H_3-12 (δ_H 1.69), H_3-13 (δ_H 1.64), H_3-14 (δ_H 1.12), H_3-15 (δ_H 1.28), each singlet]. The ^{13}C-NMR/DEPT spectra of **25** contained 15 carbon signals, comprising four CH_3, five CH_2, three CH, and three C_4 groups, which were assigned to a sesquiterpene. Its COSY correlations provided three partial units of C1–C2, C4–C6, and C8–C10, as indicated by bold lines in Fig. 9. The structure of **25** was further confirmed by HMBC analysis. The HMBC spectrum supported the C8–C13 carbon sequence due to both H_3-12 and H_3-13 signals showing correlations with two olefinic carbons C-10 (δ_C 126.1) and C-11 (δ_C 131.2), while the H_3-14 signal showed HMBC cross peaks with C-6 (δ_C 79.3), C-7 (δ_C 75.5), and C-8 (δ_C 39.4) (Fig. 9). Furthermore, HMBC correlations of H_3-15 with quaternary carbon C-3 (δ_C 73.9) and C-4 (δ_C 40.9), and of H-2 with quaternary carbon C-3 confirmed that methyl group H_3-15 and vinyl moiety C1–C2 were attached to the C-3 position. The stereochemistry of the chiral centers was elucidated using nuclear Overhauser effect spectroscopy (NOESY) correlations and by comparing the ^{13}C NMR spectrum with literature data [37, 38]. The chemical shift of the chiral center C-3 was similar to those of reference compounds 6α-hydroxycyclonerolidol and *rel*-(3*R*,6*R*,7*S*)-3,7,11-trimethyl-3,7-epoxy-1,10-dodecadien-6-ol, indicating that C-3 in **25** had the *R**-configuration [37,38]. Furthermore, the NOESY spectrum showed selective correlations between CH_3-14 and oxygen-bearing H-6, and between H_3-15 and H-8 signals, but had neither correlations between the H-6 and H_2-8 or H_3-15 signals, nor between the H_3-14 to H_3-15 signals. These NOESY data supported that two methyl groups, H_3-14 and H_3-15, had a *trans*-configuration and adopted an α-orientation with respect to the 6-OH group [37,38]. Compound **25** showed a negative optical rotation value of $[\alpha]_{25}^{D}$ –15.0° (*c* 0.02; $CHCl_3$), which was similar to that of glandulaurencianol C [39]. From these results, the structure of compound **25** was assigned to be *rel*-(3*R*,6*S*,7*S*)-6α-hydroxycyclonerolidol.

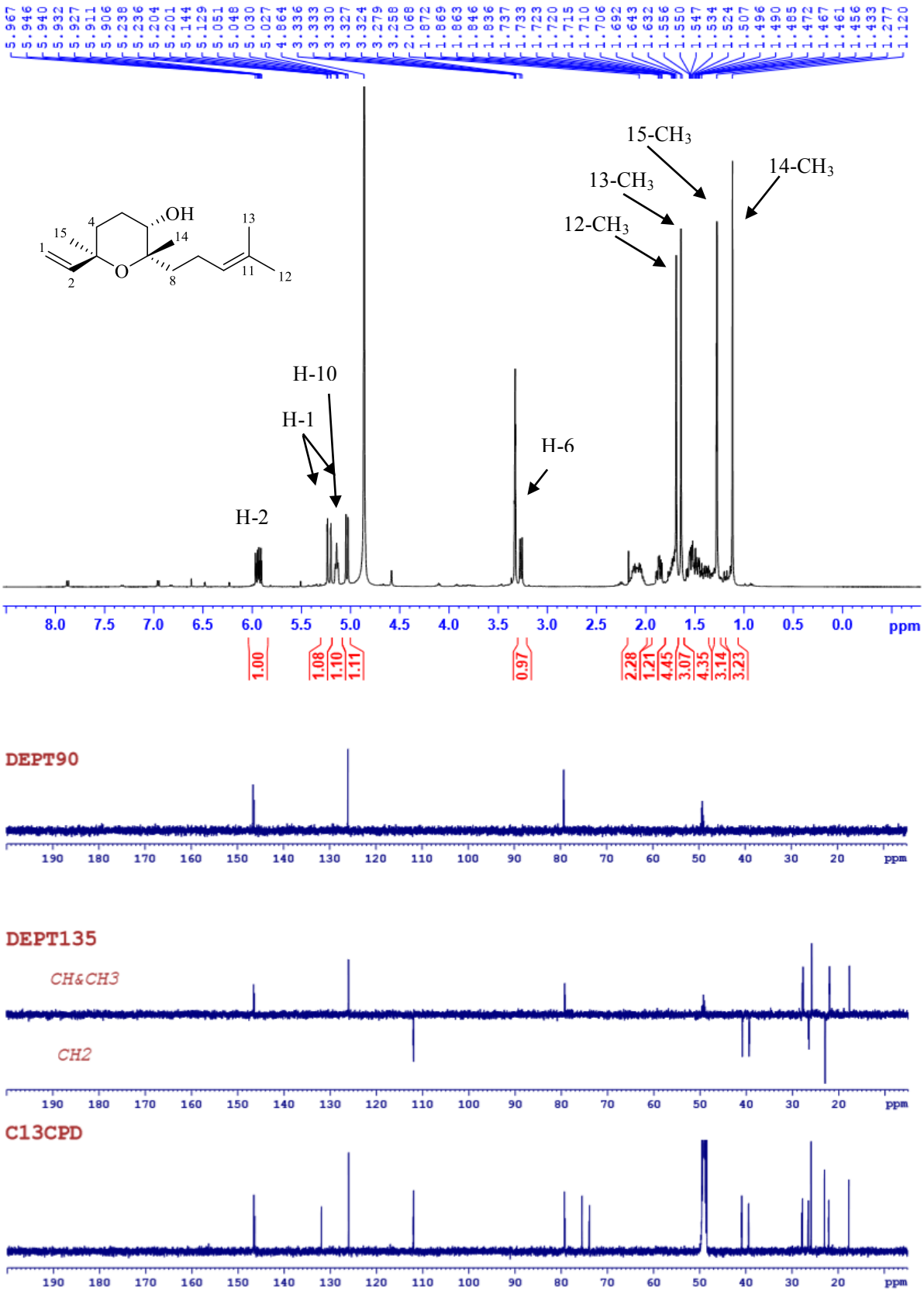
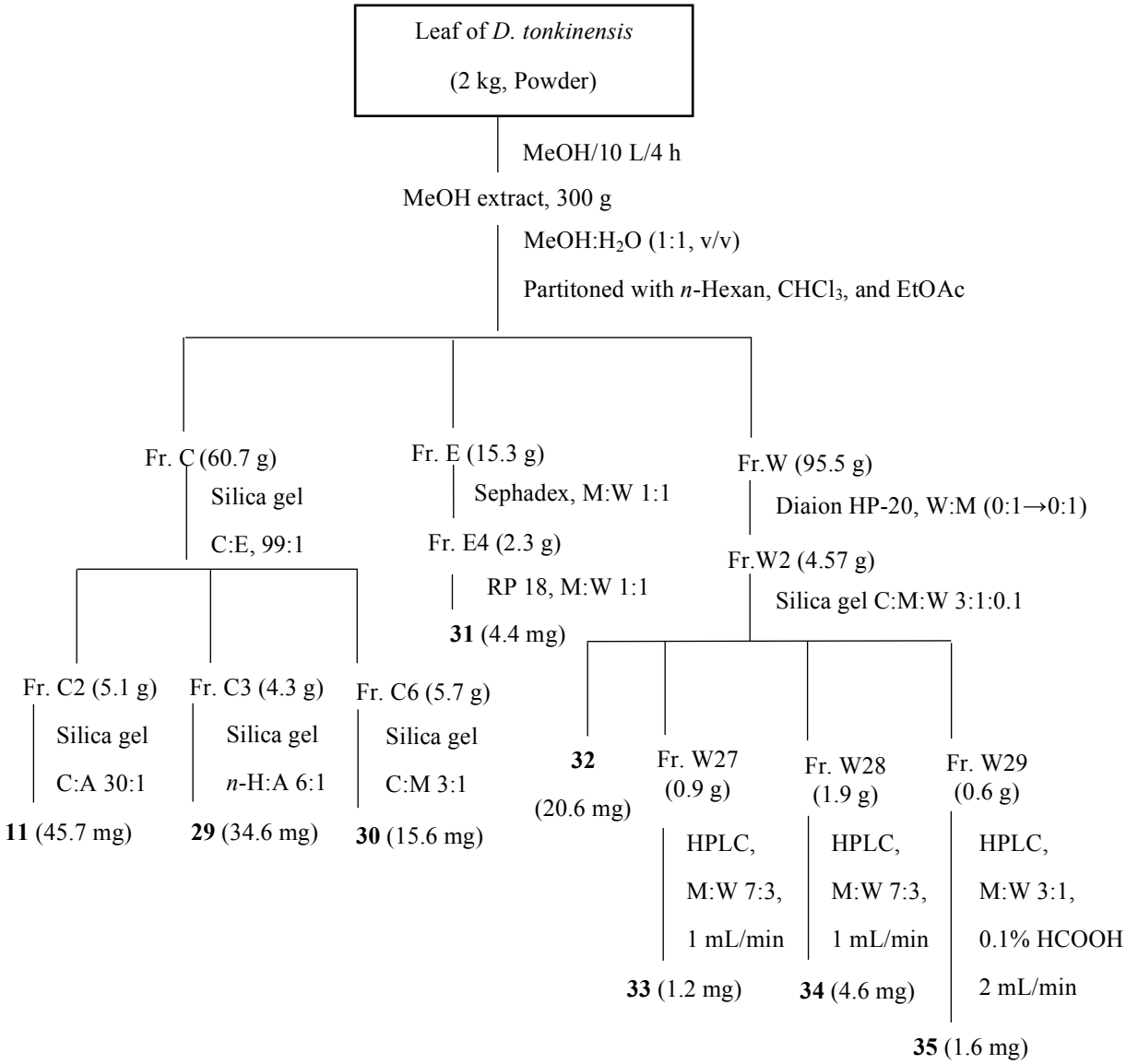


Figure 10. ^{1}H-NMR/^{13}C-NMR spectra of compound **25** in CD_3OD

2.3. Phytochemical investigation of leaf extract and structural elucidation

2.3.1. Isolation procedure



From the fr. $CHCl_3$ and aqueous residue of the methanol extract of *D. tonkinensis* leaf, two new compounds **31** and **33**, and six known compounds **11**, **29**, **30**, **32**, **34** and **35** were isolated. The chemical structures of the known compounds were identified as biochanin A (**11**), genistein (**29**), orobol (**30**), dalsissooside (**32**), ambocin (**34**), lanceolarin (biochanin A **7**-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside) (**35**) by comparison of their NMR spectroscopic data with the literature data, respectively [11, 40-43]. Among them, biochanin

A (**11**) had also been found in the heartwood, and ambocin (**34**), an isoflavone glycoside, was isolated for the first time from genus *Dalbergia*.

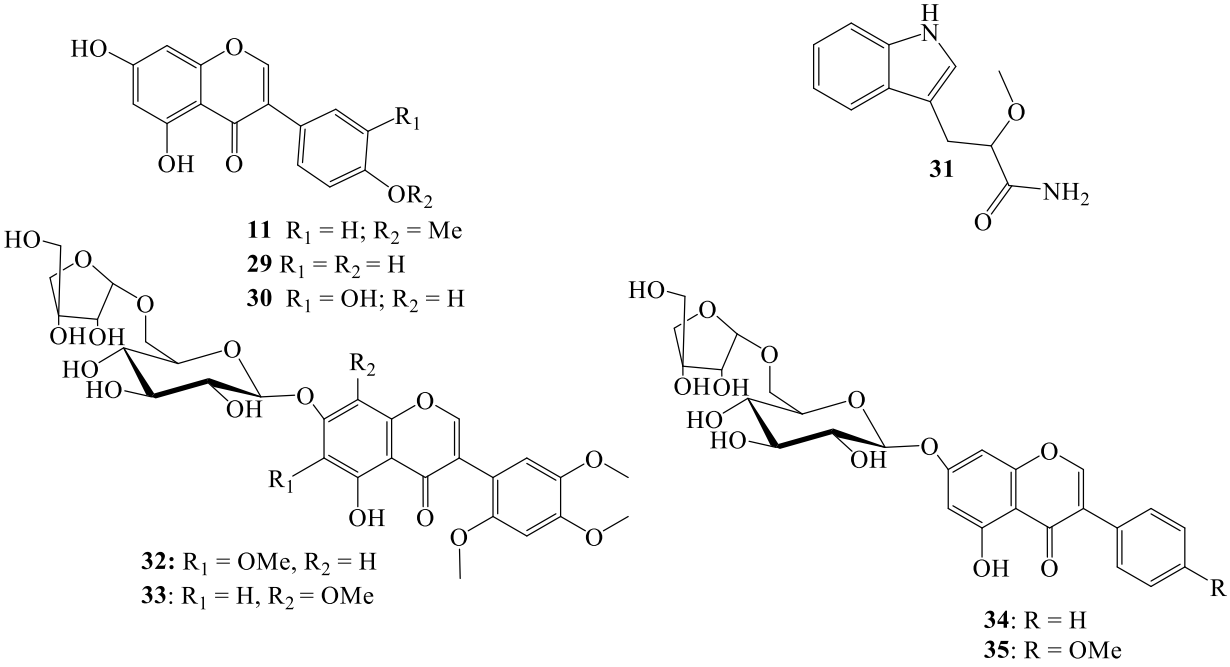


Figure 11. Compounds isolated from leaf of *D. tonkinensis*

2.3.2. Structural elucidation of new compounds 31 and 33

Compound (**31**): 3-(1*H*-indol-3-yl)-2-methoxypropanamide

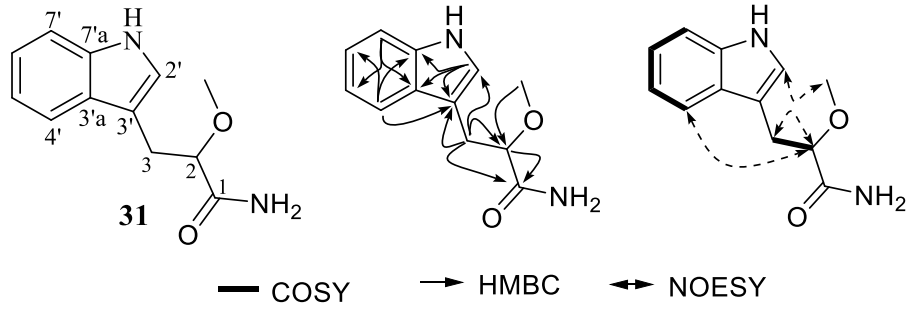


Figure 12. Structure, and COSY, HMBC and NOESY key correlations of **33**

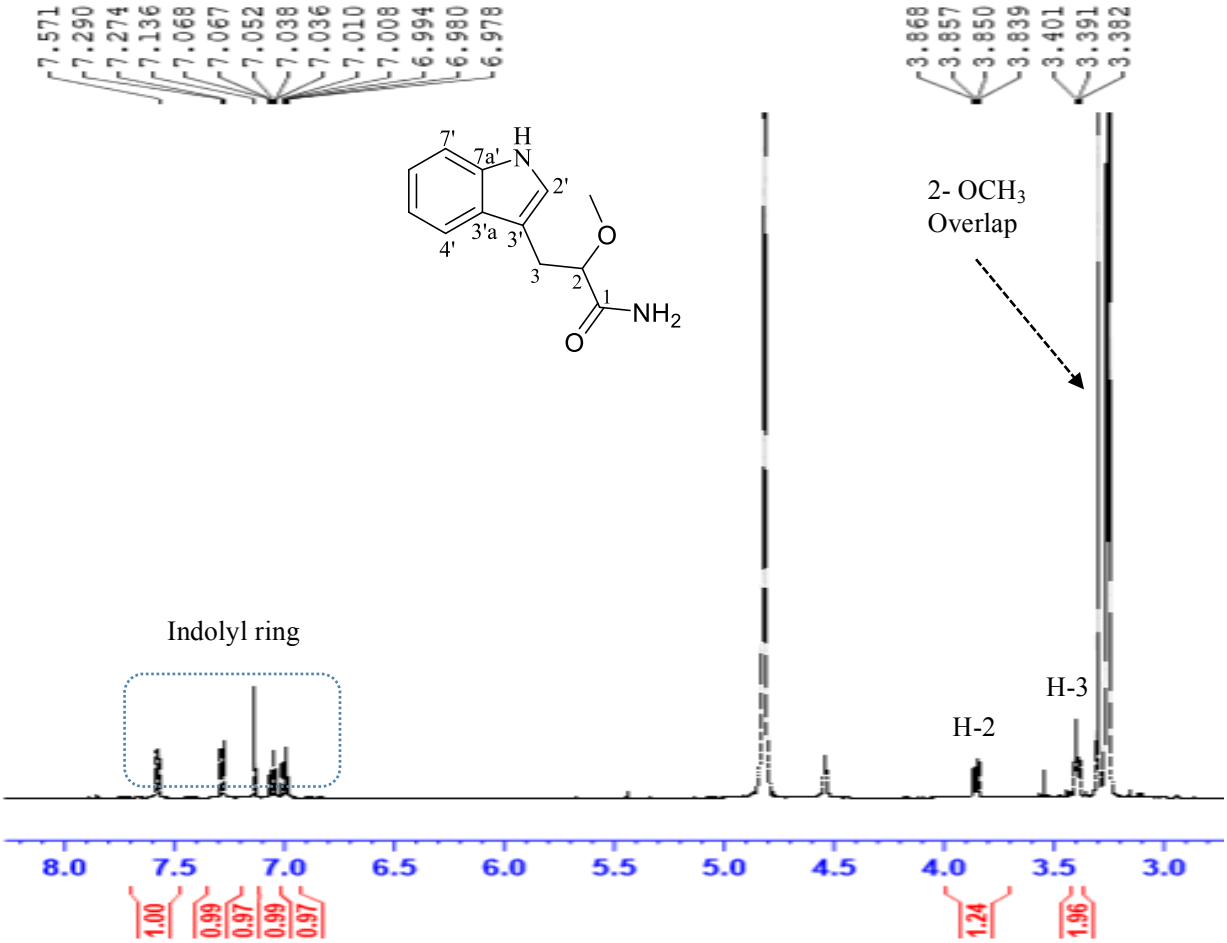
Table 4. ^{1}H-, and ^{13}C-NMR data of compound **31** in CD_3OD

3-(1*H*-indol-3-yl)-2-methoxypropanamide (**31**)					
Position	δ_H (*J* in Hz)[a)]	δ_C[b)]	**Position**	δ_H (*J* in Hz)	δ_C
1		170.3	4'	7.59 (d, 8.0 Hz)	117.6
2	3.85 (dd, 9.0, 5.5 Hz)	79.2	5'	6.98 (td, 8.0, 1.0 Hz)	118.6
3	3.39 (dd, 9.0, 5.5 Hz)	23.3	6'	7.01 (td, 8.0, 1.0 Hz)	121.2
2'	7.27 (s)	124.0	7'	7.28 (d, 8.0 Hz)	111.0
3'		107.6	7'a		136.7
3'a		126.9	2-OCH_3	3.25 (s)	51.3

[a)]500 MHz, [b)]125 MHz

Compound **31** was obtained as a white powder and its molecular formula $C_{12}H_{14}N_2O_2$ was deduced from its quasi-molecular ion peak at *m/z* 241.0953 $[M+Na]^+$ in the its positive HR-ESI-MS data. Prominent fragment ions at *m/z* 188, 144 and 118 from APCI mass were due to the loss of a methoxy group and an amide group, thereby supporting the presence of an indolyl unit. The IR spectrum of **31** showed absorption bands at 3416 and 1629 cm^{-1}, which could be attributed to amine and amide groups, respectively [44]. The UV spectrum showed absorption maxima at λ_{max} 281 and 288 nm, which are characteristic of a tryptophan derivative [44]. The ^{1}H-NMR spectrum of **31** displayed signals similar to those of 2-methoxy-3-(3-indolyl)-propionic acid [45] with resonances from an indolyl ring [H-4' (δ_H 7.59, 1H, d, 8.0 Hz), H-5' (δ_H 6.98, 1H, td, 8.0, 1.0 Hz), H-6' (δ_H 7.01, 1H, td, 8.0, 1.0 Hz), H-7' (δ_H 7.28, 1H, d, 8.0 Hz) and H-2' (δ_H 7.27, s)] and a 2-methoxypropanoyl moiety [H-2 (δ_H 3.85, 1H, dd, 9.0, 5.5 Hz), H_2-3 (δ_H 3.39, 2H, dd, 9.0, 5.5 Hz), and 2-OCH_3 (δ_H 3.25, s)]. The ^{13}C-NMR and HSQC spectra of **31** exhibited 12 carbon signals, comprising of 8 aromatic carbons from δ_C 107.6–136.7, a carboxyl carbon at δ_C 170.3 (C-1), an *O*-bearing methine carbon at δ_C 79.2 (C-2), a methylene carbon at δ_C 23.3 (C-3) and a methoxy carbon at δ_C 51.3 (2-OCH_3) (Fig. 12). The structures of **31** was also confirmed by analysis of its COSY, HMBC and NOESY spectra (Fig. 12). The ^{1}H-^{1}H COSY spectrum showed cross peaks for H-4'/H-5'/H-6'/H-7' and H-2/H_2-3 and the HMBC spectrum established the indolyl ring by the J^2 and J^3 correlations from

H-2' to C-3', C-3'a and C-7'a. Meanwhile, the HMBC cross peaks from OCH_3 to H-2, from H-2 and H_2-3 to C-1, and from H_2-3 to C-2' and C-3' confirmed the presene of a 2-methoxypropanoyl unit and its connection to carbon C-3'. In accordance with 2-methoxy-3-(3-indolyl)-propionic acid, the ^{1}H-^{1}H NOESY correlations between H-2 and H-4' and between 2-OCH_3 and H-2' were observed [45]. Based on these spectroscopic data, compound **31** was determined to be 3-(1*H*-indol-3-yl)-2-methoxypropanamide.



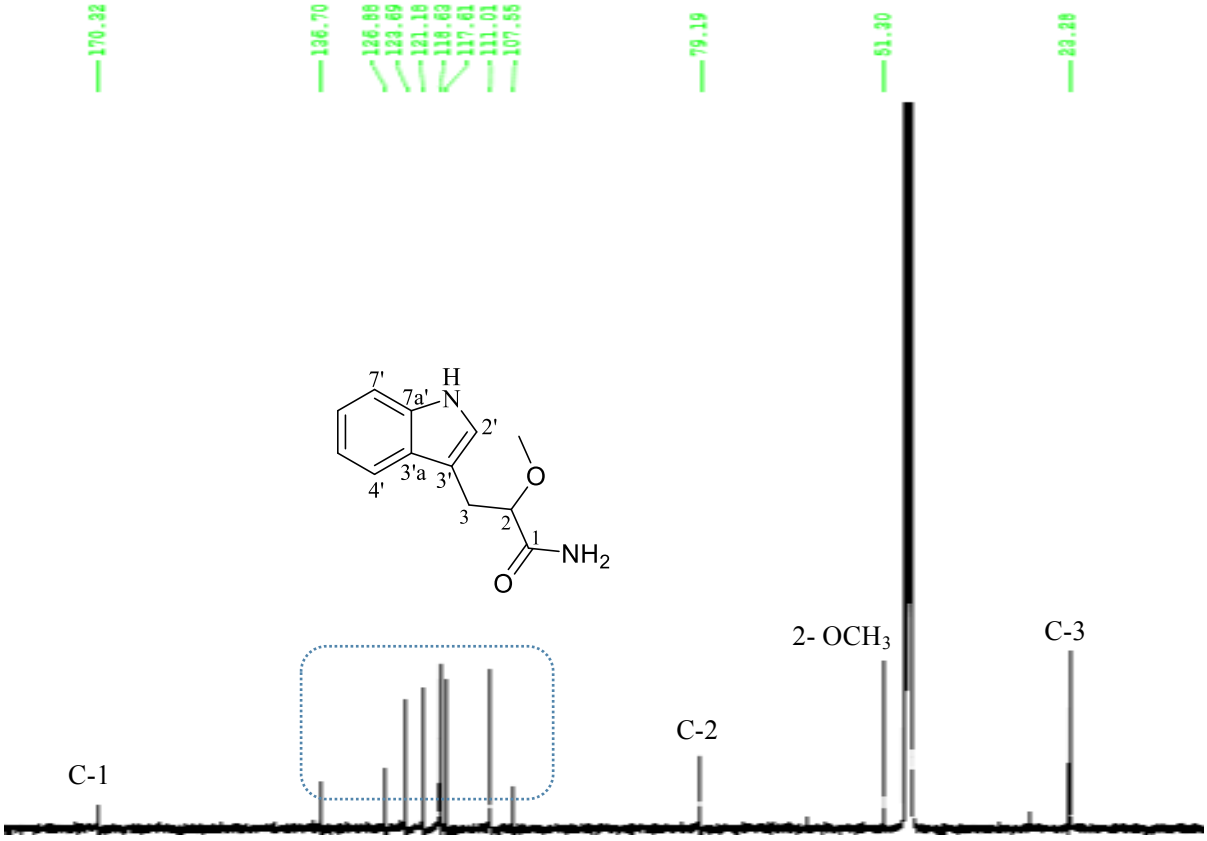


Figure 13. ^{1}H-NMR/^{13}C-NMR spectra of compound **31** in CD_3OD

Compound **33**: Isocaviunin 7-O-β-apiofuranosyl-(1→6)-β-glucopyranoside

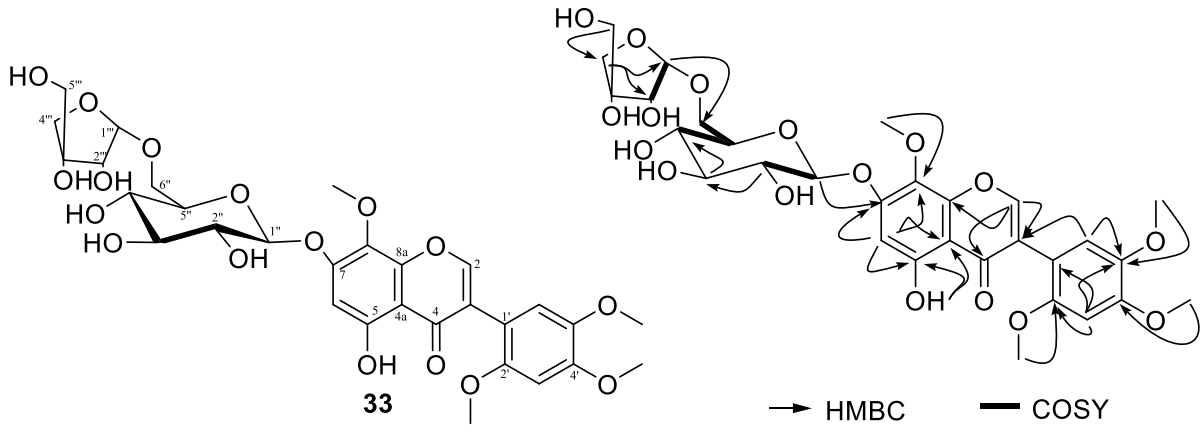


Figure 14. Structure, and COSY and HMBC key correlations of **33**

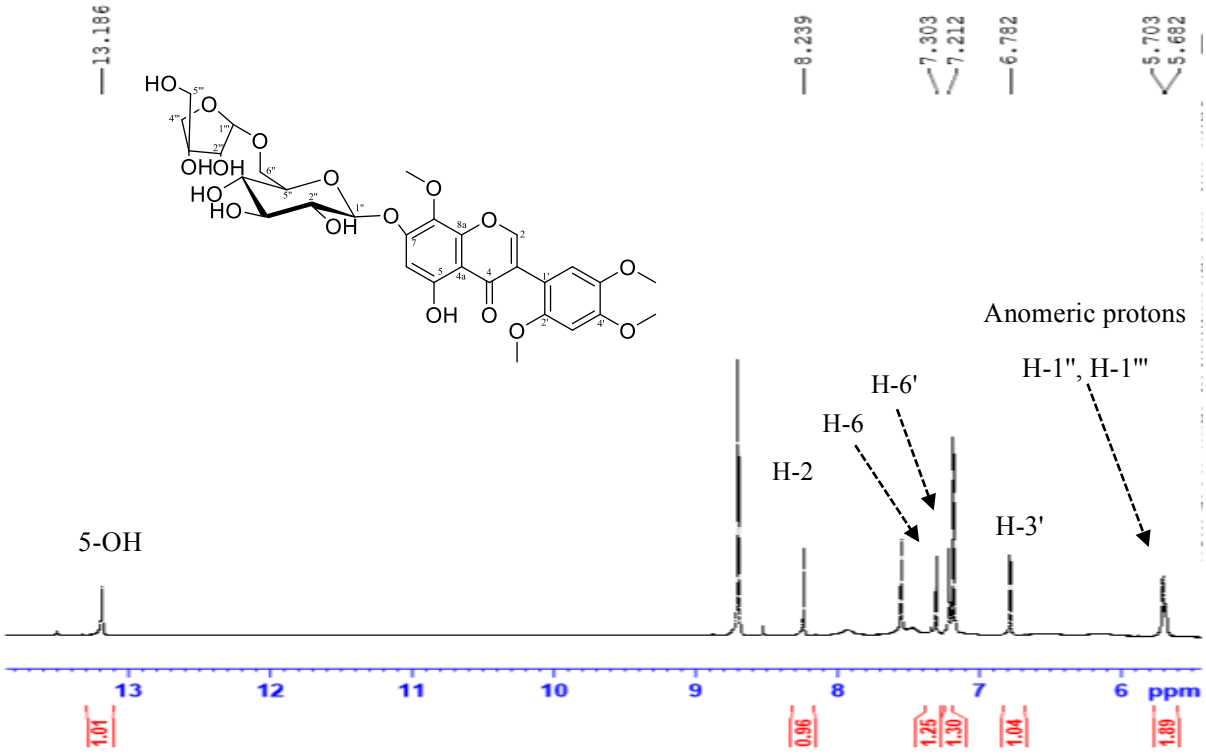
Table 5. ^{1}H-, and ^{13}C-NMR data of compound **33** in C_5D_5N

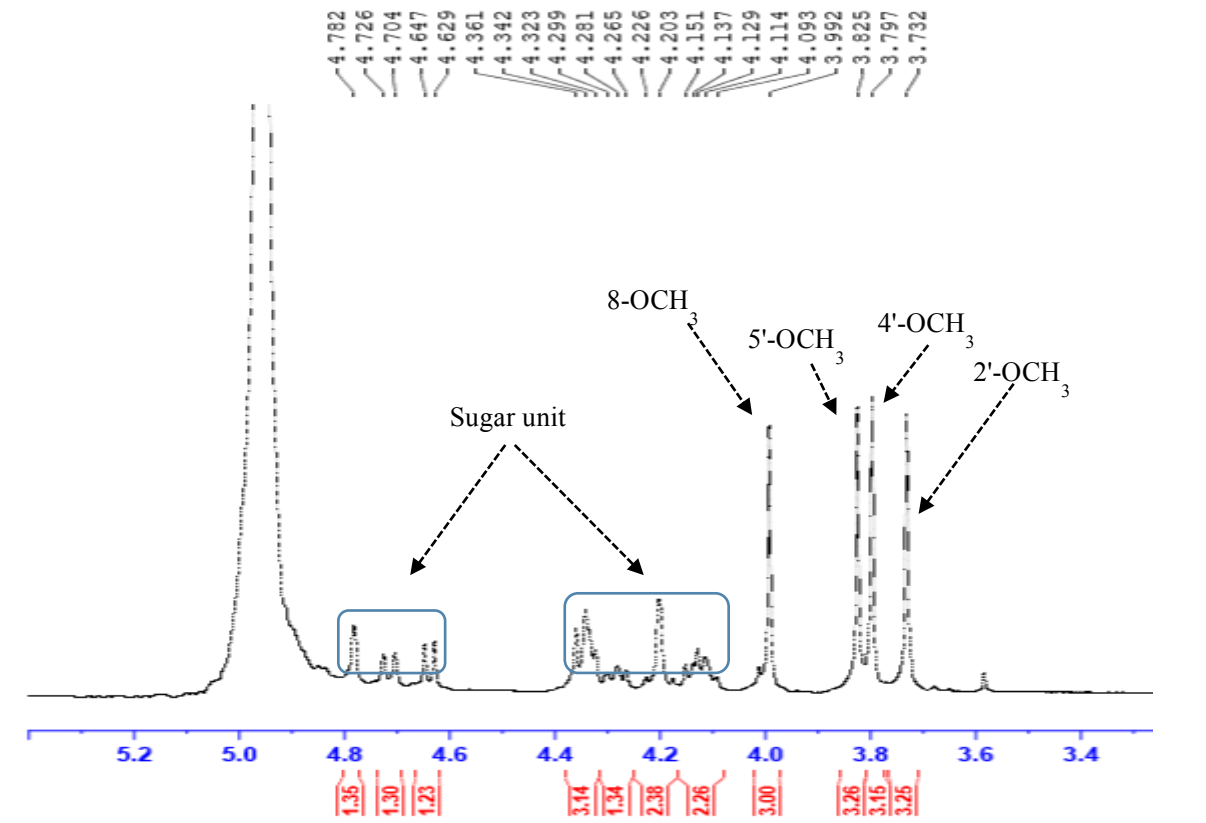
Isocaviunin 7-*O*-β-apiofuranosyl-(1→6)-β-glucopyranoside (**33**)					
Position	δ_H (*J* in Hz)[a]	δ_C[b]	**Position**	δ_H (*J* in Hz)	δ_C
2	8.24 (s)	155.7	2"	4.34 (m)	74.5
3		120.6	3"	4.78, (m)	77.7
4		181.2	4"	4.11 (m)	71.2
4a		106.9	5"	4.27 (m)	77.3
5		157.8	6"	4.71 (d, 11.0 Hz)	68.6
				4.14 (m)	
6	7.30 (s)	100.1	1‴	5.70[d] (d, 2.5 Hz)	111.0
7		157.2	2‴	4.32 (m)	78.4
8		130.0	3‴		80.2
8a		150.9[c]	4‴	4.63 (d, 9.0 Hz)	75.0
				4.36 (m)	
1'		111.3	5‴	4.20 (t, 11.5 Hz)	65.6
2'		152.6	5-OH	13.20 (s)	
3'	6.78 (s)	98.9	8-OCH_3	3.99 (s)	61.5
4'		150.9[c]	2'-OCH_3	3.73 (s)	55.9
5'		143.6	4'-OCH_3	3.80 (s)	56.3
6'	7.21 (s)	117.0	5'-OCH_3	3.83 (s)	56.7
1"	5.68[d] (d, 7.5 Hz)	102.2			

[a]500 MHz, [b]125 MHz, [c]may be interchangeable, [d]the assignments were made by HSQC and HMBC correlations and comparison with **32**

Compound **33** was obtained as white amorphous powder. Its molecular formula $C_{30}H_{36}O_{17}$ was deduced from the quasi-molecular ion peak at *m/z* 707.1577 $[M+K]^+$ in the positive HR-FAB-MS data and from the *m/z* 707 $[M+K]^+$, 669 $[M+H]^+$, and 667 $[M–H]^–$ peaks in the positive (negative) FAB-MS data. The IR spectrum of **33** showed absorption bands at 3510, 3378, 1654, and 1581 cm^{-1}, which could be attributed to hydroxyl, chelated hydroxyl, α,β-unsaturated carbonyl, and carbon-carbon double bond conjugated carbonyl groups, respectively. The UV spectrum of **33** showed absorption maxima at λ_{max} values of 205, 263 and 295 nm, which are characteristic of an isoflavone derivative [40]. The ^{1}H-NMR spectrum of **33** displayed a spectral pattern similar to that of isocaviunin 7-gentibioside (compound isolated from *D. sissoo*) with resonances from two components [40], which

include an isocaviunin nucleus [5-OH (δ_H 13.20, 1H, s), H-2 (δ_H 8.24, 1H, s), H-6 (δ_H 7.30, 1H, s), H-6' (δ_H 7.21, 1H, s), H-3' (δ_H 6.78, 1H, s), 8-OCH_3 (δ_H 3.99, 3H, s), 5'-OCH_3 (δ_H 3.83, 3H, s), 4'-OCH_3 (δ_H 3.80, 3H, s), and 2'-OCH_3 (δ_H 3.73, 3H, s)] and an *β*-apiofuranosyl-(1→6)-*β*-glucopyranosyl moiety [H-1''' (δ_H 5.70, d, 2.5 Hz), H-1'' (δ_H 5.68, d, 7.5 Hz), and 11H (δ_H 3.99-4.78)] (Table 5 and Fig. 14). The ^{13}C-NMR and HSQC spectra of **33** (C_5D_5N) contained 30 carbon signals (12×C, 11×CH, 3×CH_2, 4×CH_3), which were assignable to an isoflavone aglycone, a *β*-apiofuranosyl residue [H-1''' (J = 2.5 Hz)], and a *β*-glucopyranosyl bridge [H-1'' (J = 7.5 Hz)] [40]. Regarding the 2D-NMR analysis, the HMBC spectrum established the isocaviunin ring by the J^2 and J^3 correlations H-2/C-3, C-4 and C-8a; H-6/C-4a, C-5, C-7, and C-8; 5-OH/C-4a and C-5; H-3'/C-1' and C-5'; H-6'/C-5' and C-3; 8-OCH_3/C-8; 2'-OCH_3/C-2; 4'-OCH_3/C-4; and 5'-OCH_3/C-5, and a glycone unit was confirmed by the cross peaks H-2''/C-3''; H-3''/C-4''; H-4'''/C-1''' and C-2'''; and H-5'''/C-4''' in the HMBC spectrum as well as H-1''/H-2''; H-4''/H-5''; H-5''/H-6''; and H-1'''/H-2''' in the COSY spectrum (Fig. 14). Furthermore, the key ^{1}H-^{13}C HMBC correlation H-1'''/C-6'' evidently generated a 1→6 linkage between two sugar units, and the cross peak H-1''/C-7 indicated the connection between glycone and aglycone at C-7. In addition, the acid hydrolysis of **33** yielded the aglycone, the NMR and MS data of which were identical to those of isocaviunin [46]. Based on these findings, compound **33** was elucidated to be isocaviunin-7-*O*-*β*-apiofuranosyl-(1→6)-*β*-glucopyranoside, trivially named daltonkinensiside (**33**).


13.186
8.239
7.303
7.212
6.782
5.703
5.682
Anomeric protons
H-1", H-1'''
H-6'
H-6
H-2
H-3'
5-OH
13
12
11
10
9
8
7
6
ppm
1.01
0.96
1.25
1.30
1.04
1.89


4.782
4.726
4.704
4.647
4.629
4.361
4.342
4.323
4.299
4.281
4.265
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4.203
4.151
4.137
4.129
4.114
4.093
3.992
3.825
3.797
3.732
8-OCH3
5'-OCH3
4'-OCH3
2'-OCH3
Sugar unit
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
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3.25

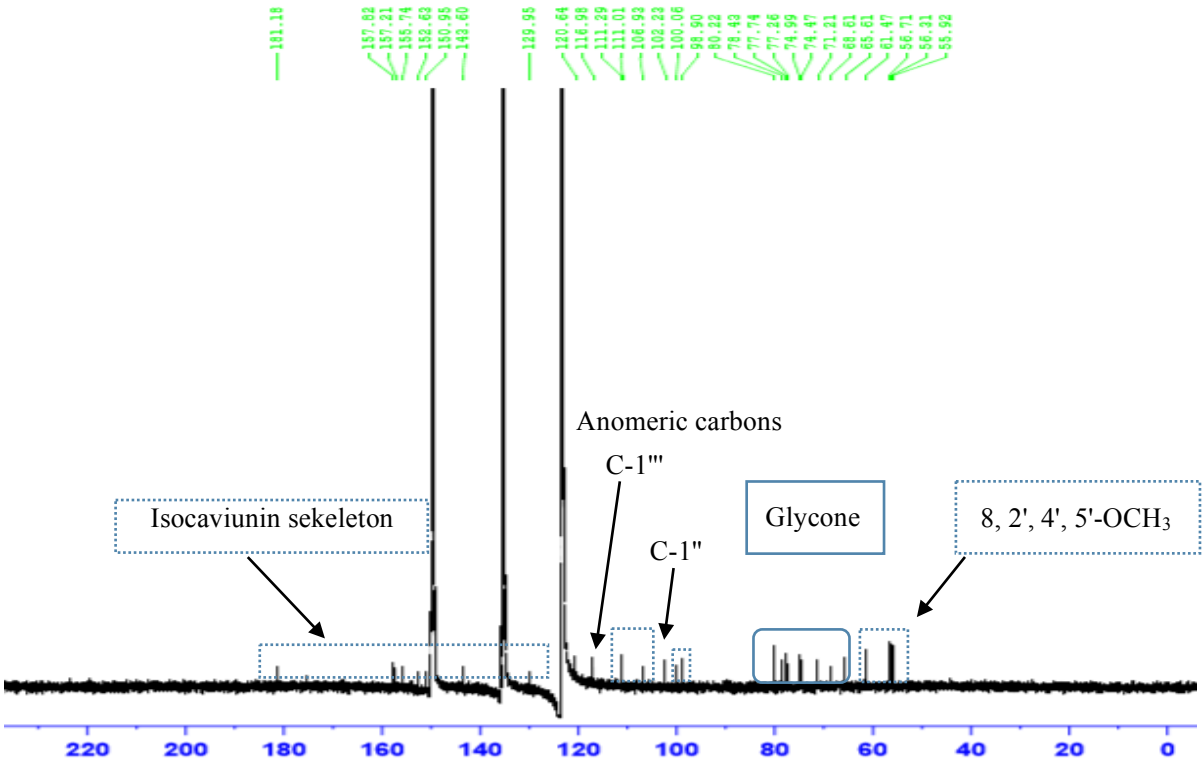
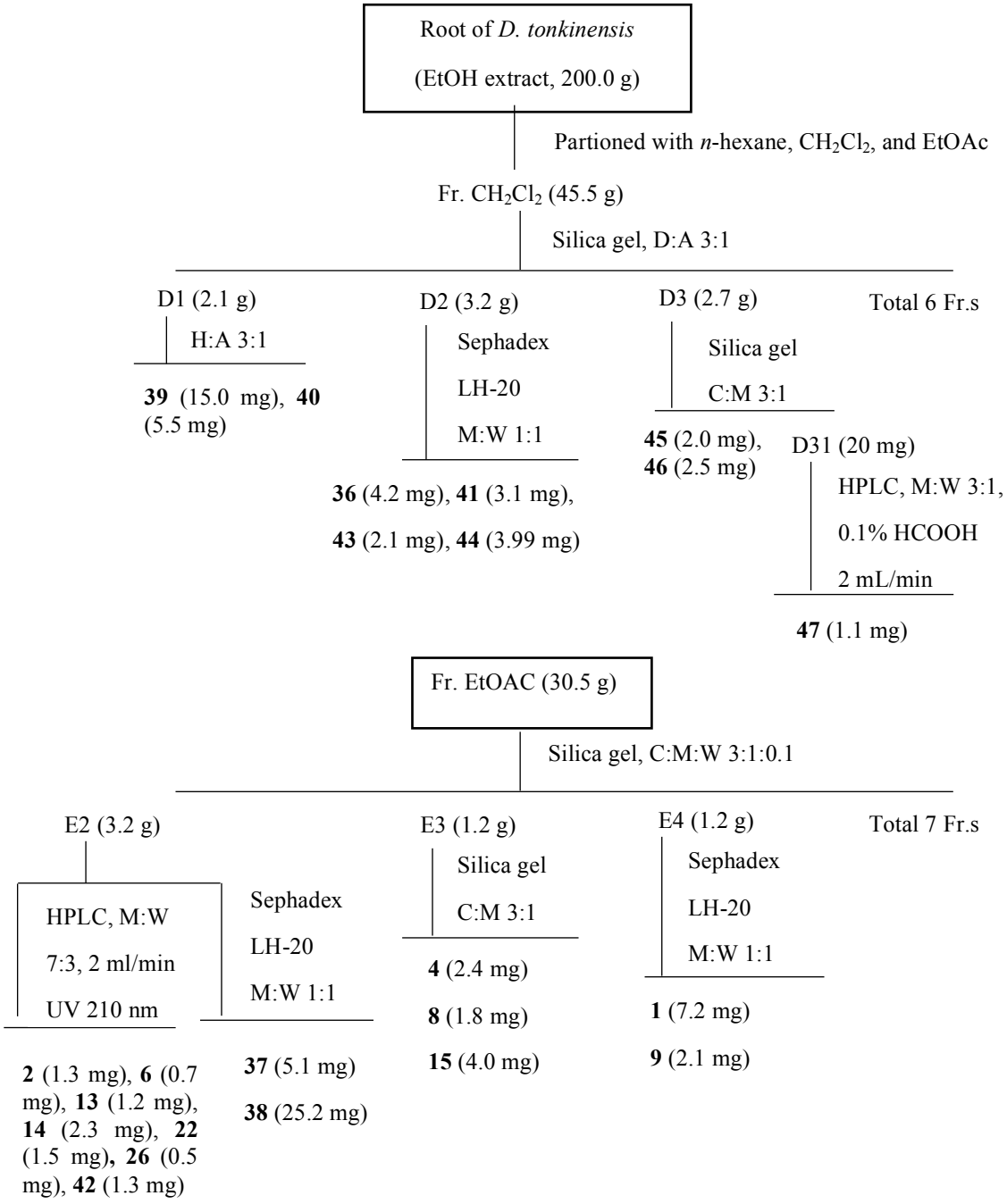


Figure 15. ^{1}H-NMR/^{13}C-NMR spectra of compound **33** in C_5D_5N

2.4. Phytochemical investigation of root extract and structural elucidation

2.4.1. Isolation procedure



From fr.s CH_2Cl_2 and EtOAc of the ethanol extract of *D. tonkinensis* root, one new compound **47** and twenty-two other known compounds were isolated. Among them, compounds **1-2**, **4**, **6**, **8**, **9**, **13-15**, **22** and **26** have already been isolated from the heartwood.

The chemical structures of the other known compounds were identified to be tectorigenin (**36**), tectoridin (**37**), tectorigenin 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**38**), 1-monolaurin (**39**), and 1-monomyristin (**40**), (*3R*)-vestitol (**41**), melilotolcarpan D (**42**), daidzein (**43**), 3'-hydroxyldaidzein (**44**), formononetin-7-*O*-*β*-D-glucopyranoside (**45**), and methyl 4-hydroxybenzoate (**46**) [1, 47-51]. Two isoflavone glycosides **37** and **38** were isolated for the first time from the root, whereas mono-acylglycerides **39** and **40** and mono-phenol **46** have never been identified in the family Fabaceae before.

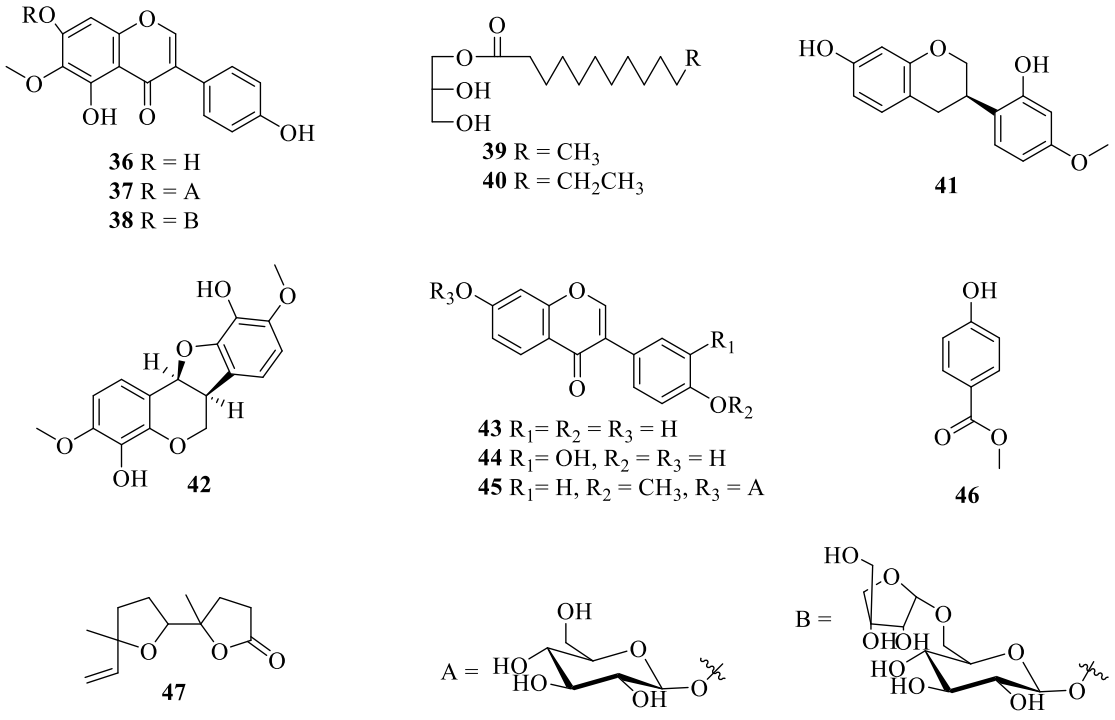


Figure 16. Compounds isolated from *D. tonkinensis* root

2.4.2. Structural elucidation of new compound 47

2,5'-Dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47**)

Rel-(2*S*,2'*S*,5'*S*)-2,5'-Dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47a**)

Rel-(2*S*,2'*S*,5'*R*)-2,5'-Dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47b**)

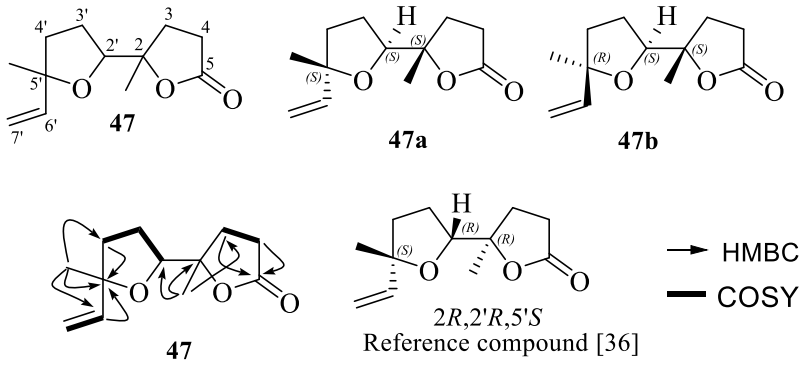


Figure 17. Structure, and COSY and HMBC key correlations of isolated compound **47**

Table 6. ^{1}H-, and ^{13}C-NMR data of compound **47** in CD_3OD

Position	δ_H (J in Hz)[a]	δ_C[b]	**Position**	δ_H (J in Hz)[a]	δ_C[b]
2		88.3	3'	1.70 (m) 1.99 (m)	26.7
2-CH_3	1.35 (s)	22.4	4'	1.76 (m) 1.95 (m)	36.9
3	1.91 (m) 2.31 (m)	28.1	5'	-	83.2
4	2.62 (m)	29.1	5'-CH_3	1.28 (s) and 1.30 (s)	25.7
5		178.4	6'	5.97 (dd, 10.5, 17.5)* 5.84 (dd, 10.5, 17.5)**	143.7
2'	4.10 (dd, 3.0, 10.0)	83.6	7'	5.18 (d, 17.5)* 4.99 (d, 10.5)**	110.6

[a]500 MHz, [b]125 MHz, ***47a**, ****47b**

Compound **47** was obtained as yellowish amorphous powder. The molecular formula of **47** corresponded to $C_{12}H_{18}O_3$, as deduced from the molecular ion peak at *m/z* 210.1253 $[M]^+$ in its HR-EI-MS data. The absorptions at 1771 and 1607 cm^{-1} in the IR spectrum showed the presence of a γ-lactone and a double bond, respectively. The ^{1}H, and ^{13}C-NMR data of **47** included two methine groups, five methylene groups, two methyl groups, two oxygenated quaternary carbons, and one lactone carbon (Table 6 and Fig. 17). Based on the HSQC, HMBC, COSY, and NOESY extensive analysis and comparison with data recorded in the literature [37], compound **47** was assigned as a stereoisomer of *rel*-(2*R*,2'*R*,5'*S*)-2,5'-dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(2*H*)-one (a component was isolated from *D. odorifera* T.

Chen) [37]. As shown in Fig. 17, the appearance of tetrahydrofuran (THP) and γ-lactone rings was confirmed by the COSY correlations H-3/H-4, H-2'/H-3', and H-3'/H-4' as well as the HMBC cross peaks H-3 and H-4/C-5 and H-4'/C-5'. Furthermore, the COSY correlation H-6'/H-7' and the HMBC interactions H-6'/C-5', 5'-CH_3/C-5' and C-4', and 2-CH_3/C-2 and C-3 indicated that the vinyl group occurred at C-5', and two methyl groups were attached at the C-2 and C-5' positions. The connection between the THP ring and the γ lactone ring can be explained by the key HMBC correlation 2-CH_3/C-2'.

Compound **47** was found to have diffential δ_H at proton 5'-CH_3 and H-6' and a small optical rotation of –1.2 (*c* 1.1, MeOH). As a consequence, we supposed that compound **47** was a diastereomeric mixture. Thus, compound **47** was further analyzed by a chiral HPLC column (OD-RH, 150 x 4.6 mm) with a mobile phase of *n*-hexane-2-propanol (80:20, v/v), thereby showing two peaks at t_R = 11.2 (35%) and t_R = 15.81 (65%). Therefore, compound **47** was identified as two diastereomers *rel*-(2*S*,2'*S*,5'*S*)-2,5'-dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47a**) and *rel*-(2*S*,2'*S*,5'*R*)-2,5'-dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47b**).

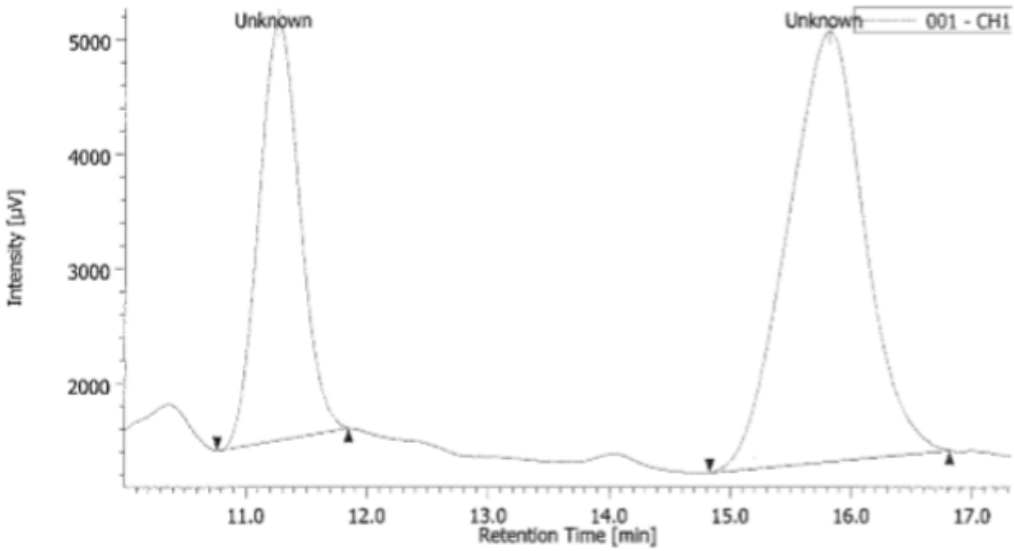
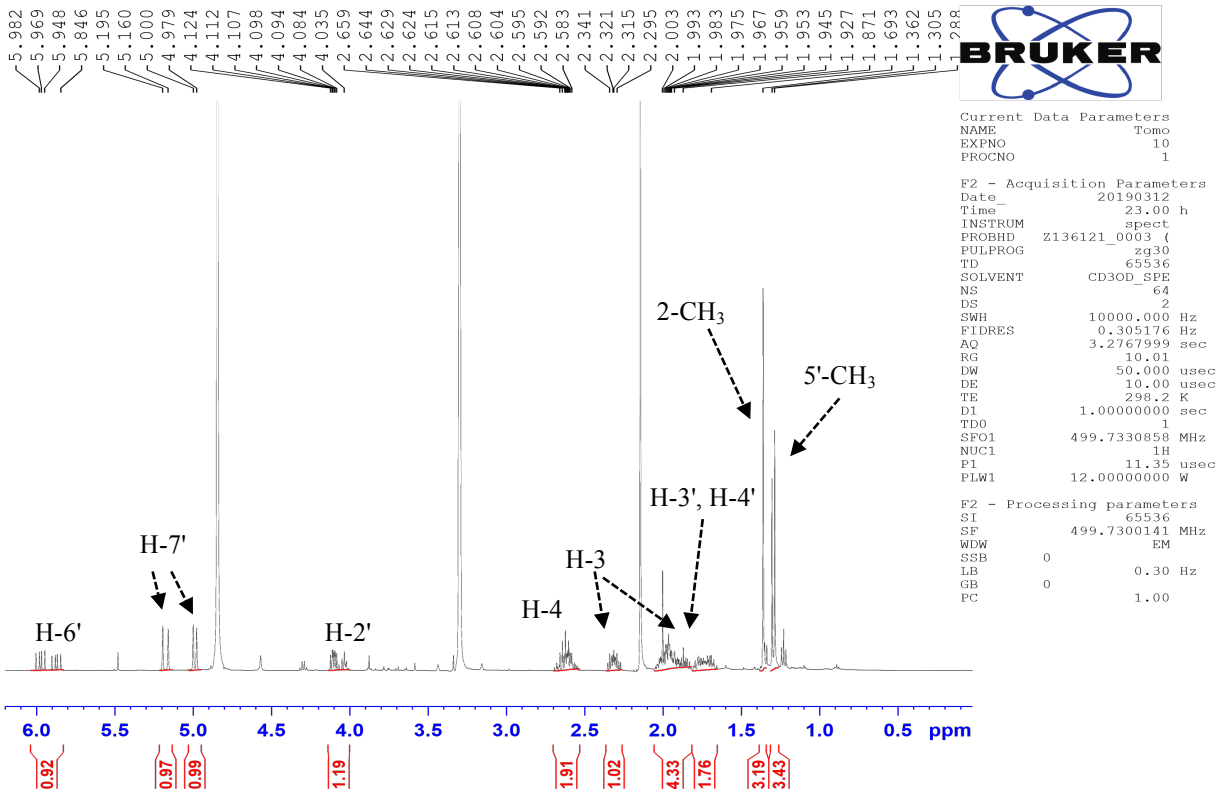


Figure 18 Chiral HPLC chromatogram of diastereomers **47a** and **47b**



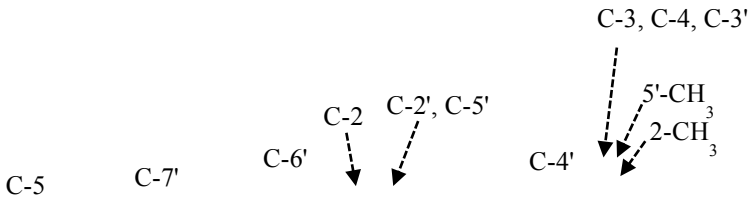


Figure 19. ^{1}H-NMR/^{13}C-NMR spectra of compound **47** in CD_3OD

Figure 20. NOESY spectrum of compound **47** in CD_3OD

2.5. Chemotaxonomic significances from the isolated constituents

A vast data evidently showed that flavonoids and their glycosides possessed the high amounts in the species of the genus *Dalbergia* and the family Fabaceae [1]. The present phytochemical studies on *D. tonkinensis* species led to the isolation and identification of 47 constituents from its several parts for the first time.

Two new unusual flavanones (2*S*)-6,8-dicarboxyethylpinocembrin (**20**) and (2*S*)-8-carboxyethylnaringenin (**21**) could be seen as the first products of mono-and/or di-substitution of carboxyethyl group at the positions C-6 and/or C-8 of pinocembrin (**5**) and naringenin (**3**). The occurrence of **20** and **21** reveals not only a key feature of chemical structure but also the significant findings regarding the species *D. tonkinensis* and the pea family for the first time. Hence, two new carboxyethylflavanones **20** and **21** may be valuable chemotaxonomic markers for the family Fabaceae.

This study also provides new information concerning isolated flavanone metabolites, such as buteaspermanol (**8**), which is characteristic of heartwood and root of *D. tonkinensis* species, since this compound had only been found in bean family [this compound was only isolated from two species of the subfamily Faboideae, *Butea monosperma* (from stem bark), and *Spatholobus suberectus* (from stem)] [52,53]. Therefore, compound **8** was isolated from the genus *Dalbergia* for the first time, and its occurrence supports the arguments to define the close releationship among the species, the genera of the subfamily Faboideae. Likewise, another flavanone glycoside naringin (**28**), which is common in plants and mostly distributed in the *Citrus* genus [54], and in *Securigera securidaca* flower (Fabaceae) [55], but it is isolated for the first time from the genus *Dalbergia*.

Of the remaining flavonoids, formononetin (**1**), liquiritigenin (**4**), medicarpin (**6**), and isoliquiritigenin (**14**) are also constituents of the bark of the Vietnamese plant *D. oliveri* [56], and sativanone (**2**), naringenin (**3**), pinocembrin (**5**), biochanin A (**11**), calycosin (**12**), 3'-*O*-methylviolanone (**15**), and genistein (**29**) are constituents of *D. parwiflora* heartwood [48]. 3'-Hydroxy-2,4,5-trimethoxydalbergiquinol (**7**), butin (**9**), 3'-hydroxymelanettin (**10**), eriodictoyl (**13**), sulfuretin (**19**), dalbergin (**22**), homoferreirin (**23**), orobol (**30**), tectorigenin (**36**), (3*R*)-vestitol (**41**), melilotocarpan D (**42**), hydroxyldaidzein (**43**), and 3'-hydroxyldaidzein (**44**) are isolated from *D. odorifera* T. Chen [1], while luteolin (**16**), and apigenin (**18**) are derived from *D. paniculata* flower [57]. Quercetin (**17**) is a constituent of *D. sericea* leaf [58] and kaemfperol (**26**) is precipitated out of tender leaf of *D. sissoo* [59].

In accordance with results of the Vietnamese plant *D. vietnamensis*, isoflavone glycosides **32-35**, **37**, and **38** were found to have 7-glycosyl moieties of *O*-β-D-glucopyranosyl, *O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl, and/or *O*-(5-*O*-trans-*p*-coumaroyl)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl [60]. Among them, isoflavone glycoside (**33**) is a new isomer of compound **32**. Ambocin (**34**), an isoflavone glycoside is also presented in the *D. tonkinensis* leaf but it is found for the first time in genus *Dalbergia.* In the mean time, tectoridin (**37**) has been isolated from *D. volubilis* flower [61], but dalsissooside (**32**), and tectorigenin 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**38**) are new compounds to date, and they have only been found in *D. sissoo* leaf [40,49]. Formononetin-7-*O*-β-D-glucopyranoside (**45**) has been identified presenting in stem bark of *D. paniculata* [62]. However, both **32**, **37** and **38** were first observed in *D. tonkinensis.*

Based on these results, a wide variety of flavonoids isolated from *D. tonkinensis* has proven the close relationship among the species of the genus *Dalbergia*, and the related genera of the family Fabaceae.

HPLC-MS and GC-MS techniques were used to detect indole-3-acetic acid derivatives in the seeds of *D. dolichopetala* [63,64], but to date, no alkaloids had been isolated from the genus *Dalbergia*. Thus, 3-(1*H*-indol-3-yl)-2-methoxypropanamide (**31**), a unique alkaloid, is likely to be an important chemotaxonomic marker for this genus. Significantly, 1*H*-indole-3-propanamide, the other indole alkaloid, showed a similar substitution pattern to **31**, but had only ever been isolated from two species of the subfamily Faboideae *Erythrina brucei* and *E. addisoniae* [65,66]. These results add to the evidence demonstrating the close relationship among the species in both subfamily.

Sesquiterpenes are rarely reported from the genus *Dalbergia*. To date, several isolated sesquiterpenes, such as (3*S*,6*S*,7*R*)-3,7,11-trimethyl-3,6-epoxy1,10-dodecadien-7-ol and *rel*-(3*S*,6S,7*R*)-3,7,11-trimethyl-3,6-epoxy-1,10-dodecadien-7-ol, or 3,7,11-trimethyldodeca-1,10-diene-3,6,7-triol, which have been identified as derivatives of nerolidol, have only been found in *D. odorifera* growing in China [37,67]. However, an isolated heterocyclic metabolite, 3,10-epoxy-3,7,11-trimethyldodeca-1,6-dien-11-ol, trivially named neroplomacrol (**24**), is one of the new compounds isolated from *Oplopanax horridus* (Sm.) Miq. (family Araliaceae), and this is the second time to report the isolation of **24** from a natural source. Compound **24** was first isolated from a plant in the legume family and the type of nerolidol derivatives present can also be seen as a phylogenetic differentiator between the Vietnamese *D. tonkinensis* species and *D. odorifera* species [68]. Last of this, *rel*-(3*R,*6*S,*7*S*)-6α-hydroxycyclonerolidol (**25**), and 2,5'-dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47**), which are new sesquiterpenes in nature, are diastereoisomers of *rel*-(3*R*,6*R*,7*S*)-3,7,11-trimethyl-3,7-epoxy-1,10-dodecadien-6-ol and *rel*-(2*R*,2'*R*,5'*S*)-2,5'-dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(2*H*)-one (components were isolated from *D. odorifera* heartwood), respectively [37]. It

therefore suggests that the environments such as geographic factors might be playing a role in affecting the native plants.

Despite the fact that phenolic compounds were found to be the second most common type of component isolated from *Dalbergia* species [1], aldehydic phenols were not available. To date, only two compounds, voludal and 2-hydroxy-3,4-dimethoxybenzaldehyde have been isolated from the non-green branches of *D. volubilis* and *D. odorifera* heartwood, respectively [69,70]. Hence, the small molecule 4-formyl phenol (**27**) is a useful agent to recognize either *D. tonkinensis* or genus *Dalbergia*. A similar compound, named killitol (**46**), was one of cytotoxic potential agents of chloroform fraction of marine plant *Thalassia testudinum* but it was found in the family Fabaceae for the first time [51].

Last but one least, mono-acylglycerides named 1-monolaurin (**39**) and tridecanoic α-monoglyceride (**40**) widely distributed in plant kindom. For instance, compound **39** presents in coconut oils, or is one of glycerols of the powder of berries of *Serenoa repens* [50]. Nevertheless, from chemotaxonomic point of view, we recognize that these compounds and related glycerides have never been found in the family Fabaceae.

In conclusion, the present studies imply that *D. tonkinensis* Prain contains interesting secondary metabolites, consisting of various typical flavonoids, along with alkaloids, sesquiterpenes, phenols, and glycerides, which provide a basic foundation for further researches.

Table 7. Chemotaxonomic summaries of phytochemicals of *D. tonkinensis*

Compounds	**Remarks**
Formononetin (**1**)	B
Sativanone (**2**)	B
Naringenin (**3**)	B
Liquiritigenin (**4**)	B
Pinocembrin (**5**)	B
Medicarpin (**6**)	B
3'-Hydroxy-2,4,5-trimethoxydalbergiquinol (**7**)	B
Buteaspermanol (**8**)	B
Butin (**9**)	B
3'-Hyrdoxymelanettin (**10**)	B
Biochanin A (**11**)	B
Calycosin (**12**)	B
Eriodictoyl (**13**)	B
Isoliquiritigenin (**14**)	B
3'-*O*-Methylviolanone (**15**)	B
Luteolin (**16**)	B
Quercetin (**17**)	B
Apigenin (**18**)	B
Sulfuretin (**19**)	B
(2*S*)-6,8-Dicarboxyethylpinocembrin (**20**)	A, B, E
(2*S*)-8-Carboxyethylnaringenin (**21**)	A, B, E
Dalbergin (**22**)	B
Homoferreirin (**23**)	B
Neroplomacrol (**24**)	B, D, F
Rel-(3*R*,6*S*,7*S*)-6α-hydroxycyclonerolidol (**25**)	A, B, F
Kaemfperol (**26**)	B
Formyl phenol (**27**)	B, D
Naringin(**28**)	B, C
Genistein (**29**)	B
Orobol (**30**)	B
3-(1*H*-indol-3-yl)-2-methoxypropanamide (**31**)	A, B, D
Dalsissooside (**32**)	B
Daltonkinensiside (**33**)	A, B
Ambocin (**34**)	B, C
Lanceolanrin (**35**)	B
Tectorigenin (**36**)	B
Tectoridin (**37**)	B
Tectorigenin 7-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**38**)	B
1-Monolaurin (**39**)	B, D
1-Monomyristin (**40**)	B, D
(3*R*)-Vestitol (**41**)	B
Melilotolcarpan D (**42**)	B
Daidzein (**43**)	B
3'-Hydroxyldaidzein (**44**)	B

Formononetin-7-O-β-D-glucopyranoside (**45**)	B
Killitol (**46**)	B, D
2,5'-Dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47**)	A, B, F

A: new compound; B: It is the first time to isolate from *D. tonkinensis*; C-D: it is reported for the first time from genus *Dalbergia*, and the family Fabaceae, respectively; E: its rare in nature, F: The class of compound only found in *D. tonkinensis* and *D. odorifera*

Chapter 3 Chemical constituents from *Cratoxylum formosum*

3.1. Introduction

Figure 21. Leaf of *C. formosum*

Scientific name: *Cratoxylum formosum* (Jack) Dyer in Hook;

Other name: *Cratoxylum formosum* subsp. *formosum*, *Cratoxylum formosum* ssp. *formosum*, *Cratoxylum formosum* Dyer, and Mampat;

Subspecies: *C. formosum* subsp. *pruniflorum* (Kurz) Gogelein;

Family: Clusiaceae (Guttiferae);

Distribution: southeast Asia countries Brunei, Burma, Cambodia, China, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, and Vietnam [71].

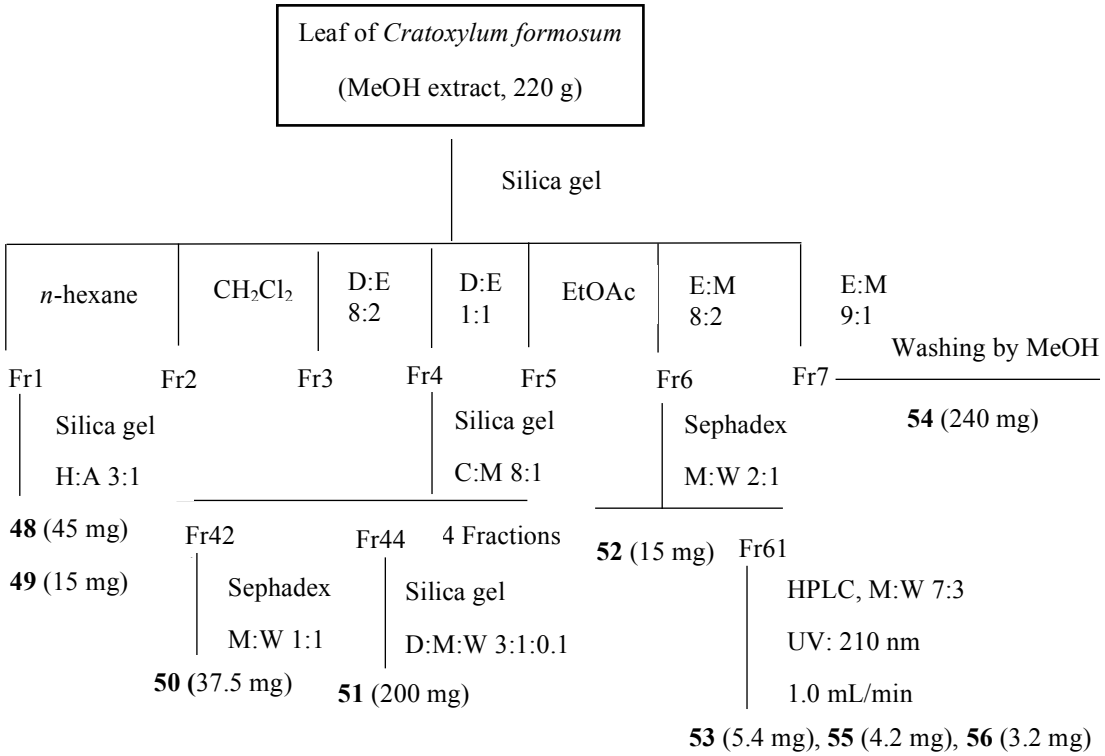
Morphology: The tropical plant *C. formosum***,** all parts of which are glabrous. Leaves elliptic to oblong (to lanceolate) $3^{1}/_{2}$-14 by 2-7 cm, petiole 6-15 mm. Connective not glandulose. Hypogynous scales linguiform often attenuate, sometimes blunt or truncate. Ecology: Granitic sand, dry, red soil, clay, rocky hill slopes, or fresh-water swamps, along rivers or near mangrove swamps, in open or dense primary forests or old secondary forests, thickets. Altitude mostly low, sometimes up to 600 m, or seldom more 1200 m. Flowering: Noted about March-July. Field characters: Trees maximum 35 m tall, young specimens and saplings spiny, base of the trunk spiny. Flowers pink or white, once noted purplish. Hypogynous scales orange-red [71].

Phytochemical investigations and biological evaluations: To the best of our knowledge, there was only one account reporting the isolation and antioxidant activities of 4 flavonol

derivatives quercetin, isoquercitrin, hyperin, and quercitrin from *C. formosum* [72]. At concentration of 1 μM, these compound could be claimed responsible for *in vitro* anti-oxidant capacity through peroxy radical scavenging experiments, and reducing powers of convert metal ion Cu^{2+} into Cu^{+}. Further, in the dose dependent manners from 1 to 10 μM, these compounds also demonstrated the cellular antioxidant behaviour when AAPH, Cu^{2+} or H_2O_2 stimulated intracellular oxidative stress on hepatoma HepG2 cell line. Its worth noting that monosaccharide such as glucose, galactose, or rhamnose in these glycoside derivatives are likely to be related to the primary reason for anti-oxidant enhancement [72].

3.2. Phytochemical investigation of leaf extract and structural elucidation

3.2.1. Isolation procedure



From methanol extract of *C. formosum* leaf, nine known compounds were isolated. The chemical structures of the known compounds were to be β-sitosterol (**48**), sitgmasterol (**49**), (+)-catechin-3-*O*-(3,4-dihydroxybenzoyl) (**50**), quercetin (**51**), quercitrin (**52**), hyperin (**53**), magniferin (**54**), afzelin (**55**), and isoquercetin **(56)** [72-76]. Despite the fact that flavans

widely distributed in *C. formosum* leaf, it is the first time to isolate (+)-catechin-3-*O*-(3,4-dihydroxybenzoyl) (**50**) from genus *Cratoxylum.*

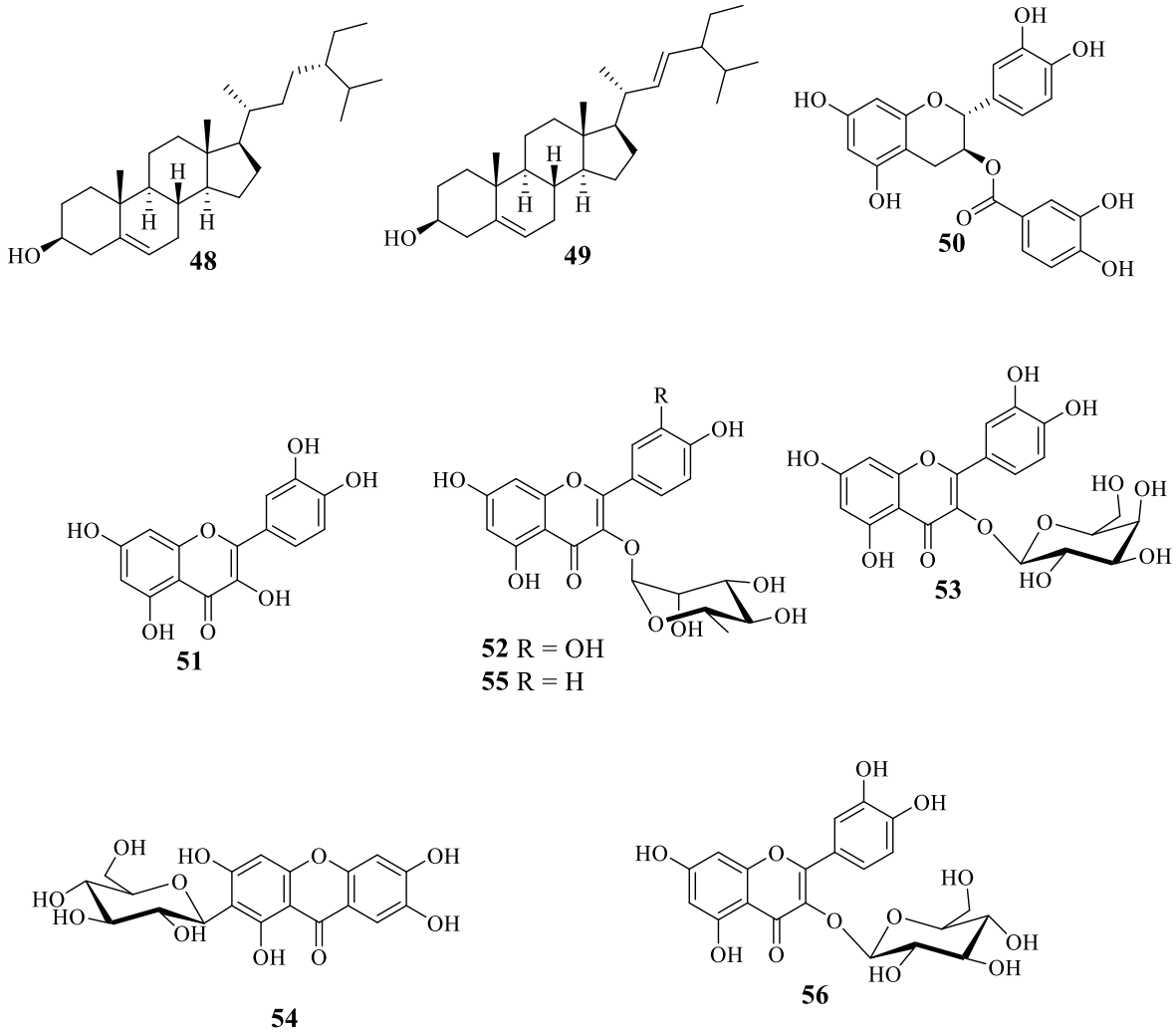


Figure 22. Chemical constituents from *C. formosum* leaf

Chapter 4 Biological activities

4.1. Introduction

Currently, infectious diseases have been emerging as the complicated public health problem around the worldwide, especially in terms of developing countries [76]. Consequently, microbacterial organisms are harmful to healthy invidiuals, leading to a further increase in infections. Considering several microbacteria, the microbacterial pathogenic strain *S. pneumoniae* is a major cause of superficial skin infection in children, infections of the ear, sinus, meningitis, and pneumonia disease, while the gram positive aerobic bacterium *B. cereus* is always considered a contaminant organism, widely existing in soil, dust, water or hospital environments, mainly causing food poisoning and necrotizing skin infection in diabetic patients [78,79]. Additionally, regarding the antimicrobial chemotherapy, clinical applications are seeking drugs and methods to treat bacterial infections. Historical records have accumulated evidence showing that the use of traditional antibiotics, which are derived from synthetic substances, is always accompanied by a long duration of treatment, high costs, and drug resistances [80]. Therefore, calls for new antibiotic drugs from natural sources in the fight against multidrug-resistant bacteria are warranted.

Serine proteases are a family of enzymes containing serine as a nucleophilic site when the peptide bonds of the proteins are cleaved. Based on substrate specificity, they are divided into three categories, including trypsin-like, chymotrypsin-like, and elastase-like proteases [81]. Serine protease enzymes can be both beneficial and harmful. They display critical roles as activators in foods and medicines, especially in milk, but are also related to numerous diseases, such as chronic inflammatory disease [82]. Much evidence shows that natural products from terrestrial plants have acted as useful serine protease inhibitors [83,84].

Among recently explored sources, Santhos and Suriyanarayanan pointed out that naturally occurring flavonoids should be the suitable priority with anti-microbacterial experimental model manipulation since they are available in quantity from plants and have the simple and small properties in molecular structure [85]. In other case, flavonoids could be a new class of candidates of serin protease inhibitors. Non-competitive inhibitory flavonoids, like agathisflavone and myricetin, were reported to be the best short-term solution for dengue virus serotype 2 NS2B-NS3 protease [86]. In the current thesis, we carry out both ani-microbacterial and serine protease assays for extracts and isolated flavonoids from *D. tonkinensis* species in anticipation of developing them into potent anti-microbacterial antibiotics as well as serine protease activators and inhibitors.

4.2. Anti-microbacterial results

4.2.1. The first model

Table 8. Plant extracts and isolated compounds for antimicrobacterial assays

-Leaf methanol extract (**DB1**)	-Leaf water decoction extract (**DB2**)
-Stem bark methanol extract (**DB3**)	-Stem bark water decoction extract (**DB4**)
-Root ethanol extract (**DB5**)	-Root methanol extract (**DB6**)
-Dichloromethane fraction of root ethanol extract (**DB7**)	-Ethyl acetate fraction of root ethanol extract (**DB8**)
-Root water decoction extract (**DB9**).	-Heartwood water decoction extract **(DB10**)
-Heartwood chloroform fraction (**DB11**)	
-Formononetin (**1**)	-Sativanone (**2**)
-Naringenin (**3**)	-Liquiritigenin (**4**)
-Pinocembrin (**5**)	-Medicarpin (**6**)
-3'-Hydroxy-2,4,5-trimethoxydalbergiquinol (**7**)	-Buteaspermanol (**8**)
-Butin (**9**)	-3'-Hyrdoxymelanettin (**10**)
-Biochanin A (**11**)	-Calycosin (**12**)
-Eriodictoyl (**13**)	-Isoliquiritigenin (**14**)
-3'-*O*-Methylviolanone (**15**)	-Luteolin (**16**)
-Quercetin (**17**)	-Apigenin (**18**)
-Sulfuretin (**19**)	-(2*S*)-6,8-Dicarboxyethylpinocembrin (**20**)
-(2*S*)-8-Carboxyethylnaringenin (**21**)	-Mixture of **2** and **15**

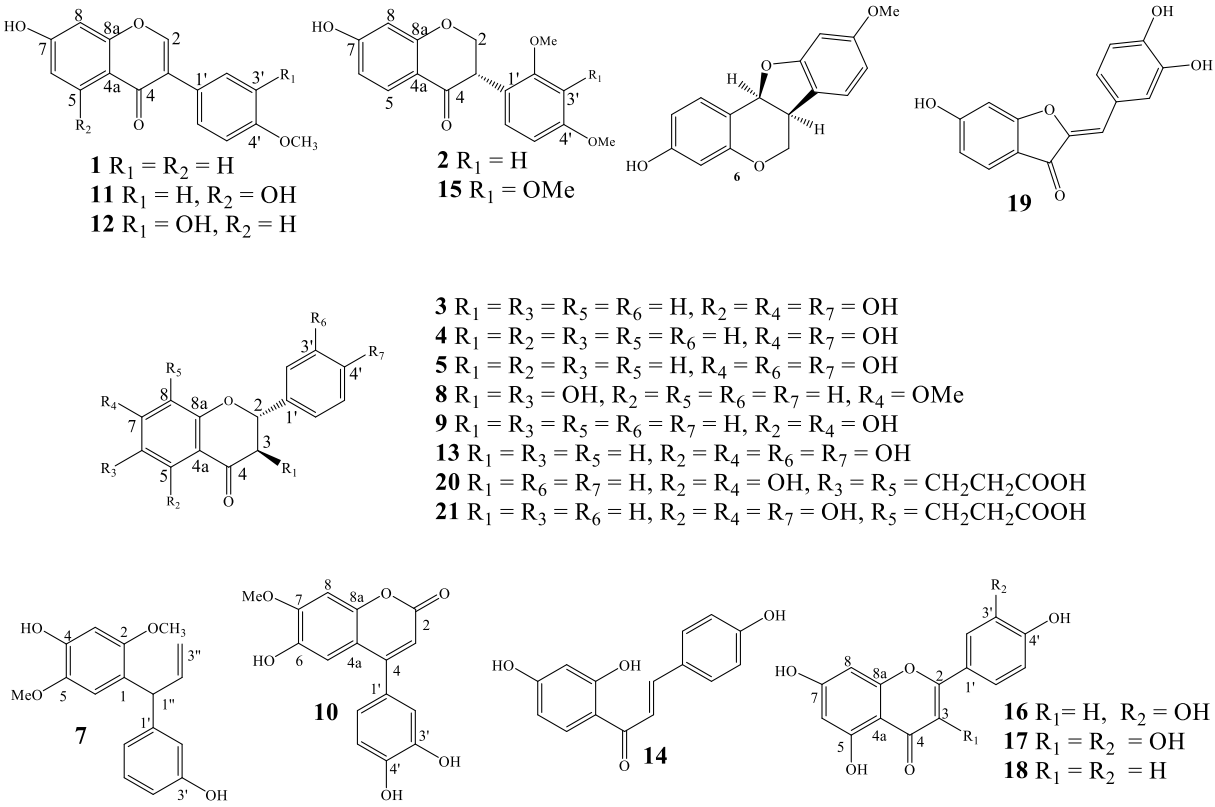


Figure 23. Chemicals for assay

The antimicrobial activities of the extracts (1.0 mg/mL) and isolated compounds (0.1 mg/mL) are presented by absorbance at λ 620 nm in Fig.s 24a-24k. The interest resuts were described as the percent observations of relative bacterial growth (or relative bacterial growth inhibition) when compared with those of the positive and negative controls, and medium only (the standard compound ampicillin sodium salt evidently generated the inhibitory percentage of greater than or equal to 80% and 50% for skin and oral microbacterial, respectively; data not shown). Among the extracts, at the concentration of 1.0 mg/mL, the crude methanol extracts of leaf (**DB1**), stem bark (**DB3**), root (**DB6**), and heartwood chloroform fraction (**DB11**) showed the significant values of 80, 70, 80, and 70% in inhibiting the microbacterium *S. pneumoniae*, respectively, whereas the extract remainders showed no activity (Fig. 24e). Additionally, the stem bark methanol extract (1.0 mg/mL) also possessed the rate of 60% for

preventing the growth of two types of oral bacteria *E. coli* and *B. Cereus* (Fig.s 24a-24b). Based on these findings, the methanol solvent and Soxhlet extraction method may be responsible for the antimicrobacterial mode, along with the latent chloroform fraction **DB11** would be further developments in phytochemical investigations. Similarly, the chloroform extract of *D. coromandeliana* stem, and the dichloromethane extract of *D. melunoxylon* bark were found to show notable activity against bacteria, and fungi, respectively [87,88].

Structural differences, stereochemistry, molecular size, the lengths of atomic bonds, extra or intramolecular hydrogen bonding, especially in terms of functional groups and their local substituents were greatly related to the outcomes in bioassays. Regarding to the isolated flavonoids, among the tested compounds (0.1 mg/mL), the flavanone pinocembrin (**5**), and isoflavone biochanin A (**11**) suppressed 80% of the growth of the two bacterial strains *B. cereus* and *S. pneumonia* compared with the positive control (Fig.s 24a and 24e). Likewise, the strongest percentage of 80% against bacterium *S. pneumonia* also accounted for the isoflavanone sativanone (**2**), chalcone isoliquiritigenin (**14**), two other flavanones naringenin (**3**), and liquiritigenin (**4**), and the mixture of two isoflavanones **2**+**15** (Figure 6). Hence, these typical flavonoids of flavanone, isoflavanone, isoflavone, or chalcone could be the best choice to argue for the mode of antimicrobacterial activity.

More recently, Cuong *et al*. found that compound **5** indicated an inhibitory effect on the filamentous fungus *A. niger* with a significant MIC value at 50 µg/mL [89]. Furthermore, in contrast to the results of the isoflavanone **15**, the mixture of **2**+**15** revealed positive signals against 70 and 50% growth inhibition of the two pathogens *B. cereus* and *S. epidermidis*, respectively (Fig.s 24a and 24d). Hence, the 2'- and 4'-methoxy groups of isoflavanones **2** and **15** could serve as the most striking feature in these efficacious aspects. Meanwhile, considering three flavones (**16-18**) and one aurone (**19**), only the flavanone luteolin (**16**)

established an inhibitory effect on the oral bacterium *S. epidermidis* with a relative growth inhibition of 50%. Presumably, regarding the flavanones, an additional hydroxyl group at C-3' produced a significant effect, whereas hydroxylation at C-2 failed to do so.

Sotres *et al*. indicated that the *n*-hexane extract of *D*. *congestiflora* heartwood, which contained a major flavonoid derivative of skeletal pterocarpan named medicarpin (**6**), caused 100% growth inhibition of the *Trametes versicolor* fungus at the dose of 250 mg/mL [90]. In the present study, as shown in numerous Figures 24a, 24c, 24g, 24h, and 24k, compound **6** showed remarkably antimicrobial activities against *B. cereus* and *V. parahaemolyticus* (70% growth inhibition), and *S. aureus* (60%), as well as the oral pathogens *S. mutans* and *P. gingivalis* (50%), whereas the inhibitory numbers of 70% growth were also calculated for a neoflavanoid **7**, and a flavanone **3** (the growth inhibition of *B. cereus*), and two other flavanones **5** (*V. parahaemolyticus*), and **9** (*S. pneumoniae*). Significantly, in the comparison, the other neoflavanoid 3'-hyrdoxymelanettin (**10**) was formed by plausible hypothesis of lactone intramolecular cyclization between the *exo*-methylene group at the carbon C-3" and 2-methoxy group, and hydroxylation at the carbon C-4' in compound **7** showed the inactive results in any antimicrobacterial experiments. In addition, the flavanone naringenin (**3**) was considerably estimated to show 60% growth suppression of two types of bacteria *S. epidermidis*, and *V. parahaemolyticus*. In general, the most striking feature was that four isolated flavanones **3-5**, and **9** indicated the mode regarding the substitution of hydroxyl groups at C-5, C-7, and C-3', or at C-4', except for compound **13**, while replacement of protons H-3, and H-5, or H-8 by hydroxyl, or carboxyethyl groups in flavanones **8**, **20**, or **21** significantly resulted in reducing the inhibitory values. On the other hand, among three isoflavones **1**, **11**, and **12**, with an opposite property of chemical structure, compounds **1** and **12** showed no significant inhibitory activity, while isoflavone **11** was not only revealed as a

latent agent that repressed the pathogenic bacteria *B. cereus* and *S. pneumoniae* but also showed 50% growth inhibition of two other types of *S. epidermidis*, and *P. gingivalis*, presumably due to the substitution at the carbon C-5 rather than C-3'.

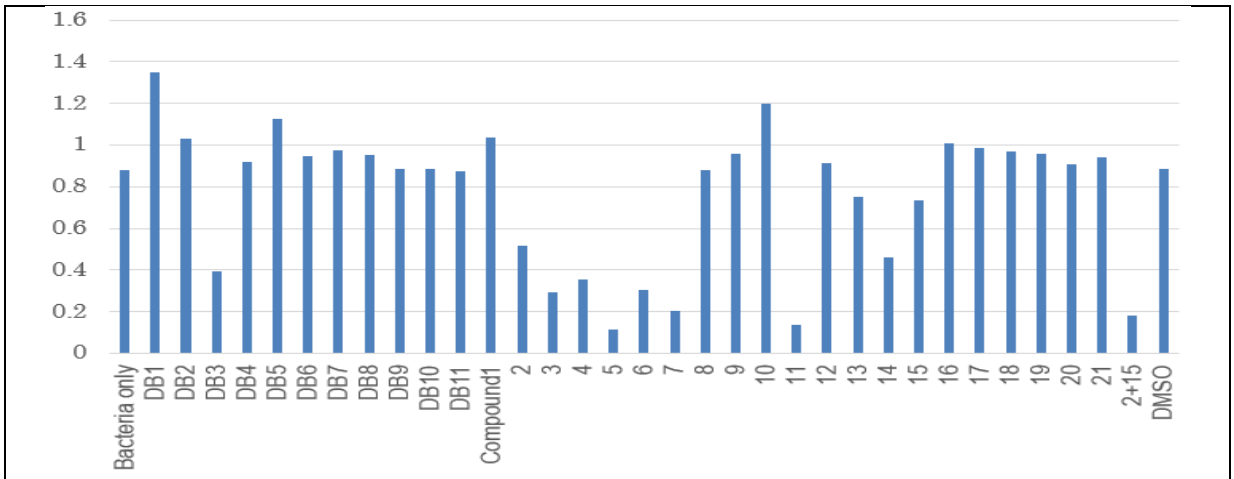


Figure 24a. The relative growth of microbacterium *B. cereus*

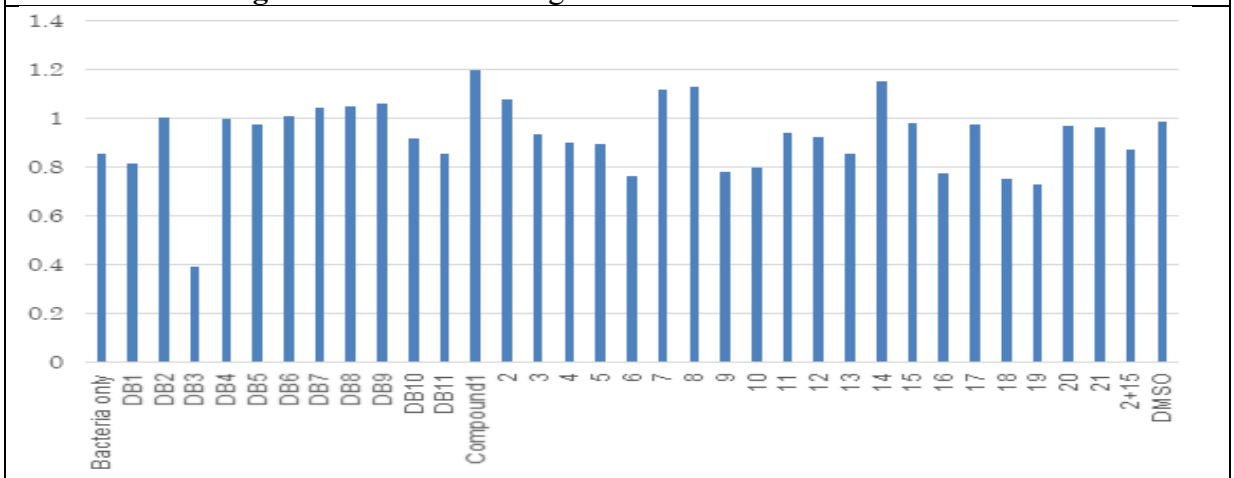


Figure 24b. The relative growth of microbacterium *E. coli*

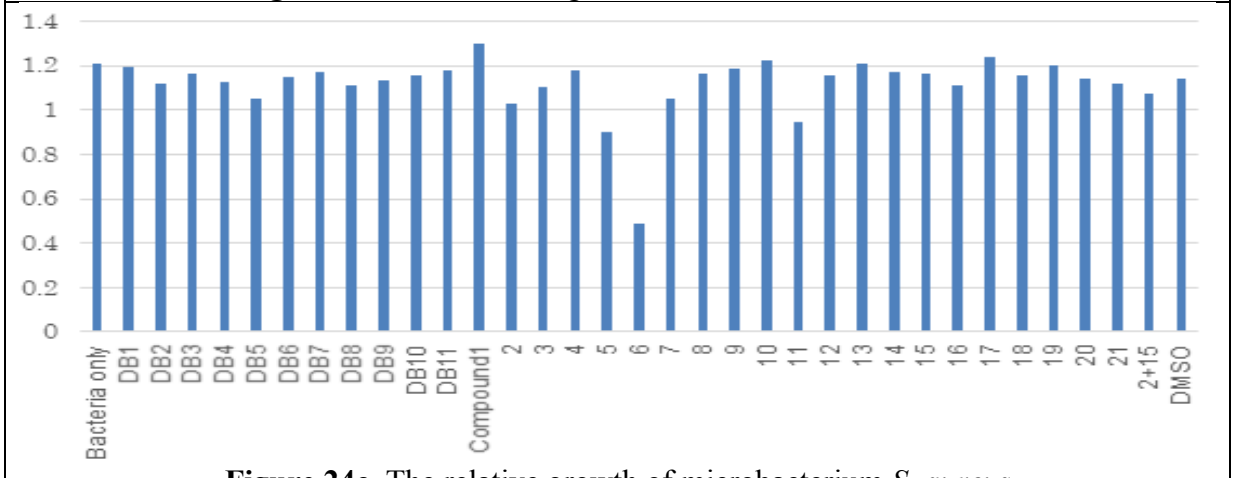


Figure 24c. The relative growth of microbacterium *S. aureus*

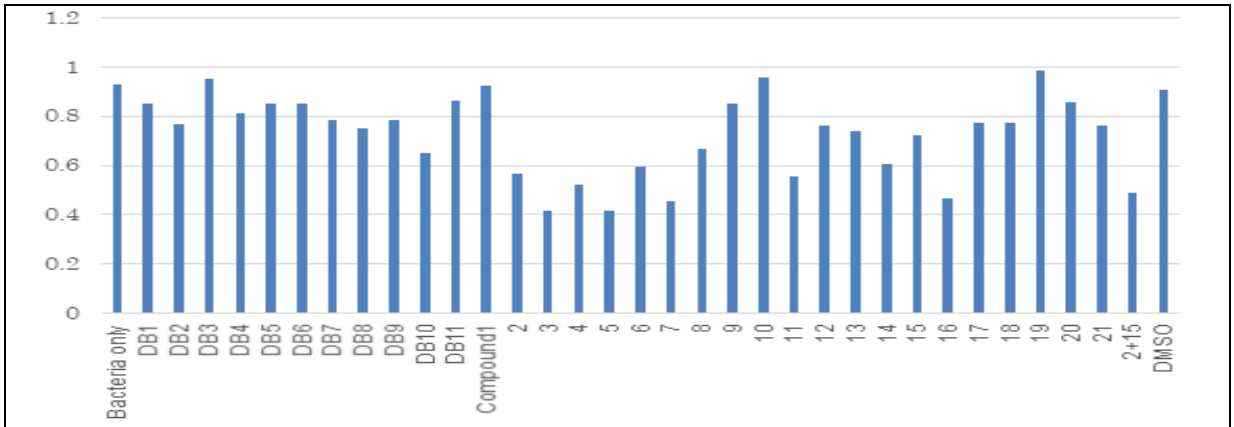


Figure 24d. The relative growth of microbacterium *S. epidermidis*

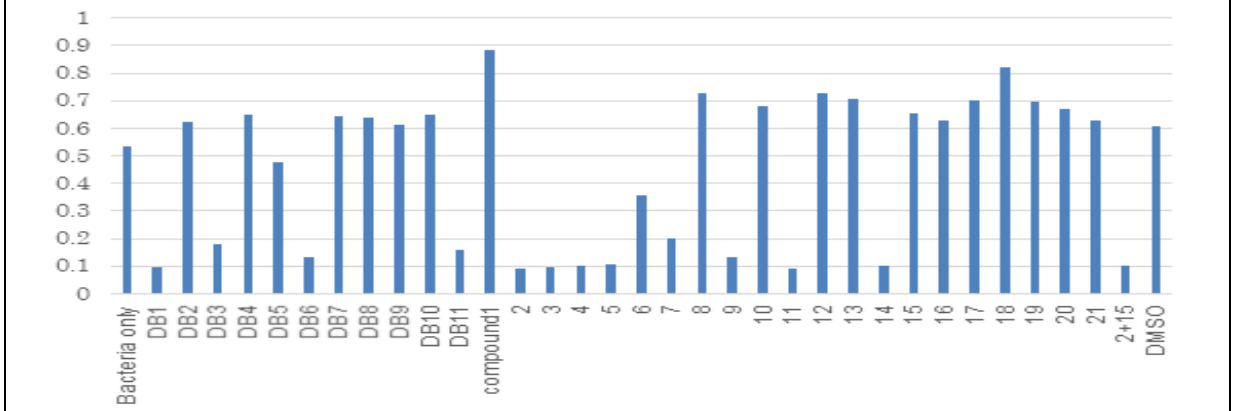


Figure 24e. The relative growth of microbacterium *S. pneumoniae*

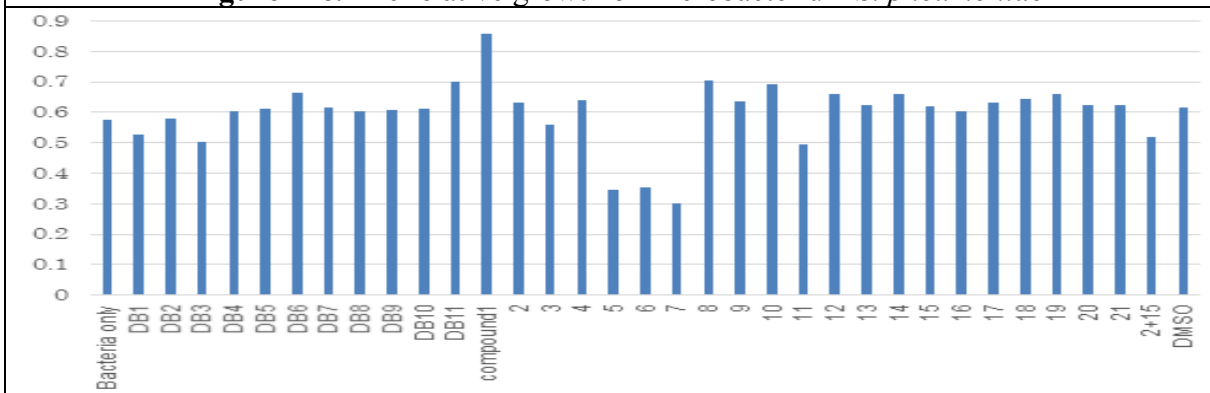


Figure 24f. The relative growth of microbacterium *S. pyogenes*

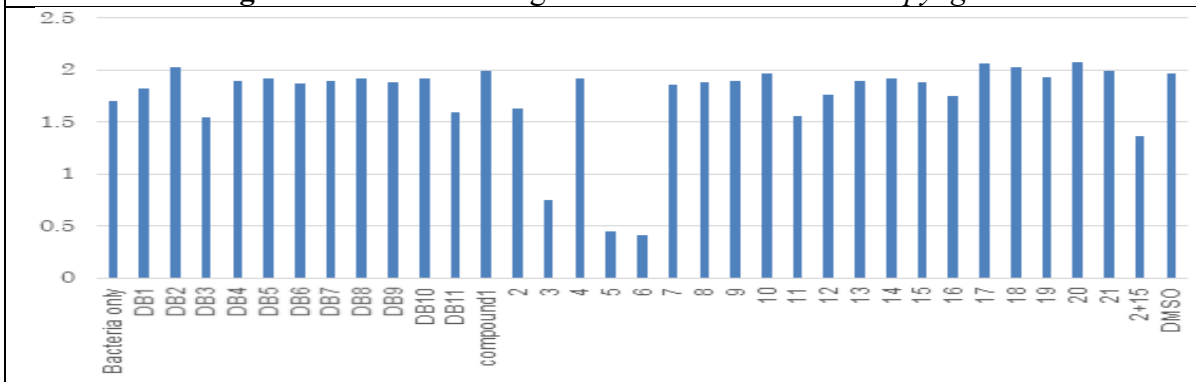


Figure 24g. The relative growth of microbacterium *V. parahaemolyticus*

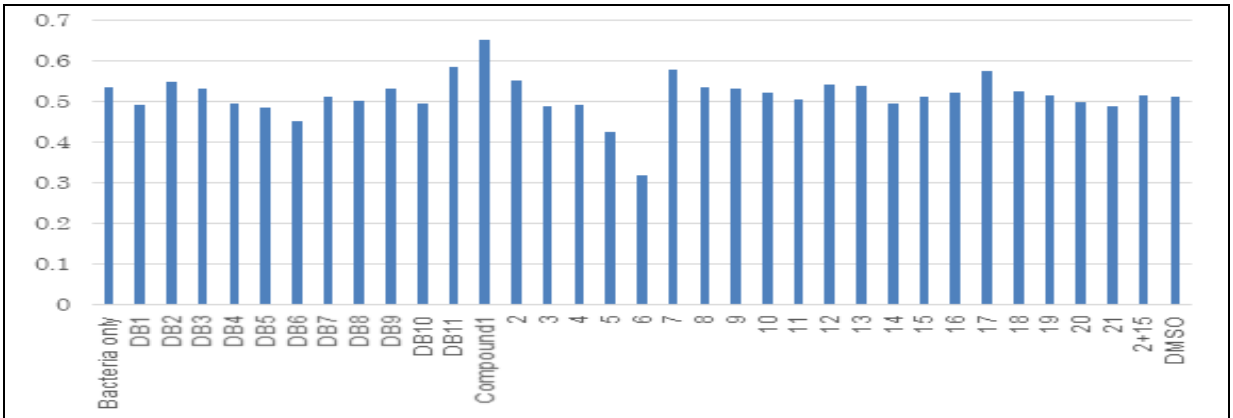


Figure 24h. The relative growth of microbacterium *S. mutans*

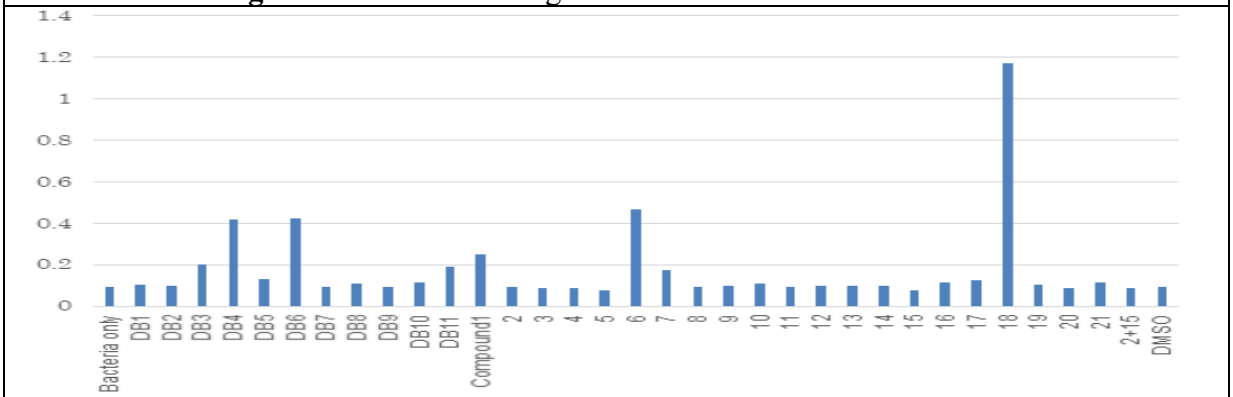


Figure 24i. The relative growth of microbacterium *S. mitis*

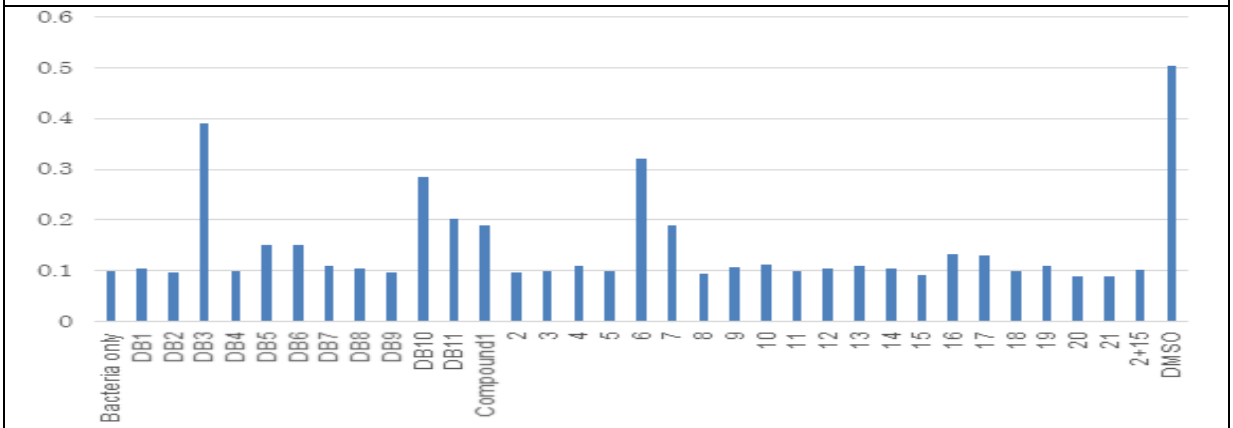


Figure 24j. The relative growth of microbacterium *S. sobrinus*

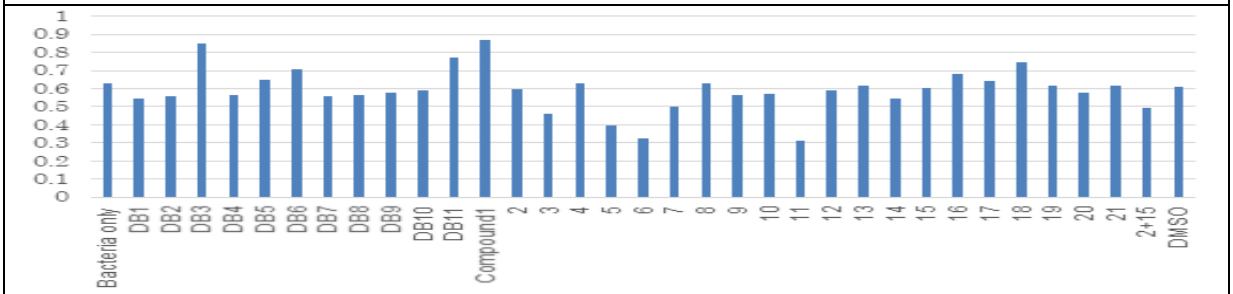


Figure 24k. The relative growth of microbacterium *P. gingivalis*

4.2.2. The second model

Table 9. Anti-microbacterial results of heartwood extracts and its isolated compounds

No	Anti-microbial acitivities (MIC: µg/mL)							
	Gram bacteria				Filamentous fungi		Yeast	
	E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*A. niger*	*F. oxyporum*	*S. cerevisiae*	*C. albicans*
CH_3OH extract*	(-)	(-)	(-)	(-)	200	(-)	(-)	(-)
$CHCl_3$ fraction*	(-)	(-)	(-)	400	200	(-)	(-)	(-)
Compound **4**	(-)	(-)	(-)	100	(-)	(-)	(-)	(-)
Compound **8**	(-)	(-)	(-)	50	100	(-)	(-)	(-)
Compound **11**	(-)	(-)	(-)	50	50	(-)	(-)	(-)
Compound **21**	(-)	(-)	(-)	(-)	100	(-)	(-)	(-)

*Extract and fraction of heartwood

Antimicrobial experiments were carried out and the results were shown in Table 9. Of the heartwood extract, and fraction, the CH_3OH extract had the weak MIC value of 200 µg/mL against only *Aspergillus niger* (439), whereas the $CHCl_3$ fraction showed the inhibition with the MIC value at 200, and 400 µg/mL against *A. niger* (439), and Gram positive bacterium *S. aureus* subsp. *aureus* (ATCC 11632), respectively. Among the isolated compounds, the known flavanone buteaspermanol (**8**), and the new flavanone (2S)-8-carboxyethylnaringenin (**21**) showed the inhibitory effects on pathogenic fungus fungus *A. niger* (439) at the weak MIC value of 100 µg/mL, and the MIC value of 50 µg/mL was assigned for biochanin A (**11**). Furthermore, the flavanone derivatives **8**, and **11** are hopeful agents against the filamentous fungus *A. niger*, revealing the effective MIC value of 50 µg/mL, whereas the remaining subtance liquiritigenin (**4**) possessed the weak MIC value of 100 µg/mL. However, none of extracts, fractions, and isolated compounds exhibited inhibitory effects on Gr negative bacteria *E. coli* and *P. aeruginosa*, Gr positive bacterium *B. subtillis* (ATCC 11774), yeast fungi, and filamentous fungus *F. oxysporum* (M42).

4.3. DPPH Radical scavenging capacity result

Table 10. Compounds for DPPH radical scavenging assay

D. tonkinensis	*C. formosum*
Medicarpin (**6**)	Catechin-3-O-(3,4-dihydroxylbenzoyl) (**50**)
Orobol (**30**)	Quercetin (**51**)
(3R)-Vestitol (**41**)	Hyperin (**53**)
Melitocarpan D (**42**)	Magniferin (**54**)
	Afzelin (**55**)
	Isoquercetin (**56**)
Standard compound: Catechin	

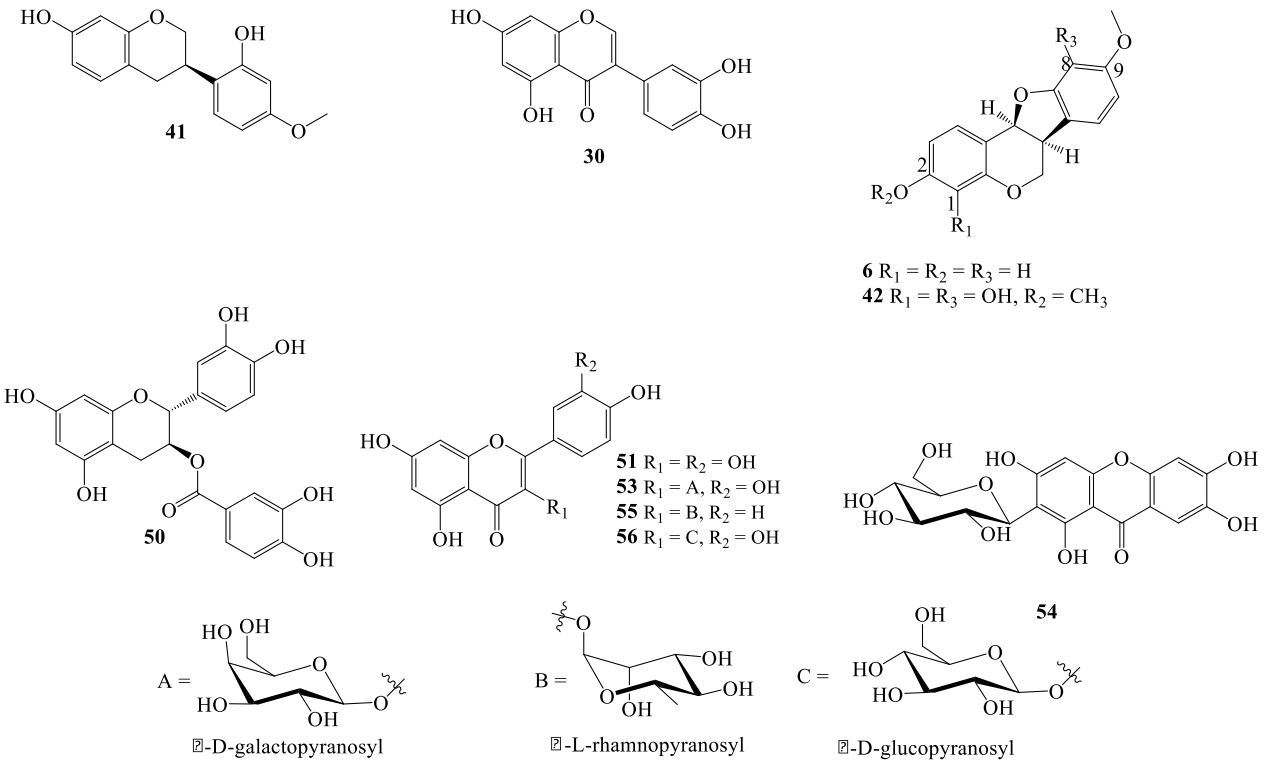


Figure 25. Chemicals for assay

The DPPH radical scavenging assay was carried out on several isolated compounds according to the protocol described by Tram and co-workers [91], and the results are given in Table 11. With regards to the several tested compounds from *D. tonkinensis* species, (3*R*)-vestitol (**41**) possessed the strongest anti-oxidative IC_{50} value of 42.20 (μg/mL), which was comparable to that of positive control catechin (IC_{50} 42.98 μg/mL). Pterocarpan **42** revealed a

strong IC_{50} value of 54.14 (μg/mL) when compared with the moderate IC_{50} value of 69.45 (μg/mL) of pterocarpan **6** and the weak one of 91.18 (μg/mL) of isoflavone **30**. It therefore can be concluded that isoflavans from *D. tonkinensis* species should be the best choice for anti-oxidant model as compared with pterocarpans or isoflavones. In addition, comparing the two pterocarpans medicarpin (**6**) and melilotolcarpan D (**42**), hydroxyl groups occurred at the carbons C-1 and C-8, and methoxyl group attached at the carbon C-2 can be claimed to be responsible for anti-oxidant increase.

Regarding to the isolated compounds from *C. formosum* leaf, the IC_{50} values established a consistent arrangement as follows: catechin (IC_{50} 42.98 μg/mL) > isoquercetin (**56**) (IC_{50} 45.63 μg/mL) > magniferin (**54**) (IC_{50} 64.03 μg/mL) > hyperin (**53**) (IC_{50} 64.22 μg/mL) > quercetin (**51**) (IC_{50} 64.54 μg/mL) > catechin-3-*O*-(3,4-dihydroxylbenzoyl) (**50**) (IC_{50} 75.37 μg/mL) > afzelin (**55**) (**IC_{50}** > 100 μg/mL, inactive). As a consequence, flavonols, flavonol glycosides, and xanthone glycosides may be regarded as promising anti-oxidant agents, but flavans cannot be considered as such. Taking flavonol derivatives into consideration, glycosylation and hydroxylation occured at the respective carbons C-3 and C-3' enhanced an increase in anti-oxidant capacity. Similarly, 3-glucopyranosylated flavonol (compound **56**) showed a better anti-oxidant poteny than 3-galactopyranosylated flavonol (compound **53**).

Table 11. DPPH Radical scavenging activity

Compounds	IC_{50} (µg/mL)
Medicarpin (**6**)	69.45
Orobol (**30**)	91.18
(3*R*)-Vestitol (**41**)	42.20
Melitocarpan D (**42**)	54.14
Catechin-3-*O*-(3,4-dihydroxylbenzoyl) (**50**)	75.37
Quercetin (**51**)	66.54
Hyperin (**53**)	66.22
Magniferin (**54**)	64.03
Afzelin (**55**)	> 100
Isoquercetin (**56**)	45.63
Catechin	42.98

4.4. Enzyme active screenings

Table 12. Plant extracts and isolated compounds for enzyme serine protease assays

-Leaf methanol extract (**DB1**)	-Leaf water decoction extract (**DB2**)
-Stem bark methanol extract (**DB3**)	-Stem bark water decoction extract (**DB4**)
-Root ethanol extract (**DB5**)	-Root methanol extract (**DB6**)
-Dichloromethane fraction of root ethanol extract (**DB7**)	-Ethyl acetate fraction of root ethanol extract (**DB8**)
-Root water decoction extract (**DB9**).	-Heartwood water decoction extract **(DB10**)
-Heartwood chloroform fraction (**DB11**)	
-Formononetin (**1**)	-Sativanone (**2**)
-Naringenin (**3**)	-Liquiritigenin (**4**)
-Pinocembrin (**5**)	-Medicarpin (**6**)
-3'-Hydroxy-2,4,5-trimethoxydalbergiquinol (**7**)	-Buteaspermanol (**8**)
-Butin (**9**)	-3'-Hyrdoxymelanettin (**10**)
-Biochanin A (**11**)	-Calycosin (**12**)
-Eriodictoyl (**13**)	-Isoliquiritigenin (**14**)
-3'-*O*-Methylviolanone (**15**)	-Luteolin (**16**)
-Quercetin (**17**)	-Apigenin (**18**)
-4-Formyl phenol (**19**)	-Sulfuretin (**20**)
-Naringin (**21**)	-(2*S*)-6,8-Dicarboxyethylpinocembrin (**22**)
-(2*S*)-8-Carboxyethylnarigenin (**23**)	7-Dehydroxy-7-(prop-2-yn-1-yl)sativanone (**2a**)
Datosativanone (**2b**)	Mixture of **2** and **15**

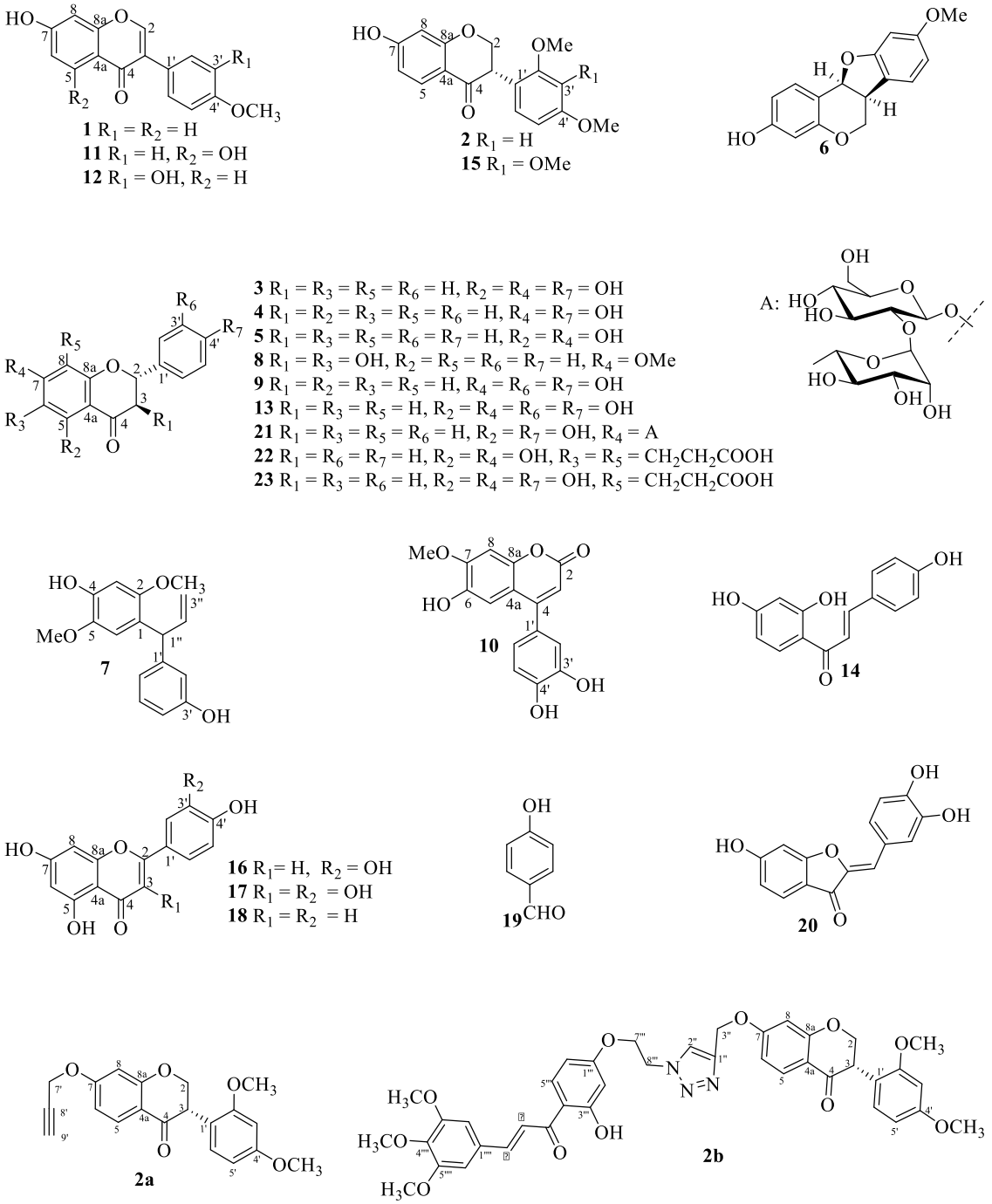


Figure 26. Chemicals for assay

The results for the serine protease assays are summarized in Table 13 and also Fig. 27. Regarding the conceptual activation, root ethanol and methanol extracts increased the activity of trypsin in a concentration-dependent manner ranging from 25 ng/mL to 250 ng/mL. In the meantime, both 3'-*O*-methylviolanone (**15**) and (2*S*)-6,8-dicarboxyethylpinocembrin (**20**)

showed the same tend at the concentration of 100 μM, in particular, medicarpin (**6**) can be seen as a good candidate for trypsin activator at concentration of 10 μM. Besides trypsin active capacity, root dichloromethane fraction of ethanol extract (**DB7**) also enhanced the activity of chymotrypsin at a concentration of 250 ng/mL, meanwhile heartwood water decoction extract (**DB6**) increased the activity of both trypsin and chymotrypsin at 25 ng/mL.

Of inhibitors, at the concentration of 100 μM, isoflavanone **2** and neoflavanoid **7** specifically prohibited the activity of chymotrypsin, and also formononetin (**1**) and butin (**9**) indicated weaker inhibition than compounds **2** and **7**. Both root water decoction extract (**DB9**) (250 ng/mL), and flavanone **5** (100 μM) showed inhibitory activities of trypsin-chymotrypsin. Similarly, root methanol extract (**DB6**) (250 ng/mL) and a flavonoid type aurone **19** (100 μM) suppressed the activity of either chymotrypsin or elastase delete. Stem bark methanol extract (250 ng/mL) exhibited inhibition for three tested enzymes when compared to methanol, and water decoction extracts of root (**DB9**). Heartwood chloroform fraction (**DB11**), which contained the enriched flavonoids, showed a trypsin-chymotrypsin-elastase inhibitory activity in a concentration-dependent manner ranging from 25 ng/mL to 100 ng/mL. Among the tested flavonoids, quercetin (**17**) was ascertained to possess the most potent activity in suppressing trypsin and chymotrypsin delete, and significant capability for elastase delete [92]. Numerous flavonoids **2b**, **3**, **4**, and **18** at the concentration of 100 μM, showed weak inhibition of three experimental serine proteases enzymes. It was noted that the leaf methanol extract merged as agonist, and antagonist, which significantly activated trypsin at 25 ng/mL, in contrast to reduce the activity of chymotrypsin at the high concentration of 250 ng/mL. Similar activity pattern is also assigned for isoflavone biochanin A (**11**). Root methanol extract (**DB6**) (25 ng/mL) can possibly be interpreted as a trypsin activator, along with the inhibitory role for chymotrypsin and elastase at the concentration of 250 ng/mL. Ethyl acetate fraction of root

ethanol extract and secondary metabolites **2a**, **2**+**15**, **8**, **10**, **12-14**, **16**, **21**, and **27-28** showed no effects on the tested three proteases.

In the model of casein cleavage activity, at the dose of 25 ng/mL, leaf water decoction (**DB2**) extract inhibited casein cleavage by trypsin, ethanol (**DB5**) and methanol (**DB6**) extracts of root activated the activity (Fig. 28). The result was same as the protease activity analysis using fluorogenic synthetic peptide substrates. However, leaf methanol extract (**DB1**) and heartwood water decoction extract (**DB10**) (25 ng/mL), and medicarpin (**6**) and quercetin (**17**) had no effect.

Fig. 27 showed that at the lowest dose of 2.5 ng/mL and 1.0 μM, no crude extracts and isolated compounds achieved the remarkable effects, but the changes corresponded to the concentration adjustment. Taking extracts into consideration, the leaf, root, and heartwood of *D. tonkinensis* may be suitable for trypsin activators in food. Furthermore, leaf, stem bark, and heartwood have significantly emerged as promising trypsin-chymotrypsin-elastase inhibitors (Table 13). Leaf water decoction extract (**DB2**) (250 ng/mL) completely controlled the activity of elastase delete to explain the great value of unique Vietnamese plant with unknown ethno-pharmacological aspect, especially in terms of water decoction traditional method.

Structural differences, stereochemistry, functional groups, and their local substituents might be related to the outcome in protease bioassays. Taking the isolates from this plant into consideration, flavonoids categorized as flavanone, isoflavanone, flavone, isoflavone, pretocarpan, aurone, and neoflavanone could be the best choice to argue for this issue. Calycosin (**12**) failed to show activity due to 3'-OH comparing to **1**. Taking a close look at flavones, quercetin (**17**) can be seen as a comparative standard one because of absolutely

attenuating actions of trypsin and chymotryspin to 100% at 100 μM, whereas apigenin (**18**), a second flavone, is not to do so, and luteolin (**16**) has no activity. The presence of hydroxyl group at C-3 and C-3' turns out to be the differential result among three flavones **16-18**, and flavononols seem generally stronger than flavones. As the third case, in accordance with our previous findings in antimicrobacterial assay, 3'-hyrdoxy-2,4,5-trimethoxydalbergiquinol (**7**), an opened ring lactone type neoflavanone, dramatically decreased chymotrypsin activity to 95% in contrast to compound **10** at the concentration of 100 μM.

Buteaspermanol (**8**), a unique flavanonol of *Dalbergia* genus showed no effect on protease activities. This means that hydroxylation at C-3 in flavanone does not show significant contribution to protease delete assay. Compound **20** having carboxylethyl groups at C-6 and C-8 was quite the reverse with compound **5**. Comparing protease inhibitory activity for naringenin (**3**), (2*S*)-8-carboxyethylnarigenin (**21**), and naringin (**28**), 7-glycosylation and 8-carboxyethylation may led to inactivation. Eriodictoyl (**13**) with an additional 5-OH resulted in reducing protease inhibitory activity, in comparison with butin (**9**). Comparing structure and activity between **4** and **9**, hydroxylation at C-3' is attributable to the attenuation of protease activity.

Naturally occurring isoflavanones **2** and **15**, their mixture **2**+**15**, and semi-synthetic products **2a** and **2b** showed similar relation in proteases delete assay. Among them, the negative and/or positive signals in outcome basically depended on the presence of methoxylation at C-3' or not. In addition, a semi-synthetic compound, datosativanone (**2b**) was prepared from **2a** and chalcone azide has been emerged as a trypsin-chymotrypsin-elastase inhibitor.

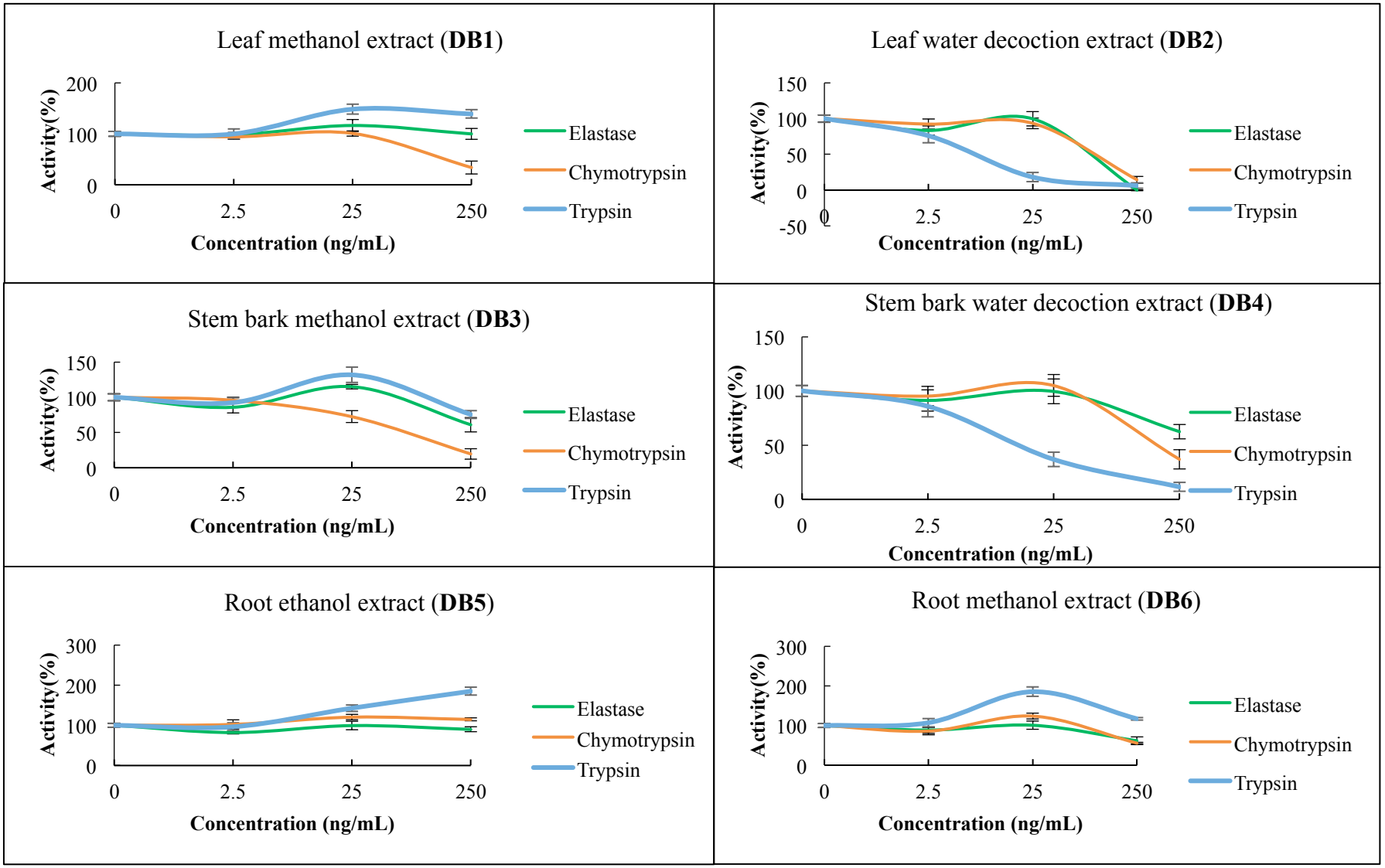
Table 13. Summary results of serine proteases analysis

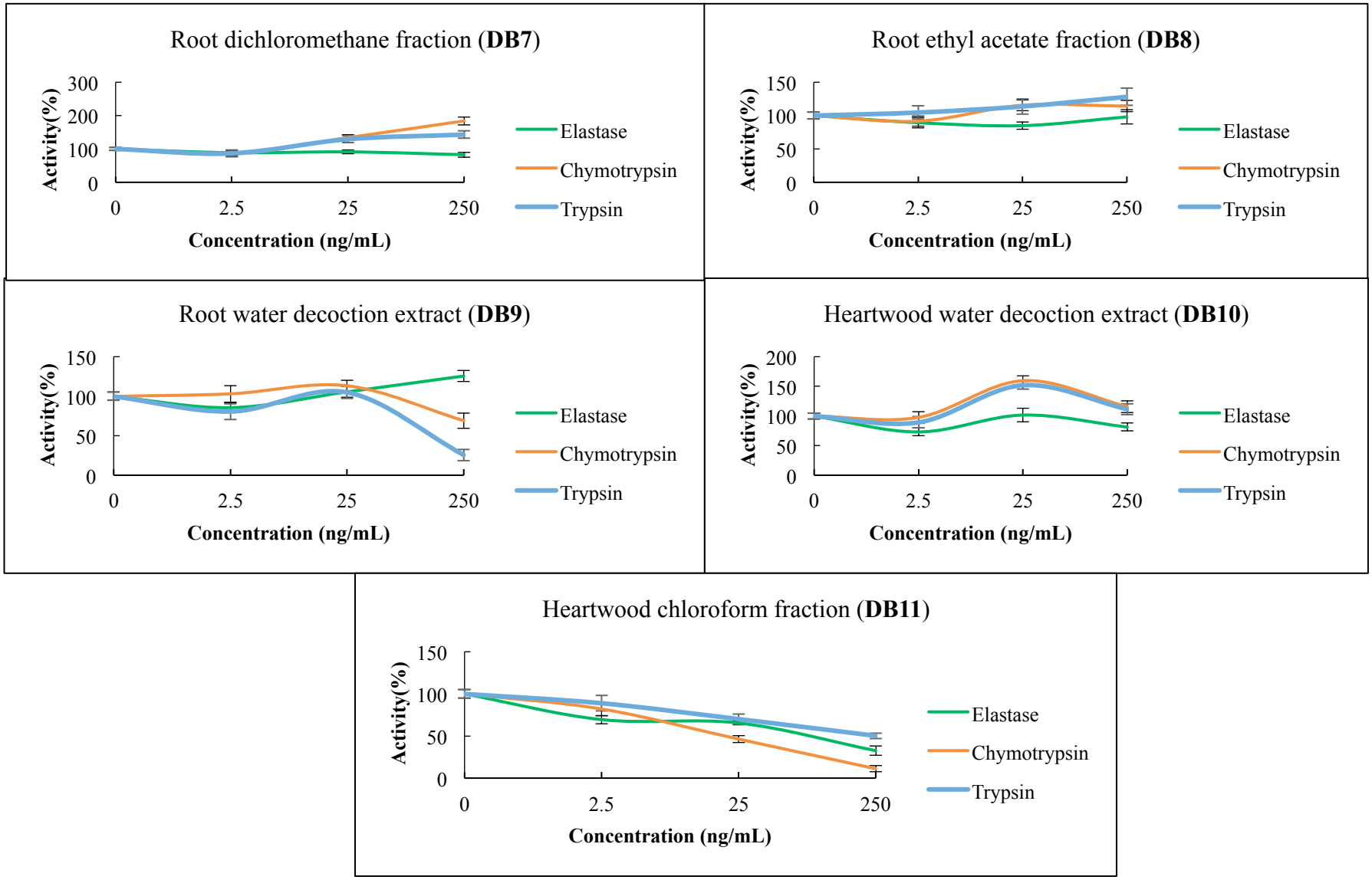
No	Activation		Inhibition		
	Trypsin	Chymotrypsin	Trypsin	Chymotrypsin	Elastase
	(Increased rate/concentration)		(Decreased rate/concentration)		
Extracts and fractions					
Leaf methanol extract (**DB1**)	1.5 times/A 1.4 times/B			66%/B	
Leaf water decoction extract (**DB2**)			82%/A 94%/B	86%/B	100%/B
Stem bark methanol extract (**DB3**)			24%/B	80%/B	39%/B
Stem bark water decoction extract (**DB4**)			63%/A 88%/B	63%/B	38%/B
Root ethanol extract (**DB5**)	1.4 times/A 1.9 times/B				
Root methanol extract (**DB6**)	1.9 times/A			44%/B	38%/B
Dichloromethane fraction (**DB7**)	1.4 times/B	1.8 times/B			
Root water decoction extract (**DB9**)			74%/B	30%/B	
Heartwood water decoction extract (**DB10**)	1.5 times/A	1.6 times/A			
Heartwood chloroform fraction (**DB11**)			30%/A 50%/B	54%/A 89%/B	35%/A 67%/B
Isolated compounds					
Formononetin (**1**)				45%/D	
Sativanone (**2**)				77%/D	
Naringenin (**3**)			55%/D	57%/D	39%/D
Liquiritigenin (**4**)			27%/D	31%/D	52%/D
Pinocembrin (**5**)			54%/D	60%/D	
Medicarpin (**6**)	1.6 times/C				
3'-Hyrdoxy-2,4,5-trimethoxydalbergiquinol (**7**)				95%/D	
Butin (**9**)				45%/D	
3'-*O*-Methylviolanone (**15**)	1.5 times/D				
Quercetin (**17**)			90%/C	86%/C	65%/D

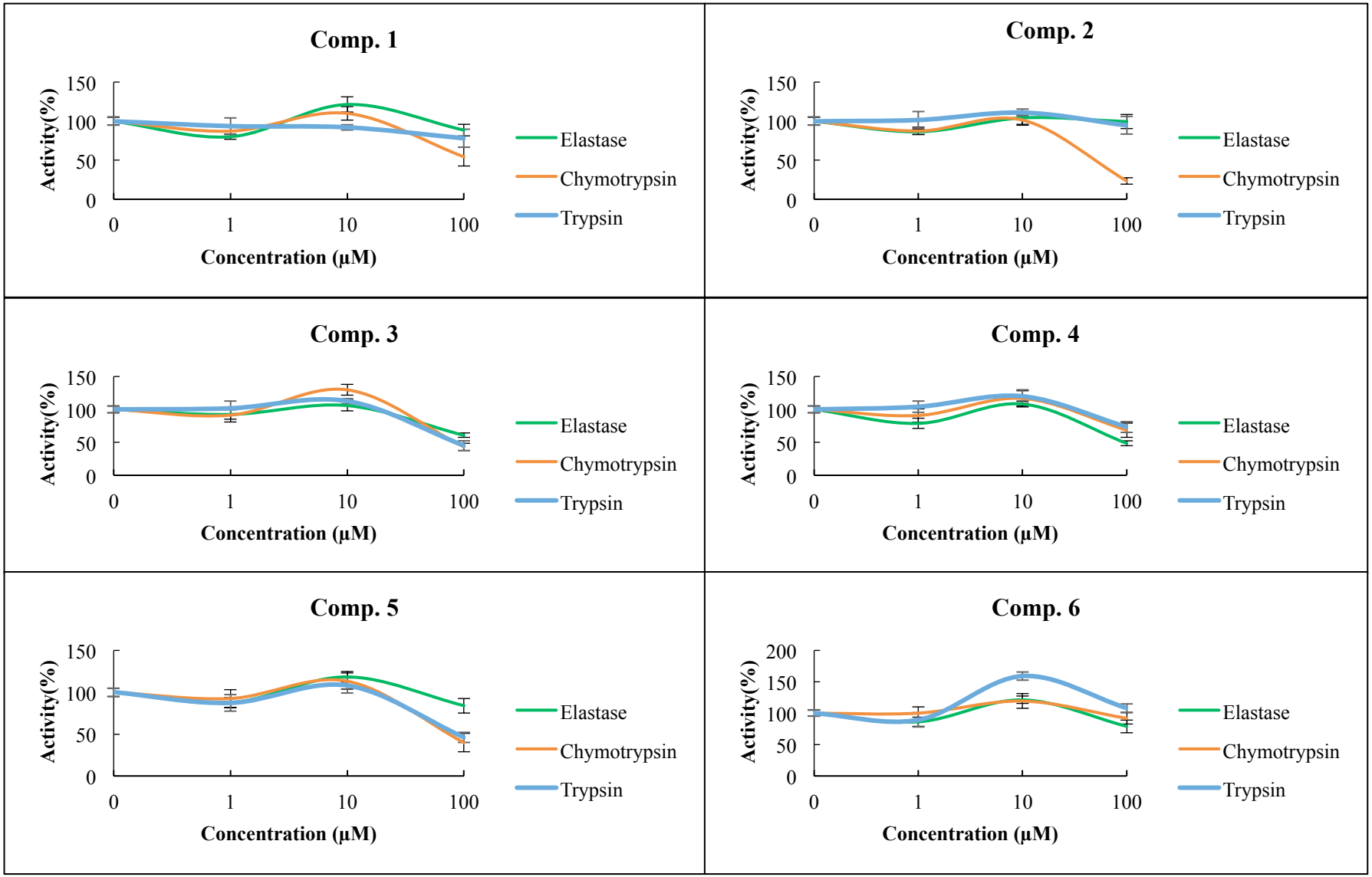
		100%/D	100%/D	
Apigenin (**18**)		79%/D	41%/D	57%/D
Sulfuretin (**20**)			74%/D	38%/D
(2*S*)-6,8-Dicarboxyethylpinocembrin (**22**)	1.6 times/D			
Datosativanone (**2b**)		39%/D	74%/D	53%/D

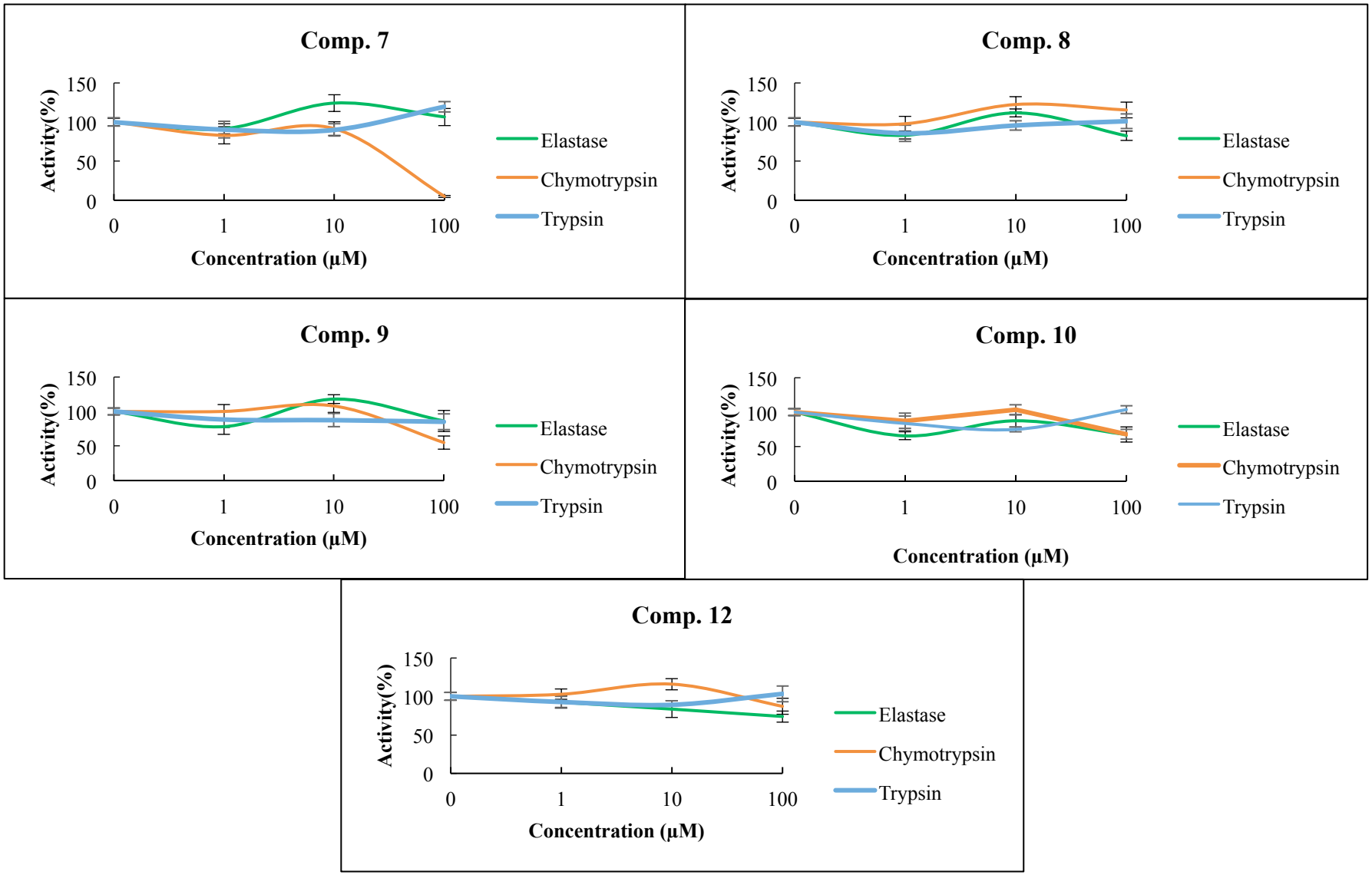
No samples increased the growth of elastase enzyme. Ethyl acetate fraction (**DB8**) of root ethanol extract, buteaspermanol (**8**), 3'-hyrdoxymelanettin (**10**), biochanin A (**11**), calycosin (**12**), eriodictoyl (**13**), isoliquiritigenin (**14**), luteolin (**16**), 4-formyl phenol (**19**), naringin (**21**), (2*S*)-8-carboxyethylnarigenin (**23**), 7-dehydroxy-7-(prop-2-yn-1-yl)sativanone (**2a**), and **2**+**15** show insignificant effects at any concentrations.

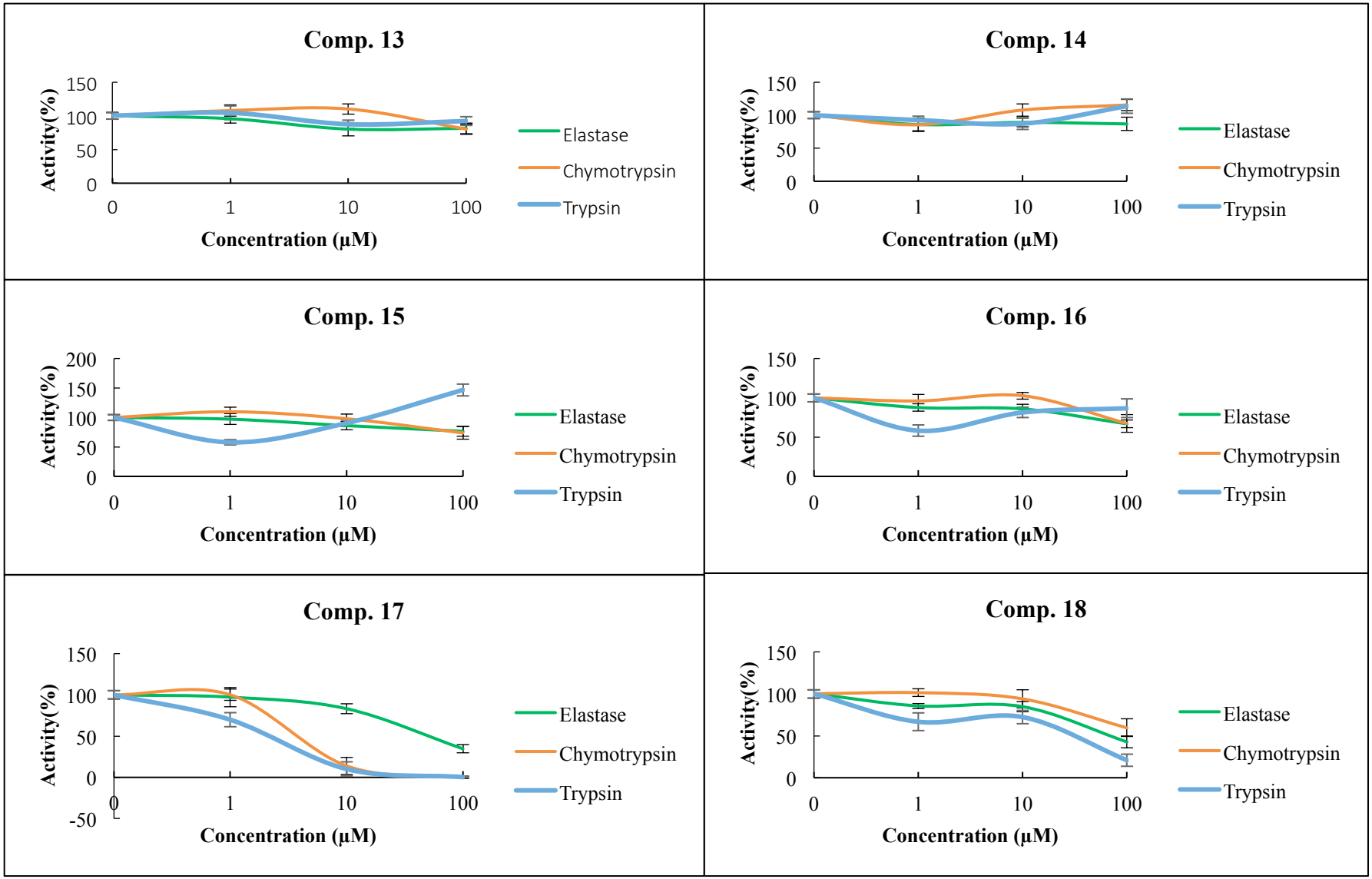
A: 25 ng/mL, B: 250 ng/mL, C: 10 μM, D: 100 μM; Increased and decreased rates compared with non-treatment group.

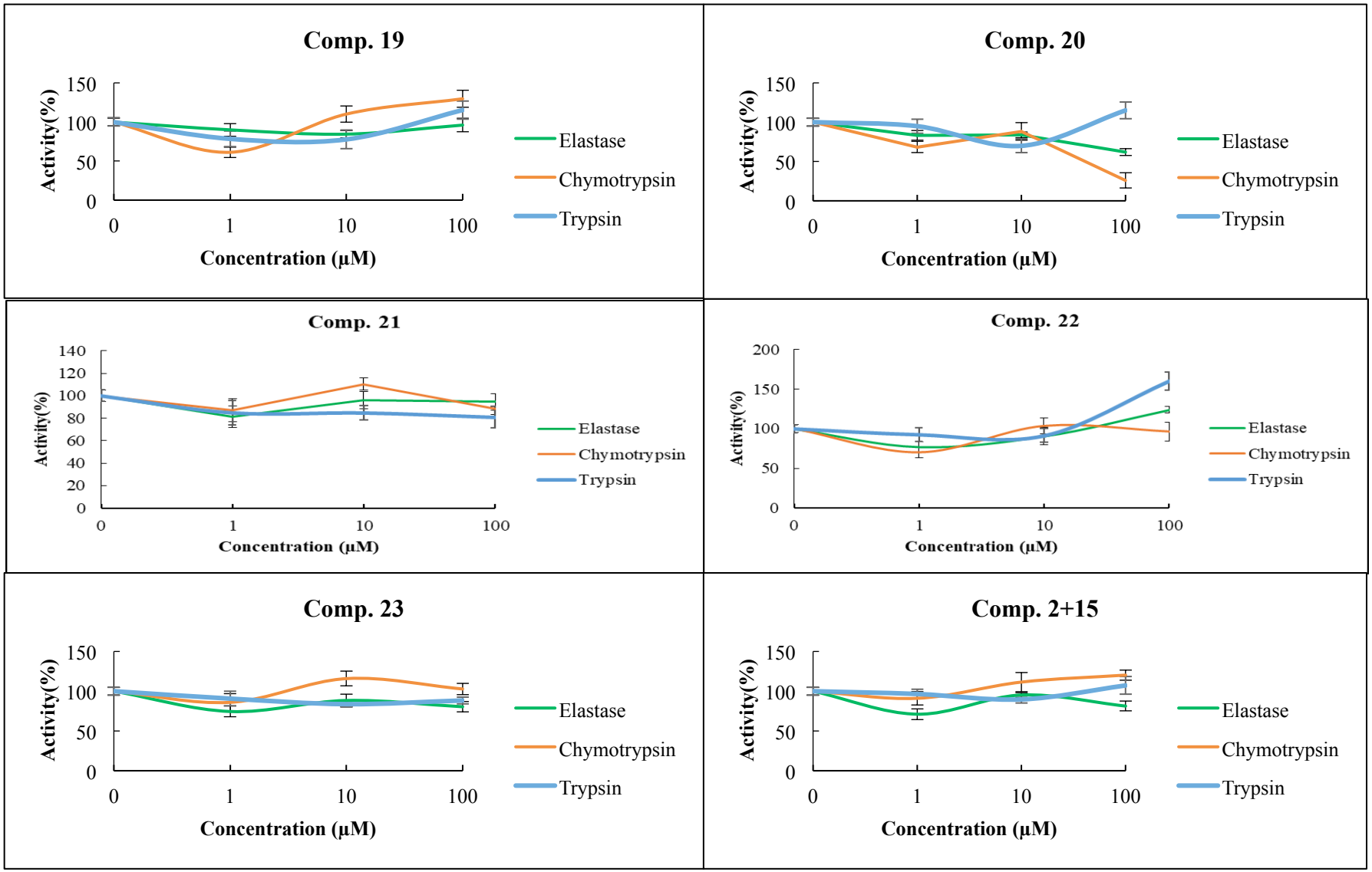

Leaf methanol extract (DB1)
Activity(%)
200
100
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Leaf water decoction extract (DB2)
Activity(%)
150
100
50
0
-50
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Stem bark methanol extract (DB3)
Activity(%)
150
100
50
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Stem bark water decoction extract (DB4)
Activity(%)
150
100
50
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Root ethanol extract (DB5)
Activity(%)
300
200
100
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Root methanol extract (DB6)
Activity(%)
300
200
100
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin


Root dichloromethane fraction (DB7)
Activity(%)
300
200
100
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Root ethyl acetate fraction (DB8)
Activity(%)
150
100
50
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Root water decoction extract (DB9)
Activity(%)
150
100
50
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Heartwood water decoction extract (DB10)
Activity(%)
200
150
100
50
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin
Heartwood chloroform fraction (DB11)
Activity(%)
150
100
50
0
0
2.5
25
250
Concentration (ng/mL)
Elastase
Chymotrypsin
Trypsin


Comp. 1
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 2
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 3
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 4
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 5
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 6
Activity(%)
200
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin


Comp. 7
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 8
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 9
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 10
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 12
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin


Comp. 13
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 14
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 15
Activity(%)
200
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 16
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 17
Activity(%)
150
100
50
0
-50
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 18
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin


Comp. 19
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 20
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 21
Activity(%)
140
120
100
80
60
40
20
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 22
Activity(%)
200
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 23
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin
Comp. 2+15
Activity(%)
150
100
50
0
0
1
10
100
Concentration (μM)
Elastase
Chymotrypsin
Trypsin

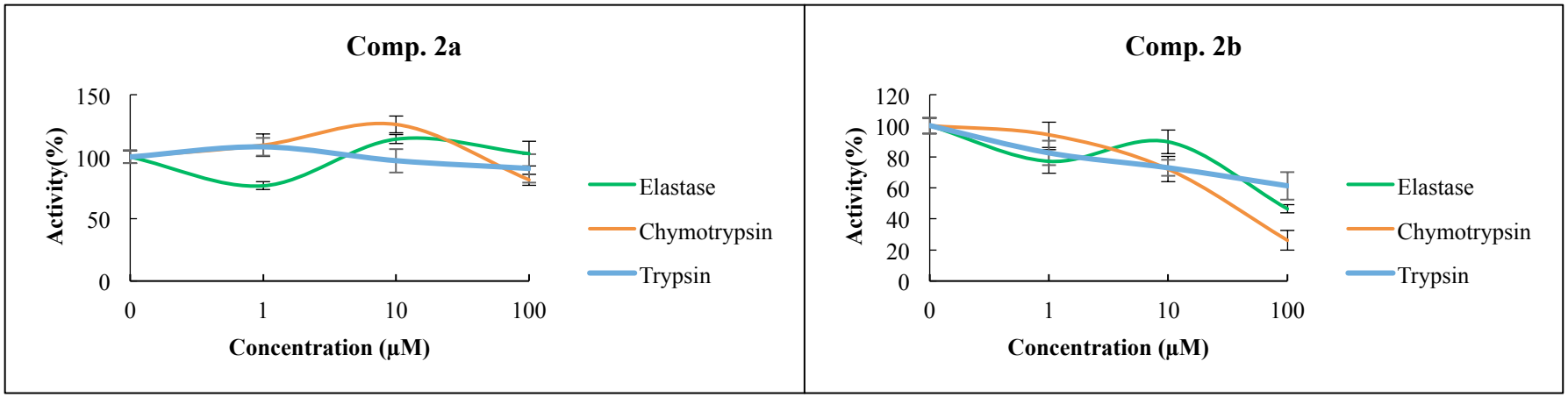


Figure 27. Effects of extracts **DB1-DB11** ((2.5, 25, and 250 ng/mL) and isolated compounds **1-10,** and **12-25** (1, 10, and 100 µM) on the amounts of serine proteases enzymes trypsin, chymotrypsin, and elastase

s

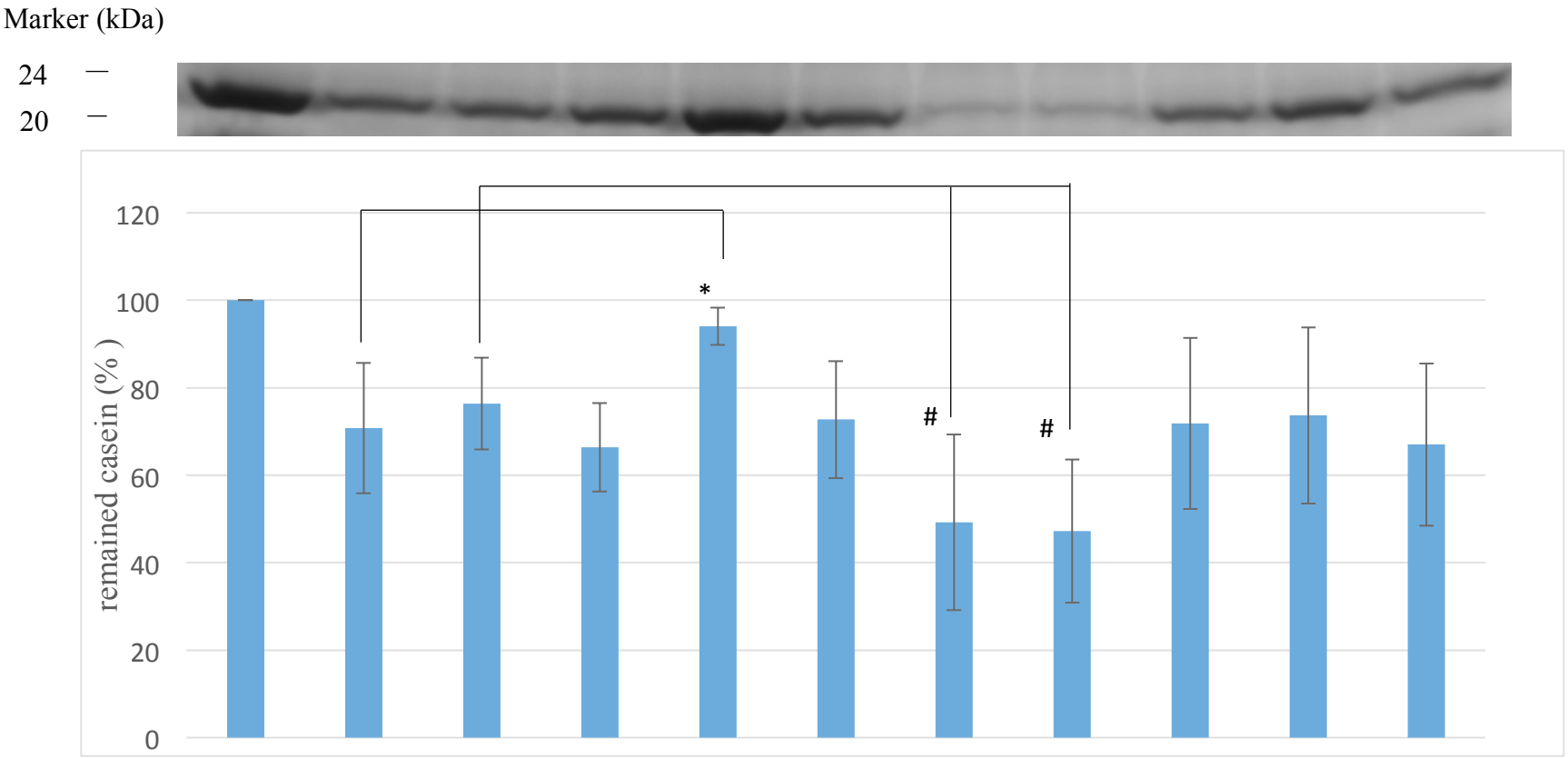


Figure 28. Effect of extracts and compounds on casein-cleavage-activity by trypsin. Data present mean ± SD values of 7 independent determinations. Differences were determined using student t-test and considered to be significant when p-value was less 0.05. *p<0.01 vs. without additives; #p<0.01 vs. DMSO

Conclusion

The current thesis have carried out the phytochemical studies of two Vietnamese plants *D. tonkinnensis* and *C. formosum* demonstrating a variety of flavonoids in both two plants. The results can remark as follows:

1) Forty seven secondary metabolites were isolated from heartwood, leaf and root of *D. tonkinnensis*, consisting of thirty nine flavonoids and flavonoid glycosides, three sesquiterpenes, two monoacylglycerides, two mono-phenols, and one alkaloid.

2) Nine compounds were isolated from *C. formosum*, comprising of six flavonols, two phytosterols, and one xanthone.

3) New compounds were found in *D. tonkinnensis*, including two flavanones (2*S*)-6,8-dicarboxyethylpinocembrin **(20**), and (2*S*)-8-carboxyethylnaringenin (**21**), one isoflavone glycoside isocaviunin 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (daltonkinensiside) (**33**), two sesquiterpenes *rel*-(3*R,*6*S,*7*S*)-6α-hydroxycyclonerolidol (**25)**, and 2,5'-dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one (**47**), and one indole alkaloid 3-(1H-indol-3-yl)-2-methoxypropanamide (**31**).

4) Flavonoid from *D. tonkinnensis* species was assigned to flavones and isoflavones, flavanones and isoflavanones, pterocarpans, neoflavonoids, chalcones, and isoflavone-7-glycosides.

5) Two new compounds **20** and **21** are rare in nature and new indole **47** is a unique alkaloid in genus *Dalbergia*. Monoacylglycerides were reported for the first time from the family Fabaceae.

6) *D. tonkinesis* extracts and fractions as well as isolated compounds were found to be promising agents against the growth of bacterial strains. For instance, at the concentration of 1.0 mg/mL, the crude methanol extracts of leaf (**DB1**), stem bark (**DB3**), root (**DB6**), and heartwood chloroform fraction (**DB11**) showed the significant values of 80, 70, 80, and 70% in inhibiting the microbacterium *S. Pneumonia*, or at the concentration of 0.1 mg/mL, the flavanone pinocembrin (**5**), and isoflavone biochanin A (**11**) suppressed 80% of the growth of the two bacterial strains *B. cereus* and *S. pneumonia*.

7) In enzyme serine protease screening, at the concentrations of 25-250 ng/mL, such as methanol extracts of leaf and root, root ethanol extract and its dichloromethane fraction, and heartwood water decoction extract of *D. tonkinensis* may be served as useful sources to stimulate trypsin enzyme activity (1.4-1.9 times increase). In addition, water decoction extracts of leaf and stem bark may explain unknown ethno-pharmacology due to the high inhibitory effects in enzyme assays using trypsin, chymotrypsin, and elastase (86-100% inhibition), or quercetin (**17**) inhibits the activities of trypsin and chymotrypsin with IC_{50} 9.7 μM.

8) Regarding DPPH radical scavenging assay, (3*R*)-vestitol (**5**) and isoquercetin (**24**) possessed the strongest antioxidative IC_{50} values of 42.20 μg/mL and 45.63 μg/mL, respectively, and their values were comparable to that of the positive control catechin (IC_{50} 42.98 μg/mL).

9) The effects of substituted groups of flavonoids accounted for biological results.

Experimental section

General analytical instruments

- Specific optical rotation ($[\alpha]_D$): JASCO P-1030 digital polarimeter.

- Infrared spectroscopy (IR): JASCO FT-IR 410 infrared spectrophotometer.

- Ultraviolet spectroscopy (UV): Shimadzu UV-1650 PC spectrophotometer.

-Circular dichroism (CD): JASCO-J-725 spectropolarimeter.

-HR-MS: MStation JMS-700 instrument.

-HPLC: JASCO PU-1580 equipped with a JASCO UV-1575 detector.

-COSMOSIL $5C_{18}$-AR-II: Nacalai tesque.

-500 NMR spectra: Bruker Avance 500 MHz spectrometer.

Reagents

The dehydrated reactions were carried out under argon atmosphere in anhydrous solvent. All other reagents were used as supplied.

Silica gel column was performed on Kieselgel 60 (40-63 mesh, MERCK); Thin-layer chromatography (TLC) was operated using Merck Silica gel 60 F_{254} pre-coated plates.

Chemical shifts are reported in δ (ppm) with reference to solvent signals [^{1}H NMR: $CDCl_3$ (7.26), CD_3OD (3.31); ^{13}C NMR: $CDCl_3$ (77.0), CD_3OD (49.0)]. The signal patterns are indicated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Plant materials

The whole plant of *D. tonkinensis* was collected in Quangbinh Province, Vietnam in 2016. The leaves of *C. formosum* were collected in Ninhbinh Province, Vietnam in 2010. This plants were identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, Hanoi, Vietnam. The voucher specimens were deposited in Department of Bioactive Products, Institute of Natural Products Chemistry, VAST, Hanoi, Vietnam.

Chapter 2 Chemical constituents from *Dalbergia tonkinensis*

2.2. Phytochemical investigation of heartwood and structural elucidation

2.2.1. Isolation procedure

Dried powdered heartwood of *D. tonkinensis* (1.0 kg) were extracted with methanol (5x1.5 L) over the period of 5 days at room temperature and concentrated under decreased pressure to yield a black crude MeOH extract (46.53 g). The crude MeOH extract was suspended in hot MeOH-H_2O (1:1, v/v) and successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and H_2O. The resulting fractions (fr.s) were concentrated under decreased pressure to give the corresponding solvent-soluble fr.s *n*-hexane (5.3 g), $CHCl_3$ (39.43 g), EtOAc (1.3 g) and H_2O (0.5 g) residue, respectively.

The fr. $CHCl_3$ (39.43 g) was separated by a silica gel column (40-63 mesh, Merck), eluting with a gradient of *n*-hexane-CH_3COCH_3 (2:1, v/v), to give 6 fr.s (C1-C6). The fr. C2 (6.1 g) was then chromatographed on a silica gel column (40-63 mesh, Merck) eluting with a solvent mixture of $CHCl_3$-CH_3COCH_3 (70:1, v/v) to produce 8 fr.s (C21-C28). Using several column chromatographies filled with silica gel or reversed RP-18, compounds **5** (45 mg), **6** (6.1 mg), **7** (18 mg), **8** (3 mg), **22** (4.2 mg), and **23** (2 mg) were obtained from the fr. C22 (1.1 g); compounds **1** (70 mg), **2** (0.2 g), and **4** (4 mg) were derived from the fr. C24 (1.5 g); compounds **24** (1.5 mg), and **25** (2 mg) were came from the fr. C27 (0.4 g); the remainder compound **3** (54 mg) was saperated from the fr. C26 (1.0 g). In the same manner, the fr. C3 (15.3 g) was subjected to a silica gel column with mobile phase of $CHCl_3$-CH_3COCH_3 (30:1, v/v) to give 8 fr.s (C31-C38). Following the applications of normal silica gel, Sephadex LH-20, and RP-18 comlumns, total 9 compounds had been isolated, including compounds **12** (5

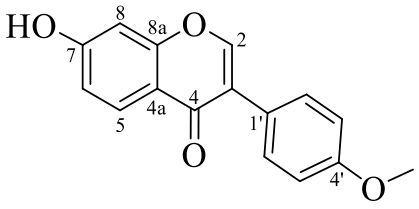
mg), **13** (5 mg), **14** (3 mg), **15** (4 mg), **16** (6 mg), **17** (2 mg), **18** (25 mg), **19** (3 mg), and **26** (1.5 mg).

In other case, to give total 3 fr.s (E1-E3), the EtOAc fr. (1.3 g) was fractionated by using the combination of a silica gel column and $CHCl_3$-CH_3COCH_3 (3:1, v/v). After that, the fr. E2 (0.8 g) was completely chromatographed on a silica gel column eluting with a solvent mixture of $CHCl_3$-EtOAc (3:1, v/v) to afford compounds **9** (5.1 mg), and **1 1** (4 mg), whereas chromatography on silica gel with CH_2Cl_2-MeOH (3:1, v/v), compound **10** (3.4 mg) had successfully been isolated from the fr. E3 (0.1 g).

With regards to power after extracting methanol, it was decocted in hot water (4 x 2.0 L) over the period of 4 h and concentrated under decreased pressure to yield brownish black crude (15.0 g), which was then subjected to a Diaion HP-20 column, eluting with gradient solvent mixture of H_2O-MeOH (1:0→0:1) to produce 5 fr.s (W1-W5). The fr. W2 (0.9 g) was further chromatographed on a silica gel column, eluting with a gradient of $CHCl_3$-MeOH-H_2O (3:1:0.1, v/v/v) to afford compounds **27** (2.5 mg) and **28** (7.4 mg). Two new carboxyethylflavanone **20** (2 mg) and **21** (4 mg) were derived from fr. W4 (1.44 g) by using a silica gel column with $CHCl_3$-MeOH-H_2O (1:1:0.1, v/v/v).

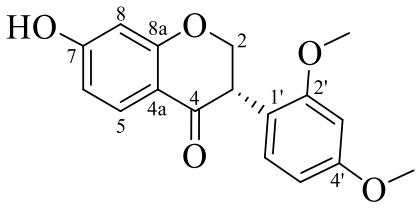
2.2.2. Chemical and physcical data of isolated compounds

Compound **1**: Formononetin



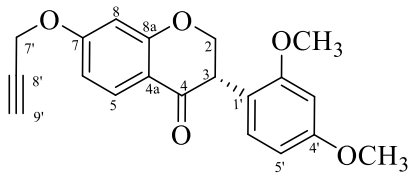
White amorphous solid, EI-MS (+): *m/z* 268 $[M]^+$, $C_{16}H_{12}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.17 (1H, s, H-2), 8.08 (1H, d, 9.0 Hz, H-5), 7.49 (2H, d, 8.5 Hz, H-2', H-6'), 7.01 (2H, d, 8.5 Hz, H-3', H-5'), 6.97 (1H, dd, 9.0, 2.0 Hz, H-6), 6.88 (1H, d, 2.0 Hz, H-8), 3.85 (3H, s, 4'-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 178.1 (s, C-4), 164.9 (s, C-7), 161.1 (s, C-4'), 159.8 (s, C-8a), 154.8 (d, C-2), 131.4 (d, C-2', C-6'), 128.5 (d, C-5), 125.7 (s, C-1'), 125.6 (s, C-3), 118.1 (s, C-4a), 116.6 (d, C-6), 114.9 (d, C-3', C-5'), 103.3 (d, C-8), 55.7 (q, 4'-OCH_3).

Compound **2**: Sativanone



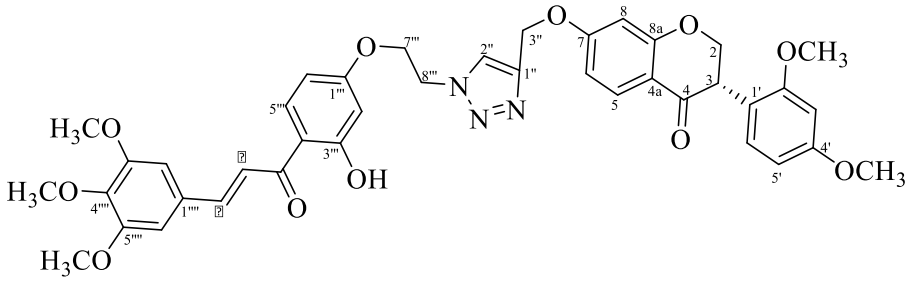
White amorphous solid, EI-MS (+): *m/z* 300 $[M]^+$, $C_{17}H_{16}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.78 (1H, d, 8.5 Hz, H-5), 7.00 (1H, d, 8.5 Hz, H-6'), 6.57 (1H, d, 2.0 Hz, H-8), 6.52 (1H, dd, 8.5, 2.0 Hz, H-6), 6.48 (1H, dd, 8.5, 2.0 Hz, H-5'), 6.35 (1H, d, 2.0 Hz, H-3'), 4.56 (1H, t, 11.0 Hz, H-2a), 4.40 (1H, dd, 11.0, 5.5 Hz, H-2b), 4.16 (1H, dd, 11.0, 5.5 Hz, H-3), 3.79 (3H, s, 2'-OCH_3), 3.76 (3H, s, 4'-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 194.4 (s, C-4), 166.4 (s, C-7), 165.7 (s, C-8a), 162.2 (s, C-4'), 159.9 (s, C-2'), 132.0 (d, C-5), 130.4 (d, C-6'), 117.4 (s, C-1'), 115.7 (s, C-4a), 111.7 (d, C-3'), 106.0 (d, C-5'), 103.6 (d, C-6), 99.9 (d, C-8), 70.0 (t, C-2), 56.0 (q, 4'-OCH_3), 55.8 (q, 2'-OCH_3), 49.0 (d, C-3).

Compound **2a**: 7-Dehydroxy-7-(prop-2-yn-1-yl)sativanone



White amorphous solid, HR-EI-MS: *m/z* 338.1151 $[M+Na]^+$ calcd. for $C_{20}H_{18}O_5Na$, found: 338.1151; ^{1}H-NMR (500 MHz, DMSO, δ ppm): 7.76 (1H, d, 8.5 Hz, H-5), 7.01 (1H, d, 8.0 Hz, H-6'), 6.71 (1H, dd, 8.0, 2.0 Hz, H-5'), 6.59 (1H, d, 2.5 Hz, H-8), 6.64 (1H, d, 2.0 Hz, H-3'), 6.49 (1H, dd, 8.5, 2.5 Hz, H-6), 4.90 (2H, d, 2.0 Hz, H-7'), 4.60 (1H, t, 11.0 Hz, H-2a), 4.47 (1H, dd, 11.0, 5.5 Hz, H-2b), 4.20 (1H, dd, 11.0, 5.5 Hz, H-3), 3.75 (3H, s, 4'-OCH_3), 3.72 (3H, s, 2'-OCH_3), 3.65 (1H, t, 2.5 Hz, H-9'); ^{13}C-NMR (125 MHz, DMSO, δ ppm): 190.6 (s, C-4), 163.1 (s, C-7), 163.0 (s, C-8a), 160.1 (s, C-4'), 158.1 (s, C-2'), 130.7 (d, C-5), 128.6 (d, C-6'), 115.8 (s, C-1'), 115.4 (s, C-4a), 110.2 (d, C-3'), 105.0 (d, C-5'), 102.0 (d, C-6), 98.9 (d, C-8), 78.8 (s, C-8'), 78.5 (d, C-9'), 70.5 (t, C-2), 55.9 (t, C-7'), 55.6 (q, 4'-OCH_3), 55.2 (q, 2'-OCH_3), 46.8 (d, C-3).

Compound **2b**:



7-Dehydroxy-7-((1-(2-(3-hydroxy-4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)sativanone, trivial name Datosativanone: Yellow amorphous solid; HR-MS-MALDI-TOF: *m/z* 760.2482 $[M+Na]^+$ calcd. for $C_{40}H_{39}N_3O_{11}Na$, found: 760.2484; ^{1}H-NMR (500 MHz, DMSO, δ ppm): 8.35 (1H, s, H-2''), 8.27 (1H, d, 9.0 Hz, H-5'''), 7.91 (1H, d, 15.5 Hz, Hα), 7.78 (1H, d, 15.5 Hz, Hβ), 7.74 (1H, d, 8.5 Hz, H-5), 7.25 (2H, s, H-2'''', H-6''''), 6.99 (1H, d, 8.5 Hz, H-6'), 6.74 (2H, d, 2.5 Hz, H-8, H-2'''), 6.56 (1H, dd, 8.5, 2.5 Hz, H-5'), 6.54 (1H, d, 2.5 Hz, H-3'), 6.53

(1H, dd, 9.0, 2.5 Hz, H-6'''), 6.47 (1H, dd, 8.5, 2.5 Hz, H-6), 5.26 (2H, s, H-3''), 4.83 (2H, t, 6.0 Hz, H-7'''), 4.60 (1H, t, 11.0 Hz, H-2a), 4.54 (2H, t, 6.0 Hz, H-8'''), 4.46 (1H, dd, 11.0, 5.5 Hz, H-2b), 4.19 (1H, dd, 11.0, 5.5 Hz, H-3), 3.86 (6H, s, 3''''-OCH_3, 5''''-OCH_3), 3.74 (3H, s, 4'-OCH_3), 3.72 (3H, s, 4''''-OCH_3), 3.70 (3H, s, 2'-OCH_3); ^{13}C-NMR (125 MHz, DMSO, δ ppm): 191.9 (s, CO), 190.5 (s, C-4), 164.4 (s, C-1'''), 166.9 (s, C-3'''), 163.9 (s, C-7), 163.2 (s, C-8a), 160.0 (d, C-4'), 130.6 (d, C-5), 158.1 (s, C-2'), 153.1 (s, C-3'''', C-5''''), 144.9 (d, Cβ), 142.0 (s, C-1''), 140.0 (s, C-4''''), 132.7 (d, C-5'''), 129.9 (d, C-1''''), 125.4 (d, C-2''), 120.9 (d, Cα), 115.8 (s, C-4a), 115.1 (s, C-1'), 114.2 (s, C-4'''), 110.2 (d, C-3'), 107.5 (d, C-6'''), 106.9 (d, C-2'''', C-6''''), 104.9 (d, C-5'), 101.8 (d, C-2'''), 101.6 (d, C-6), 98.8 (d, C-8), 72.8 (t, C-7'''), 70.4 (t, C-2), 60.1 (q, 4''''-OCH_3), 56.1 (q, 3''''-OCH_3, 5''''-OCH_3), 55.6 (q, 2'-OCH_3), 55.2 (q, 4'-OCH_3), 48.7 (t, C-8'''), 46.7 (d, C-3), 38.1 (t, C-3'').

Semi-synthesis of compounds 2a and **2b:** To a solution of compound **2** (20 mg), K_2CO_3 (18 mg), propargyl bromide (0.15 mL) in dimethylformamide (4.0 mL) was stirred at room temperature for 5h. The mixture reaction was then diluted with water and extracted three times with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. This residue was purified by silica gel column and mobile phase *n*-hexane/acetone 3:1 to give compound **2a** (14.4 mg).

A mixture of **2a** (1.0 mol) and the chalcone azide (1.0 mol) in DMSO (7 mL) was stirred at room temperature for 10 min. A catalytic amount of CuI was then added and stirred overnight at room temperature. The product was subsequently participated in distilled water, and filtered by Büsner funnel. Compound **2b** (8.25 mg) was obtained by silica gel column using CH_2Cl_2/MeOH 9:1 as eluting system.

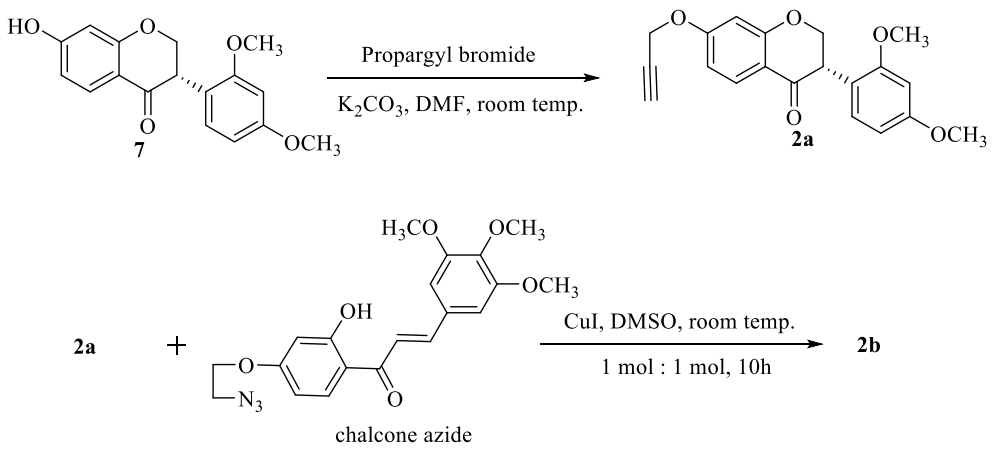
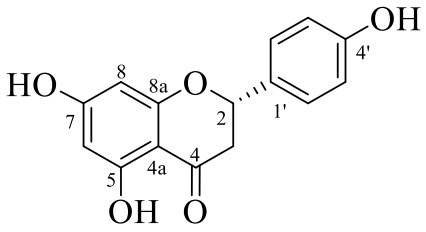


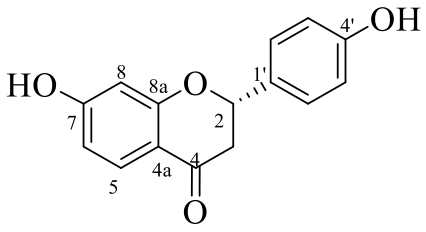
Figure 26. Semisynthetic reactions of **2a** and **2b**

Compound **3**: Naringenin



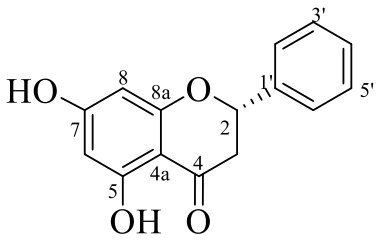
Yellow amorphous solid, EI-MS (+): *m/z* 272 $[M]^+$, $C_{15}H_{12}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.34 (2H, d, 8.5 Hz, H-2', H-6'), 6.84 (2H, d, 8.5 Hz, H-3', H-5'), 5.91 (2H, d, 2.0 Hz, H-6, H-8), 5.36 (1H, dd, 13.0, 3.0 Hz, H-2), 3.13 (1H, dd, 17.0, 13.0 Hz, H_b-3), 2.72 (1H, dd, 17.0, 3.0 Hz, H_a-3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 197.8 (s, C-4), 168.4 (s, C-7), 165.5 (s, C-5), 164.9 (s, C-8a), 159.1 (s, C-4'), 131.8 (s, C-1'), 129.1 (d, C-2', C-6'), 116.4 (d, C-3', C- 5'), 103.4 (s, C-4a), 97.1 (d, C-6), 96.2 (d, C-8), 80.5 (d, C-2), 44.1 (t, C-3).

Compound **4**: Liquiritigenin



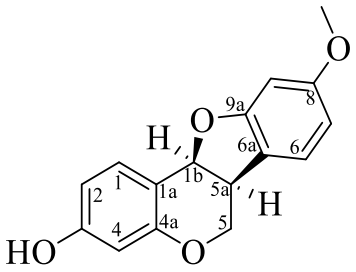
White amorphous solid, EI-MS (+): *m/z* 256 $[M]^+$, $C_{15}H_{12}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.74 (1H, d, 8.5 Hz, H-5), 7.33 (2H, d, 8.5 Hz, H-2', H-6'), 6.83 (2H, d, 8.5 Hz, H-3', H-5'), 6.51 (1H, dd, 8.5, 2.5 Hz, H-6), 6.37 (1H, d, 2.5 Hz, H-8), 5.39 (1H, dd, 13.0, 3.0 Hz, H-2), 3.06 (1H, dd, 17.0, 3.0 Hz, H_a-3), 2.71 (1H, dd, 17.0, 3.0 H_b-3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 193.5 (s, C-4), 169.9 (s, C-8a), 165.6 (s, C-7), 158.9 (s, C-4'), 131.4 (s, C-1'), 129.8 (s, C-5), 129.0 (d, C-2', C-6'), 116.3 (d, C-3', C- 5'), 114.9 (s, C-4a), 111.8 (d, C-6), 103.8 (d, C-8), 81.0 (d, C-2), 44.9 (t, C-3).

Compound **5**: Pinocembrin



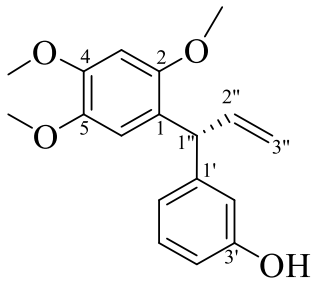
White amorphous solid, EI-MS (+): *m/z* 256 $[M]^+$, $C_{15}H_{12}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.52 (2H, d, 7.5 Hz, H-2', H-6'), 7.44 (2H, t, 8.0 Hz, H-3', H-5'), 7.39 (1H, t, 7.0 Hz, H-4'), 5.96 (1H, d, 2.0 Hz, H-6) 5.92 (1H, d, 2.0 Hz, H-8), 5.49 (1H, dd, 12.5, 3.0, H-2), 3.12 (1H, dd, 17.5, 13.0, H-3a), 2.81 (1H, dd, 17.0, 3.0, H-3b); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 197.3 (s, C-4), 168.4 (s, C-7), 165.5 (s, C-5), 140.4 (s, C-1'), 129.7 (d, C-3', C- 5'), 129.6 (d, C-4'), 103.4 (s, C-8a), 127.4 (d, C-2', C-6'), 103.4 (s, C-4a), 97.2 (d, C-6), 96.2 (d, C-8), 80.5 (d, C-2), 44.2 (t, C-3).

Compound **6**: Medicarpin



White amorphous solid, EI-MS (+): *m/z* 284 $[M]^+$, $C_{17}H_{16}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.18 (1H, d, 8.0 Hz, H-6), 7.30 (1H, d, 8.5 Hz, H-1), 6.46 (1H, dd, 8.0, 2.0 Hz, H-7), 6.40 (1H, d, 2.0 Hz, H-9), 6.32 (1H, d, 2.5 Hz, H-4), 5.48 (1H, t, 6.5 Hz, H-1b), 4.32 (1H, dd, 12.0, 6.0 Hz, H-5a), 3.76 (3H, s, 8-OCH_3), 3.54 (2H, m, H-5); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 162.6 (s, C-3), 162.1 (s, C-4a), 160.1 (s, C-8), 158.1 (s, C-9a), 133.2 (d, C-1), 128.9 (s, C-6a), 125.9 (d, C-6), 112.9 (s, C-1a), 110.7 (d, C-2), 107.3 (d, C-7), 104.1 (d, C-4), 97.6 (d, C-9), 80.1 (d, C-1b), 67.6 (t, C-5), 55.9 (8-OCH_3), 40.9 (d, C-5a).

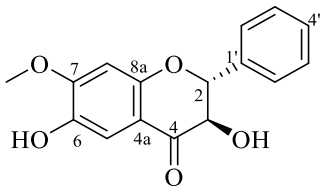
Compound **7**: 3'-Hydroxy-2,4,5-trimethoxydalbergiquinol



White amorphous solid, EI-MS (+): *m/z* 300 $[M]^+$, $C_{18}H_{20}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.08 (1H, dt, 8.0, 4.5 Hz, H-5'), 6.74 (1H, s, H-6), 6.66 (1H, s, H-3), 6.64 (1H, brd, 7.5 Hz, H-6'), 6.61 (1H, brd, 2.5 Hz, H-2'), 6.60 (1H, overlap, H-4'), 6.26 (1H, ddd, 17.0, 10.5, 7.0 Hz, H-2"), 5.16 (1H, dt, 10.0, 1.5 Hz, H_a-3"), 5.00 (1H, d, 6.5 Hz, H-1"), 4.90 (1H, dt, 17.0, 1.5 Hz, H_b-3"), 3.85 (3H, s, 4-OCH_3), 3.74 (3H, s, 5-OCH_3), 3.73 (3H, s, 2-

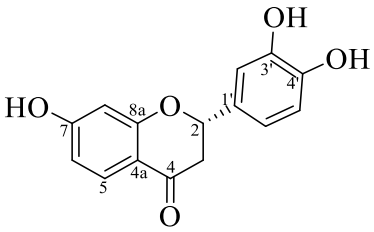
OCH_3); ^{13}C-NMR (125 MHz, CD_3OD): 158.2 (s, C-3'), 153.1 (s, C-2), 150.0 (s, C-4), 146.4 (s, C-1'), 144.3 (s, C-5), 142.0 (d, C-2''), 130.0 (d, C-5'), 125.1 (s, C-1), 120.9 (d, C-6'), 116.5 (d, C-4'), 116.0 (t, C-3''), 115.9 (d, C-6), 113.9 (d, C-2'), 99.9 (d, C-3), 57.6 (q, 2-OCH_3), 57.0 (s, 5-OCH_3), 56.8 (q, 4-OCH_3), 48.5 (d, C-1'').

Compound **8**: Buteaspermanol



White amorphous solid, EI-MS (+): *m/z* 286 $[M]^+$, $C_{16}H_{14}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.57 (2H, t, 7.0 Hz, H-2′, 6′), 7.40-7.45 (3H, m, H-3′, 4′, 5′), 7.31 (1H, s, H-5), 6.43 (1H, s, H-8), 5.09 (1H, d, 12.0 Hz, H-2), 4.53 (1H, d, 12.0 Hz, H-3), 3.88 (3H, s, 7-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 194.28 (s, C-4), 159.7 (s, C-9), 156.8 (s, C-7), 145.5 (s, C-1′),138.8 (s, C-6), 129.8 (s, C-4′), 129.3 (d, C-3′, 5′), 128.9 (d, C-2′, 6′), 112.1 (s, C-10), 108.2 (d, C-5), 104.5 (d, C-8), 85.90 (d, C-2), 74.7 (d, C-3), 56.7 (q, 7-OCH_3).

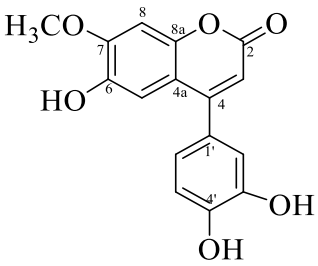
Compound **9**: Butin



White amorphous solid, EI-MS (+): *m/z* 272 $[M]^+$, $C_{15}H_{12}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.72 (1H, d, 9.0 Hz, H-5), 6.95 (1H, d, 1.5 Hz, H-8), 6.81 (2H, m, H-5', H-6'), 6.48 (1H, dd, 9.0, 2.5 Hz, H-6), 6.32 (1H, d, 2.0 Hz, H-2'), 5.32 (1H, dd, 13.0, 3.0 Hz, H-2), 3.01 (1H, dd, 17.0, 3.0 Hz, H-3a), 2.67 (1H, dd, 17.0, 3.0 Hz, H-3b). ^{13}C-NMR (125 MHz,

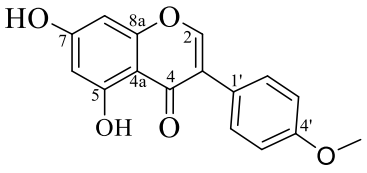
CD_3OD, δ ppm): 193.5 (s, C-4), 165.7 (s, C-7), 165.3 (s, C-4a), 146.8 (s, C-3'), 146.5 (s, C-4'), 132.2 (s, C-1'), 129.8 (s, C-5), 119.2 (d, C-6'), 116.3 (d, C- 5'), 116.2 (s, C-8a), 114.7 (d, C-2'), 111.5 (d, C-6), 104.1 (d, C-8), 81.0 (d, C-2), 45.0 (t, C-3).

Compound **10**: 3'-Hyrdoxymelanettin



White amorphous powder, EI-MS (+): *m/z* 300 $[M]^+$, $C_{16}H_{12}O_6$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.09 (1H, s, H-8), 7.05 (1H, s, H-5), 6.95 (1H, d, 8.0 Hz, H-5'), 6.94 (1H, s, H-2'), 6.88 (1H, dd, 8.0, 2.0 Hz, H-6'), 6.16 (1H, s, H-3), 3.99 (3H, s, 7-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 164.3 (s, C-2), 158.4 (s, C-4), 153.4 (s, C-7), 150.5 (s, C-8a), 148.4 (s, C-4'), 146.8 (s, C-3'), 145.0 (s, C-6), 121.5 (d, C-5'), 116.7 (d, C-2'), 116.2 (d, C-6'), 113.3 (s, C-4a), 112.2 (d, C-5), 111.3 (s, C-3), 101.5 (d, C-8), 56.9 (q, 7-OCH_3).

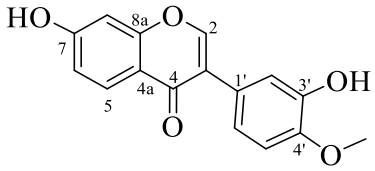
Compound **11**: Biochanin A



Yellow amorphous solid, EI-MS (+): *m/z* 284 $[M]^+$, $C_{16}H_{12}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.11 (1H, s, H-2), 7.50 (2H, d, 9.0 Hz, H-2', H-6'), 7.00 (2H, d, 8.5 Hz, H-3', H-5'), 6.38 (1H, d, 2.0 Hz, H-8), 6.25 (2H, d, 2.0 Hz, H-6), 3.85 (3H, s, 4'-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 182.2 (s, C-4), 166.0 (s, C-7), 163.9 (s, C-5), 161.2 (s, C-4'),

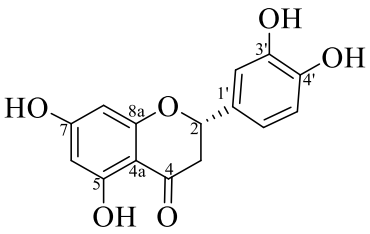
159.7 (s, C-8a), 155.0 (d, C-2), 131.3 (d, C-2', C-6'), 124.6 (s, C-1'), 124.5 (s, C-3), 114.9 (s, C-3', C-5'), 106.3 (s, C-4a), 100.2 (d,C-6), 94.8 (d, C-8), 55.8 (q, 4'-OCH_3).

Compound **12**: Calycosin



White amorphous solid, EI-MS (+): *m/z* 284 $[M]^+$, $C_{16}H_{12}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.14 (1H, s, H-2), 8.07 (1H, d, 8.5 Hz, H-5), 7.06 (1H, overlap, H-2'), 6.99 (1H, brs, H-5'), 6.98 (1H, brs, H-6'), 6.96 (1H, dd, 8.5, 2.0 Hz, H-6), 6.87 (1H, d, 2.0 Hz, H-8), 3.85 (3H, s, 4'-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 178.0 (s, C-4), 164.7 (s, C-7), 159.8 (s, C-8a), 158.4 (d, C-2), 149.2 (s, C-4'), 147.4 (s, C-3'), 128.5 (d, C-5), 126.2 (s, C-1'), 125.8 (s, C-3), 121.6 (d, C-6'), 118.2 (s, C-4a), 117.4 (d, C-2'), 112.7 (d, C-5'), 116.4 (d, C-6), 103.3 (d, C-8), 56.5 (q, 4'-OCH_3).

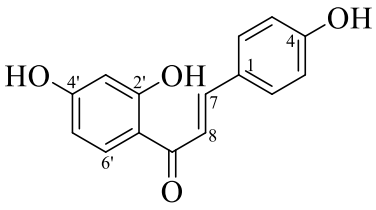
Compound **13**: Eriodictoyl



White amorphous solid, EI-MS (+): *m/z* 288 $[M]^+$, $C_{15}H_{12}O_6$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 6.94 (1H, s, H-2'), 6.81 (2H, s, H-5', H-6'), 5.92 (1H, d, 2.0 Hz, H-6), 5.90 (1H, d, 2.0 Hz, H-8), 5.31 (1H, dd, 13.0, 3.0 Hz, H-2), 3.09 (1H, dd, 17.0 Hz, 13.0, H-3a), 2.72 (1H, dd, 15.0, 5.0 Hz, H-3b); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 197.8 (s, C-4), 164.8 (s, C-5), 163.0 (s, C-7), 155.8 (s, C-8a), 146.9 (s, C-4'), 146.5 (s, C-3'), 131.8 (s, C-1'),

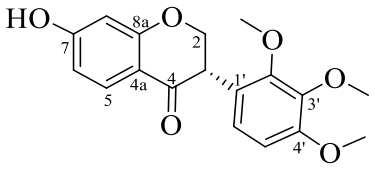
119.3 (d, C-6'), 116.3 (d, C-5'), 114.7 (d, C-2'), 103.4 (s, C-4a), 97.1 (d, C-8), 96.3 (d, C-6), 80.5 (d, C-2), 44.1 (t, C-3).

Compound **14**: Isoliquiritigenin



Yellow amorphous solid, EI-MS (+): *m/z* 256 $[M]^+$, $C_{15}H_{12}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.90 (1H, d, 9.0 Hz, H-6'), 7.81 (1H, d, 15.5 Hz, H-7), 7.64 (1H, d, 15.5 Hz, H-8), 7.65 (2H, dd, 15.5, 8.5 Hz, H-2, H-6), 6.87 (2H, d, 8.5 Hz, H-3, H-5), 6.31 (1H, dd, 9.0, 2.0 Hz, H-5'); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 193.6 (s, CO), 167.5 (s, C-4'), 166.6 (s, C-2'), 161.6 (s, C-4), 145.6 (d, C-7), 133.4 (d, C-6'), 131.8 (d, C-2, C-6), 127.8 (s, C-1), 118.4 (d, C-8), 116.7 (d, C-3, C-5), 114.7 (s, C-1'), 109.2 (d, C-5'), 103.9 (d, C-3').

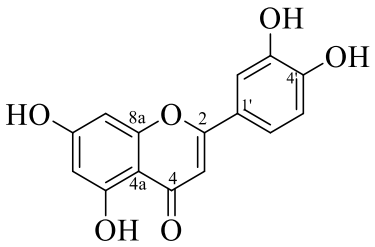
Compound **15**: 3'-*O*-Methylviolanone



White amorphous solid, EI-MS (+): *m/z* 330 $[M]^+$, $C_{18}H_{18}O_6$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.79 (1H, d, 8.5 Hz, H-5), 6.86 (1H, d, 8.5 Hz, H-5'), 6.75 (1H, d, 8.5 Hz, H-5'), 6.54 (1H, dd, 8.5, 2.0 Hz, H-6), 6.37 (1H, d, 2.0 Hz, H-8), 4.57 (1H, t, 11.5 Hz, H-2a), 4.43 (1H, dd, 11.0, 5.5 Hz, H-3), 4.14 (1H, dd, 12.0, 5.5 Hz, H-2b), 3.83 (3H, s, 4'-OCH_3), 3.83 (3H, s, 2'-OCH_3), 3.82 (3H, s, 3'-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 194.2 (s, C-4), 166.5 (s, C-7), 165.8 (s, C-8a), 153.2 (s, C-2'), 155.1 (s, C-4'), 143.6 (s, C-3'), 130.4 (d,

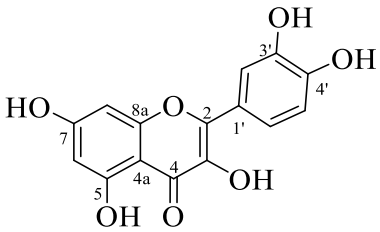
C-5), 126.0 (s, C-6'), 123.1 (s, C-1'), 111.8 (d, C-6), 115.5 (s, C-4a), 108.8 (s, C-5'), 103.7 (d, C-8), 72.4 (t, C-2), 61.2 (q, 3'-OCH_3), 61.0 (q, 2'-OCH_3), 56.6 (q, 4'-OCH_3), 49.5 (d, C-3).

Compound **16**: Luteolin



Yellow amorphous powder, EI-MS (+): *m/z* 286 $[M]^+$, $C_{15}H_{10}O_6$; ^{1}H-NMR (500 MHz, DMSO, δ ppm): 7.42 (1H, dd, 8.5, 2.0 Hz, H-6'), 7.39 (1H, d, 8.5 Hz, H-2'), 6.89 (1H, d, 8.5 Hz, H-5'), 6.66 (1H, s, H-3), 6.44 (1H, d, 2.0 Hz, H-8), 6.19 (1H, d, 2.0 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO, δ ppm): 181.6 (s, C-4), 164.1 (s, C-7), 163.9 (s, C-2), 161.5 (s, C-8a), 157.3 (s, C-5), 149.7 (s, C-4'), 145.7 (s, C-3'), 121.5 (d, C-6'), 119.0 (s, C-1'), 116.0 (d, C-6'), 113.4 (d, C-2'), 103.7 (s, C-4a), 98.8 (d, C-6), 93.8 (d, C-8).

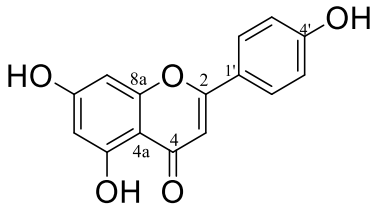
Compound **17**: Quercetin



Yellow amorphous powder, CI-MS (+): *m/z* 303 $[M+1]^+$, $C_{15}H_{10}O_7$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.75 (1H, d, 2.5 Hz, H-2'), 7.65 (1H, dd, 8.5, 2.5 Hz, H-6'), 6.91 (1H, d, 8.5 Hz, H-5'), 6.44 (1H, d, 2.0 Hz, H-8), 6.20 (1H, d, 2.0 Hz, H-6), 6.99 (1H, brs, H-5'); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 177.3 (s, C-4), 165.6 (s, C-7), 162.1 (s, C-5), 158.3 (s,

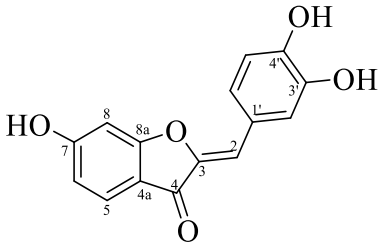
C-8a), 148.7 (s, C-4'), 148.0 (s, C-2), 146.2 (s, C-3'), 137.2 (s, C-3), 124.2 (s, C-1'), 121.7 (d, C-6'), 116.2 (d, C-2'), 116.1 (d, C-5'), 104.4 (s, C-4a), 99.3 (d, C-6), 94.4 (d, C-8).

Compound **18**: Apigenin



Yellow amorphous powder, EI-MS (+): *m/z* 270 $[M]^+$, $C_{15}H_{10}O_5$; ^{1}H-NMR (500 MHz, MeOD, δ ppm): 7.91 (2H, d, 9.0 Hz, H-2', H-6'), 6.92 (2H, d, 9.0 Hz, H-3', H-5'), 6.76 (1H, s, H-3), 6.47 (1H, d, 2.0 Hz, H-8), 6.19 (1H, d, 2.0 Hz, H-6); ^{13}C-NMR (125 MHz, MeOD, δ ppm): 181.7 (s, C-4), 164.1 (s, C-2), 163.7 (s, C-7), 161.4 (s, C-4'), 161.1 (s, C-5), 157.3 (s, C-8a), 128.4 (d, C-2', C-6'), 121.2 (s, C-1'), 115.9 (d, C-3', C-5'), 103.7 (s, C-4a), 102.8 (d, C-3), 98.8 (d, C-6), 93.9 (d, C-8).

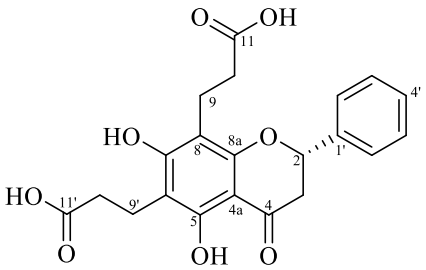
Compound **19**: Sulfuretin



White amorphous solid, EI-MS (+): *m/z* 270 $[M]^+$, $C_{15}H_{10}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.61 (1H, d, 9.0 Hz, H-5'), 7.54 (1H, d, 2.0 Hz, H-2'), 7.25 (1H, dd, 8.5, 2.0 Hz, H-6), 6.86 (1H, d, 8.5 Hz, H-5), 6.69 (3H, overlap, H-6, H-8, H-6'); ^{13}C-NMR (125 MHz, MeOD, δ ppm): 184.3 (s, C-4), 170.0 (s, C-7), 168.7 (s, C-8a), 149.4 (s, C-4'), 147.8 (s, C-3),

146.7 (s, C-3'), 126.8 (d, C-5), 126.3 (s, C-6'), 125.6 (s, C-1'), 118.9 (d, C-5'), 116.7 (d, C-6), 114.6 (d, C-2), 114.4 (d, C-2'), 114.2 (s, C-4a), 99.5 (d, C-8).

Compound **20**: (2*S*)-6,8-Dicarboxyethylpinocembrin



White amorphous solid;

$[\alpha]_D^{25}$ +45.0 (*c* 0.22, MeOH);

IR (film): 3370.12, 2931.19, 1721.05, 1683.56, 1585.53, 1444.84, 1382.38, 1216.64, 1090.62, 899.53, 693.56 cm^{-1};

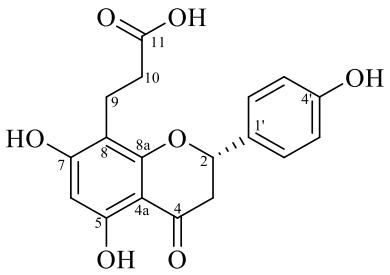
UV λ_{max} (MeOH) nm (log ε): 296 (3.64);

CD (*c* 0.2, MeOH): $\Delta\varepsilon_{290}$ (nm) −8.21, $\Delta\varepsilon_{339}$ (nm) +0.83;

HR-ESI-MS: *m/z* $[M-H]^-$ 399.1080 calcd. for $C_{21}H_{19}O_8$; found: 399.1079);

^{1}H- and ^{13}C-NMR Data of compound **20**: See Table 2.

Compound **21**: (2*S*)-8-Carboxyethylnaringenin



White amorphous solid;

$[\alpha]_D^{25}$ +37.0 (*c* 0.53, MeOH);

IR (film): 3356.96, 3089.96, 2924.09, 1737.86, 1685.78, 1576.65, 1477.47, 1377.17, 1215.15, 1035.77, 759.95, 675.09, 428.20 cm^{-1};

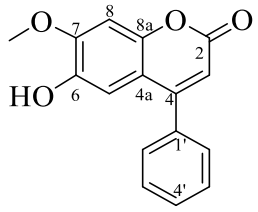
UV λ_{max} (MeOH) nm (log ε):294 (3.73);

CD (*c* 0.1, MeOH): $\Delta\varepsilon_{293}$ (nm) −0.71; $\Delta\varepsilon_{337}$ (nm) +0.19;

HR-ESI-MS: *m/z* $[M-H]^-$ 343.0815 calcd. for $C_{18}H_{15}O_7$; found: 343.0817);

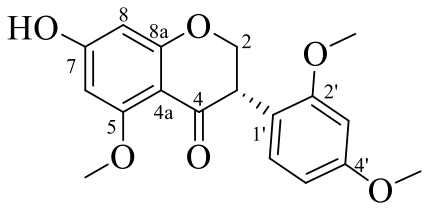
^{1}H- and ^{13}C-NMR Data of compound **21**: See Table 2.

Compound **22**: Dalbergin



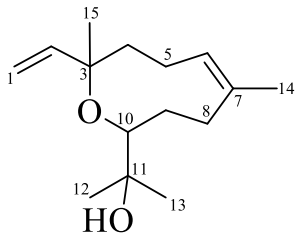
Light yellow solid, EI-MS (+): *m/z* 268 $[M]^+$, $C_{16}H_{12}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.57 (3H, m, H-3', H-4', H-5'), 7.51 (2H, m, H-2', H-6'), 7.06 (1H, s, H-8), 6.88 (1H, s, H-5), 6.21 (1H, s, H-3), 3.99 (3H, s, 7-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 163.9 (s, C-2), 158.2 (s, C-4), 153.6 (s, C-7), 113.1 (s, C-4a), 145.2 (s, C-6), 137.1 (s, C-1'), 130.8 (d, C-4'), 129.9 (d, C-3', C-5'), 129.5 (d, C-2', C-6'), 112.3 (d, C-3), 111.9 (d, C-5), 150.4 (s, C-8a), 101.1 (d, C-8), 56.9 (q, 7-OCH_3).

Compound **23**: Homoferreirin



White amorphous solid, EI-MS (+): *m/z* 330 $[M]^+$, $C_{18}H_{18}O_6$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.04 (1H, d, 8.5 Hz, H-6'), 6.59 (1H, s, H-3'), 6.52 (1H, d, 7.5 Hz, H-5'), 5.92 (1H, s, H-6), 5.90 (1H, s , H-8), 4.51 (1H, t, 11.0 Hz, H_b-2), 4.39 (1H, dd, 11.0, 5.5 Hz, H-3), 4.25 (1H, dd11.0, 5.0 Hz, H_a-2), 3.80 (3H, s, 2'-OCH_3), 3.81 (3H, s, 4'-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 199.0 (s, C-4), 168.2 (s, C-7), 165.8 (s, C-5), 165.2 (s, C-8a), 162.3 (s, C-4'), 159.9 (s, C-2'), 132.1 (d, C-6'), 116.8 (s, C-1'), 106.1 (d, C-5'), 103.3 (s, C-4a), 100.1 (s, C-3'), 97.1 (d, C-4), 96.0 (d, C-8), 71.5 (t, C-2), 56.1 (q, 2'-OCH_3), 55.8 (q, 4'-OCH_3), 48.5 (d, C-3).

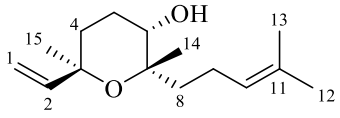
Compound **24**: Neroplomacrol



White amorphous solid, $C_{15}H_{26}O_2$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 5.93 (1H, dd,17.5, 11.0 Hz, H-2), 5.21 (2H, dd, 17.5, 1.5 Hz, H-1), 5.204 (1H, overlap, H-6), 3.25, (1H, dd, 10.5, 1.5 Hz, H-10), 2.25 (1H, m, H_a-8), 2.04 (2H, m, H-5, H_b-8), 1.64 (3H, s, H-14), 1.73, (1H, m, H_a-9), 1.54 (1H, dtd, 2.5, 3.0, 3.0 Hz, H-4), 1.35 (1H, m, H_b-9), 1.27 (3H, s, H-15), 1.18 (3H, s, H-12), 1.14 (3H, s, H-13); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 146.4 (d, C-2), 136.1 (s, C-7), 125.8 (d, C-6), 112.0 (t, C-1), 79.0 (s, C-10), 73.8 (s, C-11), 73.8 (s, C-3), 43.4

(t, C-4), 37.9 (t, C-8), 30.8 (t, C-9), 27.6 (q, C-15), 25.6 (q, C-12), 24.9 (q, C-13), 23.7 (C-5, t), 16.1 (q, C-14).

Compound **25**: *Rel*-(3*R*,6*S*,7*S*)-6α-hydroxycyclonerolidol



White amorphous solid;

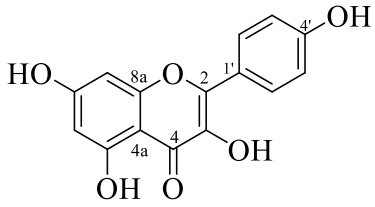
$[\alpha]_D^{25}$ −15.0 (*c* 0.02, $CHCl_3$);

IR (film) ν_{max}: 3362, 2923, 1642, 1434, 1092, 866 cm^{-1};

HR-ESI-MS: *m/z* 239.2011 $[M+H]^+$ (calcd. for $C_{15}H_{27}O_2$ 239.2010), 243.1724 $[M+Na-H_2O]^+$ (calcd. for $C_{15}H_{24}ONa$ 243.1724);

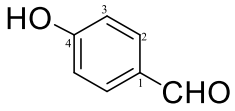
^{1}H- and ^{13}C-NMR Data of compound **25**: See Table 3.

Compound **26**: Kaemfperol



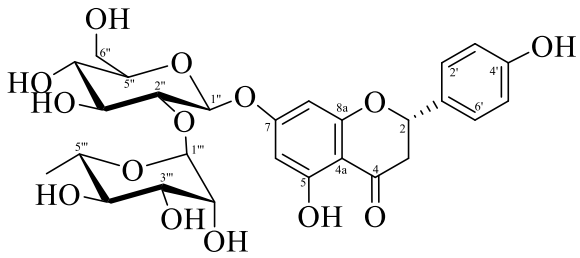
Yellow amorphous powder, EI-MS (+): *m/z* 286 $[M]^+$, $C_{15}H_{10}O_6$; ^{1}H-NMR (500 MHz, MeOD, δ ppm): 8.1 (2H, d, 8.5 Hz, H-2', H-6'), 6.91 (2H, d, 8.5 Hz, H-3', H-5'), 6.40 (1H, d, s, H-8), 6.19 (1H, s, H-6); ^{13}C-NMR (125 MHz, MeOD, δ ppm): 177.3 (s, C-4), 165.5 (s, C-7), 162.4 (s, C-5), 160.4 (s, C-4'), 158.2 (s, C-8a), 148.0 (s, C-2), 137.1 (s, C-3), 130.6 (d, C-2', C-6'), 123.7 (s, C-1'), 116.3 (d, C-3', C-5'), 104.6 (s, C-4a), 99.3 (d, C-6), 94.5 (d, C-8).

Compound **27**: 4-Hydroxybenzaldehyde **(**Formyl phenol)



White amorphous solid, EI-MS (+): *m/z* 123 $[M]^+$, $C_7H_7O_2$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 9.80 (1H, s, 1-CHO), 7.80 (2H, d, 8.5 Hz, H-2, H-6), 6.94 (2H, d, 8.5, H-3, H-5); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 191 (d, CHO), 164.1 (s, C-4), 132.4 (d, C-2, C-6), 129.0 (s, C-1), 116.1 (d, C-3, C-5).

Compound **28**: Naringin



White amorphous solid, EI-MS (+): *m/z* 580 $[M]^+$, $C_{27}H_{32}O_{14}$; ^{1}H-NMR (500 MHz, CD_3OD +DMSO, δ ppm): 12.04 (1H, s, 5-OH), 9.62 (1H, s, 4'-OH), 7.33 (2H, m, H-2', H-6'), 6.80 (2H, d, 7.0 Hz, H-3', H-5'), 6.11 (1H, d, 2.0 Hz, H-6), 6.09 (1H, t, 3.0 Hz, H-8), 5.50 (1H, ddd, 3.0, 2.5, 2.5 Hz, H-2), 5.31 (1H, d, 4.5 Hz, 3"-OH), 5.10-5.15 (3H, m, H-1", H-1'", 5"-OH), 4.72 (1H, d, 4.5 Hz, 4'"-OH), 4.66 (1H, d, 4 Hz, 2'"-OH), 4.58 (1H, s, 6"-OH), 4.47 (1H, d, 4.5 Hz, 3"-OH), 3.65-3.72 (3H, m, H-6"a, H-2'", H-5'"), 3.43 (3H, overlap, H-2", H-3", H-5"), 3.29-3.39 (2H, m, H-3a, H-6"b), 3.18-3.23 (2H, m, H-4", H-4'"), 2.73 (1H, m, H-3b), 1.16 (3H, d, 5.5 Hz, 5'"-CH_3); ^{13}C-NMR (125 MHz, MeOD, δ ppm): 198.5 (s, C-4), 166.6 (s, C-7), 165.0 (s, C-5), 164.6 (s, C-8a), 159.1 (s, C-4'), 130.9 (s, C-1'), 129.1 (d, C-2', C-6'), 116.4 (d, C-3', C-5'), 104.9 (s, C-4a), 102.5 (d, C-1'"), 99.4 (d, C-1"), 97.9 (d, C-6), 96.8 (d, C-8), 80.7

(d, C-2), 79.2 (d, C-2'''), 79.1 (d, C-3''), 78.1 (d, C-5''), 73.9 (d, C-4'''), 72.2 (d, C-2''', C-3'''), 71.3 (d, C-4''), 70.0 (d, C-5'''), 62.2 (t, C-6''), 44.1 (t, C-3), 18.2 (q, 5'''-CH_3).

2.3. Phytochemical investigation of leaf extract and structural elucidation

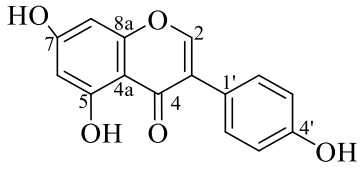
2.3.1. Isolated procedure

The leaf (2.0 kg) was extracted with MeOH (10.0 L) for 4h using the Soxhlet extractor, to produce the crude MeOH extract (300.0 g). This extract was then suspended in hot MeOH-H_2O (1:1, v/v) and successively partitioned with $CHCl_3$ and EtOAc to yield the corresponding fr.s. Using column chromatography filled with silica gel and $CHCl_3$-MeOH (99:1, v/v), the $CHCl_3$ extract (60.7 g) was separated to give 8 fr.s (C1-C8). Applying silica gel columns with mobile phases of $CHCl_3$-CH_3COCH_3 (30:1, v/v), and *n*-hexan-CH_3COCH_3 (6:1, v/v), compounds **11** (45.7 mg) and **29** (34.6 mg) were precipitated out of the fr. C2 (5.1 g) and the fr. C3 (4.3 g), respectively. Compound **30** (15.6 mg) was isolated from the fr. C6 (5.7 g) by using a silica gel column with $CHCl_3$-MeOH (3:1, v/v).

The H_2O-soluble residue (fr. W, 95.5 g) was subjected to Diaion HP-20 chromatography with gradient solvents of H_2O-MeOH (1:0→0:1, v/v) to afford 3 fr.s (W1-W3). The 25% MeOH fraction (fr. W2, 4.57 g) was separated by a normal silica gel CC, eluting with a gradient of $CHCl_3$-MeOH-H_2O (3:1:0.1, v/v/v) to yield compound **32** (20.6 mg) and 9 fr.s (W21-W29). The fr. W27 (0.9 g) was then purified by HPLC (Cosmosil 5C18-AR-II column (10x250 mm)) with MeOH-H_2O (7:3, 1 $mLmin^{-1}$) to give compound **33** (1.2 mg), while compound **34** (4.6 mg) were obtained from the fr. W28 (1.9 g) using the same manner. Finally, ultilizing HPLC (Cosmosil 5C18-AR-II column (10x250 mm)) with MeOH-H_2O-HCOOH (3:1: 0.1%, 2 $mLmin^{-1}$), compound **35** (1.6 mg) was obtained from the fr. W29 (0.6 g).

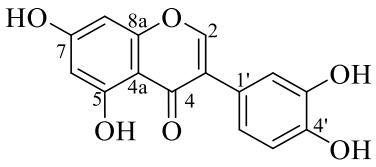
2.3.2. Chemical and physical data of isolated compounds

Compound **29**: Genistein



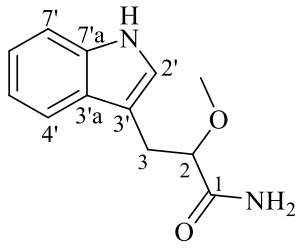
White crystal, EI-MS (+): *m/z* 270 $[M]^+$, $C_{15}H_{10}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.01 (1H, s, H-2), 7.32 (2H, d, 8.5 Hz, H-2', H-6'), 6.80 (2H,d , 8.5 Hz, H-3', H-5'), 6.30 (1H, d, 2.0 Hz, H-8), 6.17 (2H, d, 2.0 Hz, H-6); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 182.1 (s, C-4), 164.6 (s, C-7), 162.5 (s, C-5), 158.3 (s, C-4'), 157.4 (s, C-8a), 153.4 (d, C-2), 123.3 (s, C-1'), 121.9 (s, C-3), 130.0 (d, C-2', C-6'), 114.9 (s, C-3', C-5'), 104.9 (s, C-4a), 99.7 (d,C-6), 93.4 (d, C-8).

Compound **30**: Orobol



Yellow amorphous powder, EI-MS (+): *m/z* 286 $[M]^+$, $C_{15}H_{10}O_6$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.02 (1H, s, H-2), 7.01 (1H, d, 2.0 Hz, H-2'), 6.84 (1H, dd, 8.0, 2.0 Hz, H-6'), 6.84 (1H, d, 8.0 Hz, H-5'), 6.32 (1H, d, 2.0 Hz, H-8), 6.20 (2H, d, 2.0 Hz, H-6); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 180.9 (s, C-4), 164.6 (s, C-7), 162.5 (s, C-5), 158.3 (s, C-8a), 153.4 (d, C-2), 145.4 (s, C-4'), 144.8 (s, C-3'), 123.4 (s, C-1'), 122.4 (s, C-3), 120.3 (d, C-6'), 116.0 (d, C-5'), 114.9 (d, C-2'), 104.9 (s, C-4a), 98.7 (d, C-6), 93.4 (d, C-8).

Compound **31**: 3-(1H-indol-3-yl)-2-methoxypropanamide



White powder;

$[\alpha]_D^{25}$ +60.0 (*c* 0.43, MeOH);

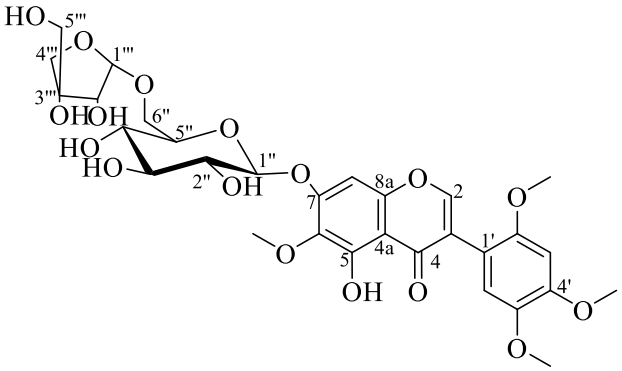
IR (film): 3416 (sh), 3045, 2980, 1629 (sh), 1548, 1514, 1453, 1355, 1199, 1146 cm^{-1};

UV (MeOH) λ_{max} nm (log ε): 223 (4.43), 281 (3.79), 288 (3.72);

HR-ESI-MS: *m/z* 241.0953 $[M+Na]^+$ (calcd. for $C_{12}H_{14}N_2O_2Na$ 241.0952), APCI-MS: *m/z* 188 $[M+H-OCH_3]^+$, 144 $[M+H-OCH_3-CONH_2]^+$, 118 [M+H-methoxypropanamide]$^+$;

^{1}H-NMR (500 MHz, CD_3OD, δ ppm) and ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): See Table 4.

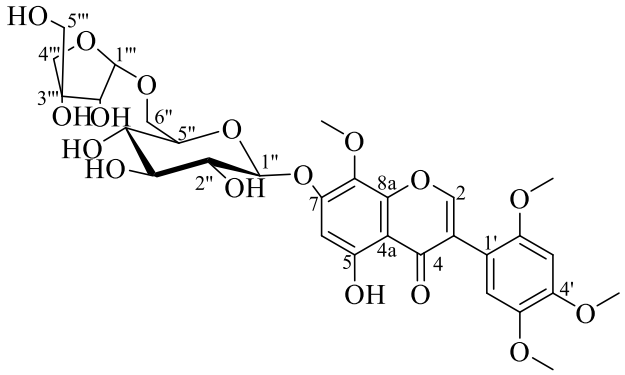
Compound **32**: Dalsissooside



White amorphous powder, EI-MS (+): *m/z* 668 $[M]^+$, $C_{30}H_{36}O_{17}$; ^{1}H-NMR (500 MHz, MeOD, δ ppm): 8.11 (1H, s, H-2), 6.93 (1H, s, H-6'), 6.91 (1H, s, H-8), 6.77 (1H, s, H-3'),

5.04 (1H, d, 7.5 Hz, H-1''), 4.95 (1H, d, 2.5 Hz, H-1'''), 3.35-4.06 (13H, sugar protons), 3.90 (3H, s, 4'-OCH_3), 3.89 (3H, s, 6-OCH_3), 3.80 (3H, s, 2'-OCH_3), 3.78 (3H, s, 5'-OCH_3); ^{13}C-NMR (125 MHz, MeOD, δ ppm): 180.6 (s, C-4), 156.7 (s, C-7), 156.2 (d, C-2), 153.3 (s, C-5), 152.8 (s, C-8a), 152.4 (s, C-2'), 150.3 (s, C-4'), 142.9 (s, C-5'), 132.9 (s, C-6), 120.4 (s, C-3), 116.3 (s, C-6'), 111.0 (s, C-1'), 109.9 (d, C-1'''), 107.1 (s, C-4a), 100.7 (d, C-1''), 98.3 (d, C-3'), 94.6 (d, C-8), 79.0 (d, C-3'''), 76.8 (d, C-2'''), 76.7 (d, C-3''), 76.0 (d, C-5''), 73.7 (t, C-4'''), 73.4 (d, C-2''), 70.3 (d, C-4''), 67.9 (t, C-6''), 64.2 (t, C-5'''), 60.2 (6-OCH_3), 56.3 (q, 2'-OCH_3), 55.9 (q, 5'-OCH_3), 55.5 (q, 4'-OCH_3).

Compound **33**: Daltonkinensiside



White amorphous powders;

$[\alpha]_D^{25}$ −266.9 (*c* 0.24, EtOH);

IR (film): 3510, 3378, 2903, 1654, 1617, 1581, 1525, 1506, 1467, 1371, 1282, 1211, 1148, 1065, 980, 905, 853, 783, 678 cm^{-1};

UV (EtOH) λ_{max} nm (log ε): 295 (3.61), 263 (3.87), 205 (3.96);

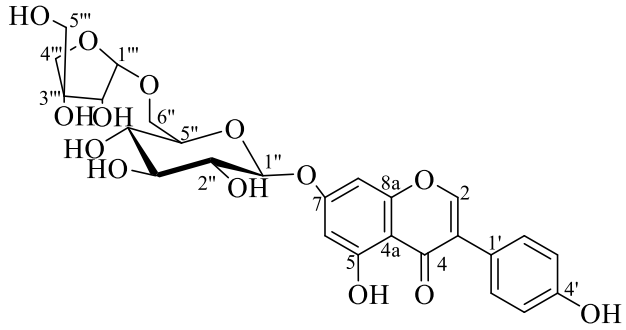
HR-FAB-MS: *m/z* 707.1577 $[M+K]^+$ (100%) (calcd. for $C_{30}H_{36}O_{17}K$ 707.1576), FAB-MS: *m/z* 707 $[M+K]^+$, 669 $[M+H]^+$, 667 $[M+H]^-$;

^{1}H-NMR (500 MHz, C_5D_5N, δ ppm) and ^{13}C-NMR (125 MHz, C_5D_5N, δ ppm): See Table 5.

Acid hydrolysis of **33**

Compound **33** (0.85 mg) was added into 1 N HCl (2 mL) and then heated to 80°C for 3.0 h. The acidic solution was extracted with chloroform (2 mL×2 times). The organic layer was washed with distilled water (2 mL×3 times), dried over Na_2SO_4, and evaporated to give an aglycone (0.45 mg) as a white amorphous powder. The aglycone was identified as isocaviunin by NMR spectroscopy techniques and HR-ESI-MS: *m/z* 397.0872 (100%) $[M+Na]^+$ (calcd. for $C_{18}H_{19}O_8Na$ 397.0899); R_f (TLC): 0.4 (CH_2Cl_2–MeOH, 6:1).

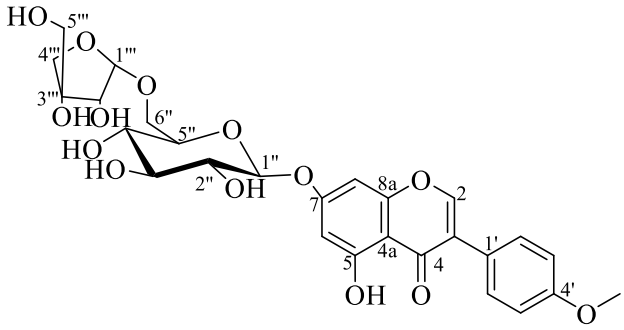
Compound **34**: Ambocin



White amorphous powder, EI-MS (+): *m/z* 564 $[M]^+$, $C_{26}H_{28}O_{14}$; ^{1}H-NMR (500 MHz, MeOD, δ ppm): 8.09 (1H, s, H-2), 7.34 (1H, d, 8.5 Hz, H-2', H-6'), 6.80 (1H, d, 8.5 Hz, H-3', H-5'), 6.66 (1H, d, 2.0 Hz, H-8), 6.46 (1H, d, 2.0 Hz, H-6), 4.94 (1H, d, 8.0 Hz, H-1''), 4.91 (1H, d, 2.5 Hz, H-1'''), 4.01 (1H, m, H- H-6''a), 3.99 (1H, d, 10.0 Hz, H-4'''a), Hz, 3.90 (1H, d, 2.5 Hz, H-2'''), 3.73 (1H, d, 10.0 Hz, H-4'''b), 3.63 (1H, t, 7.5 Hz, H-5''), 3.58 (1H, m, H-6''b), 3.56 (2H, d, 1.5 Hz, H-5'''), 3.43 (2H, m, H-2'', H-3''), 3.34 (1H, m, H-4''); ^{13}C-NMR (125 MHz, MeOD, δ ppm): 181.1 (s, C-4), 163.3 (s, C-7), 162.1 (s, C-5), 157.8 (s, C-8a), 157.5 (s, C-4'), 154.0 (d, C-2), 130.0 (d, C-2', C-6'), 123.6 (s, C-3), 121.7 (s, C-1'), 114.9 (d, C-3', C-5'),

109.8 (d, C-1'''), 106.6 (s, C-4a), 100.2 (d, C-1''), 99.8 (d, C-6), 94.6 (d, C-8), 79.1 (d, C-3'''), 76.8 (d, C-2'''), 76.8 (d, C-3''), 76.5 (d, C-5''), 73.7 (t, C-4'''), 73.3 (d, C-2''), 70.3 (d, C-4''), 67.7 (t, C-6''), 64.4 (t, C-5''').

Compound **35**: Biochanin A **7**-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosside (Lanceolanrin)



White amorphous powder, EI-MS (+): *m/z* 578 $[M]^+$, $C_{27}H_{30}O_{14}$; ^{1}H-NMR (500 MHz, MeOD, δ ppm): 8.18 (1H, s, H-2), 7.49 (1H, d, 10.0 Hz, H-2', H-6'), 6.99 (1H, d, 10.0 Hz, H-3', H-5'), 6.74 (1H, s, H-8), 6.53 (1H, s, H-6), 4.97 (1H, d, 10.0 Hz, H-1''), 4.96 (1H, d, 1.5 Hz, H-1'''), 3.83 (3H, s, 3'-OCH_3), 3.37-4.06 (13H, sugar protons); ^{13}C-NMR (125 MHz, MeOD, δ ppm): 181.3 (s, C-4), 163.3 (s, C-7), 162.1 (s, C-5), 159.9 (s, C-4'), 157.8 (s, C-8a), 154.2 (d, C-2), 129.9 (d, C-2', C-6'), 123.3 (s, C-3), 123.0 (s, C-1'), 113.5 (d, C-3', C-5'), 109.8 (d, C-1'''), 106.6 (s, C-4a), 100.2 (d, C-1''), 99.8 (d, C-6), 94.6 (d, C-8), 79.1 (d, C-3'''), 76.8 (d, C-2'''), 76.5 (d, C-3''), 75.8 (d, C-5''), 73.8 (t, C-4'''), 73.3 (d, C-2''), 70.2 (d, C-4''), 67.7 (t, C-6''), 64.4 (t, C-5'''), 54.4 (q, 5'-OCH_3).

2.4. Phytochemical investigation of root extract and structural elucidation

2.4.1. Isolation procedure

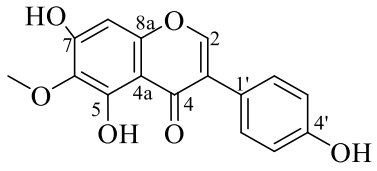
The CH_2Cl_2 root fr. (45.5 g) was chromatographed on a normal silica gel column, using a gradient of CH_2Cl_2-CH_3COCH_3 (3:1, v/v) to afford 6 fr.s (D1-D6). Compounds **39**

(15.0 mg) and **40** (5.5 mg) were separated from the fr. D1 (2.1 g) by CC with *n*-hexane-CH_3COCH_3 (3:1, v/v). The fr. D2 (3.2 g) was rechromatographed on a Sephadex LH-20, eluting with MeOH-H_2O (1:1, v/v) to yeild compounds **36** (4.2 mg), **41** (3.1 mg), **43** (2.1 mg), and **44** (3.99 mg). Compounds **45** (2.0 mg) and **46** (2.5 mg) were separated from fr. D3 (2.7 g), by using $CHCl_3$-MeOH (3:1, v/v). Compound **47** (1.1 mg) was found in a small subfr. D31 (20 mg), by using HPLC [Cosmosil 5C18-AR-II column (10x250 mm) with MeOH-H_2O-HCOOH (7:3:0.1%, 2 $mLmin^{-1}$)].

The EtOAc root fr. (30.5 g) was purified by CC with a gradient of $CHCl_3$-MeOH-H_2O (3:1:0.1, v/v/v) to afford seven fr.s. The E2 fr. (2.35 g) was then purified by HPLC [Cosmosil 5C18-AR-II column (10x250 mm) with MeOH-H_2O (7:3, 2 $mLmin^{-1}$)] to give compounds **2** (1.3 mg), **6** (0.7 mg), **13** (1.2 mg), **14** (2.3 mg), **22** (1.5 mg)**, 26** (0.5 mg), and **42** (1.3 mg). Compounds **4** (2.4 mg), **8** (1.8 mg), and **15** (4.0 mg) were precipitated out of the E3 fr. (1.2 g), obtained after CC with $CHCl_3$-MeOH (3:1, v/v). The E2 fr. (0.85 g) was also subjected to Sephadex LH-20 with MeOH-H_2O (1:1, v/v), to produce compounds **37** (5.1 mg) and **38** (25.2 mg). Compounds **1** (7.2 mg) and **9** (2.1 mg) were obtained from the fourth fraction using Sephadex LH-20 with MeOH-H_2O (1:1, v/v).

2.4.2. Chemical and physical data of isolated compounds

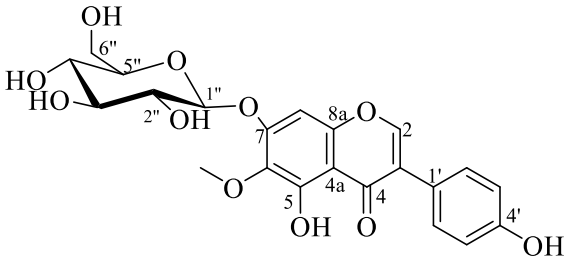
Compound **36**: Tectorigenin



Yellow amorphous powder, EI-MS (+): *m/z* 300 $[M]^+$, $C_{16}H_{12}O_6$; ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 13.10 (1H, s, 5-OH), 7.87 (1H, s, H-2), 7.40 (2H, d, 8.5 Hz, H-2', H-6'), 6.91

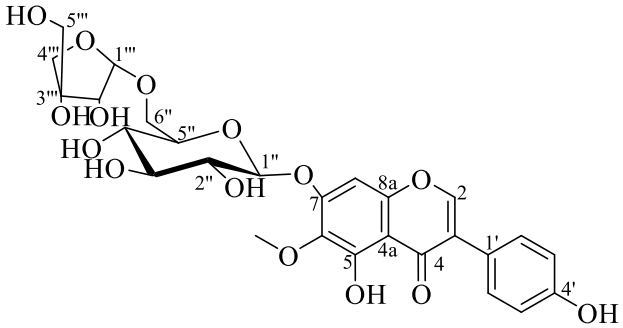
(2H, d, 8.5 Hz, H-3', H-5'), 6.51 (1H, s, H-8), 4.04 (3H, s, 6-OMe); ^{13}C-NMR (125 MHz, $CDCl_3$, δ ppm): 182.0 (s, C-4), 155.8 (s, C-4'), 155.2 (s, C-7), 153.5 (s, C-5), 152.9 (d, C-2), 152.6 (d, C-8a), 130.4 (s, C-2', C-6'), 123.1 (s, C-3), 123.0 (s, C-1'), 115.6 (s, C-3', C-5'), 106.5 (s, C-4a), 93.2 (d, C-8), 60.9 (q, 6-OMe).

Compound **37**: Tectoridin



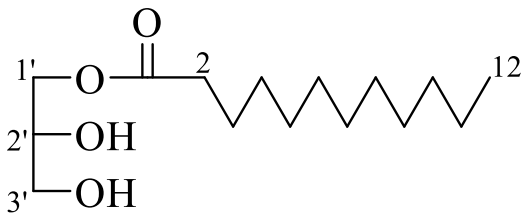
Yellow amorphous powder; EI-MS (+): *m/z* 462 $[M]^+$, $C_{22}H_{22}O_{11}$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 9.18 (1H, s, H-2), 8.18 (2H, d, 8.5 Hz, H-2', H-6'), 7.67 (1H, s, H-8), 7.62 (2H, d, 8.5 Hz, H-3', H-5'), 5.88 (1H, d, 7.0 Hz, H-1''), 4.57 (3H, s, 6-OMe), 3.92-4.53 (6H, sugar protons); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 182.1 (s, C-4), 158.8 (s, C-4'), 157.9 (s, C-7), 156.0 (d, C-2), 154.2 (s, C-5), 153.1 (s, C-8a), 133.7 (s, C-6), 131.5 (s, C-2', C-6'), 123.3 (s, C-3), 122.3 (s, C-1'), 116.4 (s, C-3', C-5'), 107.7 (s, C-4a), 101.4 (d, C-1''), 95.3 (d, C-8), 78.6 (d, C-5''), 78.0 (d, C-3''), 74.4 (d, C-2''), 70.9 (d, C-4''), 61.9 (t, C-6''), 61.6 (q, 6-OMe).

Compound **38**: Tectorigenin 7-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside



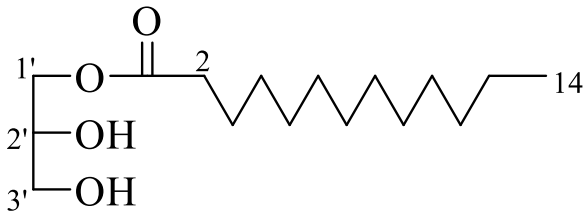
Yellow amorphous powder; EI-MS (+): *m/z* 594 $[M]^+$, $C_{27}H_{30}O_{15}$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.18 (1H, s, H-2), 7.39 (2H, d, 8.5 Hz, H-2', H-6'), 6.89 (1H, s, H-8), 6.84 (2H, d, 8.5 Hz, H-3', H-5'), 5.03 (1H, d, 7.5 Hz, H-1''), 4.95 (1H, d, 2.5 Hz, H-1'''), 3.93 (3H, s, 6-OMe), 3.48-4.03 (13H, sugar protons); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 181.1 (s, C-4), 158.2 (s, C-4'), 157.5 (s, C-7), 154.3 (d, C-2), 153.3 (s, C-5), 153.1 (s, C-8a), 132.7 (s, C-6), 130.0 (s, C-2', C-6'), 123.1 (s, C-3), 121.7 (s, C-1'), 114.9 (s, C-3', C-5'), 109.8 (d, C-1'''), 107.1 (s, C-4a), 100.6 (d, C-1''), 94.4 (d, C-8), 79.0 (d, C-3'''), 76.7 (d, C-3''), 76.6 (d, C-2'''), 75.9 (d, C-5''), 73.6 (t, C-4'''), 73.3 (d, C-2''), 70.2 (d, C-4''), 67.7 (t, C-6''), 64.3 (t, C-5'''), 60.1 (q, 6-OMe).

Compound **39**: 1-Monolaurin



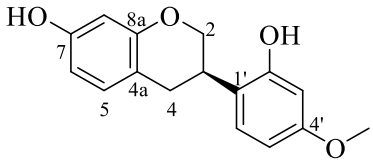
White amophour powder; EI-MS (+): *m/z* 274 $[M]^+$ $C_{15}H_{30}O_4$; ^{1}H-NMR (500 MHz, CD_3Cl_3, δ ppm): 4.20 (1H, dd, 11.0, 6.0 Hz, H-1'a), 4.14 (1H, dd, 11.0, 6.0 Hz, H-1'b), 3.93 (2H, m, H-2'), 3.66 (1H, m, H-3'a), 3.60 (1H, m, H-3'b), 2.35 (2H, t, 7.0 Hz, H-2), 1.63 (2H, t, 7.0 Hz, H-3), 1.26 (16H, brs, H-4−H-11), 0.88 (3H, t, 7.0 Hz, 12-Me); ^{13}C-NMR (125 MHz, CD_3Cl_3, δ ppm): 174.0 (C-1), 70.3 (d, C-2'), 65.2 (t, C-1'), 63.3 (t, C-3'), 34.2 (t, C-2), 22.7-29.7 (8C, C-4−C-11), 24.9 (t, C-3), 14.1 (q, 12-Me).

Compound **40**: 1-Monomyristin



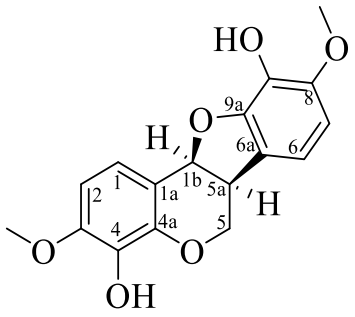
White amophour powder; EI-MS (+): *m/z* 288 $[M]^+$ $C_{16}H_{32}O_4$; ^{1}H-NMR (500 MHz, CD_3Cl_3, δ ppm): 4.20 (1H, dd, 11.0, 6.0 Hz, H-4'a), 4.14 (1H, dd, 11.0, 6.0 Hz, H-4'b), 3.93 (2H, m, H-2'), 3.67 (1H, m, H-3'a), 3.61 (1H, m, H-3'b), 2.35 (2H, t, 7.0 Hz, H-2), 1.63 (2H, t, 7.0 Hz, H-3), 1.27 (20H, brs, H-4−H-13), 0.88 (3H,t, 7.0 Hz, 14-Me); ^{13}C-NMR (125 MHz, CD_3Cl_3, δ ppm): 174.4 (C-1), 70.3 (d, C-2'), 65.2 (t, C-1'), 63.3 (t, C-3'), 34.2 (t, C-2), 31.9 (t, C-11), 29.1-29.7 (10C, C-4−C-13), 24.9 (t, C-3), 22.7 (t, C-12), 14.1 (q, 13-Me).

Compound **41**: (3*R*)-Vestitol



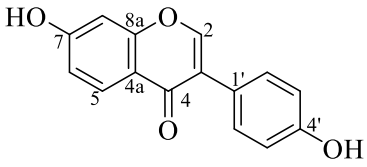
Yellow amophour powder; EI-MS (+): *m/z* 272 $[M]^+$ $C_{16}H_{16}O_4$; ^{1}H-NMR (500 MHz, CD_5N_5, δ ppm): 7.22 (1H, d, 8.5 Hz, H-6'), 7.11 (1H, d, 8.5 Hz, H-5), 6.90 (1H, d, 2.0 Hz, H-5'), 6.91 (1H, d, 2.0 Hz, H-3'), 6.88 (1H, dd, 2.5 Hz, 8.5 Hz, H-6), 6.62 (1H, d, 2.5 Hz, H-8); ^{13}C-NMR (125 MHz, CD_5N_5, δ ppm): 159.9 (s, C-4'), 158.2 (s, C-8a), 157.4 (s, C-2'), 155.9 (s, C-7), 130.7 (d, C-5), 128.3 (d, C-6'), 121.0 (s, C-1'), 113.6 (s, C-4a), 109.0 (d, C-6), 104.5 (d, C-5'), 103.9 (d, C-8), 102.5 (d, C-3').

Compound **42**: Melilotolcarpan D



Yellow amorphous solid, EI-MS (+): *m/z* 316 $[M]^+$, $C_{17}H_{16}O_6$; ^{1}H-NMR (500 MHz, CD_3Cl_3, δ ppm): 7.14 (1H, d, 8.5 Hz, H-1), 6.76 (1H, d, 8.5 Hz, H-6), 6.66 (1H, d, 8.5 Hz, H-2), 6.46 (1H, d, 8.5 Hz, H-7), 5.60 (1H, d, 7.0 Hz, H-1b), 4.35 (2H, dd, 11.0, 5.0 Hz, H-5a), 3.91 (3H, s, 3-OCH_3), 3.88 (3H, s, 8-OCH_3), 3.62 (1H, m, H-5a); ^{13}C-NMR (125 MHz, CD_3Cl_3, δ ppm): 148.1 (s, C-8), 147.4 (s, C-3), 146.1 (s, C-9a), 143.3 (s, C-4a), 133.9 (s, C-4), 130.7 (s, C-9), 121.6 (d, C-1), 121.4 (s, C-6a), 114.8 (d, C-6), 113.8 (s, C-1a), 105.3 (d, C-2), 103.8 (d, C-7), 79.2 (d, C-1b), 66.8 (t, C-5), 56.5 (q, 3-OCH_3), 56.3 (q, 8-OCH_3), 40.2 (d, C-5a).

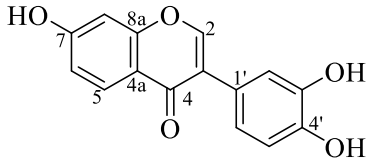
Compound **43**: Daidzein



Yellow amorphous solid, EI-MS (+): *m/z* 254 $[M]^+$, $C_{15}H_{10}O_4$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.12 (1H, s, H-2), 8.05 (1H, d, 8.5 Hz, H-5), 7.36 (2H, d, 8.5 Hz, H-2', H-6'), 6.92 (1H, dd, 2.0, 8.5 Hz, H-6), 6.85 (1H, s, H-8), 6.82 (1H, d, 8.5 Hz, H-3', H-5'); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 176.8 (s, C-4), 163.8 (s, C-7), 158.2 (s, C-4a), 157.3 (s, C-4'),

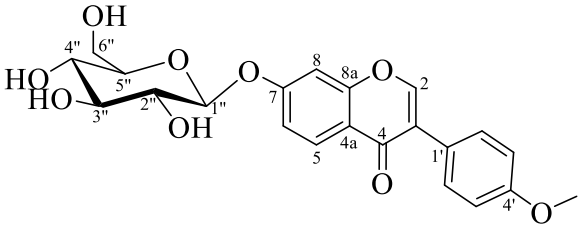
153.2 (d, C-2), 130.0 (d, C-2', C-6'), 127.1 (d, C-5), 124.5 (s, C-1'), 123.0 (s, C-3), 116.6 (s, C-4a), 114.9 (d, C-6), 114.8 (d, C-3', C-5'), 101.8 (d, C-8).

Compound **44**: 3'-Hydroxyldaidzein



Yellow amorphous solid, EI-MS (+): *m/z* 270 $[M]^+$, $C_{15}H_{10}O_5$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.12 (1H, s, H-2), 8.04 (1H, d, 8.5 Hz, H-5), 6.96 (1H, d, 2.0 Hz, H-2'), 6.93 (1H, dd, 2.0, 8.5 Hz, H-6), 6.83 (1H, dd, 2.0, 8.5 Hz, H-5'), 6.82 (1H, d, 8.5 Hz, H-6'), 6.79 (1H, d, 2.0 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 176.6 (s, C-4), 163.5 (s, C-7), 158.5 (s, C-8a), 153.5 (d, C-2), 147.7 (s, C-3'), 146.0 (s, C-4'), 127.1 (d, C-5), 124.8 (s, C-3), 124.4 (s, C-1'), 120.2 (d, C-5'), 116.7 (s, C-4a), 116.0 (d, C-2'), 115.2 (d, C-6), 114.8 (d, C-6'), 101.9 (d, C-8).

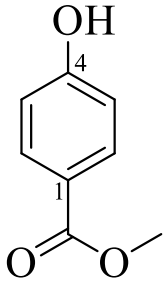
Compound **45**: Formononetin-7-O-β-D-glucopyranoside



White needle, EI-MS (+): *m/z* 430 $[M]^+$, $C_{22}H_{22}O_9$; ^{1}H-NMR (500 MHz, DMSO, δ ppm): 8.45 (1H, s, H-2), 8.08 (1H, d, 9.0 Hz, H-5), 7.55 (2H, d, 8.5 Hz, H-2', H-6'), 7.25 (1H, d, 2.0 Hz, H-8), 7.15 (1H, dd, 9.0, 2.0 Hz, H-6), 7.01 (2H, d, 8.5 Hz, H-3', H-5'), 5.11 (1H, d, 7.5 Hz, H-1"), 3.80 (3H, s, 4'-OCH_3), 3.72 (1H, dd, 5.5, 10.5 Hz, H-6"a), 3.47 (2H, m, H-6"b, H-5"), 3.37 (Overlap, H-2", H-3"), 3.18 (1H, m, H-4"); ^{13}C-NMR (125 MHz, DMSO, δ ppm):

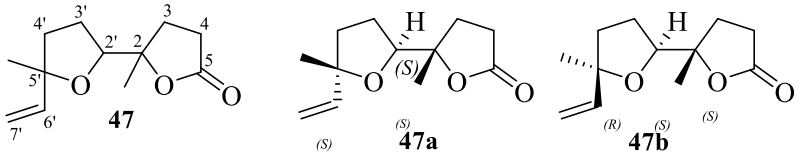
175.5 (s, C-4), 161.9 (s, C-7), 159.1 (s, C-4'), 157.5 (s, C-8a), 154.1 (d, C-2), 130.6 (d, C-2', C-6'), 127.5 (d, C-5), 124.5 (s, C-1'), 123.8 (s, C-3), 118.9 (s, C-4a), 116.1 (d, C-6), 114.1 (d, C-3', C-5'), 103.9 (d, C-8), 77.7 (d, C-5''), 76.9 (d, C-3''), 73.6 (d, C-2''), 70.1 (d, C-4''), 61.1 (t, C-6''), 55.6 (q, 4'-OCH_3).

Compound **46**: Killitol (4-Hydroxy methylbenzoate)



Yellow amorphous powder, EI-MS (+): *m/z* 152 $[M]^+$, $C_8H_8O_3$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.86 (2H, d, 8.5 Hz, C-2, C-6), 6.81 (2H, d, 8.5 Hz, C-3, C-5), 3.84 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 167.3 (s, CO), 162.1 (s, C-4), 131.3 (d, C-2, C-6), 120.8 (s, C-1), 114.7 (d, C-3, C-5), 50.8 (q, OCH_3).

Compound **47**: 2,5'-Dimethyl-5'-vinylhexahydro-2,2'-bifuran-5(*2H*)-one



Yellowish amorphous powders;

$[\alpha]_D^{25} = -1.2$ (*c* 1.1, MeOH);

IR (film): 2973.7, 1771.3, 1607.4, 1452.1, 1271.8, 1163.8, 1084.8, 944.9, 914.1, 829.2, 715.5, 617.1, 598.8, 564.1 cm^{-1};

UV λ_{max} (MeOH) nm (log ε): 206.5 (2.462), 257.5 (1.339);

HR-EI-MS: *m/z* $[M]^+$ 210.1254 calcd. for $C_{12}H_{18}O_3$; found: 210.1253), EI-MS: *m/z* $[M]^+$ 210, ^{1}H-NMR (500 MHz, CD_3OD, δ_H ppm) and ^{13}C-NMR (125 MHz, CD_3OD, δ_C ppm) are given in Table 6.

Chapter 3 Chemical constituents from *Cratoxylum formosum*

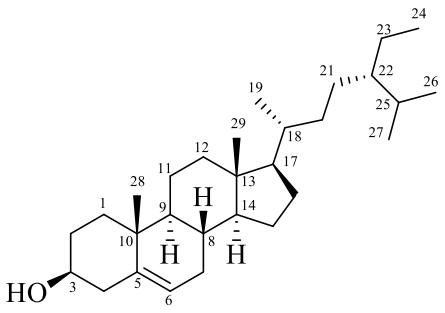
3.2. Phytochemical investigation of root extract and structural elucidation

3.2.1. Isolated procedure

Dried leaf (2.0 kg) was extracted with MeOH (10 L, 3 times), to produce the crude MeOH extract (220.0 g). This extract was then subjected to normal silica gel CC, eluting with increased mobile phase to yield the corresponding fr.s 1-7. Using silica gel CC with $CHCl_3$-MeOH (8:1, v/v), the fr.4 (16.7 g) was separated to give 4 fr.s (Fr41-Fr44). Compounds **48** (45 mg) and **49** (15 mg) were obtained from fr.1 (30.5 g) using *n*-hexane-CH_3COCH_3 (3:1, v/v) by silica gel CC. Applying Sephadex LH-20 with MeOH-H_2O (1:1, v/v), compound **50** (37.5 mg) was isolated from the fr.42 (2.1 g), meanwhile compound **51** (200 mg) was obtained from the fr.44 (2.3 g) by silica gel CC with $CHCl_3$-MeOH-H_2O (3:1:0.1, v/v/v). Similarly, the fr.6 (20.5 g) was separated by Sephadex LH-20 with MeOH-H_2O (2:1, v/v) to afford compound **52** (15.0 mg) and the fr.61 (150 mg). the fr.61 was further subjected to HPLC [MeOH-H_2O (7:3, v/v), UV 210 nm, 1.0 mL/min] to yield compounds **53** (5.4 mg), **55** (4.2 mg) and **56** (3.2 mg). From the fr.7 (30.1 g), the purified powders were obtained as a single compound **54** (240 mg) by washing with MeOH.

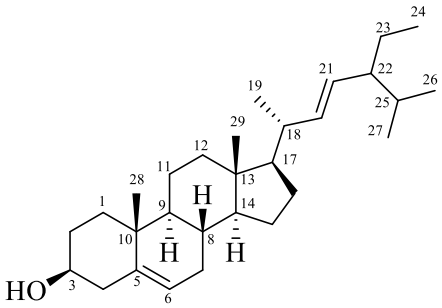
3.2.2. Chemical and physical data of isolated compounds

Compound **48**: β-Sitosterol



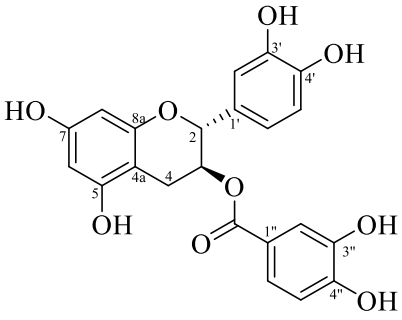
White needles, EI-MS (+): *m/z* 414 $[M]^+$; $C_{29}H_{50}O$; ^{1}H-NMR (500 MHz, $CDCl_3$): 5.36 (1H, d, 5.5 Hz, H-6), 3.51 (1H, m, H-3), 1.01 (3H, s, H-29), 0.93 (3H, d, 8.0, H-19), 0.85 (3H, d, 8.5 Hz, H-24), 0.83 (3H, d, 9.0 Hz, H-26), 0.82 (3H, d, 9.0 Hz, H-27), 0.68 (3H, d, 9.5 Hz, H-18); ^{13}C-NMR (125 MHz, $CDCl_3$): 140.8 (s, C-5), 121.7 (d, C-6), 71.8 (d, C-3), 56.8 (d, C-14), 56.1 (d, C-17), 50.1 (d, C-9), 45.8 (d, C-22), 42.4 (s, C-13), 42.3 (t, C-4), 39.8 (t, C-12), 37.3 (t, C-1), 36.5 (s, C-10), 36.1 (d, C-18), 34.0 (t, C-20), 31.9 (t, C-7) 31.9 (d, C-8), 31.7 (t, C-2), 29.1 (d, C-25), 28.3 (t, C-16), 26.1 (d, C-21), 24.3 (t, C-15), 23.1 (t, C-23), 21.1 (t, C-11), 19.8 (q, C-26), 19.4 (q, C-27), 19.0 (q, C-19), 18.8 (q, C-28), 12.0 (q, C-24), 11.8 (q, C-29).

Compound **49**: Stigmasterol



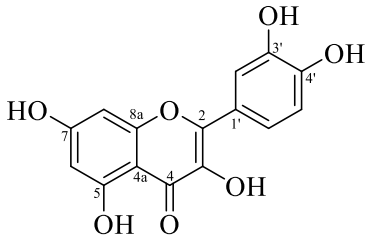
White needles, EI-MS (+): *m/z* 412 $[M]^+$; $C_{29}H_{48}O$; ^{1}H-NMR (500 MHz, $CDCl_3$): 5.36 (1H, d, 5.5 Hz, H-6), 5.14 (1H, dd, 9.0, 15.5 Hz, H-21), 5.01 (1H, dd, 9.0, 15.5 Hz, H-20), 3.51 (1H, m, H-3), 1.01 (3H, s, H-29), 0.93 (3H, d, 8.0, H-19), 0.85 (3H, d, 8.5 Hz, H-24), 0.83 (3H, d, 9.0 Hz, H-26), 0.82 (3H, d, 9.0 Hz, H-27), 0.68 (3H, d, 9.5 Hz, H-18); ^{13}C-NMR (125 MHz, $CDCl_3$): 140.8 (s, C-5), 138.3 (d, C-20), 129.2 (d, C-21), 121.7 (d, C-6), 71.8 (d, C-3), 56.8 (d, C-14), 56.1 (d, C-17), 50.1 (d, C-9), 45.8 (d, C-22), 42.4 (s, C-13), 42.3 (t, C-4), 39.8 (t, C-12), 37.3 (t, C-1), 36.5 (s, C-10), 36.1 (d, C-18), 31.9 (t, C-7) 31.9 (d, C-8), 31.7 (t, C-2), 29.1 (d, C-25), 28.3 (t, C-16), 24.3 (t, C-15), 23.1 (t, C-23), 21.1 (t, C-11), 19.8 (q, C-26), 19.4 (q, C-27), 19.0 (q, C-19), 18.8 (q, C-28), 12.0 (q, C-24), 11.8 (q, C-29).

Compound **50**: (+)-Catechin-3-O-(3,4-dihydroxybenzoyl)



Yellow amorphous powder, EI-MS (+): *m/z* 426 $[M]^+$; $C_{22}H_{18}O_9$; ^{1}H-NMR (500 MHz, CD_3OD): 7.42 (1H, d, 2.0 Hz, H-2"), 7.39 (1H, dd, 8.5, 2.0 Hz, H-6"), 6.96 (1H, d, 2.0 Hz, H-2'), 6.78 (1H, dd, 8.5, 2.0 Hz, H-6'), 6.76 (1H, d, 8.5 Hz, H-5"), 6.76 (1H, d, 8.5 Hz, H-5'), 5.93 (1H, d, 2.5 Hz, H-6), 5.90 (1H, d, 2.5 Hz, H-8), 4.80 (1H, s, H-2), 4.17 (1H, m, H-3), 2.86 (1H, dd, 17.0, 4.5 Hz, H-2a), 2.72 (1H, dd, 17.0, 3.0 Hz, H-2b); ^{13}C-NMR (125 MHz, CD_3OD): 170.9 (s, CO), 156.6 (s, C-7), 156.3 (s, C-8a), 156.0 (s, C-5), 149.1 (s, C-4"), 144.6 (s, C-3"), 144.4 (s, C-3'), 144.3 (s, C-3'), 130.9 (s, C-1'), 124.3 (s, C-1"), 122.2 (d, C-6"), 118.0 (d, C-6'), 116.4 (d, C-2"), 114.5 (d, C-5"), 114.2 (d, C-5'), 113.9 (d, C-2'), 98.7 (s, C-4a), 98.0 (d, C-6), 94.5 (d, C-8), 78.5 (d, C-2), 66.1 (d, C-3), 27.9 (t, C-4).

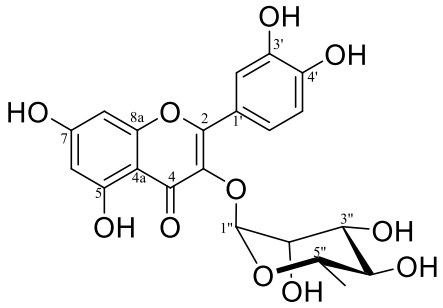
Compound **51**: Quecertin



Yellow amorphous powder, EI-MS (+): *m/z* 302 $[M]^+$; $C_{15}H_{10}O_7$; ^{1}H-NMR (500 MHz, CD_3OD): 7.69 (1H, d, 2.0 Hz, H-2'), 7.60 (1H, dd, 8.5, 2.0 Hz, H-6'), 6.83 (1H, dd, 8.5 Hz, H-5'), 6.34 (1H, d, 2.0 Hz, H-8), 6.13 (1H, d, 2.0 Hz, H-6); ^{13}C-NMR (125 MHz, CD_3OD):

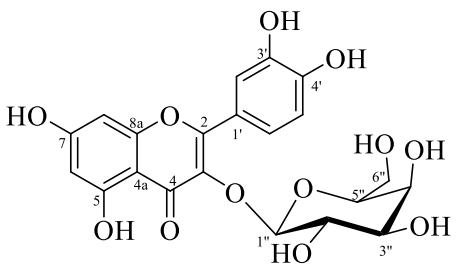
177.1 (s, C-4), 165.3 (s, C-7), 162.0 (s, C-5), 157.4 (s, C-8a), 149.0 (s, C-4'), 148.1 (s, C-2), 146.3 (s, C-3'), 137.0 (s, C-3), 123.2 (s, C-1'), 121.2 (d, C-6'), 116.9 (d, C-5'), 116.3 (d, C-2'), 104.2 (s, C-4a), 99.5 (d, C-6), 94.6 (d, C-8).

Compound **52**: Quercitrin



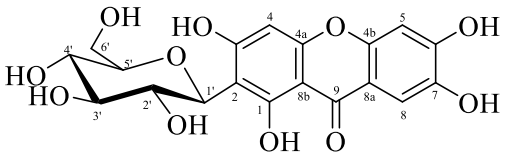
Yellow amorphous powder, EI-MS (+): *m/z* 448 $[M]^+$; $C_{21}H_{20}O_{11}$; ^{1}H-NMR (500 MHz, CD_3OD): 7.31 (1H, d, 1.5 Hz, H-2'), 7.30 (1H, dd, 8.0, 1.5 Hz, H-6'), 6.90 (1H, dd, 8.0 Hz, H-5'), 6.36 (1H, s, H-6), 6.19 (1H, s, H-8), 5.34 (1H, d, 1.5 Hz, H-1"), 4.21 (1H, brd, 1.5 Hz, H-2"), 3.75 (1H, dd, 9.0, 3.0 Hz, H-3"), 3.41 (1H, m, H-5"), 3.34 (1H, brd, 9.0 Hz, H-4"), 0.93 (3H, d, 6.0 Hz, 5"-CH_3); ^{13}C-NMR (125 MHz, CD_3OD): 178.2 (s, C-4), 164.5 (s, C-7), 161.8 (s, C-5), 157.9 (s, C-8a), 157.1 (s, C-2), 148.4 (s, C-4'), 145.0 (s, C-3'), 134.8 (s, C-3), 121.6 (s, C-1'), 121.5 (d, C-6'), 115.5 (d, C-5'), 115.0 (d, C-2'), 104.5 (s, C-4a), 102.1 (d, C-1"), 98.4 (d, C-6), 93.3 (d, C-8), 71.9 (d, C-4"), 70.7 (d, C-3"), 70.6 (d, C-2"), 70.5 (d, C-5"), 16.3 (q, 5"-CH_3).

Compound **53**: Hyperin (Quercetin-3-O-β-D-galactopyranoside)



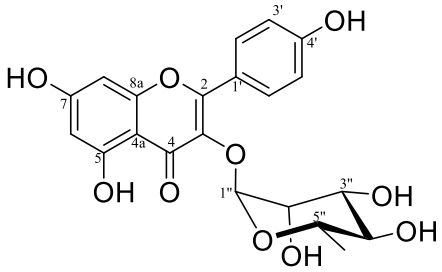
Yellow amorphous powder, EI-MS (+): *m/z* 464 $[M]^+$; $C_{21}H_{20}O_{12}$; ^{1}H-NMR (500 MHz, CD_3OD): 7.83 (1H, s, H-2'), 7.58 (1H, d, 8.5 Hz, H-6'), 6.85 (1H, d, 8.5 Hz, H-5'), 6.39 (1H, s, H-6), 6.20 (1H, s, H-8), 5.15 (1H, d, 8.5 Hz, H-1''), 3.84 (1H, d, 2.5 Hz, H-4''), 3.82 (1H, dd, 5.0, 9.5 Hz, H-2''), 3.64 (1H, dd, 6.0, 10.0 Hz, H-6''a), 3.55 (1H, dd, 3.5, 10.0 Hz, H-6''b), 3.53 (1H, m, H-3''), 3.44 (1H, m, H-5''); ^{13}C-NMR (125 MHz, CD_3OD): 178.0 (s, C-4), 164.7 (s, C-7), 161.6 (s, C-5), 157.4 (s, C-8a), 157.1 (s, C-2), 148.6 (s, C-4'), 144.0 (s, C-3'), 134.4 (s, C-3), 121.5 (d, C-1'), 121.4 (s, C-6'), 116.4 (d, C-5'), 115.7 (d, C-2'), 104.0 (s, C-4a), 102.1 (d, C-1''), 98.5 (d, C-6), 93.3 (d, C-8), 75.8 (d, C-5''), 73.7 (d, C-3''), 71.8 (d, C-2''), 68.6 (d, C-4''), 60.5 (t, C-6'').

Compound **54**: Mangiferin



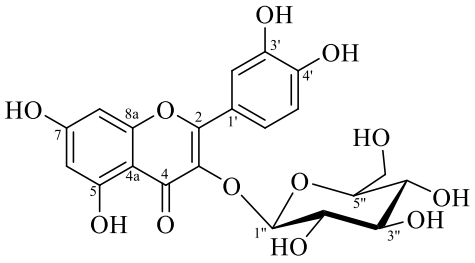
White amorphous powder, EI-MS (+): *m/z* 422 $[M]^+$; $C_{19}H_{18}O_{11}$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 7.43 (1H, s, H-8), 6.80 (1H, s, H-5), 6.35 (1H, s, H-4), 4.89 (1H, d, 10 Hz, H-1'), 4.17 (1H, t, 5.5 Hz, H-2'), 3.86 (1H, dd, 12.5, 2.5 Hz, H-6'a), 3.73 (1H, dd, 12.5, 5.5 Hz, H-6'b), 3.46 (2H, m, H-3', H-4'), 3.41 (1H, m, H-5'); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 180.1 (s, C-9), 163.8 (s, C-3), 161.9 (s, C-1), 157.4 (s, C-4a), 154.0 (s, C-6), 151.7 (s, C-4b), 143.6 (s, C-7), 112.5 (s, C-8a), 107.9 (d, C-8), 106.1 (s, C-2), 102.2 (d, C-5), 101.9 (s, C-8b), 93.5 (d, C-4), 81.3 (d, C-5'), 78.8 (d, C-3'), 73.9 (d, C-1'), 71.2 (d, C-2'), 70.5 (d, C-4'), 61.5 (t, C-6').

Compound **55**: Afzelin



Yellow amorphous powder, EI-MS (+): *m/z* 471 $[M]^+$; $C_{21}H_{20}O_{10}$; ^{1}H-NMR (500 MHz, CD_3OD): 7.72 (2H, d, 9.0 Hz, H-2', H-6'), 6.89 (2H, d, 9.0 Hz, H-3', H-5'), 6.33 (1H, brs, H-8), 6.16 (1H, brs, H-6), 5.33 (1H, brs, H-1"), 3.68 (1H, m, H-2"), 3.44 (1H, brs, H-3"), 3.16 (1H, brs, H-4"), 3.12 (1H, m, H-5"), 0.87 (3H, d, 5.5 Hz, 5"-CH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 178.2 (s, C-4), 164.8 (s, C-7), 161.0 (s, C-5), 160.3 (s, C-4'), 157.9 (s, C-8a), 157.1 (s, C-2), 134.8 (s, C-3), 130.5 (d, C-2', C-6'), 121.2 (s, C-1'), 115.1 (d, C-3', C-5'), 102.3 (s, C-4a), 102.1 (d, C-1"), 98.5 (d, C-6), 93.4 (d, C-8), 71.8 (d, C-4"), 70.7 (d, C-3"), 70.6 (d, C-2"), 70.5 (d, C-5"), 16.2 (q, 5"-CH_3).

Compound **56**: Isoquercetin (Quercetin-3-O-β-D-glucopyranoside)



Yellow amorphous powder, EI-MS (+): *m/z* 464 $[M]^+$; $C_{21}H_{20}O_{12}$; ^{1}H-NMR (500 MHz, CD_3OD): 7.70 (1H, s, H-2'), 7.58 (1H, d, 8.5 Hz, H-6'), 6.85 (1H, d, 8.5 Hz, H-5'), 6.39 (1H, s, H-6), 6.20 (1H, s, H-8), 5.24 (1H, d, 8.5 Hz, H-1"), 3.71 (1H, m, H-6"a), 3.59 (1H, m, H-6"b), 3.49 (1H, m, H-3"), 3.44 (1H, m, H-2"), 3.35 (1H, overlap, H-4"), 3.23 (1H, m, H-5"); ^{13}C-NMR (125 MHz, CD_3OD): 178.3 (s, C-4), 164.7 (s, C-7), 161.6 (s, C-5), 157.4 (s, C-8a),

157.2 (s, C-2), 148.7 (s, C-4'), 144.4 (s, C-3'), 134.4 (s, C-3), 121.7 (d, C-1'), 121.5 (s, C-6'), 116.4 (d, C-5'), 114.7 (d, C-2'), 104.0 (s, C-4a), 102.9 (d, C-1''), 98.5 (d, C-6), 93.3 (d, C-8), 75.8 (d, C-5''), 73.7 (d, C-3''), 71.8 (d, C-2''), 68.6 (d, C-4''), 61.1 (t, C-6'').

Chapter 4 Biological activities

4.1. Plant materials

The studied materials include the various extracts, fractions, and isolated compounds of *D. tonkinensis* species. The dried powdered leaf, stem bark, and root, 2.0 kg of each part was administered using methanol (10.0 L) for 4h in a Soxhlet extractor, to produce the crude extracts (300.0 g, **DB1**), (198.5 g, **DB3**), and (200.0 g, **DB6**), respectively. Similar to the procedure for heartwood, the powders after methanol extraction were then subjected to decoction in hot water (4.0 L) for 6h to afford the extracts leaf (25.8 g, **DB2**), stem bark (20.4 g, **DB4**), and root (19.7 g, **DB9**). Furthermore, the crude extract of the root (**DB5**) was obtained using ethanol (10.0 L) for 4h in a Soxhlet extractor, was resuspended in hot methanol-water (1:1, v/v) and then was successively partitioned with dichloromethane and ethyl acetate to yield the corresponding fractions dichloromethane (**DB7**), and ethyl acetate (**DB8**). Besides that, the experimental crude raws also contained the hot water decoction extract of powers after methanol extraction **DB10**, and the chloroform fraction of heartwood **DB11**. They were mentioned above.

4.2. Anti-microbacterial experiments

4.2.1. The first model

P. gingivalis (ATCC33277) was anaerobically incubated at 37^{o}C to an optical density of 1.0 at 620 nm. Precultured *P. gingivalis* was anaerobically incubated with both the extracts **DB1-DB11** at concentration of 1.0 mg/mL, the isolated compounds **1-21** and a mixture of compounds **2**+**15** at concentration of 0.1 mg/mL and in 200 µL of Gifu anaerobic medium (GAM, Nissui, Tokyo, Japan) at 37 ^{o}C for 100 h in a 96-well plate (BD Falcon, NJ, USA). The isolated compounds and the extracts of *D. tonkinensis* were dissolved in DMSO (Nacalai

Tesque, Kyoto, Japan). The degree of turbidity in the broth culture was measured at an absorbance of 600 nm using a microplate reader (Thermo Scientific, MA, USA). Similarly, the remainning bacteria were incubated at 37^{o}C to an optical density of 1.0 at 620 nm. Precultured *Bacillus cereus* (ATCC27522), *Escherichia coli* (JM109), *Staphylococcus aureus* (ATCC25923), *Staphylococcus epidermidis* (ATCC14990), *Streptococcus pneumonia* (ATCC49619), *Streptococcus pyogenes* ((ATCC12344), *Vibrio parahaemolyticus* (RIMD2210010), *Streptococcus mutans* (MT8148), *Streptococcus mitis* (ATCC903), and *Streptococcus sobrinus* (MT10186) were incubated with both the extracts **DB1-DB11** and the isolated compounds **1-21** and a mixture of compounds **2**+**15** at concentration 0.1 mg/mL in 200 µL of brain heart infusion medium (BHI, Gibco) at 37 ^{o}C for 24 h in a 96-well plate (BD Falcon, NJ, USA). The isolated compounds and the extracts were dissolved in DMSO (Nacalai Tesque, Kyoto, Japan). The degree of turbidity in the broth culture was measured at an absorbance of 600 nm using a microplate reader (Thermo Scientific, MA, USA). Ampicillin sodium salt and DMSO were used as the positive, and negative controls, respectively.

4.2.2. The second model

The antimicrobial assays were performed as described by Vanden and Vlietlinck and Xiong et al. [93,94]. Briefly, heart wood methanol extract, and its chloroform fraction, compounds **4**, **8**, **11**, and **21** were diluted with DMSO 10%. The in vitro antimicrobial experiments were carried out by the 96-well microtiter plate. The plates were then incubated at 37^{o}C in 24 hours with bacterial strains and 30^{o}C in 48 hours with fungal strains. The result was shown by MIC values (Minimum Inhibitory Concentration).

4.3. Antioxidant activity

Free radical scavenging activity of different extracts of several isolated compounds were measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (180 µL) was added to 20 µL of different samples in ethanol at different concentration (100, 50, 10, 5, 1 µg/mL). The mixture was carried out by 96-well plate at room temp for 20 min. Then, absorbance was measured at 517 nm by using spectrophotometer (UV-VIS Shimadzu). The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or percent inhibition = $[(A_O - A_1)/ A_O] \times 100$. Where A_O was the absorbance of control reaction and A_1 was the absorbance in presence of test or standard sample.

4.4. Enzyme active screenings

4.4.1. Serine protease enzyme activities

The aqueous decoction extracts were diluted by deionized water whereas DMSO (dimethyl sulfoxide) was used for the remaining samples. 80-µL aliquots of the test samples (extracts: 3.125, 31.25, and 312.5 ng/mL, compounds: 1.25, 12.5, and 125 µM) with 12.5 mM Tris-HCl (pH 8.0) were mixed with 10-µL aliquots of serine proteases (trypsin (CALBIOCHEM): 2.5 ng/mL, chymotrypsin (CALBIOCHEM): 50 µg/mL, and elastase (ELASTIN PRODUCTS CO. INC.): 25 µg/mL) in 96-well microplates and incubated at room temperature for 20 min. After preincubation at 37^{o}C for 10 min, the reaction was initiated by adding 10 µL of individual fluorogenic synthetic peptide substrates (for trypsin: Boc-Glu-Ala-Arg-methylcoumarin amide (MCA), chymotrypsin: Glt-Ala-Ala-Phe-MCA and elastase: Suc-Ala-Pro-Ala-MCA (PEPTIDE INSTITUTE, INC), 100 µM) in a total volume of 100 µL per well and incubated at 37^{o}C for 15 min. Reactions were stopped by adding 50 µL of 30% acetic acid. The amount of 7-amino-4-methylcoumarin liberated from the substrates

determined fluorometrically with excitation and emission wavelengths at 370 and 460 nm, respectively, with fluorescence spectrophotometer (Infinite 200Pro, TECAN, Tokyo, Japan). Data present mean ± SD values of 12 independent determinations. Differences were determined using student t-test and considered to be significant when *p*-value was less than 0.05.

4.4.2. Casein cleavage activity

25 ng/mL of trypsin and 25 ng/mL of extracts **DB1-DB2**, **DB4-DB6**, and **DB10** or 10 μM of compounds **6**, and **17** were mixed and incubated at room temperature for 30 min. 0.8 mg/mL of casein was added and incubated at 37□ for 3hrs. Samples were separated by poly-acrylamide gel electrophoresis (SDS-PAGE) in 12% tris-glycine gel under reducing condition using the method of Laemmli [95] and the proteins were revealed by silver stain according to the manufacture's instructions (Silver Stain Kit; Wako Pure Chemical Industries Ltd., Tokyo, Japan).

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1. **Ninh The Son**, Mari Kamiji, Tran Thu Huong, Miwa Kubo, Nguyen Manh Cuong, and Yoshiyasu Fukuyama, Chemical constituents of Vietnamese plants *Dalbergia tonkinensis* Prain and *Cratoxylum formosum* (Jack) Dyer in Hook and their antioxidative activities. *Medicinal Chemistry Research*, **2019**, *28*, 1441-1447. https://doi.org/10.1007/s00044-019-02383-9 (**SCI-E**).
2. **Ninh The Son**, Midori Suenaga, Yoiichi Matsunaga, Luu Van Chinh, Miwa Kubo, Kenichi Harada, Nguyen Manh Cuong, and Yoshiyasu Fukuyama, Serine Protease Inhibitors and Activators from *Dalbergia tonkinensis* species: Structural and Bioactive Highlights. *Journal of Natural Medicines*, **2019**, https://doi.org/10.1007/s1141 8-019-01347-y. (**SCI-E**).
3. **Ninh The Son**, Tsuyoshi Yamamoto, Yoshiyasu Fukuyama, Chemotaxonomic aspects of the constituents of the plant *Dalbergia tonkinensis*. *Biochemical Systematics and Ecology*, **2018**, *78*, 98-101 (**SCI**).
4. **Ninh The Son**, Masataka Oda, Naoki Hayashi, Daiki Yamaguchi, Yu Kawagishi, Fumi Takahashi, Kenichi Harada, Nguyen Manh Cuong, and Yoshiyasu Fukuyama, Antimicrobial activity of the constituents of *Dalbergia tonkinensis* and structural-bioactive highlights. *Natural Product Communications*, **2018**, *13*, 157–161 (**SCI-E**).
5. **Ninh The Son**, Kenichi Harada, Nguyen Manh Cuong, Yoshiyasu Fukuyama, Two new carboxyethylflavanones from the heartwood of *Dalbergia tonkinensis* and their antimicrobial activities. *Natural Product Communications*, **2017**, *12*, 1721–1723 (**SCI-E**).

6. **Ninh The Son**, Yoshiyasu Fukuyama, Nguyen Manh Cuong, chemical constituent from heartwood of *Dalbergia tonkinensis*. *Chemistry of Natural Compounds*, in press (**SCI**).