PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF *TIBOUCHINA SEMIDECANDRA* L.

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PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF *TIBOUCHINA SEMIDECANDRA* L.

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A thesis submitted in fulfillment of the
requirements for the award of the degree of
Master of Science (Chemistry)

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Dedicated to

My beloved parents

My brothers and sisters

My teachers and

My friends

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PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between December 2005 and November 2007 under the supervision of Prof. Dr. Hasnah Mohd. Sirat. Part of my work described in this thesis has been reported in the following publications:

1. Mohd Fazlin Rezali and Hasnah Mohd Sirat (2006). Phytochemical Studies of the Leaves of *Tibouchina semidecandra* L. Paper presented at the Asian Symposium on Medicinal Plants, Spices and Other Natural Products (ASOMPS) XII at Padang, West Sumatra, Indonesia. 13-18 November 2006

2. Mohd Fazlin Rezali and Hasnah Mohd Sirat (2006). Flavonoids from the Leaves of *Tibouchina semidecandra* L. Paper presented at the 22nd Annual Seminar of the Malaysian Natural Products Society 2006 at Cititel, Midvalley City, Kuala Lumpur. 8-10 November 2006.

ABSTRACT

Phytochemical studies on *Tibouchina semidecandra* L., previously known as *T. urvilleana* have resulted in the isolation of nine pure compounds comprising of one flavonol, three flavonol glycosides, two plant sterols, one fatty acid, an ester and one ellagic acid glycoside. Five compounds have been successfully isolated from the leaves, i.e quercetin, β-sitosterol-*O*-β-D-glucopyranoside, quercetin 3-*O*-α-L-(2″-*O*-acetyl) arabinofuranoside, avicularin and quercitrin. Three compounds identified as oleic acid, eicosanyl *trans-p*-coumarate and 23-ethyl-cholest-5-en-3-ol have been isolated from the roots. The stem barks gave one ellagitannin, identified as 3,3′-*O*-dimethyl ellagic acid 4-*O*-*α*-L-rhamnopyranoside. Methylation of quercetin gave quercetin tetramethyl ether, while acetylation of quercetin yielded quercetin tetraacetoxyl acetate. The structures of all compounds were established based on spectral studies using nuclear magnetic resonance, infrared and ultraviolet spectroscopies as well as mass spectrometry. Evaluation of the antioxidative activity on the crude extracts and pure compounds by electron spin resonance (ESR) and ultraviolet-visible (UV-vis) spectrophotometric assays showed that the pure isolated flavonoids and the EtOAc extract of the leaves possessed strong antioxidative capabilities. Quercetin was found to be the most active as radical scavenger in DPPH-UV and ESR methods with SC_{50} of 0.7 μM ± 1.4 and 0.7 μM ± 0.6, respectively in the antioxidant assay. A combination of quercetin and quercitrin was tested for synergistic anti-oxidative capacity. However, there was no significant improvement observed. The antimicrobial assay on the crude extracts and pure compounds were carried out against the Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* and the Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*. However, no significant activity was observed. Quercetin and quercetin tetraacetoxyl acetate exhibited strong antityrosinase agent with percent inhibition of 95.0% and 93.4% respectively, equivalent to the positive control, kojic acid in the tyrosinase enzyme assay.

ABSTRAK

Kajian fitokimia ke atas *Tibouchina semidecandra* L., dahulunya dikenali sebagai *T. urvilleana* telah berjaya mengasingkan sembilan sebatian tulen yang terdiri daripada satu flavonol, tiga flavonol glikosida, dua sterol tumbuhan, satu asid lemak, satu ester dan satu asid ellagik glikosida. Lima sebatian berjaya dipisahkan daripada bahagian daun iaitu kuersetin, β-sitosterol-*O*-β-D-glukopironosida, kuersetin 3-*O*-α-L-(2″-*O*-asetil) arabinofuranosida, avikularin dan kuersitrin. Tiga sebatian dikenalpasti sebagai asid oleik, eikosanil *trans*-*p*-koumarat dan 23-etil-kolest-5-en-3-ol berjaya diasingkan daripada bahagian akar. Kulit batang menghasilkan satu sebatian ellagitanin dikenalpasti sebagai 3,3′-*O*-dimetil asid ellagik 4-*O*-*α*-L-rhamnopiranosida. Pemetilan kuersetin memberikan kuersetin tetrametil eter, manakala pengasetilan kuersetin menghasilkan kuersetin tetraasetoksil asetat. Struktur kesemua sebatian dikenalpasti berdasarkan kepada kajian spektroskopi resonans magnet nukleus, inframerah dan ultralembayung serta kajian spektrometri jisim. Penilaian ujian antioksidan ke atas ekstrak mentah dan sebatian tulen secara RSE dan UL menunjukkan flavonoid dan ekstrak EtOAc mempunyai aktiviti antioksidan yang tinggi. Kuersetin didapati paling aktif sebagai perencat radikal bebas bagi kaedah DPPH-UL dan RSE dengan masing-masing SC_{50} 0.7 μM ± 1.4 dan 0.7 μM ± 0.6 dalam ujian antioksidan. Kajian sinergi antioksidan terhadap gabungan kuersetin dan kuersitrin didapati tidak menunjukkan kesan yang signifikan. Biocerakinan antimikrob ekstrak mentah dan sebatian tulen diuji terhadap bakteria Gram-positif, *Bacillus subtilis* dan *Staphylococcus aureus* dan bakteria Gram-negatif, *Pseudomonas aeruginosa* dan *Escherichia coli*. Walau bagaimanapun, tiada kesan signifikan dapat diperhatikan. Kuersetin dan kuersetin tetraasetoksil asetat menunjukkan aktiviti antitirosinase yang tinggi dengan peratus perencatan masing-masing, 95.0% dan 93.4%, iaitu setara dengan kawalan positif, asid kojik dalam cerakinan enzim tirosinase.

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LIST OF ABBREVIATIONS

Ac_2O	Acetic anhydride
ADP	Adenosine diphosphate
$AlCl_3$	Aluminium trichloride
^{13}C	Carbon-13
CC	Column chromatography
cm^{-1}	Per centimetre
cm	Centimeter
ºC	Degree Celcius
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
COSY	Correlation Spectroscopy
d	Doublet
dd	Doublet of doublets
DEPT	Distortionless Enhancement of Polarisation Transfer
DMSO	Dimethyl sulphoxide
DMSO-d_6	Deuterated dimethyl sulphoxide
DPPH	Diphenylpicrylhydrazyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
EIMS	Electron Impact Mass Spectrometry
ESR	Electron Spin Resonance
FABMS	Fast Atom Bombardment Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
GHz	Gigahertz

^1H	Proton
H_3BO_3	Boric acid
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HCl	Hydrochloric acid
Hz	Hertz
IC	Inhibition concentration
i.p.	Intra peritoneal
IR	Infrared
J	Coupling constant
K_2HPO_4	Potassium hydrogen phosphate
KBr	Potassium bromide
Lit.	Literature
m	Multiplet
M^+	Molecular ion
mg	Milligram
mM	Millimolar
MeOH	Methanol
MHz	Megahertz
mp	Melting point
m/z	Mass-to-charge ratio
NaOAc	Sodium acetate
NaOMe	Sodium methoxide
nm	Nanometer
NMR	Nuclear Magnetic Resonance
PAF	Platelet Activating Factor
pet. ether	Petroleum ether
ppm	Parts per million
py	Pyridine
R_f	Retention factor
RP-C_{18}	Reverse phase C_{18} silica gel
rt	Room temperature
s	Singlet

SC_{50}	Scavenging concentration to obtain 50% of the maximum scavenging capacity
t	Triplet
TLC	Thin-layer chromatography
VLC	Vacuum liquid chromatography
δ	Chemical shift
UV	Ultraviolet
μM	Micromolar
γ	Gamma
λ	Lambda
$\overline{\nu}$	Wave number

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CHAPTER I

INTRODUCTION

1.1 Medicinal Plants for Drug Discovery

Malaysia is bestowed with diverse floristic resources with nearly 20% of seed plants and 15% of ferns in the Malay Peninsular, which has been claimed to have therapeutic significances. However, the quest for unleashing the biomolecules from unexplored niches of the Malaysian rainforest is a difficult task that needs a systematic approach to relegate a discovery process. In an extraordinary move that recognizes the inevitable consequences of biotechnology, the research and development in Malaysian medicinal plants has ventured new avenues for broad screening of medicinal plants and new drugs leads [1].

The use of plants or their extracts for the healing of wounds and the treatment of diseases are as old as human history. Nearly half of today's modern medicines have originated from approximately 100 species of plants. Interests in medicinal plants throughout the world at all levels of society have grown tremendously over the past twenty years. This is the result of the use of herbal products as natural cosmetics, food supplements and self medication by the public which lead to the detailed investigations of many plant species for their biological activity effects on animals and human beings. Even pharmaceutical companies are now actively involved in this scientific investigation and a large amount of funds are allocated for these purposes.

Nowadays, the chemistry of natural products is relatively easy but the economic translation to drugs, pesticides and other high valued products remains difficult and demanding. Fortunately, many plants with ethnobotanical or ethnopharmacological activities are acceptable as supplements or botanicals, while the development to pharmaceuticals can be placed as a long-term research. The isolation and structural elucidation of natural products are not the big obstacles, which lead to compounds being quickly identified. In recent years, a rich harvest of novel natural products have been made, some of which possess cytotoxic or insecticidal activities. Some of the diverse classes of natural products including alkaloids, flavonoids, terpenoids and xanthonoids isolated from Malaysian plant families such as Annonaceae, Moraceae, Piperaceae, Zingiberaceae and Melastomataceae have been encountered.

1.2 Melastomataceae Family

The Melastomataceae or locally known as “sendudok” is a dicotyledon tropical plant family with about 4500 species; 3000 species in South America, 250 species in tropical west and east African harbour, and 250 species in Madagascar. The remaining 1000 species can be found in Asia Oceania and northern Australia with a concentration in central Malaysia (Borneo) [2]. In Malaysia, there are 25 genera and 180 species which are usually found in lowlands and mountains. Among them are: *Tibouchina, Melastoma, Huberia, Lavoisiera, Microlicia, Trembleya, Memycelon, Heterocentron* and *Osbeckia.*

Melastomataceae mainly consists of herbs, shrubs, and climbers. The tree members are mostly rather small, few reaching as much as 60 feet high [3]. The botanical characters of this family are opposite leaves, simple, generally with three prominent longitudinals veins. Flowers are small to large, clustered, regular or bilaterally symmetrical; four or five sepals, or apparently absent; four or five petals, separate, pink, purple or blue, rarely white; stamens twice as many as petals, eight or ten, with rather thick, pink or blue stalks (rarely white) and large yellow, pink or blue

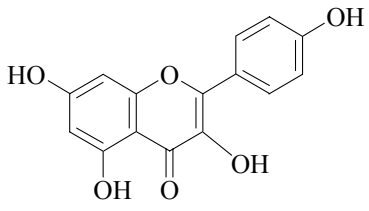
anthers; ovary inferior. The fruit is a berry with many small seeds or with one large seed; in some other cases capsular and opening, with dry or pulpy contents.

In traditional medicines, Melastomataceae has been used for the treatment of diarrhea, puerperal infection, dysentery, leucorrhea, wound healing, post-partum treatment and hemorrhoids [4].

1.2.1 A Review of Phytochemicals and Biological Properties of Melastomataceae Family

There has only been a few phytochemical studies reported from the Melastomataceae family. The family is characterized by the presence of tannins which is very common, flavonoids (common) and alkaloids (rare). Acylated anthocyanins have been isolated from the fruits and flowers [5].

A wide diversity of flavonoid structures has been found in this family, with the predominance of flavonol glycosides mainly kaempferol (**1**) and quercetin (**2**). Glycosides of isorhamnetin (**3**), rhamnetin (**4**) and myricetin (**5**) were also found, although less frequently. Derivatives of apigenin (**6**), luteolin (**7**) and chrysoeriol (**8**) were among the flavones isolated in this family [5].



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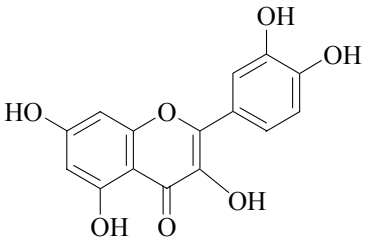

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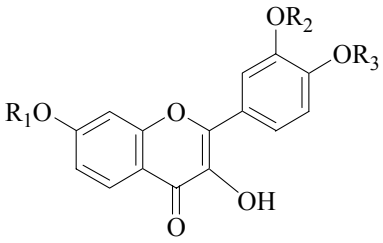
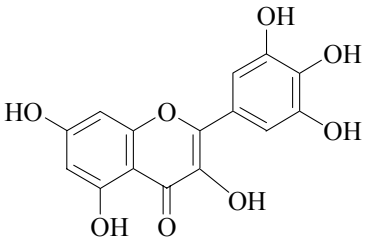


(**3**) R_1, R_3 = H, R_2 = CH_3
(**4**) R_1 = CH_3, R_2, R_3 = H



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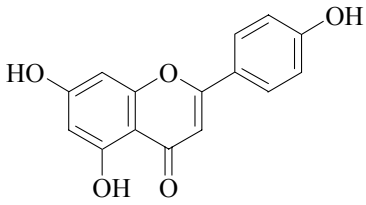

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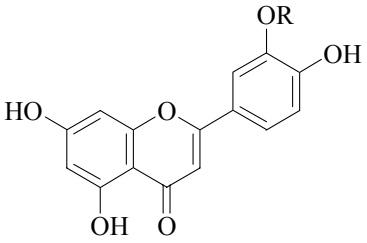
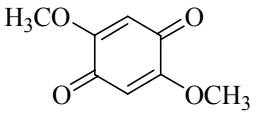


(**7**) R = H
(**8**) R = CH_3

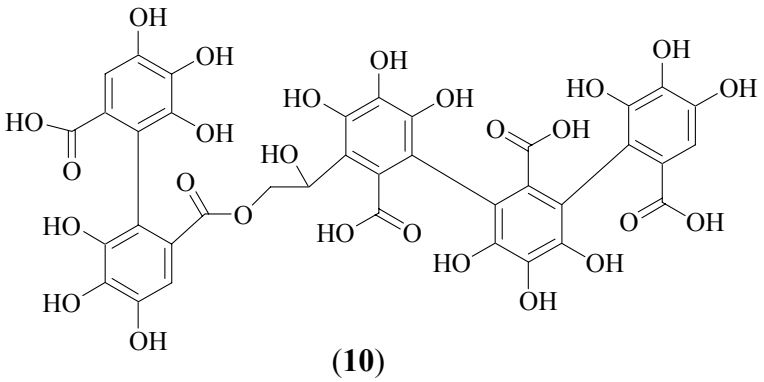
An anticancer agent, 2,5-dimethoxybenzoquinone (**9**) was determined as a cytotoxic constituent of *Tibouchina pulchra.* This compound showed an ED_{50} of 2.5 μg/mL in the KB cell culture [6].



(**9**)

Three active principles were isolated from the leaves of *Melastoma candidum* using the screening of hypertensive effect on spontaneously hypertensive rats (SHR). Intravenous injection of castalagin (**10**), procyanidin (**11**) and helichrysoside (**12**) into SHR lowered the mean blood pressure through a decrease of sympathetic tone as well as due to direct vasodilatation in a dose-dependent manner, with helichrysoside (**12**) being the most potent compound [7].

The acetone extract of *M. candidum* revealed a good bactericidal effect with minimum bactericidal concentrations value of 0.08 to 5.12 mg/ml, good thermal stability (heating at 121 ºC for 15 minutes), and broad antibacterial activity in the pH range of 5–8 [8].



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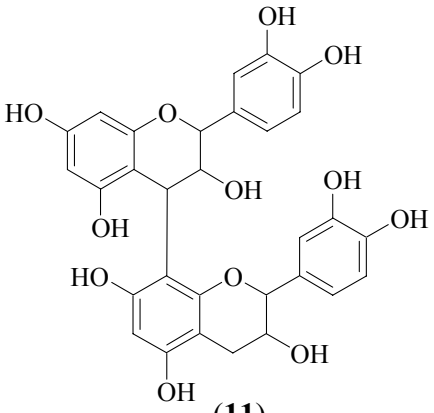

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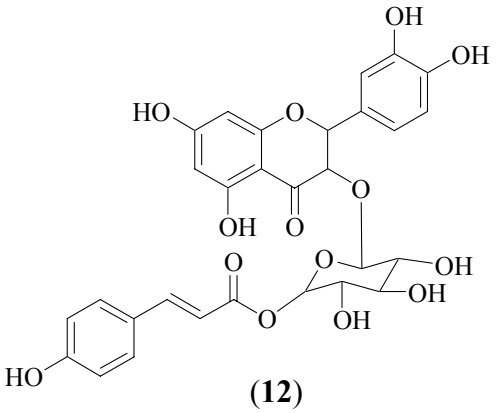
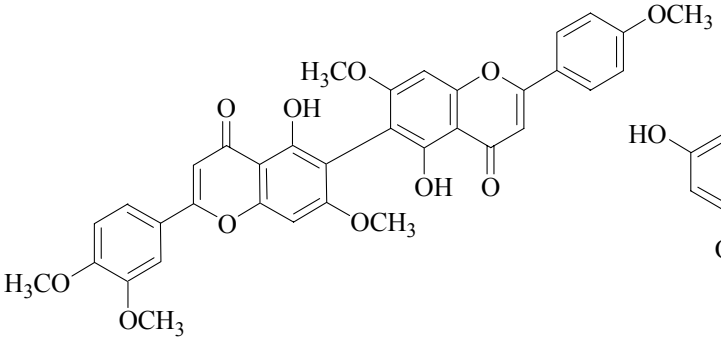


(**12**)

Chromatographic fractionation of the methanolic extract from the leaves of *Miconia cabucu* Hoehne by Juliana *et al.* afforded the first example of a C6-C6″ linked flavone dimer, 5-hydroxy-4′,7-dimethoxyflavone-(6-C-6″)-5″-hydroxy-3‴,4‴,7″-trimethoxyflavone (**13**) together with the known compounds, quercetin-3-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-xylopyranoside (**14**), quercitrin (**15**), myricetin-3-*O*-α-L-rhamnopyranoside (**16**), isoquercitrin (**17**), kaempferol-3-*O*-β-D-(6″-coumaroyl)-glucopyranoside (**18**) and gallic acid (**19**) [9].



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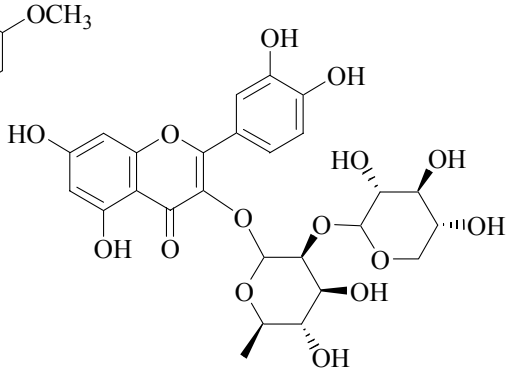

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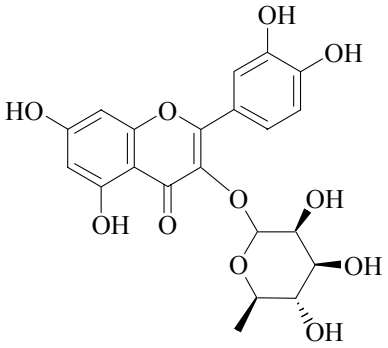

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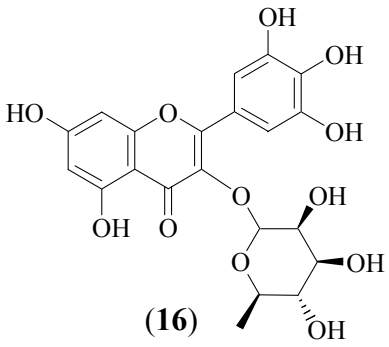

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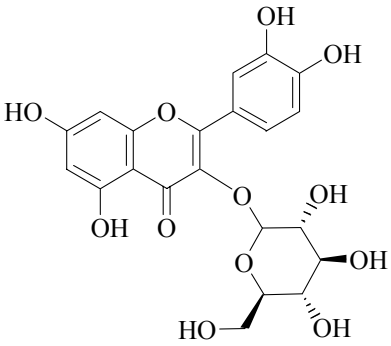

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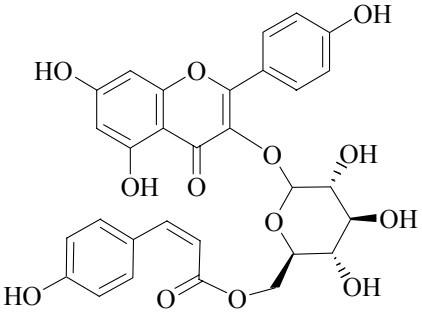

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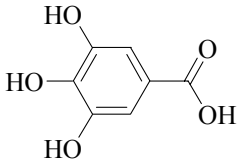
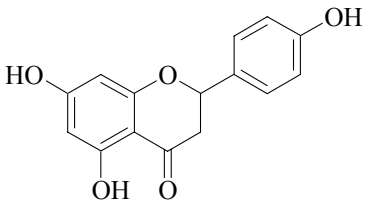


(**19**)

Phytochemical and bioactivity studies of the flowers of *Melastoma malabathricum* L. have been carried out by Deny Susanti *et al.* [10]. The ethyl acetate extract yielded three compounds, identified as naringenin (**20**), kaempferol (**1**) and kaempferol-3-*O*-D-glucopyranoside (**21**) while the methanol extract gave kaempferol-3-*O*-(2″,6″-di-*O*-*p*-trans-coumaroyl)-glucopyranoside (**22**) and kaempferol-3-*O*-D-glucopyranoside (**21**). All these compounds as well as their crude extracts were found to be active as free radical scavengers in the DPPH radical-scavenging electron spin resonance spectroscopic method. Naringenin (**20**) and kaempferol-3-*O*-(2″,6″-di-*O*-*p*-*trans*-coumaroyl)-glucopyranoside (**22**) were also found to be active in inhibiting cell proliferation of human cell line from breast carcinoma with IC_{50} values of 0.28 μM and 1.3 μM, respectively.



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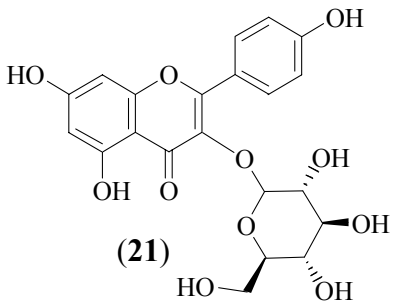

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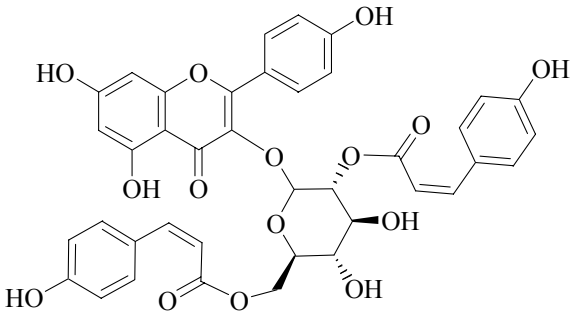
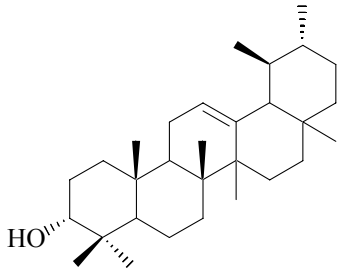


(**22**)

In the search for natural compounds useful against anti-inflammatory activity, α-amyrin (**23**), betulinic acid (**24**), quercetin (**2**) and quercitrin (**15**) which were also isolated from *M. malabathricum* L. had been assessed *in vitro* by determining their inhibitory effects on platelet activating factor (PAF) binding to rabbit platelets using ^{3}H-PAF as a ligand. The results indicated that quercetin (**2**), quercitrin (**15**), α-amyrin (**23**), and betulinic acid (**24**) showed inhibition of PAF receptor binding with IC_{50} values of 33.0, 45.4, 20.0 and 22.2 μM, respectively. The IC_{50} values of these compounds were comparable to cedrol (13.1 μM), which was a known PAF receptor antagonist. These results suggested that natural flavonoids and pentacyclic triterpenes from *M. malabathricum* L. possess selective antagonistic activity towards PAF and could be an attractive candidate as natural anti-inflammatory compounds [11].



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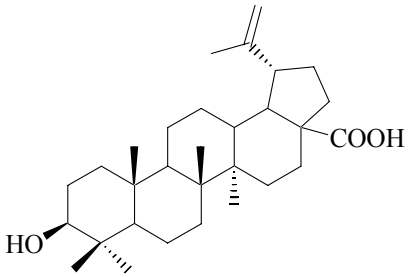
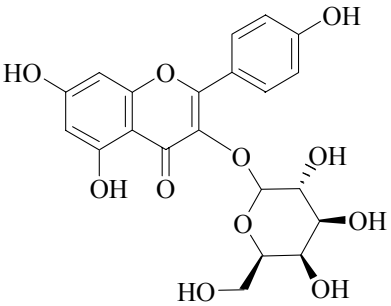


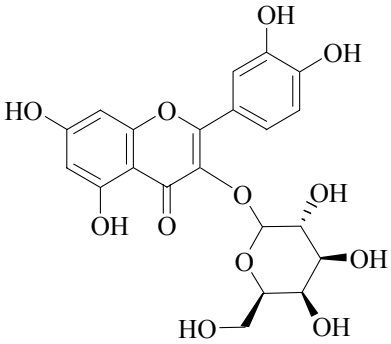
(**24**)

The antinociceptive effect of ethanolic extract of *M. malabathricum* using acetic acid-induced abdominal writhing test and hot-plate test in mice has been carried out by Sulaiman *et al.* It was demonstrated that the extract (30-300 mg/kg, i.p.) strongly and dose-dependently inhibited the acetic acid-induced writhing test with an ED_{50} of 100 mg/kg i.p., suggesting that, the ethanolic extract of *M. malabathricum* has a potential as antinociceptive agent that acts at both peripheral and central level of nerves [12].

The chemical investigation on *Monochaetum multiflorum* yielded trifolin (**25**), hyperin (**26**), quercetin 3-(6′-*O*-caffeoyl)-β-D-galactopyroside (**27**), isoquercitrin (**17**), quercetin 3-(6′-*O*-caffeoyl)-β-D-glucopyroside (**28**), 4-*O*-β-D-glucopyranosyl-2-*O*-methylphloroacetophenone (**29**), 4-*O*-(6′-*O*-galloyl-β-gluco pyranosyl)-*cis*-*p*-coumaric acid (**30**), 6′-*O*-galloylprunasin (**31**), benzyl 6′-*O*-galloyl-β-glucopyranoside (**32**) and a novel diester of tetrahydroxy-μ-trunixic acid with 2 mole of hyperin (monochaetin) (**33**) [13].



(**25**)



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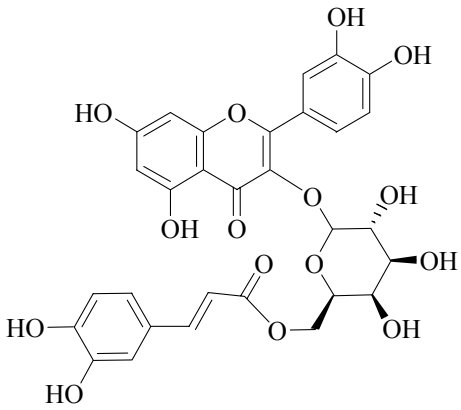
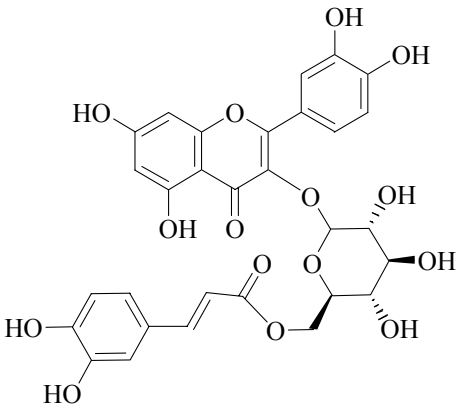
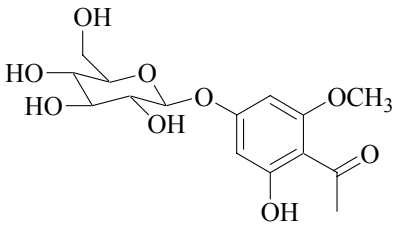


(**27**)



(**28**)



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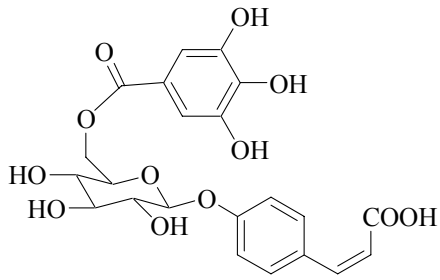

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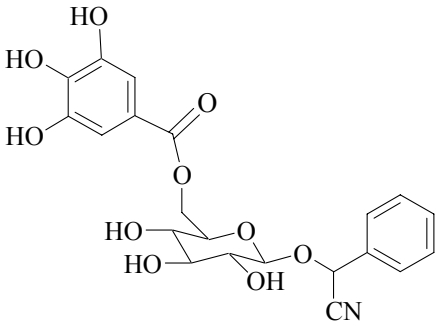
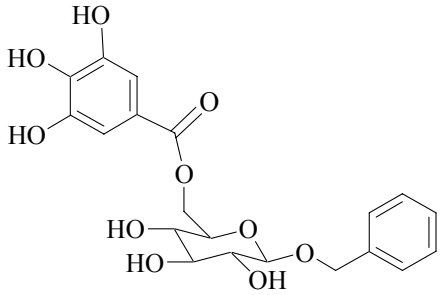
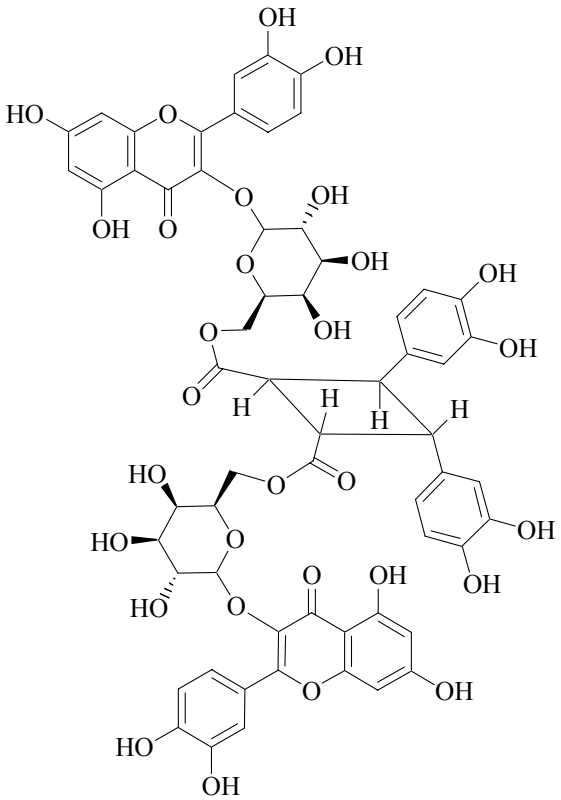


(**31**)

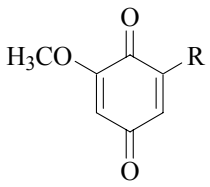


(**32**)



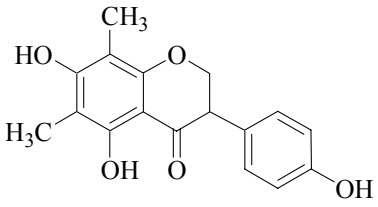
(**33**)

Bioactivity-directed isolation of an EtOAc extract from the leaves of *Miconia lepidota*, afforded two benzoquinones, namely 2-methoxy-6-heptyl-1,4-benzoquinone (**34**) and 2-methoxy-6-pentyl-1,4-benzoquinone (**35**) which showed potential as an anticancer agents [14].

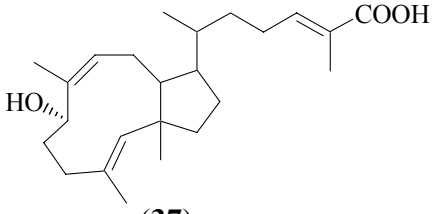


(**34**) R = C_7H_{15}
(**35**) R = C_5H_{11}

The chemical investigation of *Henriettella fascicularis* has led to the isolation of 4′,5,7-trihydroxy-6,8-dimethoxylisoflavone (**36**) and sesterterpenoic acid (**37**) [15].

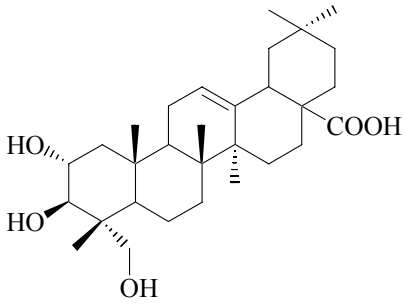


(**36**)



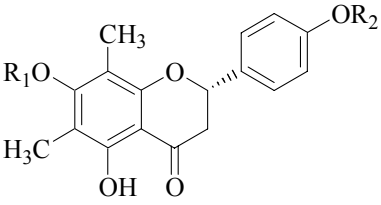
(**37**)

The ethanol extract of *Miconia pilgeriana* yielded a triterpene compound which was identified as arjunolic acid (**38**) [16].

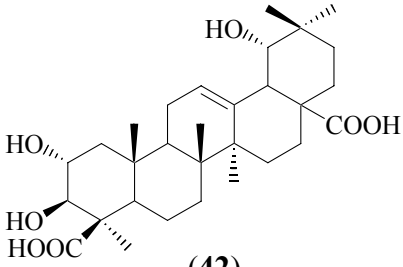


(**38**)

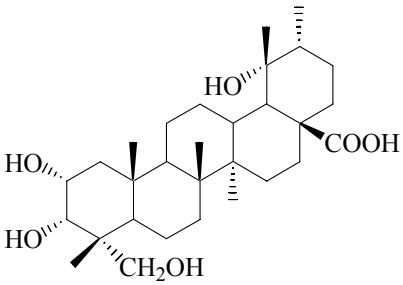
Bioactivity-guided isolation of the ethanol extract of *Miconia trailii* yielded miconioside A (**39**), miconioside (**40**), matteucinol (**41**), bartogenic acid (**42**), arjunolic acid (**38**), and myrianthic acid (**43**) [17].



(**39**) R_1 = α-L-Ara(1→6)-β-D-Glc, R_2 = CH_3
(**40**) R_1 = β-D-Api(1→6)-β-D-Glc, R_2 = H
(**41**) R_1 = H, R_2 = H

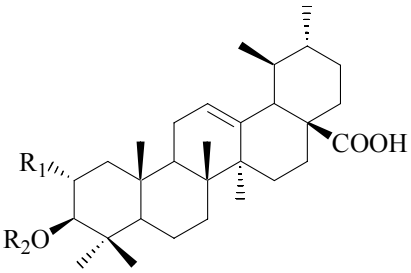


(**42**)

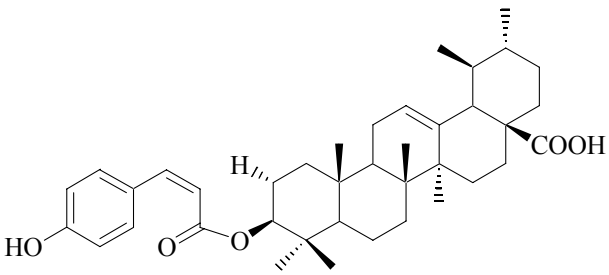


(**43**)

The chemical investigation of *Monochaetum vulcanicum* resulted in the isolation of 3β-acetoxy-2α-hydroxyurs-12-en-28-oic acid (**44**), ursolic acid (**45**), 2α-hydroxy-ursolic acid (**46**) and 3-(*p*-coumaroyl)ursolic acid (**47**) [18].

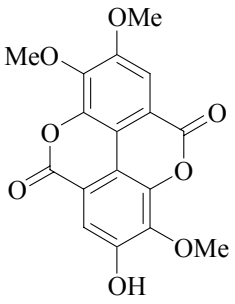


(**44**) R_1 = OH, R_2 = Ac
(**45**) R_1 = H, R_2 = H
(**46**) R_1 = OH, R_2 = H

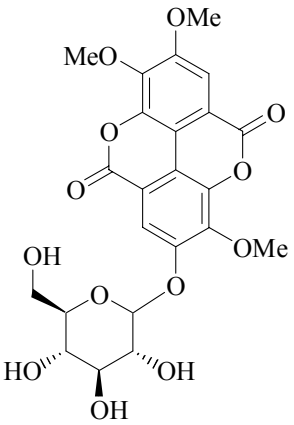


(**47**)

Two ellagic acids identified as tri-*O*-methyl ellagic acid (**48**) and tri-*O*-methyl ellagic acid glucoside (**49**) were successfully isolated from *Melastoma polyanthum* [19].

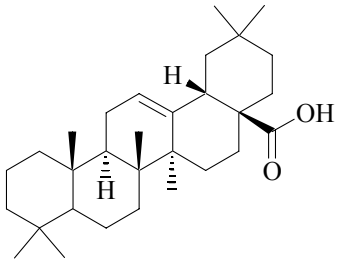


(**48**)

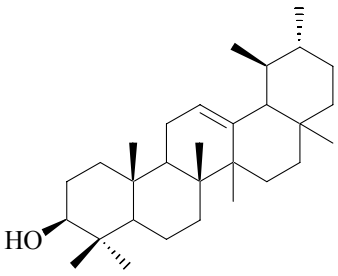


(**49**)

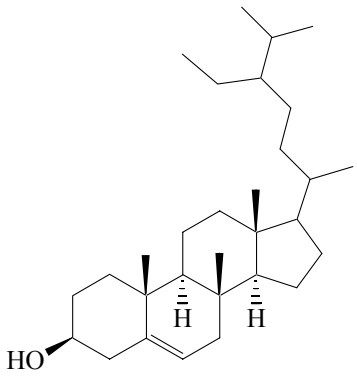
The hexane, dichloromethane and ethanol extracts of *Miconia rubiginosa* which were evaluated for their analgesic effects showed a significant inhibition in mice and rats ($p < 0.05$ and $p < 0.01$) using the acetic acid-induced writhing and hot plate tests. These extracts (200 mg/kg body wt.) showed a significant ($p < 0.05$) antinociceptive effect, lower than that produced by morphine (4 mg/kg wt.). The fractionation of the dichloromethane extract yielded ursolic acid (**45**) and oleanoic acid (**50**) as the major compounds. Three triterpenes from the hexane extract was identified using gas chromatography as α-amyrin (**23**), β-amyrin (**51**), and β-sitosterol (**52**) [20].



(**50**)



(**51**)



(**52**)

Dissotis brazae Cogn. which was traditionally used to treat malaria in Kenya was tested for *in vivo* antiplasmodium activity against chloroquin-resistant (ENT-36). The aqueous extract of the stems showed the strong inhibitory activity with IC_{50} 6.4 µg/mL [21].

1.3 *Tibouchina* Genus

Tiobouchina is a genus of about 350 species of neotropical plants. Members are shrubs or subshrubs, and are known as "glory bushes" or "glory trees". They are native to rainforest of Mexico, the West Indies, and South America, especially Brazil. The name comes from an adaptation of the native Guiana term for these shrubs. Several species are cultivated for their large bright flowers such as *T. multiflora, T. organensis, T. maudhiana* and *T. semidecandra.*

1.3.1 *Tibouchina Semidecandra* L.

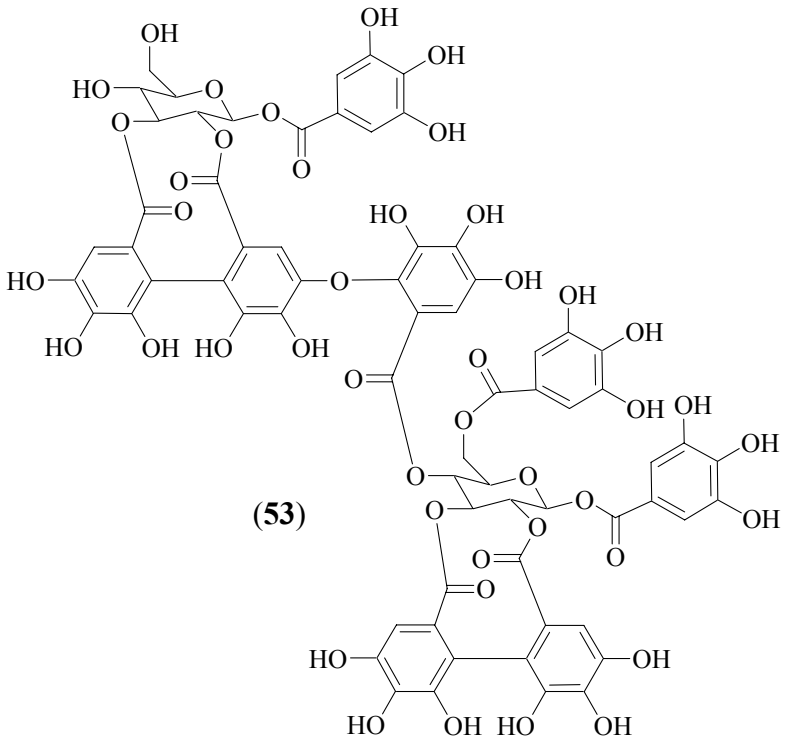
Tibouchina semidecandra L. is a shrub that has been introduced to Malaya from Brazil [3]. It bears beautiful dark purple flowers throughout the year and grows well in frost-free areas around the world. The pristine purple flower makes it a valuable ornamental plant and a potential source for the extraction of natural colourants. This plant is also used traditionally for both medicinal and food purposes [22].

Figure 1.1 *Tibouchina semidecandra* L.

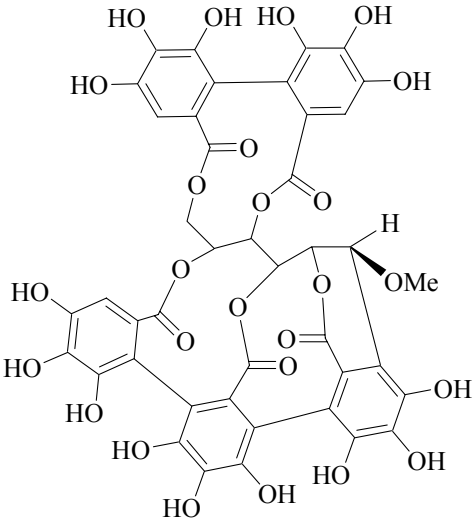
1.3.2 Chemical Investigation of *Tibouchina*

The chromatographic survey of the tannins in this family revealed that *Tibouchina* is rich in tannins, particularly in oligomeric hydrolysable tannins. Repeated chromatography of the *n*-BuOH extract of the leaves of *Tibouchina multiflora* over polystyrene and polyvinyl gel afforded two new oligomeric hydrolysable tannins named nobotanin O (**53**) and nobotanin P (**54**) [23].

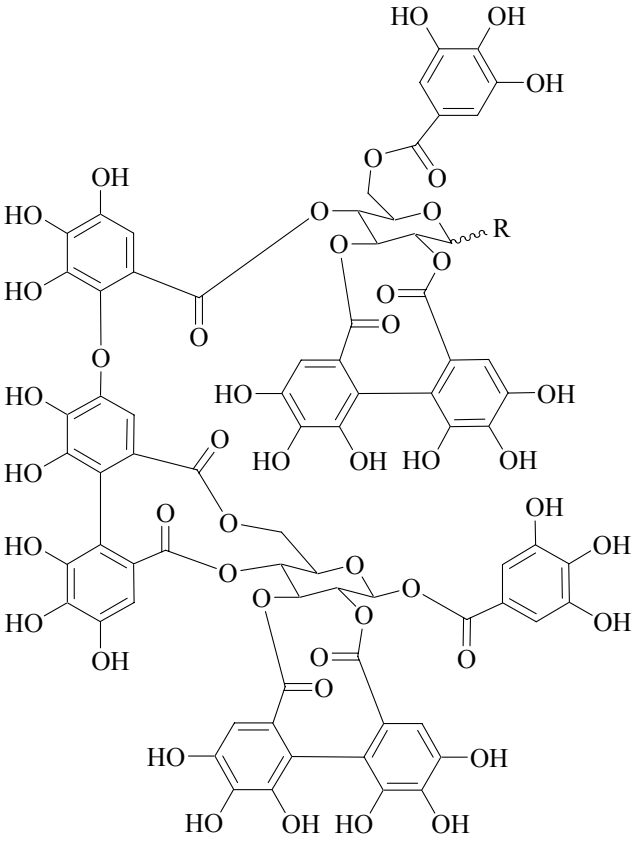
Yoshida *et al.* found by means of a chromatographic survey that *T. semidecandra* (collected from Japan) was also rich in tannins, particularly in oligomeric hydrolysable tannins. Seven new hydrolysable tannins, named as methylvescalagin (**55**)**,** nobotanin A (**56**), nobotanin B (**57**), nobotanin C (**58**), nobotanin D (**59**), nobotanin E (**60**) and nobotanin F (**61**). **S**everal flavonoid compounds such as quercetin **(2)**, myricetin **(5)**, leucodelphinidin (**62**), leucocyanidin (**63**)**,** quercetin-3-*O*-(6″-*O*-galloylgalactoside) (**64**), avicularin (**65**) and tibouchinin (**66**) have also been isolated from this plant [24-28].

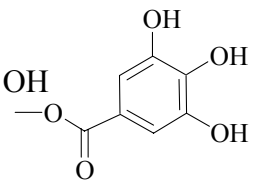


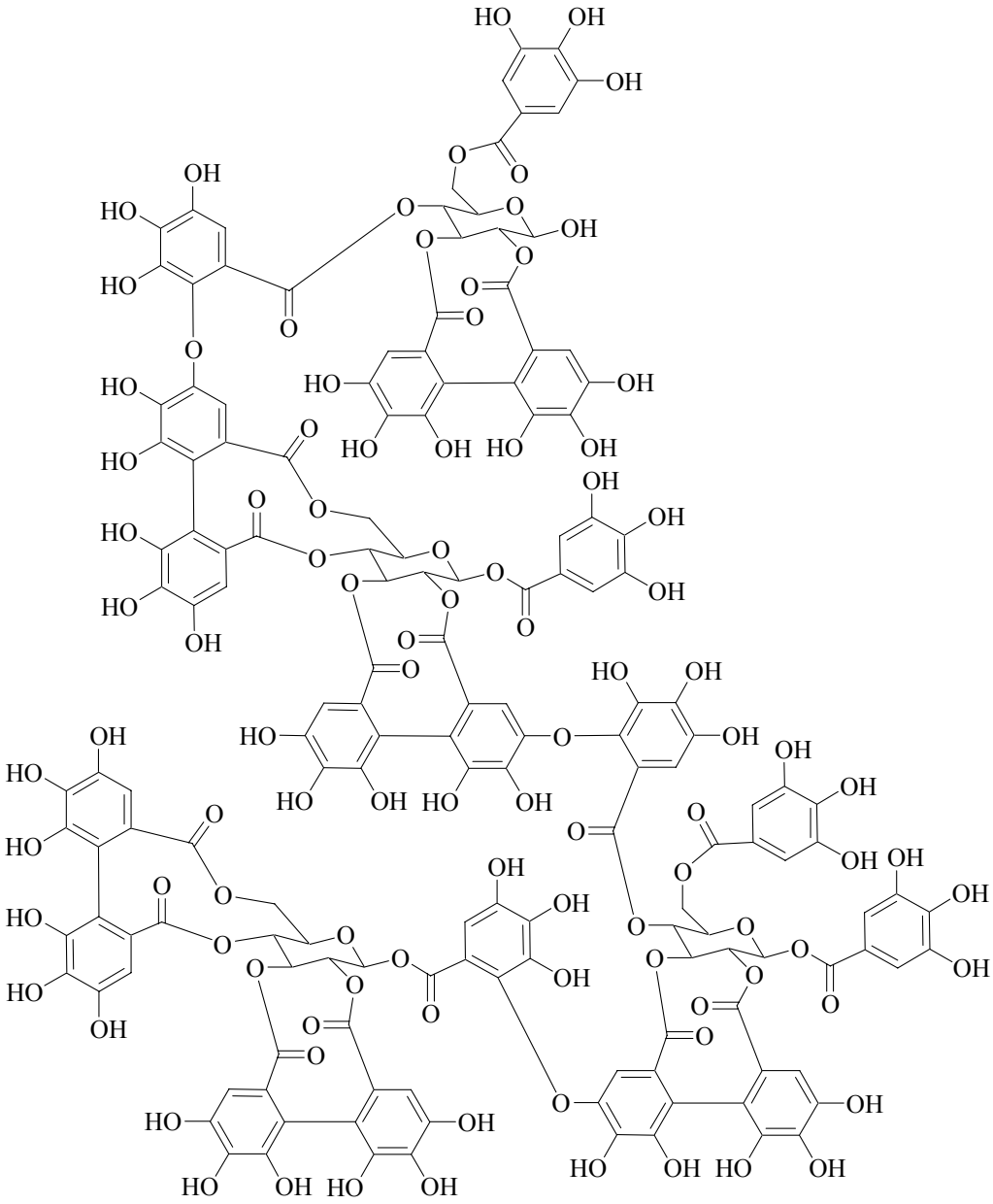
(**53**)



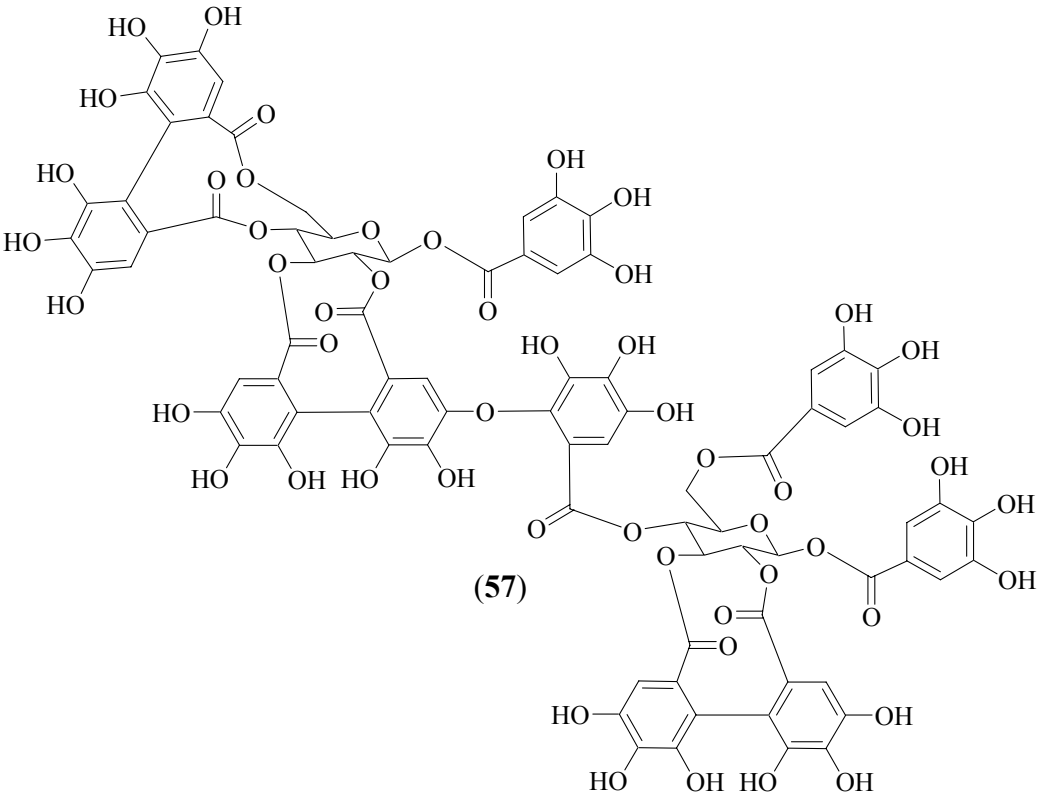
(**55**)



(**56**) R = OH
(**61**) R = 



(**54**)



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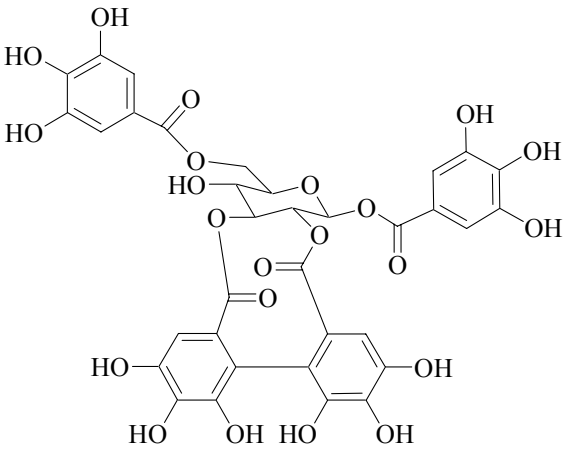
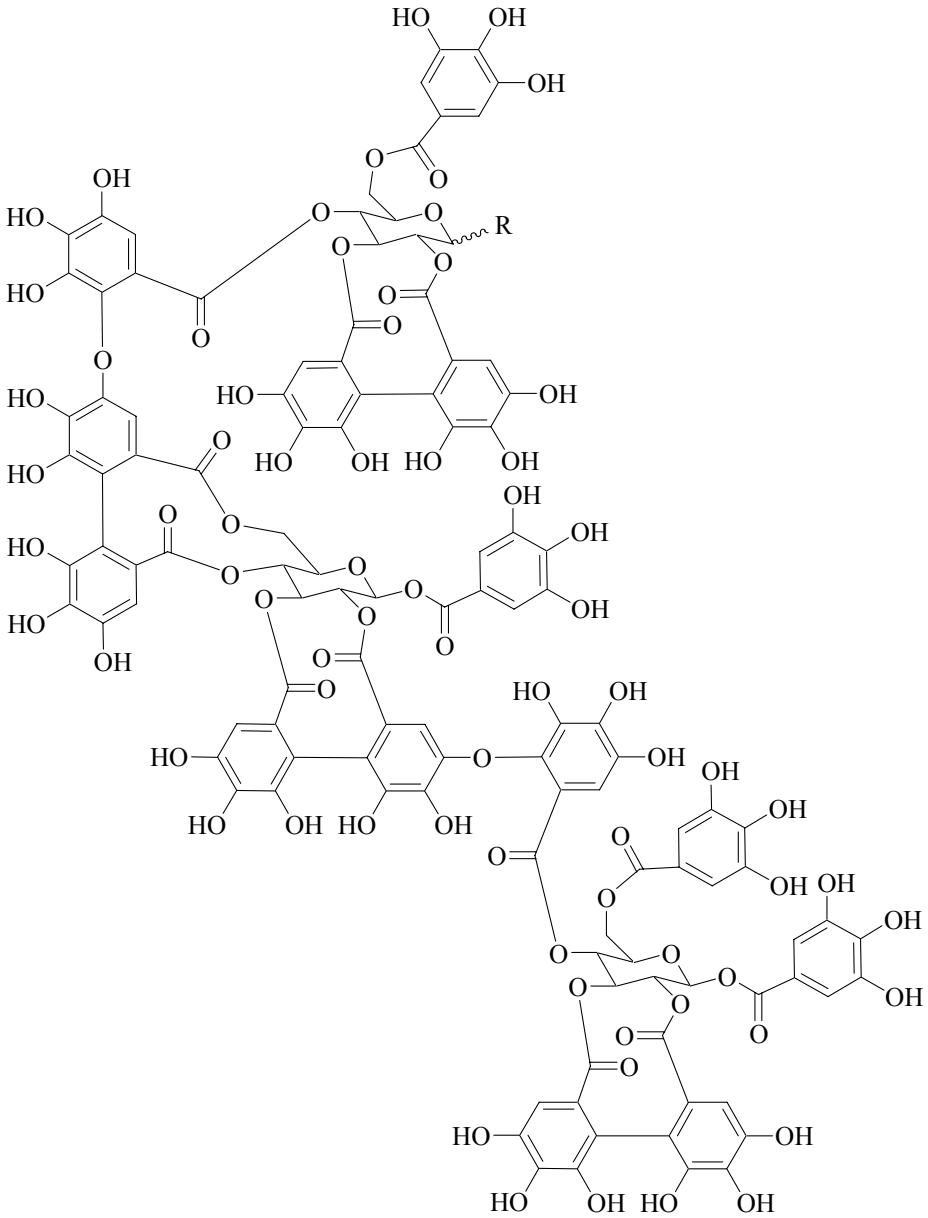
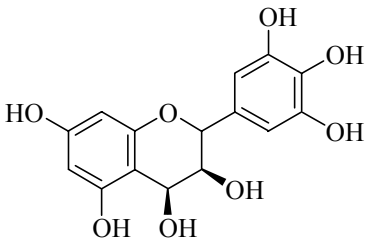


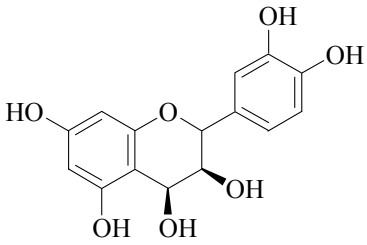
(**59**)



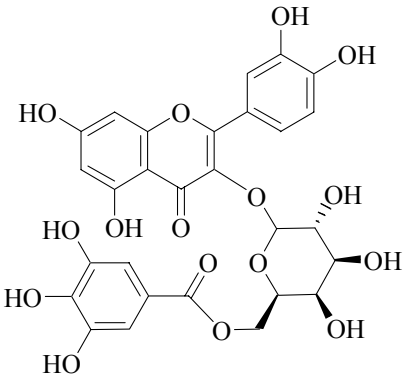
(**58**) R = OH
(**60**) R =



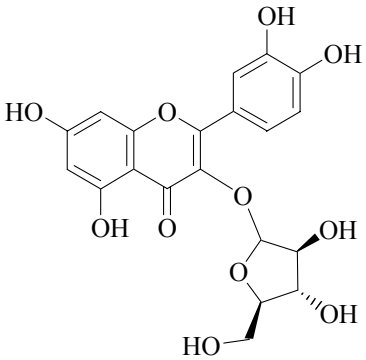
(**62**)



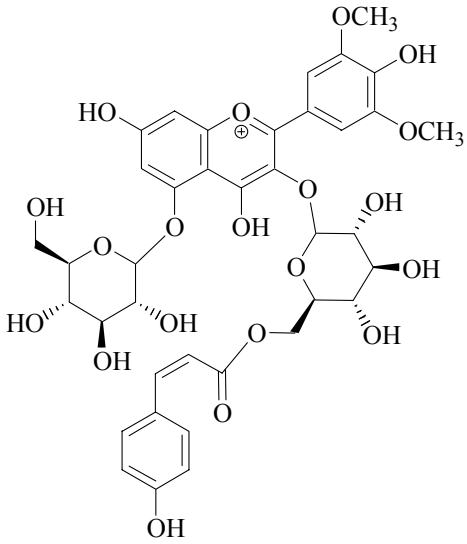
(**63**)



(**64**)

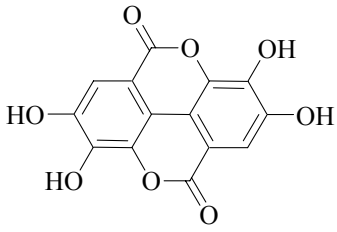


(**65**)

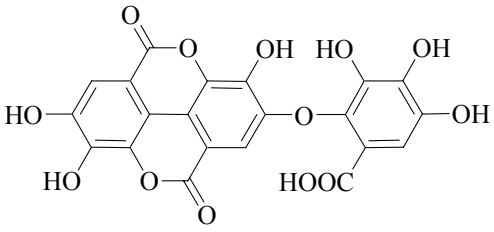


(**66**)

Acid hydrolysis of nobotanin F (**61**) gave gallic acid (**19**), ellagic acid (**67**), valoneic acid dilactone (**68**) and glucose [27].

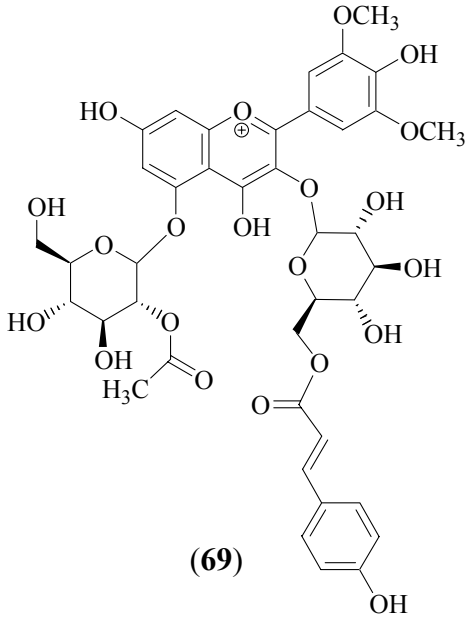


(**67**)



(**68**)

The structure of the major pigment in the purple flowers of *T. semidecandra* has been identified as maldivin 3-(*p*-coumaroylglucoside)-5-acetylglucoside (**69**)**,** a new anthocyanin by using chromatographic and various NMR techniques [29].



(**69**)

1.4 Biosynthesis of Flavonoids

In recent years, flavonoids have attracted the interest of researchers because they show promise of being powerful antioxidants which can protect the human body from free radicals [30]. Flavonoids cannot be produced by the human body and thus, have to be taken in, mainly through the daily diet. The evidence reported in the chemistry, biochemistry and pharmacy literature supports the view that flavonoids play a vital biological role, including the function of scavenging reactive oxygen species.

Chemically, there are three features that confer on flavonoids and their remarkable antioxidant properties:

- the hydrogen donating substituents (hydroxyl groups), attached to the aromatic ring structures of flavonoids, enable the flavonoids to undergo a redox reaction that helps them to scavenge free radicals more easily;
- a stable delocalization system, consisting of aromatic and heterocyclic rings as well as multiple unsaturated bonds, which helps to delocalize the resulting free radicals, and
- the presence of certain structural groups which are capable of forming transition metal-chelating complexes that can regulate the production of reactive oxygen species such as $OH^{\bullet}$ and $O^{-1\bullet}$.

Flavonoids represent a highly diverse class of secondary plant metabolites with about 9000 structures which have been identified up to now. All flavonoids derive their 15-carbon skeleton from two basic metabolites, malonyl-CoA and *p*-coumaroyl-CoA. Basically, flavonoids are derivatives of 1,3-diphenylpropan-1-one (C6-C3-C6). The crucial biosynthetic reaction is the condensation of three molecules malonyl-CoA with one molecule *p*-coumaroyl-CoA to a chalcone intermediate. Chalcones and dihydrochalcones are classes of flavonoids that consist of two phenolic groups which are connected by an open three carbon bridge. Derived from the chalcone structure, a flavonoid-class containing three rings, the flavanones, can be formed. Here, the three-carbon bridge is part of an additional heterocyclic six-membered ring that involves one of the phenolic groups on the adjacent ring. Based

on these flavanones, all other flavonoid-classes are generated, including isoflavones, flavanols, anthocyanidins, flavonols and flavones as shown in **Figure 1.2**. This latter flavonoid-class is characterized by the presence of a double bond between C2 and C3 in the heterocycle of the flavan skeleton. The B-ring is attached to C2 and usually no substituents are present at C3. This differentiates them from flavonols where a hydroxyl group can be found at that C3 position [31].

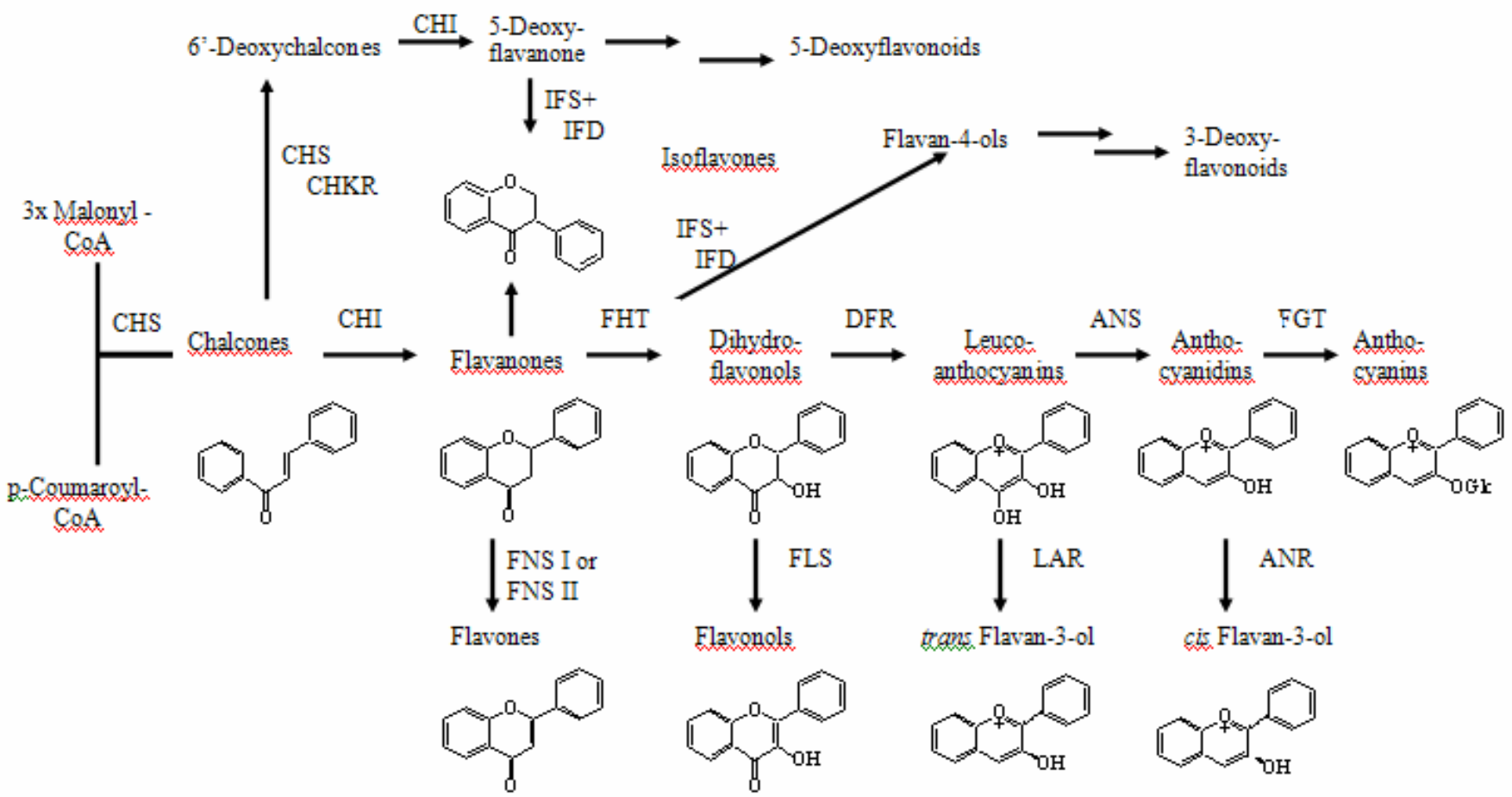


Figure 1.2. Scheme of general flavonoid pathway. Enzymes are abbreviated as follows:CHS, chalcone synthase; CHKR, chalcone polyketide reductase; CHI, chalcone isomerase; FHT, flavanone 3-β-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; FGT, flavonoid glycosyltransferase; FNS, flavone synthase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; IFS, isoflavone synthase; IFD, isoflavone dehydratase.

1.5 Research Objectives

Phytochemical investigations reported in the literature are mostly carried out on the *Tibouchina semidecandra* of Japan. A thorough literature search did not reveal any report on the chemical constituents of *T. semidecandra* found in Malaysia, except on the anthocyanin stability in the flower [22] and the chemical manipulation of growth and flowering of this plant [32]. Furthermore, there has been no report on bioactivity studies of this species. Therefore, this research will focus on the phytochemical and biological activity studies of *Tibouchina semidecandra* L.

The objectives of this research are to extract three parts of the plant (leaves, roots and stem barks) using organic solvents of different polarity either by soxhlet or cold extraction, followed by fractionation of the extracts using vacuum liquid chromatography technique. The next objectives are to isolate the phytochemical compounds using various chromatographic techniques either on silica gel or Sephadex LH-20 followed by structural identification using various spectroscopic methods including high field 1D NMR (^{1}H NMR, ^{13}C NMR, DEPT), 2D NMR (COSY, HMQC, HMBC), FTIR, UV spectroscopies and Mass spectrometry. The final objective is to evaluate the biological activities of the crude extracts and the pure compounds using several bioassays including antioxidant (free radical scavenging on 2,2-diphenyl-1-picrylhidrazyl (DPPH) method), antibacterial and antityrosinase assays.

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