

**PHYTOCHEMISTRY AND BIOACTIVITY OF
MAYTENUS ROYLEANUS AND *FICUS FAVEOLATA***



**BY
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**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
PESHAWAR, PAKISTAN
NOVEMBER, 2011**

**PHYTOCHEMISTRY AND BIOACTIVITY OF
MAYTENUS ROYLEANUS AND *FICUS FAVEOLATA***



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fulfillment of the degree of

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in

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by

Ala Ud Din

**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
PESHAWAR, PAKISTAN
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"With Him are the keys of the unseen, the treasures that none knoweth but He. He knoweth whatever there is on the earth and in the sea. Not a leaf doth fall but with His knowledge. There is not a grain in the darkness (or depths) of the earth, nor anything fresh or dry (green or withered) but is (inscribed) in a record clear (to those who can read)."
(V^oJ. 59)

CERTIFICATE

This is to certify that this dissertation entitled “**PHYTOCHEMISTRY AND BIOACTIVITY OF *MAYTENUS ROYLEANUS* AND *FICUS FAVEOLATA***” submitted by Mr. **Ala Ud Din** is hereby accepted in the present form by the Institute of Chemical Sciences, University of Peshawar as a partial fulfillment for the degree of **Doctor of Philosophy in Chemistry**.

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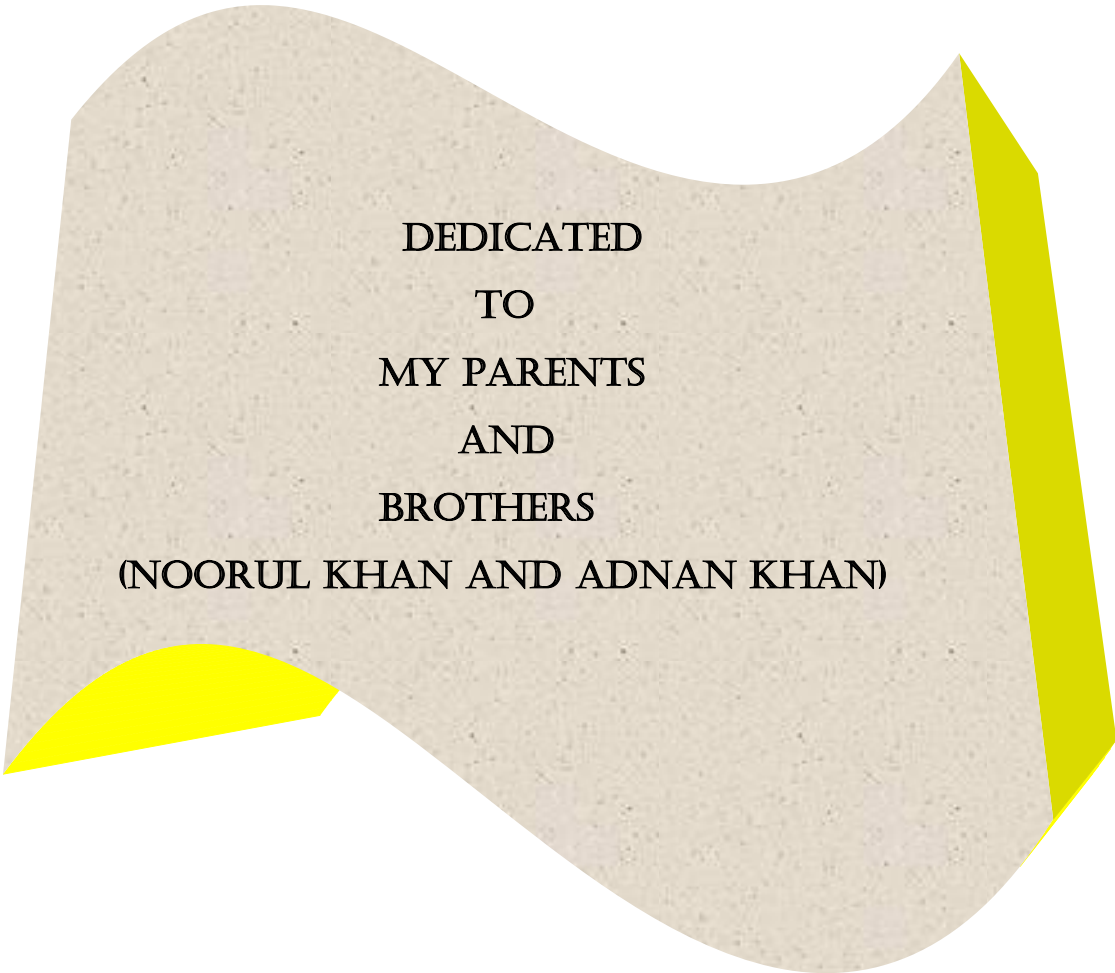
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DEDICATED
TO
MY PARENTS
AND
BROTHERS
(NOORUL KHAN AND ADNAN KHAN)

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– Sir Isaac Newton, circa 1676

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ABSTRACT

This dissertation deals with phytochemical and bioactivities studies on indigenous medicinal plants; *Maytenus royleanus* (Celastraceae) (Part **A**), *Ficus faveolata* (Moraceae) (Part **B**) and additional miscellaneous part **C** (Cyclotetrapeptides from *Aspergillus ochraceopetaliformis*).

Part-A

Part **A** deals with phytochemical and bioactivities studies on the chemical constituents of the roots of *Maytenus royleanus*. The systematic phytochemical studies have led to the isolation and structure elucidation of three new compounds (**1-3**). In addition seven known compounds (**4-10**) have also been isolated for the first time from this species. Structures have been elucidated by advanced spectroscopic techniques.

Royleanine A (**1**)

1 β ,2 β ,5 β ,7 β ,8 β ,11-Hexaacetoxy-4-hydroxy-3 α ,15-[2',3'-dimethyl-3'(5"-carboxy-2"-pyridyl)-propanoic acid] dicarbollactone-dihydro-agarofuran

Royleanine B (**2**)

1 β ,2 β ,5 β ,7 β ,8 β ,11-Hexaacetoxy-3 α ,15-[2'-methyl-4'(5"-carboxy-2"-pyridyl)-butanoic acid] dicarbollactone-dihydroagarofuran

Royleanine C (**3**)

1 β ,2 β ,5 β ,7 β ,8 β ,11-Hexaacetoxy-3 α ,15-[2'-methyl-2' β -benzoate-4'(4"-carboxy-2"-pyridyl)-butanoic acid] dicarbollactone-dihydroagarofuran

Salaspermic acid (4)

Orthosphenic acid (5)

3 α ,22 β -Dihydroxyolean-12-en-29-oic acid (6)

Oleuropein (7)

Ligutroside (8)

Quercetin (9)

β -Sitosterol-3-O- β -D-glycopyrannoside (10)

Part-B

Part **B** deals with isolation and characterization of chemical constituents from *Ficus faveolata*. Five compounds were isolated from the stem methanolic extract of *Ficus faveolata*; two of them are new (**11**, **12**) and three known compounds (**13-15**) hitherto unreported from this source. Their structures have been elucidated by spectroscopic techniques.

Ficusonic acid (**11**)

3 β ,21 β -Dihydroxy-olean-12-en-29-oic acid

Ficusonolide (**12**)

3 α -Hydroxyolean-12-en-29,19 α -olide

Gallocatechine (**13**)

β -Sitosterol-3-O- β -D-glycopyrannoside (**14**)

3 β ,22 β -Dihydroxyolean-12-en-29-oic acid(**15**)

Part-C

Part **C** is the miscellaneous section which describes the isolation and characterization of two new Cyclotetrapeptides; ochrine A (**16**) and ochrine B (**17**) from the fungal strain *Aspergillus ochraceopetaliformis* collected from Hawaii shallow water sediment. Their structures have been elucidated by modern spectroscopic techniques.

Chapter 1

General Introduction

In a very broad sense the term natural products research can be define as the search for and description of substances found in nature.¹ There are various types of motivations behind natural products research, such as drugs discovery for human ailments like antibiotics, antiseptic and anticancer, commercial purpose as a anti fouling paint for boat hulls, to understand the nature, arresting power as an efficient synthetic chemist and in order to understand the producer organism nature for example the microorganism produced iron sequestering siderophores.²

On the basis of their biosynthesis (**Fig. 1.1**), natural products can be classified into six different groups of compounds such as terpenes, steroids, peptides, aromatic compounds, polyketides and alkaloids.¹ These secondary metabolites are actually synthesized from their primary metabolites such as amino acids and sugars. An organism does not require these secondary metabolites for their immediate survival but rather these compounds play important role in the producer life as chemical defense, as a signal of maturity for reproduction and settlement.¹

From very early time man has been depended on nature in different ways such as shelter, food, clothing, transportation, fertilizers etc. With the passage of time they started using natural resources; plants, animals and minerals for curing of diseases and food as well. The modern medicine is based on gradual development of observation, scientific efforts and the history of medicine is as long as human civilization. However, the basis of these developments is traditional medicines and therapies.

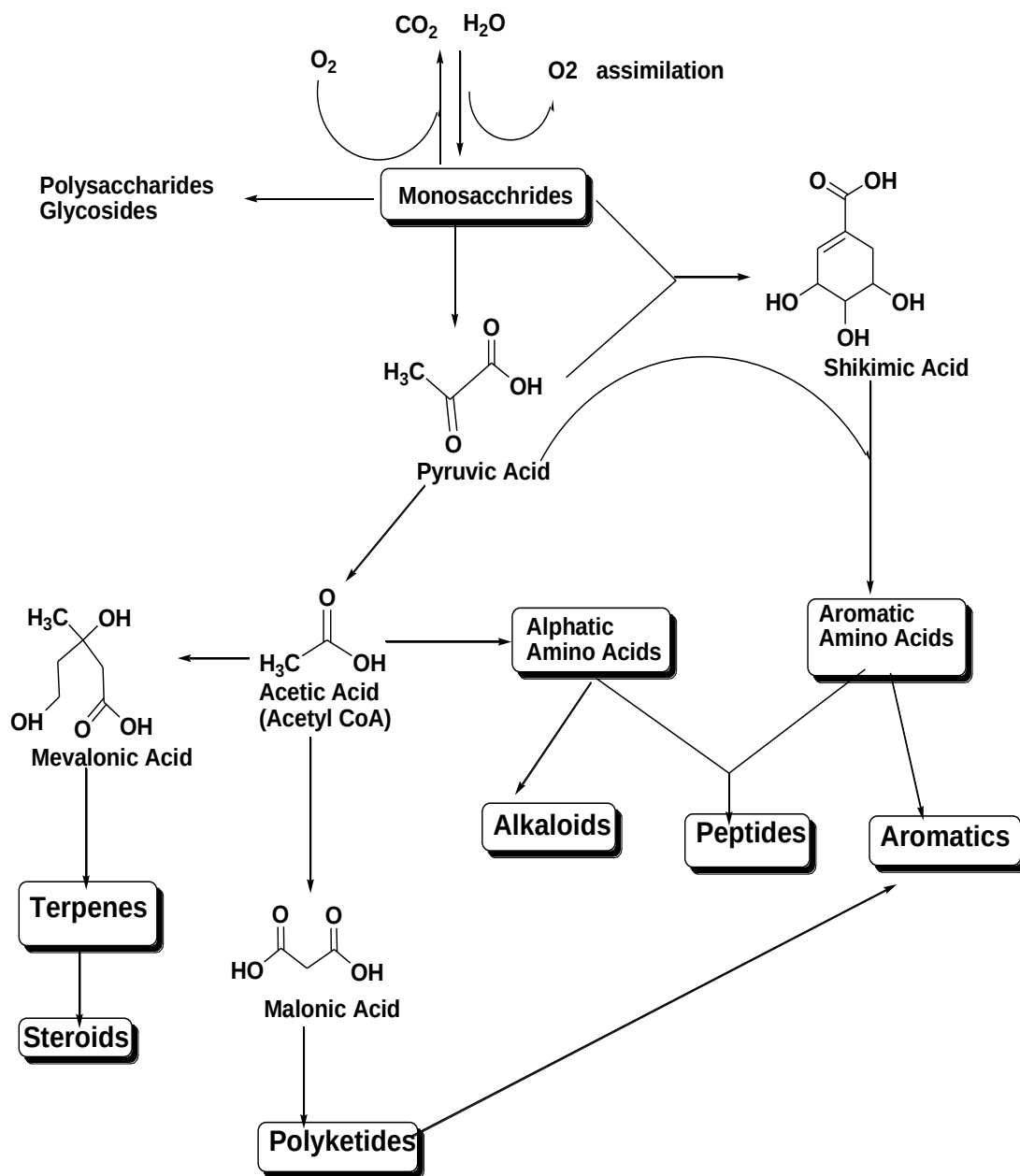


Fig.1.1. Biosynthetic pathways of natural products.

The traditional medicines based on plants which have been existed for thousands of years. Among 250,000-500,000 species of plants; 300 species are cultivated for food purposes, few of them are using as staple foods such as rice, wheat, maize, beans, potatoes etc., while small percentage of flowering plants 270,000 on the earth have been studied for their medicinal value and chemical composition.

Plants have been used as source of medicines in all cultures from ancient times. Egyptain pharmacopeia which is known as “Ebers paprus” described about 1000 medicines and their preparation formulae.

South Asia Ayurveda has strong scientific approaches to established traditional medicines for the treatment of ailments. The detail description has been given in the Atharveda, Charak Samhita and Sushita Samhita (1000- 500 BC).

The Chinese traditional medicines have also been used for centuries. The Materia Medica (1100 BC), Shenong Herbal (100BC-365 AD) and Tang Herbal (659-850 AD) have a detail record of medicines and prescriptions. Among Chinese system of medicine; Fullis (2950 BC) is the forerunner and was modified later on by the emperor Hong Ti and developed the treatment for complicated diseases such as blood pressure, asthma etc.

In the ancient Europe, the work of some Greeks and Roman scientists are worth mentioning and developed the rational basis for the development of herbal drugs. The famous philosopher and scientist, Heophrastus (300 BC) described medicinal qualities of herbs in his book “History of plants”. Another Greeks physician Dioscorridos (100 BC) recorded the use of medicinal herbs. Galen (130-200 BC) wrote more than 300 books on herbal medicine, in which he documented their formulae and prescriptions.

Arab muslims developed their system purely on Islamic basis. Ali Ibn Rabban al Tabri (782-855 AD) wrote a book *Firdous al Hikmat*,³ on herbal medicines in seven parts, while Abu Bakr Bin Zakarya (835-932 AD) has written more than 250 books most of them are purely on the herbal medicine. He used opium for the first time as an anesthetic. His famous book *Kitab Al Mansoor* has thoroughly recorded Unani (Greek) medicines and his another book *Kitab Al Hawi* considered as a good collection of Indian, Greek and Arabic plants. Abdullah Ibn Sina (980-1037) has extensively described more than 1000 drugs in his book “*Connon*” while his other important book “*Kitab Ash Shifa*” is supposed to be a scientific encyclopedia. AL-Idrisei (1099-1166 AD) has also written several books on medicinal plants. One of his famous book “*Kitab al Jamili Ashiat al Nabatat*” in which he demonstrated plants origin drugs and their uses.

To mankind herbal medicine is the most ancient form of health care to man. The rapid advancement in the field of combinatorial chemistry and synthetic medicine which replaced by the plants derived medicine. Current data showed that about 25% of the drugs used in the world are derived from the plant extract or synthetic derivatives of plants derived medicines.

In many developing countries according to World Health Organization (WHO), more than 70% people depend upon traditional medicines taken from the plants. The rural people in Asia and Africa are still using the herbal medicines or plant-derived drugs for the treatment of different diseases. However, the role of traditional medicines and their uses is a controversial subject, as one group supports the traditional system of medicine and consider as a remedy for all diseases while the other group supports the replacement of traditional medicines with modern medicine or synthetic. However, the truth lies in

between the two systems and there is a great need to learn from each other experiences. So the need of the time is that the supporters of modern medicines must look into the effectiveness of herbal medicines in impartial way.

Indo-Pak sub-continent is an example for the use of Unani medicine system. Hakim Syed Mohammad Hussain described 1500 drugs in his book *Makhzaml Advia* which was based on plants origin and considered as the father of Unani medicines. Hakim Raza Ali khan has translated the Sanskrit name of herbal plants and medicines with modification and new entries in his book *Tadhkirat-ul-Hiud*. Hakim Ajmal Khan established Ayurvedic and Unani Tibbi College in 1920 at Delhi solely about herbal medicine and treatment, where the traditional medicines were studied in great detail with their physiological effect on human bodies.

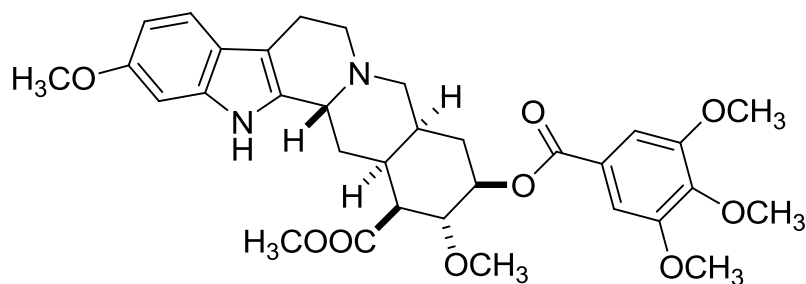
Morphine was the first compound isolated by Serturmer from *Papaver somniferum*, opened the vista of natural products chemistry. Later on many scientists isolated important compounds like quinine, cocaine and reserpine.

Before the discovery of antibiotics, infections were the major cause of high death rate in the past but in 1630 a revolutionary change was brought by natural products when infectious diseases were controlled by chemical constituents isolated from the *Cinchona* tree bark.⁴ Later on it was considered as a strong remedy specifically for malaria, a deadly disease in the past, and realized that quinine alkaloid was active compound which is one of the most important medicine among thousand extracted from plants.⁴

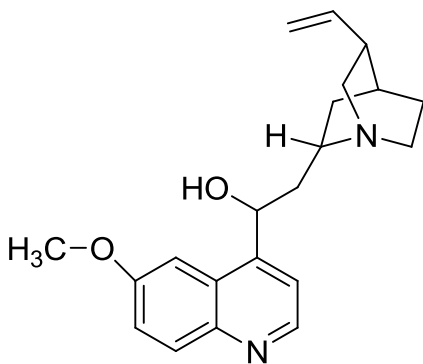
The discovery of aspirin is well documented, in 18th century Edward Stone used the bark of *Salix alba* for the treatment of fever, which have same bitter taste like

Cinchona plant. The work led to an important discovery as the active compound in the bark extract was salicin; a compound related to salicylic acid.⁵ More related compounds were discovered from other sources for the treatment of pain, fever, rheumatic fever and arthritis. On the basis of traditional uses of plants for the treatment of ailments led the Bayer in 1897 for the discovery of *o*-acetylsalicylic acid (aspirin).⁵

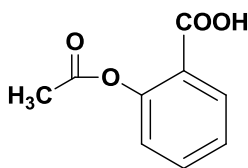
Reserpine is an alkaloid isolated from the roots of *Rauvolfia serpentine*, for the treatment of hypertension which is also used for the treatment of snake bite and mental disorder.⁶ An important compound prostratin was isolated from *Homalanthus nutans* for the treatment HIV.⁷



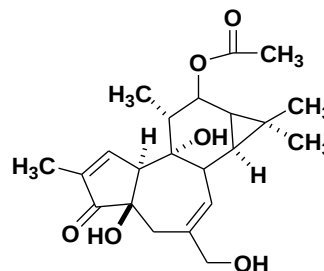
Reserpine



Quinine



Aspirin



Prostratin

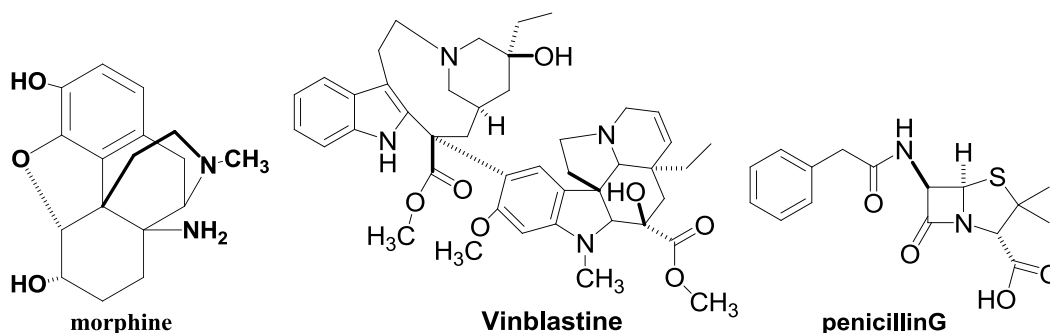
Flavonones used for the treatment of skin inflammation by Samoans, was isolated from the roots bark of *Erythrina variegata*.⁸ *C. comosa* roots used for relieving stomach pain and other gastrointestinal disorders.⁹ Active constituent of this plant was found significant anti-parasitic activity.⁹

In the last few decades, several bioactive compounds were discovered such as clavulanic acid, cyclosporine A, pilocarpine, ivermectin, vincristine and vinblastine.¹⁰ These discoveries stimulated pharmaceutical companies for the plant derived medicines. In 1996 Clark wrote a review on the natural products derived drugs¹¹ while Cragg. et al wrote a review on natural products drugs for the treatment of cancer and infection.¹² Many of the published manuscripts give an account of bioorganic chemistry;^{13,14} searching natural products derived drugs,^{15,16} new techniques and approaches in the drugs discovery from natural resources¹⁷ and the role of natural products for the improvement of life.¹⁸

Some of the natural products have high therapeutic indications which are the secondary metabolites of the plants and microorganisms produced for their own survival. Some of these have proved as life saving drugs in the history of drugs discovery. For example taxol and vinblastin (cancer),¹⁰ avermectine (parasitic-infection), cyclosporine (immunesuppression) and mevinoline (hyper-cholesterolaemia).¹⁹

Plants have resulted in the isolation of large number of compounds, like alkaloids, flavonoids, lignins, saponins, glycosides, terpenoids, essential oils, fatty acids etc. Researchers are still trying to isolate bioactive compounds from the natural sources of plants, fungi, animals and microorganisms.

Microorganisms were being recognized as a new and the inexhaustible source of new bioactive compounds,^{20,21} as later on compounds like penicillin a pay-dirt antibiotic²² and bleomycine an antitumor agent.²³



The bioassay guided isolation and structure elucidation of targeted compounds have greatly increased due to advancement in the instruments and bioassay guided technology which has made the natural products chemistry as a center of interest rather than synthetic chemistry. Synthetic chemistry in a single experiment can produce many compounds for bioassay screening but plants and microbes are producing compounds in unpredictable ways which involved different types of genes and enzymes.

Since 2005 more than thirteen natural products derived drugs are available in the market (**Table 1.0**) however the number can increase as 32 natural products derived compounds are in the 3rd clinical trial. The importance of natural products is still evident from 20 compounds (52%) evaluated for screening of oncology, 10-13 compounds (27%) for the treatment of bacterial and fungal infection, and remaining compounds are evaluated for different diseases. So the natural product chemistry is still a very prolific research in the production of medicine and without it there would be some therapeutic deficit.⁴¹

Table 1.0 Natural products derived-drugs launched since 2005

Year	Generic name (trade name)	Lead compound	Classification	Disease area
2005	Dronabinol / cannabidiol (Sativex [®]) ^{24,25}	Dronabinol / cannabidiol	NP	Pain
2005	Fumagilin (Flisint [®]) ^{26,27}	Fumagillin	NP	Antiparasitic
2005	Doripenem ²⁸ (Finibax [®] / Doribax [™])	Thienamycin	NP-derived	Antibacterial
2005	Tigecycline (Tygacil [®]) ²⁹	Tetracycline	Semi-synthetic NP	Antibacterial
2005	Ziconotide (Prialt [®]) ^{30,31}	Ziconotide	NP	Pain
2005	Zotarolimus- (Endeavor [™] stent) ³²	Sirolimus	Semi-synthetic NP	Cardiovascular surgery
2006	Anidulafungin (Eraxis [™] / Ecalta [™]) ^{33,34}	Echinocandin B	Semi-synthetic NP	Antifungal
2006	Exenatide (Byetta [™]) ³⁵	Exenatide-4	NP	Diabetes
2007	Lisdexamfetamine (Vyvanse [™]) ³⁶	Amphetamine	NP-derived	ADHD
2007	Retapamulin ³⁷ (Altabax [™] /Altargo [™])	Pleuromutilin	Semi-synthetic NP	Antibacterial
2007	Temsirolimus (Torisel [™]) ³⁸	Sirolimus	Semi-synthetic NP	Oncology
2007	Trabectedin (Yondelis [™]) ³⁹	Trabectedin	NP	Oncology
2007	Ixabepilone (Ixempra [™]) ⁴⁰	Epothilone B	Semi-synthetic NP	Oncology

NP = Natural products

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(Part A)

Chapter 2

Literature review on the genus *Maytenus*



Maytenus royleanus



Maytenus royleanus fruits and leaves

2.1 Introduction to family Celastraceae

2.1.1 Morphological characters of Celastraceae plants

The family Celastraceae (also called Chingithamnaceae, Canotinceae, Goupiaceae and Siphonodontaceae) belongs to the order Celastrales, including 90-100 genera and about 1300 species. Four genera and ten species are present in Pakistan. Most of the genera are tropical while some like *Maytenus*, *Celastrus* and *Euonymus* are found in temperate climate.¹

Plants of this family are trees (evergreen or deciduous, erect or scandent shrubs), and lianas, while stems sometimes thorny or rootlets,¹ and very rarely with buttressed trunk. Plants of Celastraceae have simple leaves which can be alternate or opposite, petiolate; venation pinnate, leaf blade laminar, with reticulate secondary veins, having entire margins, crenate, serrate, or dentate; with small stipules which may be caduous or estipulate.¹ Inflorescences terminal or axillary, few or many flowered, cymose, recemose, thyrsoid, fasciculate, or flowers solitary and rarely panicle. Flowers actinomorphic,¹ unisexual or bisexual, free sepals and petals. Flowers with intrastaminal, stamens may be either on disk or extrastaminal, annular, margins unturned, pulvinate, or copular, conspicuous, rarely obscure, lobed, entire, or angular. 3-5 stamens¹ which are alternate with petals, ovaries 3-5 superior to half inferior, often partially immersed in disk,¹ placentation axil, with erect oval, style terminal, simple, short or absent; stigma lobed or simple. Fruit is dehiscent capsule loculicidally,¹ berry, or samara, with a single surrounding wing,¹ with bonny pericarp, leathery fleshy, or chartaceous. Seeds 1-12 which are commonly smooth and rarely furrowed, basal, sometimes winged, exarillate or

aril basal to completely enveloping the seed, aril membranous, fleshy, foliaceous or thick, connate, germination epigeous.¹

2.1.2 Selected genera of Celastraceae

- *Maytenus* (*Gymnosporia*) Wall.
- *Cassine* Linn.
- *Celastrus* Linn.
- *Euonymus* Linn.
- *Mangifera* Linn.
- *Elaeodendron* Linn.
- *Catha* Linn.

2.2 The genus *Maytenus*

2.2.1 General morphological characters of *Maytenus* plants

The plants of genus *Maytenus* are represented by trees, dwarf trees and shrubs, having exstipulate leaves which are often alternate but rarely in cluster. Species of *Maytenus* have axillary inflorescence which may either dichotomous flowers or cymes and very rarely solitary. Unisexual flowers are common, however bisexual flowers are also present. Each flower has 4-5 sepals, 4-5 petals, 4-5 stamens, which are attached just below the disc, with slightly broad filament and anther is dithecous. Disc may be either sinuate or 5-10 lobed. Ovary consists of 2-3 cells which is embedded in the disc, each of which is 2-ovuled; with short style and 2-3 lobed stigma. Up to four seeds in its fruit which may be either partially or completely covered.² The genus *Maytenus* is consisting 260 species which are distributed throughout the world and can be found in tropical, subtropical and temperate region. Species of the genus *Maytenus* are found in the continent of America, South East Asia, Africa and Australia.³ Three of them are present in Pakistan;

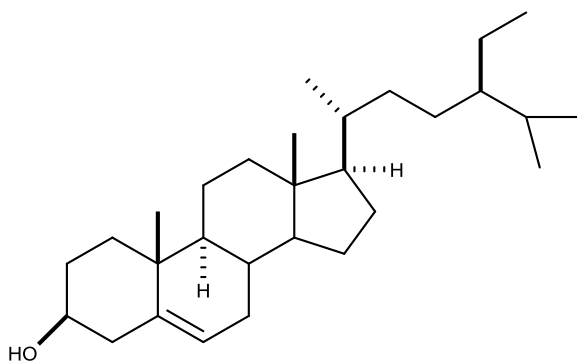
- *Maytenus royleanus* (Wall. ex Lawson) Cufodontis,
- *Maytenus senegalensis* (Lam.) Exell
- *Maytenus wallichiana* (Spreng.) Raju & Babu.

2.2.2 Phytochemical review on the genus *Maytenus*

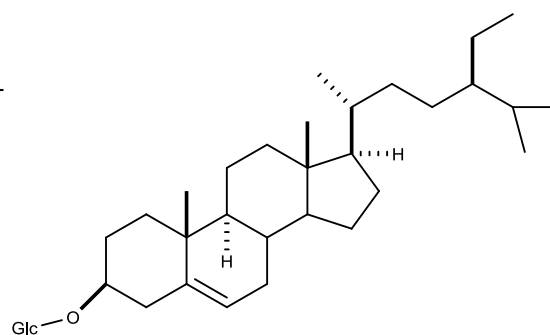
A variety of compounds have been isolated from the *Maytenus* species which have shown important biological activities described below:

2.2.2.1 Steroids

Many steroids have been isolated from different parts of the genus *Maytenus*; like β -sitosterol from the arial parts of *M. forsskaoliana*⁴ and *M. buchananii*,⁵ and stem bark of *M. laevis*⁶ while β -sitosterol-glucosoid has been isolated from the stem of *M. illicifolia*.⁷



β -Sitosterol

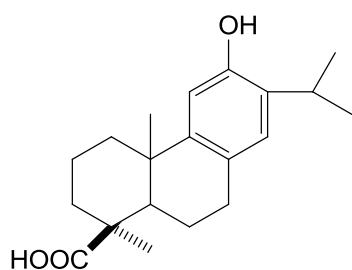


β -Sitosterol-glucosoid

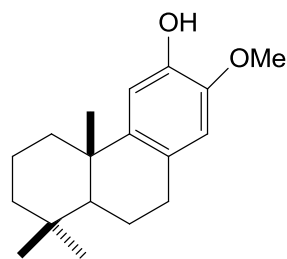
2.2.2.2 Diterpenes

The diterpenoids derived from geranylgeraniol pyrophosphate make a big group of C-20 substances. These diterpenoids are often of the plants or fungal origin which

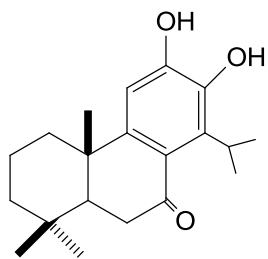
includes the plant growth stimulating hormones and resin acid. The diterpenes can be classified, on the basis of biogenetic principles.⁸ A variety of diterpenes have been isolated from the genus *Maytenus* so far, such as lambertic acid,⁶ dispermol,⁹ dispermone,¹⁰ and maytenoquinone.¹¹



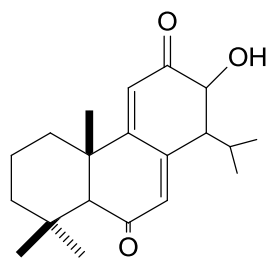
Lambertic acid



Dispermol



Dispermone



Maytenoquinone

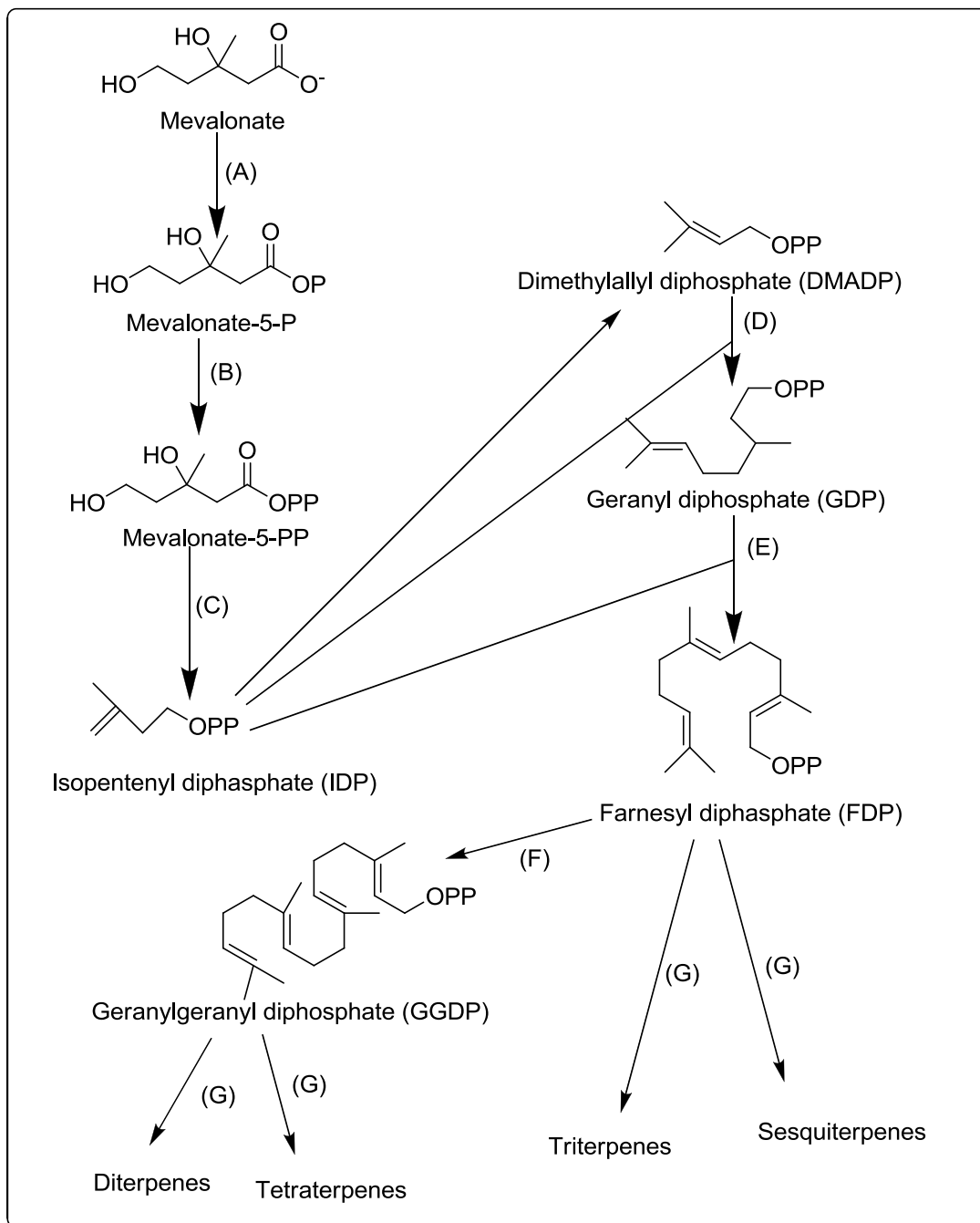


Fig.2.1. Biosynthesis of terpenes.

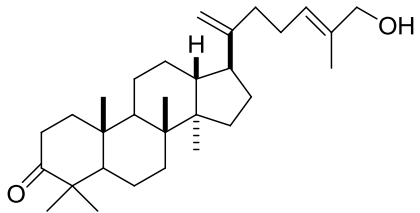
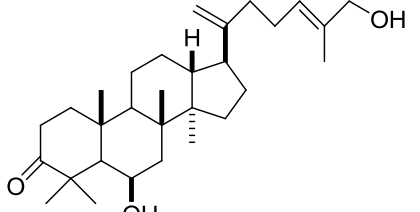
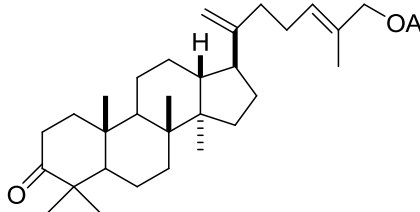
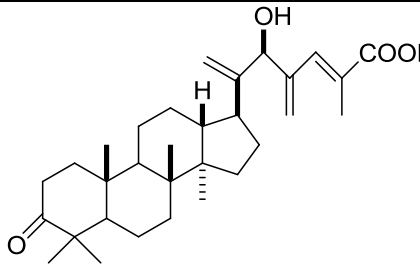
(A) mevalonate kinase (B) mevalonate-5-P kinase, (C) mevalonate-5-pyrophosphate decarboxylase, (D) isopentenyl pyrophosphate isomerase, (E) farnesyl pyrophosphate synthetase, (F) geranylgeranyl pyrophosphate synthetase (G) terpenes synthetase.

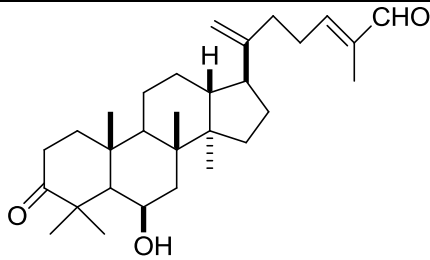
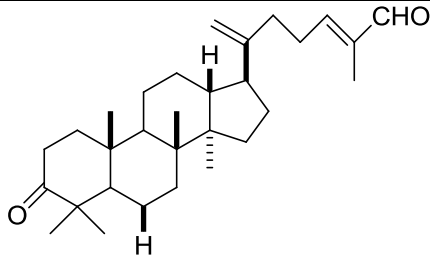
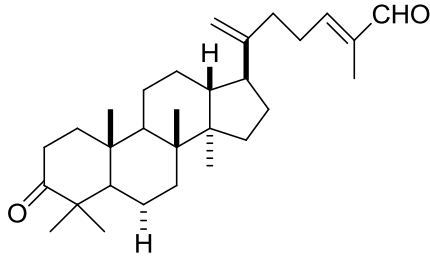
2.2.2.3 Triterpenes

A variety of triterpenes have been isolated from different plant members of the genus *Maytenus*, which are summarized in the following tables.

2.2.2.3.1 Dammarane

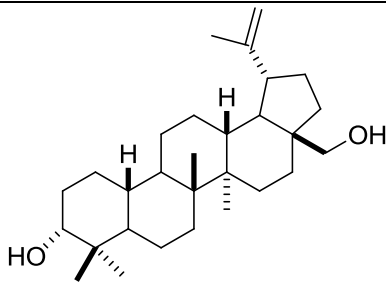
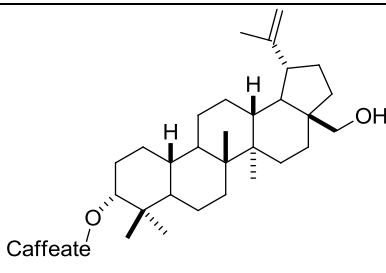
Table 2.1 Dammarane triterpenes isolated from the genus *Maytenus*

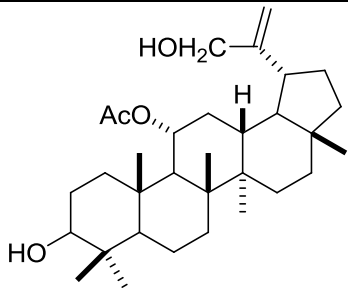
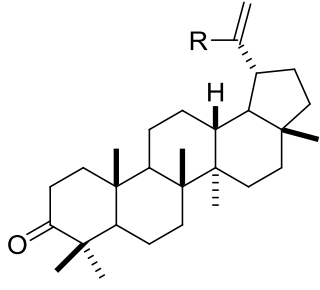
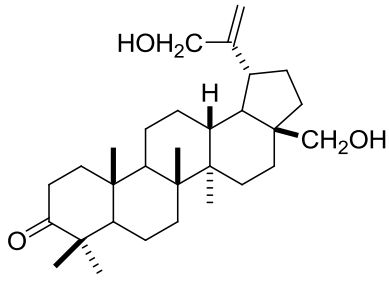
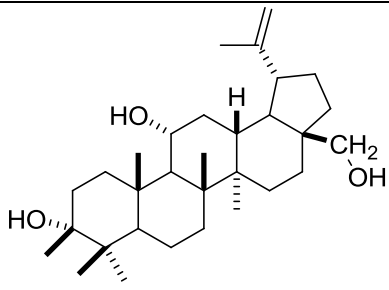
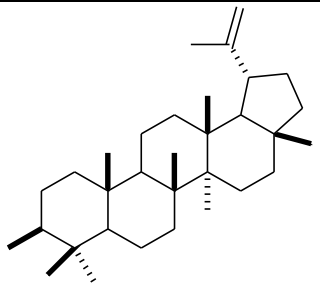
No.	Compounds	Structures	Source and references
1	26-Hydroxy-20,24-dammaradien-3-one.		Stem bark of <i>M. macrocarpa</i> ¹²
2	6,26-Dihydroxy-20,24-dammaradien-3-one.		Stem bark of <i>M. macrocarpa</i> ¹²
3	26-Acetoxy-20,24-dammaradien-3-one.		Stem bark of <i>M. macrocarpa</i> ¹²
4	22-Hydroxy-23-methyl-ene-3-oxodammara-20,24-dien-26-oic acid.		Stem bark of <i>M. macrocarpa</i> ¹²

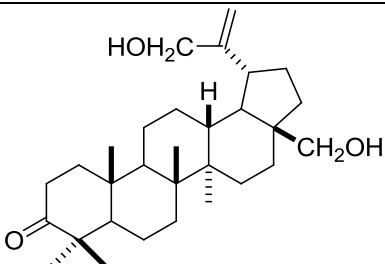
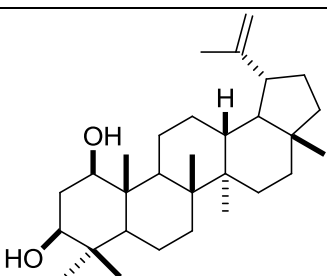
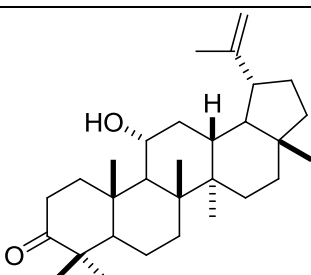
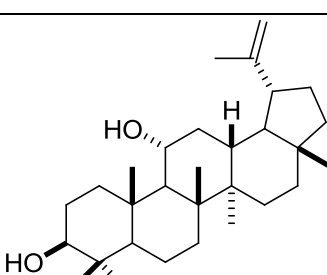
5	6-Hydroxy-3-oxo-20,24-dammaradien-26-al.		Stem bark of <i>M. macrocarpa</i> ¹²
6	24 (Z)-3-oxo-20,24-dammaradien-26-al.		Stem bark of <i>M. macrocarpa</i> ¹²
7	24 (E)-3-oxo-20,24-dammaradien-26-al.		Stem bark of <i>M. macrocarpa</i> ¹²

2.2.2.3.2 Lupane

Table 2.2. Lupane triterpenes isolated from the genus *Maytenus*

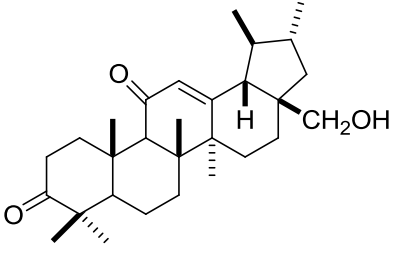
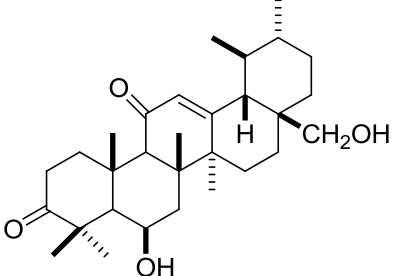
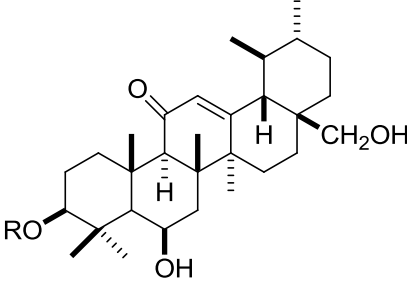
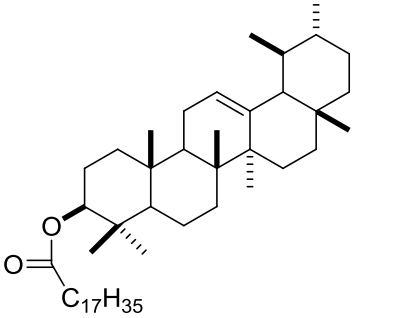
No.	Compounds	Structures	Source and references
8	Betulin.		Roots of <i>M. cuzcoina</i> ^{14,15} Leaves of <i>M. chiapensis</i> ^{14,15} Stem of <i>M. forsskaoliana</i> ¹⁶
9	Betulin-3 β -caffeate.		Roots of <i>M. cuzcoina</i> ^{14,15} Leaves of <i>M. chiapensis</i> ^{14,15} Stem of <i>M. forsskaoliana</i> ¹⁶

10	Lup-20(29)-ene-3 β ,30-diol.		Stem heartwood of <i>M. nemerosa</i> ¹⁷
11	Lup-20(29)-ene-11 α -hydroxy-30-(4'-hydroxybenzoyloxy)-3-one.	 R=4'-hydroxybenzoyloxy	Twigs of <i>M. putterlickoides</i> ¹⁸
12	Lup-20(29)-ene-28,29-dihydroxy-3-one.		Aerial stem of <i>M. canariensis</i> ¹⁹
13	Lup-20(29)-ene-3 α ,11 α ,28 β -triol.		Roots of <i>M. cuzcoina</i> ¹⁴ Leaves of <i>M. chiapensis</i> ¹⁴
14	Lupeol.		Roots of <i>M. cuzcoina</i> ¹⁴ and <i>M. horrid</i> ²⁰ Leaves of <i>M. chiapensis</i> , ¹⁴ <i>M. macrocarpa</i> ²¹ and <i>M. salicifolia</i> ²²

15	Lupan-20(29)-ene-3-oxo28,30-diol.		Roots of <i>M. cuzcoina</i> ¹⁴ Leaves of <i>M. chiapensis</i> ¹⁴
16	3- <i>epi</i> - Glochidiol.		Roots of <i>M. cuzcoina</i> ^{14,15} Leaves of <i>M. chiapensis</i> ^{14,15}
17	Regidinol.		Roots of <i>M. cuzcoina</i> ^{14,15} Leaves of <i>M. chiapensis</i> ^{14,15} and <i>M. rigida</i> ²³
18	Nepeticin.		Roots of <i>M. cuzcoina</i> ¹⁴ Leaves of <i>M. chiapensis</i> ¹⁴

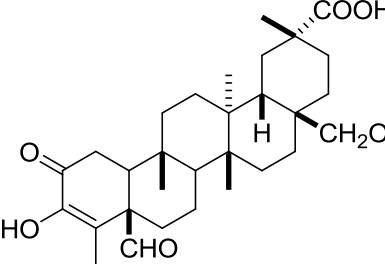
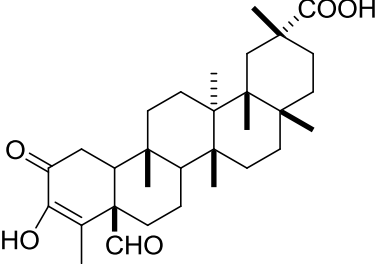
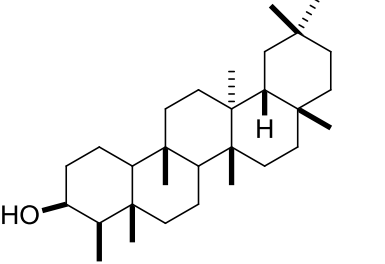
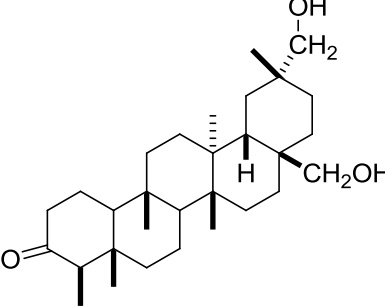
2.2.2.3.3 Ursane

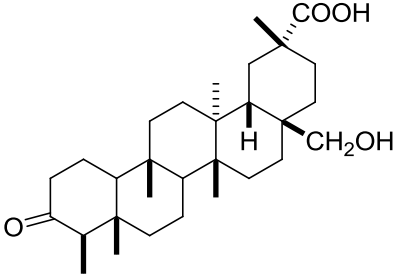
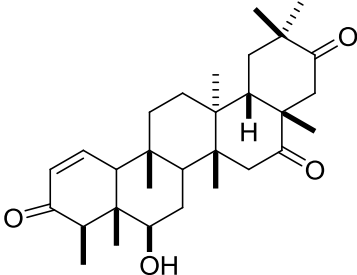
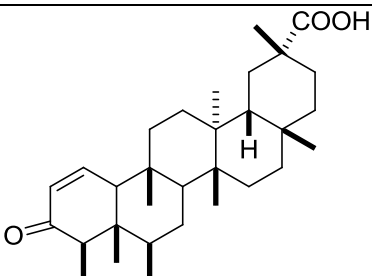
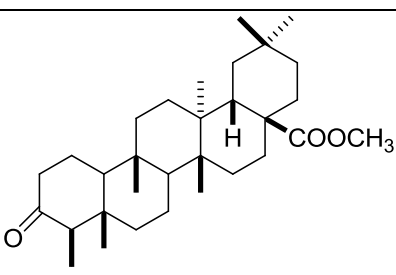
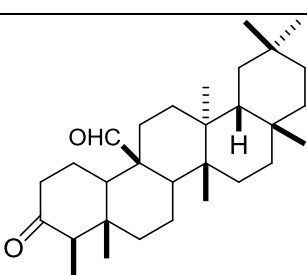
Table 2.3 Ursane triterpenes isolated from the genus *Maytenus*

No.	Compounds	Structures	Source and references
19	Krukovine B.		Stem bark of <i>M. krukovii</i> ²⁴
20	Krukovine D.		Stem bark of <i>M. krukovii</i> ²⁴
21	Uvaol-3-caffeae.	 R=Caffeate	Leaves of <i>M. ilicifolia</i> ¹³ Stem bark of <i>M. laevis</i> ²⁵
22	3 β -Stearyloxy-urs-12-ene.		Leaves of <i>M. salicifolia</i> ²²

2.2.2.3.4 Friedelin

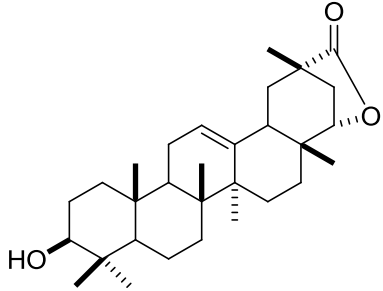
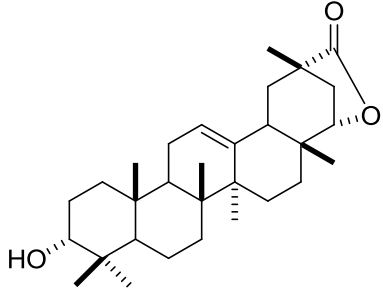
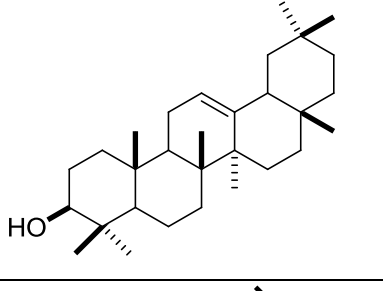
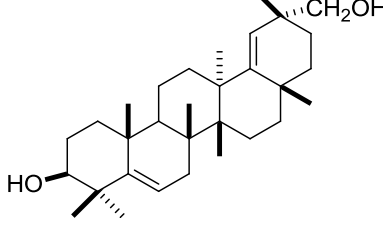
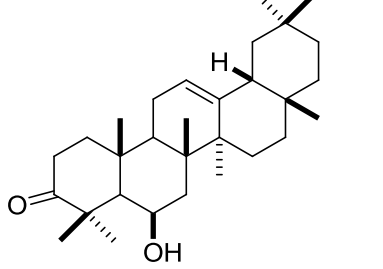
Table 2.4 Friedelin triterpenes isolated from the genus *Maytenus*

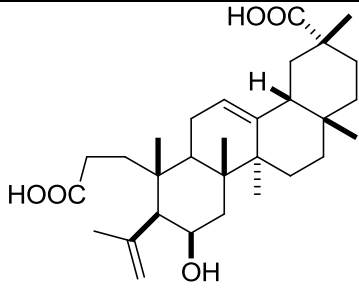
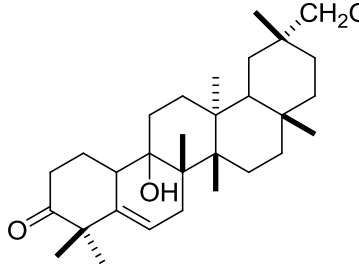
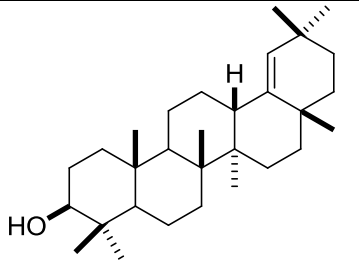
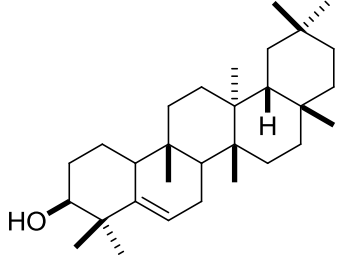
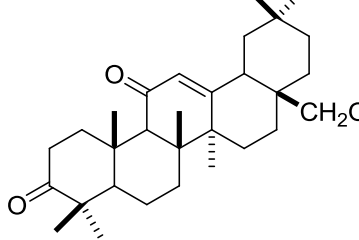
No.	Compounds	Structures	Source and references
23	Canophyllol.		Stem of <i>M. diversifolia</i> ²⁶ and <i>M. macrocarpa</i> ²⁷ Leaves of <i>M. laevis</i> . ²⁵
24	Cangoronine.		Roots of <i>M. ilicifolia</i> ²⁸
25	3 β -Friedelinol.		Leaves of <i>M. aquifolium</i> ²⁹ and <i>M. salicifolia</i> ²²
26	Maytenfoliol.		Stem of <i>M. diversifolia</i> ³⁰

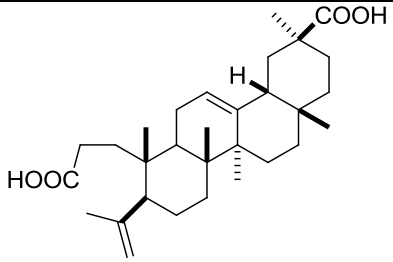
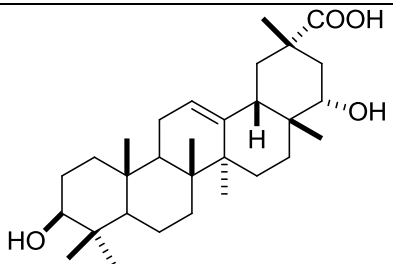
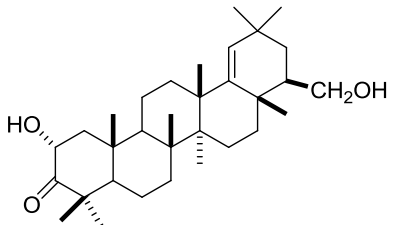
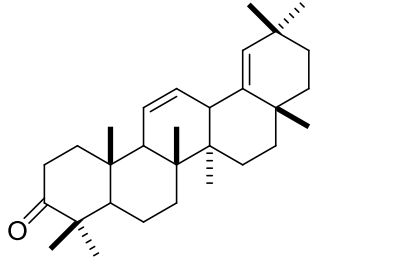
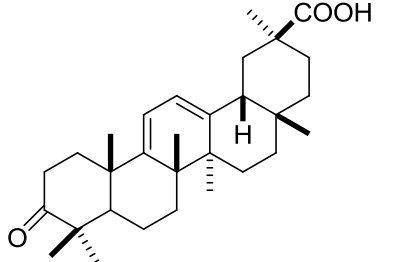
27	Maytenfolone.		Stem <i>M. diversifolia</i> ³¹
28	Maytensifolin C.		Stem of <i>M. diversifolia</i> ³²
29	Polpunic acid.		Leaves and stem of <i>M. buchanai</i> ³³ Roots of <i>M. ilicifolia</i> ²⁸ and <i>M. senegalensis</i> ^{28,34}
30	3-Oxo-28-friedelanoic methyl ester.		Stem of <i>M. octogona</i> ³⁵
31	3-Oxofriedelan-25-al.		Stem bark of <i>M. macrocarpa</i> ²⁷

2.2.2.3.5 Oleanane and glutin

Table 2.5 Oleanane and glutin class of triterpenes isolated from the genus *Maytenus*

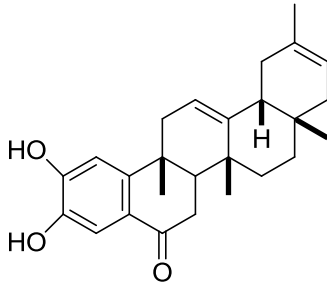
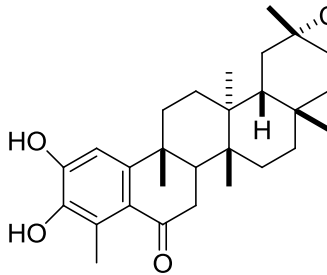
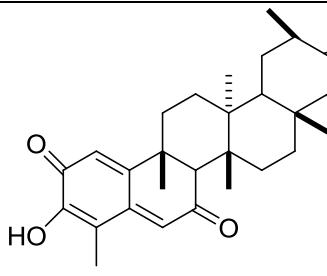
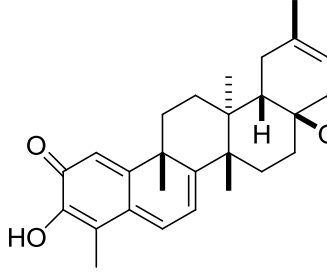
No.	Compounds	Structures	Source and references
32	Abruslactone A.		Stem bark of <i>M. leavis</i> ²⁵
33	3-Epi-abruslactone A.		Stem bark of <i>M. leavis</i> ²⁵
34	β -Amyrin.		Stem of <i>M. forsskaoliana</i> , ³⁶ <i>M. numerosa</i> , ¹⁷ <i>M. undata</i> ³⁷ and <i>M. diversifolia</i> ²⁶ Leaves of <i>M. salicifolia</i> ²² and <i>M. arbutifolia</i> ³⁶
35	3 β ,29-Dihydroxy-glutin-5-ene.		Stem bark of <i>M. macrocarpa</i> ³⁹ Roots of <i>M. ilicifolia</i> ³⁸ and <i>M. horrida</i> ²⁰
36	Daturaolone.		Stem bark of <i>M. macrocarpa</i> ³⁹

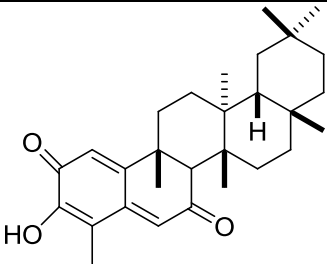
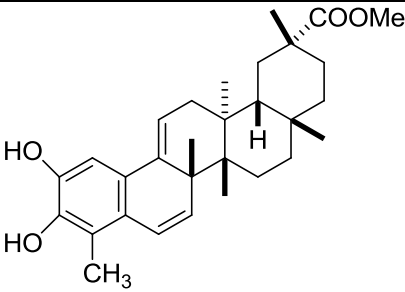
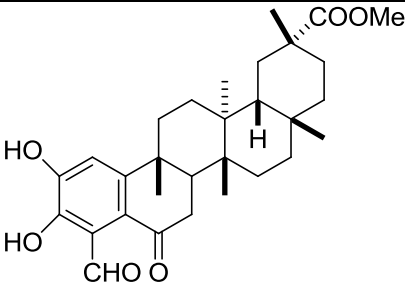
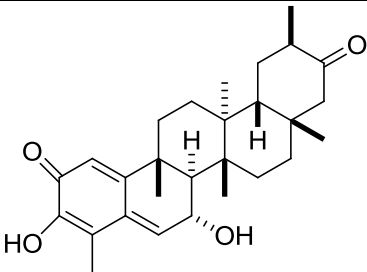
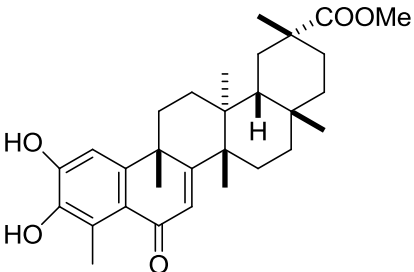
37	<i>Epi</i> -koatjapic acid.		Stem of <i>M. undata</i> ³⁷
38	Friedoolean-9-ol-3-one.		Roots of <i>M. horrida</i> ²⁸
39	Germanicol.		Roots of <i>M. horrida</i> ²⁰
40	3 β ,11 α -Hydroxy-glutin-5-ene.		Roots of <i>M. horrida</i> ²⁰
41	Krukovine A.		Stem bark of <i>M. krukuvii</i> ²⁴

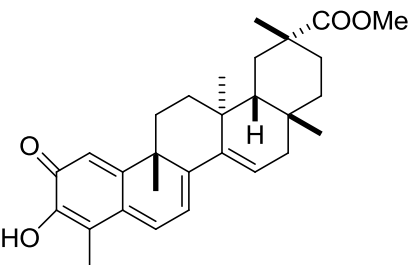
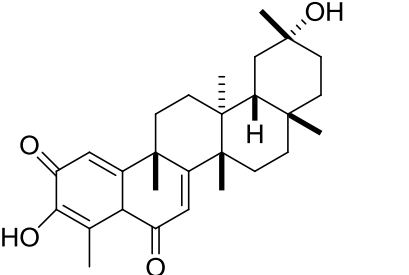
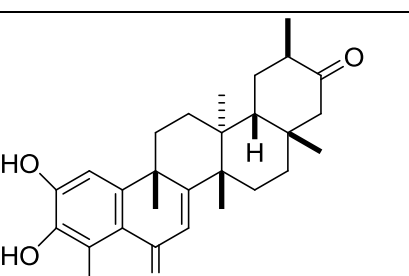
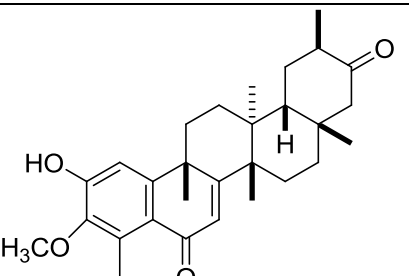
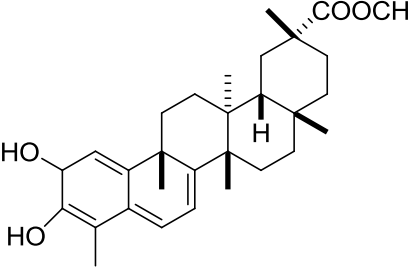
42	Koetjapic acid.		Stem of <i>M. undata</i> ³⁷
43	Maytenofolic acid.		Leaves of <i>M. heterophylla</i> ³⁶ Stem bark of <i>M. leavis</i> ²⁵ Stem of <i>M. diversifolia</i> ³⁰
44	Maytefolin B.		Leaves of <i>M. ilicifolia</i> ^{28,40}
45	11, 13(18)-Oleanadien-3-one.		Stem of <i>M. dispersus</i> ⁴¹
46	3-Oxo-olean-9(11), 12-diene-30-oic acid.		Aerial parts of <i>M. undata</i> ^{37,24}

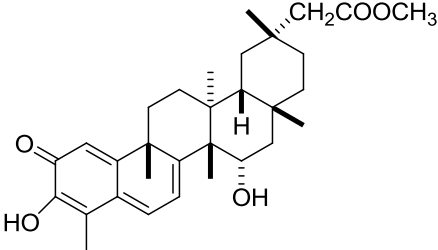
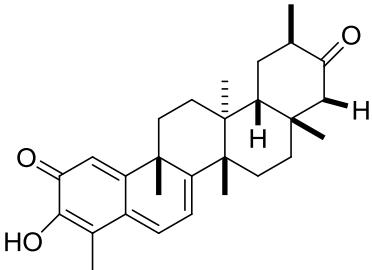
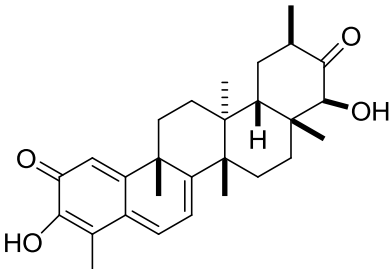
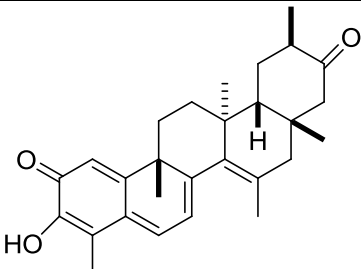
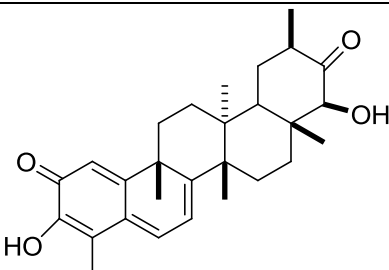
2.2.2.3.6 Quinine methide

Table 2.6 Quinine methide class of triterpenes isolated from the genus *Maytenus*

No.	Compounds	Structures	Source and references
47	Canarol.		Roots of <i>M. canariesis</i> ⁴²
48	Blepharodol.		Roots of <i>M. blepharode</i> ⁴³
49	3,22-Dihydroxy-24,29-dinor-1(10),3,5-friedelatriene-2,7,21-trione.		Roots of <i>M. amazonica</i> ⁴⁴
50	16-Hydroxyigues-terin.		Roots bark of <i>M. canariensis</i> ⁴⁵

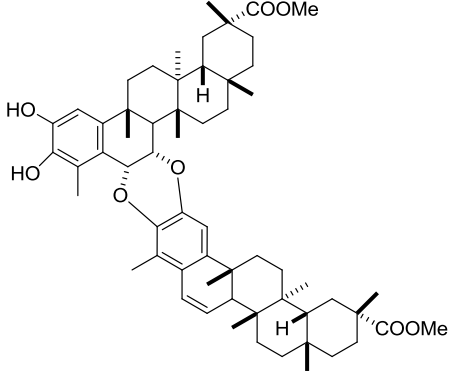
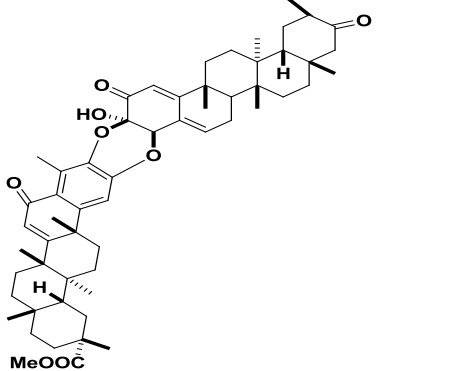
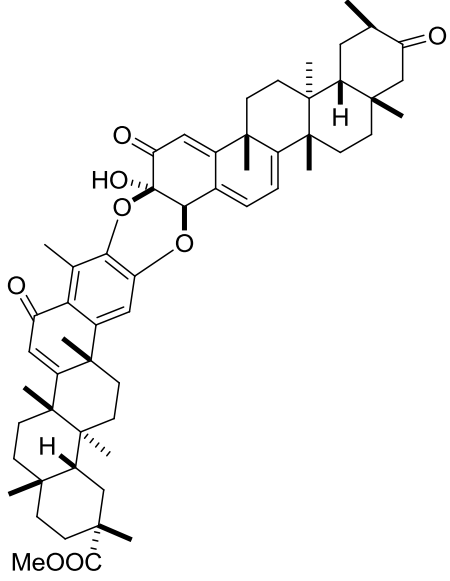
51	Dispermoquinone.		Roots bark of <i>M. dispermus</i> ⁴⁶
52	Isopristimerin III.		Roots of <i>M. ilicifolia</i> ⁴⁷
53	Macrocarpin A.		Roots of <i>M. macrocarpa</i> ⁴⁸
54	7 α -Hydroxy-amazoquinone.		Roots of <i>M. amazonica</i> ⁴⁹
55	6-Oxopristimerol.		Stem bark of <i>M. chuchuhuasca</i> ⁵⁰

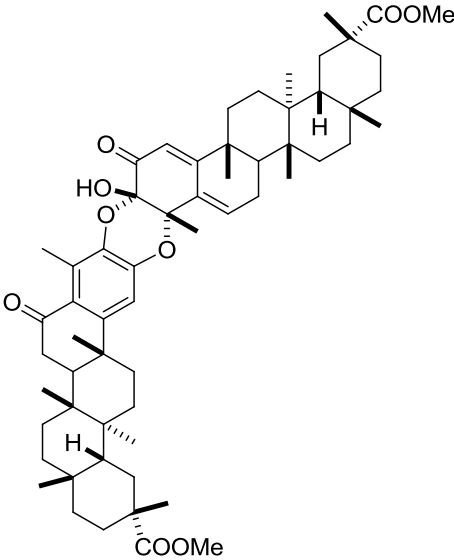
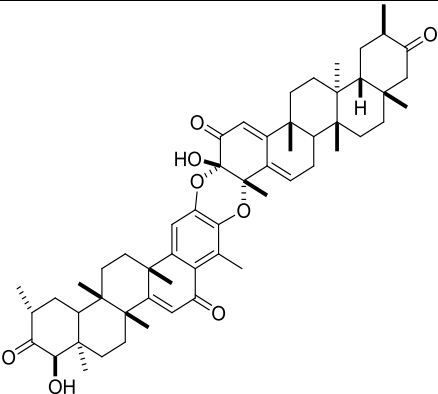
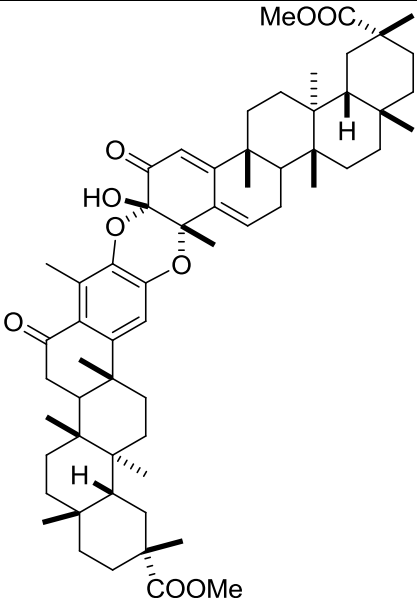
56	Netzahualcoyene. Pristimerinene.		Stem of <i>M. horrid</i> , ⁵¹ <i>M. macrcarpa</i> ³⁹ and <i>M. scutioides</i> ⁴²
57	20 α -Hydroxy-6-oxoiguesterol.		Roots of <i>M. ilicifolia</i> ³⁴
58	6-Oxotingenol.		Roots of <i>M. canariensis</i> ⁵² and <i>M. ilicifolia</i> ³⁴
59	3-O-Methyl-6-Oxotingenol.		Roots of <i>M. canariensis</i> ⁵²
60	Pristimerin.		Leaves of <i>M. aquifolium</i> ²⁹ Roots bark of <i>M. catingaram</i> , ⁵³ <i>M. chuchuhuasca</i> ⁵⁰ and <i>M. ilicifolia</i>

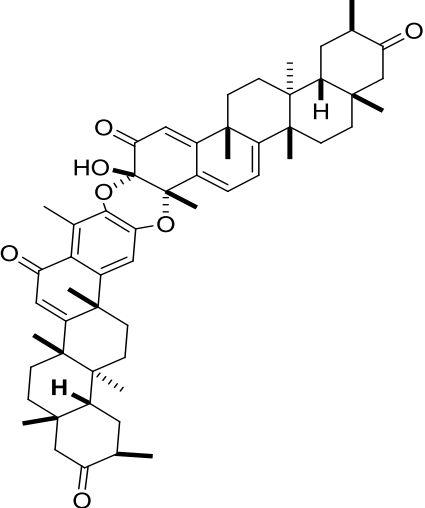
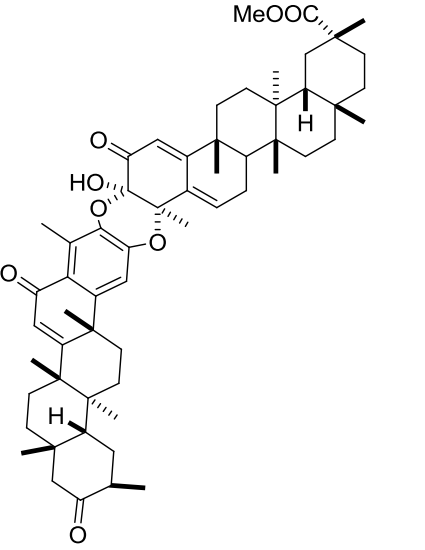
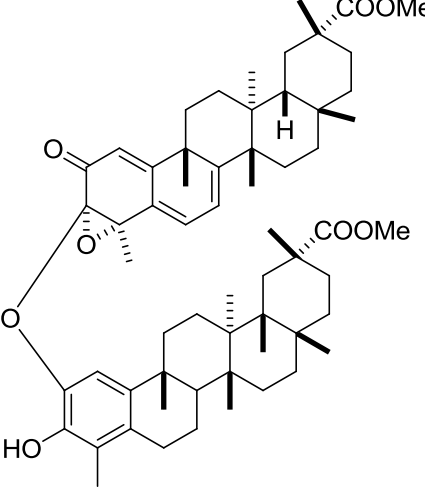
61	15-Hydroxypristimerine.		Roots of <i>M. scutioides</i> ⁵⁴
62	Tingenin A.		Stem of <i>M. macrocarpa</i> ⁵⁵
63	Tingenin B.		Stem of <i>M. scutioides</i> ⁵⁶
64	Scutione.		Stem of <i>M. macrocarpa</i> ³⁹ Roots of <i>M. scutioides</i> ⁵⁷
65	20 β -Hydroxytingenone.		Stem of <i>M. nemerosa</i> ¹⁷ and <i>M. buchananii</i> ³³ Roots bark of <i>M. chuchhuasca</i> ⁵⁰

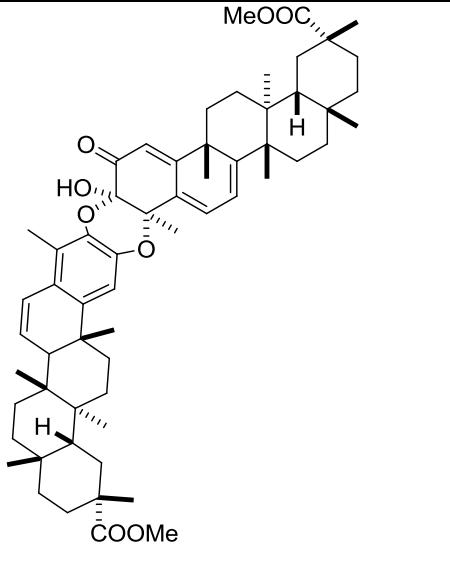
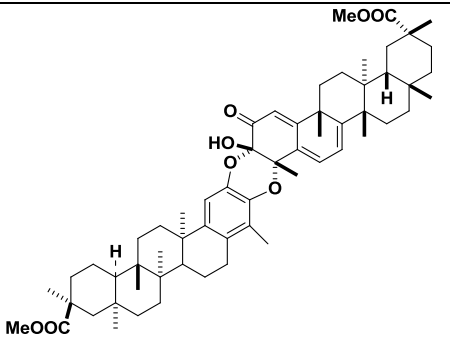
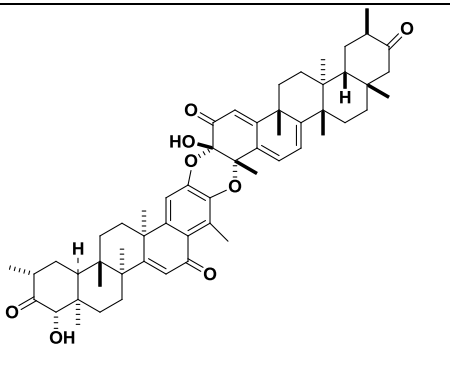
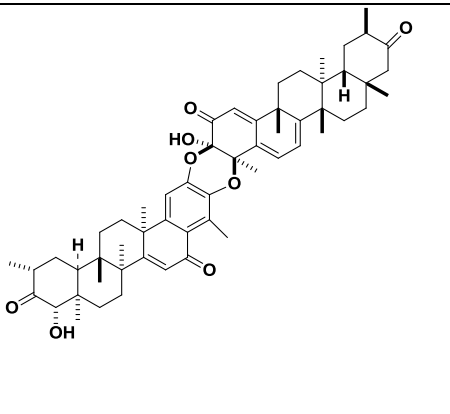
2.2.2.3.7 Triterpene dimers

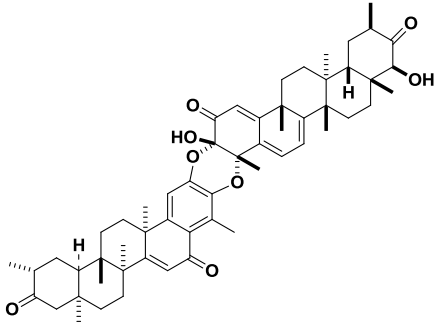
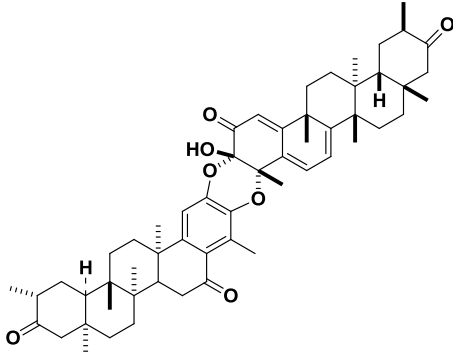
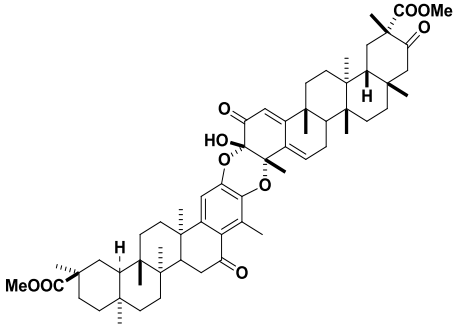
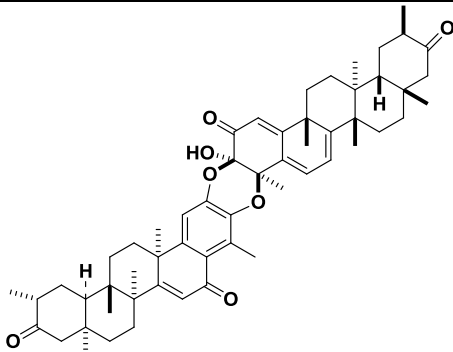
Table 2.7 Dimeric triterpenes isolated from the genus *Maytenus*

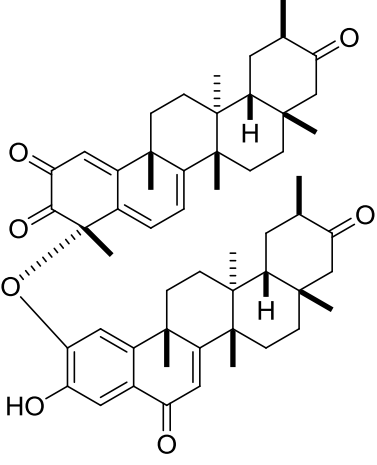
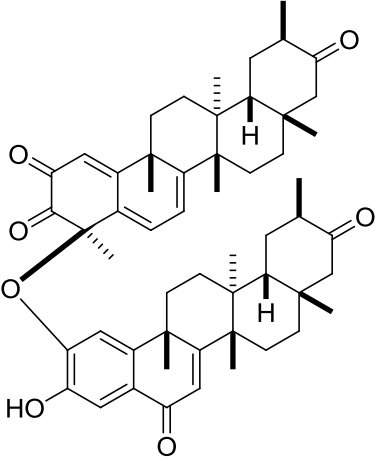
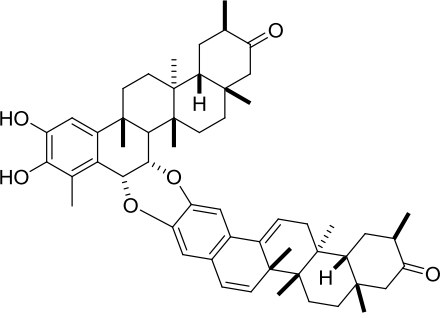
No.	Compounds	Structures	Source and references
66	Cangorosin A.		Roots of <i>M. blepharodes</i> ⁴³ and <i>M. ilicifolia</i> ⁵⁸
67	7,8-Dihydro-cangorosin B.		Stem of <i>M. chuchuh-uasca</i> ⁵⁹
68	Cangorosin B (Isoxuxuarine Fa).		Stem of <i>M. chuchuh-uasca</i> ⁵⁹

69	7,8-Dihydroxyxuxarine E α .		Stem of <i>M. chuchhuasca</i> ⁶⁰
70	7,8-Dihydroxyxuxarine D α .		Stem of <i>M. chuchhuasca</i> ⁵⁹
71	7 α -Hydroxyxuxarine E α .		Stem of <i>M. chuchhuasca</i> ⁵⁹

72	Isoxuxuarine A α .		Stem bark of <i>M. chuchhuasca</i> ⁶⁰
73	7,8-Dihydro-Isoxuxuarine G β .		Roots of <i>M. chuchhuasca</i> ⁵⁹
74	Magellanin.		Stem <i>M. magellanica</i> ⁶¹

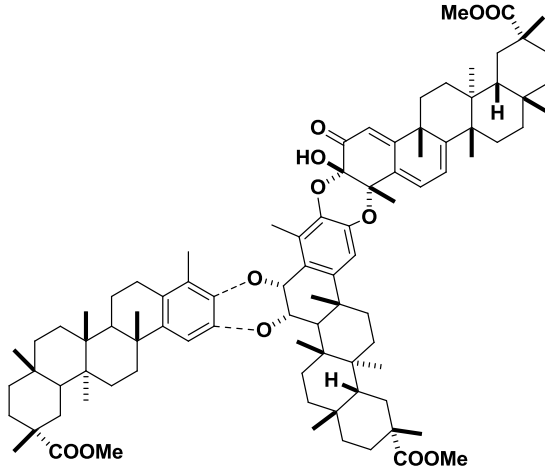
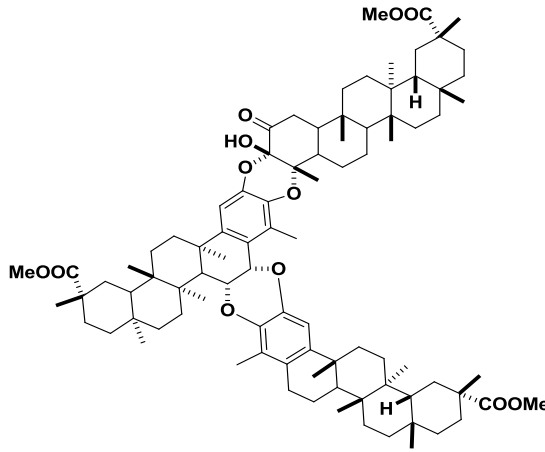
75	Scutionin α A.		Stem of <i>M. scutioides</i> ⁴²
76	7',8'-Dihydro-scutionin α B.		Roots bark of <i>M. blepharodes</i> and <i>M. magellanica</i> ⁴³
77	Xuxuarine A α .		Stem of <i>M. chuchhuasca</i> ⁶²
78	Xuxuarine C β .		Stem of <i>M. chuchhuasca</i> ⁶²

79	Xuxuarine Ca.		Stem of <i>M. chuchhuasca</i> ⁶²
80	7,8-Dihydro Xuxuarine Aα.		Stem bark of <i>M. chuchhuasca</i> ⁶⁰
81	Xuxuarine Eα.		Roots bark of <i>M. blepharodes</i> ⁴³
82	Xuxuarine Aβ.		Stem of <i>M. chuchhuasca</i> ⁶⁰

83	Umbellatin β .		Roots of <i>M.umbellate</i> ⁶³
84	Umbellatin α .		Roots of <i>M. umbellate</i> ⁶³
85	Xuxuasin A.		Roots bark of <i>M. chuchuhuasca</i> ⁶⁴

2.2.2.3.8 Triterpene trimmers

Table 2.8 Trimmeric triterpenes isolated from the genus *Maytenus*

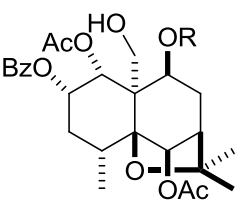
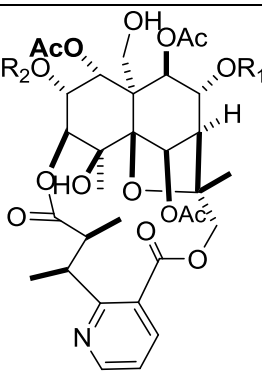
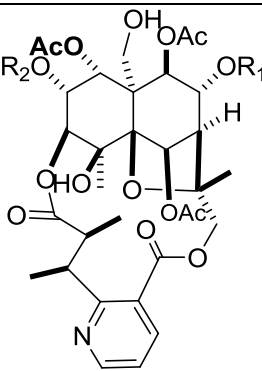
No.	Compounds	Structures	Source and references
86	Triscutin A		Roots of <i>M. scutioides</i> ⁶⁵
87	Triscutin B		Roots of <i>M. scutioides</i> ⁶⁵

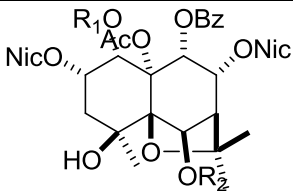
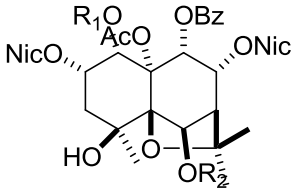
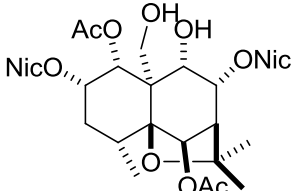
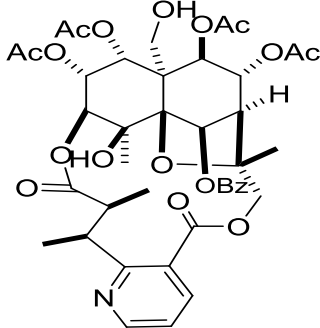
2.2.2.4 Sesquiterpenes

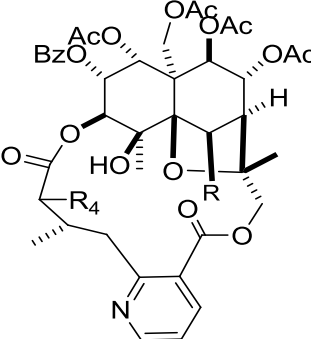
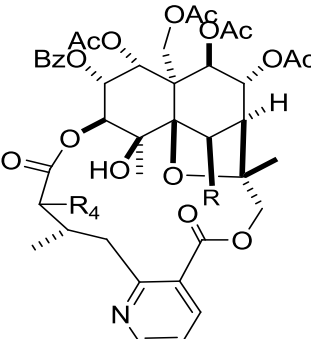
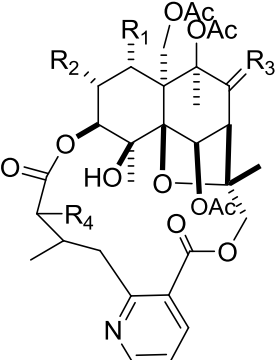
The family *Celastraceae* is a good source of sesquiterpene having dihydro- β -agarofuran [5,11-epoxy-5 β ,10 α -eudesman-4(14)-ene] skeleton.

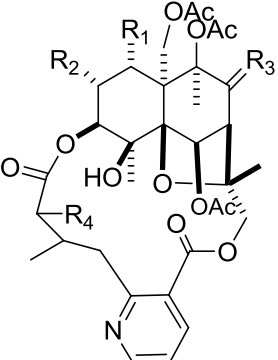
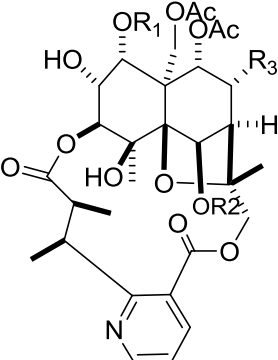
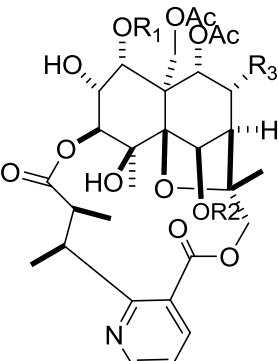
2.2.2.4.1 Dihydro- β -agarofurane sesquiterpene alkaloids

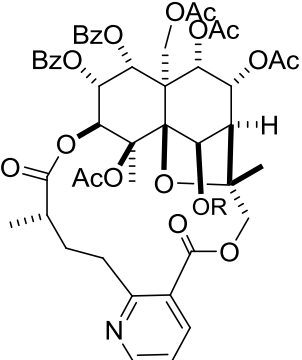
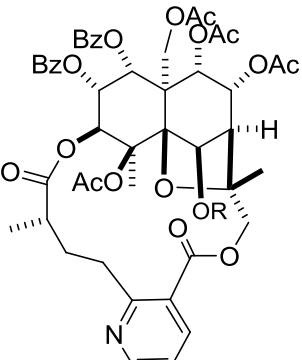
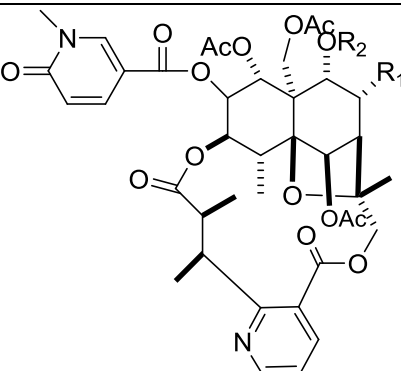
Table 2.9 Dihydro- β -agarofurane sesquiterpene alkaloids isolated from the genus *Maytenus*

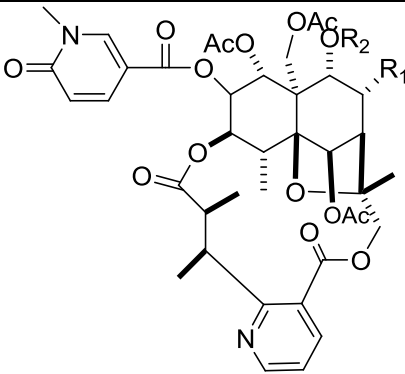
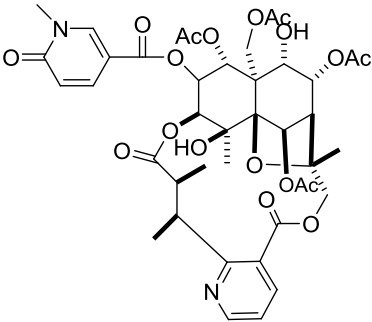
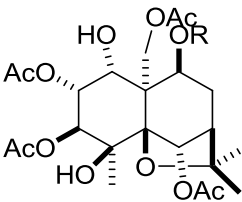
No.	Compounds	Structures	Source and references
88	Maymyrisine.	 R=3-pyridinecarbonyl	Fruits of <i>M. myrrisoids</i> ⁶⁶
89	Aquifoliunine EII.	 R₁=H, R₂=β-acetyl	Roots of <i>M. aquifolium</i> ⁶⁷
90	Aquifoliunine EIIV.	 R₁=acetyl, R₂=α-(3-	Roots of <i>M. aquifolium</i> ⁶⁷

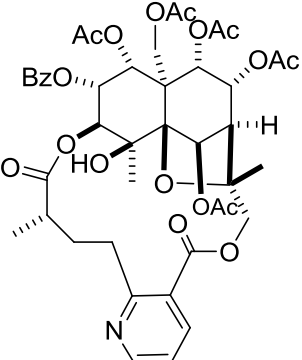
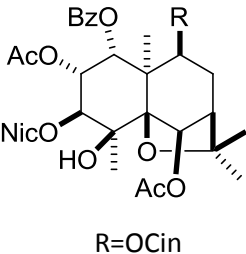
		pyridinecarbonyl)	
91	Cangorin A.	 R₁=acetyl, R₂=nicotyl	Roots of <i>M. ilicifolia</i> ⁶⁸
92	Cangorin B.	 R₁=Nic, R₂=benzoyl	Roots of <i>M. ilicifolia</i> ⁶⁸
93	Cangorin J.		Roots of <i>M. ilicifolia</i> ⁶⁹
94	Cangorinine EI.		Roots bark of <i>M. ilicifolia</i> ⁷⁰

95	Cangorinine WI.	 R=Benzoyl	Stem bark of <i>M. ilicifolia</i> ⁷¹
96	Cangorinine WII.	 R=pyridinecarbonyl	Stem bark of <i>M. ilicifolia</i> ⁷¹
97	Chiapenine ES-I.	 R₁= R₂=OBz, R₃=α-OAc,β-H, R₄=OH	Leaves of <i>M. chiapensis</i> ⁷²

98	Chiapenine ES-II.	 $R_1 = R_2 = \text{OBz}$, $R_3 = \text{O}$, $R_4 = \text{OH}$	Leaves of <i>M. chiapensis</i> ⁷²
99	Ebenifoline EI.	 $R_1 = \text{benzoyl}$, $R_2 = \text{acetyl}$	Stem of <i>M. ebenifolia</i> ⁷³ Leaves of <i>M. chiapensis</i> ⁷²
100	Mayteine.	 $R_1 = R_2 = \text{acetyl}$	Stem of <i>M. ebenifolia</i> Roots of <i>M. quaianensis</i> , ⁷⁴ <i>M. krukurvii</i> ⁷⁵ and <i>M. putterlickoides</i> ⁷⁵ Leaves of <i>M. chiapensis</i> ⁷²

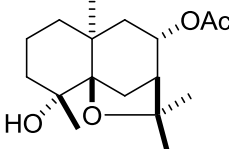
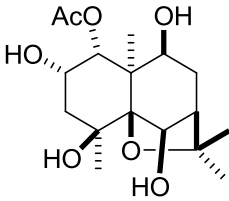
101	Ebenifoline WI.	 R=acetyl	Stem of <i>M. ebenifolia</i> ⁷⁶
102	Ebenifoline WII.	 R=H	Stem bark of <i>M. ebenifolia</i> ⁷⁷
103	Emargunatine A.	 R₁=α-acetoxy, R₂= acetyl	Roots and stem of <i>M. emarginata</i> ^{75,31}

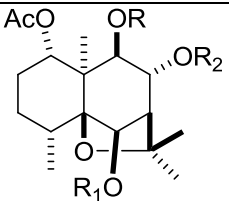
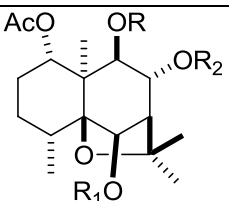
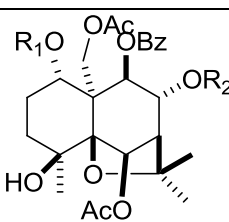
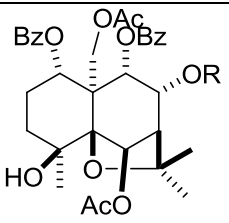
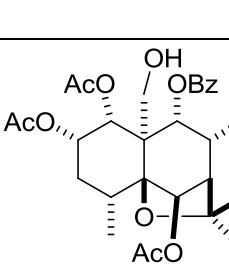
104	Emargunatine B.	 $R_1 = \beta\text{-acetoxy}$, $R_2 = \text{benzoyl}$	Stem of <i>M. emarginata</i> ⁷⁸
105	Emargunatine C.		Stem of <i>M. ebenifolia</i> ⁷⁹
106	Maytine.	 $R = 3\text{-pyridinecarbonyl}$	Stem heartwood of <i>M. serrata</i> ⁸⁰

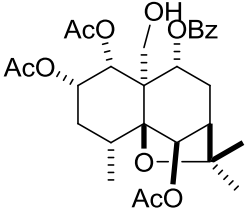
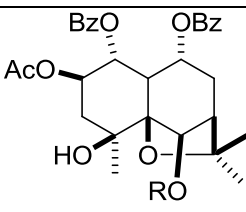
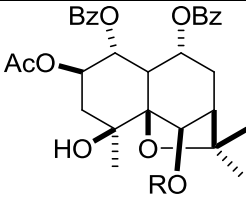
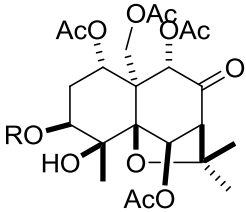
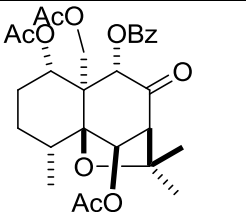
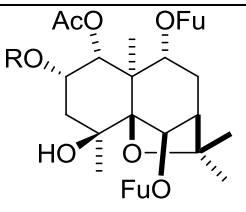
107	Wilforine.		Roots of <i>M. senegalensis</i> ⁸¹
108	2 α ,6 β -Diaceto-xy- 1 α -benzoyl-oxy- 9 β -cinnam-oyloxy- 3 β -nico- tinoyloxydihydro- β -agarofu-ran. R=OCin		Roots of <i>M. magellanica</i> ⁸²

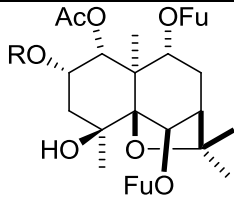
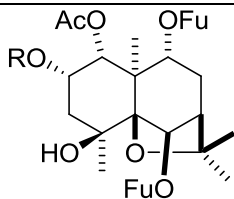
2.2.2.4.2 Dihydro- β -agarofurane sesquiterpene polyesters

Table 2.10 Dihydro- β -agarofurane sesquiterpene polyesters isolated from the genus *Maytenus*

No.	Compounds	Structures	Source and references
109	Boariol.		Seeds of <i>M. boaria</i> ⁸³
110	Acetoxyeumaitenol.		Stem of <i>M. boaria</i> ⁸⁴

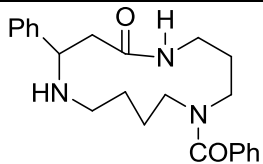
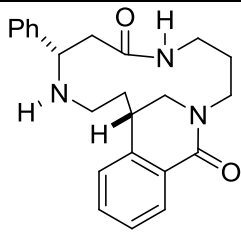
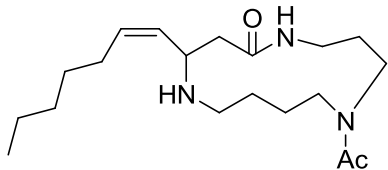
111	1 α -Acetoxy-9 β -(3-furancarboxyl)-6 β ,8 α -dihydroxy-dihydro- β -agarofuran.	 R=3-furancarboxyl, R₁=R₂=H	Seeds of <i>M. boaria</i> ⁸⁵
112	Eumaitenin.	 R=3-furancarboxyl, R₁=R₂=acetyl	Stem of <i>M. boaria</i> ⁸⁴
113	Isoalato(1 α ,6 β ,15-triacetoxy-9 α -benzoyloxy-4 β ,8 α -dihydroxydihydro- β -agarofuran.	 R₁= acetyl, R₂=H	Stem of <i>M. canariensis</i> ⁵²
114	6 β ,15-diacetoxy-1 α ,9 α -dibenzoyloxy- 8 α -tigloyloxy-4 β - hydroxydihydro- β -agarofuran.	 R=tigloyl	Stem of <i>M. canariensis</i> ⁵²
115	1 α ,2 α ,6 β -triacetoxy-9 α -benzoyloxy-8 α ,15 - dihydroxydihydro- β -agarofuran.		Stem of <i>M. chubutensis</i> ⁶⁶

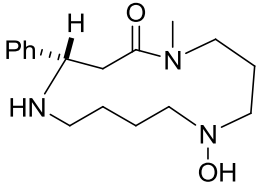
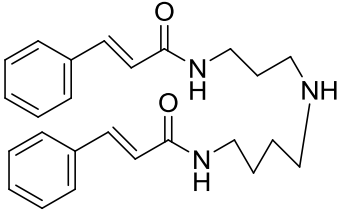
116	1 α ,2 α ,6 β - triacet- oxy-9 β -benzo- yloxy-15-dihydro- xydihydro- β - agarofuran.		Stem of <i>M. chubute- nsis</i> ⁶⁶
117	2 β ,6 β -diacetyl- 1 α ,9 α -dibenzoyl- celorbicol.		Stem of <i>M. magella- nica</i> ⁸⁶
118	2 β -acetyl-1 α ,6 β , 9 α -tribenzoyl- celorbicol.		Stem of <i>M. magella- nica</i> ⁸⁶
119	1 α ,3 β ,6 β ,9 α ,15- pentaacetoxy-4 β - hydroxy- β - agarofuran.		Stem of <i>M. cheapen- sis</i> ¹⁹
120	1 α , 6 β ,8 α ,15- tetraacetoxy-9 β - benzoyloxydihydro - β -agarofuran.		Roots of <i>M. chubuten- sis</i> ⁸²
121	1 α - acetox-6 β ,9 β - difuroyloxy-2 α ,4 β - dihydroxydihydro- β -agarofuran.		Fruits of <i>M.cuzconia</i> ⁸⁷

122	1 α - acetoxy-2 α -propionyl-6 β ,9 β -difuroyloxy-4 β -hydroxydihydro- β -agarofuran.	 R=propionate	Fruits of <i>M. cuzco-nia</i> ⁸⁷
123	1 α - acetoxy-6 β ,9 β -difuroyloxy-4 β -hydroxydihydro- β -agarofuran-1 α -methylbutyrate.	 R=methylbutyrate	Fruits of <i>M. cuzco-nia</i> ⁸⁷

2.2.2.5 Spermidine alkaloids

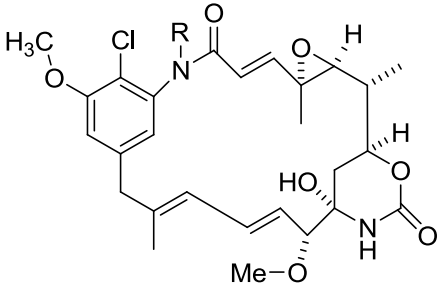
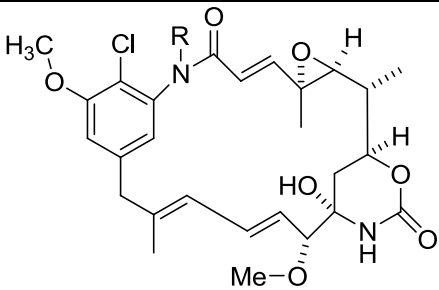
Table 2.11 Spermidine alkaloids isolated from the genus *Maytenus*

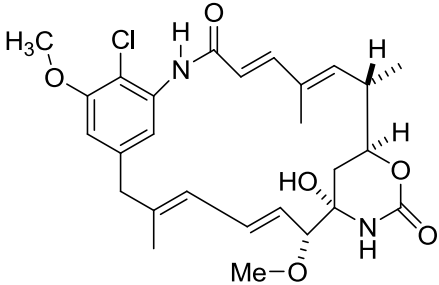
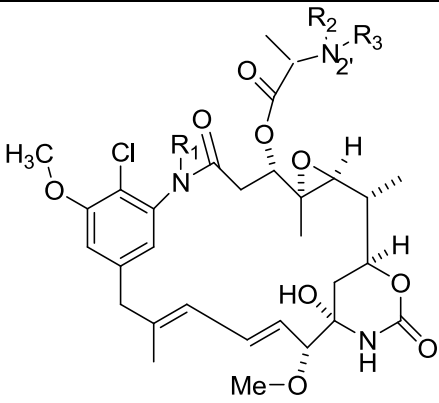
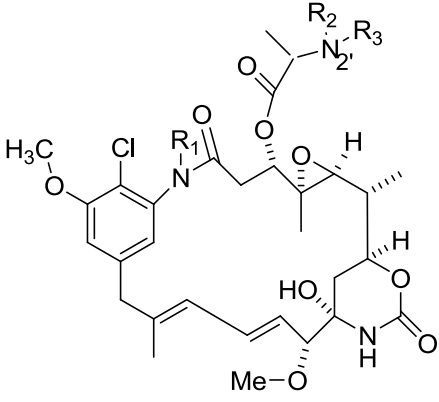
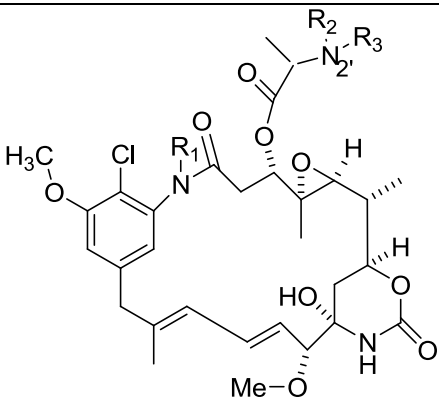
No.	Compounds	Structures	Source and references
124	Celabenzine.		Stem heartwood of <i>M. mossambicensis</i> ⁸⁸
125	Isocyclocelabenzine.		Leaves of <i>Maytenus mossambicensis</i> ⁸⁹
126	Loesenerine.		Leaves of <i>M. loeseneri</i> ⁹⁰

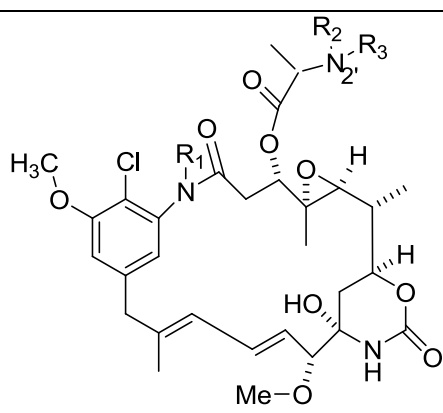
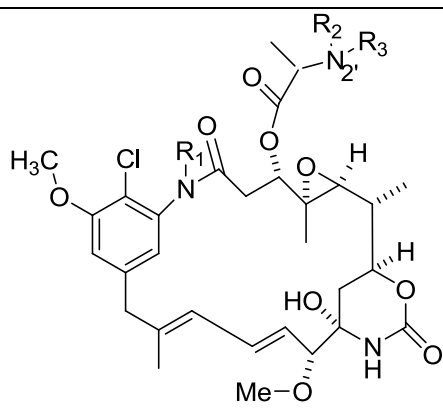
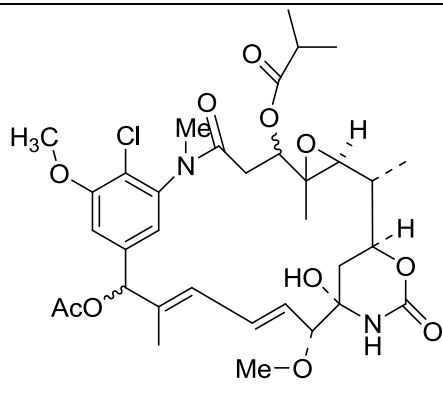
127	Mayfoline.		Stem of <i>M. buxifolia</i> ⁹¹
128	Maytenine.		Leaves of <i>M. chuchuhuasha</i> ⁹²

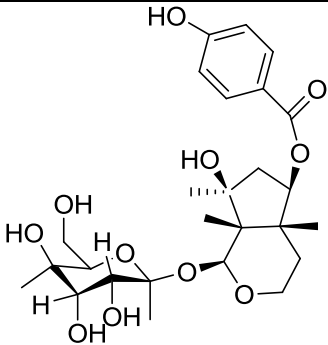
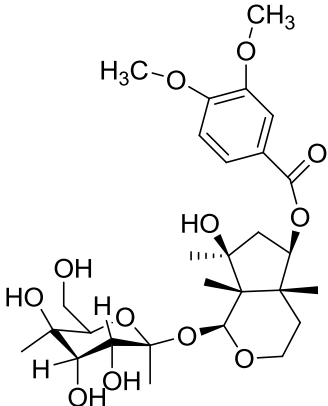
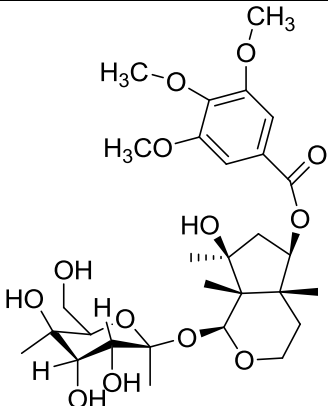
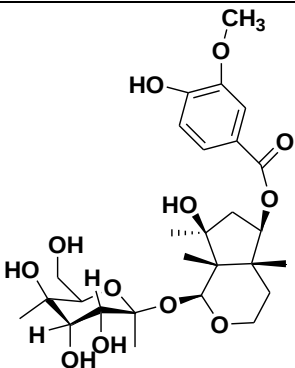
2.2.2.6 Ansa macrolides

Table 2.12 Ansa macrolides isolated from the genus *Maytenus*

No.	Compounds	Structures	Source and references
129	Maysine.	 R=CH ₃	Stem of <i>M. Buchanania</i> ⁹³
130	Normaysine.	 R=H	Stem of <i>M. Buchanania</i> ⁹³

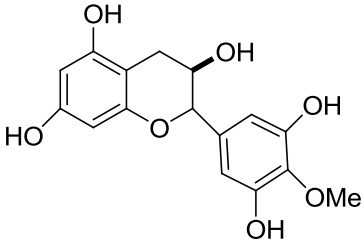
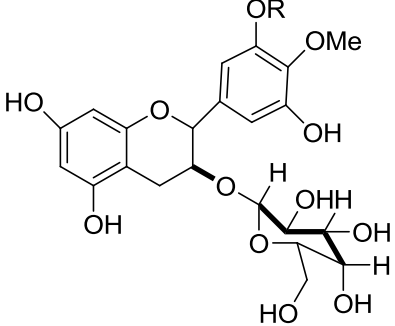
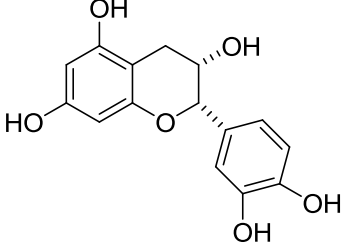
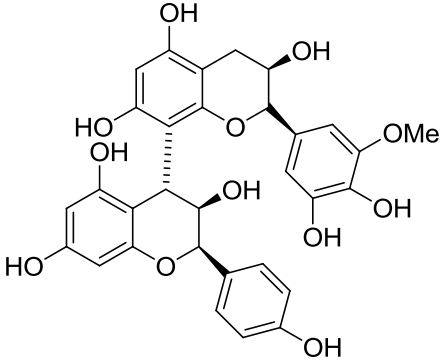
131	Maysenine.		Stem bark of <i>M. buechananii</i> . ⁹³
132	Maytanprine.	 $R_1=CH_3, R_2=propionyl, R_3=CH_3$	Stem of <i>M. Buchananii</i> , ⁹⁴ <i>M. diversifolia</i> ⁹⁵ and <i>M. ilicifolia</i> ⁹⁶
133	Maytanvaline.	 $R_1=CH_3, R_2=methylpentanoyl, R_3=CH_3$	Stem heartwood of <i>M. buechananii</i> . ⁹⁶
134	Maytanbutine.	 $R_1=2-methylprpanoyl, R_2=propionyl,$	Stem heartwood of <i>M. buechananii</i> . ⁹⁴ and <i>M. ilici-folia</i> ⁹⁶

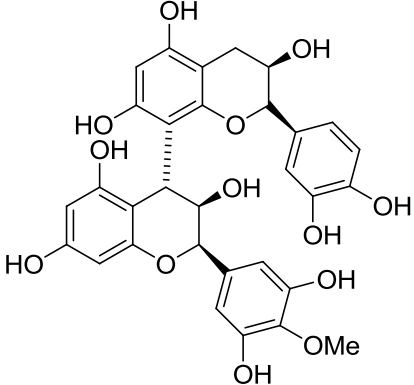
		$R_3 = \text{CH}_3$	
135	Normaytancy-prine.	 $R_1 = R_2 = \text{H}, R_3 = 2,3\text{-dimethylcyclopropylcarbonyl}$	Stem heartwood and barks of <i>M. buchananii</i> ⁹⁷
136	Normaytansine	 $R_1 = \text{CH}_3, R_2 = \text{propionyl}, R_3 = \text{H}$	Stem heartwood of <i>M. buchananii</i> ⁹⁸
137	Maytanbutacine.		Stem heartwood of <i>M. serrata</i> ⁹⁹

141	6- <i>O</i> -Hydroxybenzoylajugol.		Stem bark of <i>M. laevis</i> ²⁵
142	6- <i>O</i> -3',4'-Dimethoxybenzoyl ajugol.		Stem bark of <i>M. laevis</i> ²⁵
143	(6- <i>O</i> -(3',4',5'-Trimethoxybenzoyl) ajugol.		Stem bark of <i>M. laevis</i> ²⁵
144	6- <i>O</i> -4'-Hydroxy-3'-methoxybenzoylajugol.		Stem bark of <i>M. laevis</i> ²⁵

2.2.2.8 Flavonoids and flavonoid glycosides

Table 2.14 Flavonoids and flavonoid glycosides isolated from the genus *Maytenus*.

No.	Compounds	Structures	Source and references
145	4'-O-methyl-(-)-epigallocatechin.		Leaves of <i>M. aquifolium</i> ³⁶ and <i>M. arbutifolia</i> ³⁶
146	Hyprine 2''-O-[- β -D-Glucopyranosyl-(1-3)- α -L-rhamnopyranosyl], 6''-O- α -L-rhamnopyranosyl.	 R=O-[-β-D-glucopyranosyl-(1-3)-α-L-rhamnopyranosyl], 6''-O-α-L-rhamnopyranosyl	Leaves of <i>M. aquifolium</i> ¹⁰⁰
147	(-)-Epi-catechin.		Leaves of <i>M. heterophylla</i> ³⁶
148	Ouratea proanthocyanidin A.		Roots bark of <i>M. leavis</i> ¹⁰²

149	<i>Epi</i> -catechin. (4 β)-4'-O-methyl- epigallocatechin.		Leaves of <i>M. senegalensis</i> ¹⁰³
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2.2.3 Biological activities reported from the genus *Maytenus*

2.2.3.1 Traditional uses

Species of this genus are used, as antiseptic, antiasthmatic, antiulcer,¹⁰⁵ antitumor¹⁰⁴ and antiacid.¹⁰⁶ In South American countries *Maytenus ilicifolia* and *Maytenus aquifolium* are known as holly spines.¹⁰⁵

2.2.3.2 Antibacterial and antifungal activities

The ethanolic crude extract of *Maytenus macrocarpa* roots showed antibacterial activity against a variety of bacteria,¹⁰⁷ which was confirmed by the isolation of antibacterial compounds; Scutione.¹⁰⁸ The crude fractions from *M. senegalensis* showed both antibacterial and anti-inflammatory activities.¹⁰⁹ Oxotingenol⁵² and koetjabic acid³⁷ isolated from *M. senegalensis* have antibacterial activities while antifungal tetraterpenoid maytenone was isolated from the same plant.¹¹⁰

2.2.3.3 Antiparasitic activity

The water fraction from the leaves and roots of *M. heterophylla* and *M. senegalensis* showed moderate antiparasitic activity,¹¹¹ while methanolic extract of leaves and roots of *Maytenus acuminata* exhibited significant antiparasitic activities.¹¹²

2.2.3.4 Antioxidant activity

The crude methanolic bark extract from *M. krukovii* inhibited the mutagenic activity of promutagen 2-aminoanthracene, while methanolic roots extract of *M. ilicifolia* has strong antioxidant activity.^{113,114}

2.2.3.5 Effects on CNS

The chloroform fraction obtained from the methanolic extract of *M. obtusifolia* strongly inhibited the amphetamine toxicity in mice. It can also lower the conditioned response on dose related manner in rats.¹¹⁵

2.2.3.6 Gastro-intestinal usefulness

M. ilicifolia tea/infusion efficiently inhibited gastric lesions induced by ethanol in rat¹¹⁶ while its dried crude methanolic extract can reduce gastric ulcer and increased the P^H and quantity of gastric juice in rats.¹¹⁷ Similarly antiulcer activity has also shown by dried extract of *M. aquifoliuimi* which were induced by stress, indomethacin and ethanol.¹⁰⁸ which was confirmed by isolation of antiulcer compounds; 3 β -Friedelanol, Friedelin and triterpenes.¹⁰⁹

2.2.3.7 Muscle relaxation action

The crude methanolic extract of leaves of *M. ilicifolia* produced relaxation effect of the pre-contracted aorta rings of rats and guinea pigs.¹¹⁸

2.2.3.8 Contraceptive activities

The leaves extract of the *M. ilicifolia* produced abortion effect in guinea pigs.¹¹⁹

2.2.3.9 Antitumor and cytotoxic activities

Maytenus ilicifolia tea has cytotoxic and produced antimutagenic effect in salmonella assay against mutagenic compounds; aflatoxin B, sodium azide and others.¹²⁰ The triterpenes,^{19,20} triterpenes dimmer,³⁵ dihydro- β -agarofurane,^{75,76} dihydro- β -agarofurane alkaloids⁷⁸ and ansa macrolides^{93,97} isolated from *Maytenus* species having anti tumor and cytotoxic activities.

2.2.3.10 Antidiabetic activity

M. leavis bark's tea has been used for the treatment of diabetic in Latin America due to the presence of lambertic acid isolated from the bark of *M. leavis*.¹²⁰

2.2.3.11 Insecticidal and antifeedant actions

Insecticidal compounds have been isolated from genus *Maytenus*; dihydro- β -agarofuran sesquiterpenes (eumaitenin)¹⁰² dihydro- β -agarofuran sesquiterpenes polyesters (isoalato),⁵² which showed antifeedant action on Egyptian cotton worm larva, while dihydro- β -agarofuran sesquiterpenes pyridine alkaloids (alatusinine and wilfordine) showed insecticidal activity against *Spodoptera littoralis*.⁷²

2.3 Rational

Maytenus royleanus belongs to the genus *Maytenus* and common in the northern part of Pakistan (Buner). Literature survey revealed; no previous phytochemical study on this specie has been done so far, however phytochemical investigation was carried out on other species of *Maytenus* and isolated bioactive compounds.

On the basis of above phytochemical review, bioactivities of compounds isolated from other species of *Maytenus* and considering change geographical and environmental

conditions of Pakistan, that *Maytenus royleanus* will be a good source of new bioactive secondary metabolites.

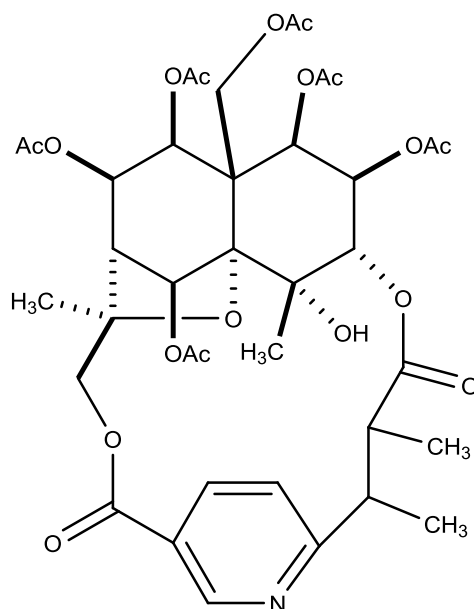
Chapter 3

Results and Discussion

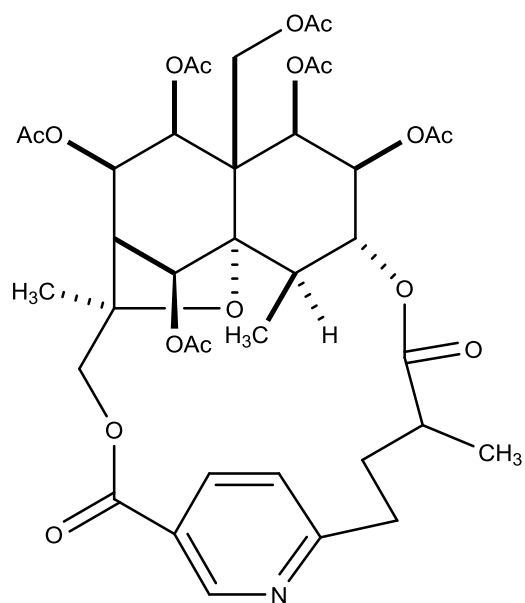
3.1 Chemical constituents isolated from *Maytenus royleanus*

Three new; royleanine A (**1**), royleanine B (**2**), royleanine C (**3**) and seven known compounds; salaspermic acid (**4**), orthosphenic acid (**5**), 3 α ,22 β -dihydroxyolean-12-en-29-oic acid (**6**), oleuropein (**7**), ligutroside (**8**), quercetin (**9**) and β -sitosterol-3-O- β -D-glycopyrannoside (**10**) were isolated from the roots of methanolic extract of *Maytenus royleanus* (*vide* experimental). Their structures were elucidated by advance spectroscopic techniques and comparison with related compounds.

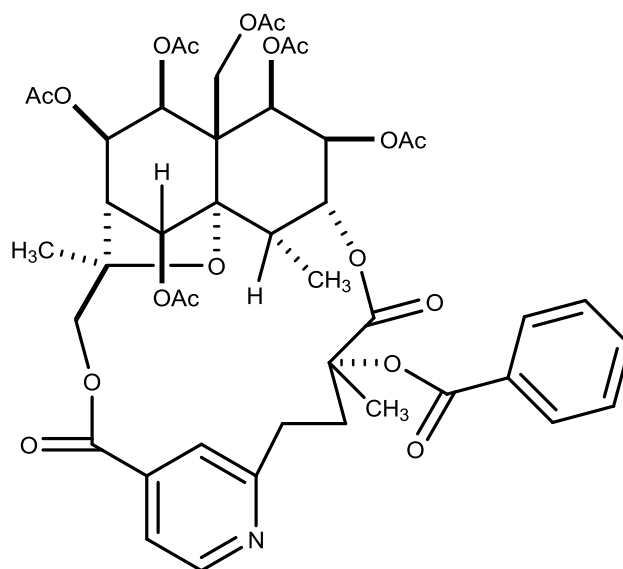
Royleanine A (**1**)



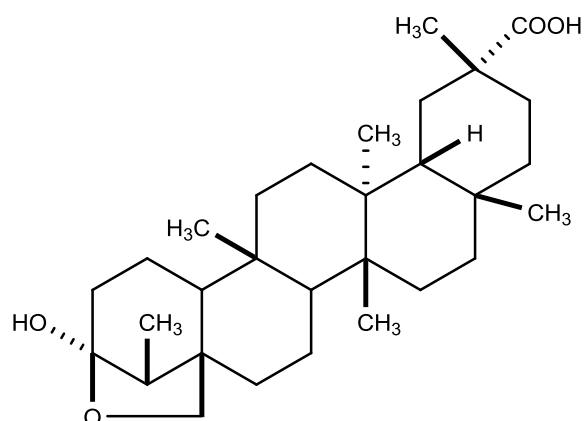
Royleanine B (2)



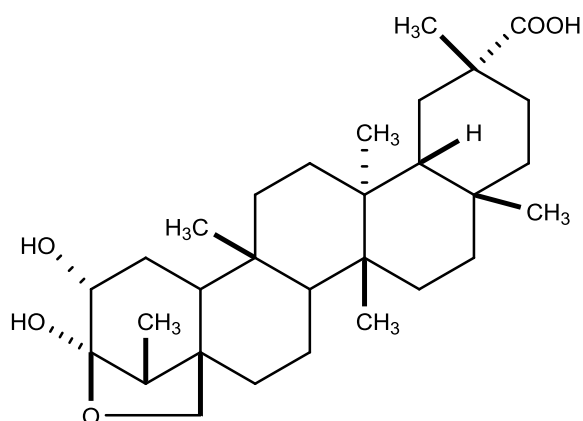
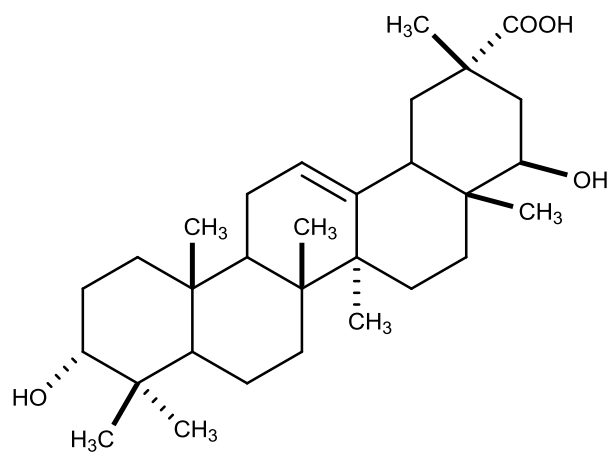
Royleanine C (3)



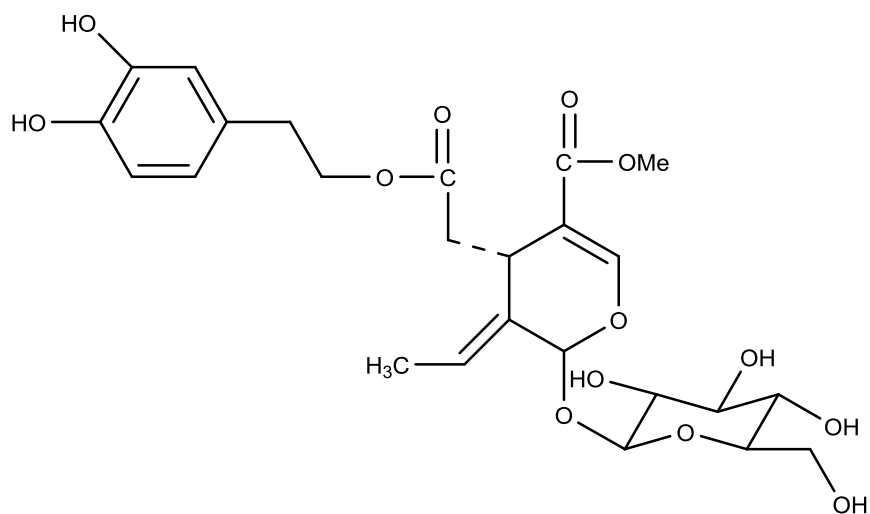
Salaspermic acid (4)



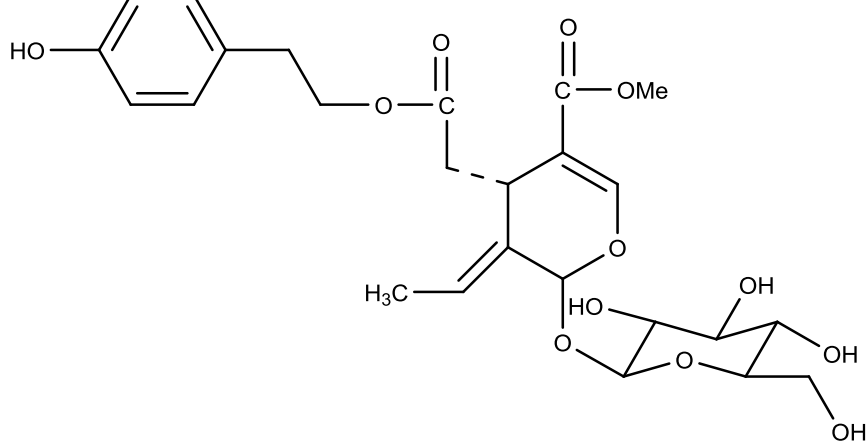
Orthosphenic acid (5)

3 α ,22 β -dihydroxyolean-12-en-29-oic acid (6)

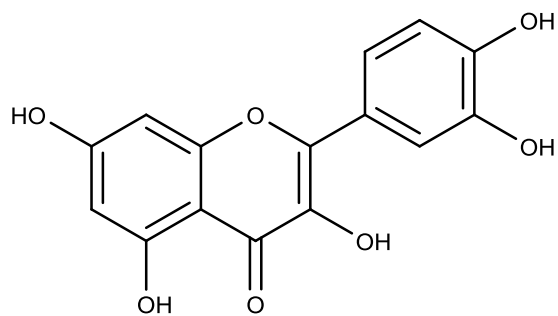
Oleuropein (7)

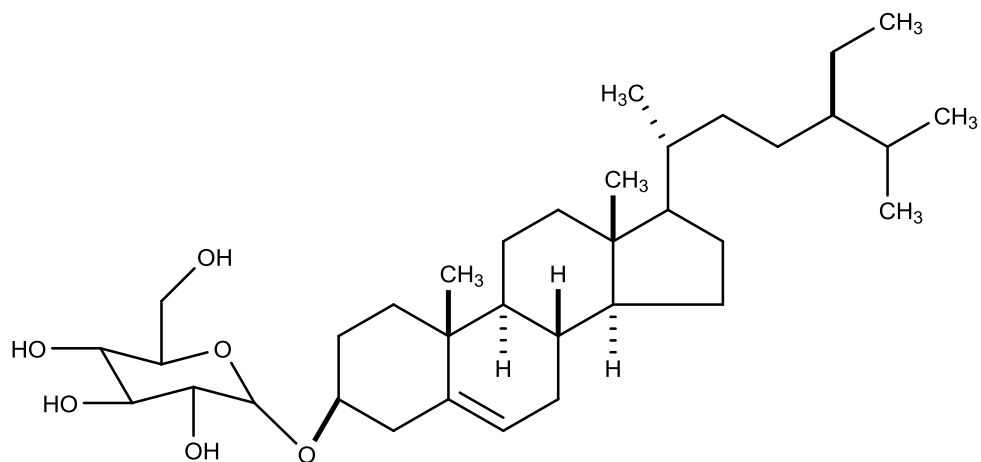


Ligutroside (8)



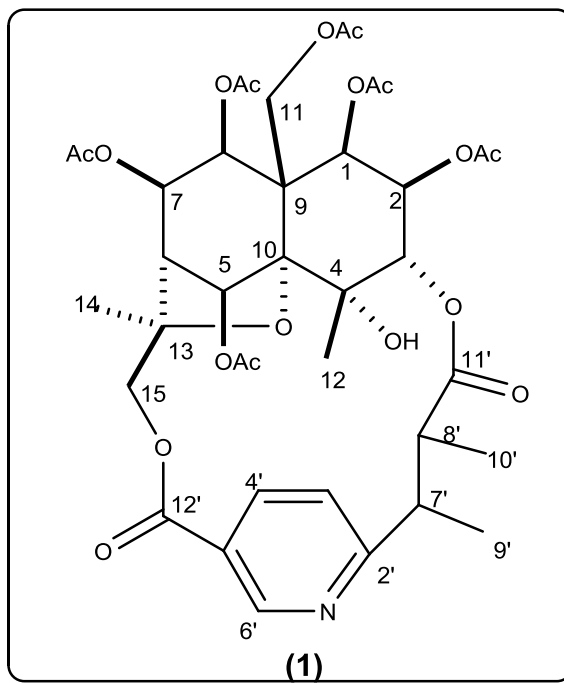
Quercetin (9)



β -Sitosterol-3-O- β -D-glycopyrannoside (**10**)

3.2.1 New compounds isolated from the roots of *Maytenus royleanus*

3.2.1.1 Royleanine A (1)



Compound **1** was isolated as yellow crystals. Its molecular formula $C_{38}H_{47}NO_{18}$ was established by high resolution ESIMS which showed m/z 805.2916 (calcd. 805.2851 for $C_{38}H_{47}NO_{18}$), suggesting the presence of sixteen degree of unsaturations. The IR spectrum of indicated absorption bands at 3487 (hydroxyl group), 1750.5 (ester carbonyl), 1675 (α,β -unsaturated carbonyl), 1630 and 1585 cm^{-1} (pyridine ring), while the UV spectrum of showed an absorption maxima at 264 nm which confirmed the presence of pyridine moiety. The ^{13}C NMR spectra (broad band and DEPT; **Table 3.1**) of **1** showed 38 carbon atoms corroborated the presence of fourteen quaternary carbons; twelve CH, two CH_2 and ten CH_3 type carbon atoms.

The ^1H NMR spectrum (**Table 3.1**) of **1** displayed the presence of two quaternary, two tertiary, and six acetyl methyl groups resonated at δ 1.61 (s, H-14), 1.51 (s, H-12),

1.42 (d, $J=7.0$ Hz; H-9'), 1.16 (d, $J=7.1$ Hz; H-10'), 2.19 (OAc-1), 2.16 (2×OAc; OAc-2 and OAc-7), 1.84 (OAc-5), 1.98 (OAc-8) and 2.13 (OAc-11) respectively. Two oxygenated methylenes and six oxygenated methine groups appeared at δ 5.22 (d, $J=13.4$ Hz; H-11a), 4.41 (d, $J=13.4$ Hz; H-11b), 5.91 (d, $J=11.4$ Hz; H-15a) and 3.93 (d, $J=11.4$ Hz; H-15b), 5.62 (d, $J=3.6$ Hz; H-1), 5.20 (dd, $J=3.6, 1.8$ Hz; H-2), 4.65 (d, $J=1.8$ Hz; H-3), 7.0 (brd. s; H-5), 5.54 (dd, $J=6.0, 4.2$ Hz; H-7) and 5.40 (d, $J=6$ Hz; H-8). The aromatic protons appeared downfield at δ 8.68 (d, $J=1.8$ Hz; H-6'), 8.11 (dd, $J=7.8, 1.8$ Hz; H-4') and δ 7.42 (d, $J=7.8$ Hz; H-3') were assigned to the disubstituted pyridine ring. The ^{13}C NMR spectrum (**Table 3.1**) disclosed the presence of eight ester carbonyls resonating at δ 171.8 (OAc-1), 170.5 (OAc-2), 171.0 (OAc-5), 171.4 (OAc-7), 171.2 (OAc-8), 172.0 (OAc-11), 170.2 (-COO-11') and 175.6 (-COO-12'), pyridine carbons δ 165.8 (C-2'), 123.0 (C-3'), 139.0 (C-4'), 127.0 (C-5') and 152.5 (C-6'), four methyl carbons δ 12.1 (C-9'), 9.9 (C-10'), 23.6 (C-12) and 18.5 (C-14), two oxygenated methylene carbons δ 61.2 (C-11) and 71.3 (C-15) and nine methine carbons δ 74.8 (C-1), 69.8 (C-2), 77.6 (C-3), 75.4 (C-5), 51.6 (C-6), 70.2 (C-7), 72.1 (C-8), 36.6 (C-7') and 46.4 (C-8').

The presence of dibasic acid moiety of isomeric evoninic acid was confirmed by the coupling of two methyl groups resonating at δ 1.42 (d, $J=7.0$ Hz; H-9') and 1.16 (d, $J=7.1$ Hz; H-10') with two methine protons at δ 2.54 (d, $J=7.1$ Hz; H-8') and δ 4.65 (d, $J=7.0$ Hz; H-7'). All the assignments were made on the basis of HMBC and COSY correlations (**Fig. 3.1**). The COSY correlations between H-2' and H-3' was strong however meta correlation between H-3' and H-6' was weak, which were confirmed by long range HMBC correlations, H-3' exhibited correlation with C-5' and C-7', H-4' with C-

2' while C-6' and C-12', H-6' with C-2', C-4' and C-12'. The presence of two methylene groups were confirmed from both ID and 2D NMR spectra and assigned to C-11 and C-15. The COSY (^1H - ^1H) indicated two sets of isolated spin system due to H-5, H-6, H-7 and H-1, H-2, H-3. All the spectral data confirmed the presence of cyclodiester of evoninic acid which was linked to hexa-acetyl euonyminol.¹²¹ The evoninic acid linkage to the sesquiterpene confirmed by HMBC long range correlations of carbonyl carbon at δ 170.2 (C-12') with protons at δ 8.11 (H-4'), δ 5.91 (Ha-15), δ 3.93 (Hb-15) and the carbonyl carbon at δ 175.6 (C-11') long rang correlations with protons at δ 4.65 (q, $J=7.0$ Hz; H-7') and 1.16 (d, $J=7.1$ Hz; H-10').

The stereochemistry of different stereocenters was assigned by NOESY correlations and showing resemblance with related compounds.^{121,122}

All the above discussion suggested that the structure of **1** as 1 β ,2 β ,5 β ,7 β ,8 β ,11-hexaacetoxy-4-hydroxy-3 α ,15-[2',3'-dimethyl-3'(5"-carboxy-2"-pyridyl)-propanoic acid] dicarbollactone-dihydro-agarofuran (royleanine A).

Table-3.1: ^1H (600 MHz) and ^{13}C NMR (125 MHz) spectral data of royleanine A (**1**) in CD_3OD .

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C-1	74.8 CH	5.62	d, $J=3.6$	C9, 11, COO-1
C-2	69.8 CH	5.20	dd, $J=3.6, 1.8$	C4, 11, COO-2
C-3	77.6 CH	4.65	d, $J=1.8$	C1, 4, 10
C-4	71.9 C	-	-	-
C-5	75.4 CH	7.0	brd. s	-
C-6	51.6 CH	2.52	d, $J=4.2$	C7, 8, 10
C-7	70.2 CH	5.54	dd, $J=6.0, 4.2$	C9, COO-7
C-8	72.1 CH	5.40	d, $J=6.0$	C1, 7, 11
C-9	53.5 C	-	-	-
C-10	95.0 C	-	-	-
C-11	61.2 CH_2	5.22 4.41	d, $J=13.4 \text{ H}_a$ d, $J=13.4 \text{ H}_b$	C8, 9, COO-11 C8, 9, 10
C-12	23.6 CH_3	1.51	s	C3, 4, 10
C-13	85.8 C	-	-	-
C-14	18.5 CH_3	1.61	s	-
C-15	71.3 CH_2	5.91 3.93	d, $J=11.4 \text{ H}_a$ d, $J=11.4 \text{ H}_b$	C12' C12', 13, 14
C-2'	165.8 C	-	-	-
C-3'	123.0 CH	7.42	d, $J=7.1$	C7,5'
C-4'	139.0 CH	8.11	dd, $J=7.1, 1.8$	C2',5',12'
C-5'	127.0 C	-	-	-
C-6'	152.5 CH	8.68	d, $J=1.8$	C2',4',12'
C-7'	36.6 CH	4.65	q, $J=7.0$	C2', 9', 11'
C-8'	46.4 CH	2.54	q, $J=7.1$	C9', 10'
C-9'	12.1 CH_3	1.42	d, $J=7.0$	C2', 8'
C-10'	9.9 CH_3	1.16	d, $J=7.1$	C7', 8', 11'
C-11'	175.6 C	-	-	-
C-12'	170.2 C	-	-	-
1-Ac	20.9 CH_3	2.19	s	COO-1
2-Ac	20.8 CH_3	2.16	s	COO-2
5-Ac	21.2 CH_3	1.84	s	COO-3
7-Ac	20.8 CH_3	2.16	s	COO-4
8-Ac	20.9 CH_3	1.98	s	COO-5
11-Ac	21.8 CH_3	2.13	s	COO-6
1-CO	171.8 -	-	-	-
2-CO	170.5 -	-	-	-
5-CO	171.0 -	-	-	-
7-CO	171.4 -	-	-	-
8-CO	171.2 -	-	-	-
11-CO	172 -	-	-	-

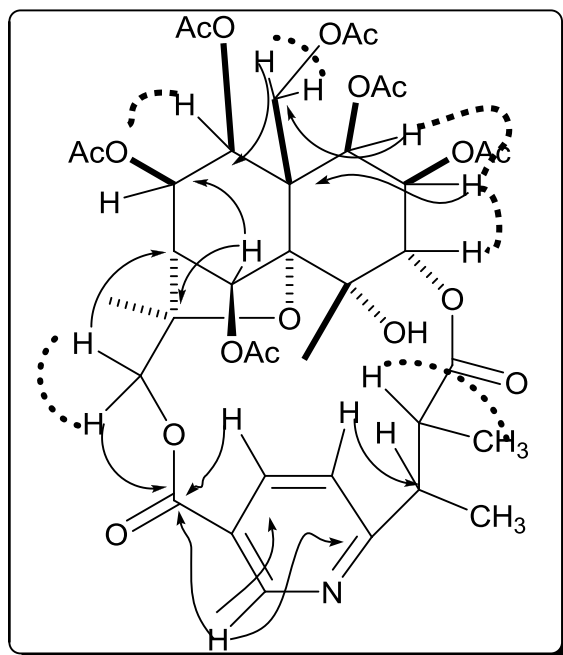
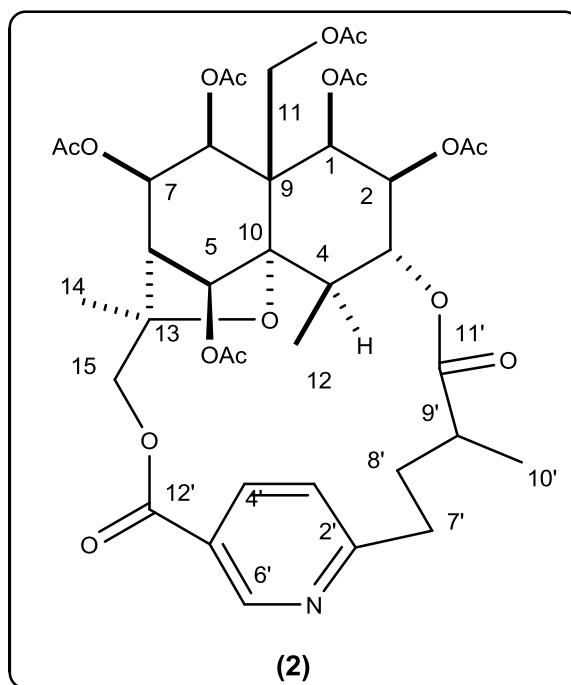


Fig. 3.1. Important HMBC (————→) and COSY (-----) correlations observed in **1**.

3.2.1.2 Royleanine B (2)

Compound **2** was purified as yellow amorphous powder. The molecular formula $C_{38}H_{47}NO_{17}$ was consistent with HRESIMS molecular ion peak at m/z 789.2693 (calcd. 789.2795 for $C_{38}H_{47}NO_{17}$). The formula $C_{38}H_{47}NO_{17}$ suggested the presence of sixteen degrees of unsaturation. The IR spectrum of **2** exhibited the presence of ester carbonyl (1750.5 cm^{-1}), α,β -unsaturated carbonyl (1673 cm^{-1}) and pyridine ring (1641 and 1550 cm^{-1}) while UV spectrum showed maximum absorption at 260 nm confirmed the presence of aromatic moiety.



The ^1H and ^{13}C NMR spectra (BB and DEPT; **Table 3.2**) indicated the presence of 38 carbons; thirteen quaternary, twelve methine, four methylene and nine methyl groups in **2** while COSY and HMBC spectra established the carbon connectivity pattern. The ^1H NMR spectrum (**Table 3.2**) of **2** indicated the presence of one tertiary, two

secondary and six acetyl methyls resonated at δ 1.63 (s, H-14), 1.22 (d, $J=9.6$ Hz; H-12), 1.16 (d, $J=9.6$ Hz; H-10'), 2.18 (OAc-1), 2.16 (OAc-2), 1.57 (OAc-5), 2.16 (OAc-7) and 2.18 (OAc-8) and 2.18 (OAc-11) respectively. The ^1H NMR spectrum (**Table 3.2**) further showed peaks for two oxygenated methylenes at δ 5.23 (d, $J=15$ Hz; H-11a), 4.41 (d, $J=15$ Hz; H-11b), 5.83 (d, $J=13.8$ Hz; H-15a), 3.72 (d, $J=13.8$ Hz; H-15b), three methines at δ 2.75 (d, $J=9.6$ Hz; H-4), 2.61 (d, $J=4.2$ Hz; H-6), 2.93 (m, H-9') and six oxygenated methine groups at δ 4.92 (d, $J=4.2$ Hz; H-1), 5.61 (dd, $J=4.2, 1.8$ Hz; H-2), 4.93 (d, $J=1.8$ Hz; H-3), 6.62 (brd. s, H-5), 5.53 (dd, $J=7.2, 4.2$ Hz; H-7) and 5.31 (d, $J=7.2$ Hz; H-8). The ^{13}C NMR spectrum (**Table 3.2**) indicated the presence of eight ester carbonyls resonating at δ 169.1 (OAc-1), 168.8 (OAc-2, 7), 168.5 (OAc-5), 169.1 (OAc-8), 167.7 (OAc-11), 174.4 (-COO-11') and 169.0 (-COO-12'), pyridine carbons δ 164.3 (C-2'), 120.5 (C-3'), 138.0 (C-4'), 126.0 (C-5') and 151.1 (C-6'), three methyl carbons δ 15.8 (C-10'), 13.1 (C-12) and 18.5 (C-14), two oxygenated methylene carbons δ 59.0 (C-11) and 67.9 (C-15) and nine methine carbons δ 73.6 (C-1), 69.9 (C-2), 72.6 (C-3), 72.9 (C-5), 49.2 (C-6), 68.8 (C-7), 70.0 (C-8), 68.8 (C-7') and 35.8 (C-9'). These characteristic peaks confirmed the presence of polyester type sesquiterpene skeleton. In the ^1H NMR the proton resonating at δ 8.71 (d, $J=1.8$ Hz; H-6'), 8.33 (dd, 8.7, 1.8 Hz; 4') and 7.34 (d, $J=8.7$ Hz; H-3') indicated the 2', 5'-disubstituted pyridine ring of dibasic acid moiety of evoninic acid.¹²¹ The two methylene proton in the ^1H NMR spectrum at δ 3.91 (ddd, $J=11.6, 6.8, 5.6$ Hz; H-7') and δ 1.92 (m, H-8') were assigned to C-7' and C-8' while protons appeared at δ 2.93 (m, H-9') and 1.16 (d, $J=8.4$ Hz; H-10') were assigned to methine and methyl groups of the dibasic acid moiety of the isomeric evoninic acid. The downfield carbons signals appeared at δ 169.1 $\times$ 2, 168.8 $\times$ 2, 168.5, 167.7, 174.4 and

169.1, which were assigned to ester functionalities (**Table 3.2**). Both ^1H and ^{13}C NMR spectra of **2** suggested many similarities with compound **1**, indicating that both compounds have the same basic skeleton. However the ^1H and ^{13}C NMR spectra of **2** indicated the absence of methyl group attached at position C-7' and extra signals for methine (C-4) and methylene groups (C-7' and C-8') were observed.

In the COSY spectrum strong correlation was observed between H-3' and H-4' protons (**Fig. 3.2**), while weak correlations were observed for H-6' proton with H-3' and H-4' of 2',5'-disubstituted pyridine ring. The HMBC spectrum of **2** indicated long range correlations observed between H-4'/C-2', C-6, C-12, H-3'/C-5', C-7', H-6'-C-4', C-12' (**Table 3.2 and Fig. 3.2**). The relative stereochemistry of **2** was also confirmed by NOESY and comparison of spectral data with related compounds in literature.^{121,122}

On the basis of spectral data and supporting evidences the structure of **2** as 1 β ,2 β ,5 β ,7 β ,8 β ,11-hexaacetoxy-3 α ,15-[2'-methyl-4'(5"-carboxy-2"-pyridyl)-butanoic acid] dicarbollactone-dihydroagarofuran (royleanine B).

Table-3.2: ^1H (600 MHz) and ^{13}C NMR (125 MHz) spectral data of royleanine B (2) in CD_3OD

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C-1	73.6 CH	4.92	d, $J=4.2$	C9, 11, COO-1
C-2	69.9 CH	5.61	dd, $J=4.2, 1.8$	C1, 4, 9, COO-2
C-3	72.9 CH	4.93	d, $J=1.8$	C1, 2, 4, 5
C-4	35.9 CH	2.75	d, $J=9.6$	C2, 5, 9
C-5	72.9 CH	6.62	brd. s	
C-6	49.2 CH	2.61	d, $J=4.2$	C7, 8, 10
C-7	68.8 CH	5.53	dd, $J=7.2, 4.2$	C9, COO-7
C-8	70.0 CH	5.31	d, $J=7.2$	C1, 7, 11
C-9	49.5 C	-	-	-
C-10	89.7 C	-	-	-
C-11	59.0 CH_2	5.23H _a 4.41H _b	d, $J=15$ d, $J=15$	C8, 9, 10 C8, 9, COO-11
C-12	13.1 CH_3	1.22	d, $J=9.6$	C3, 5
C-13	82.0 C	-	-	-
C-14	18.5 CH_3	1.63	s	
C-15	67.9 CH_2	5.83H _a 3.72H _b	d, $J=13.8$ d, $J=13.8$	C12' C12', 13, 14
C-2'	164.3 C			
C-3'	120.5 CH	7.34	d, $J=8.7$	C7,5'
C-4'	138.0 CH	8.33	dd, $J=8.7, 1.8$	C2',5',12'
C-5'	126.0 C			
C-6'	151.1 CH	8.71	d, $J=1.8$	C2',4',12'
C-7'	32.16 CH_2	3.91	ddd, $J=11.6, 6.8, 5.6$	C3',9'
C-8'	30.8 CH_2	1.92	m	C2',10',11'
C-9'	35.8 CH	2.93	m	C7', 10'
C-10'	15.8 CH_3	1.16	d, $J=8.4$	C8', 11'
C-11'	174.4 C	-	-	-
C-12'	169.0 C	-	-	-
1-Ac	18.4 CH_3	2.18	s	CO-1
2-Ac	18.9 CH_3	2.16	s	CO-2
5-Ac	17.1 CH_3	1.57	s	CO-5
7-Ac	18.8 CH_3	2.16	s	CO-7
8-Ac	18.3 CH_3	2.18	s	CO-8
11-Ac	19.1 CH_3	2.18	s	CO-11
1-CO	169.1 C	-	-	-
2-CO	168.8 C	-	-	-
5-CO	168.5 C	-	-	-
7-CO	168.8 C	-	-	-
8-CO	169.1 C	-	-	-
11-CO	167.7 C			

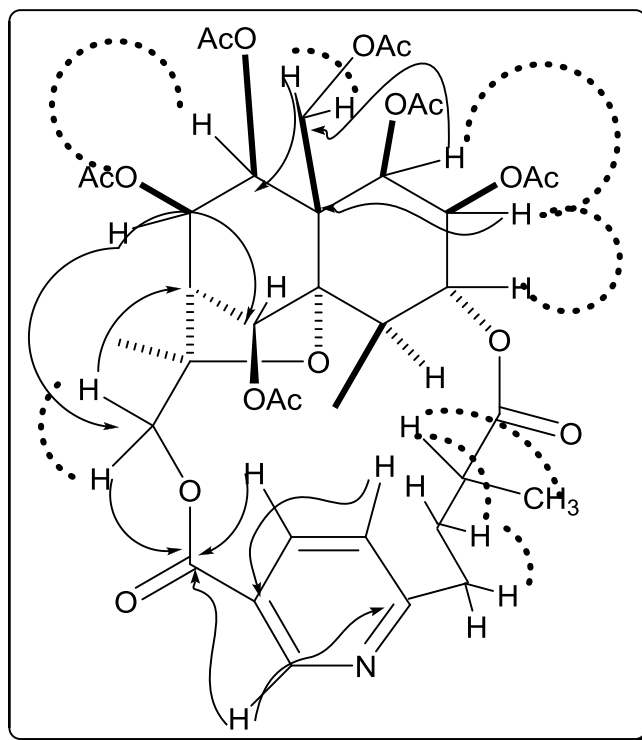
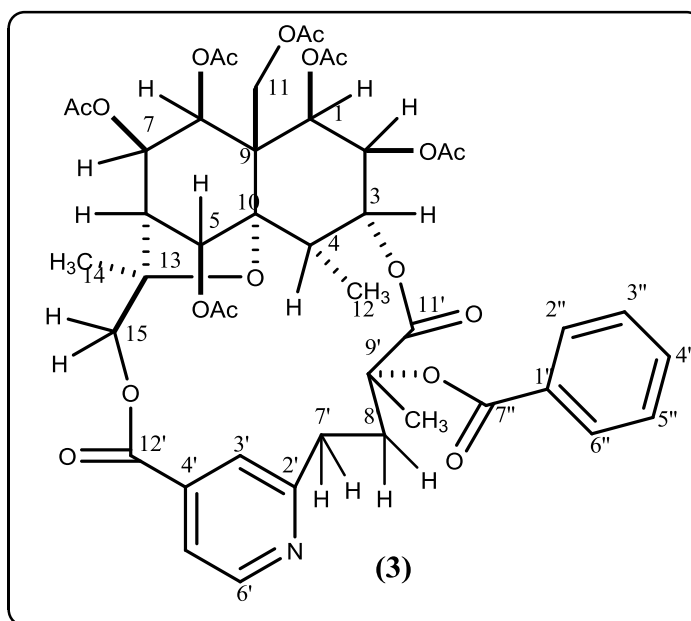


Fig.3.2. Important HMBC (————→) and COSY (-----) correlations observed in **2**.

3.2.1.3 Royleanine C (3)

Compound **3** was isolated as white powder. The ESI(+ve)MS spectrum showed molecular ion peak at m/z 910 $[M^+ + H]$ corresponding to molecular formula $C_{45}H_{51}NO_{19}$ which was developed by HRESIMS m/z 909.3078 (calcd. 909.3171 for $C_{45}H_{51}NO_{19}$).

The UV spectrum showed maximum absorption at 240 nm indicated the presence of aromatic moiety in compound **3**, while IR spectrum exhibited absorption bands at 2922 (methylene group), 1735.6 (ester carbonyl), 1592 cm^{-1} (aromatic moiety). The ^{13}C NMR spectra (broad band and DEPT; **Table 3.3**) of **3** indicated the presence of nine CH_3 , four CH_2 , sixteen CH and sixteen quaternary carbon atoms, corresponding to 45 carbons.



The ^1H NMR spectrum of **3** showed aromatic protons signals at δ 8.87 (brd. s, H-3'), 7.50 (d, $J=4.8$ Hz; H-5'), 8.61 (d, $J=4.8$ Hz; H-6') assigned to 2',4'-disubstituted pyridine moiety, present in majority of *Maytenus* alkaloids.¹²¹ Assignments to respective carbons were confirmed by 2D NMR spectra (**Table 3.3** and **Fig. 3.3**). The ^1H NMR

spectrum also displayed aromatic protons signals at δ 8.07 (dd, $J=1.2, 7.8$ Hz; H-2'', 6''), 7.54 (t, $J=7.2$ Hz; H-3'', 5'') and 7.67 (t, $J=7.8$ Hz; H-4'') were assigned to phenyl ring of benzoate moiety. The 2',4'-disubstituted pyridine ring and presence of benzoate moiety in **3** differentiate it from **1** and **2**. The ^1H NMR spectrum of **3** further showed six acetyl methyls appeared at δ 2.22 (s, OAc-1), 1.85 (s, OAc -2), 1.74 (s, OAc -5), 2.06 (s, OAc -7), 2.01 (s, OAc -8), 2.28 (s, OAc -11) while three methyl groups resonated at δ 1.38 (d, $J=7.8$ Hz; H-12), 1.63 (s, H-14), 1.97 (s, H-10'). Two methine protons appeared at δ 2.76 (d, $J=7.8$ Hz; H-4), 2.76 (d, $J=3.0$ Hz; H-6) and six oxygenated methines protons resonated at δ 5.79 (d, $J=3.6$ Hz; H-1), 5.47 (dd, $J=2.4, 3.6$ Hz; H-2), 4.80 (d, $J=2.8$ Hz; H-3), 6.4 (brd. s, H-5), 5.71 (dd, $J=9.6, 3.6$ Hz; H-7) and 5.73 (d, $J=9.6$ Hz; H-8) (**Table 3.3**) were characteristic peaks of dihydroagarofuran polyester sesquiterpene skeleton.¹²² Assignments to respective positions were made by both COSY and HMBC correlations (**Fig 3.3**). The dibasic acid moiety of evoninic acid in compound **3** is different from **1** and **2** due to unusual attachment of benzoate group at position C-9' which was absent in other analogues. The ^{13}C NMR spectrum of **3** showed downfield signals characteristic for ester carbonyls appearing at δ 171.5, 170, 171, 171.9, 171.2, 171.7, 172.6, 168 and 172.8 assigned to C-1, 2, 5, 7, 8, 11, 11', 12' and 7'' respectively. The pyridine carbons resonated at δ 153.6 (C-2'), 152.0 (C-3'), 128.7 (C-4'), 127.5 (C-5'), 153.0 (C-6') and six phenyl carbons at δ 166.6 (C-1''), 131.1 (C-2'', 6''), 130.1 (C-3'', 5''). 2D NMR spectra (**Table 3.3** and **Fig.3.3**) of **3** indicated the pyridine exhibited as 2,4-disubstituted instead of 2,5-disubstituted pyridine in **2**.

In the HMBC spectrum of compound **3** long range correlations were also observed H-1/C-11, COO-1; H-2/C-9, COO-2; H-3/C-10, 12, 11'; H-4/C-2, 5; H-5/C-7;

H-6/C-10; H-7/C-9, 13; H-11/C-1, 8, COO-11; and H-15/C-13, COO-12', which confirmed the presence of dihydroagarofuran sesquiterpene skeleton. The relative stereochemistry of **3** was confirmed by NOESY spectrum and comparison of spectral data reported compounds in the literature.^{121,122}

All spectral data and comparison with related compounds, with in agreement that the structure of **3** as 1 β ,2 β ,5 β ,7 β ,8 β ,11-hexaacetoxy-3 α ,15-[2'-methyl-2' β -benzoate-4'(4"-carboxy-2"-pyridyl)-butanoic acid] dicarbollactone-dihydroagarofuran (royleanine C).

Table-3.3: ^1H (600 MHz) and ^{13}C NMR (125 MHz) spectral data of royleanine C (3) in CD_3OD

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C-1	75.8 CH	5.79	d, $J=3.6$	C 11, COO-1
C-2	71.3 CH	5.47	dd, $J=2.4, 3.6$	C 9, COO-2
C-3	77.3 CH	4.80	d, $J=2.8$	C 10, 12, 11'
C-4	39.1 CH	2.76	d, $J=7.8$	C 2, 5, 9
C-5	76.5 CH	6.41	brd. s	C 7, 13,
C-6	50.6 CH	2.76	d, $J=3.0$	C 10, 15
C-7	74.2 CH	5.71	dd, $J=9.6, 3.0$	C9, 13, COO-7
C-8	74.7 CH	5.73	d, $J=9.6$	C1, 6, COO-8
C-9	51.1 C	-	-	-
C-10	92.4 C	-	-	-
C-11	62.3 CH_2	5.05 H_a 4.61 H_b	d, $J=12.6$ d, $J=12.6$	C 1, 8, COO-11 C 8, 9, COO-11
C-12	16.1 CH_3	1.38	d, $J=7.8$	C 3, 10
C-13	85.9 C	-	-	-
C-14	19.3 CH_3	1.63	s	C 6, 15
C-15	71.9 CH_2	4.14 5.24	d, $J=11.4$ d, $J=11.4$	C 6, COO-12' C13, COO-12'
C-2'	153.6 C	-	-	-
C-3'	152.0 CH	8.87	brd. s	C 5', 7', 12'
C-4'	128.7 C	-	-	-
C-5'	127.5 CH	7.50	d, $J=4.8$	C 3', 12'
C-6'	153.0 CH	8.61	d, $J=4.8$	C2', 4', 12'
C-7'	32.2 CH_2	3.30 H_a 2.76 H_b	ddd, $J=12.6, 5.5, 5.4$ ddd, $J=12.6, 3.6, 3.0$	C 2', 3', 9'
C-8'	41.1 CH_2	2.42 H_a 2.00 H_b	m m	C 2', 10', 11' C 7', 10', 11'
C-9'	81.8 C	-	-	-
C-10'	21.0 CH_3	1.97	-	C 8', 11'
C-11'	172.6 C	-	-	-
C-12'	168.0 C	-	-	-
C-1''	166.6 C	-	-	-
C-2'',6''	131.1 $\text{CH}\times 2$	8.07	dd, $J=1.2, 7.8$	C 4', 6', 7'
C-3'',5''	130.1 $\text{CH}\times 2$	7.54	t, $J=7.2$,	C 1''
C-4''	135.1 CH	7.67	t, $J=7.2$	C 2'', 6''
C-7''	172.8 C	-	-	-
1-Ac	21.3 CH_3	2.22	s	COO-1
2-Ac	21.0 CH_3	1.85	s	COO-2
5-Ac	20.9 CH_3	1.74	s	COO-5
7-Ac	21.3 CH_3	2.06	s	COO-7
8-Ac	21.3 CH_3	2.01	s	COO-8
11-Ac	21.4 CH_3	2.28	s	COO-11
1-CO	171.5 C	-	-	-
2-CO	170.0 C	-	-	-
5-CO	171.0 C	-	-	-
7-CO	171.9 C	-	-	-
8-CO	171.2 C	-	-	-
11-CO	171.7 C	-	-	-

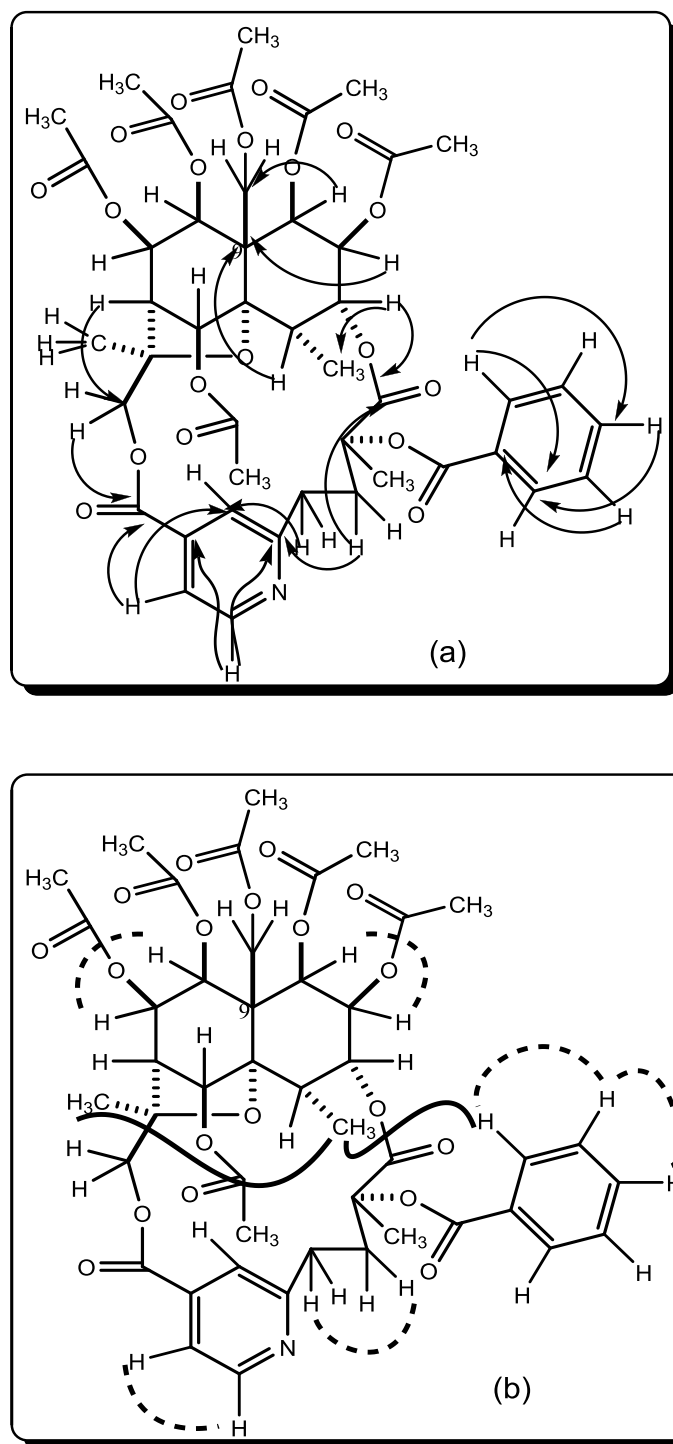


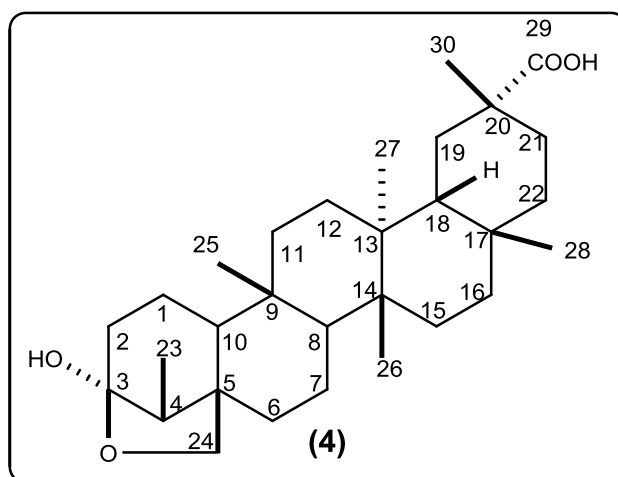
Fig.3.3. a) Important HMBC correlations observed in **3**.

b) COSY (dotted arrow) and NOESY correlations (plane line) observed in **3**.

3.2.2 Known compounds isolated from the roots of *Maytenus royleanus*

3.2.2.1 Salaspermic acid (4)

Compound **4** was isolated as white powder. The molecular formula $C_{30}H_{48}O_4$ was established through HREIMS showing molecular ion peak at m/z 472.2353 (calcd. 472.3553). The EIMS spectrum of compound **4** showed diagnostic peaks at m/z 125.0, 135.1, 235.1, 289.1 and 272.2 characteristic for 3-oxofriedelane skeleton.¹²³ The IR spectrum exhibited the presence of carbonyl and hydroxyl group at 1685 and 3400 cm^{-1} respectively.



The ^1H NMR spectrum (**Table 3.4**) of compound **4** displayed the presence of a secondary methyl, five tertiary methyls, an oxymethylene moiety and absence of unsaturations confirmed the presence of friedelane skeleton,¹²⁴ which was confirmed by ^{13}C NMR spectrum (**Table 3.4**).

The ^1H and ^{13}C NMR assignments are based on 2D experiments (**Table 3.4** and **Fig. 3.4**) and comparison of the data with reported compounds in the literature^{123,124} suggested structure **4** as 3-oxo-friedelan-29-oic acid hemiacetal.

Table 3.4: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in $\text{C}_5\text{D}_5\text{N}$ and HMBC correlations of salaspermic acid (**4**)

C.No	¹³ CNMR(δ)		¹ HNMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C1	20.6	CH ₂	1.84 H _a 1.57 H _b	m m	C3, C5 C3, C5
C2	39.2	CH ₂	2.19 H _a 1.89 H _b	dd, J=12.0, 5.5 dt, J=12.0, 5.5,	C4,C10
C3	106.0	C			
C4	54.0	CH	1.49	q, J=7.0	C2, C10
C5	47.2	C			
C6	34.1	CH ₂	1.68 H _a 1.35 H _b	m m	C6, C10
C7	19.6	CH ₂	1.29 H _a 1.01 H _b	m m	C5, C9, C14
C8	50.3	CH	1.23	m	C6, C10, C15
C9	37.7	C			
C10	57.4	CH	1.14	m	C2, C4, C6, C8
C11	34.8	CH ₂	1.32 H _a 1.19 H _b	m m	C8, C10, C13, C25
C12	29.7	CH ₂	1.66 H _a 1.31 H _b	m m	C9, C14
C13	39.6	C			
C14	39.3	C			
C15	29.5	CH ₂	1.72 H _a 1.07 H _b	m m	C8, C13, C17
C16	36.7	CH ₂	1.64 H _a 1.39 H _b	m m	C14, C18
C17	30.5	C			
C18	44.8	CH	1.55	m	C14, C16
C19	30.9	CH ₂	2.53	d, J=13.5	
C20	40.7	C			
C21	30.5	CH ₂	2.68	dd, J=14.0, 4.0	
C22	37.5	CH ₂	2.36	dt, J=14.0, 4.0	
C23	8.6	CH ₃	1.19	d, J=7.0	C3, C4, C5
C24	73.1	CH ₂	4.25 H _a 3.70 H _b	d, J=8.0 d, J=8.0	C4, C6, C10 C4, C6, C10
C25	16.9	CH ₃	0.82	s	C8, C10, C11
C26	18.1	CH ₃	1.25	s	C8, C13, C14, C15
C27	16.7	CH ₃	0.89	s	C12, C13, 18
C28	32.1	CH ₃	1.12	s	C17, C18, C22
C29	181.5	C			
C30	32.4	CH ₃	1.42	s	C19, C20, C21

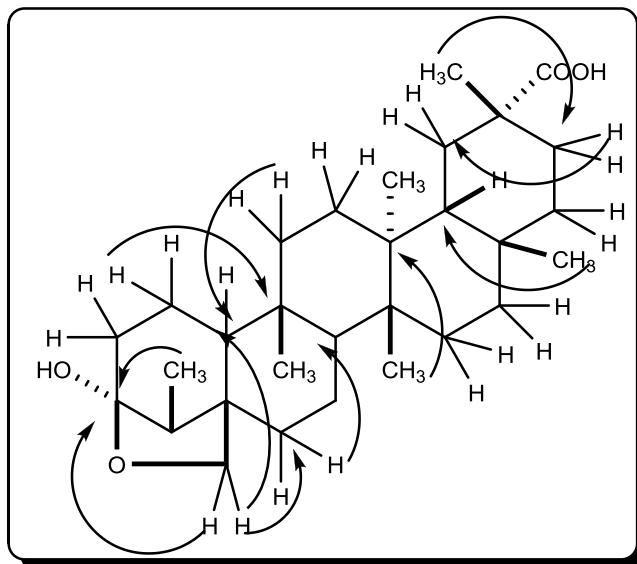
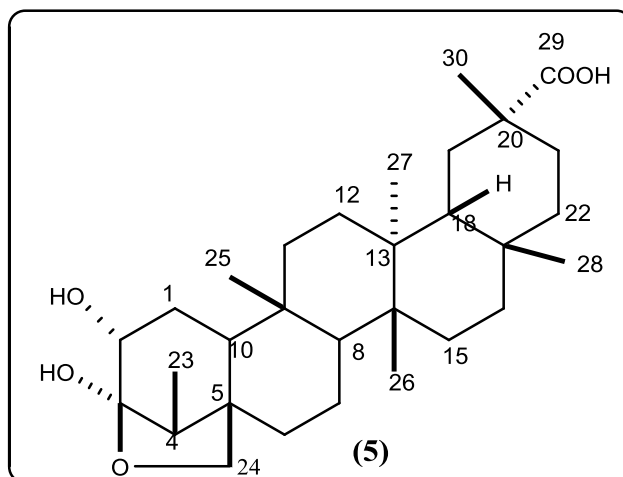


Fig.3.4. Important HMBC correlations observed in **4**.

3.2.2.2 Orthosphenic acid (5)



Compound **5** was isolated as white amorphous solid. The EIMS exhibited molecular ion peak at m/z 488 for $C_{30}H_{48}O_5$ which was depicted from HREIMS 488.3532 (calcd. 488.3502 for $C_{30}H_{48}O_5$). Characteristic peaks of compound **5** in the EIMS at m/z 125, 189.1, 305 and 383. The IR spectrum showed the presence of carboxyl and hydroxyl group.

Both ^1H and ^{13}C (**Table 3.5**) NMR spectra confirmed the presence of thirty carbon atoms in compound **5**; five methines, eleven methylenes, six methyls and eight quaternary carbon atoms in compound **5**. The assignments were made on the basis of 2D NMR spectra (**Table 3.5** and **Fig. 3.5**).

All the spectral data (**Table 3.5**) in comparison with reported compounds^{123,124} suggested that compound **5** as 2*R*,3*R*,7*bR*,9*aS*,12*R*,13*bS*,15*aR*,16*R*)-2,3-dihydroxy-7*b*,9*a*,12,13*b*,15*a*,16-hexamethyl-cosahydro-3,5*a*-methanochryseno[2,1-*c*]oxepine-12-carboxylic acid (orthosphenic acid).

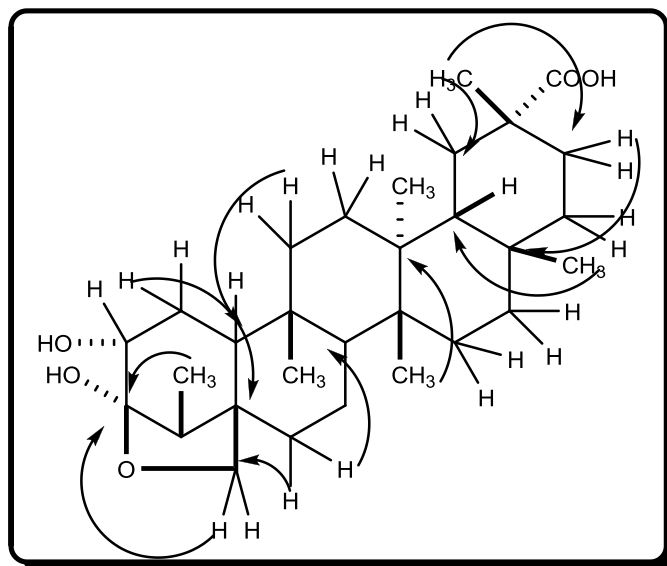
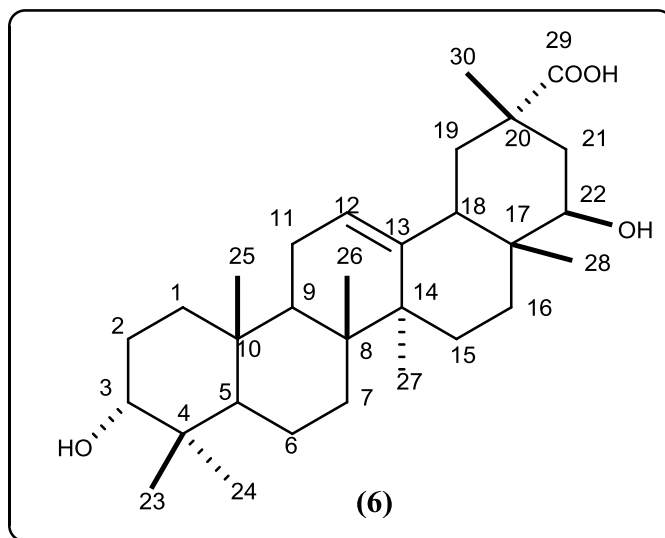


Fig.3.5. Important HMBC correlations observed in 5.

Table 3.5: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in CD_3OD and HMBC correlations of orthosphenic acid (**5**)

C.No	¹³ C NMR(δ)		¹ H NMR(δ)	Multiplicity & <i>J</i> (HH in Hz)	HMBC
C1	28.9	CH ₂	1.87 H _a 1.60 H _b	m m	C3, C5 C3, C5
C2	74.4	CH	2.19	d, <i>J</i> =3.5	C4,C10
C3	108.2	C			
C4	51.3	CH	1.35	q, <i>J</i> =7.0	C2, C10
C5	48.3	C			
C6	34.8	CH ₂	1.77 H _a 1.50 H _b	m m	C6, C10
C7	20.4	CH ₂	1.47 H _a 1.37 H _b	m m	C5, C9, C14
C8	47.5	CH	1.95	q, <i>J</i> =7.0	C6, C10, C15
C9	38.2	C			
C10	53.9	CH	1.53	m	C2, C4, C6, C8
C11	35.4	CH ₂	1.38 H _a 1.32 H _b	m m	C10, C13, C25
C12	30.3	CH ₂	1.54 H _a 1.33 H _b	m m	C9, C14
C13	40.4	C			
C14	40.3	C			
C15	30.2	CH ₂	1.45 H _a 1.23 H _b	m m	C8, C13, C17
C16	37.5	CH ₂	1.74 H _a 1.36 H _b	m m	C14, C18
C17	31.0	C			
C18	46.0	CH	1.55	m	C14, C16
C19	31.5	CH ₂	2.37	d, <i>J</i> =13.0	
C20	41.4	C			
C21	31.3	CH ₂	2.04	dd, <i>J</i> =13.0, 4.0	
C22	37.8	CH ₂	2.07	dt, <i>J</i> =13.0, 4.0	
C23	7.7	CH ₃	0.91	d, <i>J</i> =7.5	C3, C4, C5
C24	72.9	CH ₂	4.03 H _a 3.57 H _b	d, <i>J</i> =8.0 d, <i>J</i> =8.0	C4, C6, C10 C4, C6, C10
C25	17.3	CH ₃	0.96	s	C8, C10, C11
C26	18.5	CH ₃	1.00	s	C8, C13, C15
C27	16.9	CH ₃	0.86	s	C12, C13, 18
C28	32.3	CH ₃	1.08	s	C17, C18, C22
C29	182.8	C			
C30	32.6	CH ₃	1.16	s	C19, C20, C21

3.2.2.3 **3 α ,22 β -Dihydroxyolean-12-en-29-oic acid (6)**

Compound **6** was isolated as white amorphous powder. The molecular formula $C_{30}H_{48}O_4$ was established through HREIMS which showed molecular ion peak at m/z 472.3519 (calcd. 472.3474). The EIMS spectrum of compound **6** showed peaks at m/z 95, 246, 254.3 and 472.4 characteristic for 3 α ,22 β -dihydroxyolean-12-en-29-oic acid. The IR spectrum showed the presence of carboxyl and hydroxyl group at 1705 and 3438 cm^{-1} respectively.

The ^1H and ^{13}C spectra (**Table 3.6**) of compound **6** indicated the presence of seven methyls, eight quaternary, nine methylenes and five methine groups. The ^1H NMR spectrum showed two oxygenated methine groups resonated at δ 3.42 (dd, $J=15.0, 5.0$ Hz; H-3), 4.02 (dd, $J=5.0, 2.5$ Hz; H-22) and one olefinic proton resonated at δ 5.40 (brd. s, H-12), while ^{13}C NMR spectrum (**Table 3.6**) of compound **6** displayed characteristic peaks of two oxygenated methine carbons at δ 3.42 (C-3), 4.02 (C-22), and one

carboxylic acid carbon resonated at δ 180.9. All these assignments were made by HMBC long range correlations (**Table 3.6** and **Fig. 3.6**).

On the basis of spectral data and comparison with known compounds¹²⁵⁻¹²⁷ the structure of **6** as 3 α ,22 β -dihydroxyolean-12-en-29-oic acid.

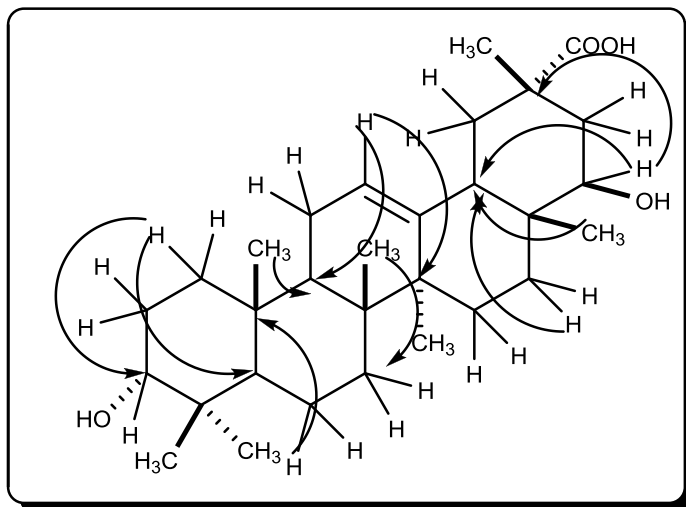
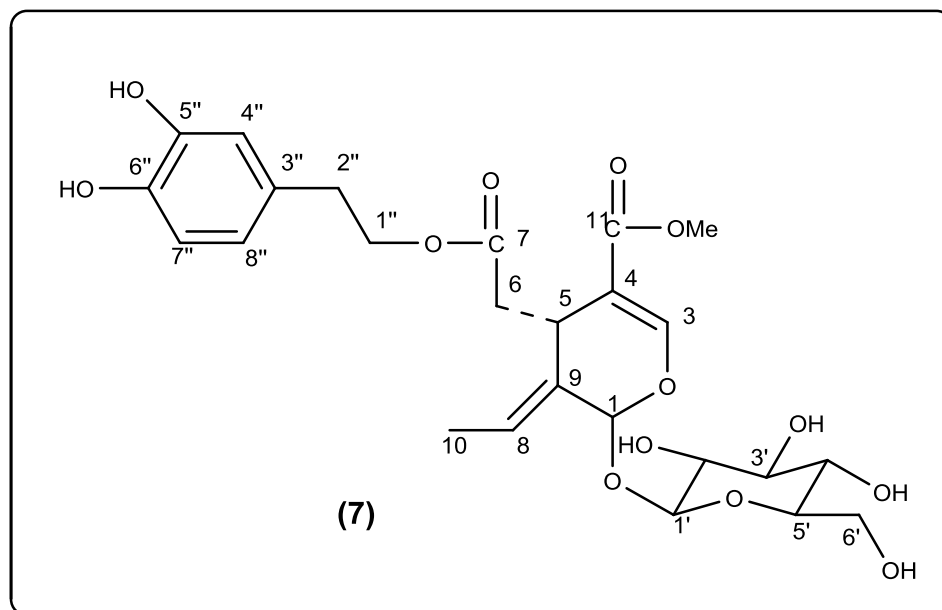


Fig.3.6. Important HMBC correlations observed in **6**.

Table 3.6: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in $\text{C}_5\text{D}_5\text{N}$ and HMBC correlations of $3\alpha,22\beta$ -dihydroxyolean-12-en-29-oic acid (**6**)

C.No	¹³ C NMR(δ)		¹ H NMR(δ)	Multiplicity & <i>J</i> (HH in Hz)	HMBC
C1	38.7	CH ₂	2.62 H _a 2.31 H _b	m m	C3, C5
C2	26.2	CH ₂	2.12 H _a 1.83 H _b	m m	C4,C10
C3	78.0	CH	3.42	dd, <i>J</i> =15.0, 5.0	
C4	37.3	C			
C5	48.0	CH	1.67	t, <i>J</i> =5.0	
C6	18.8	CH ₂	1.57 H _a 1.42 H _b	m m	C8, C10
C7	32.9	CH ₂	1.54 H _a 1.33 H _b	m m	C5, C9, C14
C8	39.4	C			
C9	47.3	CH	2.33	m	
C10	39.2	C			
C11	19.9	CH ₂	2.09 H _a 1.89 H _b	m m	C8, C13,
C12	123.2	CH	5.40	br. s	C9, C14
C13	144.2	C			
C14	42.5	C			
C15	23.9	CH ₂	1.94	m	C13, C17
C16	28.2	CH ₂	1.87 H _a 1.78 H _b	m m	C14, C18
C17	40.2	C			
C18	55.7	CH	2.33	m	C14, C16
C19	39.1	CH ₂	1.54 H _a 1.01 H _b	t, <i>J</i> =13.8 m	
C20	43.3	C			
C21	41.2	CH ₂	2.72 H _a 1.78 H _b	m m	
C22	74.6	CH	4.02	dd, <i>J</i> =5.0, 2.5	
C23	28.7	CH ₃	1.05	s	C3, C5
C24	17.0	CH ₃	1.22	s	C3, C5
C25	15.8	CH ₃	0.97	s	C1, C9
C26	16.6	CH ₃	1.05	s	C9, C14
C27	26.4	CH ₃	1.28	s	C7, C15
C28	21.3	CH ₃	1.58	s	C16, C22
C29	180.9	C			
C30	25.4	CH ₃	1.37	s	C19, C21, C29

3.2.2.4 Oleuropein (7)



Compound **7** was shown to have the molecular formula $C_{25}H_{32}O_{13}$ by analysis of the HRESIMS m/z 540.5138 (calcd. 540.1843). The IR spectrum indicated the presence of two carbonyl and hydroxyl groups at 1630, 1695 and 3400 cm^{-1} respectively. The ^{13}C NMR spectra (BB and DEPT; **Table 3.7**) of **7** confirmed the presence of seven quaternary carbons, twelve methine carbons, four methylene carbons and two methyl carbons. The ^1H NMR spectrum the olefinic proton resonated at δ 7.50 (s, H-3), an acetalcarbinol proton appeared at δ 5.90 (brd. s; H-1), two methine protons at δ 3.96 (m, H-5), 6.06 (q, $J=6.5\text{ Hz}$; H-8), one methylene group at δ 2.65 (dd, $J=9.0, 14.0\text{ Hz}$; H-6_a), 2.42 (dd, $J=4.5, 14.0\text{ Hz}$; H-6_b), a methyl group δ 1.65 (d, $J=7.0\text{ Hz}$; H-10) and one methoxy group at δ 3.70 (s, OMe-11) confirmed the presence of aglycone moiety. The peaks of glucose moiety and phenylethyl protons were also observed in the ^1H NMR spectrum (**Table 3.7**) at δ 4.79 (d, $J=8.0\text{ Hz}$; H-1'), 6.53 (dd, $J=7.0, 2.0\text{ Hz}$; H-4''), 6.67 (d, $J=8.0, \text{ Hz}$; H-7'') and 6.65 (d, $J=2.0, \text{ Hz}$; H-8''). The carbon connectivity and

assignment of protons were established by COSY, HMQC and HMBC spectra (**Fig. 3.7**). The spectral data of **7** is identical to oleuropein:¹²⁸ as (4*S*,*E*)-methyl 4-(2-(3,4-dihydroxyphenethoxy)-2-oxoethyl)-3-ethylidene-2-((2*S*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)-3,4-dihydro-2*H*-pyran-5-carboxylate.

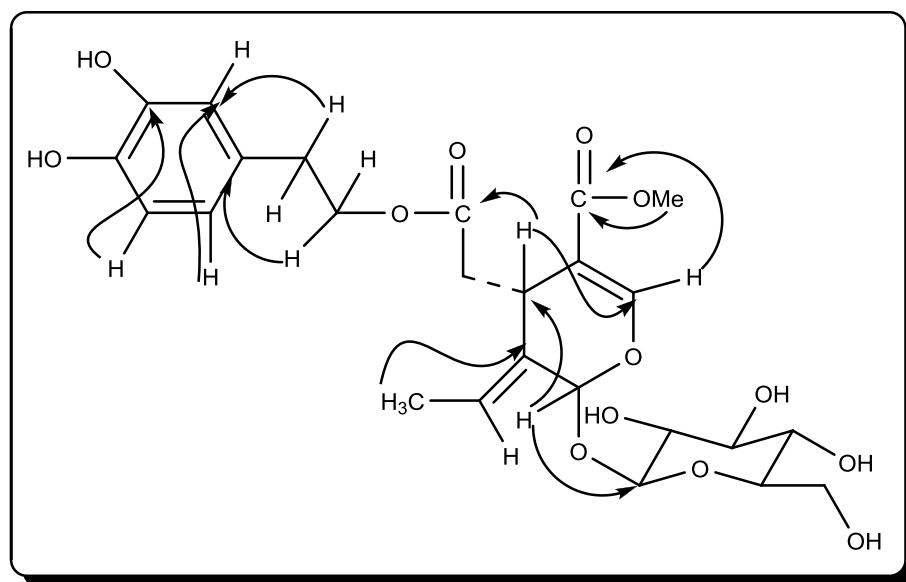
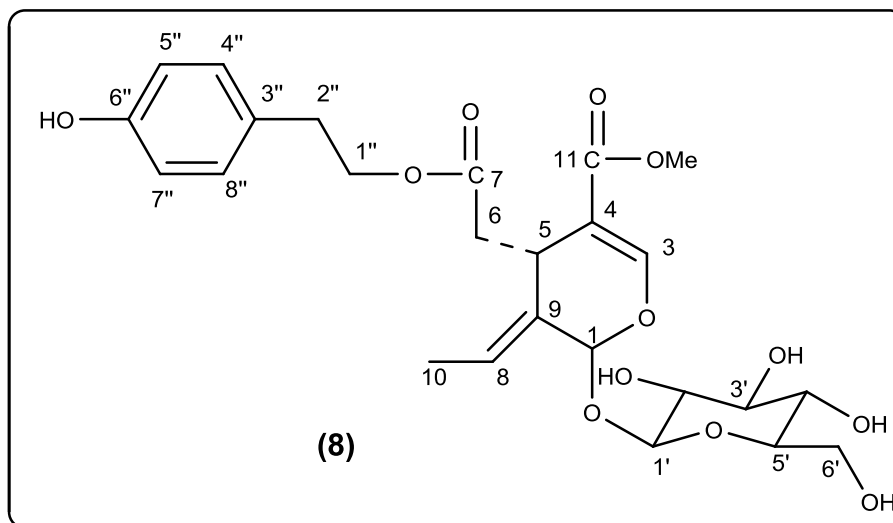


Fig.3.7. Important HMBC correlations observed in **7**.

Table 3.7: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in CD_3OD and HMBC correlations of oleuropein (7)

C.No	^{13}C NMR(δ)		^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
Aglycone					
C1	95.1	CH	5.90	brd. s	C3, C5, C1'
C3	155.2	CH	7.50	s	C5, C11
C4	109.4	C			
C5	31.8	CH	3.96	dd, $J=9.0, 4.5$	C7, C11
C6	41.3	CH ₂	2.65 H _a 2.42 H _b	dd, $J=9.0, 14.0$ dd, $J=4.5, 14.0$	C4, C9
C7	173.2	C			
C8	124.9	CH	6.06	q, $J=7.0$	C1
C9	130.4	C			
C10	13.6	CH ₃	1.65	d, $J=7.0$	C9
C11	168.7	C			
OMe	52.0		3.70	s	C11
Glucose					
C1'	100.9	CH	4.79	d, $J=8.0$	
C2'	71.4	CH			
C3'	74.7	CH			
C4'	77.9	CH			
C5'	78.4	CH			
C6'	62.7	CH ₂	3.86 H _a 3.67 H _b	m m	
Phenylethyl					
C1''	64.9	CH ₂	3.67	m	C7, C3''
C2''	35.4	CH ₂	2.75	t, $J=7.0$	C4'', C8''
C3''	130.7	C			
C4''	121.3	CH	6.53	dd, $J=7.0, 2.0$	C6'', C8''
C5''	144.9	C			
C6''	146.2	C			
C7''	116.3	CH	6.67	d, $J=8.0$	C1, C9
C8''	117.1	CH	6.65	d, $J=2.0$	C9, C14

3.2.2.5 Ligutroside (**8**)

Compound **8** was isolated as white powder and IR spectrum displayed the presence of carbonyl groups and hydroxyl group at 1625, 1700 and 3400 cm^{-1} respectively. The molecular formula $\text{C}_{25}\text{H}_{32}\text{O}_{12}$ was established by HREIMS showing molecular ion peak at m/z 524.5144 (calcd. 524.1894 for $\text{C}_{25}\text{H}_{32}\text{O}_{12}$). The ^1H NMR spectra of compound **8** showed the presence of thirteen methine groups and five quaternary carbons. The ^1H NMR further showed spectrum the *p*-substituted phenyl protons resonated at δ 7.04 (d, $J = 8.5$ Hz; H-4'', 8''), 6.70 (d, $J = 6.5$ Hz; H-5'', 7''), ethyl protons of the phenylethyl moiety appeared at δ 4.21 (m, H_a-1''), 4.10 (m, H_b-1'') and 2.81 (t, $J = 7.0$ Hz; H-2''). The ^{13}C NMR spectrum showed aglycone carbons resonated at δ 95.1 (C-1), 155.2 (C-3), 109.4 (C-4), 31.8 (C-5), 41.2 (C-6), 173.2 (C-7), 124.9 (C-8), 130.4 (C-8), 13.6 (C-10), 168.6 (C-11) and 52.0 (OMe). All the spectral data (**Table 3.8** and **Fig. 3.8**) of **8** were in agreement to ligutroside.¹²⁸

Table 3.8: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in $\text{C}_5\text{D}_5\text{N}$ and HMBC correlations of ligutroside (**8**)

C.No	¹³ C NMR(δ)		¹ H NMR(δ)	Multiplicity & <i>J</i> (HH in Hz)	HMBC
Aglycone					
C1	95.1	CH	5.92	s	C3, C5
C3	155.2	CH	7.50	s	
C4	109.4	C			
C5	31.8	CH	3.96	m, <i>J</i> =9.5, 4.5	C8, C10
C6	41.2	CH ₂	2.68 H _a	dd, <i>J</i> =4.5, 14.0	
			2.43 H _b	dd, <i>J</i> =9.5, 14.0	
C7	173.2	C			
C8	124.9	CH	6.06	q, <i>J</i> =7.0	
C9	130.4	C			
C10	13.6	CH ₃	1.63	d, <i>J</i> =7.5	
C11	168.6	C			
OMe	52.0		3.70	s	C9, C14
Glucose					
C1'	100.8	CH	4.80	d, <i>J</i> =8.0	
C2'	71.5	CH			
C3'	74.8	CH			
C4'	77.9	CH			
C5'	78.5	CH		m	
C6'	62.8	CH ₂	3.85 H _a	dd, <i>J</i> =6.0, 12.0	C14, C16
			3.67 H _b	dd, <i>J</i> =1.0, 12.0	
Phenylethyl					
C1''	66.9	CH ₂	4.21 H _a	m	
			4.10 H _b	m	
C2''	35.2	CH ₂	2.81	t, <i>J</i> =7.0	
C3''	130.0	C			
C4''	131.0	CH	7.04	d, <i>J</i> =8.5	
C5''	116.3	CH	6.70	d, <i>J</i> =8.5	
C6''	157.1	C			
C7''	116.3	CH	6.70	d, <i>J</i> =8.5	C1, C9
C8''	131.0	CH	7.04	d, <i>J</i> =8.5	C9, C14

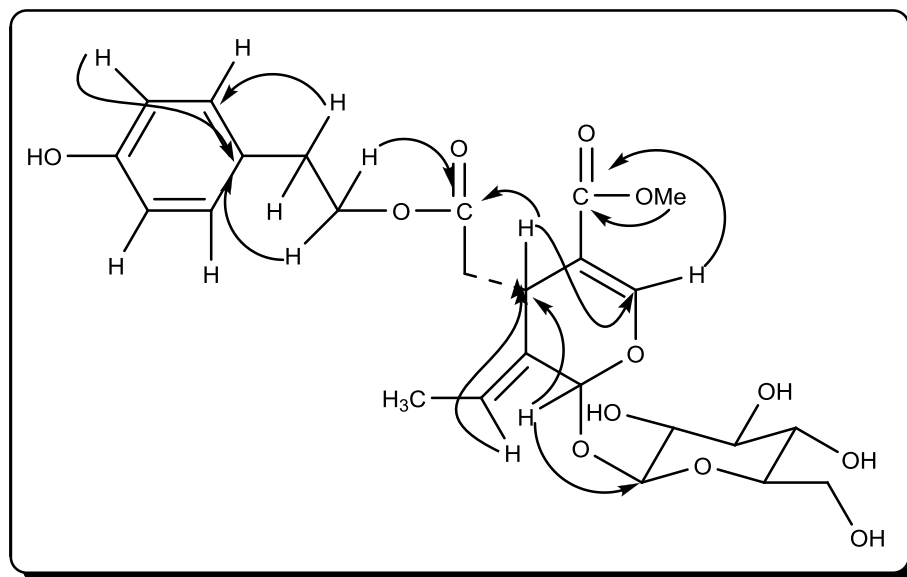
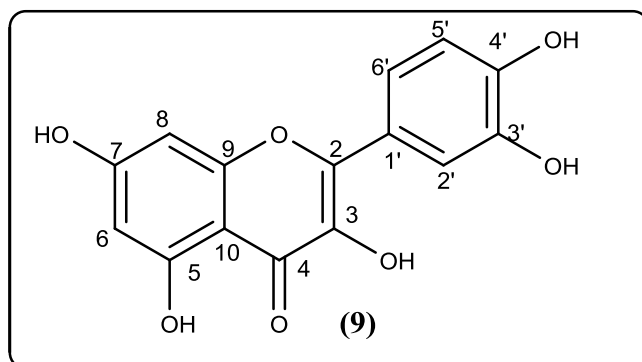


Fig.3.8. Important HMBC correlations observed in **8**.

3.2.2.6 Quercetin (9)



Compound **9** isolated as yellow powder. The EIMS and ESIMS of **9** showed molecular ion peak at m/z 302.1 and 303.0 $[M^+ + H]$ respectively, while the molecular formula $C_{15}H_{10}O_7$ was established by HREIMS m/z 302.2357 (calcd. 302.0427). The IR displayed maximum absorption at 3293, 1616, 1511 and 1166 indicated the presence of hydroxyl, carbonyl, aromatic ring and ether group respectively. The ^1H and ^{13}C (BB and DEPT) NMR spectra (**Table 3.9**) of **9** showed the presence of ten quaternary and five methines carbons. 3,5,7-Trihydroxy-chromen-4-one skeleton¹²⁹ was confirmed by appearing of two one proton singlet(s) at δ 6.17 (H-6), 6.38 (H-8) while the remaining downfield signals appeared at δ 7.73 (s, H-2'), 6.87 (d, $J=8.5$ Hz; H-5') and 6.62 (d, $J=8.5$ Hz; H-6') were assigned to the disubstituted phenyl ring attached at C-2 to chromen skeleton¹²⁹ in the ^1H NMR spectrum which was further confirmed by ^{13}C NMR (**Table 3.9**). All these assignments were made by HMBC and COSY correlations (**Table 3.9** and **Fig. 3.9**).

The spectral data and comparison with quercetin¹³¹ indicated that compound **9** as 2-(3',4'-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one.

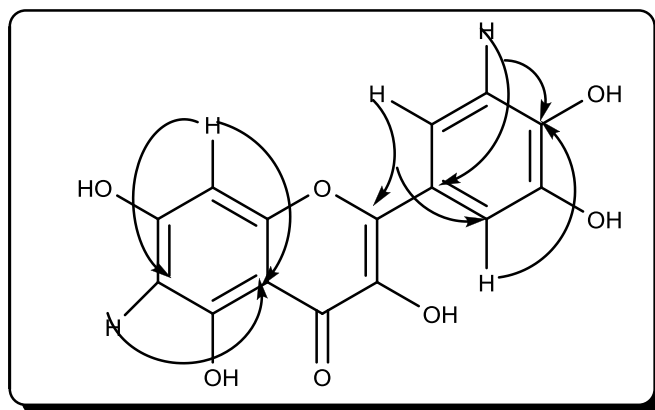
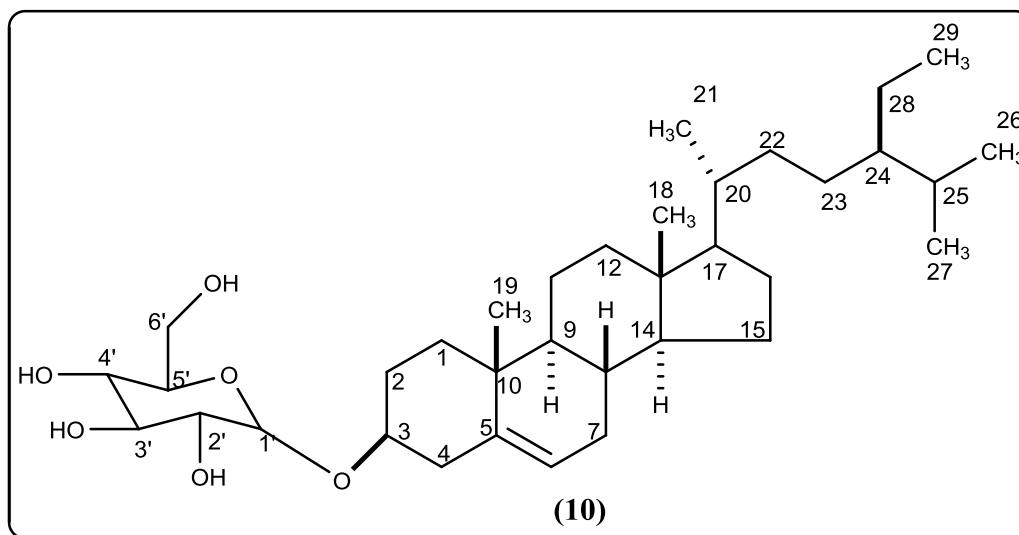


Fig.3.9. Important HMBC correlations observed in **9**.

Table 3.9: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in CD_3OD and HMBC correlations of quercetin (**9**).

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C2	146.2 C			
C3	137.3 C			
C4	177.4 C			
C5	162.5 C			
C6	99.3 CH	6.17	s	C8, C10
C7	165.6 C			
C8	94.4 CH	6.38	s	C10
C9	158.3 C			
C10	104.5 C			
C1'	124.2 C			
C2'	116.0 CH	7.73	s	C2, C4', C-6'
C3'	148.0 C			
C4'	148.8 C			
C5'	116.2 CH	6.87	d, $J=8.5$	C2, C-4'
C6'	121.7 CH	6.62	d, $J=8.5$	

3.2.2.7 β -Sitosterol-3-O- β -D-glycopyrannoside (**10**)

Compound **10** was isolated as white powder. The molecular formula $C_{35}H_{60}O_6$ was established through HRESIMS showing molecular ion peak at m/z 576.4390 (calcd. 576.8473 for $C_{35}H_{60}O_6$) while the EIMS m/z 397 $[M^+ + H]$ indicated the loss of glycoside moiety. The IR spectrum showed absorption peaks at 3449, 3045 and 1620 cm^{-1} indicated the presence of hydroxyl group, C-H olefinic and C=C olefinic respectively. The ^1H and ^{13}C (BB and DEPT; **Table 3.10**) spectra of **10** indicated the presence of three quaternary carbons, six methyls, twelve methylenes and fourteen methine groups; characteristic of β -sitosterol-3-O- β -D-glycopyrannoside.¹³² The spectral data (1D and 2D NMR; **Table 3.10** and **Fig. 3.10**) of **10** was tantamount to the reported data of the β -sitosterol-3-O- β -D-glycopyrannoside in literature.¹³²

Table-3.10: ^1H (600 MHz) and ^{13}C NMR (125 MHz) spectral data of β -sitosterol-3-*O*- β -D-glycopyrannoside (**10**) in $\text{C}_5\text{D}_5\text{N}$

C.No	¹³ C NMR(δ)		¹ H NMR(δ)	Multiplicity & <i>J</i> (HH in Hz)	HMBC
C-1	37.5	CH ₂	1.73 H _a /1.68 H _b	m	C9, 5, 19
C-2	30.3	CH ₂	2.14 H _a 2.10 H _b	m	C4,2'
C-3	78.5	CH	3.97	m	C1', 5
C-4	40.0	CH ₂	1.98 H _a /1.94 H _b	brd. s	
C-5	141.0	C			
C-6	122.0	CH	5.34	d, <i>J</i> =4.8	C8, 10
C-7	32.2	CH ₂	1.83 H _a /1.37 H _b	m	C5,9
C-8	32.1	CH	1.56	m	
C-9	50.4	CH	0.91	m	
C-10	37.0	C			
C-11	21.3	CH ₂	1.39 H _a /1.34 H _b	m	C8, 10, 12
C-12	39.3	CH ₂	2.72 H _a 2.47 H _b	dd, <i>J</i> =13.2, 2.7 t, <i>J</i> =11.4	C17, 18
C-13	42.5	C	-	-	
C-14	56.8	CH	1.10	m	
C-15	24.5	CH ₂	1.52 H _a	m	
C-16	28.5	CH ₂	1.38 H _a 1.23 H _b	m m	
C-17	56.3	CH	1.07	m	C15, 22
C-18	12.0	CH ₃	0.65	s	
C-19	19.4	CH ₃	0.92	s	
C-20	36.4	CH	1.35	m	C16, 23
C-21	19.0	CH ₃	0.95	d, <i>J</i> =6.0	
C-22	34.2	CH ₂	1.40 H _a 1.01 H _b	m m	C21, 24
C-23	26.4	CH ₂	1.27	m	C28
C-24	46.0	CH	0.98	m	
C-25	29.5	CH	1.85	m	
C-26	19.2	CH ₃	0.84	d, <i>J</i> =6.3	
C-27	21.3	CH ₃	0.86	s	C24
C-28	24.5	CH ₂	1.32 H _a 1.29 H _b	m m	C23, 25
C-29	12.2	CH ₃	0.88	s	C24
C-1'	102.6	CH	5.05	d, <i>J</i> =7.8	C3
C-2'	75.3	CH	4.05	m	
C-3'	78.6	CH	4.28	t, <i>J</i> =5.1	
C-4'	71.7	CH	4.27	t, <i>J</i> =5.1	
C-5'	78.1	CH	3.93	m	
C-6'	62.8	CH ₂	4.56 H _a 4.41 H _b	d, <i>J</i> =11.4 dd, <i>J</i> =11.4, 5.4	

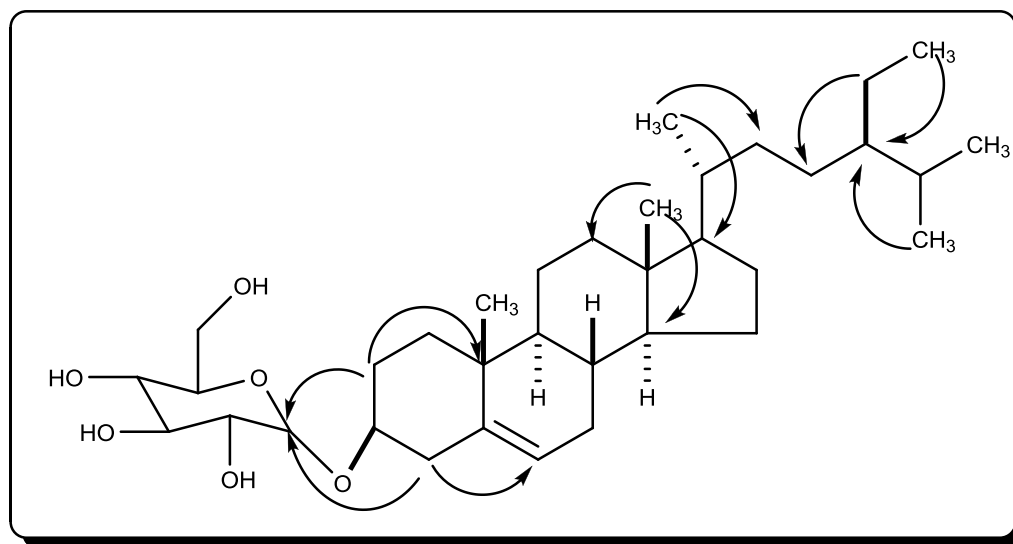


Fig.3.10. Important HMBC correlations observed in **10**.

Chapter 4

Experimental Section

4.0 General spectroscopic and physical methods

4.1 General experimental procedures

NMR spectra of pure compounds were recorded on Varian 500, 600 INOVA MHz (cold probe) and Bruker AV 500 spectrometers, operated at 500, 600 (^1H NMR) and 125 MHz (^{13}C NMR) respectively. EI mass spectra were recorded on JEOL JMS600 and Finnigan-Mat-311 mass spectrometer. Low and high resolution ESI mass spectra were recorded on Mariner ESI-TOF mass spectrometer. IR spectra were recorded on Perkin-Elmer 2000 and Nicolet-380 FT-IR spectrophotometers. The optical rotation was recorded on JASCO P-2000 Polarimeter. UV spectra were recorded on Kontron-Uvikon 930 spectrophotometer (Kontron instruments AG, Zürich, CH). Melting point was determined on BUCHI 535 (SWISS) instrument.

Column chromatography (CC) silica gel 60 (70-230 mesh, Merck) was used and the purity of compounds were checked by Merck TLC aluminum sheets of 0.2 mm thickness (20×20 cm, Kieselgel 60 F254 specification). Detection of constituents were carried out under UV (254 and 365 nm), spraying with 1% $\text{Ce}(\text{SO}_4)_2$ in 10% aqueous H_2SO_4 , Dragendorff's reagent and with I_2 vapors/solution. The LCMS data for confirming the purity of the compounds (MeOH/ H_2O with 0.1% FA) was recorded by using a Phenomenex Luna column C_{18} RP (5 μm , 150 × 4.6) on Sedex 55 ELS detector, 996 photodiode array detector in combination with ESI-TOF-MS (+ve). Phenomex 5 μm Luna C_{18} RP column (250 × 10) was used in the HPLC connected with UV detector. Organic solvents such as methanol (MeOH), ethanol (EtOH), dichloromethane (DCM), n-hexane, petroleum ether, acetone, ethyl acetate (EtOAc) and pyridine were used for extraction, fractionation, column chromatography, and purification. Analytical grade

solvents (acetonitrile (MeCN) and methanol) were used in HPLC while deuterated solvents were used for NMR analysis.

4.2 Reagents

4.2.1 Dragendorff's reagent

Dragendorff's reagent was used for the identification of alkaloids, appearing as orange/light brown on TLC strip. Solution 1 was prepared by dissolving KI (8 gm) in 20 ml distilled water while solution 2 prepared by dissolving basic bismuth nitrate (0.85 mg) in a mixture of 40 ml distilled water and 10 ml acetic acid. Both solutions were mixed in 1:1 ratio to prepare the stock solution. Spraying reagent was prepared by diluting 5 ml of the stock solution with 90 ml of distilled water and 10 ml acetic acid.

4.2.2. Ceric sulphate-sulphuric acid

It is common visualizing reagent and all type of oxidizable compounds were located which appeared in different colors. The reagent prepared by mixing saturated ceric sulphate solution with 65 % sulphuric acid (0.26 gm in 100 ml H₂SO₄).

4.2.3. Anisaldehyde-sulphuric acid

The reagent prepared by mixing 0.5 ml of *p*-anisaldehyd with 10 ml glacial acetic acid and 85 ml of methanol was added to the mixture followed by the addition of 5 ml conc. sulphuric acid.

4.2.4. Iodine solution

Iodine solution was prepared by dissolving a mixture of 1g iodine and 10 g potassium iodide (KI) in 100 ml distilled water, followed by the addition of 100 ml of sulphuric acid (20 %). Spraying solution on TLC strip, the unsaturated compound appeared as charred spots after heating TLC strip.

4.3 Methods and materials

4.3.1 Plant material

The roots of *Maytenus royleanus* were collected from the district Buner, Khyber Pakhtunkhwa, Pakistan during the month of June 2008. The plant was identified by taxonomist Mr. Ambara Khan (Degree College Daggar, Buner). The voucher specimen (Bot.10068) was deposited in the Herbarium of Department of Botany, University of Peshawar, Peshawar, Pakistan.

4.3.2 Extraction and isolation

The roots of *Maytenus royleanus* shad dried (13 Kg) and powder by local mechanical grinder were extracted three times at room temperature with methanol. The combined extract was filtered and concentrated under reduced pressure to give thick brownish syrup (380 gm), which was partitioned between n-hexane and water. The n-hexane phase FH1 (20 gm) was concentrated under reduce pressure (**Scheme 4.1**), which was not pursued in the present work. The water soluble phase was subjected for further fractionation. The polarity of the aqueous phase was changed to 10% MeOH/H₂O by addition of 200 ml of MeOH which was extracted with n-hexane (3X, 2L each time) to

obtain FH2 (20 mg) followed by extraction with dichloromethane (DCM) (3X, 2L each time) to obtain DCM soluble phase FD1 (20 gm) concentrated under vacuum. The DCM insoluble aqueous layer polarity was changed to 50% MeOH/H₂O, which was further partitioned into 5% EtOAc/n-hexane and DCM afforded fraction FH3 (7gm) and FD2 (22 gm) respectively (**Scheme 4.2**). The insoluble methanolic aqueous phase was further changed to 70% MeOH/H₂O which was extracted with 10% EtOAc/n-hexane (3X, 2L) and DCM (3X, 2L) obtained fractions FH4 (10 mg) and FD3 (30 mg) respectively. The remaining 70% MeOH/H₂O (FM) phase was not pursued in the present work (**Scheme 4.2**).

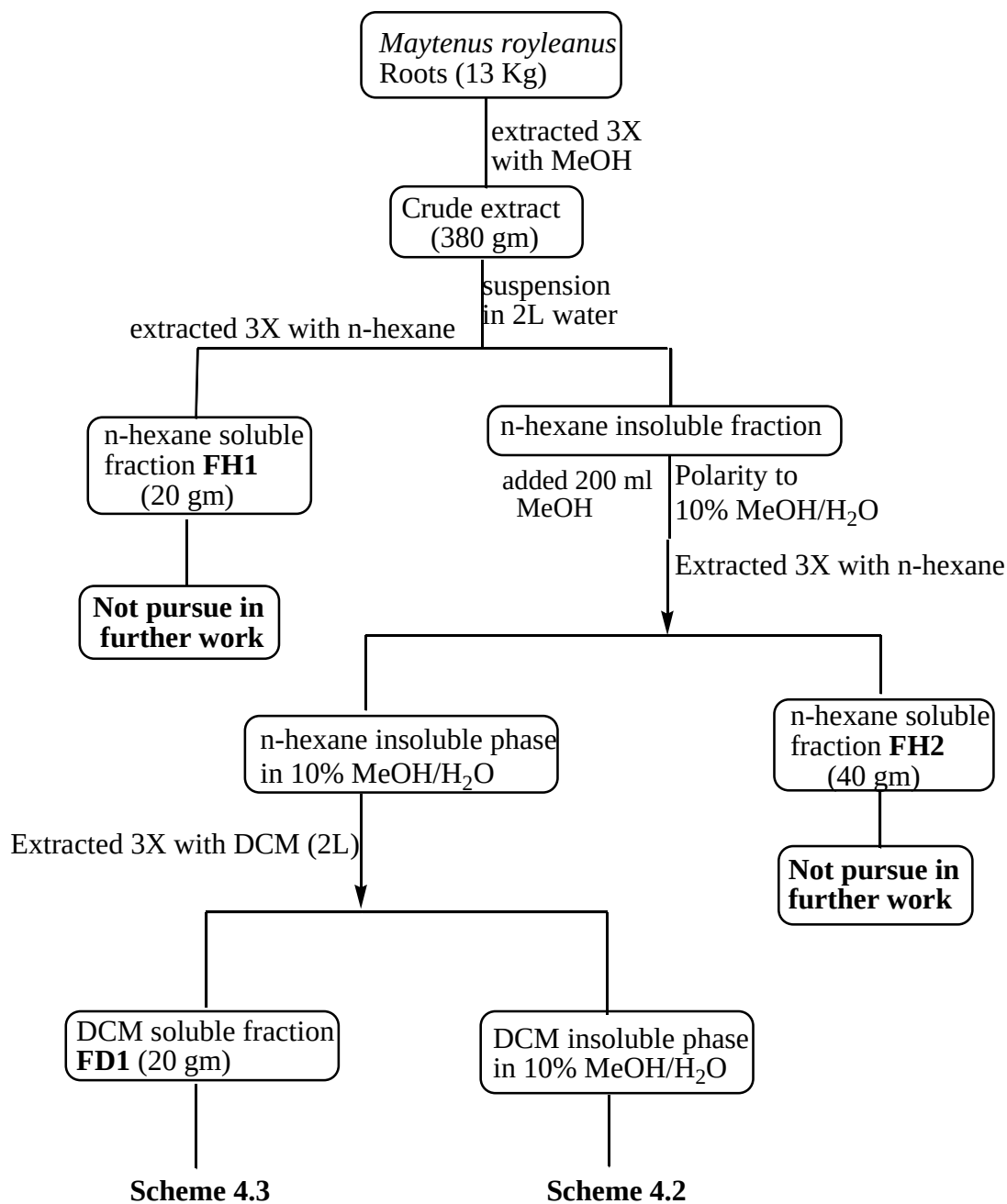
FD1 (20 mg) was subjected to column chromatography (CC) on silica gel 60 (70-230 mesh, Merck) and eluted with solvents n-hexane and ethyl acetate increasing in order polarity. As a result, seven sub-fractions (FD1F1 to FD1F7) were obtained after combination of the fractions on the basis of TLC profile. Sub-fraction FD1F6 was subjected to further CC on silica gel 60 (70-230 mesh, Merck), n-hexane, n-hexane-EtOAc, EtOAc solvent systems and elute 23% n-hexane/EtOAc furnished orthosphenic acid (**4**) (22 mg). Fraction FD1F3 was a mixture of three compounds based on LCMS profile which was subjected to HPLC (Phenomenex Luna column C₁₈ RP (5 µm, 150 × 4.6; 0.1 % acidic (formic acid) gradient solvent system (10-100 MeCN/H₂O in 30 minutes) furnished three compounds; royleanine A (**1**) (8 mg), royleanine B (**2**) (5 mg) and salaspermic acid (**3**) (20 mg) (**Scheme 4.3**).

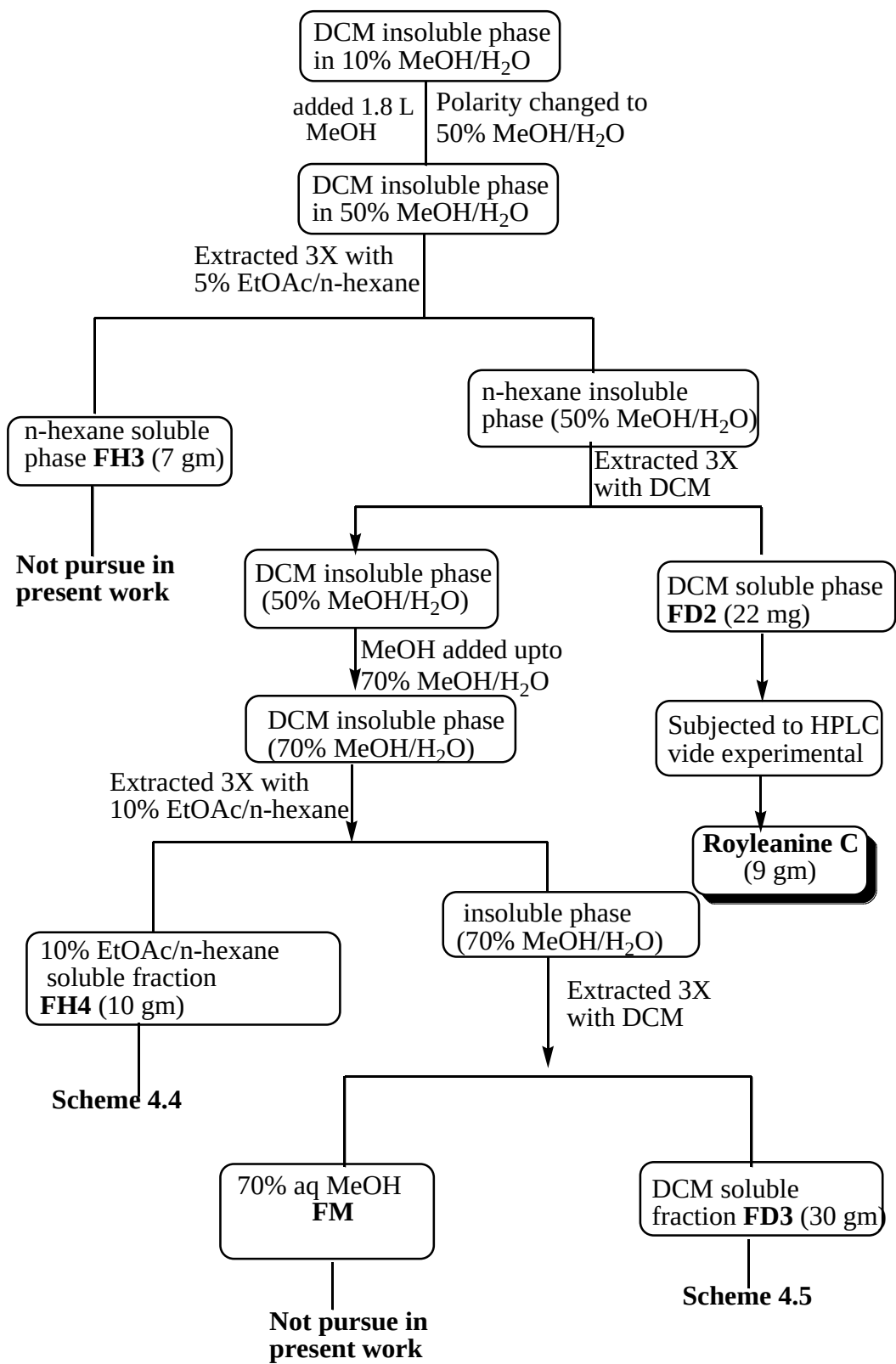
Fraction FD2 (22 mg) was mixture with one major compound which was purified by HPLC (Phenomenex Luna column C₁₈ RP, 5 µm, 150 × 4.6) with gradient solvent

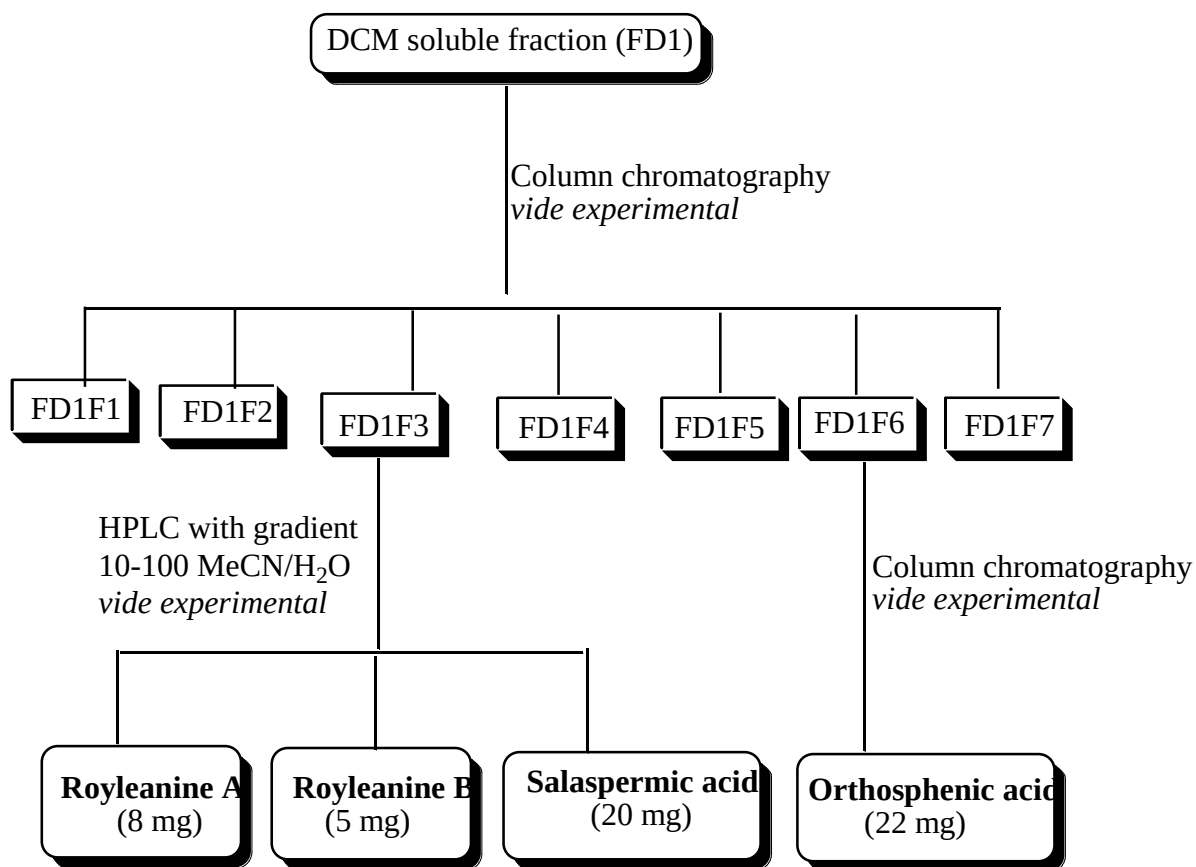
system (10-100 MeCN/H₂O in 30 minutes) resulted into the isolation of pure compound; royleanine C (**3**) (**Scheme 4.2**).

Compounds 3 α ,22 β -dihydroxyolean-12-en-29-oic acid (**6**), quercetin (**9**) and β -sitosterol-3-O- β -D-glycopyrannoside (**10**) were isolated from the fraction FH4 (10 gm) by column chromatography silica gel 60 (70-230 mesh, Merck) eluted with n-hexane/EtOAc solvent system with increasing polarity and preparative TLC (12%, 15% and 42% EtOAc/n-hexane respectively) (**Scheme 4.4**).

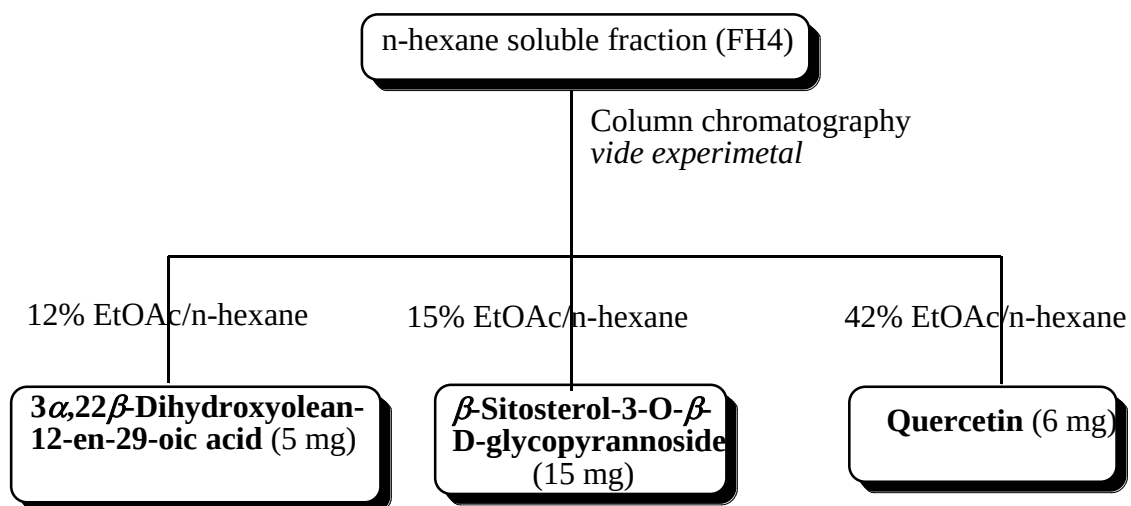
Fraction FD3 (30 gm) was a mixture of many compounds based on TLC profile which was subjected to CC on silica gel 60 (70-230 mesh, Merck) eluted with DCM, DCM-MeOH, MeOH solvent system. As a result 17 fractions were collected and combined on the basis of TLC profile to ultimately afford five fractions FD3F1 to FD3F5. Fractions FD3F1, FD3F3 and FD3F5 were not pursued in the present work due to small quantity and mixture of many compounds. Compounds oleuropein (**7**) and ligutroside (**8**) were obtained from fractions FD3F2 and FD3F4 respectively, by preparative TLC in the solvent system DCM-MeOH (49-1) (**Scheme 4.5**).

**Scheme 4.1.** Extraction and isolation.

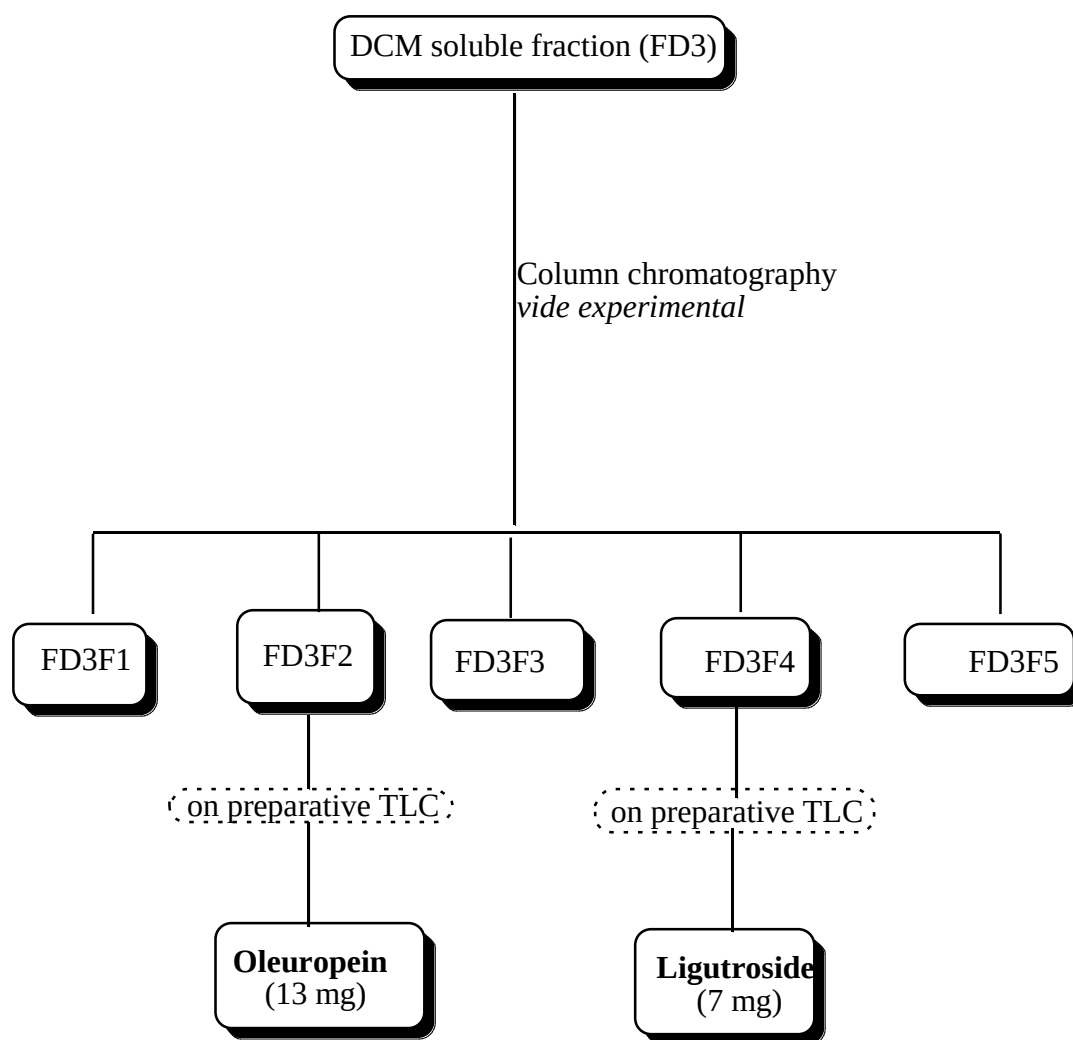
**Scheme. 4.2.** Extraction and isolation.



Scheme 4.3. Extraction and isolation.



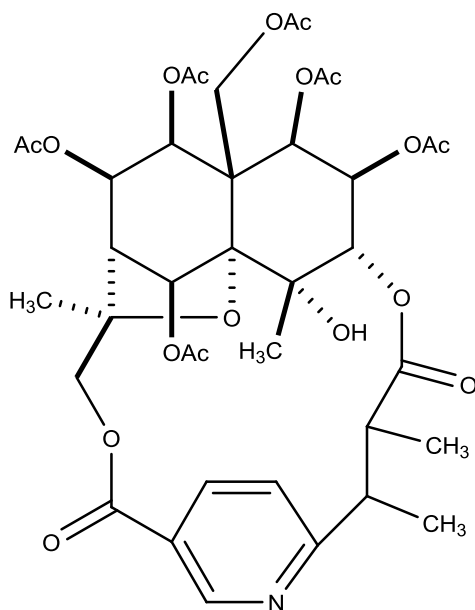
Scheme. 4.4. Extraction and isolation.



Scheme 4.5. Extraction and isolation.

4.4 Physical data of the compounds:

4.4.1 Royleanine A (1)



1 β ,2 β ,5 β ,7 β ,8 β ,11-Hexaacetoxy-4-hydroxy-3 α ,15-[2',3'-dimethyl-3'(5''-carboxy-2''-pyridyl)-propanoic acid] dicarbollactone-dihydroagarofuran

Yellow crystalline solid (8 mg)

Molecular Formula:

C₃₈H₄₇NO₁₈

Melting Point:

160-164 °C

IR (KBr): cm⁻¹

3487 (OH), 1750.5 (CO), 1585.7 (C=C aromatic)

UV (MeOH): $\lambda_{\max}$ (nm)

229, 264

ESIMS: m/z (rel.int. %)

806 [M + H]⁺ (100), 828.2 [M + Na]⁺ (25), 1611.9 [2M + H]⁺ (19)

HRESIMS: m/z

805.2916 (calcd. 805.2851 for C₃₈H₄₇NO₁₈)

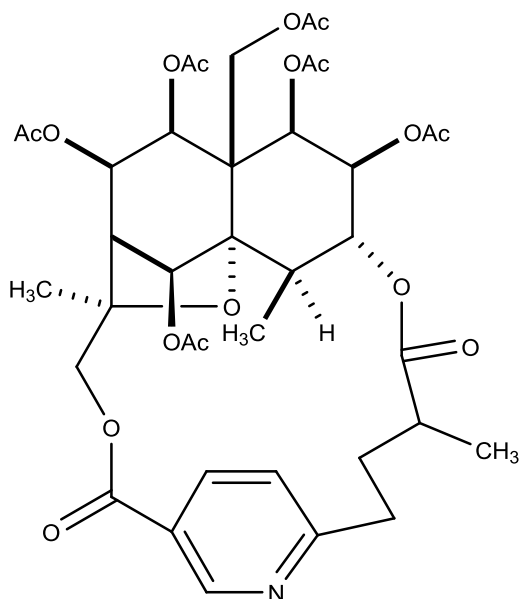
¹H NMR:

Table 3.1

¹³C NMR:

Table 3.1

4.4.2 Royleanine B (2)



1 β ,2 β ,5 β ,7 β ,8 β ,11-Hexaacetoxy-3 α ,15-[2'-methyl-4'(5''-carboxy-2''-pyridyl)-butanoic acid] dicarbollactone-dihydroagarofuran

Yellow crystalline solid (5 mg)

Molecular Formula:

C₃₇H₄₃NO₁₈

Melting Point:

184-190 °C

IR (KBr): cm⁻¹

3450.9 (OH), 2935.1 (C-H saturated), 1751 (CO), 1591 (C=C aromatic)

UV (MeOH): λ_{max} (nm)

226, 266

ESIMS: m/z (rel. Int. %)

790 [M + H]⁺ (100), 812 [M + Na]⁺ (20%), 1580 [2M + H]⁺ (12), 1601 [2M + Na]⁺ (8)

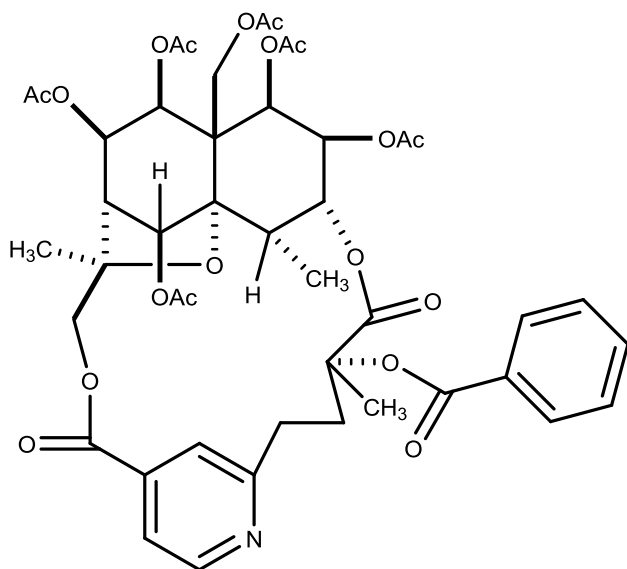
¹H NMR:

Table 3.2

¹³C NMR:

Table 3.2

4.4.3 Royleanine C (3)



1β,2β,5β,7β,8β,11-Hexaacetoxy-3α,15-[2'-methyl-2'β-benzoate-4'(4''-carboxy-2''-pyridyl)-butanoic acid] dicarbollactone-dihydroagarofuran

White solid (9 mg)

Molecular Formula:

C₄₅H₅₁NO₁₉

Melting Point:

166-170 °C

IR (KBr): cm⁻¹

2922.3 (C-H saturated), 1731.5 (CO), 1592 (C=C aromatic)

UV (MeOH): λ_{max} (nm)

240

ESIMS: *m/z* (rel. Int. %)

910 [M + H]⁺ (100), 932 [M + Na]⁺ (60), 1820 [2M + H]⁺ (55), 1843.9 [2M + Na]⁺ (10)

HRESIMS: *m/z*

909.3078 (calcd. 909.3171 for C₄₅H₅₁NO₁₉)

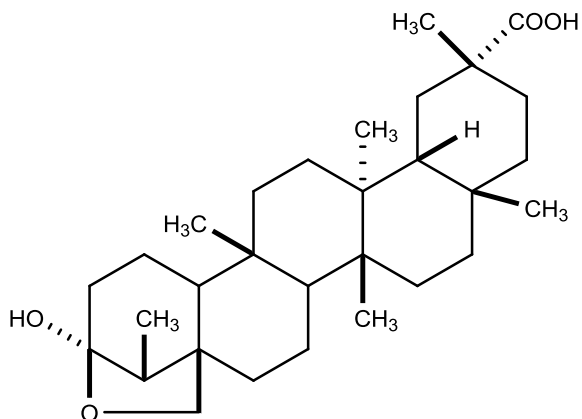
¹H NMR:

Table 3.3

¹³C NMR:

Table 3.3

4.4.4 Salaspermic acid (4)



3-oxo-Friedelan-29-oic acid hemiacetal

White powder (20 mg)

Molecular Formula: $C_{30}H_{48}O_4$ **Melting Point:**

320-330 °C

IR (KBr): cm^{-1}

3400 (OH), 3300 (OH), 2650-2500 (C-H saturated), 1685 (CO)

UV (MeOH): λ_{max} (nm)

204

EIMS: m/z (rel. Int. %)

125 (100), 81 (18), 472.2 (15)

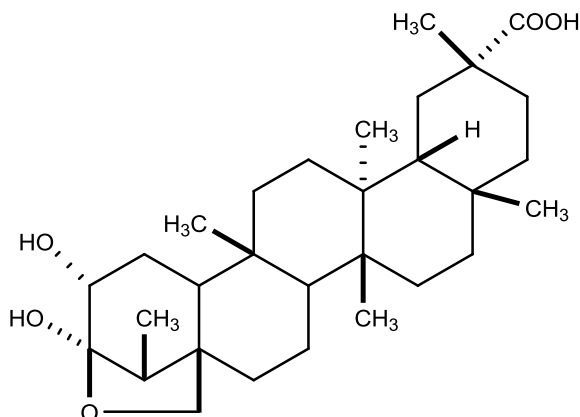
HRMS: m/z 472.3537 (calcd. 472.3552 for $C_{30}H_{48}O_4$) **^1H NMR:**

Table 3.4

 ^{13}C NMR:

Table 3.4

4.4.5 Orthosphenic acid (5)



2*R*,3*R*,7*bR*,9*aS*,12*R*,13*bS*,15*aR*,16*R*)-2,3-Dihydroxy-7*b*,9*a*,12,13*b*,15*a*,16-hexamethylcosahydro-3,5*a*-methanochryseno[2,1-*c*]oxepine-12-carboxylic acid

White amorphous solid (22 mg)

Molecular Formula:

C₃₀H₄₈O₅

Melting Point:

298-300°C

IR (KBr): cm⁻¹

3450.9 (OH), 2935.1 (C-H saturated), 1751 (CO)

EIMS: *m/z* (rel. Int. %)

125 (100), 488.3 (60), 109 (30), 81 (20)

HRMS: *m/z*

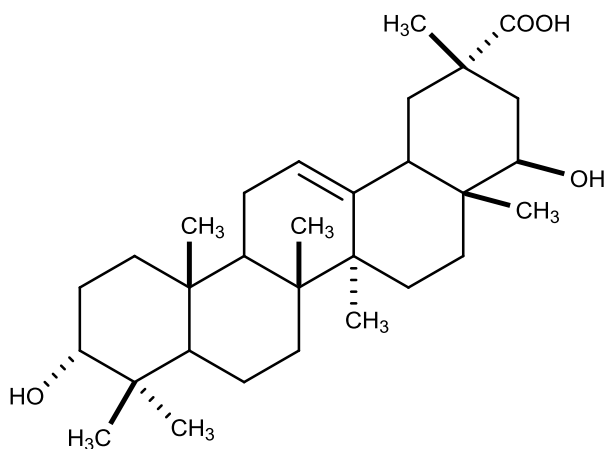
488.3498 (calcd. 488.3502 for C₃₀H₄₈O₅)

¹H NMR:

Table 3.5

¹³C NMR:

Table 3.5

4.4.6 3 α ,22 β -Dihydroxyolean-12-en-29-oic acid (**6**)

White amorphous powder (5 mg)

Molecular Formula:

C₃₀H₄₈O₄

IR (KBr): cm⁻¹

3438 (Hydroxyl), 2945 (C-H saturated), 1705 (Carbonyl)

EIMS: *m/z* (rel. Int. %)

55 (100), 95 (89), 246 (73), 131 (51), 217 (39) 185 (43) 472.4 (6)

HRMS: *m/z*

472.3537 (calcd. 472.3552 for C₃₀H₄₈O₄)

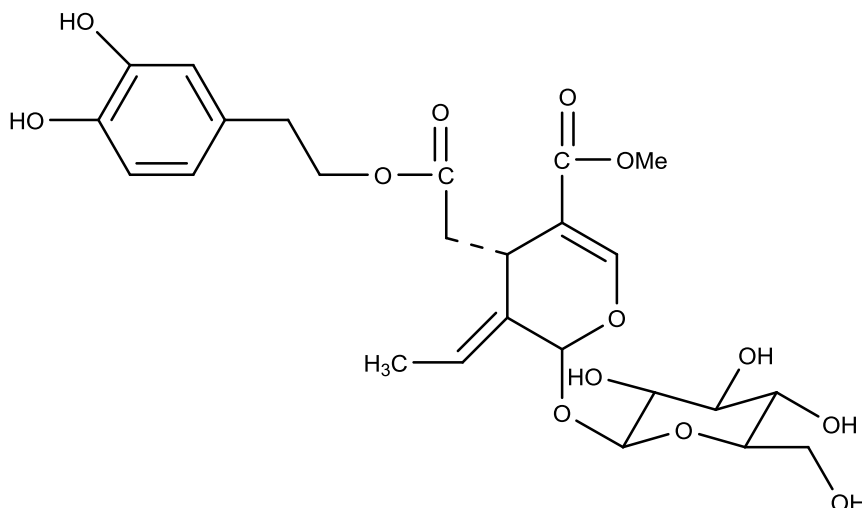
¹H NMR:

Table 3.6

¹³C NMR:

Table 3.6

4.4.7 Oleuropein (7)



(4*S*,*E*)-Methyl 4-(2-(3,4-dihydroxyphenethoxy)-2-oxoethyl)-3-ethylidene-2-((2*S*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)-3,4-dihydro-2*H*-pyran-5-carboxylate

Colorless powder (13 mg)

Molecular Formula:

C₂₅H₃₂O₁₃

IR (KBr): cm⁻¹

3400 (OH), 1695 (CO), 1630 (CO)

UV (EtOH): λ_{max} (nm)

238, 279

[α]_D²⁰: MeOH

+93 (c 0.41, MeOH)

EIMS: *m/z* (rel. int. %)

123 (100), 165 (75), 136 (70), 167 (69), 137 (40), 359 (10)

HREIMS: *m/z*

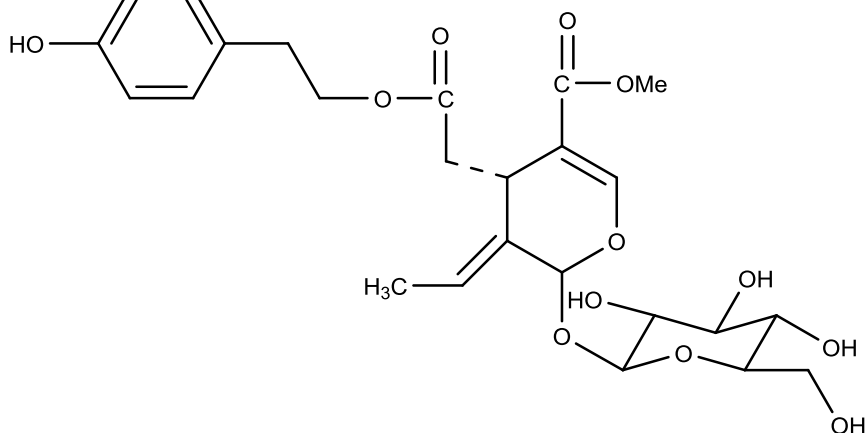
482.2300 (calcd. 482.2304 for C₂₈H₃₄O₇)

¹H NMR:

Table 3.7

¹³C NMR:

Table 3.7

4.4.8 Ligutroside (**8**)

(4*S*,*E*)-Methyl 3-ethylidene-4-(2-(4-hydroxyphenethoxy)-2-oxoethyl)-2-((2*S*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy)-3,4-dihydro-2H-pyran-5-carboxylate

Colorless Powder (7 mg)

Molecular Formula:

C₂₅H₃₂O₁₂

IR (KBr): cm⁻¹

3400 (OH), 1700 (CO), 1625 (CO)

[α]_D²⁰: MeOH

+97 (*c* 0.41, MeOH)

UV (EtOH): λ_{max} (nm)

239, 280

EIMS: *m/z* (rel. Int. %)

121 (100), 107 (70), 167 (48), 362 (10)

HREIMS: *m/z*

456.341 917 (calcd. 425.341 956 for C₃₀H₄₈O₃)

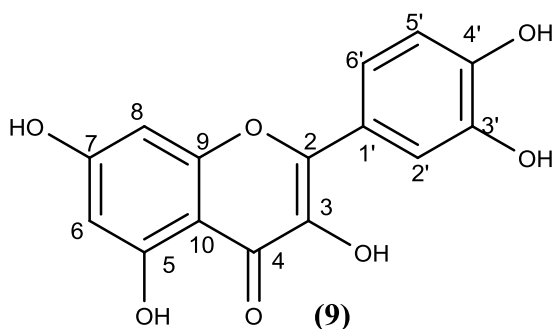
¹H NMR:

Table 3.8

¹³C NMR:

Table 3.8

4.4.9 Quercetin (9)



2-(3',4'-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one

Yellow powder (6 mg)

Molecular Formula:

C₁₅H₁₀O₇

IR (KBr): cm⁻¹

3405 (OH), 1657 (CO), 1553 (C=C aromatic)

UV (EtOH): λ_{max} (nm)

350

EIMS: *m/z* (rel. int. %)

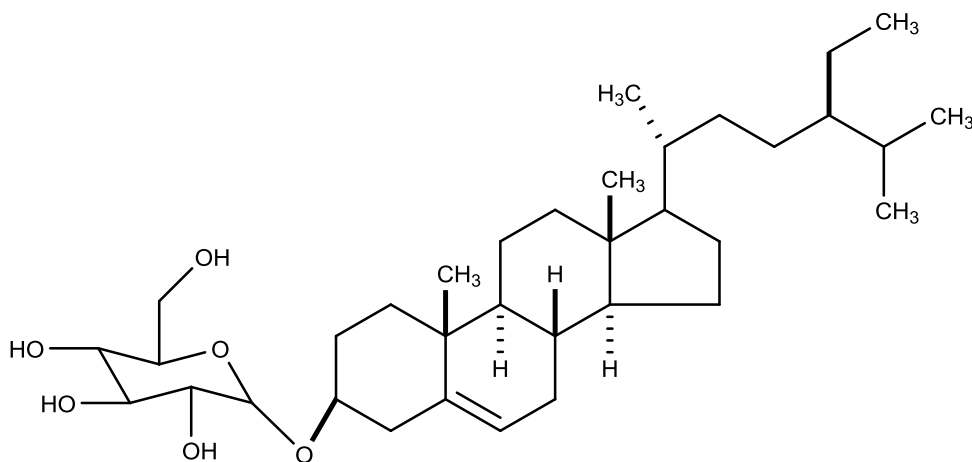
302.1 (100), 274.2 (20), 137 (18).

¹H NMR:

Table 3.9

¹³C NMR:

Table 3.9

4.4.10 β -Sitosterol-3-O- β -D-glycopyrannoside (**10**)

(3*R*,4*S*,5*S*,6*R*)-2-((3*S*,8*S*,9*S*,10*R*,13*R*,14*S*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yloxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol

White powder (15mg)

Molecular Formula:

C₃₅H₆₀O₆

Melting Point:

235-236°C

IR (KBr): cm⁻¹

3449 (OH), 3045 (C-H olefinic), 1620 (C=C olefinic)

EIMS: *m/z* (rel. int. %)

397 (100) [M⁺+H-gly]

HREIMS: *m/z*

576.4390 (calcd. 576.8473 for C₃₅H₆₀O₆)

¹H NMR:

Table 3.10

¹³C NMR:

Table 3.10

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(Part B)

Chapter 5

Phytochemical studies on *Ficus faveolata*



Ficus faveolata



Ficus faveolata fruits and leaves

5.1 Introduction to the family Moraceae

5.1.1 The genus *Ficus*

The genus *Ficus* (Fig genus) is one of the important and large genus among 40 genera of the Moraceae family (mulberry), and consists of more than 800 species.¹ The plants of this genus are evergreen and deciduous. Most of these plants are tall trees while some are shrubs and rarely climbers. Majority of these species have aerial roots called auxiliary roots which arise from its branches. Few important species of this genus are *F. carica* (common fig which has different variety with gene diversity),² *F. religiosa*, *F. elastica*, *F. benghalensis*, *F. recemosa*, *F. salicifolia*, *F. glomerata*, *F. padifolia* and *F. maxima*.. The genus is represented in Pakistan by:

- *F. religiosa*
- *F. faveolata*
- *F. palmate*
- *F. recemosa*
- *F. bangalensis*

5.1.2 General morphological characters of *Ficus faveolata*

“Leaves petiolate simple, entire, serrate or lobed, palmately or pinnately 3-5-costate; stipules 2, lateral, fugaceous and leaving a distinct annular scar. Inflorescence axillary cymes usually in the form of racemes, often catkins, globose heads, spikes or hypenthodium. Flowers minute, unisexual, actinomorphic, hypogynous to epigynous. Sepals (2-3) 4 (-6), occasionally absent, biseriate, free, or valvate or $\pm$ united, enveloping the carpel in female flowers, persistent, petals absent, stamens as many as sepals, often less, antisepalous, filaments or erect in bud, free with ditheous, versatile, longitudinally

dehiscent anthers, pistillode present or absent in male flowers. Ovary bicarpellate, one carpel frequently abortive, superior or inferior, unilocular, ovule solitary, anatropous campylotropous, pendulous from the apex, rarely basal and upright; styles 2 (-1), filiform, simple or forked. Fruits are simple drupe, nut or achene and sorosis or syconium. Seeds surrounded by endocarp, endospermous or exalbuminous; embryo mostly curved”.¹

5.1.3 Pharmacological importance of the genus *Ficus*

The leaves of *Ficus pumila* have been traditionally consumed by some Okinawan elders as a beverage, while the folks used as an medicinal herb to treat diabetes, dizziness, high blood pressure, and neuralgia.³ The stem bark powdered of *Ficus sycomorus* was soaked in water for at least 6 hours and the aqueous solution given orally to patient for the treatment of ailments, such as mental illness, wound dressing and diarrhea, while sedative and anticonvulsant effects on rats was also reported.^{4,5} Fruits and stem bark of *Ficus indica* have been traditionally used in folk medicines to treat diabetes, hypertension, asthma, burns, edema and indigestion. The methanolic extracts of both fruits and stem bark exhibited hypoglycemic,⁶ anti-ulcer, anti-allergic,⁷ analgesic⁸ and anti-inflammatory activities.⁹ Ethanolic crude extract of stem bark of *F. microcarpa* has shown anti-plateletic and cytotoxic activities.^{10, 11} The isolation of secondary metabolites such as triterpenoids,¹² phenoids,¹³ lignans,¹⁴ isoflavone¹⁵ and α -lactone¹⁶ from the stem heartwood and bark confirmed the biological activities. Mathanolic extracts of both leaves and stem bark of *F. fistulosa* showed anti-plasmodial activity led to the isolation of triterpenes from mathanolic extract of the stem bark.^{17,18}

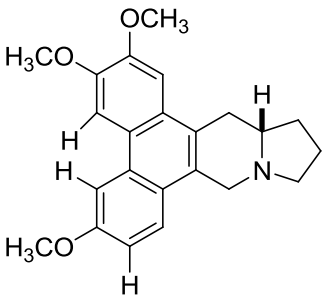
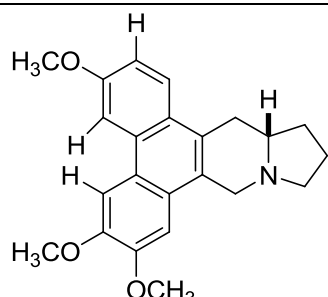
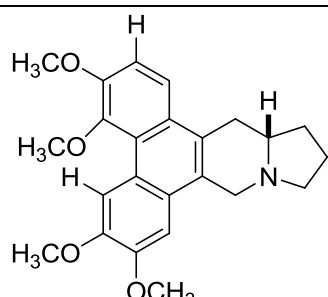
Table 5.1. Ethnomedical uses of the different parts of the *Ficus* spp. for different disorders

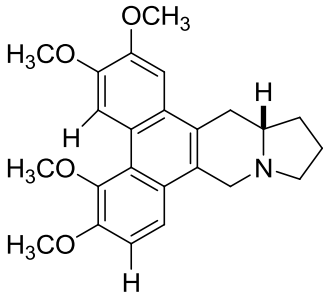
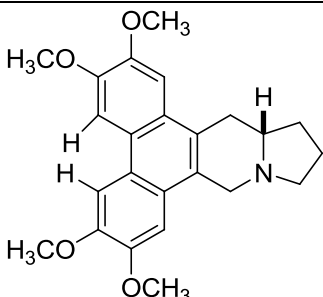
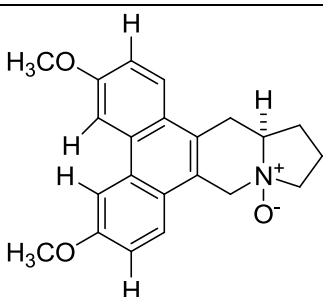
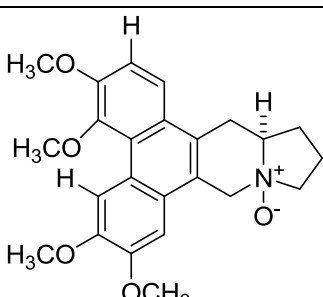
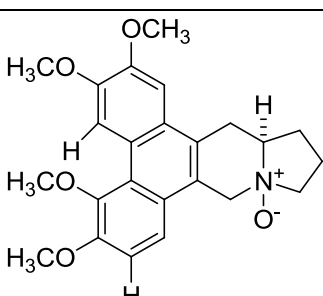
<i>Ficus</i> species	Plant part	Country	Use
<i>F. racemosa</i>	Fruits	India	Soar throat ¹⁹
<i>F. racemosa</i>	Leaves with honey	India	Bilious affection ¹⁹
<i>F. racemosa</i>	Powder bark	India	Diabetes ¹⁹
<i>F. racemosa</i>	Fruits	India	Laxative, digestive ¹⁹
<i>F. religiosa</i>	Latex with milk	India	Cooling, ulcer ¹⁹
<i>F. benghalensis</i>	Latex	India	External wart ²⁰
<i>F. carica</i>	Fruit	Japan	Cancer ²¹
<i>F. carica</i>	Fresh Fruit	USA	Oral cancer ²²
<i>F. pachyrrachis</i>	Whole plant	Tanga	Breast cancer ²³
<i>F. aurantiaceae</i>	Leaves	Malaysia	Pruritis ²⁴
<i>F. maxima</i>	Latex	Peru	Rheumatism ²⁵
<i>F. maxima</i>	Wood sap	Handuras	Gingivitis oral ²⁶
<i>F. obtusifolia</i>	Latex	Mexico	Toothache ²⁷
<i>F. reflexa</i>	Bark	Rodrigues Island	Intestinal ulcer ²⁸
<i>F. religiosa</i>	Dried leaf juice	Fiji	Unconsciousness ²⁹
<i>F. salicifolia</i>	Leaves	Oman	Bruised finger, toe ³⁰
<i>F. pungens</i>	Leaf	Papua New Guinea	Body pain ³¹
<i>F. carica</i>	Latex	Turkey	Bee sting ³²
<i>F. carica</i>	Leaf	Oman	Darmetitis ³⁰
<i>F. carica</i>	Fruit	Turkey	Mastitis ³³
<i>F. scabra</i>	Bark	Fiji	Ophthalmia ³⁴
<i>F. thunbergii</i>	Leaf	Japan	Lumbago ³⁵
<i>F. toxicaria</i>	Leaf	Indonesia	Gastroenteritis ³⁶
<i>F. toxicaria</i>	Latex	Indonesia	Toothache ³⁷
<i>F. subcuneata</i>	Latex	Papua New Guinea	Dermatitis ³⁸
<i>F. septica</i>	Leaves	Papua New Guinea	Cephalgia ³⁹
<i>F. septic</i>	Dried bud	Papua New Guinea	Headache ⁴⁰
<i>F. septic</i>	Leaf	Buka Island	Gastralgia ⁴¹
<i>F. racemosa</i>	Leaf juice	India	Pneumonia ⁴²
<i>F. maxima</i>	Dried Leaf	Brazil	Rheumatism ⁴³
<i>F. carica</i>	Stem latex	Turkey	Warts ⁴⁴
<i>F. glomerata</i>	Latex	India	Ophthalmia ⁴⁵

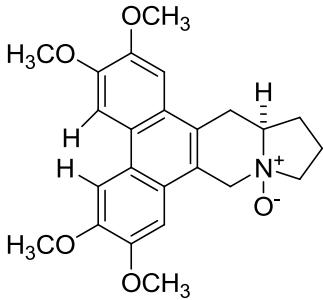
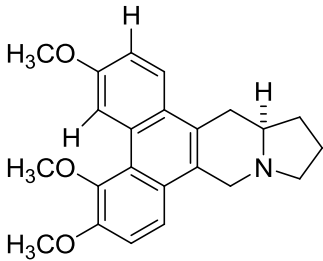
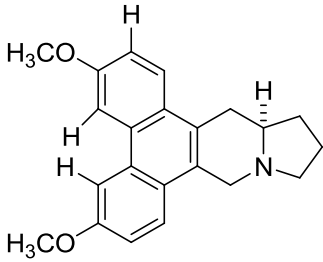
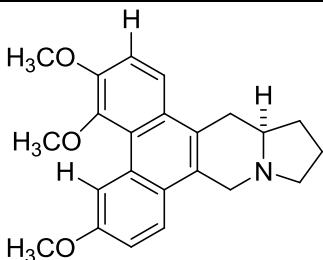
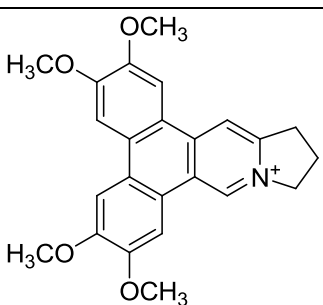
5.1.4 Phytochemical review of the genus *Ficus*

The literature studies on the genus *Ficus* revealed variety of compounds have been isolated such as flavonoids, triterpenes, alkaloids, coumarins, phenolic and peptides. Some of these compounds are presented in **Table 5.2**.

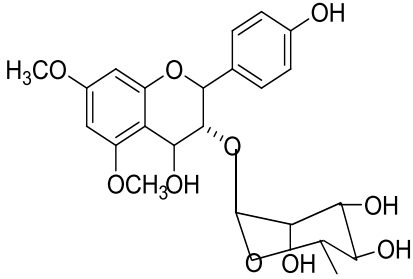
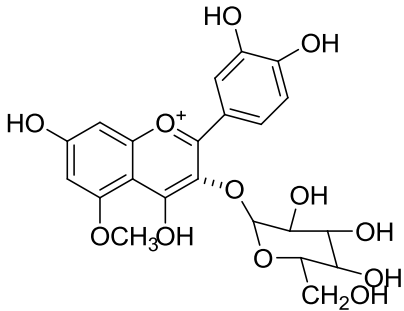
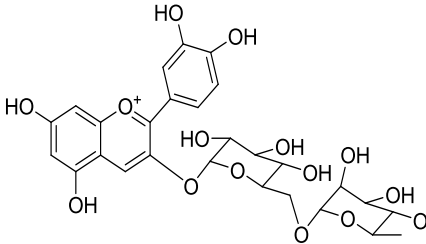
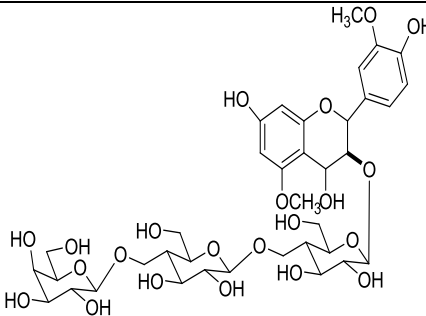
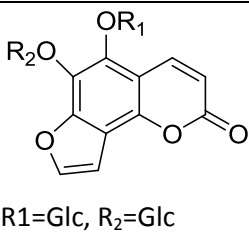
Table 5.2: Previously isolated compounds from the genus *Ficus*

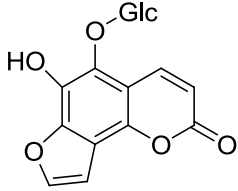
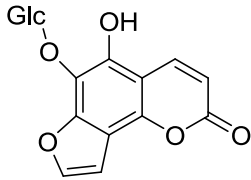
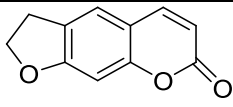
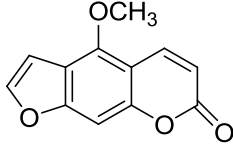
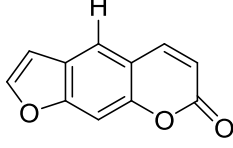
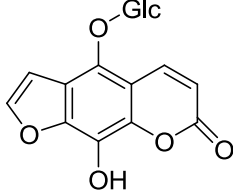
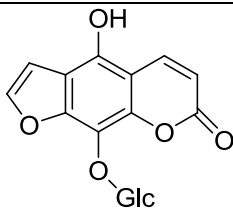
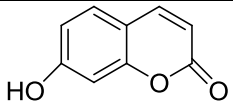
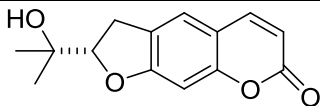
No.	Compounds	Structures	Source and references
01	Antofine		<i>F. septica</i> ⁴⁶
02	2-Demethoxytylophorine		<i>F. hispida</i> ⁴⁷
03	Tylophorine		<i>F. septica</i> ⁴⁸

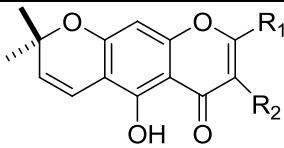
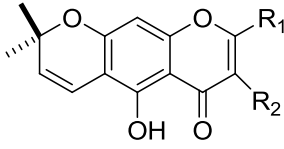
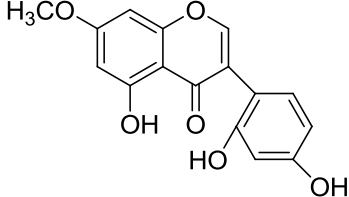
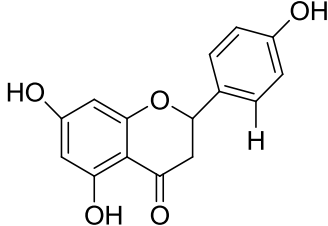
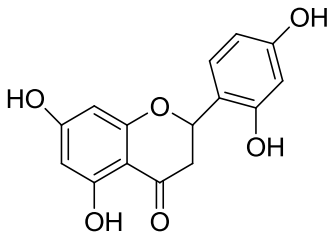
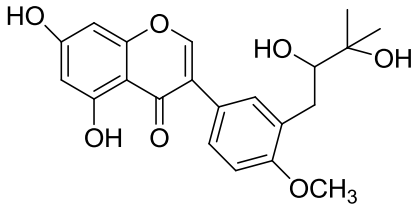
04	Isotylocrebrine		<i>F. septica</i> ⁴⁸
05	Tylocrebrine		<i>F. septica</i> ⁴⁸
06	10S,13aR-Antofine N-oxide		<i>F. septica</i> ⁴⁹
07	10S,13aR-Isotylocrebrine N-oxide		<i>F. septica</i> ⁴⁹
08	10S,13aR-Tylocrebrine N-oxide		<i>F. septica</i> ⁴⁹

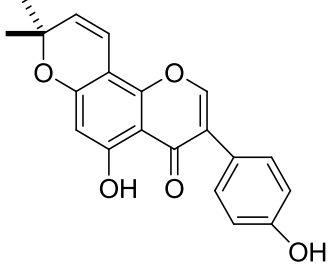
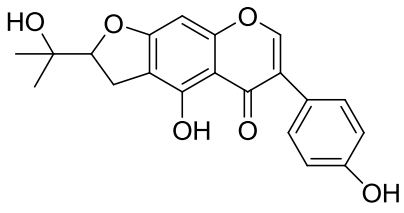
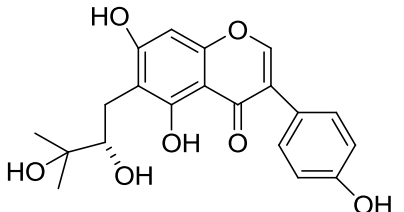
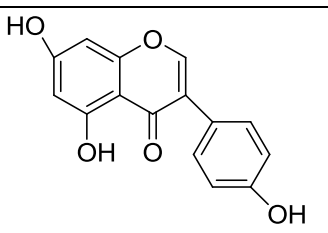
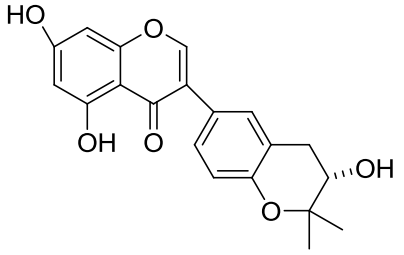
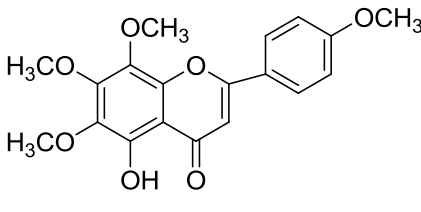
09	10S,13 α R-Tylophorine N-oxide		<i>F. septica</i> ⁴⁹
10	Ficuseptine B		<i>F. septica</i> ⁴⁹
11	Ficuseptine C		<i>F. septica</i> ⁴⁹
12	Ficuseptine D		<i>F. septica</i> ⁴⁹
13	Dehydrotylophorine		<i>F. septica</i> ⁵⁰

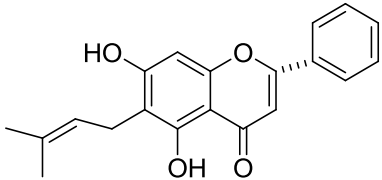
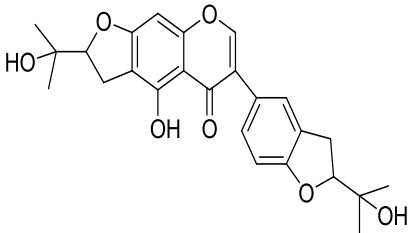
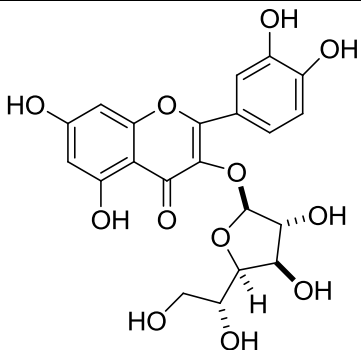
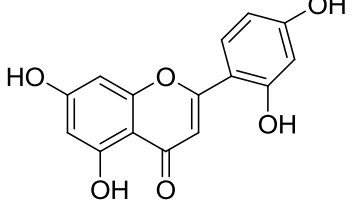
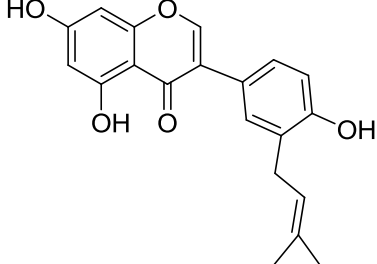
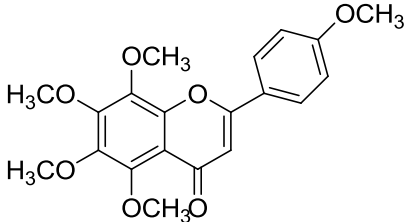
14	10 <i>S</i> ,13 <i>αS</i> -Isotylocrebrine <i>N</i> -oxide		<i>F. septica</i> ⁴⁹
15	Ficuseptine A		<i>F. septica</i> ⁴⁸
16	Hispidine		<i>F. septica</i> ⁴⁷
17	10 <i>R</i> ,13 <i>αR</i> -Tylocrebrine <i>N</i> -oxide		<i>F. septica</i> ⁴⁸
18	10 <i>R</i> ,13 <i>αR</i> -Tylophorine <i>N</i> -oxide		<i>F. septica</i> ⁴⁸

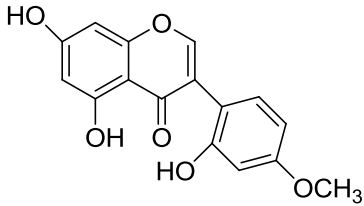
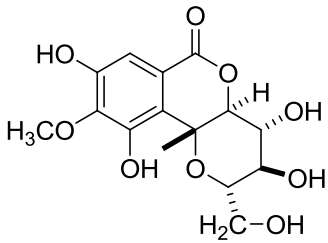
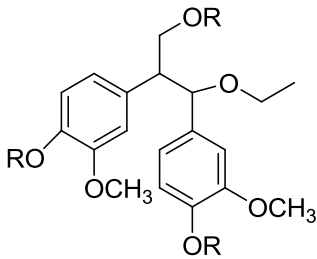
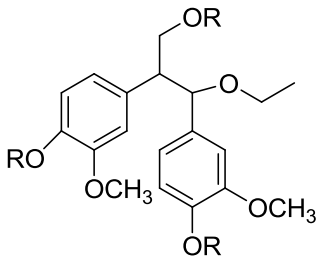
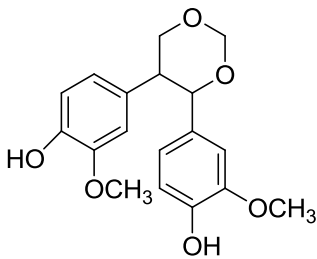
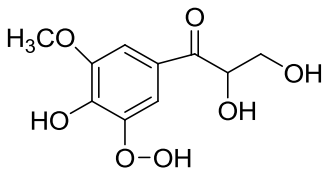
19	5,6-Dimethyl ether of leucopelargonidin-3-O- α -l-rhamnoside		<i>F. benghalensis</i> ⁵¹
20	Cyanidin-3-O-glucoside		<i>F. carica</i> ⁵²
21	Cyanidin-3-O-rhamnoglucoside		<i>F. carica</i> ⁵²
22	5,3'-Dimethyl leucocyanidin-3-O- β -galactosyl cellobioside		<i>F. benghalensis</i> ⁵¹
23	5,6-O- β -D-Digluco-pyrano-sylangelicin	 R ₁ =Glc, R ₂ =Glc	<i>F. ruficaulis</i> ⁵³

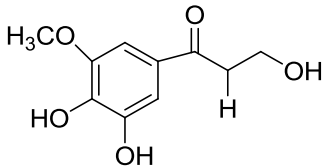
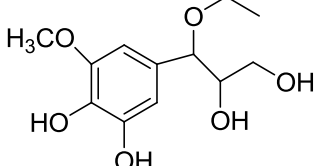
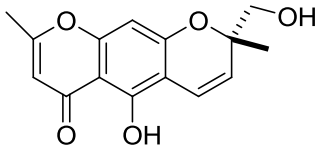
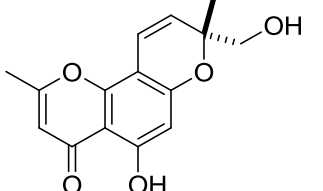
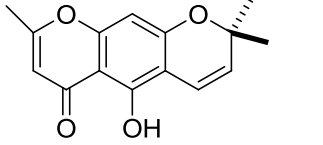
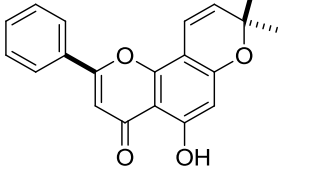
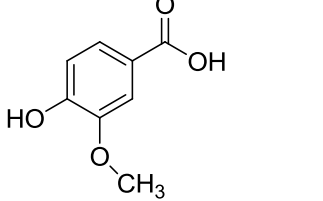
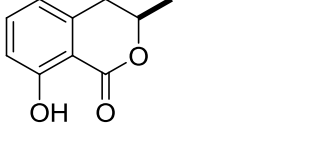
24	5- <i>O</i> - β -D-Glucopyranosyl-6-hydroxyangelicin		<i>F. ruficaulis</i> ⁵³
25	6- <i>O</i> - β -D-Glucopyranosyl-5-hydroxyangelicin		<i>F. ruficaulis</i> ⁵³
26	4',5'-Dihydropsoralen		<i>F. carica</i> ⁵⁴
27	Bergapten		<i>F. carica</i> ⁵⁵
28	Psoralen		<i>F. carica</i> ⁵⁵
29	5- <i>O</i> - β -D-Glucopyranosyl-8-hydroxypsoralen		<i>F. ruficaulis</i> ⁵³
30	8- <i>O</i> - β -D-Glucopyranosyl-5-hydroxypsoralen		<i>F. ruficaulis</i> ⁵³
31	Umbelliferone		<i>F. carica</i> ⁵⁴
32	Marmesin		<i>F. carica</i> ⁵⁴

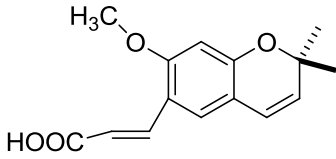
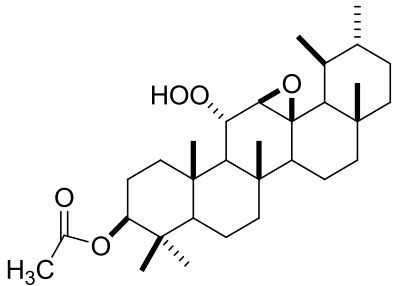
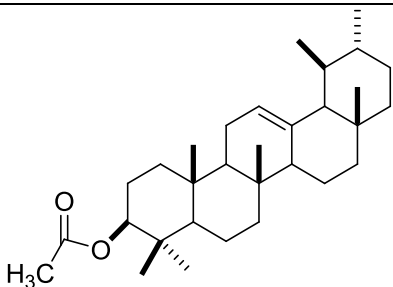
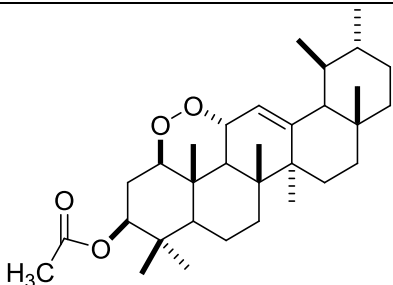
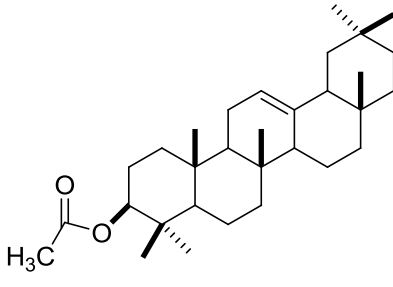
33	Alpinumisoflavone	 R₁= H, R₂=para-hydroxyl phenol	<i>F. nymphaeifolia</i> ⁵⁶
34	Carpachromene	 R₁= para-hydroxyl phenol, R₂=H	<i>F. formosana</i> ⁵⁷
35	Cajanin		<i>F. nymphaeifolia</i> ⁵⁶
36	Apigenin		<i>F. formosana</i> ⁵⁷
37	Norartocarpetin		<i>F. formosana</i> ⁵⁷
38	5,7-Dihydroxy-4-methoxy-3'-(2,3-dihydroxy-3-methylbutyl)isoflavone		<i>F. nymphaeifolia</i> ⁵⁶

39	Derrone		<i>F. nymphaeifolia</i> ⁵⁶
40	Erythrinin		<i>F. nymphaeifolia</i> ⁵⁶
41	Erycibenin		<i>F. nymphaeifolia</i> ⁵⁶
42	Genistein		<i>F. nymphaeifolia</i> ⁵⁶
43	Ficuisoflavone		<i>F. microcarpa</i> ⁵⁸
44	5-Hydroxy-4',6,7,8-tetramethoxyflavone		<i>F. hirta</i> ⁵⁹

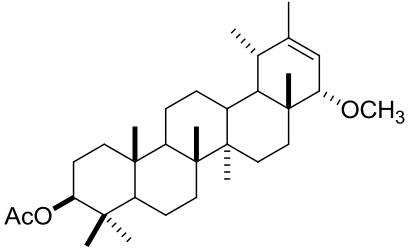
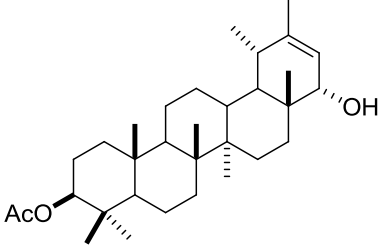
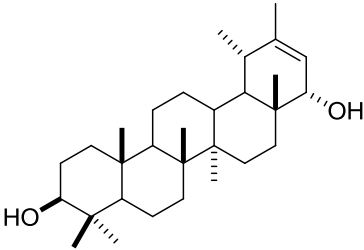
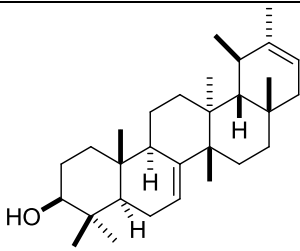
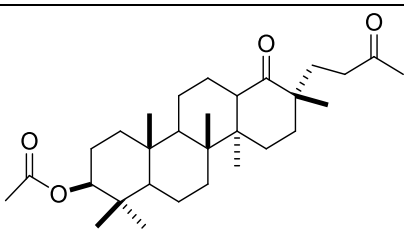
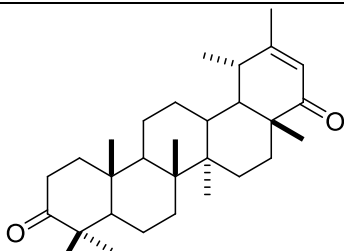
45	Isoglabranin		<i>F. formosana</i> ⁵⁷
46	Isolupinisoflavone E		<i>F. microcarpa</i> ⁵⁸
47	Isoquercitrin		<i>F. ruficaulis</i> ⁵³
48	Norartocarpetin		<i>F. formosana</i> ⁵⁷
49	3β-(3-Methylbut-2-enyl)biochanin A		<i>F. nymphaeifolia</i> ⁵⁶
50	4',5,6,7,8-Pentamethoxyflavone		<i>F. hirta</i> ⁵⁹

51	5,7,2'-Trihydroxy-4'-methoxyisoflavone		<i>F. nymphaeifolia</i> ⁵⁶
52	Berginin		<i>F. recemosa</i> ⁶⁰
53	<i>erythro</i> -2,3-Bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol	 R=H, <i>erythro</i>	<i>F. beecheyana</i> ⁶¹
54	<i>threo</i> -2,3-Bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol	 R=H, <i>threo</i>	<i>F. beecheyana</i> ⁶¹
55	<i>trans</i> -4,5-Bis(4-hydroxy-3-methoxyphenyl)-1,3-dioxacyclohexane		<i>F. beecheyana</i> ⁶¹
56	2,3-Dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone		<i>F. beecheyana</i> ⁶¹

57	3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone		<i>F. beecheyana</i> ⁶¹
58	<i>threo</i> -3-(4-Hydroxy-3,5-dimethoxyphenyl)-3-ethoxypropane-1,2-diol		<i>F. beecheyana</i> ⁶¹
59	Ficuformodiol B		<i>F. formosana</i> ⁵⁷
60	Ficuformodiol A		<i>F. formosana</i> ⁵⁷
61	Vanillic Acid		<i>F. septica</i> ⁴⁸
62	Racemosic acid		<i>F. recemosa</i> ⁶⁰
63	Spatheliachromene		<i>F. formosana</i> ⁵⁷
64	3-(7-Methoxy-2,2-dimethyl-2H-6-chromenyl)-(E)-propenoic acid		<i>F. formosana</i> ⁵⁷

65	Obovatin		<i>F. formosana</i> ⁵⁷
66	3 β -Acetoxy-12 β ,13 β -epoxy-11 α -hydroperoxyursane		<i>F. micricarpa</i> ⁶²
67	3 β -Acetoxy- β -amyrin		<i>F. hirta</i> ⁵⁹
68	<i>threo</i> -3-(4-Hydroxy-3,5-dimethoxyphenyl)-3-ethoxypropane-1,2-diol		<i>F. beecheyana</i> ⁶¹
69	3 β -Acetoxy- α -amyrin		<i>F. hirta</i> ⁵⁹

70	3 β -Acetoxy-12,19-dioxo-13(18)-oleanene		<i>F. micricarpa</i> ⁶²
71	3 β -Acetoxy-11 α ,12 α -epoxy-16-oxo-14-taraxerene		<i>F. micricarpa</i> ⁶²
72	3 β -Acetoxy-11 α ,12 α -epoxy-14-taraxerene		<i>F. micricarpa</i> ⁶²
73	(20S)-3 β -Acetoxy-20-hydroperoxy-30-norlupane		<i>F. micricarpa</i> ⁶²
74	(20S)-3 β -Acetoxylupan-29-oic acid		<i>F. micricarpa</i> ⁶²

75	3 β -Acetoxy-22 α -methoxy-20-taraxastene		<i>F. micricarpa</i> ⁶³
76	3 β -Acetoxy-20-taraxasten-22 α -ol		<i>F. micricarpa</i> ⁶³
77	20-Taraxastene-3 β ,22 α -diol		<i>F. micricarpa</i> ⁶³
78	Baurinol		<i>F. carica</i> ⁶⁴
79	29,30-Dinor-3 β -acetoxy-18,19-dioxo-18,19-secolupane		<i>F. micricarpa</i> ⁶⁵
80	3,22-Dioxo-20-taraxastene		<i>F. micricarpa</i> ⁶⁶

5.1.5 Classification of *Ficus faveolata*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Rosales
Family:	Moraceae
Tribe:	Ficeae
Genus:	<i>Ficus</i>
Species:	<i>faveolata</i>

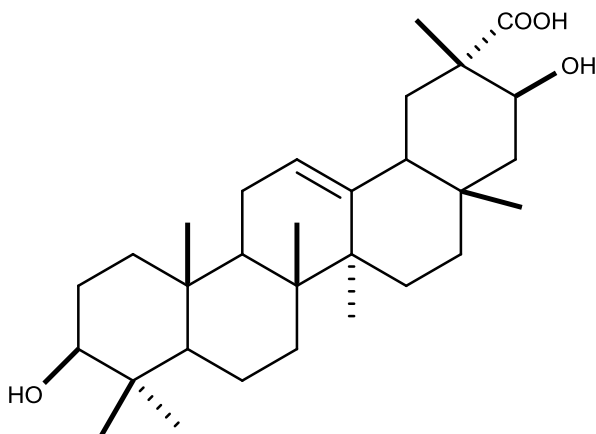
Chapter 6

Results and Discussion

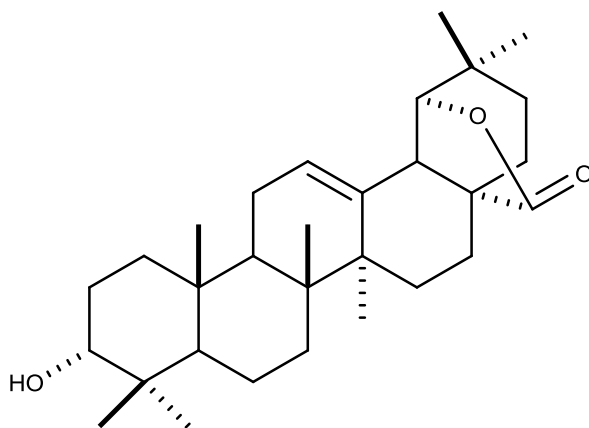
6.1 Chemical constituents isolated from *Ficus faveolata*

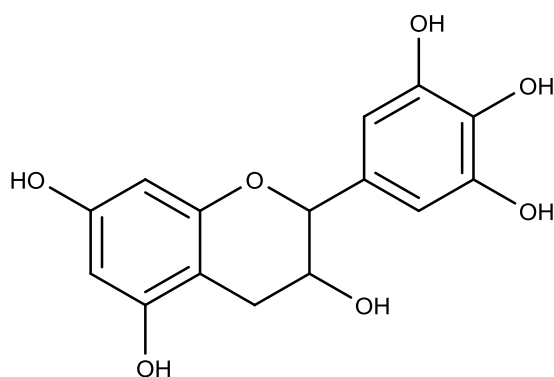
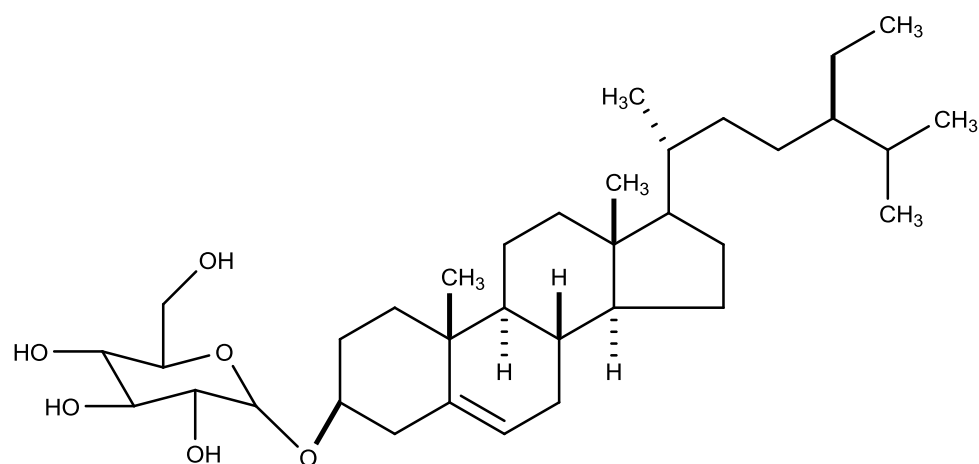
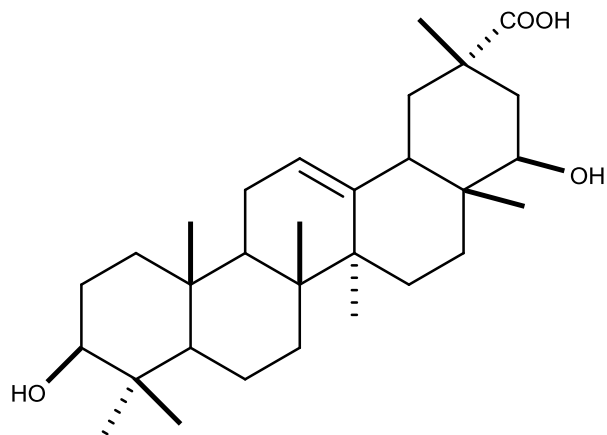
Two new; Ficusonic acid (**11**), Ficusonolide (**12**) and three known compounds; gallocatechine (**13**), β -sitosterol-3-*O*- β -D-glycopyrannoside (**14**) and 3 β ,22 β -dihydroxyolean-12-en-29-oic acid (**15**) were isolated from the methanolic extract of *Ficus faveolata* (*vide experimental*). Their structures were elucidated by spectral studies such as IR, UV, MS, ^1H , ^{13}C NMR and comparison with related compounds.

Ficusonic acid (**11**)



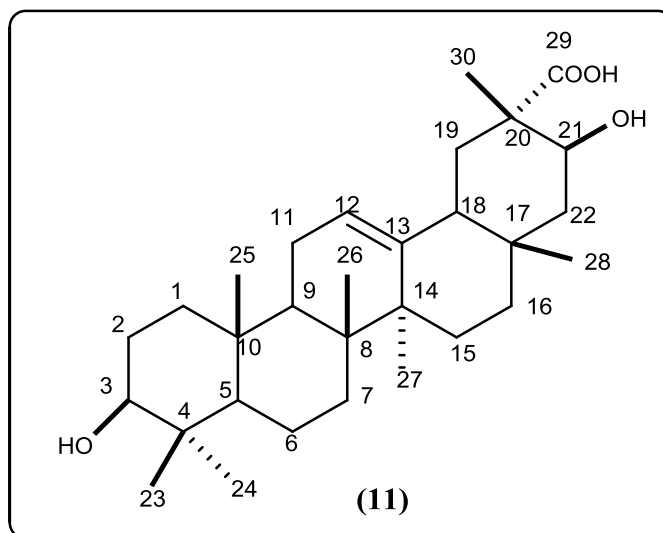
Ficusonolide (**12**)



6.3.1 Gallocatechine (**13**) β -Sitosterol-3-O- β -D-glycopyrannoside (**14**)3 β ,22 β -dihydroxyolean-12-en-29-oic acid(**15**)

6.2 New compounds isolated from *Ficus faveolata*

6.2.1 Ficusonic acid (11)



The structure of ficusonic acid (**11**) commenced by establishing the molecular formula $C_{30}H_{48}O_4$ through HREIMS m/z 472.6997 (calcd. 472.3553 for $C_{30}H_{48}O_4$). The IR spectrum showed absorption bands at 3358 (hydroxyl), 2945 (C-H olefinic), 1760 (carboxylic carbonyl) and 1381 (*gem*-dimethyl group) cm^{-1} . The 7 degrees of unsaturation evident in the molecular formula were; one carbonyl group at δ_c 182.3, one olefinic bond at δ_c 124.3 (C-12), 144.7 (C-13) and five rings. The ^1H and ^{13}C (BB and DEPT; **Table 6.1**) NMR spectra indicated seven methyls, seven quaternary, nine methylene and six methine carbons including hydroxyl and carboxylic groups. The ^1H NMR spectrum (**Table 6.1**) showed seven tertiary methyls signals as expected for olean-12-en skeleton with a secondary hydroxyl substituent.⁶⁸ In the ^1H NMR spectrum one proton triplet at δ 5.25 ($J=3.5$ Hz; H-12) displayed the presence of a trisubstituted double bond and two oxygenated methines resonated at δ 3.14 (dd, $J=11.5, 4.5$ Hz; H-4) and

3.51 (dd, $J=12.5, 4.5$ Hz; H-21) (**Table 6.1**). The characteristic pentacyclic triterpene fragmentation pattern was observed in the EIMS spectrum of **11** (**Fig. 6.1c**). All the spectral data suggested that **11** was oleanane-type pentacyclic terpenoid derivatives.⁶⁸ The equatorial positions of two hydroxyl groups at C-3 and C-21 were confirmed by respective carbinol protons appearing as doublet of doublet instead of triplet for axial position. Most of the ^1H and ^{13}C NMR spectral data of **11** were identical to related compounds,⁶⁹ however the ^1H NMR spectrum indicated hydroxyl group attached at C-21 (δ 3.51, dd, $J=12.5, 4.5$ Hz; H-21) instead of C-22 which was confirmed by COSY, HMBC correlations and mass fragments (**Fig. 6.1a-c**). The carbons connectivity was developed and protons were assigned to their respective carbons by 2D NMR. A proton resonated at δ 3.51 (dd, $J=12.5, 4.5$ Hz; H-21) showed long range correlations with carbon atoms appeared at δ 182.3 (C-29), 25.2 (C-30), 40.0 (C-19) and 41.5 (C-22) which was confirmed by COSY correlations for proton at δ 3.51 (dd, $J=12.5, 4.5$ Hz; H-21) with δ 1.23 (s, H-30), 2.24 (dd, $J=14.0, 12.5$ Hz; H-22_a) and 2.03 (dd, $J=14.0, 4.5$ Hz; H-22_b). On the basis of these spectral data oxygenated methine group resonated at δ 76.0 in the ^{13}C NMR spectrum was assigned to position C-21. The relative stereochemistry of **11** was confirmed by NOESY, COSY and HMBC experiments.

The 1D and 2D NMR spectral data and comparison with related spectral data in the literature⁶⁹⁻⁷¹ suggested that the structure of **11** as $3\beta,21\beta$ -dihydroxy-olean-12-en-29-oic acid (ficusonic acid).

Table 6.1: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in CD_3OD and HMBC correlations of ficusonic acid (**11**)

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C1	38.2	CH_2	1.90 H_a 1.62 H_b	m m C3, C5
C2	26.6	CH_2	1.73 H_a 1.05 H_b	d, $J=9.0$ m C4, C10
C3	79.7	CH	3.14	dd, $J=11.5, 4.5$
C4	38.0	C		
C5	48.0	CH	2.03	dd, $J=13.5, 4.0$
C6	19.5	CH_2	1.56 H_a 1.43 H_b	m m C10
C7	33.7	CH_2	1.58 H_a 1.35 H_b	m m C5, C14
C8	39.6	C		
C9	49.6	CH	1.63	m
C10	39.9	C		
C11	20.2	CH_2	1.24 H_a 1.20 H_b	m m C13,
C12	124.3	CH	5.25	t, $J=3.5$ C9, C14
C13	144.7	C		
C14	43.2	C		
C15	24.7	CH_2	1.88	m C13, C17
C16	27.9	CH_2	1.62 H_a 1.57 H_b	m m C14, C18
C17	41.2	C		
C18	56.6	CH	0.85	brd. s C14, C16
C19	40.0	CH_2	1.64 H_a 1.01 H_b	m m
C20	43.9	C		
C21	76.0	CH	3.51	dd, $J=12.5, 4.5$ C17, C19, C29
C22	41.5	CH_2	2.24 H_a 2.03 H_b	dd, $J=14.0, 12.5$ dd, $J=14.0, 4.5$ C18, C20
C23	28.7	CH_3	0.97	s C3, C5
C24	17.4	CH_3	0.96	s C3, C5
C25	16.1	CH_3	0.78	s C1, C9
C26	16.3	CH_3	1.00	s C9, C14
C27	26.7	CH_3	1.18	s C7, C15
C28	21.0	CH_3	0.98	s C16, C22
C29	182.3	C		
C30	25.2	CH_3	1.23	s C29

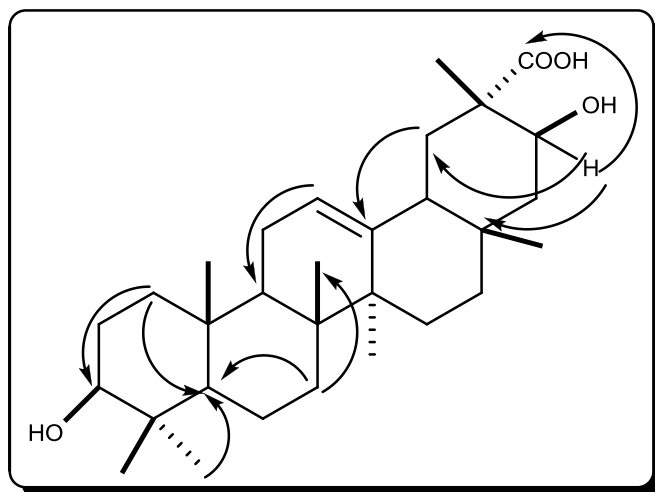


Fig.6.1a. Important HMBC correlations observed in **11**.

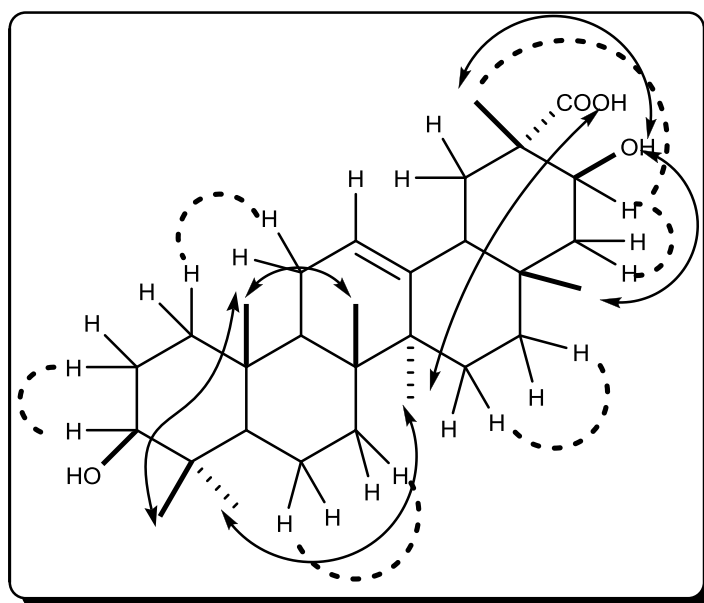
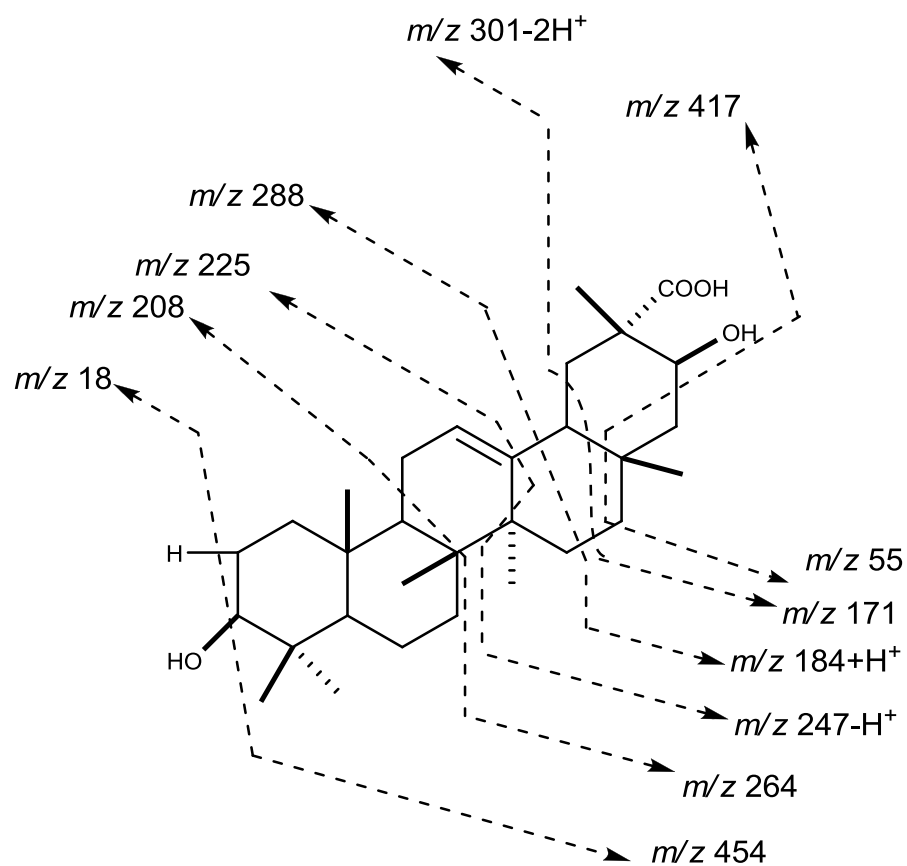


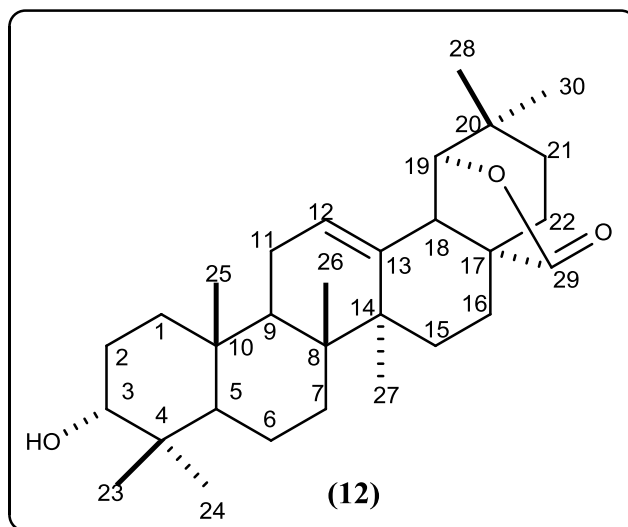
Fig.6.1b. COSY (dotted line) and NOESY correlations (double headed arrow) observed in **11**.



(c)

Fig.6.1c. Mass fragmentation pattern of 11.

6.2.2 Ficusonolide (12)



High resolution ESIMS of ficusonolide (**12**) gave molecular ion peak at m/z 454.6844 (calcd. 454.3447 for $C_{30}H_{46}O_3$), corresponding to molecular composition $C_{30}H_{46}O_3$, having 8 degrees of unsaturation corresponded to six rings, one olefinic bond and an ester carbonyl. The IR spectrum displayed strong absorption bands at 3490, 2945, 1748 and 1377 cm^{-1} , indicating the presence of hydroxyl, C-H saturated, ester carbonyl and *gem*-dimethyl functionalities respectively. The ^{13}C NMR spectrum (**Table 6.2**) of **12** indicated 30 resolved peaks corresponding to 30 carbon atoms which were classified as seven methyls, nine methylenes, six methines, seven quaternary and one carbonyl carbons by DEPT spectrum analysis. The ^1H NMR spectrum (**Table 6.2**) of **12** showed seven tertiary methyl singlets at δ 0.80, 0.90, 0.94, 1.04, 1.07, 1.21 and 1.22, compared to olean triterpenoid skeleton⁶⁸ while a vinylic proton resonated at δ 5.30 (t, $J=3.6\text{ Hz}$; H-12) and a carbinol proton triplet displayed at δ 3.43 ($J=3.0\text{ Hz}$; H-3) showing the axial position of hydroxyl group attached at C-3 confirmed the presence olean-12-en type triterpenoid skeleton.⁶⁸ which was supported by characteristic signals at δ_c 77.9 (C-3),

124.9 (C-12), 140.5 (C-13) and 42.6 (C-17) in ^{13}C NMR spectrum. The HMBC (**Table 6.2, Fig. 6.2a**) experiment and mass fragments pattern of **12** presented retro-Diels Alder standard fragmentation [m/z 246 and 207; **Fig. 6.2c**] indicating that the lactone ring was contained at rings D and/or E. The placement of lactone ring at ring E (between C17 and C-19) was made by COSY and HMBC spectra supported by mass fragment [m/z 300] (**Fig. 6.2a-c**) and ^1H NMR spectrum displayed doublet at 4.13 (d, $J=5.4$ Hz; H-19) instead of doublet of doublet in 3-*epi*abruslactone.⁷² All these assignments were made by long range HMBC correlations of H-19 with carbons at C-17 (δ 42.6), C-28 (24.8), C-29 (182.3) and C-30 (17.1) confirmed by COSY correlations of proton at δ 4.13 (d, $J=5.4$ Hz; H-19) with proton resonated at δ 0.85 (brd. s; H-18) (**Fig. 6.2a-b**).

The spectral data and comparison with related compounds in the literature;^{72,73} the structure of **12** as 3 α -hydroxylean-12-en-29,19 α -olide (ficusonolide).

Table 6.2: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in CD_3OD and HMBC correlations of ficusonolide (**12**)

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C1	33.8	CH_2 2.23 H_a 1.82 H_b	brd. s m	C3, C5
C2	25.6	CH_2 2.01 H_a 1.18 H_b	m m	C4, C10
C3	77.9	CH 3.43	t, $J=3.0$	
C4	37.3	C		
C5	55.7	CH 0.82	m	
C6	18.7	CH_2 1.65 H_a 1.45 H_b	d, $J=3,6$ d, $J=3.0$	C8, C10
C7	33.4	CH_2 1.46 H_a 1.34 H_b	m m	C5, C9, C14
C8	39.6	C		
C9	47.8	CH 1.57	m	
C10	35.4	C		
C11	23.7	CH_2 1.84	m	C8, C13
C12	124.9	CH 5.30	t, $J=3.6$	C9, C14
C13	140.5	C		
C14	39.5	C		
C15	28.1	CH_2 1.80	m	C13, C17
C16	24.6	CH_2 0.99 1.57 H_b	m m	C14, C18
C17	42.6	C		
C18	43.3	CH 0.85	brd. s	C14, C16
C19	83.1	CH 4.13	d, $J=5.4$	
C20	39.3	C		
C21	40.3	CH_2 1.95 H_a 1.47 H_b	m m	
C22	39.1	CH_2 1.63 H_a 1.52 H_b	m m	
C23	16.6	CH_3 1.04	s	C3, C5
C24	28.7	CH_3 1.22	s	C3, C5
C25	15.8	CH_3 0.94	s	C1, C9
C26	21.2	CH_3 1.21	s	C9, C14
C27	24.0	CH_3 1.07	s	C7, C15
C28	24.8	CH_3 0.80	s	C16, C22
C29	182.3	C		
C30	17.1	CH_3 0.90	s	C19, C21, C29

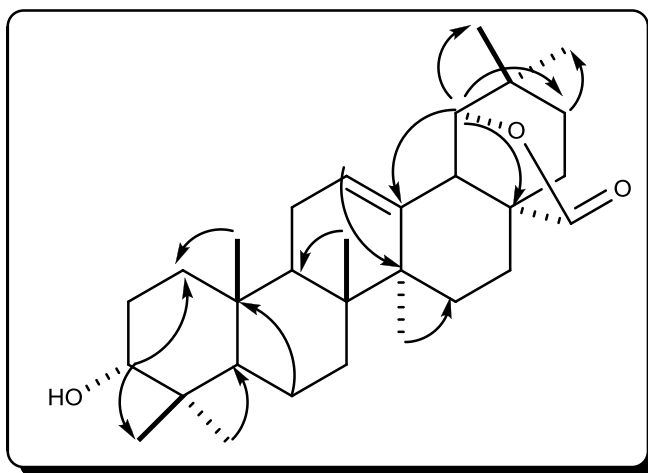


Fig.6.2a. Important HMBC correlations observed in 12.

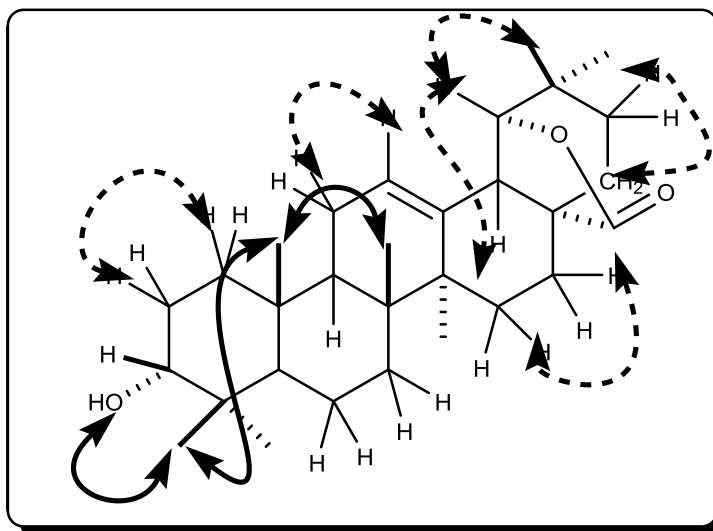


Fig.6.2b. COSY (dotted line) and NOESY correlations (double headed arrow) observed in 12.

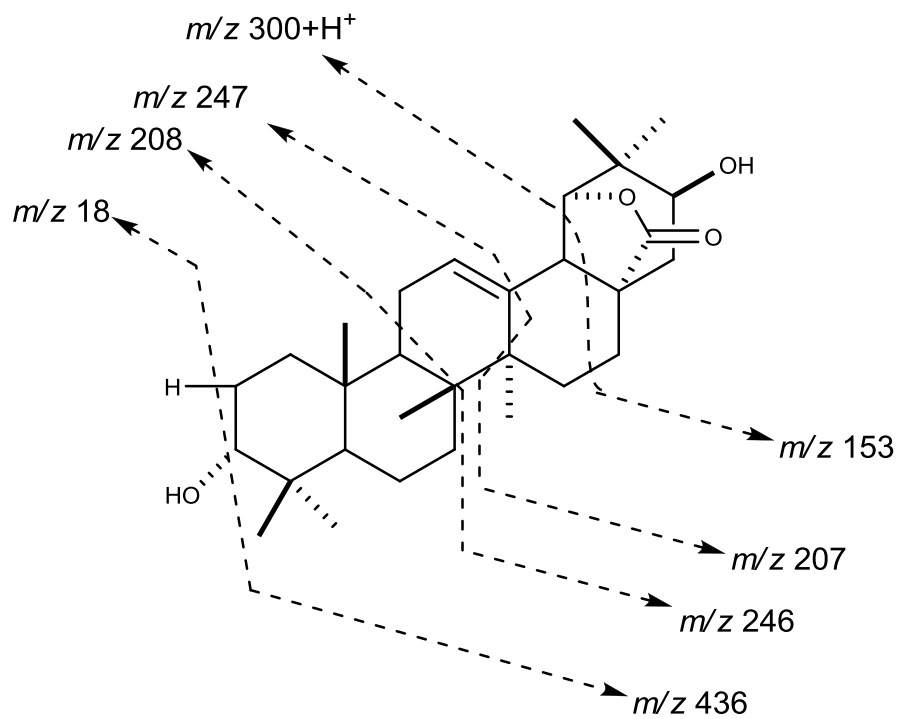
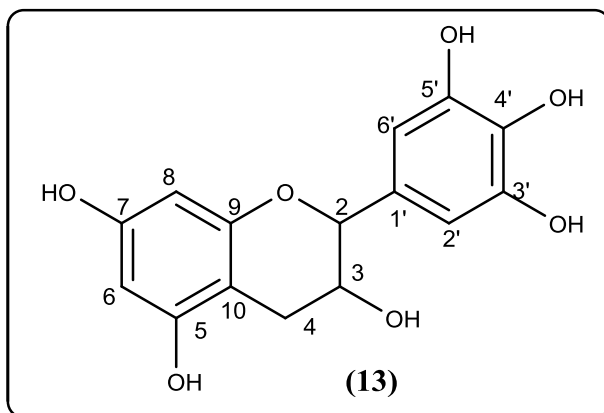


Fig.6.2c. Mass fragmentation of 12.

6.3 Known compounds isolated from *Ficus faveolata*

6.3.1 Gallocatechine (13)



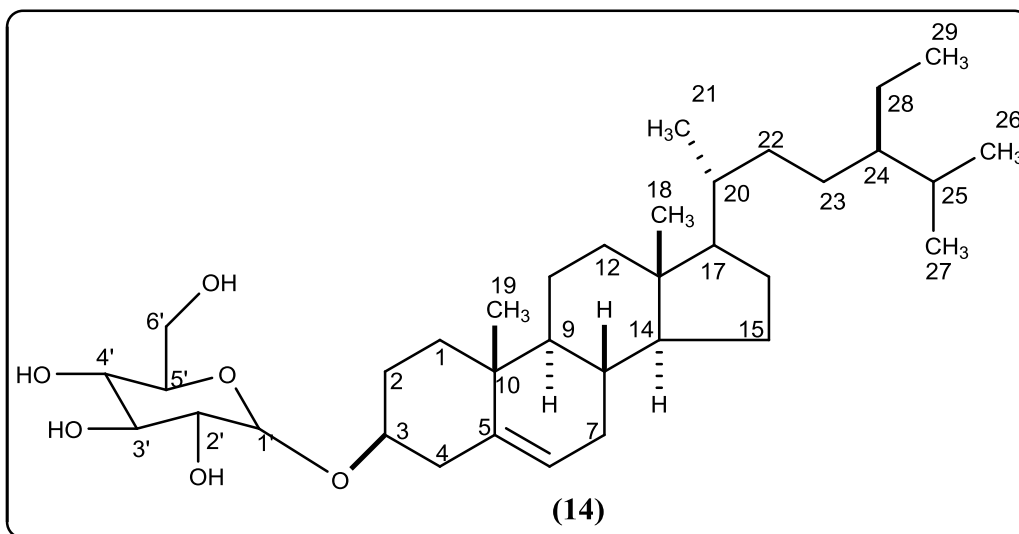
The ESIMS spectrum of compound **13** showed molecular ion peak at m/z 329 $[M^+ + Na]$ corresponding to the molecular formula $C_{15}H_{14}O_7$. The IR spectrum displayed absorption peaks at 3480 (hydroxyl) and 1550 cm^{-1} (C=C aromatic). The ^1H and ^{13}C NMR spectrum (**Table 6.3**) of **13** displayed the presence of a methylene, six methine and eight quaternary carbons. The assignments were made on by 2D NMR experiment.

All the spectral data coincided with reported compound; gallocatechine (2-(3',4',5'-trihydroxyphenyl)chroman-3,5,7-triol).⁷⁴

Table 6.3: ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) in CD_3OD and HMBC correlations of gallocatechine (**13**).

C.No	¹³ C NMR(δ)	¹ H NMR(δ)	Multiplicity & <i>J</i> (HH in Hz)	HMBC	
C2	79.9	CH	4.75	s	C2, C2', C6'
C3	67.5	CH	4.16	brd. s	C1', C10
C4	29.2	CH ₂	2.83 H _a	dd, <i>J</i> =4.5, 16.5	C-9, C-5
			2.72 H _b	dd, <i>J</i> =4.5, 15.5	C-2, C-5
C5	157.3	C			
C6	69.4	CH	5.92	d, <i>J</i> =2.5	C8, C10
C7	168.0	C			
C8	95.9	CH	5.85	d, <i>J</i> =2.5	C10
C9	157.7	C			
C10	100.1	C			
C1'	131.5	C			
C2', 6'	107.0	CH×2	6.51	s	C2, C4', C-6'
C3', 5'	146.7	C×2			
C4'	133.5	C			

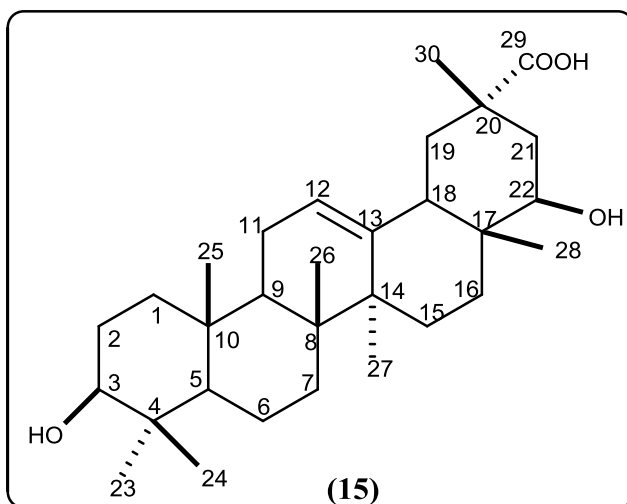
6.3.2 β -Sitosterol-3-O- β -D-glycopyrannoside(14)



The molecular formula $C_{35}H_{60}O_6$ of **14** established through HRESIMS showing molecular ion peak at m/z 576.4383 (calcd. 576.8473 for $C_{35}H_{60}O_6$). The EIMS m/z 396 [M^+ -gly] indicated the loss of glycoside moiety. The ^1H and ^{13}C (BB and DEPT; **Table 6.4**) spectra of **14** displayed the presence of three quaternary carbons, six methyls, twelve methylenes and fourteen methine groups. The experimental data; 1D, 2D NMR, IR and MS of **14** were in agreement with the reported data of the β -sitosterol-3-O- β -D-glycopyrannoside in literature.⁷⁵

Table-6.4: ^1H (600 MHz) and ^{13}C NMR (125 MHz) spectral data of β -sitosterol-3-*O*- β -D-glycopyrannoside (**14**) in $\text{C}_5\text{D}_5\text{N}$

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
C-1	37.5	CH_2 1.72H _a 1.67H _b	m m	C9, 5, 19
C-2	30.3	CH_2 2.40 H _a /2.11 H _b	m	C4,2'
C-3	78.5	CH 3.9	m	C1', 5
C-4	39.4	CH_2 1.99 H _a /1.94 H _b	brd. s	
C-5	140.9	C		
C-6	121.9	CH 5.33	brd. s	C8, 10
C-7	32.2	CH_2 1.81H _a /1.36H _b	m	C5,9
C-8	32.1	CH 1.57	m	
C-9	50.4	CH 0.91	m	
C-10	37.0	C		
C-11	21.3	CH_2 1.39H _a /1.32 H _b	m	C8, 10, 12
C-12	40.0	CH_2 2.72H _a 2.46H _b	brd. d, $J=12.9$ t, $J=11.1$	C17, 18
C-13	42.5	C -	-	
C-14	56.9	CH 1.13	m	
C-15	24.5	CH_2 1.53H _a	m	
C-16	28.6	CH_2 1.48 H _a /1.23H _b	m	
C-17	56.3	CH 1.10	m	C15, 22
C-18	12.0	CH_3 0.65	s	
C-19	19.4	CH_3 0.93	s	
C-20	36.4	CH 1.35	m	C16, 23
C-21	19.0	CH_3 0.97	d, $J=6.3'$	
C-22	34.2	CH_2 1.39 H _a 1.01 H _b	m m	C21, 24
C-23	26.4	CH_2 1.35	m	C28
C-24	46.1	CH 1.01	m	
C-25	29.5	CH 1.85	m	
C-26	19.2	CH_3 0.84	d, $J=6.3$	
C-27	20.0	CH_3 0.85	s	C24
C-28	23.4	CH_2 1.32 H _a 1.30 H _b	m m	C23, 25
C-29	12.2	CH_3 0.95	s	C24
C-1'	102.6	CH 5.05	d, $J=7.8$	C3
C-2'	75.4	CH 4.06	m	
C-3'	78.7	CH 4.29	t, $J=4.8$	
C-4'	71.7	CH 4.41	m	
C-5'	78.1	CH 3.91	m	
C-6'	62.9	CH_2 4.56 H _a 4.44 H _b	d, $J=11.4$ m	

6.3.3 $3\beta,22\beta$ -Dihydroxyolean-12-en-29-oic acid (**15**)

The molecular formula $C_{30}H_{48}O_4$ of **15** was established through HREIMS showing molecular ion peak at m/z 472.3620 (calcd. 472.3474 for $C_{30}H_{48}O_4$). The ^1H , ^{13}C NMR (broad band and DEPT; **Table 6.5**), IR, MS spectra were identical to reported compound; $3\beta,22\beta$ -dihydroxyolean-12-en-29-oic acid.⁷⁶

Table 6.5: ^1H NMR (600 MHz), ^{13}C NMR (125 MHz) in $\text{C}_5\text{D}_5\text{N}$ and HMBC correlations of $3\beta,21\beta$ -dihydroxyolean-12-en-29-oic acid (**15**)

C.No	¹³ C NMR(δ)	¹ H NMR(δ)	Multiplicity & <i>J</i> (HH in Hz)	HMBC	
C1	38.9	CH ₂	1.55 H _a 0.98 H _b	m m	C3, C5, C25
C2	27.9	CH ₂	1.78 H _a 1.50 H _b	m	C4,C10
C3	77.9	CH	3.39	m	C1, C23, C24
C4	39.1	C			
C5	55.5	CH	0.80	d, <i>J</i> =13.5	
C6	18.6	CH ₂	1.57H _a 1.51 H _b	m m	C8, C4
C7	32.7	CH ₂	1.48 H _a 1.27 H _b	brd. d <i>J</i> =6.0 brd. d <i>J</i> =6.0	C9, C26
C8	40.1	C			
C9	47.8	CH	1.62	m	
C10	37.1	C			
C11	23.8	CH ₂	1.88	m	C13, C8, C10
C12	123.1	CH	5.35	m	C9, C18, C14
C13	144.0	C			
C14	42.3	C			
C15	26.0	CH ₂	1.79 H _a 1.50 H _b	m m	C13
C16	19.7	CH ₂	2.04 H _a 1.86 H _b	m m	C14, C18
C17	39.3	C			
C18	47.1	CH	2.29	brd. s	C20, C28
C19	41.0	CH ₂	2.48 H _a 1.73 H _b	m m	
C20	43.1	C			
C21	38.5	CH ₂	2.57H _a 2.26 H _b	m m	C17, C19
C22	74.5	CH	3.99	brd. d, <i>J</i> =14.0	C18, C20
C23	28.8	CH ₃	1.19	s	C3, C5
C24	16.5	CH ₃	1.03	s	C3, C5
C25	15.6	CH ₃	0.93	s	C1, C9
C26	16.9	CH ₃	1.01	s	C9, C14
C27	26.2	CH ₃	1.23	s	C7, C15, C26
C28	25.3	CH ₃	1.32	s	C16
C29	180.8	C			
C30	21.2	CH ₃	1.54	s	C29

Chapter 7

Experimental Section

7.1 Methods and materials

7.1.1 Plant materials

Ficus faveolata was collected from Chagharzi, district Buner during the month of July 2007. The plant was identified by taxonomist Mr. Ambara Khan (Degree College Daggar, Buner). A voucher specimen (Bot.15077) was deposited in the Herbarium of Department of Botany, University of Peshawar, Peshawar, Pakistan.

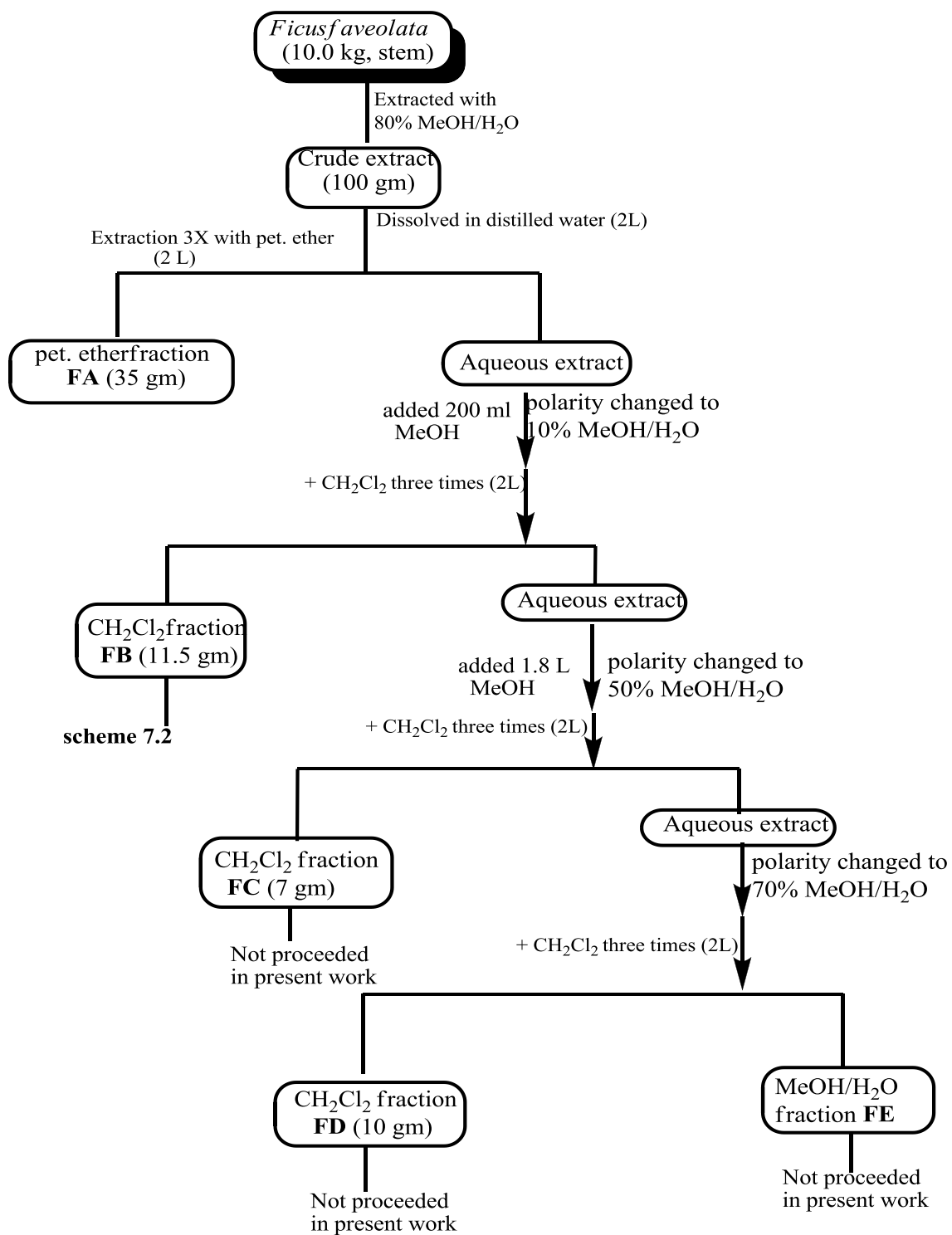
7.1.2 Extraction and isolation

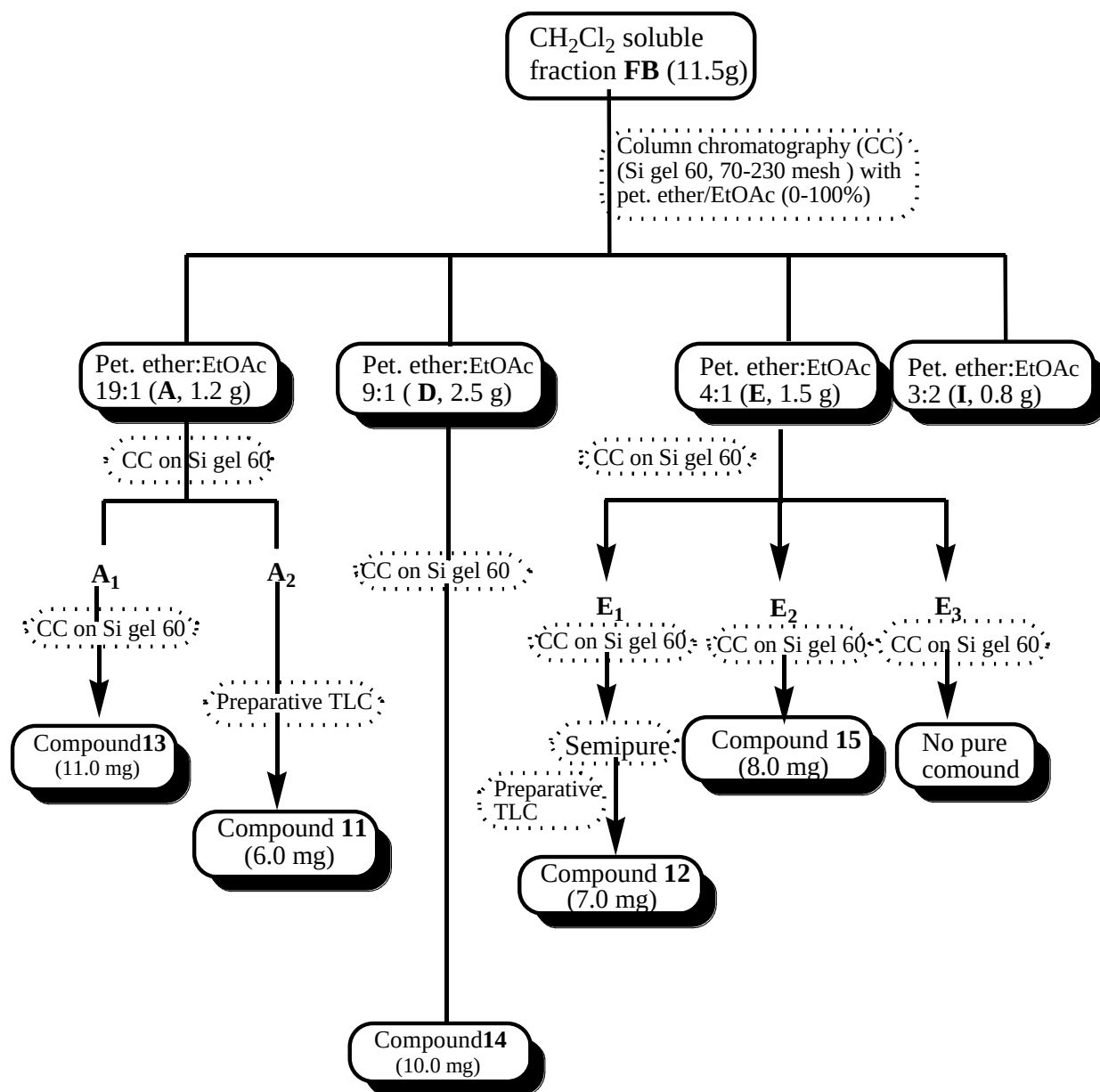
The air dried roots and stem of *Ficus faveolata* were repeatedly extracted (X3) with 80% MeOH/H₂O at room temperature. The combined extract was concentrated under vacuum to obtain brownish thick syrup that constituted the crude aqueous methanolic extract (100 gm). The crude extract was suspended in distilled water and defatted (X3) with petroleum ether afforded fraction **FA** (35 gm; **Scheme 7.1**). The polarity of aqueous phase was change to 10% MeOH/H₂O by addition of MeOH (200 ml) followed by extraction with dicholomethane (DCM) and concentrated under vacuum to obtain DCM soluble fraction **FB** (11.5 gm). The polarity of aqueous layer was changed to 50% MeOH/H₂O by addition of MeOH (1.8 L) and extracted with DCM to obtain fraction **FC** (7 gm). By addition of MeOH in the aqueous layer was changed to approximately 70% MeOH/H₂O which was further extracted with DCM to get DCM afforded DCMsoluble fraction **FD** (10 gm), while the DCM insoluble phase (aqueous methanolic phase) was not pursued in the present study.

The fraction **FB** (11.5 gm) was subjected to column chromatography over silica gel 60 (70-230 mesh, Merck), eluted with petroleum ether, petroleum ether-ethyl acetate

and ethyl acetate. As a result various fractions were obtained and combined on the basis of TLC profile to afford fractions **A-I** (**Scheme 7.1**). Fraction **A** (1.2 gm) was further subjected to column chromatography over silica gel 60 ((70-230 mesh, Merck) eluted with EtOAc/petroleum ether resulted into a number of fractions **A1-A8**, Fraction **A1** was further purified by CC (30% EtOAc/petroleum ether) to furnish compound **13** (11 mg) while compound **11** (6.0 gm) was purified by preparative TLC (3% MeOH/H₂O) from fraction **A2**.

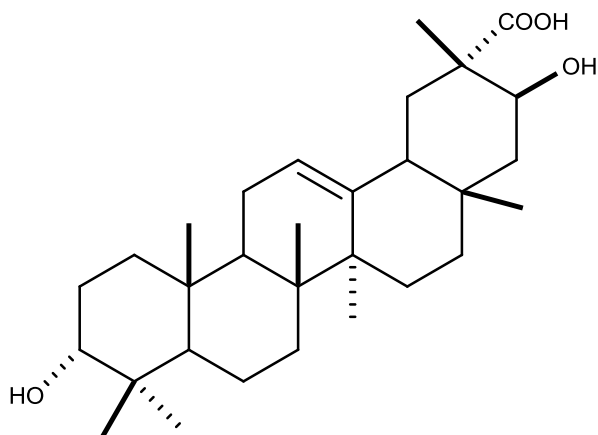
Compound **14** was furnished as an amorphous powder (10 mg) from fraction **D** by CC silica gel 60 (70-230 mesh, Merck) eluted with petroleum ether, petroleum ether-acetone and acetone in order of increasing polarity. Fraction **E** was collected at 20% EtOAc/petroleum ether which was rechromatographed by CC (silica gel 60, 70-230 mesh, Merck, solvent system petroleum ether, petroleum ether- acetone and acetone). As a result, six sub-fractions (**E1-E6**) were obtained on the basis of TLC profile. Sub-fraction **E1** furnished compounds **12** as white amorphous powder (7 mg) by preparative TLC (35% EtOAc/n-hexane) while compound **15** furnished as white amorphous powder (8 mg) by column chromatography from **E2** (**Scheme 7.2**).

**Scheme 7.1.** Extraction and isolation.

**Scheme 7.2.** Extraction and isolation.

7.2 Physical data of the compounds

7.2.1 3 α ,21 β -Dihydroxyolean-12-en-29-oic acid (**11**)



White amorphous powder (6 mg)

Molecular Formula:

C₃₀H₄₈O₄

Melting Point:

185-189 °C

IR (KBr): cm⁻¹

3358 (OH), 2945 (C-H olefinic), 1760 (CO carboxylic)

EIMS: *m/z* (rel. Int. %)

55 (100), 95 (90), 120 (80), 246 (80), 264 (45), 217 (45), 185 (35), 454 (5)

HRESIMS: *m/z*

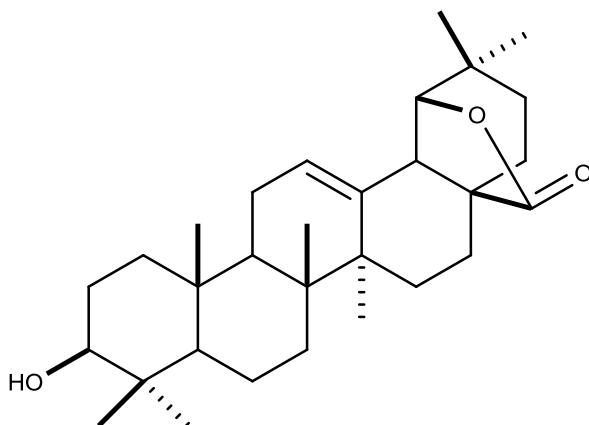
472.6997 (calcd. 472.3553 for C₃₀H₄₈O₄)

¹H NMR:

Table 6.1

¹³C NMR:

Table 6.1

7.2.2 3 α ,21 β -Dihydroxyolean-12-en-29-oic acid (**12**)

White amorphous powder (7 mg)

Molecular Formula:

C₃₀H₄₆O₃

IR (KBr): cm⁻¹

3490 (OH), 2945 (C-H olefinic), 1748 (CO ester)

EIMS: *m/z* (rel. Int. %)

246.1 (100), 190 (50), 207 (40), 454 (17)

HRESIMS: *m/z*

455.3522 [M⁺+H] (calcd. 454.6844 for C₃₀H₄₆O₃)

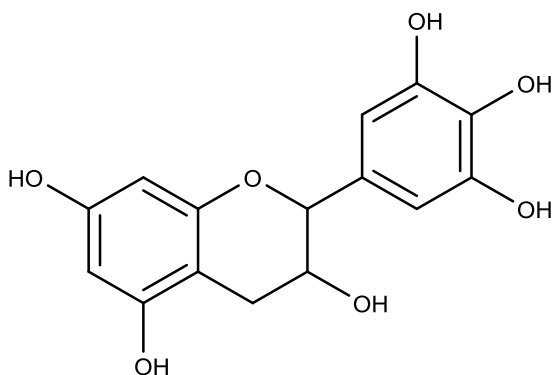
¹H NMR:

Table 6.2

¹³C NMR:

Table 6.2

7.2.3 Gallocatechine (13)



2-(3',4',5'-Trihydroxyphenyl)chroman-3,5,7-triol

White yellow powder (11 mg)

Molecular Formula: $C_{15}H_{14}O_7$ **IR (KBr):** cm^{-1} 3405 (OH), 2945 (C-H aromatic), 1550
(C=C aromatic)**ESIMS:** m/z (rel. Int. %)329 $[M + Na]^+$ (100), 635 $[2M + Na]^+$ (83),
942.5 $[3M + Na+H]^+$ (8)**ESIMS:** m/z (rel. Int. %)

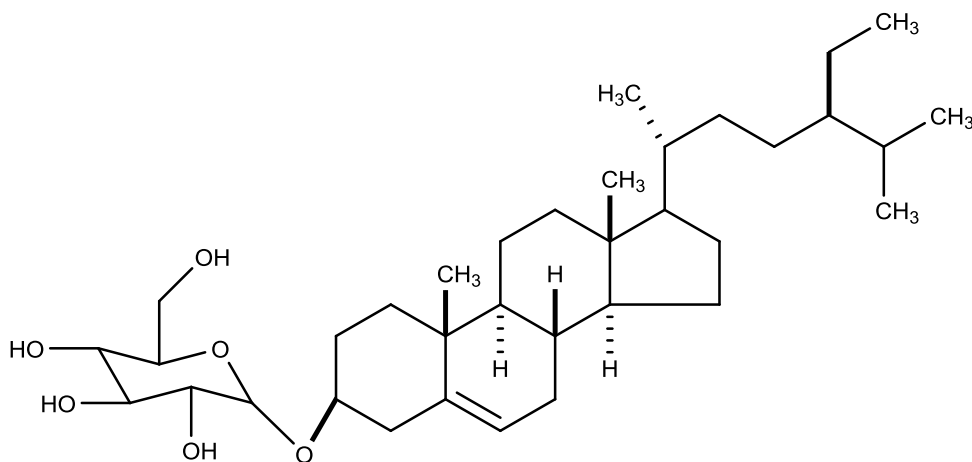
44 (100), 126 (20), 139 (15), 454 (10)

HRESIMS: m/z 306.0740 (calcd. 306.0773 for $C_{15}H_{14}O_7$) **^1H NMR:**

Table 6.3

 ^{13}C NMR:

Table 6.3

7.2.4 β -Sitosterol-3-*O*- β -D-glycopyrannoside (**14**)

(3*R*,4*S*,5*S*,6*R*)-2-((3*S*,8*S*,9*S*,10*R*,13*R*,14*S*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yloxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol

White powder (15mg)

Molecular Formula:

C₃₅H₆₀O₆

Melting Point:

234-240°C

IR (KBr): cm⁻¹

3400 (OH), 2935 (C-H olefinic), 1600 (C=C olefinic)

HRESIMS: *m/z*

576.4383 (calcd. 576.8473 for C₃₅H₆₀O₆)

EIMS: *m/z* (rel. int. %)

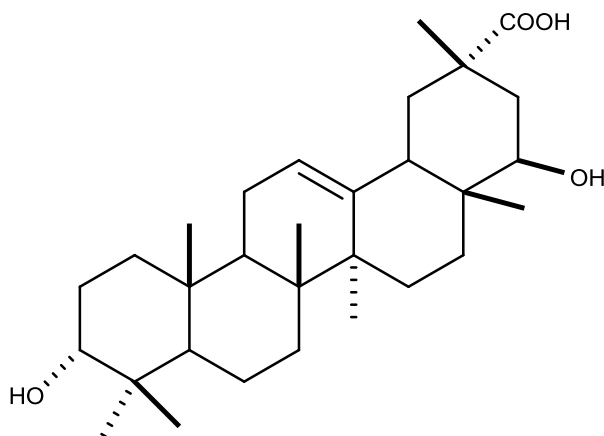
396.5 (100) [M⁺-gly], 147 (20), 381 (12), 255 (11)

¹H NMR:

Table 6.4

¹³C NMR:

Table 6.4

7.2.5 3 β ,22 β -Dihydroxyolean-12-en-29-oic acid (**15**)

(2*S*,4*R*,4*aR*,6*aS*,6*bR*,10*R*,12*aR*)-4,10-Dihydroxy-2,4*a*,6*a*,6*b*,9,9,12*a*-heptamethyl-1,2,3,4,4*a*,5,6,6*a*,6*b*,7,8,8*a*,9,10,11,12,12*a*,12*b*,13,14*b*-icosahydricene-2-carboxylic acid

White amorphous powder (8 mg)

Molecular Formula:

C₃₀H₄₈O₄

IR (KBr): cm⁻¹

3465 (OH), 2990 (C-H olefinic), 1755 (CO carboxylic)

EIMS: *m/z* (rel. Int. %)

246 (100), 95 (85), 55 (81), 131 (53), 190 (44), 217 (42), 454 (03), 472 (01)

HRESIMS: *m/z*

472.3620 (calcd. 472.3553 for C₃₀H₄₈O₄)

¹H NMR:

Table 6.5

¹³C NMR:

Table 6.5

7.3 References

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(Part C)

Chapter 8

(Miscellaneous)

Cyclotetrapeptides from *Aspergillus ochraceopetaliformis*

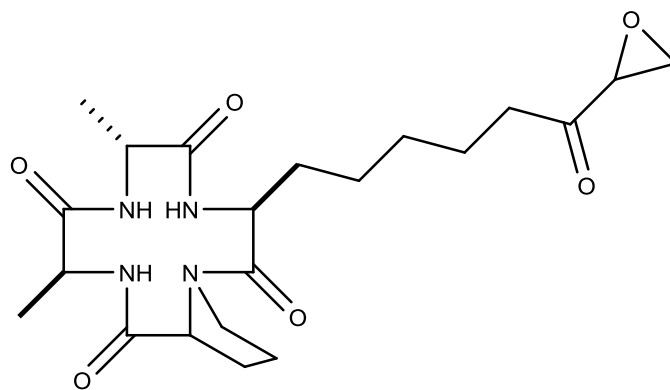
NOTE

Part C of the thesis has been accomplished under the auspices of Higher Education Commission, International Research Support Initiative Program (IRSIP) at University of California, Santa Cruz, USA under the supervision of Prof. Dr. Phillip Crews. Modification of research topic has been submitted to the Advance Studies and Research Board (ASRB), University of Peshawar. Unfortunately, it was not approved without any authentic reason. As per HEC rule any research work under IRSIP will be a part of Ph.D. thesis. To fulfill HEC requirement; miscellaneous chapter (Cyclotetrapeptides from *Aspergillus ochraceopetaliformis*) has been included under the approved Ph.D. research topic “Phytochemistry and Bioactivity of *Maytenus royleanus* and *Ficus faveolata*”.

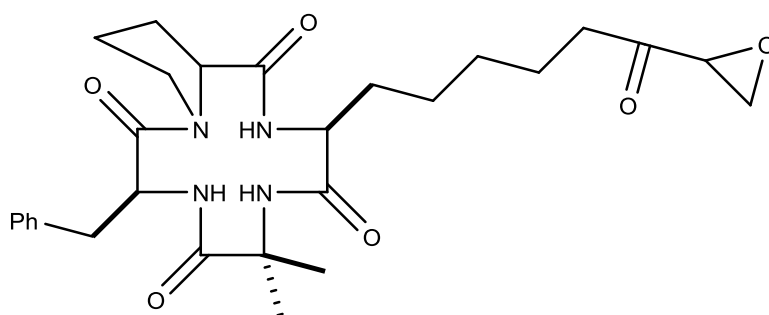
8.1 Introduction

There are approximately 80,000 species of fungi which may be single-cell or multi-cells heterotrophic organisms and can reproduce both sexually and asexually.¹ The classification was made on the basis of their morphology, modes of nutrition, reproductive strategies and DNA or gene sequencing.^{3,4} The morphological and phylogenetic study has built a strong foundation for the classification of all fungal species.

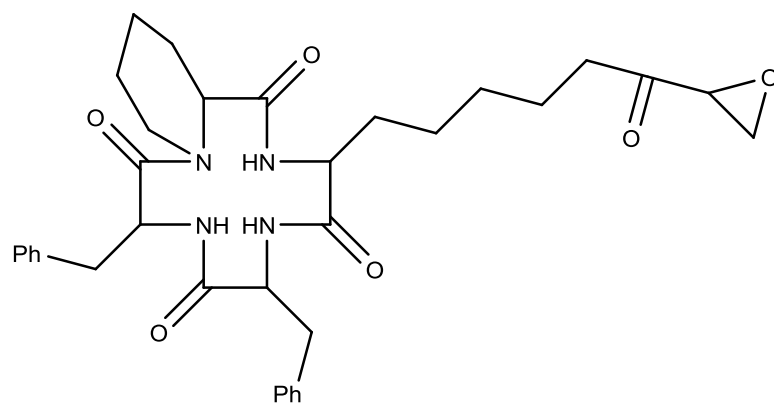
A large number of peptides particularly cyclic peptides have been isolated from fungi, bacteria, plants and animals, some of these cyclic peptides are found to be potent bioactive.⁵ Among these sources, Fungi has been found as the rich source of bioactive cyclic peptides. The importance of cyclotetrapeptides are based on cytotoxic or phytotoxic activities, however these peptides are control by special structural features like D-L amino acids, secondary amide bonds and sometimes exotic amino acids residues which is often found in the fungi derived cyclotetrapeptides.⁶ Some of the major representatives of the biologically active cyclotetrapeptides are phytotoxic tentoxin from *Alternaria alternate*,⁷ AM-toxin I from *Alternaria mali*,⁸ HC-toxin from *Cochliobolus carbonum*,⁹ cytotoxic chlamydocin from *Diheterosporia clamydosporia*,¹⁰ trapoxin A from *Helicoma ambiens*,¹¹ WF-3161 from *Petriella guttulata*,¹² and enzyme inhibitors like apicidine from *Fusarium* species.¹³



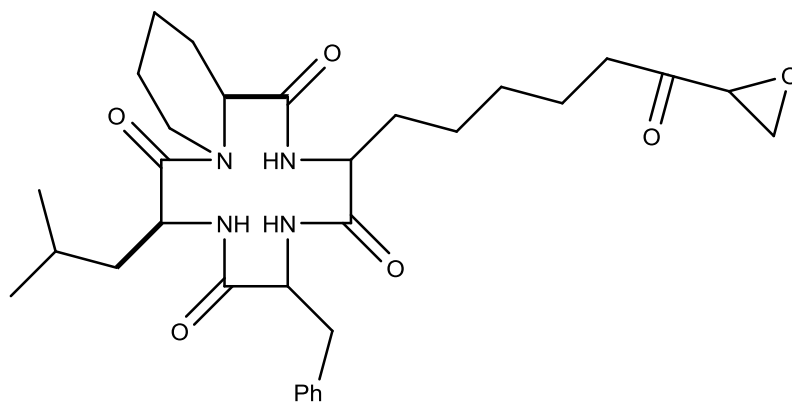
HC-Toxin



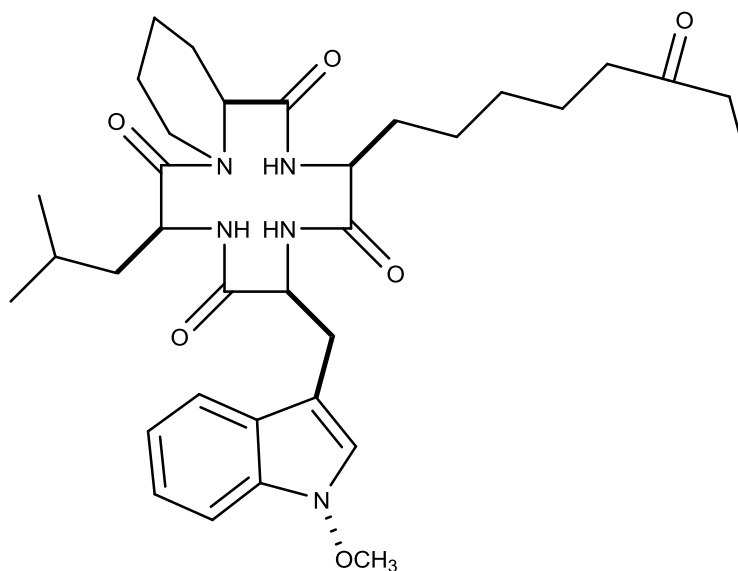
Chlamydocin



Trapoxine A

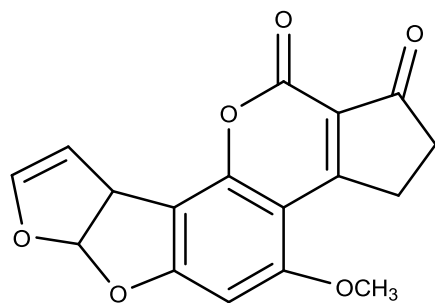


WF3161

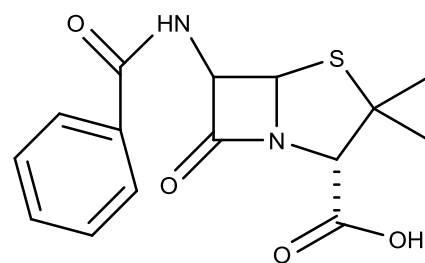


Apicidine

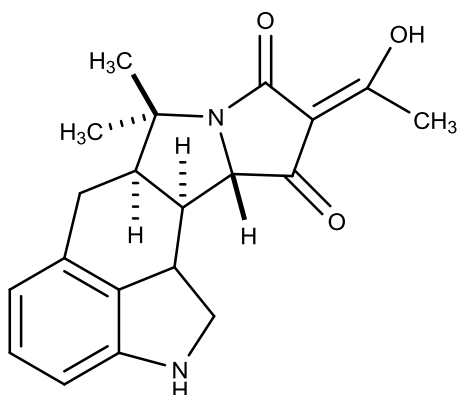
The genus *Aspergillus* merine derived fungi has considerable numbers of cytotoxic and bioactive secondary metabolites,¹⁴ such as aflatoxin (*A. flavus*),¹⁵ penicillic acid (*A. Nidulans*),¹⁶ cyclopiazonic acid (*A. flavus*),¹⁷ gliotoxin (*A. fumigatus*)¹⁸ and sclerotiotide A (*A. Sclerotiorum*).¹⁹ Up till now more than 200 strains of *Aspergillus* have been identified.²⁰



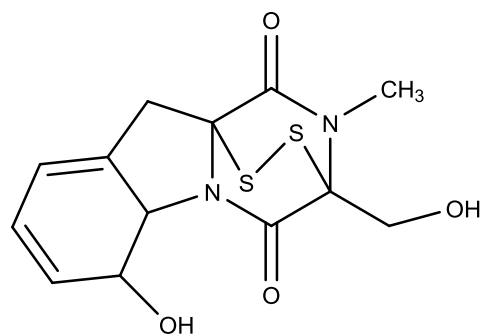
Aflatoxin



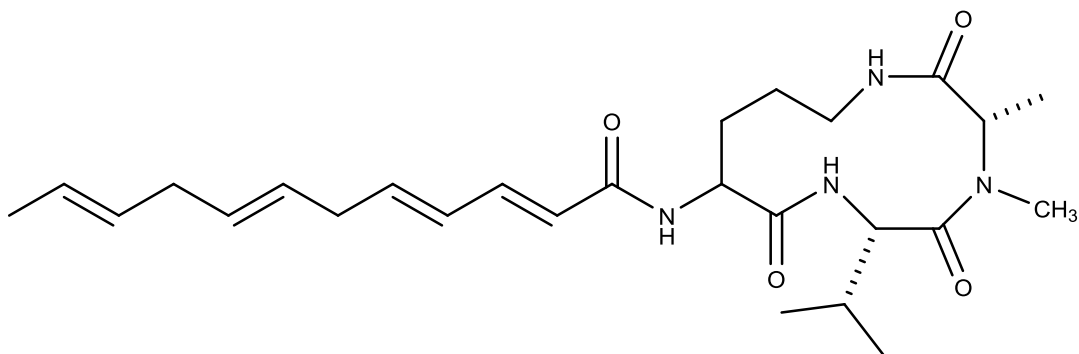
penicillic acid



cyclopiozonic acid



Gilotoxin



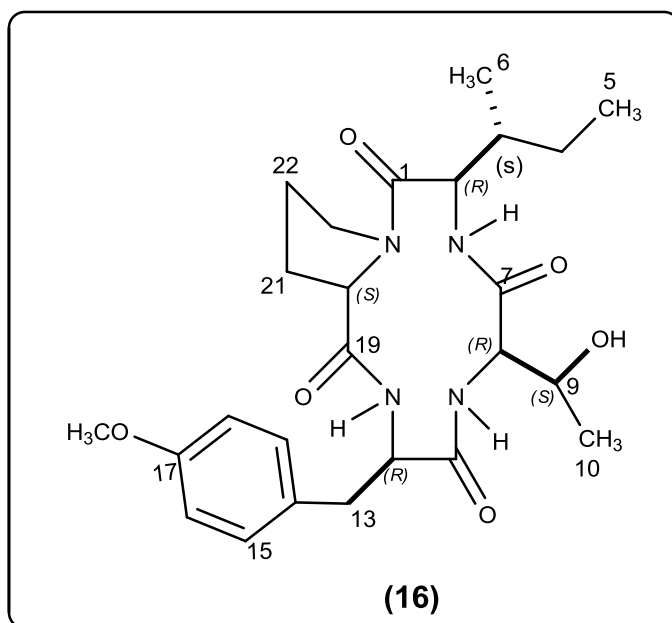
Sclerotiotide A

8.2 Results and Discussion

The present work has been done on the basis of high selectivity of ethyl acetate crude extract of marine sediment-derived fungal strain code # 088702A against solid tumor in a soft-agar based disk diffusion assay,²¹ as resulted into the isolation of two new cyclotetrapeptides; ochrine A (**16**) and ochrine B (**17**) from *Aspergillus ochraceopetaliformis*. Their structures were elucidated by spectral and chemical studies such as IR, UV, MS, ¹H, ¹³C NMR and marfey's method.

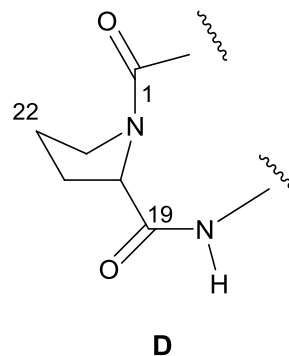
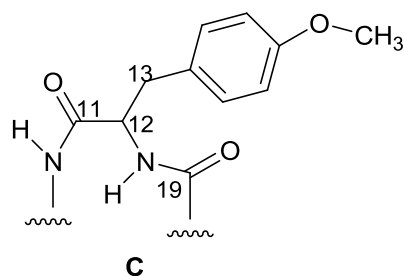
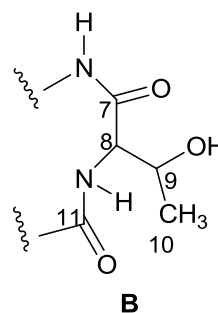
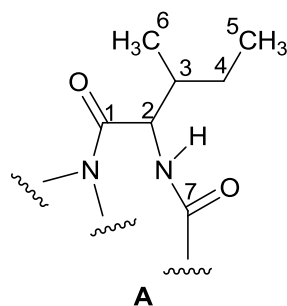
8.3 New compounds isolated from *Aspergillus ochraceopetaliformis*

8.3.1 Ochrine A (**16**)



Compound **16** was isolated as white amorphous powder. The molecular formula $C_{25}H_{36}N_4O_6$ was established through HRESIMS analysis showing a pseudo molecular ion at m/z 511.2527 $[M + Na]$ indicating 10 degrees of unsaturation. The ¹³C NMR (**Table 8.1**) data showed the presence of four amide carbonyl groups at δ 170.4 (C-1), 174.7 (C-

7), 175.1 (C-11), 175.3 (C-19) and a di-substituted phenyl ring at δ 134.3 (C-14), 116.2 (2 $\times$ C-15), 133.1 (2 $\times$ C-16) and 160.9 (C-17) confirmed by ^1H NMR experiment showing NH protons at δ 8.86 (m), 7.53 (m) and 10.14 (d, $J=6.6$ Hz) and phenyl protons at δ 7.17 (d, $J=8.4$ Hz; 2H-15), δ 7.28 (d, $J=8.4$ Hz; 2H-16). On the basis of COSY and HMBC substructures **A**, **B**, **C** and **D** were developed. The ^1H and ^{13}C spectra (in Pyridine- d_5) indicated that **16** was a cyclic tetra peptide consisting of Ile (Isoleucine), Thr (Threonine), Tyr (Tyrosine) and Pro (Proline) while Tyr residue was observed to be *O*-methylated. All these amino acids account for 8 of the 10 degrees of unsaturation but the cyclic nature of **16** and presence of Proline can fully justify 10 numbers of unsaturations. The sequence of amino acids in the cyclic form was developed by 2D NMR data (**Fig. 8.1**, **Table 8.1**). The HMBC and COSY data revealed correlations sequence: Ile 2-NH (δ 8.86) to Thr carbonyl C-7; Thr 8-NH (δ 10.14) to O-Me Tyr C-11 and C-12; OCH_3 Tyr 12-NH (δ 7.53) to Pro C-19 and Pro (Proline) methine proton (δ 1.75) to Ile C-1 supported by mass fragments (**Fig. 8.2**).



Detail analysis of the spectral data and proposed mass fragmentation led to the conclusion that compound **16** as a cyclotetrapeptide with sequence [Ile-Thr-O-Me-Tyr-pro].

Marfey's method²² was employed (*Vide* experimental) for absolute configuration of the chiral carbons in the **16**. By acid hydrolysis free amino acids were yielded. ESI(+)MS showed the M+H ions at m/z 116 (Pro), 120 (Thr), 132 (Ile) and 182 (Tyr). The absolute stereochemistry of compound **16** was assigned by LC-MS analysis of the Marfey's derivatized²²⁻²⁴ acid hydrolysate in comparison to the reference complex of standard amino acids (D and L). The analysis showed the presence of L-Ile, L-Thr, L-Pro and L-O-Me-Tyr in compound **16**. By employing the NMR data, HRESIMS, Marfey's method and molecular models, the absolute configuration of ochrine A **16** was 2*R*, 3*S*, 8*R*, 9*S* and 12*R* with sequence [Ile-Thr-O-Me-Tyr-pro].

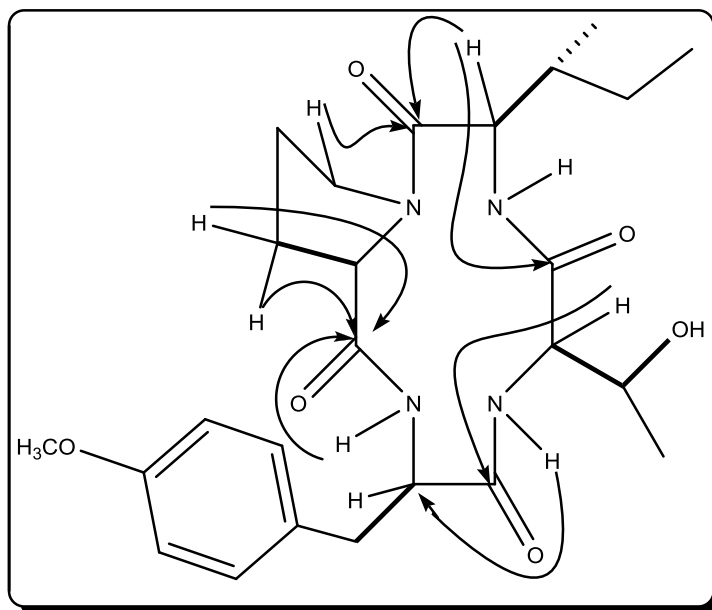


Fig. 8.1. Important HMBC correlations observed in **16**.

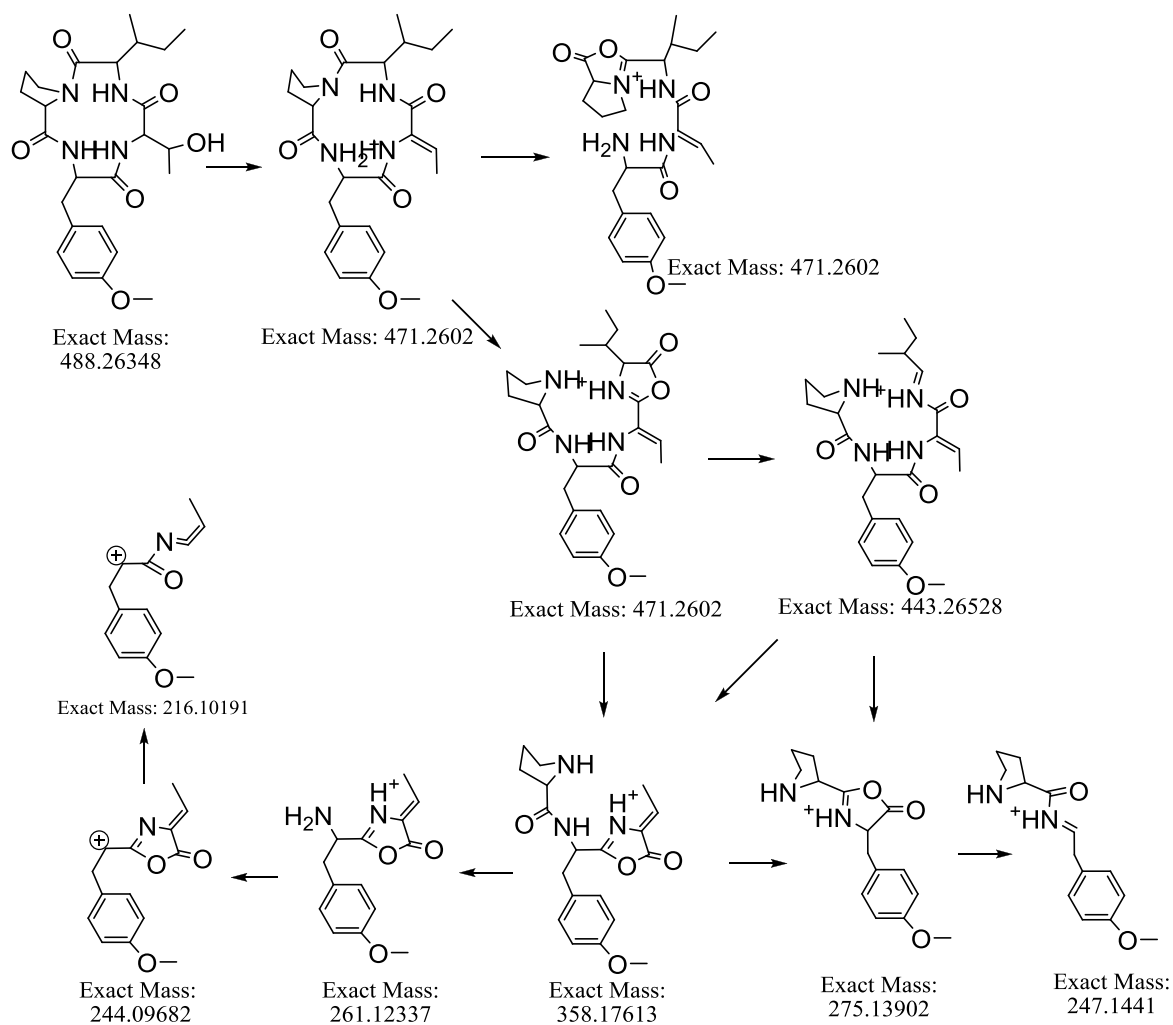
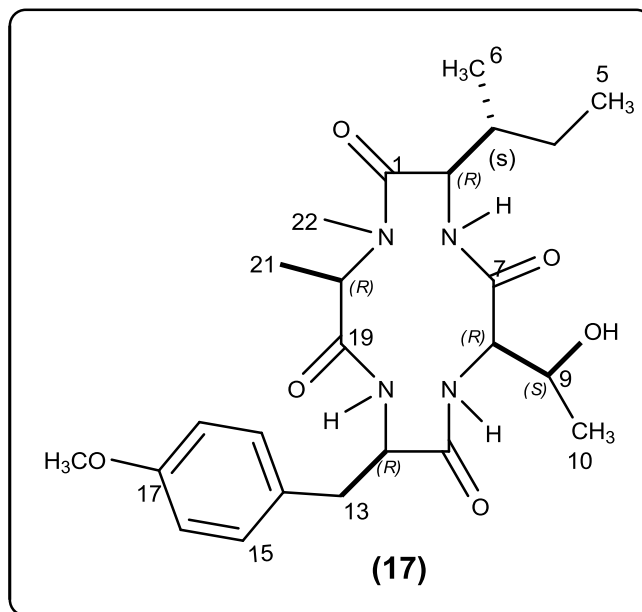


Fig. 8.2. Mass fragmentation pattern of **16**.

Table 8.1: ^1H NMR (600 MHz), ^{13}C NMR (125 MHz) in $\text{C}_5\text{D}_5\text{N}$ and HMBC correlations of ochrine A (**16**)

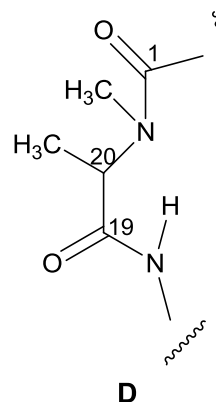
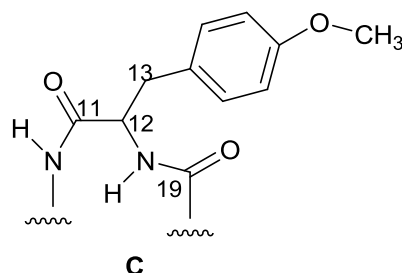
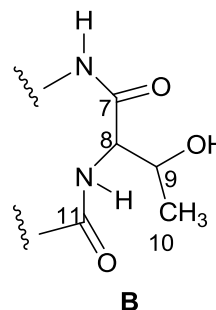
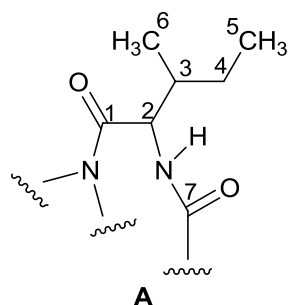
C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
L-Ile				
C1	170.4 C			
C2	58.3 CH	5.10	t, $J=10.5$	C1,3,4,6,7
C3	38.6 CH	2.43	m	C2,3,8
C4	26.5 CH_2	1.35 H_a 2.00 H_b	m m	C4,5,6,7 C4,5,6
C5	14.1 CH_3	0.91	t, $J=7.5$	C3,4
C6	19.0 CH_3	1.21	d, $J=7.5$	C2,3,4
NH		8.86	m	C2,7
L-Thr				
C7	174.7 C			
C8	66.9 CH	4.50	dd, $J=1.8, 9.0$	C7,9
C9	69.6 CH	4.87	brd. d	
C10	23.7 CH_3	1.41	d, $J=6.5$	C8,9
NH		10.14	brd. m	
OH				
L-O-Me-Tyr				
C11	175.1 C			
C12	57.6 CH	4.48	m	C11,13
C13	37.0 CH_2	3.75 H_a 3.99 H_b	m m	C11,12 C11,14
C14	134.3 C			
C15	116.2 $\text{CH}\times 2$	7.17	d, $J=8.4$	C14,16,17
C16	133.1 $\text{CH}\times 2$	7.28	d, $J=8.4$	C14,17
C17	160.9 C			
C18	57.2 OCH_3	3.7	s	C17
NH		7.53	m	C12,13
L-Pro				
C19	175.3 C			
C20	61.1 CH	4.61	d, $J=7.8$	C21,22,23
C21	33.4 CH_2	2.02 H_a 1.75 H_b	m m	C1,22,23 C1,20,22
C22	23.3 CH_2	1.77 H_a 2.21 H_b	m m	C9,20 C23
C23	49.8 CH_2	3.68 H_a 4.13 H_b	dd, $J=13.8, 3.0$ m	C22 C21

8.3.2 Ochrine B (17)



Compound **17** was obtained as white amorphous powder. The molecular formula $C_{24}H_{36}N_4O_6$ was consistent with HRESIMS showed pseudo molecular ion peak at m/z 499.2527 (calcd. 499.2526 for $C_{24}H_{36}N_4O_6Na$). The formula $C_{24}H_{36}N_4O_6$ suggested the presence of nine degrees of unsaturation. UV spectrum showed absorption maxima at 274nm (λ_{max}) indicated the presence of aromatic ring. The ^1H NMR spectrum (**Table 8.2**) displayed the presence of three amide NH groups resonated at δ 9.20, 9.91, 7.32 and methyls signals at δ 7.32 (m), δ 3.79 (s, H-18) attributed to N-Me and O-Me, while phenyl ring protons displayed at δ 7.05 (d, $J=12.0$ Hz; H-15 and 15') and 7.31 (d, $J=12.0$ Hz; H-16 and 16'). The ^{13}C NMR spectra (**Table 8.2**) exhibited the presence of four amide carbonyl carbons appeared at δ 171.3 (C-1), 173.5 (C-7), 173.7 (C-11) and 174.0 (C-19). These characteristic signals appeared in both ^1H and ^{13}C NMR spectra indicated

the peptide nature. On the basis of COSY and HMBC correlations four sub-structures **A**, **B**, **C** and **D** were developed for **17**.



The presence of L-N-Me-Ala in **17** was confirmed by LC-MS analysis of the Marfey's derivatized acid hydrolysate and ESI(+)MS analysis of the derivatized complex which revealed $[M+H]^+$ ions at m/z 104 (N-Me-Ala), 120 (Thr), 132 (Ile) and 182 (Tyr), while the sequence of amino acids in peptide **17** was confirmed by 2D NMR (**Fig. 8.3**, **Table 8.2**) data and mass fragmentation pattern (**Fig. 8.4**). The HMBC and COSY data revealed correlation sequence; Ile 2-NH (δ 9.92) to Thr carbonyl C-7 (173.5); Thr 8-NH (δ 9.91) to O-Me Tyr C-11 (173.7) and C-12 (55.7); O-Me Tyr 12-NH (δ 7.32) to N-Me-Ala C-19 (174.0) and N-Me-Ala n-methyl proton (δ 3.38) to Ile C-1 (171.3) (**Table 8.2**).

To determine the L (L or L-allo) and D (D or D-allo) configuration of amino acids present in **17**, Marfey's method²² was employed (*vide experimental*). A mixture of free amino acids of **17** was yielded by acid hydrolysis, indicated by ESI(+)MS, [M+H]⁺ ions at m/z 104 (-N-Me-Ala), 120 (Thr), 132 (Ile) and 182 (Tyr): the methyl group of O-Me-Tyrosine was lost by acid hydrolysis. The absolute stereochemistry of compound **17** was assigned by LC-MS analysis by Marfey's derivatized^{22,23} acid hydrolysate in comparison to the reference complex of standard amino acids (D or D-allo and L or L-allo) indicated the presence of L-Ile, L-Thr, L-N-Me-Ala and L-O-Me-Tyr. By using the NMR data, HRESIMS, Marfey's method and molecular models; the absolute configuration of **17** as 2R, 3S, 8R, 9S, 12R and 21R with amino acids sequence [L-Ile-L-Thr-L-O-Me-Tyr-L-N-Me-Ala] (ochrine B).

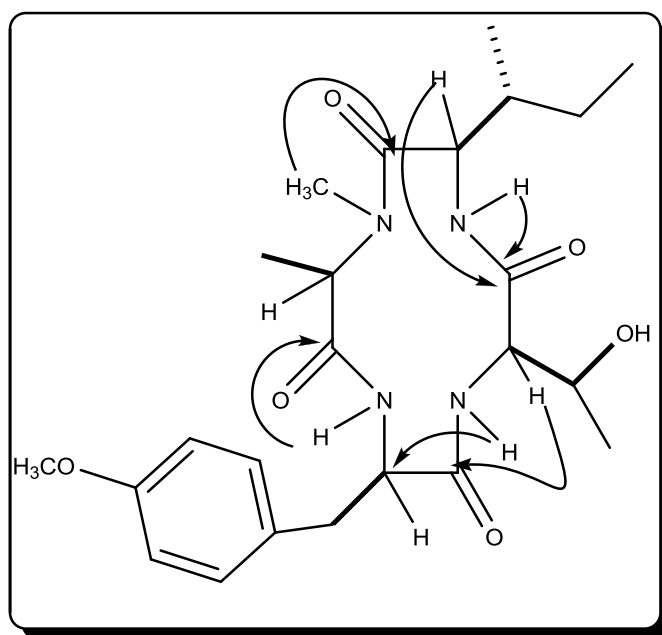


Fig. 8.3. Important HMBC correlations observed in **17**.

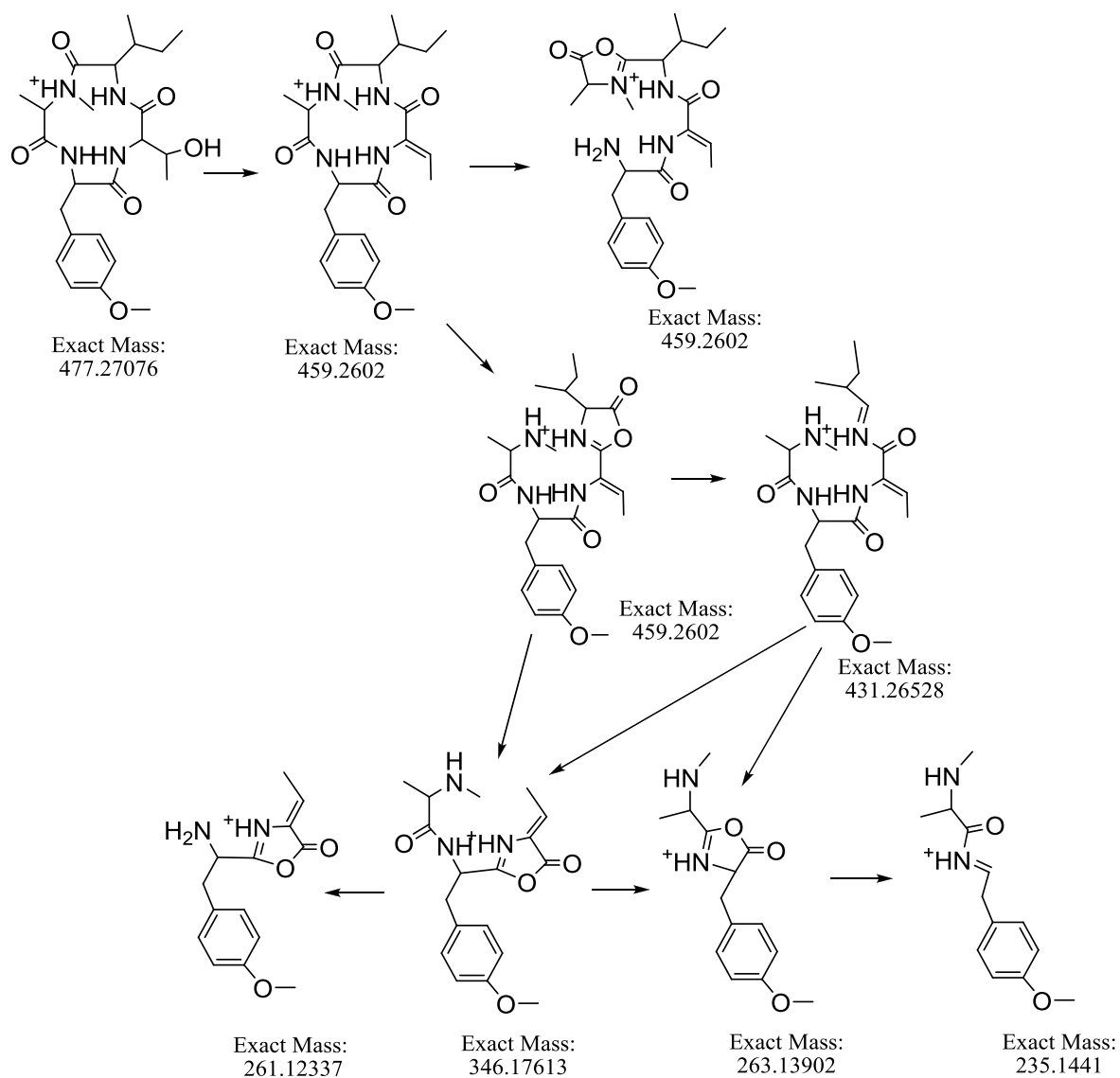


Fig. 8.4. Mass fragmentation pattern of 17.

Table 8.2: ^1H NMR (600 MHz), ^{13}C NMR (125 MHz) in $\text{C}_5\text{D}_5\text{N}$ and HMBC correlations of ochrine B (**17**)

C.No	^{13}C NMR(δ)	^1H NMR(δ)	Multiplicity & J (HH in Hz)	HMBC
L-Ile				
C1	171.3 C			
C2	55.8 CH	5.17	t, $J=8.5$	C1,3, 6
C3	37.5 CH	2.40	m	C1,2,4,5
C4	25.1 CH_2	1.17 H_a 2.01 H_b	d, $J=6.0$ m	C3,5,6
C5	12.8 CH_3	0.92	t, $J=7.5$	C3,4
C6	17.8 CH_3	1.17	d, $J=6.5$	C2,3,4
NH		9.20	brd. m	
L-Thr				
C7	173.5 C			
C8	65.4 CH	4.41	dd, $J=1.8, 9.0$	C7,9
C9	68.1 CH	4.81	brd. d	
C10	22.3 CH_3	1.38	d, $J=6.0$	C8
NH		9.91	brd. m	
OH				
L-O-Me-Tyr				
C11	173.7 C			
C12	55.7 CH	5.15	brd. m	C11
C13	35.6 CH_2	3.64 H_a 3.92 H_b	dd, $J=13.8, 3.6$ brd, m	C14,15 C14
C14	134.3 C			
C15	114.8 $\text{CH}\times 2$	7.05	d, $J=12.0$	C14,16,17
C16	132.0 $\text{CH}\times 2$	7.31	d, $J=12.0$	C15,17
C17	159.5 C			
C18	55.8 OCH_3	3.79	s	C17
NH		7.32	m	C19
L-N-Me-Ala				
C19	174.0 C			
C20	54.7 CH	4.75	m	C19,21
C21	17.2 CH_3	1.45	d, $J=7.2$	C1,20
C22	30.9 N-CH_3	3.38	s	C1,20

8.4 Experimental section

8.4.1 General experimental procedures

Varian Inova 600/ cold probe was used for ^1H (600MHz) and ^{13}C (125MHz) NMR spectra, while both high and low resolution mass spectra were taken ESI-TOF mass spectrometer. Phenomex 5 μm Luna C₁₈ RP column (250 $\times$ 10) was used in the HPLC with a single wave length 230 nm. LCMS data was obtained for both peptides by using Sedex 55 ELS detector, 996 photodiode array detector and C18 RP (Phenomenex Luna), 5 μm column (150 $\times$ 4.6) on ESITOFMS. Optical rotation was recorded on a Jasco P-2000 polarimeter. The purity of compounds was determined with analytical HPLC by using MeCN and H₂O with 0.1% formic acid.

8.4.2 Determination of (D/L) amino acid configuration by using marfey's method

1mg of each compound **16** and **17** was hydrolyzed in 6N HCl (0.5ml) at 110 °C for 16 hours in a sealed, thick-walled vial. The hydrolysate was evaporated under N₂ stream and then dissolved in 50 μL acetone/water which was first treated with 100 μL solution of (Marfey's reagent) N-(5-Fluoro-2,4-dinitrophenyl)-D-Lecinamide (D-FDLA, 1.4 mg/100 μL of Acetone) and then added 20 μL of 1M NaHCO₃ solution.^{26,27} The reaction mixture was heated at 40°C for 1 hour, cooled at room temperature and the reaction was quenched by adding 10 μL of 2M HCl,²⁵ and dried under N₂ stream. The residue of both compounds **16** and **17** were dissolved in 0.5 mL of DMSO, for further analysis on LC-ELSD-MS. Each standard amino acid (1 mg) was also derivatized by same procedure with D-FDLA. The aliquots (10 μL) of both compounds **16** and **17** were run on reversed phase LCMS (Column: Phenomenex, Luna, 150 $\times$ 4.60mm, 5 μm , linear

gradient of 40% CH₃CN/H₂O: 0.1% FA per 30 minutes, flow rate 1mL/min) was used to separate the derivatized products. Retention time of D-FDLA derivatives of commercially standard amino acids are: L-Pro (12.5 minutes), L-O-Me-Tyr (23.3 minutes) and L-N-Me-Ala (12.3 minutes). The LCMS chromatogram of D-FDLA derivatized hydrolysate of **16** confirming the presence of L-O-Me-Tyr and L-Pro while the LCMS chromatogram of D-FDLA derivatized hydrolysate of **17** showed corresponding peaks for L-O-Me-Tyr and L-N-Me-Ala. To further confirmed the presence of L or L-allo isomers of both lle and thr, the D-FDLA derivatives of four isomers of two standard amino acids and compounds **16** and **17** were subjected to LCMS on chiral column (Phenomenex, Chirex (S), 50×4.60mm, 5 µm, linear gradient 30-65 CH₃CN/0.1M Ammonium Acetate_(aq): 0.3% TFA, per 30 minutes, flow rat 1mL/min), indicated the presence of L-lle (6.55 min) and L-Thr (5.02 min) in both Ochrine A (**16**) and B (**17**).

8.4.3 Biological materials

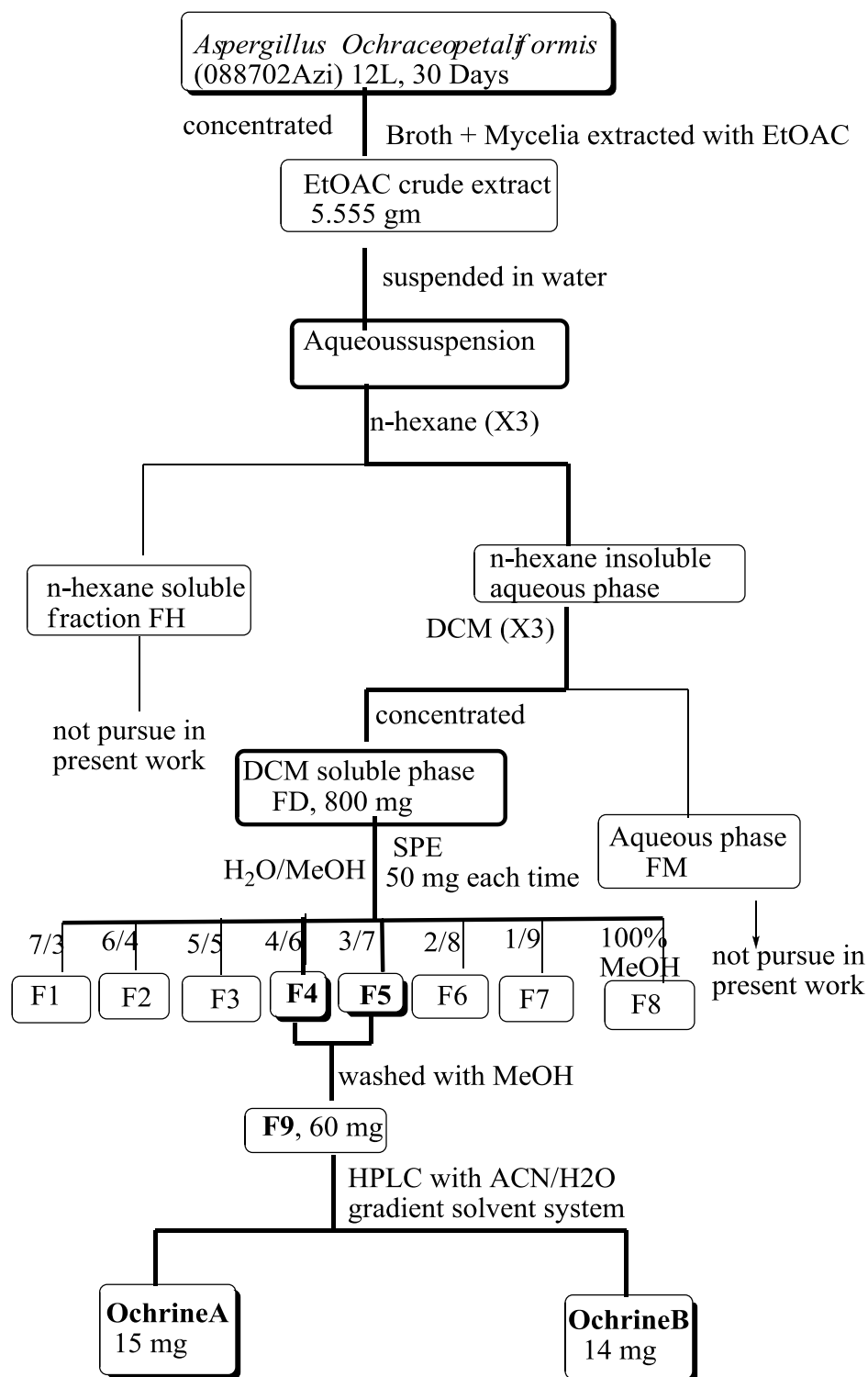
The *A. ochraceopetaliformis* (collection # 088702A) was collected from shallow water sediment in Hawaii in 2008, during an expedition to Hawaii. The strain was taxonomically identified by morphological method and molecular analysis (ITS and D1/D2) using DNA sequencing at UTHSCSA, University of Texas. The DNA sequencing experiment was performed by Dr. Brain Wickes. The strain was preserved in the glycerol stocks in liquid N₂ in Crews Laboratory University of California Santa Cruz, USA, as per standard laboratory procedure.

8.4.4 Culture conditions

The strain 088702A was grown in twelve liters deionized water in Czapek-Dox media (3.5 and 0.5% yeast, autoclaved for 30 minutes, PH 7). Culture was inoculated and shaken at 150 rpm at 25°C on shaker (New Brunswick Scientific Co. Inc. Model G-53) for 30 days.

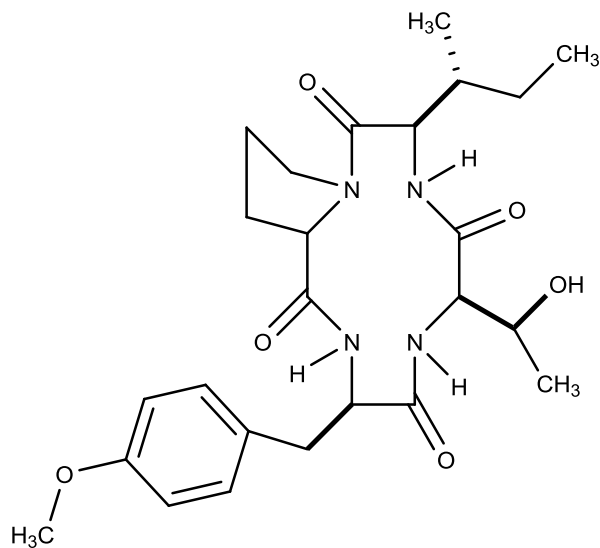
8.4.5 Extraction and isolation

Collective mycelia and broth was repeatedly (X3) extracted with ethyl acetate. The combined extract was concentrated under vacuum at 40°C and extract was partitioned into hexane (**EFH**), dichloromethane (**EFD**) and MeOH (**EFM**) and concentrated under vacuum (**Scheme 8**). Fraction **EFD** (800 mg) was fractionated into eight major fractions **F1-F8** by Solid Phase Extraction (SPE) using Strata-X 33U Polymeric Reversed Phase (500mg/6ml) Phenomenex column, with H₂O-MeOH and MeOH (**Scheme 8.0**). The fractions **F4** (60% MeOH) and **F5** (70% MeOH) were combined based on LCMS profile and concentrated under N₂ gas stream to obtained solid white material (**F9**, 60 mg), washed with MeOH. Fraction **F9** furnished compounds **16** (15 mg) and **17** (14 mg) as white amorphous powder by reverse phase semi preparative HPLC (10-100% MeCN/H₂O per 30 minutes; 225 nm wavelength) (**Scheme 8.0**).

**Scheme 8.0** Extraction and isolation

8.5 Physical data of the compounds

8.5.1 Ochrine A (16)



White amorphous powder (15 mg)

Molecular Formula:

$C_{25}H_{36}N_4O_6$

ESIMS: m/z (rel. Int. %)

179 (100), 511 (85), 245 (68), 225 (55), 223 (50), 351 (38), 512 (28), 207 (25)

$[\alpha]_D^{25}$: (C_5H_5N)

+68 (c 0.1, C_5H_5N)

UV: λ_{max} (MeCN, nm)

274

HRESIMS: m/z

511.2527 (calcd. 499.2526 for $C_{24}H_{36}N_4O_6Na$)

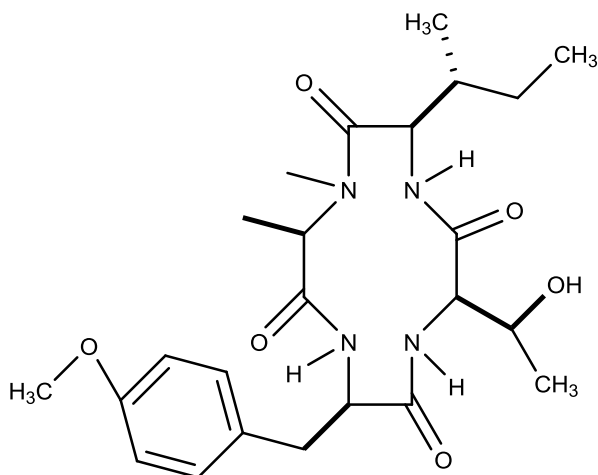
^1H NMR:

Table 8.1

^{13}C NMR:

Table 8.1

8.5.2 Ochrine B (17)



White amorphous powder (14 mg)

Molecular Formula:

$C_{24}H_{36}N_4O_6$

ESIMS: m/z (rel. Int. %)

167 (100), 499 (80), 233 (70), 213 (61), 211 (55), 339 (30), 500 (22), 195 (25)

$[\alpha]_D^{25}$ (C_5H_5N)

−100 (c 0.1, C_5H_5N)

UV: λ_{max} (MeCN, nm)

274

HRESIMS: m/z

499.2527 (calcd. 477.2526 for

$C_{25}H_{36}N_4O_6Na$)

^1H NMR:

Table 8.2

^{13}C NMR:

Table 8.2

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