PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION ON *ARTEMISIA ELEGANTISSIMA* PAMP AND *ARTEMISIA INCISA* PAMP (ASTERACEAE)

BY

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UNIVERSITY OF PESHAWAR, PAKISTAN

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BY

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In the name of ALLAH, the most gracious, the most merciful

"Were they created by nothing, or were they themselves the creators?

Or did they create the heavens and the earth? Nay, they

Have no firm belief." (Al-Qur'an 52:35-36)

"It is He Who made the sun radiate a brilliant light and the moon reflect a

luster, and ordained for it stages, that you might know the

number of years and reckoning of time."

(Al-Qur'an 10:5)

DECLARATION

This is to certify that this dissertation prepared by **Mamoon Ur Rashid** entitled "**Phytochemical and biological investigation on *Artemisia elegantissima* Pamp *and Artemisia incisa* Pamp (Asteraceae)"** is accepted in the present form by the Institute of Chemical Sciences, University of Peshawar as fulfilling this part of the requirement for the degree of **Doctor of philosophy in Chemistry**.

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DEDICATED

To

My Father (Late)

The First Good Man I Ever Loved

For earning an honest life for us and for inspiring, shaping my life as a self-confident person. He was an amiable and gentle father who taught me to believe in Allah and rely on hard work. He was very Kind to me, his provision and devotions have made me able to complete my PhD thesis.

(May Allah shower His Mercy upon him and fill his grave with Noor, save him from Ahzaab and the fire of Jahannum and grant him Jannat-ul-Firdus.

(Ameen)

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LIST OF ABBREVIATIONS

^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
1D NMR	One Dimensional Nuclear Magnetic Resonance Spectroscopy
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
2D NMR	Two Dimensional Nuclear Magnetic Resonance Spectroscopy
A20/2J	Murine B Lymphoma-derived Cell Line
A549	Adenocarcinoma Human Alveolar Basal Epithelial Cells
AIDS	Acquired Immune Deficiency Syndrome
ATP	Adenosine Triphosphate
BSA	Bovine Serum Albumin
CC	Column Chromatography
COSY	Correlation Spectroscopy
CR	Completely Randomized
DEPT	Distortionless Enhancement by Polarization Transfer
DiFMUP	6,8-Difluoro-4-methylumbelliferyl Phosphate
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetraacetate
EI-MS	Electron Impact-Mass Spectrometry
EMRSA	Endemic Methicillin Resistant *Staphylococcus aureus*
ESI-MS	Electron Spray Ionization-Mass Spectrometry
FAB-MS	Fast Atom Bombardment Mass Spectrometry

FACS	Fluorescence-Activated Cell Sorter
FBS	Foetal Bovine Serum
FITC	Fishery Industrial Technology Center
GC-MS	Gas Chromatography-Mass Spectrometry
HCT116	Human Colon Carcinoma Cell Line
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation
HMI	Human-Machine Interface
HR-EIMS	High Resolution-Electron Impact Mass Spectrometery
HR-ESIMS	High Resolution-Electro Spray Ionization Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	Inhibitory Concentration 50
IR (ATR)	Infra-Red (Attenuated Transmitted Radiation)
L	Levo
LC_{50}	Lethal Concentration 50
LC-MS	Liquid Chromatography-Mass Spectrometry
LDH	Lactose Dehydrogenase
LSD	Least Significant Difference Test
MCF7	Michigan Cancer Foundation-7
MIC	Minimum Inhibitory Concentration
MS	Mass Spectrometry
MTT	Methyl Thiazol Tetrazolium

NOESY	Nuclear Overhauser Effect Spectroscopy
PCR	Polymerase Chain Reaction
PTP 1B	Protein Tyrosine Phosphatase 1B
P-value	Probability value
R	Rectus
R_f	Retardation Factor
RPMI	Roswell Park Memorial Institute
SAR	Structure Activity Relationship
SPHK1	Sphingosine Kinase 1
SPHK2	Sphingosine Kinase 2
TFMS	Bis(4-Trifluoromethylsulfonamidophenyl)-1,4-diisopropylbenzine
TLC	Thin Layer Chromatography
t_R	Retention Time
UV-Vis	Ultraviolet-Visible
VLC	Vacuum Liquid Chromatography

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ABSTRACT

This dissertation consists of two parts; "**Part A**" and "**Part B**". The "**Part A**" gives details of the phytochemical studies on two indigenous medicinal plants; *Artemisia elegantissima* Pamp and *Artemisia incisa* Pamp (Asteraceae). The selected species of *Artemisia* have been investigated for the first time for their phytochemical constituents. Four new (artemglycerol **(1)**, artemceramide-A **(2)** santolynilol-3-acetate **(3)** and artemceramide-B **(4)**) and thirty three known compounds (5,7,8,3',4'-pentahydroxy-6-methoxyflavone **(5)**, bonanzin **(6)**, 3′,4′-dihydroxybonanzin **(7)**, apigenin **(8)**, (*S*)-dihydroluteolin **(9)**, scoparol **(10)**, nepetin **(11)**, *bis*-dracunculin **(12)**, dracunculin **(13)**, scopoletin **(14)**, allograciolone acetate **(15)**, betulinic acid **(16)**, triacontanoic acid **(17)**, nonacosanol **(18)**, (*Z*)-*p*-hydroxycinnamic acid **(19)**, stigmasterol **(20)**, β-sitosterol **(21)**, β-sitosterol-3-*O*-β-D-glucopyranoside **(22)**, *cis*-dehydromatricaria ester **(23)**, eicosanoic acid **(24)**, octacosanoic acid (25), eupatorin **(26)**, santolinylol **(27)**, *trans*-ethyl caffeate **(28)**, scoparone **(29)**, isoscopoletin **(30)**, esculetin **(31)**, isofraxidin **(32)**, ethyl-(*E*)-*p*-hydroxy cinnamate **(33)**, taraxerol **(34)**, taraxerol acetate **(35)**, benzoic acid **(36)** and linoleic acid **(37)**) have been isolated for the first time from these species.

The two new compounds; artemglycerol **(1)** and artemceramide-A **(2)** were isolated from *A. elegantissima* while two new compounds; santolynilol-3-acetate **(3)** and artemceramide-B **(4)** were isolated from *A. incisa*. The flavonoid (5,7,8,3',4'-pentahydroxy-6-methoxyflavone) has been presented with full spectral data and physicochemical constant for the first time. The structural characterizations of the

compounds have been achieved by using spectroscopic techniques (EI-MS, ESI-MS, UV-Vis, IR, ^{1}H-NMR, ^{13}C-NMR, DEPTq, DEPT-135, HMBC, HSQC, NOESY and COSY).

In "**Part B**" of the dissertation, anticancer, antidiabetic, antibacterial, antitrypanosomal, nematicidal and sphingokinase inhibition studies of the fractions and the compounds isolated from *A. elegantissima* and *A. incisa* have been discussed.

The preliminary results obtained from the anticancer screening of apigenin (**8**), scopoletin (**14**), *trans*-ethyl caffeate (**28**) and taraxerol acetate (**35**) performed against the three cancer cell lines (Human colon carcinoma cell line; HCT116, Adenocarcinomic human alveolar basal epithelial cells; A549 and Michigan cancer foundation-7; MCF7) in the MTT assay have produced no good results.

The antidiabetic screening was performed against Protein Tyrosine Phosphatase 1B (PTP 1B) at a conc. of 100 µg/ml. The two compounds; bonanzin (**6**) and 3′,4′-dihdroxy bonanzin (**7**) were highly active which showed inhibition of 22 and 18 % of the control respectively. While *bis*-dracunculin (**12**), dracunculin (**13**), scopoletin **(14)**, nepetin (**11**), scoparol (**10**) and apigenin (**8**) also showed good inhibition (33, 35, 32, 30, 26, and 29 % of the control respectively).

Antibacterial activities were carried out against four microbial strains i.e. *Mycobacterium marinum*, sporadic methicillin resistant *Staphylococcus aureus* (SMRSA) and endemic methicillin resistant *Staphylococcus aureus* (EMRSA) while antitrypanosomal activities were performed against *Trypanosoma brucei brucei* (*T.b. brucei*).

In the antibacterial screening at a conc. of 200 µg/ml, 3′,4′-dihydroxybonanzin **(7)** was the most active compound against both *SMRSA* and *EMRSA* which showed -2.7 and -10.8 % D control respectively while scoparol **(10)** and apigenin **(8)** also showed good activities against *EMRSA* (-3.2 and -1.2 % D control respectively). At a conc. of 100 µg/ml, *bis*-dracunculin **(12)** (-1.2 % of D control) showed selective inhibition of *M. marinum***.**

In the antitrypanosomal screening, scopoletin (**14**) was found to be the most active compound (MIC: <0.19 µg/ml) followed by 3′,4′-dihydroxy bonanzin **(7)** (MIC: 6.25 µg/ml). These results can help in directing the rational design of 3′,4′-dihydroxy bonanzin **(7)** and scopoletin **(14)** as other similar secondary metabolites or synthetic analogs as potent and effective antiprotozoal agents against *T.b. brucei*.

In the nematicidal screening performed against root knot nematodes (% eggs hatching of *Meloidogyne javanica*), esculetin **(31)** (LC_{50}: 57.6 µg ml^{-1}), dracuculin **(13)** (LC_{50}: 63.5 µg ml^{-1}) and linoleic acid **(37)** (LC_{50}: 82.6 µg ml^{-1}) showed good activities while against stage two juvenile (J2s) of *M. javanica* (% mortality), esculetin **(31)** showed high activity (LC_{50}: 90.4 µg ml^{-1}) followed by isofraxidin **(32)** (LC_{50}: 93.0 µg ml^{-1}) and *trans*-ethyl caffeate **(28)** (LC_{50}: 99.5 µg ml^{-1}).

In sphingokinase (SPHK) inhibition studies, the two new compounds; artemceramide-A **(2)** and artemceramide-B (**4**) were found to be SPHK2-specific inhibitor showed inhibition of 43 and 49 % respectively at a conc. of 4 mM. Possibly due to unsaturation, artemceramide-A **(2)** showed better inhibition than the artemceramide-B **(4)**. It is demonstrated that artemceramide-A **(2)** and artemceramide-B **(4)** induce apoptosis via

phosphorylation by SPHK2 and they are considered to be SPHK2 specific inhibitor, can exert its effect on cell death independently. While againt SPHK1, the compounds; artemceramide-A **(2)** and artemceramide-B **(4)** showed minimum inhibition.

Part A

Chapter 1

Introduction

1.1 General.

Natural products obtained from medicinal plants are generally practiced and progressively used in management of many diseases. It has produced significant drugs both in their natural form as well as templates for synthetic modifications.[1]

To date, more than 200,000 of secondary metabolites have been reported from higher plants. Out of the various classes of natural products reported, phenyl propanoids, polyketides, terpenoids and alkaloids are the most important.[2]

Due to variety of geographical locations in Pakistan, it has rich flora of medicinal plants. There are about 6000 plant species reported in Pakistan, out of which 600 to 700 are regarded of medicinal values.

1.1.1 Natural products as a source of drugs.

The inevitability of natural products research and their traditional based therapeutic value exploration can be judged from the history and one can mention the discovery of the pain relievers such as the morphine **(38) (Figure 1.1a**, **Appendix A)**, (page 261) an alkaloid of the plant; *papaver somniferum*, antimalarial drug such as the quinine **(39)** isolated from the bark of *Cinchona succirubra*, as well as the active principle from the leaves of *Salix alba,* the salicin **(41)** which was eventually developed in the form of salicylic acid (aspirin) **(40)**.[3] Similarly, artemisinin **(42)** an antimalarial isolated from *Artemisia annua* in the form of semisynthetic derivatives; artemether **(43)**, arteether **(44)** and artesunate **(45)** are used as a magic bullet against the multidrug resistant strains of *Plasmodium*.[4] More recently, the anticancer agents; vinblastine **(46)** and vincristine **(47)** were isolated

as antineoplastic agents from *Catharanthus roseus* and consequently derivatized and optimized to vinorelbine and vindesine.[5]

These few accounts emphasize the prospective of natural products as a source of drugs as well as an important linkage between the folk medicine and the drug development.[3] The current plants under phytochemical and biological investigations were from the family Asteraceae.

1 . 2 The family Asteraceae.

The family Asteraceae (also known as aster, daisy or sunflower family) is having 1500 genera and 23000 species which have importance due to its economical, biological, chemical and medical application worldwide.[6] Terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes are the major classes of the compounds reported from the family. Sesquiterpene lactones are the major constituents of the family and by the year 1994, more than 4000 sesquiterpenes were isolated.[6-7]

The chemical constituents of the family Asteraceae have various medicinal uses like relieving cough, inducing sweat, reducing phlegm, invigorating blood circulation, diuretic, anthelminthic, antihypertension, cytotoxic, antimalarial, antitoxic, analgesic and antiallergic.[8] Some of the important biological investigations reported on the family are discussed in **Part B, Sections 1-3.**

Due to important bioactive constituents isolated from the family Asteraceae, the genus *Artemisia* was selected as a topic of the current phytochemical and biological investigation.

1.3 The genus *Artemisia* Linn.

The genus *Artemisia* L. is one of the largest genus of the family with over 500 species and wide spread in nature.[9] The plants are mostly herbs; however, some are shrubby and woody. General morphological features are capitula small, usually racemouse, paniculate or capitate, leaves alternate, inflorescence, rarely solitary; involucral bracts in few rows, receptacle flat to hemispherical, without scales and sometimes hirsute; florets all tubular, achenes obovoid, pappus absent or sometimes a small scarious ring.[6]

A number of *Artemisia* species are used in the treatment of various ailments such as cancer, malaria, hepatitis, infections and inflammation.[10] The artemisinin; an antimalarial currently used against resistant strain of malaria was isolated from *A. annua.*[11]

Some of the important biological investigations reported in the literature on *Artemisia* are discussed in **Part B, Section 1-3.**

Due to the unique chemistry and biology of the genus *Artemisia,* the two virgin species; *Artemisia elegantissima* Pamp and *Artemisia incisa* Pamp were subjected to phytochemical and biological investigation.

1.3.1 *Artemisia elegantissima* Pamp.

Distribution. *Artemisia elegantissima* is a perennial herb, grows in pebbly hill slopes above 1500 m. It is distributed in Pakistan (Chitral and Kashmir) and India.





Description. The plant is glabrous, 50–100 cm tall herb with ± spreading striate-sulcate shoots. Long petiolate with basal leaves, narrowly winged, rachis winged, sparse forked. The upper side is dark green with appressed hairs while the lower side is greyish-green with moderately to densely appressed hair. The leaves on the upper and middle stem are 2-4 cm long. Lamina is 6-12×3-8 cm long with remote primary segments, acuminate.

1.3.2 *Artemisia incisa* Pamp.

Distribution. *Artemisia incisa* is perennial herb, grows along the field borders in wet soils or salty-clay, common between altitude of 1500 and 3000 m. It is distributed in India (Himachal Pradesh), N.W. Himalayas, Hindu Kush (N.E. Afghanistan) and Pakistan (Kashmir).





Description. The plant is 60–125 cm tall with solitary, erect, branched above, sparsely appressed-puberulous to closely glabrous. The stems are angular-ridged from prostrate, ± woody rootstock. The leaves are green above with T-shaped sparse hairs, shortly petiolate

or almost sessile, oblong to sketchily ovate, 7-12×5-7 cm, densely to lightly greyish-green arachnoid below, primary segments 7-9, acute and lanceolate, 4-6×2-3.5 cm.

1.4 Literature review of the genus *Artemisia*.

1.4.1 Phytochemicals reported.

So far, 296 *Artemisia* species are phytochemically explored. A number of metabolites (around 1100) including; flavonoids, terpenoids, coumarins, polyacetylenes and glycosides have been reported from the genus and these are briefly discussed here.[12]

1.4.1.1 Aliphatic hydrocarbons, alcohols, ketones, aldehydes, fatty acids and esters.

Most of these compounds were reported from the GC-MS analysis of the essential oils of different *Artemisia* species. These were further classified as given below.

i. **Unbranched saturated hydrocarbons.**
ii. **Unbranched saturated alcohols, ketones and aldehydes.**
iii. **Unbranched saturated fatty acids and esters.**
iv. **Unbranched alkenic hydrocarbons and alcohols.**
v. **Unbranched alkenic aldehydes, ketones, fatty acids and esters.**
vi. **Branched aliphatic hydrocarbons and alcohols.**
vii. **Branched aliphatic aldehydes, ketones, fatty acids, esters and anhydrides.**
viii. **Branched alkenic hydrocarbons and aldehydes.**
ix. **Polyacetylenic compounds.**

The saturated fatty acids are expected to be the precursors of the hydrocarbons, alcohols, ketones and aldehydes. The unbranched saturated hydrocarbons (C_5–C_{34}),[13] alcohols (C_5, C_6, C_8, C_9, C_{23}, C_{28} and C_{30}),[14] aldehydes (C_4, C_6, C_8, C_{13}),[14] ketones (C_3, C_7, C_9 and C_{27}),[15] fatty acids (C_3–C_{34}),[16] and esters (C_3, C_5, C_9, C_{14}, C_{18}, C_{19} and C_{61})[14, 17] have been reported from *Artemisia*. Palmitic and stearic acids were the most abundant saturated fatty acids, while oleic acid was the most abundant unsaturated fatty acid.[18]

In case of unbranched alkenic hydrocarbons, several small molecules (C_{20}) with a maximum of three olefinic bonds located both at the terminal and nonterminal positions[19] and alkenic alcohols (C_{10}) with both the terminal and nonterminal hydroxyl substitution have been reported. Aldehydes with α,β-unsaturation (C_3–C_{11})[18] were more common than their ketones analogues (C_3–C_9). Carboxylic acids (C_3–C_{18}) were more common than their esters analogues.[20]

The branched aliphatic hydrocarbons (C_2–C_{32}) and alcohols (C_2–C_{24}) were less common than their linear analogues. In case of branched aliphatic alcohols, the terminal hydroxyl substitution was more common as compared to the nonterminal substitution.[14]

The branched fatty acids, aldehydes and ketones with the carbon chain of C_{32}, C_{10} and C_8 have also been reported from *Artemisia*.[15, 21]

Among the cyclic hydrocarbons, cyclopropane derivatives were most common. Various furan derivatives substituted with alkenic, acetylenic, oxygenated side chains and linked with oxygenated rings have also been reported from *Artemisia.*[22]

Polyacetylenes were common in the genus *Artemisia* like other members of the Asteraceae family. The representative compounds of acetylenes, cyclic hydrocarbons and furan derivatives are summarized in **Table 1.1.**

Table 1.1. Acetylenes, cyclic hydrocarbons and furan derivatives.

Name of the compound	**Name of the compound**
Acetylenes derivatives	
*****1-((*2R,5R*)-1,6-Dioxaspiro[4.5]dec-3-en-2-yl)hexa-2,4-diyn-1-one **(51)**[23]	*****2,7-Dimethyl-3-octen-5-yne **(57)**[14]
*****Prop-1-ynylthiophen-2-yl ethanone **(52)**[24]	*****4,6-Heptadiyne-1,3-diol **(58)**[25]
*****Butyl-1,3-diynylbenzene **(53)**[26]	*****2,4-Hexadiynal **(59)**[25]
*****1-Dodecyne **(54)**[14]	*****Capillanol **(60)**[27]
*****1-Hepten-6-yne **(55)**[26]	*****Capillene **(61**)[28]
*****10-Dodecyn-1-ol **(56)**[24]	*****Dehydromatricaria ester **(23)**[29]
Cyclic hydrocarbons	
*****2,2′-Dimethyl-1,1′-bicyclopropane **(62)**[14]	*****Methyl cyclopentane **(68)**[30]
*****1,4-Dipropyl-cyclooctane(**63**)[15, 31]	*****Hexylcyclohexane **(69)**[32]
*****3,5-Cycloheptadienone **(64)**[33]	*****Cyclopropene, 3-ethenyl-3-methyl **(70)**[15]
*****Jasmone **(65)**[30]	*****15-Hydroxy-3-longipinen-5-one **(71)**[15]
*****Artepillin A **(66)**[34]	*Artemisia* lactone **(72)**[35]
*****1,9-*seco*-1,9-Presilphiperfolanedione **(67)**[36]	*****bicyclo[2.2.2]-1,2,3,6-Tetramethyl octa-2,5-diene **(73)**[32]
Furan derivatives	
*****4-Methyl-2,3-dihydrofuran **(74)**[26]	*****Artemone **(77)**[37]
*****5-Methylfuran-2-carbaldehyde **(75)**[15]	*****Davana ether **(78)**[38]
*****1-Hydroxybisaboloxide B **(76)**[26]	*****Isosecotanapartholide **(79)**[39]

*****Structures are given in **Figure 1.2a (Appendix A)** (page 262).

1.4.1.2 Aromatic hydrocarbons, alcohols, ketones, aldehydes, fatty acids and esters.

The aromatic compounds constitute small group of secondary metabolites reported from *Artemisia*.[22] Some of the important compounds of this class are given in **Table 1.2.**

Table 1.2. Simple phenols, benzylic alcohols, phenolic hydrocarbons, chalcones, polyaromatic and polyphenolic derivatives.

Name of the compound	**Name of the compound**
Simple phenols and phenolic hydrocarbons	*Benzylic derivatives*
*****(*E*)-3-(3-Hydroxyphenoxy)acrylaldehyde **(80)**[13]	*****Benzyl 2-phenylacetate **(82)**[26]
*****6-Methyl-2-phenylhept-5-en-2-ol **(81)**[40]	*****4-Phenylbutan-2-one **(83)**[41]
Aryl ketones	
*****2′,4′-Dimethylether-2′,4′,6′-trihydroxyacetophenone **(84)**[42]	*****1-Phenylhexa-2,4-diyn-1-one **(86)**[43]
*****2′-Methylether-2′,4′,6′-trihydroxyacetophenone **(85)**[42]	*****(*2E*,*4E*)-1-Phenylhexa-2,4-dien-1-one **(87)**[44]
Benzoic acid derivatives	
*****2-Hydroxybenzoic acid **(88)**[45]	*****4-(*O*-β-D-Glucosyloxy)-3-hydroxy benzoic acid **(90)**[46]
*****Isopentyl 2-hydroxybenzoate **(89)**[31]	*****Phenyl propanoic acid **(91)**[31]
Simple phenyl propanoids	
*****1-(5-Acetyl-2-hydroxy-4-methoxyphenyl)-3-hydroxy-3-methylbutan-1-one **(92)**[31]	*****4-Allyl-2-methoxyphenol **(97)**[13]
Polyaromatic and polyphenolic	*Chalcones derivatives*
*****1,2-Tetrahydro-acenaphthylene **(93)**[22]	*****Davidigenine **(95)**[22]
*****Colletotric acid **(94)**[22]	*****7′-Methoxydavidigenine **(96)**[22]

*Structures are given in **Figure 1.3a** (**Appendix A)** (page 263).

1.4.1.3 Cinnamyl, caffeoyl, ferruloyl, coumarins, isocoumarins and bicoumarins derivatives.

Some of the important ester formed by various combination of the four hydroxyl groups of quinic acid with cinnamic and ferrulic acid and the compounds consist of benzo-α-lactone rings are given in **Table 1.3.**

Table 1.3. Cinnamyl, caffeoyl, ferruloyl, coumarins, isocoumarins and bicoumarins derivatives.

Name of the compound	**Name of the compound**
Cinnamyl, caffeoyl and ferruloyl derivatives	
*****Methyl cinnamate **(98)**[31]	*****3,5-Diferuoylquinic acid **(107)**[46]
*****Benzyl cinnamate **(99)**[31]	*****Isochlorogenic acid C **(108)**[46]
*****Chlorogenic acid **(100)**[31]	*****4-Caffeoyl-5-feruloylquinic acid **(109)**[46]
*****Isochlorogenic acid B **(101)**[46]	*****4-Feruloyl-5-caffeoylquinic acid **(110)**[46]
*****3-Caffeoyl-4-feruloylquinic acid **(102**)[46]	*****4,5-Diferuoylquinic acid **(111)**[46]
*****3,4-Diferuoylquinic acid **(103)**[46]	*****1-Caffeoyl-5-feruoylquinic acid **(112)**[46]
*****Isochlorogenic acid A **(104)**[46]	*****_trans_-Ethyl caffeate **(28)**[47]
*****3-Caffeoyl-5-feruloylquinic acid **(105)**[46]	*****Ethyl-(*E*)-*p*-hydroxy cinnamate **(33)**[48]
*****3-Feruloyl-5-caffeoylquinic acid **(106)**[46]	*****(*Z*)-*p*-Hydroxycinnamic acid **(19)**[49]
Coumarins, isocoumarins and bicoumarins derivatives	
*****Scopoletin **(14)**[50]	*****_2H_-1-Benzopyran-2,2,6-triol **(113)**[51]
*****Scoparone **(29)**[52]	*****Benzotetronic acid **(114)**[51]
*****Isofraxidin **(32)**[53]	*****Farnochrol **(119)**[49]
*****Isoscopoletin **(30)**[54]	*****Arteminorin A **(115)**[55]
*****Esculetin **(31)**[56]	*****Arteminorin B **(116)**[55]
*****Dracunculin **(13)**[49]	*****Arteminorin C **(117)**[55]
*****_bis_-Dracunculin **(12)**[49]	*****Scopodrimol A **(118)**[57]

*****Structures are given in **Figure 1.4a** (**Appendix A)**, (page 264).

1.4.1.4 Flavonoids.

The genus *Artemisia* is a rich source of flavonoids[58] which have been further sub-divided into various groups based on the number and orientation of hydroxyl groups. (a) The flavonones, flavonols and tetrahydroxyflavonols related to kaempferol **(124)**,[59] pentahydroxyflavonols derived from quercetin **(127)**,[60] hexahydroxyflavonols based on quercetagetin derivatives **(135)**[61] and miscellaneous hexahydroxyflavonols are reported[62] and these are summarized in **Table 1.4.**

Table 1.4. Flavonoids.

Name of the compound	**Name of the compound**
Flavones derivatives	
*****Apigenin **(8)**[60]	
Luteolin tetrahydroxyflavones	*Pentahydroxyflavones*
*****Luteolin **(120)**[51]	*****Cirsiliol **(123)**[63]
*****Luteolin-7-methyl ether **(121)**	*****Eupatorin **(26)**[64]
*****Dihydroluteolin **(9)**[62]	
Other tetrahydroxyflavones	*Hexahydroxyflavones*
*****Cirsimaritin **(122)**[62]	*****5,′6,7-Trimethoxy-2,4′,5′-trihydroxy-flavone **(124)**[63]
Tetrahydroxy flavonols	
Kaempferols derivatives	*Other Tetrahydroxyflavonols*
*****Kaempferol **(125)**[60]	*****3-Hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-*4H*-1-benzopyran-4-one **(127**)[46]
*****Rhamnocitrin **(126)**[62]	
Pentahydoxy flavonols (*quercetin derivatives*)	

Continued on next page

Name of the compound	**Name of the compound**
*****Quercetin **(128)**[60]	*****Rhamnetin **(131)**[60]
*****Isoquercitrin **(129)**[63]	*****Isorhamnetin **(132)**[62]
*****Isorhamnetin -3′-*O*-β-D-glucoside **(130)**[51]	*****Tamarixetin **(133)**[60]
Other pentahydroxyflavonols	
*****Penduletin **(134)**[51]	*****Mikanin **(135)**[46]
Hexahydroxy flavonols (*Quecetagetin derivatives*)	
*****3-Methoxyquercetagetin **(136)**[51]	*****Chrysosplenetin **(139)**[59]
*****Axillarin **(137)**[60]	*****Casticin **(140)**[62]
*****Bonanzin **(6)**[64]	*****Patuletin-3′-*O*-β-D-glucoside **(141)**[62]
*****Chrysosplenol D **(138)**[64]	
Other hexahydroxyflavones	
*****Patuletin **(142**)[60]	*****6,7,3′,4′-Tetramethoxyquercetagetin **(145)**[65]
*****Cirsilineol **(143**)[62]	*****4′-Methoxyquercetagetin **(146)**[51]
*****Eupatin **(144**)[65]	
Other hexahydroxyflavonols	
*****Mearnsetin **(147)**[46]	*****Chrysosplenol E **(148)**[46]
*****3′,5,7,8-Tetrahydroxy-3,4′-dimethoxyflavone **(149)**[62]	

*Structures are given in **Figure 1.5a and 1.5b** (**Appendix A)**, (pages 265-266).

1.4.1.5 Terpenoids.

The oxygenated, reduced, and dehydrated products of hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}), sterols (C_{29}) and saponins have been reported from the genus.[1,22] Among these, the sesquiterpenes (C_{15}), triterpenes (C_{30}) and sterols (C_{29}) are the major class of the compounds isolated from the genus.[22] The "head-to-tail" condensation of a "precursor molecule" dimethylallypyrophosphate (DMAPP; $(CH_3)_2C=CHCH_2OHPO_3\text{-}OH_2PO_3$), with a "chain extender" of isopentenyl pyrophosphate (IPP; $CH_2=CH(CH_3)CH_2CH_2OHPO_3\text{-}OH_2PO_3$), both ($C_5$) gives rise to the regular monoterpenes while the "head-to-middle" condensation of two (C_5) precursors give rise to irregular monoterpenes. Myrcene **(150)** was the most abundant regular acyclic monoterpene accounting for about 5 % while, *Artemisia* ketone **(153)** was the most abundant irregular monoterpene accounting for about 50 % of all the irregular monoterpenes isolated from the essential oil of *A. annua*.[31]

Sesquiterpenoids (C_{15}) constituted the most diverse and most abundant group of secondary metabolites reported from *Artemisia*. These are biosynthesized from farnesyl pyrophosphate (FPP) **(167)**, which is produced by "head-to-tail" condensation of three isoprene units (IPP; $CH_2=CH(CH_3)CH_2CH_2OHPO_3\text{-}OH_2PO_3$ and DMAPP; $(CH_3)_2C=CHCH_2OHPO_3\text{-}OH_2PO_3$). These were further categorized as acyclic sesquiterpenes (e.g., farnesane), monocyclic sesquiterpenes (e.g., bisabolanes, germacranes, elemanes, humulanes and caryophyllanes), bicyclic sesquiterpenes (e.g., eudesmanes, eremophilanes, cadinanes, muurolanes, amorphanes, seco-cadinanes, guaianes, aromadendrane), tricyclic sesquiterpenes (e.g., cedrane, silphinane, isocomane,

α-santalane, copaene, bourbonane, opentene and *α-trans*-bergamotol classes of sesquiterpenoid). Some of the major terpenes types are summarized in **Table 1.5.**

Table 1.5. Monoterpenes, sesquiterpenes, sterols and triterpenes.

Name of the compound	**Name of the compound**
Monoterpenes (C_{10})	
Regular acyclic monoterpenes	*Irregular acyclic monoterpenes*
*****Citronellol **(150)**[31]	******Artemisia* ketone **(154)**[66]
*****Myrcene **(151)**[14]	*****Santolina alcohol **(155)**[13]
******cis*-Epoxyocimene **(152)**[26]	*****Santolinylol **(27)**[67]
*****Perillene **(153)**[13]	*****3-Hydroxy santolinate **(156)**[68]
Cyclic monoterpe derivatives	*Cyclic monoterpe derivatives*
******p-Menthane*	*Camphane*
*****α-Phellandrene **(157)**[13]	*****Borneol **(159)**[69]
******trans*-Carvyl **(158)**[70]	*****Camphor **(160)**[66]
Pinane Monoterpenoids	*Thujane Monoterpenoids*
*****α-Pinene **(161)**[71]	*****α-Thujene **(164)**[19]
*****Pinocarvone **(162)**[69]	*****Sabinol **(165)**[14]
*****β-Pinene oxide **(163)**[66]	******trans*-Sabinyl acetate **(166)**[66]
Sesquiterpenes (C_{15})	
Farnesane sesquiterpenoids	*Bisabolanes sesquiterpenoids*
*****Farnesol (**167**)[72]	*****α-Bisabolol **(170)**[70]
*****Farnesyl pyrophosphate **(168)**[73]	******cis*-Lanceol **(171)**[74]
*****Farnesal **(169)**[73]	
Germacrane sesquiterpenoids	*Elemane sesquiterpenoids*
***** Germacrene A (**172**)[71]	*****γ-Elemene (**174)**[19]
*****Pregeijerene **(173)**[75]	*****Elemol **(175)**[16]

Continued on next page

Name of the compound	**Name of the compound**
Humulane sesquiterpenoids	*Caryophyllane sesquiterpenoids*
*****α-Humulene (**176**)[4]	*****β-Caryophyllene **(177**)[4]
Eudesmane (selinane) sesquiterpenoids	*Cadinane*
*****1-β-Hydroxy-4(15),7-eudesmadiene **(178)**[16]	*****Artemisinol **(180)**[76]
*****Kongol **(179)**[66]	*****α-Cadinene **(181)**[65]
Murolane sesquiterpenoids	*Amorphane sesquiterpenoids*
*****γ-Murolene **(182**)[13]	*****Arteannuin A **(184)**[77]
*****_t_-Murolol **(183**)[66]	*****Artemisinic acid **(185)**[78]
Seco-Cadinane sesquiterpenoids	*Guaiane sesquiterpenoids*
*****Artemisinin **(5)**[79]	*****α-Guaiene **(186)**[80]
Aromadendrane sesquiterpenoids	*Cedrane sesquiterpenoids*
*****α-Aromadendrene **(187)**[81]	*****Cedra-8(15)-en-9a-ol **(188)**[16]
	*****3α,15-Dihydroxy cedrane **(189**)[82]
Miscellaneous tricyclic sesquiterpenes	
*****Silphinane **(190)**[16]	*****β-Isocomene **(191)**[16]
Diterpenes (C_{20})	
Phytane diterpenoids	*Cyclic diterpenes*
*****Phytol **(192)**[70]	*****Abscisic acid **(194)**[83]
*****Phytene-1-ol-2-hydroperoxide **(193)**[22]	*****13-*epi*-Manool **(195)**[13]
	*****15-Isopimaradiene **(196)**[13]
Sterols (C_{29})	
*****β-Sitosterol **(21)**[52]	*****Stigmasterol **(20)**[64]
*****β-Spinasterol **(197)**[84]	*****β-Sitosterol-*O-D*-glucopyranoside) **(22)**[85]
Triterpenes (C_{30})	
Oleananes (Olean-12-enes)	

Continued on next page

Name of the compound	**Name of the compound**
*****β-Amyrin **(198)**[20,86]	*****3-β-Hydroxy-12-oleanen-28-oic acid) **(199)**[64]
Friedalanes (D: *A fiiedooleanane)*	
*****Friedelan-3- β-ol **(200)**[85]	*****Friedelin **(201)**[85]
Ursanes (Urs-12-enes)	
*****α-Amyrin **(203)**[87]	*****α-Amyrenone **(204**)[21]
Taraxterone	
*****Taraxasterone (**205)**[20]	*****Baurenol **(206)**[21]
Lupanes and hopanes triterpenes	
*****Lanoscopariol (**207)**[88]	*****Betulinic acid (**16)**[20,89]

*****Structures of mono, sesqui and diterpenes are given in **Figure 1.6a** (page 267) **and 1.6b** (page 268) while triterpenes and sterols are given in **Figure 1.6c (Appendix A)**, (page 269).

1.4.1.6 Nitrogen containing compounds.

Few alkamides, peptides and alkaloids have also been isolated from *Artemisia*. However, we are reporting ceramides (sphingolipids) for the first time from the genus *Artemisia*. The names of some of the important compounds are given in **Table 1.6.**

Table 1.6. Nitrogen containing compounds.

Name of the compound	Name of the compound
*****Aurantinamide acetate **(208)**[90]	*****6-Amino-8,9-dihydro-7*H*-purin-2-ol **(209)**[91]
*****Benzo[*d*]thiazole **(210)**[15]	*****(6-(3-Methylbut-2-enyl)-1H-indole-3-*****carboxylic acid) **(211)**[92]
*****Pyrrolidin-2-one **(212)**[93]	*****2-((*5R,8R*)-3,5-Dimethyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[c]pyridin-8-yl)acrylic acid) **(213)**[94]
*****Pellitorine **(214)**[95]	*****Neopellitorine A **(215)**[95]
*****Neopellitorine B **(216)**[95]	

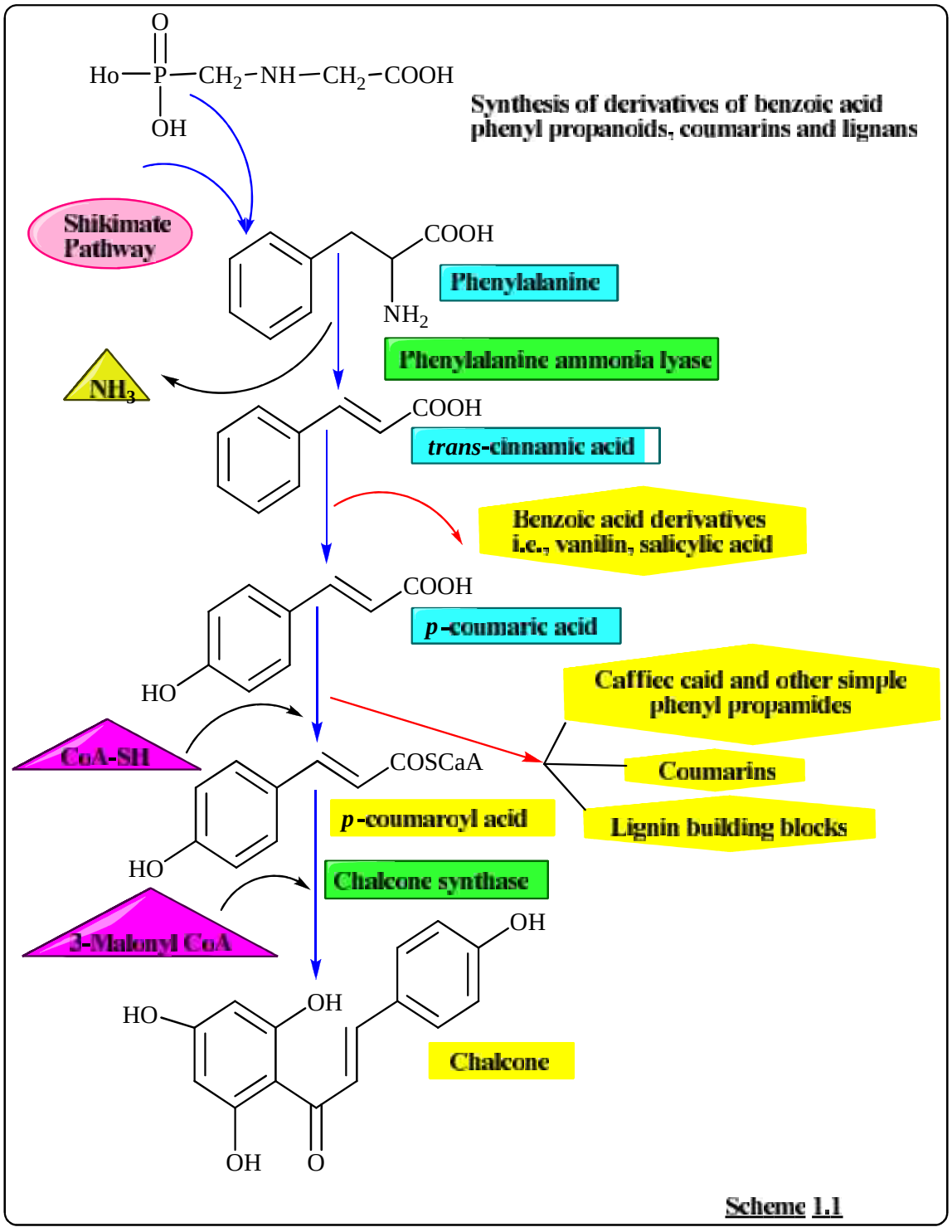
*****Structures are given in **Figure 1.7a** (**Appendix A)**, (page 270).

1.5 Biosynthetic pathways of some important classes of the compounds isolated from the genus *Artemisia*.

1.5.1 Shikimate pathway for the biosynthesis of benzoic acid, phenyl propanoids, coumarins and lignins derivatives.[22,96]

Phenylalanine is supposed to be the key precursor for virtually all phenolic compounds.[97]

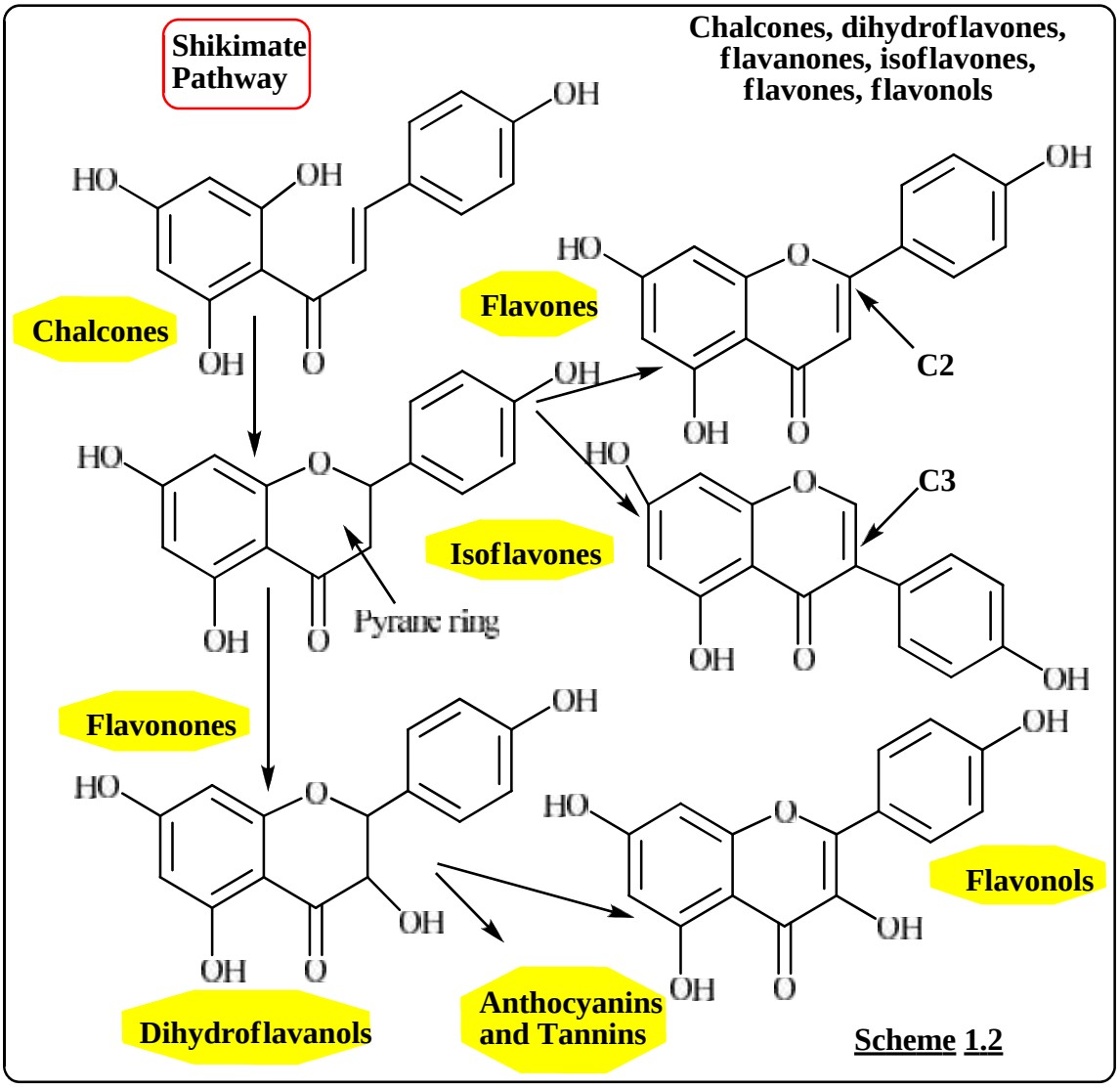
The sequence of different steps of shikimate pathway are given in **Scheme 1.1.**



Scheme 1.1. Shikimate pathway for the biosynthesis of benzoic acid, phenyl propanoids, coumarins and lignins derivatives.

1.5.2 Shikimate pathway for the biosynthesis of chalocones, dihydroflavones, flavones, isoflavones, flavononesand flavonols derivatives.[22,96]

A molecule of *p*-coumaroyl reacts with 3-malonyl-CoA under the action of chalcones synthase (CHS), yields chalcone, which acts as a precursor for other phenolic derivatives.[97] The various steps are given in **Scheme 1.2.**

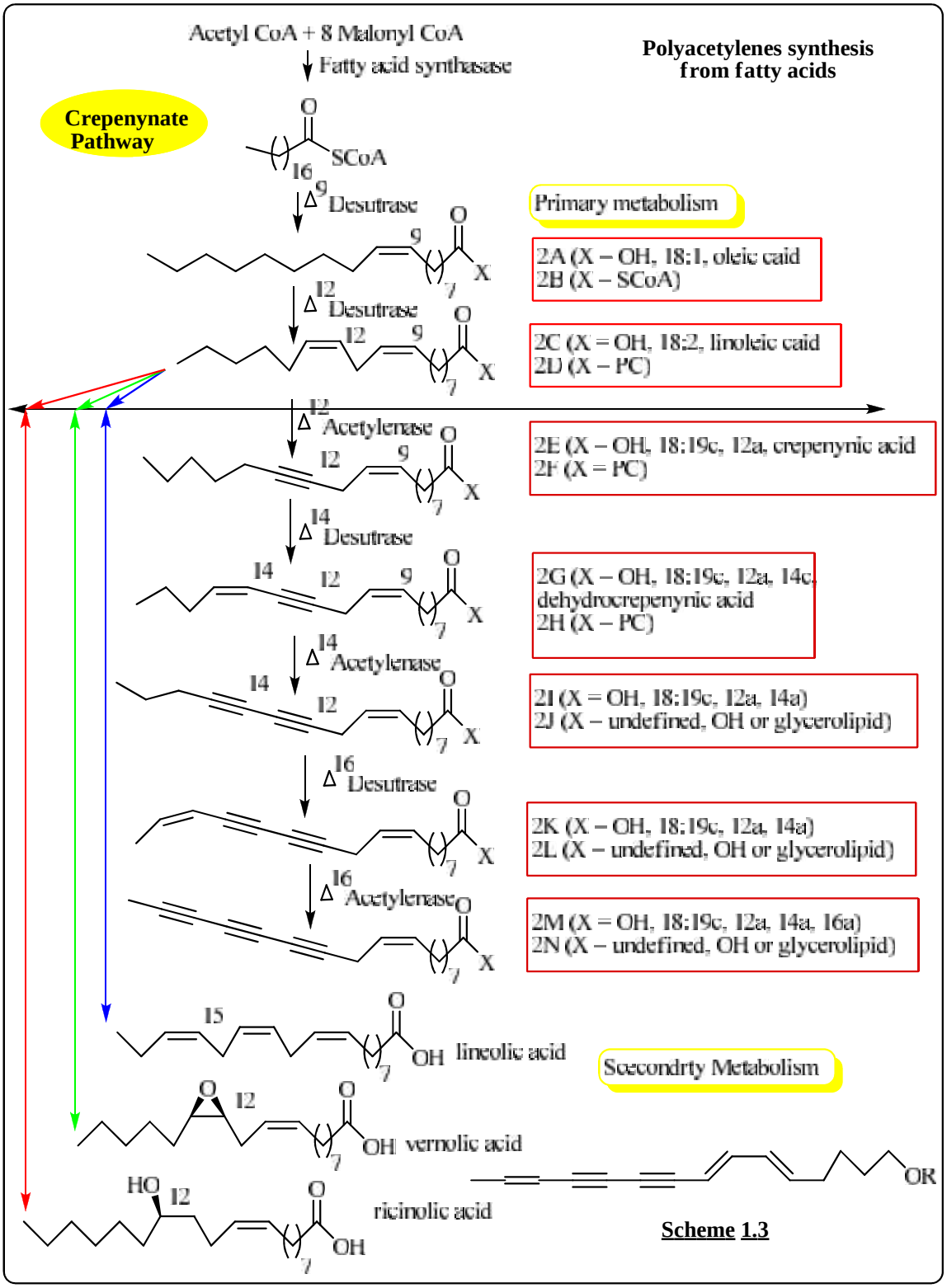


Scheme 1.2. Shikimate pathway for the biosynthesis of chalocones, dihydroflavones, flavonones, isoflavones, flavones and flavonols derivatives.

1.5.3 The crepenynate pathway for polyacetylenes biosynthesis.[98]

Acetylenic fatty acids are produced through crepenynate pathway.[98] Primary metabolism offers the group of fatty acid precursors for the biosynthesis of acetylenes derivatives.[99]

The different steps of the crepenynate pathway are given in **Scheme 1.3.**



Scheme 1.3. The crepenynate pathway for the biosynthesis of polyacetylenes.

Part A

Chapter 2

Results and Discussion

2.1 Phytochemicals isolated from the two species of *Artemisia* (*A. elegantissima* and *A. incisa*).

Thirty seven compounds were isolated from the aerial parts (stem and leaves) and roots of *Artemisia elegantissima* and *Artemisia incisa*. Among the isolated compounds, four were new **(1-4)** and thirty three **(5-37)** were a new source compounds. However, the compound; 5,7,8,3',4'-pentahydroxy-6-methoxyflavone **(5)** isolated from the aerial parts of *A. elegantissima* was presented with full data of 1D and 2D-NMR and physicochemical constants for the first time.

Various fractions of the aerial parts (stem and leaves) of *A. elegantissima* have resulted in the isolation of eighteen compounds. Compound **1** was a new, while compounds **(5–18)** were a new source compounds.

Ten compounds were isolated from various fractions of the roots of *A. elegantissima*. Compound **2** was a new and the other nine compounds **(14-16, 19, 21-25)** were a new source compounds.

Similarly, from various fractions of the aerial parts (stem and leaves) of *A. incisa* were isolated sixteen compounds. Compound **3** was a new and the rest of the compounds **(13, 20, 21, 26-37)** were a new source compounds.

Various fractions of the roots of *A. incisa* have resulted in the isolation of nine compounds. Compound **4** was a new and the rest of the compounds **(13, 20, 21, 30, 32-35)** were a new source compounds.

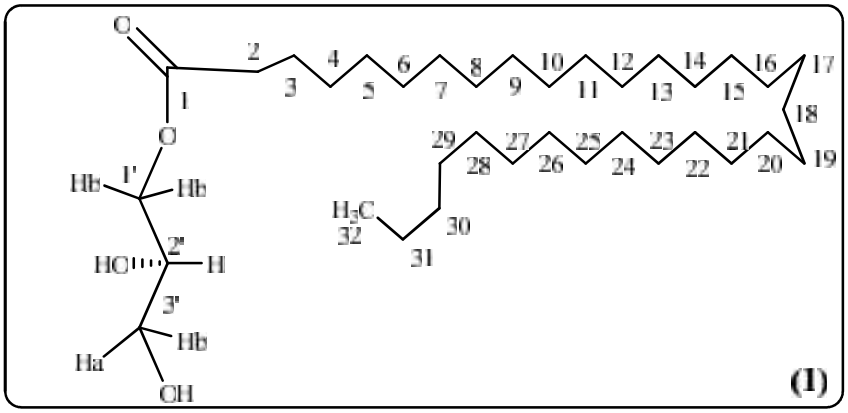
The results of the 37 compounds have been discussed in the following sections where the new compounds were discussed first followed by the new source compounds in each section. Similarly, the polar compounds were discussed first followed by the nonpolar compounds in each section.

From the systematic phytochemical investigation on the two *Artemisia* species, it was evident that monoterpenes and phenolic compounds were the major constituents of the aerial parts while triterpenes and sterol were present both in the aerial parts as well as in the roots of the two *Artemisia* species. The ceramides were only isolated from the roots of the two species.

Various chromatographic and spectroscopic techniques were applied to purify and elucidate the structures of the compounds isolated from the two species. Most of the isolated compounds were tested for their biological activities which are discussed in **Chapter 5 (Sections 5.1.1-5.1.6).** The extraction and isolation procedures of the compounds are discussed in **Chapter 3 (Sections 3.5.1-3.5.4).**

2.2 New compound isolated from the aerial parts (leaves and stems) of *Artemisia elegantissima*.

2.2.1 (*R*)-2,3-Dihydroxypropyl dotriacontanoate (artemglycerol) (1).



Compound **1** was obtained as a white powder, m.p. 81–82 °C. The molecular formula of $C_{35}H_{70}O_4$ was determined by HR-ESIMS (positive ion mode) from the quasimolecular ion peak at *m*/*z* 555.527 [M^++H]. The IR spectrum showed absorption bands for OH st. at $\bar{\upsilon}$ 3460.11 and C=O st. at $\bar{\upsilon}$ 1732.60 $cm.^{-1}$

Adjacent protons coupling ^1H-^1H were established through COSY, the ^1H-^{13}C correlation were established through HSQC, while the long range ^1H-^{13}C correlations were established through HMBC experiments.

The ^1H and ^{13}C-NMR spectra of the compound **1 (Table 2.1)** showed signals characteristic of fatty acid and glycerol moieties.

In the ^1H-NMR spectrum of the compound **1 (Table 2.1),** the terminal methyl was resonating at δ 0.85 (3H, *J* = 6.4 Hz, H-32).

Table 2.1. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of (*R*)-2,3-dihydroxypropyl dotriacontanoate **(1)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	174.33	C	---	---
2	34.15	CH_2	2.33 (2H, t, *J* = 7.5)	C-1, C-29
3	24.92	CH_2	1.62 (2H, m)	---
4–29	29.01–29.61	$26CH_2$	1.23 (52H, m)	---
30	31.92	CH_2	1.59 (2H, m)	---
31	22.68	CH_2	1.56 (2H, m)	---
32	14.10	CH_3	0.85 (3H, t, *J* = 7.0)	C-33, C-31
1′	65.18	CH_2	4.13 (1H, dd, *J* = 16.5, 4.5, Ha);	C-2′, C-1′, C-1
			4.19 (1H, dd, *J* = 16.5, 6.0, Hb)	
2′	70.28	CH	3.91 (1H, br. s)	C-1′, C-3
3′	63.33	CH_2	3.58 (1H, dd, *J* = 17.0, 6.0, Ha);	C-1′, C-2′
			3.68 (1H, dd, *J* = 17.0, 3.5, Hb)	
2′-OH	---	H	2.40 (1H, br. s, 2′-OH)	C-3′, C-2′
3′-OH	---	H	2.02 (1H, br. s, 3′-OH)	C-2′, C-3′, C-1′

The four methylenes were resonating at δ 1.56 (1H, m, H-31), 1.59 (1H, m, H-30), 2.33 (2H, t, *J* = 7.5 Hz, H-2) and 1.62 (2H, m, H-3). The broad signal (integrated for 26 methylenes protons) was resonating at δ 1.23 (54H, m, H-4−H-29).

The glycerol moiety, in the ^{1}H-NMR spectrum **(Table 2.1)** showed resonances for two methylenes protons at δ 4.13 (diastereotopic protons) (1H, dd, *J* = 16.5, 5.0 Hz, Ha-1′), 4.19 (1H, dd, *J* = 17.5, 6.0 Hz, Hb-1′) and δ 3.58 (diastereotopic protons) (1H, dd, *J* = 17.0, 6.0 Hz, Ha-3′), 3.68 (1H, dd, *J* = 15.0, 3.5 Hz, Ha-3′). The oxymethine proton was resonating at δ 3.91 (1H, br. s, H-2′). The hydroxyl protons were resonating at δ 2.40 (1H, br. s, OH-2′) and 2.02 (1H, br. s, OH-3′) respectively.

In ^{13}C-NMR spectrum, the terminal methyl of the fatty acid moiety was resonating at δ 14.10 (C-32). The four methylenes were resonating at δ 22.68 (C-31) and 31.92 (C-30), δ 24.92 (C-2) and 34.15 (C-3) respectively. A series of twenty six methylenes were resonating at δ 29.01–29.61 (C-4–C-29) and the carbonyl was resonating at δ 174.33 (C-1). The two oxymethylenes and oxymethine of the glycerol moiety were resonating at δ 65.18 (C-1′), 63.33 (C-3′) and 70.28 (C-2′) respectively.

The above information obtained from ^{1}H and ^{13}C-NMR data **(Table 2.1)** revealed that the compound **1** was a fatty acid-1-glyceride. This data was auxiliary supported by the HMBC spectrum. In the HMBC spectrum **(Table 2.1)**, the two oxymethylene protons resonating at δ_H 4.13 (Ha-1′); 4.19 (Hb-1′) and 3.58 (Ha-3′); 3.68 (Hb-3′) showed correlations with each other resonating at δ_C 65.18 (C-1′), 63.33 (C-3′) and with the methine carbon resonating at δ_C 70.28 (C-2′). The oxymethine proton at δ_H 3.91 (H-2′) showed correlations with the two oxymethylene carbons resonating at δ_C 65.18 (C-1′) and

63.33 (C-3′). The hydroxyl protons at δ_H 2.02 (OH-3′) showed two and three bond correlations with the carbons resonating at δ_C 70.28 (C-2′) and 63.33 (C-3′). Similarly, the hydroxyl proton resonating at δ_H 2.40 (OH-2′) showed two and three bond correlations with carbons resonating at δ_C 65.18 (C-1′), 70.28 (C-2′) and 63.33 (C-3′) which confirm their position of attachment and the unsymmetrical substitution of the glycerol moiety.[84] Furthermore, the oxymethylene protons resonating at δ_H 4.13–4.19 (H-1′) showed correlation with the acyl carbon resonating at δ_C 174.33 (C-1) which specified that the acyl group of the fatty acid moiety was terminally substituted at the oxymethylene (C-1′) of the glycerol moiety.

Similarly, the methylenes protons of the fatty acid moiety resonating at δ_H 2.33 (H-2), and 1.61 (H-3) showed correlations with the carbons resonating at δ_C 24.92 (C-2), 34.15 (C-3) and 174.33 (C-1). These correlations proved the presence of an ester linkage.

In the COSY spectrum, oxymethine proton resonating at δ 3.91 (H-2′) showed correlations with the diastereotopic protons resonating at δ 4.13 (Ha-1′); 4.19 (Hb-1′), and 3.58 (Ha-3′); 3.68 (Ha-3′) which also explained the terminal substituted glycerol moiety.

According to the HR-ESIMS spectrum, which showed the quasimolecular ion peak at *m/z* 555.527 [M^++H], the fatty acid moiety should be dotriacontanoic acid.[100]

The relative stereochemistry of the compound **1** was based on the literature which has reported that the glyceride analogue with levo (-) specific rotation $^{20}[\alpha]_D$ - 0.011 (*c* 0.075, $CHCl_3$) had an absolute configuration of *R*,[101] therefore, the structure of the compound **1** was elucidated as (*R*)-1-*O*-dotriacontanoyl glycerol or (*R*)-2,3-dihydroxypropyl

dotriacontanoate (**Figure 2.1**). The key COSY and HMBC correlation of (*R*)-2,3-dihydroxypropyl dotriacontanoate (**1)** are given in **Figure 2.1.**

To the best of our knowledge, it is a new compound. The compound was named as artemglycerol.

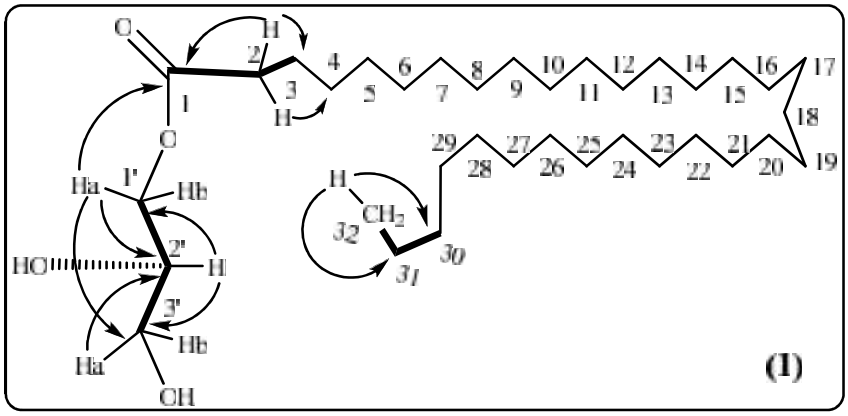
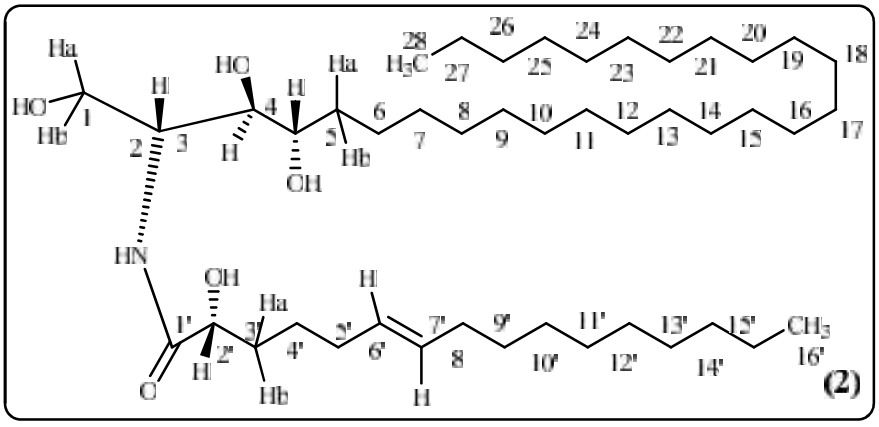


Figure 2.1. Key HMBC (→) and COSY (—) correlations of (*R*)-artemglycerol **(1).**

2.3 New compound isolated from the roots of *Artemisia elegantissima*.

2.3.1 (*R,E*)-2-Hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadec-6-enamide (artemceramide-A) (2).



Compound **2** was obtained as an optically active amorphous white powder, m.p. 139–140 °C and $^{20}[\alpha]_D$ + 8.88 (*c* 0.6, C_5H_5N). The IR spectrum of the compound **2** showed absorptions for OH and NH st. at ῡ 3600-3400, 3328.5, 3246.6, 3218.6, NHC=O st. at ῡ 1621.8 and C=C st. at ῡ 1563.0 cm.$^{-1}$ Its molecular formula was determined by HR-ESIMS (positive ion mode) which showed pseudo molecular ion peak at *m/z* 710.5922 [M^++H], corresponding to $C_{44}H_{87}NO_5$.

Protons coupling; ^{1}H-^{1}H were established through COSY, the ^{1}H-^{13}C correlation were established through HSQC, while the long range ^{1}H-^{13}C correlations were established through HMBC experiments.

The ^{1}H and ^{13}C-NMR spectra of the compound **2 (Table 2.2)** showed characteristic signals of α-hydroxy fatty acid and sphingosine base moieties.

In the ^{1}H-NMR spectrum **(Table 2.2)**, the NH proton was resonating at δ 8.58 (1H, d, *J* = 8.5 Hz, NH). The oxymethine protons were resonating at δ 4.35 (1H, dd, *J* = 11.0, 4.5 Hz, H-3), 4.29 (1H, m, H-4) and 4.62 (1H, dd, *J* = 7.6, 3.5 Hz, H-2′). The amido methine proton was resonating at δ 5.12 (1H, m, H-2). The methylene protons were resonating at δ 2.01 (diastereotopic) (1H, m, Ha-3′) and 2.23 (1H, m, Hb-3′) while the oxymethylene protons were resonating at δ 4.51 (diastereotopic) (1H, dd, *J* = 17.5, 5.0 Hz, Ha-1) and 4.42 (1H, dd, *J* = 17.5, 5.0 Hz, 1Hb-1). The olefinic protons were resonating at δ 5.53 (1H, dt, *J* = 15.5, 7.0 Hz, H-6′) and 5.49 (1H, dt, *J* = 15.5, 7.0 Hz, H-7′). The two methylenes protons adjacent to the olefinic bond were resonating at δ 1.97 (2H, m, H-8′) and 2.18 (2H, m, H-5′). The two pair of terminal methylene protons were resonating at δ 1.59 (4H, m, H-14′/H-26) and 1.56 (4H, m, H-15′/H-27). A series of 24 methylene protons were resonating at δ 1.24–1.29 (48H, br. s, H-9′–H-13′/H-7–H-25). The protons of the two terminal methyls were resonating at δ 0.85 (6H t, *J* = 6.5 Hz, H-16′/H-28).

Correspondingly, in the ^{13}C-NMR spectrum **(Table 2.2)**, the amide carbon (C-1′) was resonating at δ 175.29. The amido methine (C-2) and the three oxymethines (C-4, C-3 and C-2′) were resonating at δ 53.07, 72.98, 76.94 and 72.52 respectively.

The olefinic methines; C-7′ and C-6′ were resonating at δ 130.75 and 130.80 respectively. The methylenes; C-8′ and C-5′ were resonating at δ 33.1 and 33.3 respectively. The methylenes; C-1 and C-3′ were resonating at δ 62.12 and 35.90 respectively. The two pair of methylenes; C-14′/C-26 and C-15′/C-27 were resonating at δ 31.92 and 22.68 respectively. A series of 24 methylenes; C-7–C-25/ C-9′–C-13′ were resonating at δ 28.34–30.14.

Table 2.2. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of (*R,E*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadec-6-enamide **(2)** in C_5D_5N.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
NH	---	H	8.58 (1H, d, *J* = 8.5)	---
1	62.12	CH_2	4.51 (1H, dd, *J* = 17.5, 5.0, Ha);	C-2, C-3
			4.42 (1H, dd, *J* = 17.5, 4.0, Hb)	
2	53.07	CH	5.12 (2H, m)	C-1′, C-1, C-3, C-4
3	76.94	CH	4.35 (1H, dd, *J* = 11.0, 4.5)	C-1, C-2, C-4
4	72.98	CH	4.29 (1H, m)	C-2, C-3, C-5
5	35.75	CH_2	2.01 (1H, m, Ha);	---
			2.20 (1H, m, Hb)	
6	30.3	CH_2	2.15 (2H, m)	---
1-OH	---	H	6.25 (1H, br. s)	C-1
3-OH	---	H	6.72 (1H, br. s)	C-3
4-OH	---	H	6.70 (1H, br. s)	C-4
1′	175.29	C	---	---
2′	72.53	CH	4.62 (1H, dd, *J* = 7.6, 3.5)	C-1′, C-3′
3′	35.90	CH_2	2.20 (1H, m, Ha);	---
			2.23 (1H, m, Hb)	
4′	26.9	CH_2	1.70 (m)	---
5′	33.89	CH_2	2.04 (2H, m)	---
6′	130.85	CH	5.53 (1H, dt, *J* = 15.5, 7.0)	C-4′, C-7′, C-8′
7′	130.98	CH	5.49 (1H, dt, *J* = 15.5, 7.0)	C-5′, C-6′, C-9′
8′	33.30	CH_2	1.98 (2H, m)	---

Continued on next page

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
9′–13′	and			
7–25	29.52–30.01	24CH_2	1.29 (48H, m)	---
26/14′	32.03	2CH_2	1.78 (4H, m)	C-13′/C-25, C-15′/C-27
27/15′	22.94	2CH_2	1.70 (4H, m)	C-14′/C-26, C-16′/C-28
28/16′	14.26	2CH_3	0.85 (6H, t, J = 7.0)	C-15′/C-27, C-14′/C-26
2′-OH	---	H	7.64 (1H, br. s)	C-2′

From the 1D-NMR spectral data, compound **2** was declared to be a phytosphingosine-type ceramide (consist of *α*-hydroxy fatty acid and a long chain base; LCB).[102] Furthermore, 2D-NMR analysis were carried out in order to finalize the structure for compound **2**.

In the COSY spectrum, the amidomethine proton at δ_H 5.12 (H-2) showed cross peaks with the diastereotopic oxymethylene protons at δ_H 4.51 (Ha-1) and 4.42 (Hb-1), amido proton at δ_H 8.58 (CONH) and oxymethine proton at δ_H 4.35 (H-3). The later also correlated with the oxymethine proton at δ_H 4.29 (H-4). Similarly, in the COSY spectrum, the oxymethine proton at δ_H 4.62 (2′) showed cross peaks with the diastereotopic methylene protons at δ_H 2.20 and 2.23 (H-3′) which in turn showed correlation with methylene protons at δ_H 1.78 (4′).

In the HMBC spectrum, the proton at δ_H 4.62 (H-2′) showed correlations with the carbons resonating at δ_C 175.29 (C-1′) and 35.90 (C-3′). The hydroxyl proton at δ_H 6.98 (OH-2′) showed correlation with the carbon resonating at δ_C 72.52 (C-2′) which confirmed the structure of *α*-hydroxy fatty acid moiety.

The diastereotopic protons at δ_H 4.51 (Ha-1) and 4.42 (Hb-1) showed correlations with the carbons resonating at δ_C 53.07 (C-2) and 76.94 (C-3). The proton at δ_H 5.12 (H-2) showed correlations with the carbons resonating at δ_C 175.29 (C-1′), 62.12 (C-1) and 76.94 (C-3) which confirmed an amide linkage between the sphingosine base and *α*-hydroxy fatty acid. The proton at δ_H 4.35 (H-3) showed correlations with the carbons resonating at δ_C 53.07 (C-2) and 72.98 (C-4). The proton at δ_H 4.29 (H-4) showed correlations with the carbons resonating at δ_C 76.94 (C-3) and 35.98 (C-5). The hydroxyl

protons at δ_H 6.4 (OH-1) showed correlation with the carbon resonating at δ_C 62.12 (C-1). The hydroxyl protons at δ_H 6.72 and 6.70 (OH-3/OH-4) showed correlations with the carbons resonating at δ_C 76.94 (C-3) and 72.98 (C-4). These correlations confirmed the attachment of hydroxyl groups at carbons; C-1, C-3 and C-4 of the sphingosine base moiety.

The olefinic protons at δ_H 5.53 (H-6′) and 5.49 (H-7′) showed correlations with the carbons resonating at δ_C 130.73, 130.85, 33.1 (C-5′) and 33.3 (C-8′), which showed their *trans* orientation and location at the middle of the *α*-hydroxy fatty acid moiety. Thus the HMBC correlations also agreed with the 1D-NMR assigned structure for compound **2.**

2.3.1.1 Chemical studies on compound 2.

In order to determine the chain length of the fatty acid and sphingosine moieties and the double bond position, the structure of the compound **2** was also analyzed with the help of chemical methods of methanolysis and oxidation.

2.3.1.1a Methanolysis of the compound 2.

Methanolysis of the compound **2** yielded an unsaturated *α*-hydroxy fatty acid methyl ester and a long chain base (LCB). The unsaturated methyl ester of *α*-hydroxy fatty acid (*n*-hexane extract) after subjecting to GC-MS analysis was identified as (*E*)-methyl-2-hydroxyhexadec-6-enoate[103] attributed to the molecular ion peak at *m/z* 284.23 [M^+] which required the molecule to be consist of two double bond equivalents (C=O and C=C functional groups). The part of the LCB was therefore, a C_{28} aliphatic amino alcohol unit with an amino group and three hydroxyl groups.

The absolute configuration at C-2 was determined to be *R* with $^{20}[\alpha]_D$ -1.1 (*c* 0.07, $CHCl_3$).[104]

2.3.1.1b Oxidation of (*R,E*)-methyl 2-hydroxyhexadec-6-enoate.

The $KMnO_4$ oxidation of unsaturated methyl ester of *α*-hydroxy fatty acid; (*R,E*)-methyl 2-hydroxyhexadec-6-enoate and subsequent GC-MS analysis of the *n*-hexane extract afforded decanis acid[87] (capric acid),[26] attributed to the characteristic molecular ion peak at *m/z* 172.1 [M^+]. The decanoic acid obtained allowed the location of the double bond at carbons; C-6′ and C-7′ of the (*E*)-methyl-2-hydroxyhexadec-6-enoate. The 6′,7′ alkene bond was found to be *trans*, which was demonstrated by the large vicinal coupling constants (*J* = 15.5 Hz) and the absence of NOESY correlation between the protons: H-6′; δ_H 5.53 (dt, *J* =15.5, 7.0 Hz) and H-7′; δ_H 5.49 (dt, *J* =15.5, 7.0 Hz).[105] The trans geometry of the 6′,7′-olefinic bond was also supported by the chemical shift of the allylic carbons resonating at δ_C 33.5 (C-5) and 33.1 (C-8). Usually, the signals of the allylic carbon for *cis* double bond appeared at δ_C 27–28, while for a *trans* double bond appeared at δ_C 32–33.[106]

The configurations of the artemceramide-A **(2)** was assigned by comparing the characteristic 1D-NMR **(Table 2.2.1)**, and ^1H-^1H NOESY correlations with the analogues as reported in the literature, in which the specific rotation $^{20}[\alpha]_D$ + 8.88 (*c* 0.07, C_5H_5N) and the NOESY correlation of protons; H-2 with H-4 and *vice versa*, were close to that of the natural ceramide; (*R,Z*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxynonadecan-2-yl)pentacos-4-enamide (psychotramide) with $^{20}[\alpha]_D$ + 8.0 (*c* 0.05, $CHCl_3$).

Table 2.2.1. Key data comparison of ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) in C_5D_5N of (*2′R*)-2′-hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadecanamide **(2)** and literature analogue; (*R,Z*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxynonadecan-2-yl)pentacos-4-enamide (psychotramide)**.**[107]

Artemceramide-A **(2)**			Literature analogue (psychotramide)		
C. No.	^{13}C- and ^{1}H-NMR (δ)		C.No.	^{13}C- and ^{1}H-NMR (δ)	
NH	---	8.58 (1H, d, *J* = 8.5)	NH	---	8.54 (1H, d, *J* = 8.0)
1	62.12	4.51 (1H, dd, *J* = 17.5, 5.0, Ha); 4.42 (1H, dd, *J* = 10.5, 4.0, Hb)	1	62.4	4.48 (1H, dd, *J* = 17.5, 5.0, Ha) 4.39 (1H, dd, *J* = 10.5, 4.0, Hb)
2	53.07	5.12 (1H, m)	2	53.4	5.08 (1H, m)
3	76.94	4.35 (1H, dd, *J* = 11.0, 4.5)	3	77.3	4.30 (1H, m)
4	72.98	4.29 (1H, m)	4	72.9	4.60 (1H, m)
5	35.75	2.01 (1H, m); 2.20 (1H, m)	5	33.7	2.21 (1H, m, Ha); 2.02 (1H, m, Hb)
6	30.3	2.15 (2H, m)	6	30.3	2.20 (2H, m)
1-OH	---	6.25 (1H, br. s)	1-OH	---	---
3-OH	---	6.72 (1H, br. s)	3-OH	---	---
4-OH	---	6.70 (1H, br. s)	4-OH	---	---
1′	175.29	---	1′	175.7	---
2′	72.52	4.62 (1H, dd, *J* = 7.6, 3.5)	2′	73.3	4.26 (1H, m)
3′	35.90	2.01 (1H, m, Ha); 2.22 (1H, m, Hb)	3′	36.5	2.21 (1H, m, Ha); 2.02 (1H, m, Hb)

Continued on next page

Artemceramide-A **(2)**			Literature analogue (psychotramide)		
C. No.	^{13}C- and ^{1}H-NMR (δ)		C.No.	^{13}C- and ^{1}H-NMR (δ)	
4′	26.9	1.70 (2H, m)	4′	131.1	5.49 (1H, dt, 8.0, 4.0)
5′	33.89	2.04 (2H, m)	5′	131.1	5.53 (1H, dt, 8.0, 4.0)
6′	130.85	5.53 (1H, dt, *J* = 15.5, 7.0)	6′	26.3	1.92 (2H, m)
7′	130.98	5.49 (1H, dt, *J* = 15.5, 7.0)			
8′	33.30-	1.98 (1H, m),	7′-24′ and		
9′-13′ and			6-16	30.3-34.3	1.26 (54H, m)
7-25	29.01–30.0				
		11.30 (48H, m)			
26/14′	32.03	1.70 (4H, m)	17/23′	32.6	1.26 (4H, m)
27/15′	22.94	1.78 (4H, m)	18/24′	23.4	1.26 (4H, m)
28/16′	14.26	0.85 (6H, t, *J* = 7)	19/25′	14.7	0.88 (6H, t, *J* = 7.0)
2′-OH	---	7.64 (1H, br. s)	2′-OH	----	---

For the purpose of comparison, the ^{1}H and ^{13}C-NMR spectra of the compound **2** and the literature reference compound[107] are given in **Table 2.2.1.**

The results of the comparison of 1D-NMR data and NOESY correlation indicated that the absolute configurations of the LCB carbons; C-2, C-3, and C-4 were *2R*, *3R*, and *4S* respectively.[108]

As a result of the spectral data and physicochemical constants discussed above, the structure of the compound **2** was established as (*2′R,E*)-2′-hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadec-6-enamide.

To the best of our knowledge it is a new compound. The compound was named as artemceramide-A.

The key HMBC, COSY and NOESY correlations of artemceramide-A **(2)** are given in **Figure 2.2.**

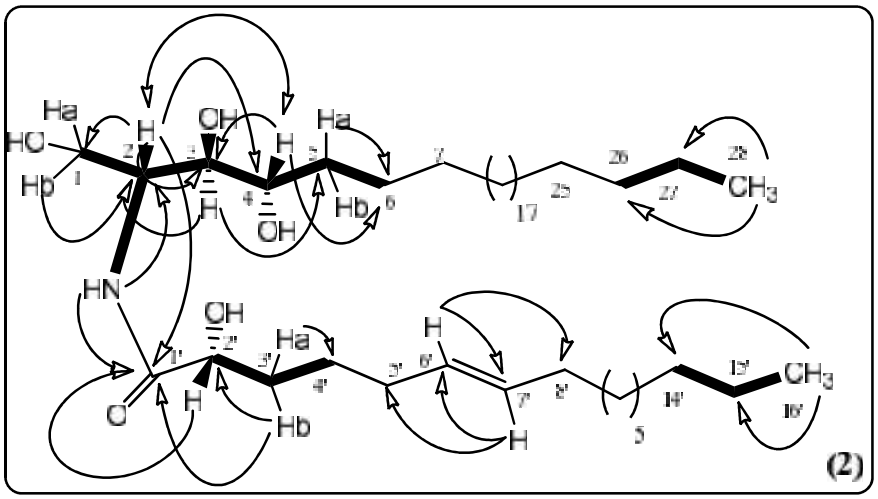
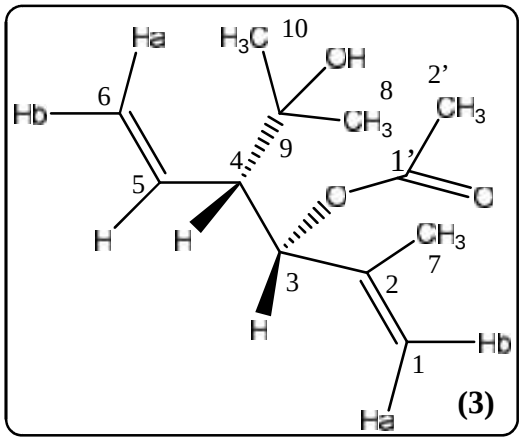


Figure 2.2 Key ($^{1}H \longrightarrow {}^{13}C$ HMBC) (^{1}H ▬▬ ^{1}H COSY) and ($^{1}H \leftrightarrows {}^{1}H$ NOESY) correlations of artemceramide-A **(2)**

2.4 New compound isolated from the roots of *Artemisia incisa*.

2.4.1 (*3R,4S*)-4-(2-Hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl acetate) ((*3R,4S)*-santolinylol-3-acetate) (3).



(3)

Compound **3** was obtained as gummy oil, off white color with $^{20}[\alpha]_D$ - 9.1 (*c* 1.0, $CHCl_3$). The IR spectrum showed absorption for OH st. at $\bar{\upsilon}$ 3295, O-C=O st. at $\bar{\upsilon}$ 1737 and C=C st. at $\bar{\upsilon}$ 1656 $cm.^{-1}$ The LR-EIMS showed molecular ion peak at *m/z* 212.14 $[M^+]$ corresponding to the molecular formula of $C_{10}H_{20}O_3$.

Protons coupling; ^1H-^1H were established through COSY, the ^1H-^{13}C correlation were established through HSQC, while the long range ^1H-^{13}C correlations were established through HMBC experiments.

The ^1H and ^{13}C-NMR spectra of the compound **3** gave signals characteristic of an irregular monoterpene ester.

Table 2.3. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of (*3R,4S*)-4-(2-hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl acetate) **(3)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	116.41	CH_2	4.95 (1H, br. s, Ha) 5.04 (1H, br. s, Hb)	C-2, C-3
2	143.46	C	---	---
3	81.54	CH	5.36 (1H, d, *J* = 9.6)	C-1, C-2, C-4, C-1′
4	53.45	CH	2.46 (1H, dd, *J* = 9.6, 6.8)	C-3, C-5, C-6, C-9
5	135.76	CH	5.50 (1H, ddd, *J* = 16.6, 12.0, 6.8)	C-4, C-6, C-3, C-9
6	120.23	CH_2	5.16 (1H, dd, *J* = 17.0, 2.0, Ha) 5.13 (1H, dd, *J* = 12.0, 2.0, Hb)	C-4, C-5
7	18.23	CH_3	1.76 (3H, s)	C-1, C-2, C-3
8	22.41	CH_3	1.21 (3H, s)	C-4, C-9, C-10
9	76.77	C	---	---
10	27.05	CH_3	1.24 (3H, s)	C-4, C-8, C-9
1′	170.22	C	---	---
2′	29.05	CH_3	2.09 (3H, s)	C-1′

In the ^{1}H-NMR spectrum of the compound **3 (Table 2.3)**, the four methyl groups were resonating at δ 1.76 (3H, s, H-7), 1.99 (3H, s, H-8), 1.24 (3H, s, H-10) and 2.09 (3H, s, H-2′). The axial methylene protons were resonating at δ 4.94 (1H, br. s, Ha-1); 5.04 (1H, br. s, Hb-1) and δ 5.13 (1H, dd, *J* = 12.0, 1.8 Hz, Ha-6); 5.16 (1H, dd, *J* = 17.0, 2.0 Hz, Hb-6). The methine protons were resonating at δ 5.2 (1H, d, *J* = 9.6 Hz, H-3), 2.45 (1H, dd, *J* = 6.8, 9.6 Hz, H-4) and 5.502 (1H, ddd, *J* = 15.6, 6.2, 2.0 Hz, H-5). The position of the hydroxyl proton was not clear.

In the ^{13}C-NMR spectrum **(Table 2.3)**, the methyl groups were resonating at δ 18.23 (C-7), 22.41 (C-8), 27.05 (C-10) and 29.04 (C-2′). The axial methylenes were resonating δ 116.43(C-1) and 120.23 (C-6). The methines were resonating at δ 81.54 (C-3), 53.45 (C-4) and 135.76 (C-5). The quaternary carbons were resonating at δ 146.43 (C-2), 76.77 (C-9) and 170.2 (C-1′).

For the purpose of comparison, the ^{1}H and ^{13}C-NMR spectra of both the compound **3** and **27** (santolinylol) are given in **Table 2.3.1.**

In compound **3** as compared to compound **27**, the hydroxyl group at carbon; C-3 was replaced by an acetoxy group which was evident from the additional resonances of acetyl group (CH_3CO) in ^{1}H and ^{13}C-NMR spectra resonating at δ_H 2.09 (3H, s, H-2′), δ_C 29.05 (C-2′) and 170.2 (C-1′). This fact was further supported by the downfield shift of the methine proton by 1ppm, resonating at δ_H 4.35 (1H, d, *J* = 10 Hz, H-3) in compound **27** as compared to compound **3** where it was resonating at δ_H 5.37 (1H, d, *J* = 9.6 Hz, H-3).

Table 2.3.1. Key data comparison of ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$ of (*3R,4S*)-4-(2-hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl acetate) **(3)** in $CDCl_3$ and santolinylol **(27).**[67]

Santolinylol-3-acetate **(3)**			Santolinylol **(27)**	
C. No.	^{13}C- and ^{1}H-NMR (δ)		C. No.	^{13}C- and ^{1}H-NMR (δ)
1	116.41	4.95 (1H, br. s, Ha) 5.04 (1H, br. s, Hb)	114.21	4.93 (1H, br. s, Ha) 5.02 (1H, br. s, Hb)
2	143.46	---	145.6	---
3	81.54	5.36 (1H, d, *J* = 9.6)	78.52	4.35 (1H, d, *J* =10.0)
4	53.45	2.46 (1H, dd, *J* = 6.8, 9.6)	56.71	2.38 (1H, dd, *J* =10.0, 10.0)
5	135.76	5.50 (1H, ddd, *J* = 16.6, 12.0, 6.8)	133.9	5.50 (1H, ddd, *J* =16.6, 12.0, 6.8)
6	120.23	5.16 (1H, dd, *J* = 17.0, 2.0, Ha) 5.13 (1H, dd, *J* = 12.0, 2.0, Hb)	117.9	5.07 (1H, dd, *J* =19.2, 2.0, Ha) 5.03 (1H, dd, *J* =12.0, 2.0, Hb)
7	18.23	1.76 (3H, s)	16.24	1.70 (3H, s)
8	22.41	1.21 (3H, s)	24.35	0.90 (3H, s)
9	76.77	---	73.93	---
10	27.05	1.24 (3H, s)	30.83	0.98 (3H, s)
1′	170.22	---	---	---
2′	29.05	2.09 (3H, s)	---	---

The characteristic ^{1}H and ^{13}C-NMR values of the methine group (CH-3) resonating at δ_H 5.37 (H-3) and δ_C 81.54 (C-3) and the HMBC correlation of the proton resonating at δ_H 5.37 (H-3) with the carbon resonating at δc 170.2 (C-1′) confirmed the presence of acetyl group at position C-3 in compound **3.** Similarly, the position of the hydroxyl proton in compound **3** was not clear, however, its presence was deduced from the characteristic resonance of the hydroxylated carbon; C-9 at δ_C 76.77 (C-9).

In the COSY spectrum, the methine proton resonating at δ 5.2 (H-3) showed correlation with the methine protons resonating at δ 2.45 (H-4) and 5.502 (H-5). The later have also correlation with the axial methylene (CH_2-6) protons resonating at δ_H 5.16 (diastereotopic) (Ha-6); 5.13 (Hb-6). These correlation confirmed the substitution of allylic and acetyl groups at the methine carbon (CH-3) while the substitution of vinylic and 2-hydroxy propanyl groups at the methine carbon (CH-4). The methines (CH-3) and (CH-4) are directly coupled to each other.

The HMBC correlations between the two methyl groups; CH_3-8 and CH_3-10 with each other and the carbons; C-9 and C-4 deduce the presence of 2-hydroxy propanol group substituted at the methine carbon (C-4).

The relative stereochemistry of the compound **3** was deduced from the NOESY correlation between the methine protons; H-3 and H-4 which were the same like in case of (*3R,4S*)-santolinylol (**27)**[67] and thus the relative stereochemistry was consequently assigned as *3R* and *4S* configuration. This was further supported by comparing the specific rotation; $^{20}[\alpha]_D$ - 9.1 (*c* 1.0, $CHCl_3$) of the compound **3** with other reference

compounds; (*3R,4S*)-3-hydroxy santolinate **(172)**[109]; $^{20}[\alpha]_D$ - 6.7 (*c* 0.70, $CHCl_3$) and (*R*)-artemisyl acetate **(170)**[67]; $^{20}[\alpha]_D$ - 5.8 (*c* 0.67, $CHCl_3$).[41]

Thus, from the information obtained from spectral data and physicochemical constants, the structure of the compound **3** was established as (*3R,4S*)-4-(2-hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl acetate.

To the best of our knowledge, it is a new compound. The compound was named as (*3R,4S*)-santolinylol-3-acetate.

The key HMBC and COSY correlations of the compound **3** are given in **Figure 2.3a** while its NOESY correlation are given in **Figure 2.3b** respectively.

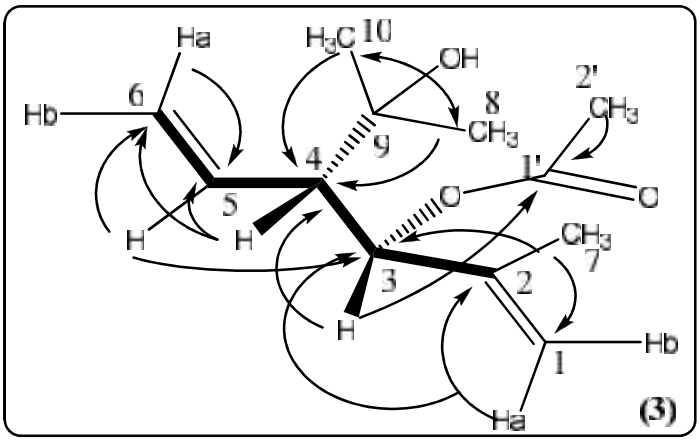


Figure 2.3a. Key HMBC (→) and COSY (—) correlations of santolinylol-3-acetate **(3).**

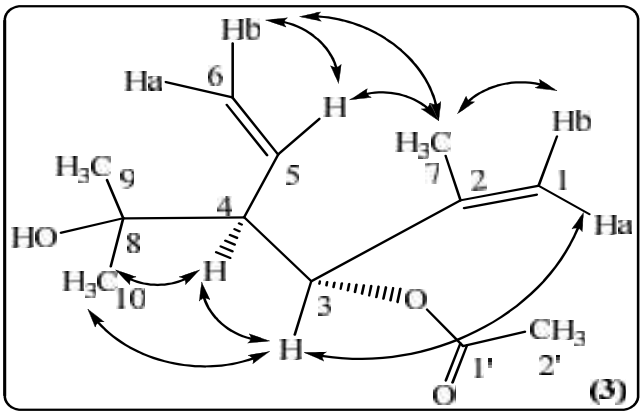
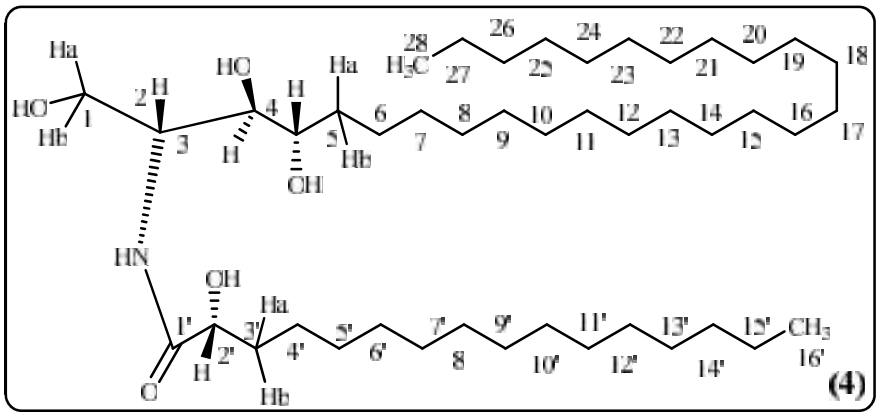


Figure 2.3b. Key NOESY (↔) correlation of santolinylol-3-acetate **(3).**

2.5 New compound isolated from the roots of *Artemisia incisa*.

2.5.1 (*2′R*)-2′-Hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadecanamide (artemceramide-B) (4).



Compound **4** was obtained as an amorphous white powder m.p. 141–142 °C. The IR spectrum of the compound **4** showed absorptions for OH and NH st. at ῡ 3328.5, 3246.6 and 3218.6 and NHC=O st. at ῡ 1621.8 cm.$^{-1}$ The molecular formula of $C_{44}H_{89}NO_5$ was determined by HR-ESIMS (positive ion mode) which showed the pseudo molecular ion peak at *m/z* 712.6093 [M^++H].

Protons coupling; ^{1}H-^{1}H were established through COSY, the ^{1}H-^{13}C correlation were established through HSQC, while the long range ^{1}H-^{13}C correlations were established through HMBC experiments.

The ^{1}H and ^{13}C-NMR spectra of the compound **4 (Table 2.4)** showed characteristic signals of α-hydroxy fatty acid and sphingosine base moieties. Compound **4** was the saturated analogue of the compound **2**.

The ^{1}H-NMR spectrum (**Table 2.4**) showed amide proton (CONH) resonance at δ 8.58 (1H, d, *J* = 8.5 Hz, NH). The oxymethine protons were resonating at δ 4.62 (1H, dd, *J* = 7.6, 3.5 Hz, H-2′), 4.35 (1H, dd, *J* = 11.0, 4.5 Hz, H-3) and 4.29 (1H, m, H-4) while the aminated methine proton was resonating at δ 5.12 (1H, m, H-2). The oxymethylene protons were resonating at δ 4.51 (diastereotopic) (1H, dd, *J* = 17.5, 5.0 Hz, Ha-1) and 4.42 (1H, dd, *J* = 17.5, 5.0 Hz, Hb-1). The two methylene protons were resonating at δ 2.01 (diastereotopic) (1H, m, Ha-3′); 2.23 (1H, m Hb-3′) and 1.99 (diastereotopic) (1H, m, Ha-5); 2.20 (1H, m, Hb-5). The two pair of methylenes protons were resonating at δ 1.78 (4H, m, H-26/H-14′) and 1.70 (4H, m, H-27/H-15′). A sequence of 27 methylenes protons were resonating at δ 1.24–1.29 (54H, br s, H7–H-25/H-5′–H-13′). The two terminal methyls protons were resonating at δ 0.85 (6H, t, *J* = 6.5 Hz, H-28/H-16′).

In the ^{13}C-NMR spectrum of the compound **4 (Table 2.4)**, the amide carbon was resonating at δ 175.29 (C-1′). The aminated methine was resonating at δ 53.07 (C-2) and the three oxymethines were resonating at 72.98 (C-3), 76.94 (C-4) and 72.52 (C-2′).

Table 2.4. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of (*2′R*)-2′-hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadecanamide **(4)** in C_5D_5N.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
NH	---	H	8.59 (1H, d, *J* = 9.0)	---
1	61.74	CH_2	4.50 (1H, dd, *J* = 17.5, 5.0, Ha);	C-2, C-3
			4.43 (1H, dd, *J* = 17.5, 3.8, Hb)	
2	52.59	CH	5.11 (2H, m, H-2)	C-1′, C-1, C-3, C-4
3	76.54	CH	4.36 (1H, dd, *J* = 11.0, 4.5)	C-1, C-2, C-4
4	72.72	CH	4.30 (1H, m)	C-2, C-3, C-5
5	33.84	CH_2	2.19 (1H, m, Ha);	---
			1.98 (1H, m, Hb)	
6	30.36	CH_2	2.21 (2H, m)	---
1-OH	---	H	6.24 (1H, br. s)	C-1
3-OH	---	H	6.71 (1H, br. s)	C-3
4-OH	---	H	6.70 (1H, br. s,)	C-4
1′	174.95	C	---	---
2′	72.16	CH	4.63 (1H, dd, *J* = 7.6, 3.5)	C-1′, C-3′
3′	35.37	CH_2	2.20 (1H, m, Ha);	---
			2.23 (1H, m, Hb)	
4′	25.48	CH_2	1.98 (2H, m)	---
5′–13′ and 7–25	29.16–29.99	$27CH_2$	1.26 (56H, m)	---

Continued on next page

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
26/14′	31.92	$2CH_2$	1.78 (4H, m)	C-13′/C-25, C-15′/C-27
27/15′	22.55	$2CH_2$	1.71 (4H, m)	C-14′/C-26, C-16′/C-28
28/16′	13.77	$2CH_3$	0.84 (6H, t, J = 7.0)	C-15′/C-27, C-14′/C-26
2′-OH	---	H	7.65 (1H, br. s)	C-2′

The two pair of methylenes were resonating at δ 22.68 (C-27/C-15′) and 31.92 (C-26/C-14′). The four methylenes were resonating at δ 35.90 (C-3′) 25.48 (C-4), 33.84 (C-5) and 30.36 (C-6). The sequence of 27 methylenes were resonating at δ 28.34–30.14 (C-7-C-25/C-5′-C-13′). The two terminal methyls were resonating at δ 14.10 (C-28/C-16′).

The spectral data of 1D-NMR revealed that compound **4** was a phytosphingosine type ceramide. Furthermore, analysis of the correlation established through HMBC, COSY and NOESY spectra led to the exact structure assignment for compound **4**.

In the COSY spectrum, the amidomethine proton at δ_H 5.11 (H-2) showed cross peaks with the diastereotopic oxymethylene protons at δ_H 4.51 (Ha-1); 4.43 (Hb-1), amido proton at δ_H 8.59 (NH), oxymethine proton at δ_H 4.36 (H-3). The later also correlated with the oxymethine proton at δ_H 4.30 (H-4). Similarly, in the COSY spectrum, the oxymethine proton at δ_H 4.62 (H-2′) showed cross peaks with the diastereotopic methylene protons at δ_H 2.01 (Ha-3′); 2.23 (Hb-3′) which in turn showed correlation with the methylene protons at δ_H 1.98 (H-4′).

In the HMBC spectrum, the diastereotopic protons at δ_H 4.51 (Ha-1) and 4.42 (Hb-1) showed correlations with the carbons resonating at δ_C 53.07 (C-2) and 76.94 (C-3). The proton at δ_H 5.12 (m, H-2) showed correlations with the carbons resonating at δ_C 175.29 (C-1′), 62.12 (C-1) and 76.94 (C-3) which showed the attachment of sphingosine with *α*-hydroxy fatty acid through amide linkage. The proton resonating at δ_H 4.35 (H-3) showed correlation with the carbons resonating at δ_C 53.07 (C-2) and 72.98 (C-4). The proton at δ_H 4.29 (H-4) showed correlations with the carbons resonating at δ_C 76.94 (C-3) and 35.98 (C-5). The proton at δ_H 6.4 (OH-1) showed correlation with the carbon resonating

at δ_C 62.12 (C-1). The protons at δ_H 6.8 (OH-3/OH-4) showed correlation with the carbons resonating at δ_C 76.94 (C-3) and 72.98 (C-4). These HMBC correlations confirmed the attachment of hydroxyl groups at carbons; C-1, C-3 and C-4 of sphingosine moiety.

2.5.1.1 Chemical studies on compound 4.

In order to determine the chain length of the fatty acid and sphingosine moieties. The structure of the compound **4** was also analyzed with the help of chemical method of methanolysis.

2.5.1.1a Methanolysis of the compound 4.

Methanolysis of the compound **4** produced *α*-hydroxy fatty acid (FAME) and a long chain base (LCB). The methyl ester of *α*-hydroxy fatty acid produced (*n*-hexane extracted) was subjected to GC-MS analysis and was identified as (*R*)-methyl 2-hydroxyhexadecanoate at *m/z* 286.25 [M^+], while the absolute configuration at C-2 was determined to be *R*, from the specific rotation $^{20}[\alpha]_D$ -1.40 (*c* 0.10, $CHCl_3$).[104, 110] The part of LCB was therefore, a C_{28} aliphatic amino alcohol (a unit with an amino group and three hydroxyl groups). Consequently, the structure of the compound **4** was determined to be (*2′R*)-2′-hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2yl)hexadecanamide (**Figure 2.5**).

The configurations of the compound **4** were assigned by comparing the ^{1}H-NMR, ^{13}C-NMR (**Table 2.4**) and physicochemical constants as well as ^{1}H-^{1}H NOESY correlation

Table 2.4.1. Key data comparison of ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) in C_5D_5N of (*2′R*)-2′-hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadecanamide **(4)** and literature analogue; (*R*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxynonacosan-2-yl-pentacosanamide.[110]

Artemceramide-B **(4)**			literature analogue; (*R*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxynonacosan-2-yl-pentacosanamide		
C. No.	^{13}C-	^{1}H-NMR (δ)	C. No.	^{13}C-	^{1}H-NMR (δ)
NH	---	8.59 (1H, d, *J* = 9.0)	NH	---	8.58 (1H, d, *J* = 9.0)
1	61.74	4.50 (1H, dd, *J* = 17.5, 5.0, Ha); 4.43 (1H, dd, *J* = 17.5, 3.8, Hb)	1	62.9	4.50 (1H, dd, *J* = 17.5, 5.0, Ha) 4.42 (1H, dd, *J* = 17.5, 4.0, Hb)
2	52.59	5.11 (1H, m)	2	53.9	5.10 (1H, m)
3	76.54	4.36 (1H, dd, *J* = 11.0, 4.5)	3	77.7	4.34 (1H, m)
4	72.72	4.30 (1H, m)	4	73.3	4.27 (1H, m)
5	33.84	2.19 (1H, m, Ha); 1.98 (1H, m, Hb)	5	35.0	1.99 (1H, m, Ha); 2.20 (1H, m, Hb)
6	30.36	2.21 (2H, m)	6	30.3	2.20 (2H, m)
1-OH	---	6.24 (1H, br. s)	1-OH	---	---
3-OH	---	6.71 (1H, br. s)	3-OH	---	---
4-OH	---	6.70 (1H, br. s)	4-OH	---	---
1′	174.95	---	1′	176.1	---
2′	72.16	4.63 (1H, dd, *J* = 7.6, 3.5)	2′	73.9	4.61 (1H, m)
3′	35.37	2.01 (1H, m, Ha); 2.23 (1H, m, Hb)	3′	36.6	1.99 (1H, m, Ha); 2.24 (1H, m, Hb)
4′	25.48	1.98 (2H, m)	4′	26.6	1.69 (2H, m)
5′–13′ and 7–25		1.26 (m, 56H) 29.16–29.99	5′-22′ and 7-26		1.24-1.29 (72H, m) 30.5-31.2
26/14′	31.92	1.78 (4H, m)	27/23′	33.0	1.24-1.29 (4H, m)

Continued on next page

Artemceramide-B **(4)**			literature analogue; (*R*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxynonacosan-2-yl-pentacosanamide		
C. No.	^{13}C	^{1}H-NMR (δ)	C. No.	^{13}C	^{1}H-NMR (δ)
27/15′	22.50	1.71 (4H, m)	28/24′	23.8	1.24-1.29 (4H, m)
28/16′	13.77	0.85 (6H, t, 7.0)	29/25′	15.1	0.85 (6H, t, *J* = 6.5)
2′-OH	---	7.65 (1H, br. s)	2′-OH	----	---

with the analogue reported in the literature **(Table 2.4.1)** as well as the unsaturated analogue; compound **2 (Table 2.2)**.

In case of the compound **4,** the optical rotation; $^{20}[\alpha]_D$ +7.98 (*c* 0.07, C_5H_5N) and NOESY correlation of methine protons; H-2 and H-4 were in close agreement with the literature analogue; (*R*)-2-hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxynonacosan-2-yl-pentacosanamide; $^{20}[\alpha]_D$ +10.2 (*c* 0.09, C_5H_5N).[110] This evidence indicates that the absolute configurations at the carbons; C-2, C-3, and C-4 of sphingosine moiety were *2R*, *3R*, and *4S* respectively.

From the information obtained from spectral data and physicochemical constants, the structure of the compound **4** was established as (*2′R*)-2′-hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadecanamide**.**

To the best of our knowledge, it is a new compound. The compound was named as artemceramide-B.

It is interesting to know that this is the first report of ceramides isolation from the genus *Artemisia* which is phytochemically the most explored genus of the family Asteraceae, till date more than 1000 compounds has been isolated.[1,18,22,111] However, ceramides have been reported from the family compositeae[112] and alkamides from *Artemisia dracunculus*.[95] These ceramides were isolated from the roots of the *Artemisia* plants. The two *Artemisia* species (*A. elegantissima* and *A. incisa*) have produced the same type of ceramide with very close values of 1D-NMR and correlations of 2D-NMR with the only difference of saturation in compound **4** and unsaturation (olefinic bond) at position C-6′ and C-7′ in compound **2**.

The key HMBC, COSY and NOESY correlations of artemceramide-B **(4)** is given in **Figure 2.4.**

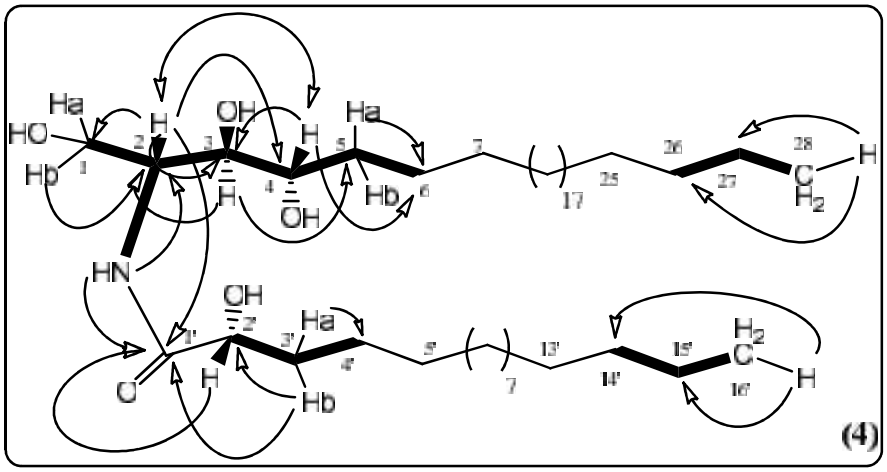
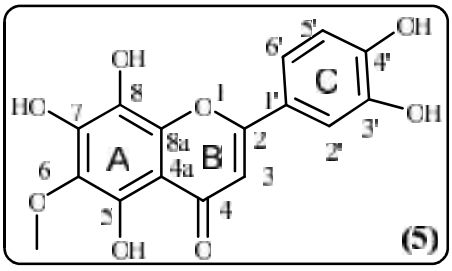


Figure 2.4 Key ($^1H \longrightarrow {}^{13}C$ HMBC) (^1H ——— ^1H COSY) and ($^1H \leftrightarrows {}^1H$ NOESY) correlations of artemceramide-B **(4)**

2.6 New source compounds isolated from the aerial parts (leaves and stems) of *Artemisia elegantissima.*

2.6.1 5,7,8,3',4'-Pentahydroxy-6-methoxyflavone (5).[113]



Compound **5** was obtained as yellow prism like crystals, m.p. 283–285 °C (lit. m.p. not reported). The molecular formula of $C_{16}H_{12}O_8$ was deduced from HR-ESIMS (positive ion mode) which exhibited a pseudo molecular ion peak at *m/z* 333.062 [M^++H].

The ^{1}H and ^{13}C-NMR **(Table 2.5)** spectra give signals characteristic of flavone skeleton. In the ^{1}H-NMR spectrum **(Table 2.5)**, the methoxy protons were resonating at δ 3.87 (3H, s, OCH_3-6). The olefinic proton of ring **B** was resonating at signal at δ 6.57 (1H, s, H-3). The aromatic protons of ring **C** were resonating at δ 7.59 (1H, dd, *J* = 8.4, 2.0, Hz, H-6′), 6.99 (1H, d, *J* = 8.4 Hz, H-5′) and 7.69 (1H, d, *J* = 2.0 Hz, H-2′). The hydroxyl proton was resonating at δ 12.0 (1H, br. s, OH-5) while the resonances for the rest of the hydroxyl protons (OH-6, OH-7, OH-8, OH-3′ and OH-4′) were not clear however, their presence at the particular position was deduced from the ^{13}C-NMR values of carbons at δ 132.1 (C-6), 145.9 (C-7), 134.5 (C-8), 145.9 (C-3′) and 150.0 (C-4′) respectively.

The ^{13}C-NMR spectrum of the compound **5** showed sixteen carbon atoms which according to DEPTq, there were four methines, eleven quaternary and one methyl carbon.

In ^{13}C-NMR spectrum, the carbonyl was resonating at δ 182.5 (C-4) and the methoxy group was resonating at δ 59.7 (OCH_3-6). The olefinic carbons were resonating at δ 102.5 (C-3) and 164.4 (C-2). The aromatic carbons were resonating at δ 115.8 (C-5′), 119.1 (C-6′), 113.0 (C-2′), 145.9 (C-3′), 150.0 (C-4′), 122.2 (C-1′), 153.1 (C-5), 132.1 (C-6), 145.9 (C-7), 134.5 (C-8), 105.8 (C-4a) and 140.2 (C-8a).

From the spectral information of ^{1}H, ^{13}C-NMR and MS and other physical data, the structure of the compound **5** was partially established as 2-(3,4-dihydroxyphenyl)-5,7,8-trihydroxy-6-methoxy-4H-chromen-4-one (5,7,8,3',4'-pentahydroxy-6-methoxyflavone).[1]

Table 2.5. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of 5,7,8,3',4'-pentahydroxy-6-methoxyflavone **(5)** in $(CD_3)_2CO$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	164.4	C	---	---
3	102.5	CH	6.57 (1H, s)	C-2, C-4, C-4a, C-1′
4	182.5	C	---	---
5	153.1	C	---	---
6	132.1	C	---	---
7	145.9	C	---	---
8	134.5	C	---	---
4a	105.8	C	---	---
8a	140.2	C	---	---
1′	122.2	C	---	---
2′	113.0	CH	7.69 (1H, d, J = 2)	C-1, C-2′, C-3′, C-4′, C-6′
3′	145.9	C	---	---
4′	150.0	C	---	---
5′	115.8	CH	6.99 (1H, d, J = 8.4)	C-1′, C-3′ C-4′, C-6′
6′	119.1	CH	7.59 (1H, dd, J = 8.4, 2.0)	C-1, C-2′, C-4′, C-5′
6-OCH_3	59.7	CH_3	3.87 (3H, s)	C-6
5-OH	---	H	12.0 (1H, br. s)	C-5

The complete structure elucidation of the compound **5** was achieved by applying 2D-NMR experiments (HMBC and COSY) (**Table 2.5**).

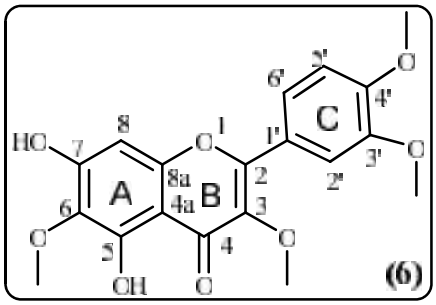
In the HMBC spectrum **(Table 2.5)**, the methoxy protons at δ_H 3.87 (OCH_3-6) showed correlations with the carbon resonating at δ_C 132.1 (C-6) while the hydroxyl proton at δ_H 12.0 (OH-5) showed correlations with the carbon resonating at δ_C 153.1 (C-5) which gave indication of its substitutions at the carbons; C-6 and C-5 of ring **A**. The olefinic proton at δ_H 6.57 (H-3) showed correlations with the carbons resonating at δ_C 182.5 (C-4), 164.4 (C-2), 105.8 (C-4a) and 122.2 (C-1′). The proton at δ_H 7.69 (H-2′) showed correlation with the carbons resonating at δ_C 119.1 (C-6′), 150.0 (C-4′), 145.9 (C-3′), 164.4 (C-2) and 122.2 (C-1′). The proton at δ_H 7.59 (H-6′) showed correlations with the carbons resonating at δ_C 115.8 (C-5′), 113.0 (C-2′), 150.0 (C-4′), 122.2 (C-1′) and 164.4 (C-2). The proton at δ_H 6.99 (H-5′) showed correlations with the carbons resonating at δ_C 145.9 (C-3′), 150.0 (C-4′), 122.2 (C-1′) and 119.1 (CH-6′).

From the above HMBC correlation **(Table 2.5)**, the compound **5** was finally established as 5,7,8,3',4'-pentahydroxy-6-methoxyflavone.[113]

In the literature, compound **5** has been reported by the synonym: (a) 5,7,8,3',4'-pentahydroxy-6-methoxyflavone.[114] from the genus *Hemizonia* (Asteraceae) but without information on the spectral data and physicochemical constants.[1,113-114]

So hereby, it is presented with full data of 1D and 2D-NMR **(Table 2.5)** and physicochemical constants as well as a new source compound from *A. elegantissima*.

2.6.2 Bonanzin (6).



Compound **6** was obtained as an orange yellow crystals, m.p. 156–157 °C (lit. m.p. 157–158 °C).[64] The HR-ESIMS (positive ion mode) showed a pseudo molecular ion peak at *m/z* 375.100 [M^++H] corresponding to the molecular formula of $C_{19}H_{18}O_8$.

The ^{1}H and ^{13}C-NMR spectra of the compound **6**, give signals characteristic of flavonol skeleton.

In the ^{1}H-NMR spectrum **(Table 2.6),** the aromatic protons of ring **C** were resonating at δ 7.48 (1H, d, *J* = 2.0 Hz, H-2′), 7.38 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′) and 6.86 (1H, d, *J* = 8.4 Hz, H-5′). The aromatic proton of ring **A** was resonating at δ 6.25 (1H, s, H-8). The two pairs of methoxy protons were resonating at δ 3.70 (6H, s, OCH_3-3/OCH_3-3′) and 3.78 (6H, s, OCH_3-6/OCH_3-4′). The hydroxyl proton was resonating at δ 12.0 (1H, s, OH-12) while the proton signal of OH-7 was not clear and its position was deduced from the characteristic ^{13}C-NMR values of the carbon resonating at δ 152.3 (C-7).

In the ^{13}C-NMR spectrum (**Table 2.6**), seventeen carbon atoms were present which were differentiated by DEPT-135 as eleven quaternary, four methine and four methyl carbons.

The ring **B** carbons were resonating at δ 137.4 (C-3), 154.7 (C-2) and 177.5 (C-4).

The ring **A** carbons were resonating at δ 152.1 (C-8a), 152.9 (C-5), 132.9 (C-6), 152.3 (C-7), 95.22 (C-8) and 102.1 (C-4a). The ring **C** carbons were resonating at δ 149.2 (C-3′) 149.7 (C-4′), 112.3 (C-2′), 111.6 (C-5′), 120.9 (C-6′) and 121.5 (C-1′). The methoxy carbons were resonating at δ 59.5 (OCH_3-3), 59.8 (OCH_3-3′), 60.1 (OCH_3-6) and 60.7 (OCH_3-4′).

From the above evidence of ^{1}H and ^{13}C-NMR spectra and physicochemical constants, the structure of the compound **6** was interpreted as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,6-dimethoxy-4H-chromen-4-one (bonanzin).[64] The structure was further confirmed by applying 2D-NMR experiments.

In the HMBC spectrum **(Table 2.6),** the proton at δ_H 6.25 (H-8) showed correlations with the carbons resonating at δ_C 102.1 (C-4a), 132.9 (C-6) and 152.3 (C-7). These correlations confirmed the presence of methine group at C-8 position of ring **A**. The proton at δ_H 6.86 (H-5′) showed correlation with the carbons resonating at δ_C 149.2 (C-3′), 149.7 (C-4′), 121.5 (C-1′) and 120.9 (C-6′). The proton at δ_H 7.48 (H-2′) showed correlations with the carbons resonating at δ_C 120.9 (C-6 149.6 (C-4′), 149.2 (C-3′), 154.7 (C-2) and 121.5 (C-1′). The proton at δ_H 7.38 (H-6′) showed correlations with the carbons resonating at δ_C 111.6 (C-5′), 112.3 (C-2′), 149.7 (C-4′), 121.5 (C-1′) and 154.7 (C-2). The hydroxyl proton at δ_H 12.0 (OH-5) showed correlation with the carbon resonating at δ_C 152.9 (C-5) and the methoxy protons at δ_H 3.70 (OCH_3-3), 3.70 (OCH_3-3′), 3.78 (OCH_3-6) and 3.88 (OCH_3-4′) showed correlations with the carbons resonating at δ_C 137.4 (C-3), 149.2 (C-3′), 132.9 (C-6) and 149.7 (C-4′) respectively.

Table 2.6. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of bonanzin **(6)** in $(CD_3)_2SO$.

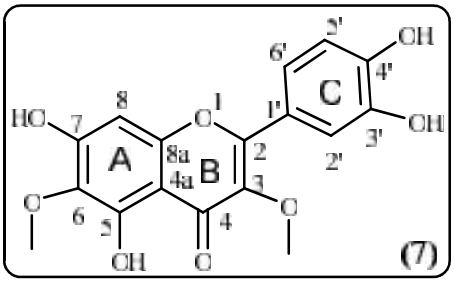
C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	154.7	C	---	---
3	137.4	C	---	---
4	177.5	C	---	---
5	152.9	C	---	---
6	132.9	C	---	---
7	152.3	C	---	---
8	95.22	CH	6.25 (1H, s)	C-6, C-7, C-4a
4a	102.1	C	---	---
8a	152.1	C	---	---
1′	121.5	C	---	---
2′	112.3	CH	7.48 (1H, d, *J* = 2)	C-2, C-2′, C-3′, C-4′
3′	149.2	C	---	---
4′	149.6	C	---	---
5′	111.6	CH	6.86 (1H, d, *J* = 8.4)	C-1′, C-3′
6′	120.9	CH	7.38 (1H, dd, *J* = 8.4, 2.0)	C-1, C-2′, C-4′, C-5′
3-OCH_3	59.5	CH_3	3.70 (3H, s)	C-3
6-OCH_3	60.1	CH_3	3.78 (3H, s)	C-6
3′-OCH_3	59.8	CH_3	3.70 (3H, s)	C-3′
4′-OCH_3	60.7	CH_3	3.78 (3H, s)	C-4′
5-OH	---	H	12.0 (1H, br. s)	C-5
7-OH	---	H	Not clear	---

These correlations confirmed that the methoxy groups were substituted at positions C-3′ and C-4′ of ring **C** and positions C-6 and C-3 of ring **A** and **B** respectively. The carbons; C-5 and C-7 were found to be the hydroxylated carbons of ring **A**.

Thus, from the spectral **(Table 2.6)** and physical data, the structure of the compound **6** was elucidated as bonanzin,[1,64] which is hereby reported as new source compound from *A. elegantissima*.

In the literature, compound **6** was known by synonyms: (a) bonanzin, (b) 2-(3,4-dimethoxyphenyl)-5,7-dihydroxy-3,6-dimethoxy-4H-1-benzopyran-4-one and (c) 5,7-dihydroxy-3,3′,4′,6-tetramethoxyflavone. Bonanzin (**6)** has been isolated from *Bahia oppositifolia*, *Artemisia* species,[64] *Brickellia* species, *Parthenium ligulatum,* S*tevia cuzcoensis* and *Vitex trifoliate*.[1,61]

2.6.3 3′,4′-Dihydroxybonanazin (7).



Compound **7** was isolated as yellow crystals, m.p. 286–287 °C (lit. m.p. 284–288 °C). The HR-ESIMS (positive ion mode) showed a pseudo molecular ion peak at *m/z* 347.069 [M^++H] corresponding to the molecular formula of $C_{17}H_{14}O_8$.

The ^{1}H and ^{13}C-NMR spectra for compound **7** give characteristic signals of flavonol skeleton.

In the ^{1}H-NMR spectrum (**Table 2.7),** the ring **C** protons were resonating at δ 7.70 (1H, d, *J* = 2.0 Hz, H-2′), 6.99 (1H, d, *J* = 8.4 Hz, H-5′) and 7.61 (1H, dd, *J* = 8.4, 2.0, Hz, H-6′). The ring **A** proton was resonating at δ 6.57 (1H, s, H-8). The methoxy protons were resonating at δ 3.88 (3H, s, OCH_3-3) and 3.86 (3H, s, OCH_3-6). The hydroxyl proton was resonating at δ 12.93 (1H, s, OH-5) while the rest of the hydroxyl protons signals were not clear in the spectrum but their positions were deduced from the characteristic ^{13}C-NMR values of the carbons resonating at δ_C 146.0 (C-3′), 149.6 (C-4′) and 151.9 (C-7).

Similarly, the ^{13}C-NMR (BB) spectrum (**Table 2.7**) showed seventeen carbon atoms which were distinguished by DEPT-135 into eleven quaternary and four methine carbons.

The ring **B** carbons were resonating at δ 176.9 (C-4), 153.8 (C-2) and 136.9 (C-3). The ring **A** carbons were resonating at δ 152.7 (C-5), 131.8 (C-6), 151.9 (C-7), 151.8 (C-8a), 94.8 (C-8) and 101.7 (C-4a). The ring **C** carbons were resonating at δ 115.5 (C-2′), 116.3 (C-5′), 120.7 (C-6′), 146.0 (C-3′), 149.6 (C-4′) and 121.2 (C-1′). The methoxy carbons were resonating at δ 59.8 (OCH_3-3) and 60.1 (OCH_3-6).

From the above information of 1D-NMR (**Table 2.7)** and the physical data, the structure of the compound **7** was interpreted as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,6-dimethoxy-4H-chromen-4-one (3′,4′-dihydroxybonanzin).[64] However, the 1D-NMR proposed structure was further analyzed by 2D-NMR experiments.

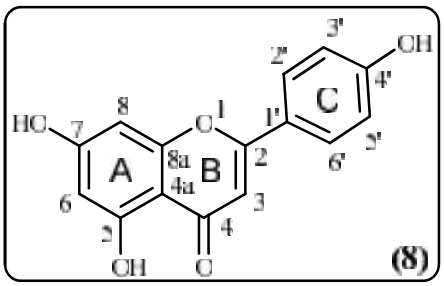
Table 2.7. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of 3′,4′-dihydroxybonanazin **(7)** in $(CD_3)_2CO$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	153.8	C	---	---
3	136.9	C	---	---
4	176.5	C	---	---
5	152.7	C	---	---
6	131.8	C	---	---
7	151.9	C	---	---
8	94.8	CH	6.57 (1H, s)	C-6, C-7, C-4a
4a	101.7	C	---	---
8a	151.8	C	---	---
1′	121.2	C	---	---
2′	115.5	CH	7.70 (1H, d, J = 2.0)	C-1, C-2′, C-3′, C-4′
3′	146.0	C	---	---
4′	149.6	C	---	---
5′	116.3	CH	6.99 (1H, d, J = 8.4)	C-1′, C-3′
6′	120.7	CH	7.61 (1H, dd, J = 8.4, 2.0)	C-1, C-2′, C-4′, C-5′
3-OCH_3	59.3	CH_3	3.86 (3H, s)	C-3
6-OCH_3	60.5	CH_3	3.88 (3H, s)	C-6
5-OH	---	H	12.93 (1H, br. s)	C-5

In the HMBC spectrum, the proton at δ_H 6.57 (H-8) showed correlation with the carbons resonating at δ_C 131.8 (C-6), 151.9 (C-7) and 101.7 (C-4a). These correlations proved the presence of only single methine; CH-8 in ring **A**. The methoxy protons at δ_H 3.86 (OCH_3-3) and 3.88 (OCH_3-6) showed correlation with the carbons resonating at δ_C 136.9 (C-3) and 131.8 (C-6) respectively, which showed there substitution position in ring **B** and A respectively. Similarly, the hydroxyl proton at δ_H 12.93 (OH-5) showed correlation with the carbon resonating at δ_C 152.7 (C-5). The proton at δ_H 6.98 (d, 1H-5′) showed correlations with the carbons resonating at δ_C 146.0 (C-3′), 149.6 (C-4′), 121.2 (C-1′) and 120.7 (C-6′). The proton at δ_H 7.68 (H-2′) showed correlations with the carbons resonating at δ_C 120.7 (C-6′), 149.6 (C-4′), 146.0 (C-3′), 154.7 (C-2) and 121.2 (C-1′). The proton at δ_H 7.58 (H-6′) showed correlations with the carbons resonating at δ_C 116.3 (C-5′), 115.5 (C-2′), 149.6 (C-4′), 121.2 (C-1′) and 153.8 (C-2).

From the spectral **(Table 2.7)** and physical data, the structure of the compound **7** was elucidated as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,6-dimethoxy-4*H*-chromen-4-one (3′,4′-dihydroxybonanzin),[1,64] which is hereby reported as new source compound from *A. elegantissima*. Compound **7** has been reported from *Artemisia copa.*[1]

2.6.4 Apigenin (8).



Compound **8** was isolated as yellow needles like crystals, m.p. 351–353 °C (lit. m.p. 352 °C).[51] The HR-ESIMS (positive ion mode) exhibited a pseudo molecular ion peak at *m/z* 271.052 [M^++H] corresponding to the molecular formula of $C_{15}H_{10}O_5$.

The ^{1}H and ^{13}C-NMR spectra of the compound **8** give characteristic signals of flavone skeleton.[46]

In the ^{1}H-NMR spectrum **(Table 2.8)**, the ring **A** protons were resonating at δ 6.26 (1H, d, *J* = 1.6 Hz, H-8) and 6.54 (1H, d, *J* = 1.6 Hz, H-6). The ring **B** proton were resonating at δ 6.62 (1H, s, H-3). The two pairs of equivalent protons of ring **C** were resonating at δ 7.94 (2H, d, *J* = 7.2, H-2′/H-6′) and 7.03 (2H, d, *J* = 7.2, H-3′/H-5′).

Correspondingly, the ^{13}C-NMR (BB) spectrum **(Table 2.8)**, indicated fifteen carbon atoms which were differentiated by DEPT-135 as eight quaternary and seven methine carbons (including; the two pair of equivalent methine).

In the ^{13}C-NMR spectrum, the ring **B** carbons were resonating at δ 181.8 (C-4), 163.8 (C-2) and 103.2 (C-3). The ring **A** carbons were resonating at δ 159.1 (C-5), 161.4 (C-7), 156.7 (C-8a), 98.9 (C-6), 93.6 (C-8) and 101.9 (C-4a). The ring **C** carbons were resonating at δ 128.7 (C-2′/C-6′), 115.8 (C-3′/C-5′), 161.2 (C-4′) and 121.9 (C-1′).

Table 2.8. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of apigenin **(8)** in $(CD_3)_2CO$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	163.8	C	---	---
3	103.2	CH	6.62 (1H, s)	C-4, C-2, C-4a
4	181.8	C	---	---
5	159.1	C	---	---
6	98.9	CH	6.26 (1H, d, *J* = 1.6)	C-4, C-7, C-8a
7	161.4	C	---	---
8	93.6	CH	6.54 (1H, d, *J* = 1.6)	C-4a, C-6, C-7
4a	101.9	C	---	---
8a	156.7	C	---	---
1′	121.9	C	---	---
2′/6′	128.7	2CH	7.94 (2H, d, *J* = 7.2)	C-1, C-2′, C-6′, C-3′, C-4′, C-5′
3′/5′	115.8	2CH	7.03 (2H, d, *J* = 7.2)	C-1′, C-2′, C-6′, C-3′, C-4′, C-5′
4′	161.2	C	---	---
5-OH	---	H	12.0 (1H, s)	---
7-OH	---	H	Not clear	---
4′-OH	---	H	Not clear	---

From 1D-NMR and physical data, the structure of the compound **8** was elucidated as 4′,5,7-trihydroxy flavone (apigenin).[51] However, the structure of the compound **8** was further analyzed by applying 2D-NMR experiments.

In the HMBC spectrum, the proton at δ_H 6.62 (H-3) showed correlation with the carbons resonating at δ_C 181.8 (C-4), 163.8 (C-2) and 121.9 (C-1′). The proton at δ_H 6.26 (H-6) showed correlations with the carbons resonating at δ_C 181.8 (C-4), 161.4 (C-7) and 156.7 (C-8a). The proton at δ_H 6.26 (H-6) showed correlation with the carbons resonating at δ_C 98.9 (C-6), 161.4 (C-7) and 101.9 (C-4a).

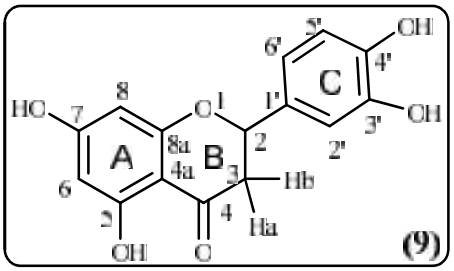
In the COSY spectrum, the protons at δ_H 7.94 (H-2′/H-6′) and 7.03 (H-3/H-5) showed correlations with each other which defined the 4-hydroxyphenyl moiety substituted at the C-2 position of the 5,7-dihydroxy-4H-chromen-4-one.

Thus, from the physicochemical constants and 1D, 2D-NMR spectral data **(Table 2.8)**, the structure of the compound **8** was elucidated as 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (apigenin) which was comparable with the literature.[1,51]

In the literature, compound **8** was known by synonyms: (a) 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, (b) apigenin, (c) apigenol, (d) pelargidenone and (e) versulin. It has been isolated from various biological sources and found free or as glycosides in the roots, leaves, stems, seeds or fruits of a number of plant species.[51] It has also been reported from leaf tissues of some fossil fuel.[1,58]

It is hereby reported as new source compound from *A. elegantissima*.

2.6.5 (*S*)-Dihydroluteolin (9).



Compound **9** was isolated as milky yellow needles, m.p. 265–266 °C (lit. m.p. 267 °C)[60] and optical activity; $^{20}[\alpha]_D$ - 47.1 (*c* 0.49, MeOH).[60]

The molecular formula of $C_{15}H_{12}O_6$ was deduced from the HR-ESIMS (positive ion mode) which exhibited a pseudo molecular ion peak at *m/z* 289.063 [M^++H].

The ^{1}H and ^{13}C-NMR spectra for compound **9** give characteristic signals of dihydroflavone skeleton.

In the ^{1}H-NMR spectrum of the compound **9**, the ring **B** protons were resonating at δ 5.41 (1H, dd, *J* = 10.0, 3.0 Hz, H-2) and 3.14 (diastereotopic) (1H, dd, *J* = 13.0, 3.0 Hz, Ha-3); 2.94 (1H, dd, *J* = 13.0, 3.0 Hz, Hb-3). The ring **A** protons were resonating at δ 5.94 (1H, d, *J* = 2.5 Hz, H-6) and 5.96 (1H, d, *J* = 2.5 Hz, H-8). The ring **C** protons were resonating at δ 7.03 (1H, br. s, H-2′), 6.87 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′) and 6.86 (1H, d, *J* =8.4 Hz, H-5′). The hydroxyl protons were resonating at δ 12.1 (1H, s, OH-5), 9.69 (1H, s, OH-7), 8.12 (1H, s, OH-3′) and 8.12 (1H, s, OH-4′).

The ^{13}C-NMR spectrum of the compound **9** showed fifteen carbon atoms which according to the DEPTq experiment there was one methylene, six methines and eight quaternary carbons.

Table 2.9. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of (*S*)-dihydroluteolin **(9)** in $(CD_3)_2CO$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	79.44	CH	5.41 (1H, dd, *J* = 10.0, 3.0)	C-3, C-4, C-1′, C-8a
3	42.57	CH_2	3.14 (1H, dd, *J* = 13.0, 3.0, Ha); 2.74 (1H, dd, *J* = 13.0, 3.0, Hb)	C-2, C-4, C-4a
4	195.5	C	---	---
5	164.4	C	---	---
6	95.85	CH	5.94 (1H, d, *J* = 2.5)	C-5, C-8, C-7, C-4a
7	166.6	C	---	---
8	94.83	CH	5.96 (1H, d, *J* = 2.5)	C-6, C-7, C-4a, C-8a
4a	102.8	C	---	---
8a	164.4	C	---	---
1′	131.0	C	---	---
2′	113.9	CH	7.03 (1H, br. s)	C-1, C-3′, C-4′, C-6′
3′	145.9	C	---	---
4′	146.2	C	---	---
5′	115.4	CH	6.86 (1H, d, *J* = 8.4)	C-1′, C-3′, C-4′, C-6′
6′	118.4	CH	6.87 (1H, dd, *J* = 8.4, 1.9)	C-1, C-2′, C-3′, C-5′
5-OH	---	H	12.1 (1H, s)	C-5, C-4, C-6, C-4a
7-OH	---	H	8.12 (1H, s)	C-7
3′-OH	---	H	8.12 (1H, s)	C-3′
4′-OH	---	H	9.69 (1H, s)	C-4

In the ^{13}C-NMR spectrum, the ring **B** carbons were resonating at δ 195.5 (C-4), 79.44 (C-2) and 42.57 (C-3). The ring **A** carbons were resonating at δ 95.85 (C-6), 94.83 (C-8) 164.4 (C-5), 166.6 (C-7), 102.8 (C-4a) and 164.4 (C-8a). The ring **C** carbons were resonating at δ 113.9 (C-2′), 115.4 (C-5′), 118.4 (C-6′), 145.9 (C-3′), 131.0 (C-1′) and 146.2 (C-4′).

The information obtained from ^{1}H and ^{13}C-NMR (**Table 2.9**) and MS spectra partially assigned the structure of the compound **9** as dihydroluteolin[60] but it was further analyzed by applying 2D-NMR experiments.

In the HMBC spectrum (**Table 2.9**), the proton at δ_H 5.41 (H-2) showed correlation with the carbons resonating at δ_C 42.57 (C-3), 195.5 (C-4), 164.4 (C-8a) and 131.0 (C-1′). The protons at δ_H 3.14-2.74 (H-3) showed correlations with the carbons resonating at δ 79.44 (C-2) 195.5 (C-4) and 102.8 (C-4a). The proton at δ_H 5.94 (H-6) showed correlations with the carbons resonating at δ_C 94.83 (C-8), 164.4 (C-5), 166.6 (C-7) and 102.8 (C-4a). The proton at δ_H 5.96 (d, 1H-8) showed correlations with the carbons resonating at δ_C 95.85 (C-6), 166.6 (C-7), 164.4 (C-5) and 164.4 (C-8a). The proton at δ_H 7.03 (H-2′) showed correlations with the carbons at δ_C 79.44 (C-2), 118.4 (C-6′), 145.9 (C-3′) and δ 146.2 (C-4′). The proton at δ 6.87 (H-6′) showed correlations with the carbons resonating at δ_C 113.9 (C-2′), 115.4 (C-5′), 131.0 (C-1′) and 145.9 (C-3′). The proton at δ_H 6.86 (H-5′) showed correlations with the carbons resonating at δ_C 118.4 (C-6′), 131.0 (C-1′), 145.9 (C-3′) and 146.2 (C-4. The hydroxyl proton at δ_H 12.1 (5-OH) showed correlations with the carbons at δ_C 95.85 (C-6), 195.5 (C-4), 164.4 (C-5) and 102.8 (C-4a). The hydroxyl proton at δ_H 9.69 (7-OH), 8.12 (3′-OH) and 8.12 (4′-OH) showed correlations with the carbons; C-7, C-3′ and C-4′ respectively, to which they are attached.

The HMBC correlation discussed above also elucidated the structure of the compound **9** as dihydroluteolin.[60]

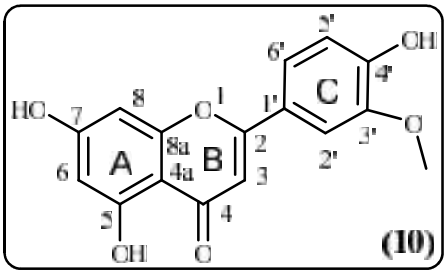
The relative stereochemistry of the compound **9** at C-3 position was assigned as (*S*) configuration by comparing the optical rotation values; $^{20}[\alpha]_D$ - 47.1 (*c* 0.49, MeOH) and NOESY correlation of the proton; H-2 of ring **B** with the ring **C** protons; H-2′, H-5′ and H-6′ which were absent in this case.

The spectral and physical data (**Table 2.9**) were in close agreement with the reported values for (*S*)-3′,4′,5,7-tetrahydroxyflavon-4-one (dihydroluteolin)[60] which is hereby reported as a new source compound from *A. elegantissima*.

Dihydroluteolin **(9)** has been isolated from a number of plants families and is one of the widespread flavone of the family Compositeae, also found in many dicotyledons families (Rutacae, Guttiferae, Labiatae, Rhamnacae, Papaveracae, Verbenacae, Rubiacae, Hydrangeacae, Myoporacae, Capparacae, Ericacae, Euphorbiacae, Rosacae and Hydrophyllacae, Leguminosae, Crassulacae, Cruciferae, Balanophoracae, Eucryphiaceae) and in monocotyledons (Graminae), ferns (Aspleniacae) and conifers).[1,58]

In the literature, compound **9** was reported by synonyms: (a) (2-(3,4-dihydroxyphenyl)-2′,3′-dihydro-5,7-dihydroxy-4-1-benzopyran-4-one), (b) (3′,4′,5,7-tetrahydroxyflavone), (c) eriodictyol, (d) huazhongilexone and (e) dihydroluteolin.[1,61]

2.6.6 Scoparol (10).



Compound **10** was isolated as yellow crystals, m.p. 330–332 °C (lit. m.p. 330–331 °C).[115] The molecular formula of $C_{16}H_{12}O_6$ was deduced from HR-ESIMS (positive ion mode) which showed the pseudo molecular ion peak at *m/z* 301.06 [M^++H].

The ^{1}H and ^{13}C-NMR spectra for the compound **10** give characteristic signals of flavone skeleton.

In the ^{1}H-NMR spectrum (**Table 2.10**), the ring **B** protons were resonating at δ 6.26 (1H, d, *J* = 1.6, H-6) and 6.52 (1H, d, *J* = 1.6 Hz, H-8). The ring **C** proton was resonating at δ 6.99 (1H, s, H-3). The ring **C** protons were resonating at δ 7.70 (1H, d, *J* = 2.04 Hz, H-2′), 7.81 (1H, dd, *J* = 8.2, 2.04 Hz, H-6′) and 7.03 (1H, d, *J* = 8.2 Hz, H-5′). The methoxy protons were resonating at δ 3.87 (3H, s, OCH_3-3′). The two hydroxyl protons were resonating at δ 8.01 (1H, s, OH-7), 8.43 (1H, s, OH-4′) and 12.18 (1H, s, OH-5).

The spectral (ESIMS and ^{1}H-NMR) (**Table 2.10**) and physical data of the compound **10** were in close agreement with the reported values for 4′,5,7-trihydroxy-3′-methoxyflavone (scoparol),[115] which is hereby reported as a new source compound from *A. elegantissima*.

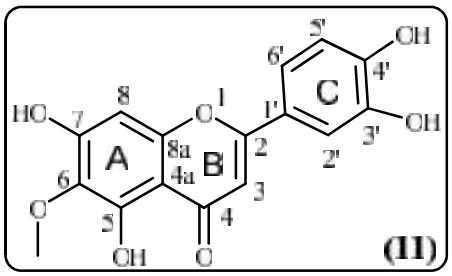
Table 2.10. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) spectra of scoparol **(10)** in $(CD_3)_2CO$.

C. No.	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
3	CH	6.99 (1H, s)
6	CH	6.26 (1H, d, *J* = 1.6)
8	CH	6.52 (1H, d, *J* = 1.6)
2′	CH	7.70 (1H, d, *J* = 2.1)
5′	CH	7.03 (1H, d, *J* = 8.2)
6′	CH	7.81 (1H, dd, *J* = 8.2, 2.1)
5-OH	H	12.17 (1H, s)
7-OH	H	8.01 (1H, s)
3′-OCH_3	H	3.87 (3H, s)
4′-OH	H	8.43 (1H, s)

Compound **10** has been isolated from various plants families including; Compositeae, Hydrophyllacae, Passifloracae, Penaeacae, Labiatae, Scrophulariacae and other.[1,58]

The different synonyms used in the literature for compound **10** were: (a) 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one, (b) chrysoeriol, (c) scoparol (d) luteolin and (e) 4′,5,7-trihydroxy-3′-methoxyflavone.[1,61]

2.6.7 Nepetin (11).



Compound **11** was isolated as yellow needles like crystals, m.p. 272–273 °C (lit. m.p. 272–274 °C).[116] The molecular formula of $C_{16}H_{12}O_7$ was deduced from HR-ESIMS which showed a pseudo molecular ion at *m/z* 316.067 peak [M^++H].

The ^{1}H and ^{13}C-NMR spectra for compound **11** give characteristic signals of a typical flavone skeleton.

In the ^{1}H-NMR spectrum **(Table 2.11)**, the proton H-3 of ring **B** was resonating at δ 6.57 (1H, s, H-3) while the proton H-8 of ring **A** was resonating at δ 6.63 (1H, s, H-8). The others aromatic protons were resonating at δ 7.67 (1H, d, *J* = 1.2 Hz, H-2′), 7.58 (1H, dd, *J* = 8.4, 1.2 Hz, H-6′) and 6.99 (1H, d, *J* = 8.4 Hz, H-5′). The methoxy protons were resonating at δ 3.87 (3H, s, OCH_3-6) while the chelated hydroxyl proton was resonating at δ 12.0 (1H, br. s, OH-5). The signals of hydroxyl protons; OH-7, OH-3′ and OH-4′

were not clear, however, their presence at the particular positions were evidenced from the characteristic ^{13}C-NMR values of the carbons resonating at δ 157.3 (C-7), 146.2 (C-3) and 150.0 (C-4) respectively.

The ^{13}C-NMR (DEPTq) spectrum **(Table 2.11)** of the compound **11** showed sixteen carbons including; one methyl, five methines and ten quaternary carbons.

In ^{13}C-NMR spectrum, the ring **B** carbons were resonating at δ 182.3 (C-1), 103.1 (C-3) and 164.4 (C-2). The ring **A** carbons were resonating at δ 95.22 (C-8), 152.8 (C-5), 131.9 (C-6), 157.3 (C-7), 150.0 (C-8a) and 104.6 (C-4a). The ring **C** carbons were resonating at δ 113.0 (C-2′), 115.8 (C-5′), 119.1 (C-6′), 146.2 (C-3′), 122.2 (C-1′) and 150.0 (C-4′). The methoxy carbon was resonating at δ 59.23 (OCH_3-6).

From the information of MS, ^{1}H and ^{13}C-NMR spectra along with physical data, the partially assigned structure of the compound **11** was elucidated as nepetin.[116] Additionally it was subjected to HMBC analysis.

In the HMBC spectrum **(Table 2.11)**, the methoxy protons at δ_H 3.87 (OCH_3-6) showed correlation with the carbons resonating at δ_C 131.9 (C-6) and the hydroxyl proton at δ_H 12.0 (OH-5) showed correlation with the carbon resonating at δ_C 152.8 (C-5) which gave a clue of its point of attachment at ring **A**. The proton at δ_H 6.58 (H-3) showed correlation with the carbons resonating at δ_C 182.3 (C-4), 164.4 (C-2), 122.2 (C-1′) and 104.6 (C-4a). The proton at δ_H 6.63 (H-8) showed correlations with the carbons resonating at δ_C 131.9 (C-6), 157.3 (C-7) and 104.6 (C-4a). The proton at δ_H 7.67 (H-2′) showed correlations with the carbons resonating at δ_C 119.1 (C-6′), 146.2 (C-3′), 150.0 (C-4′), 122.2 (C-1′) and 164.4 (C-2).

Table 2.11. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of nepetin **(11)** in $(CD_3)_2CO$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	164.4	C	---	---
3	103.1	CH	6.58 (1H, s)	C-2, C-4, C-4a, C-1′
4	182.3	C	---	---
5	152.8	C	---	---
6	131.9	C	---	---
7	157.3	C	---	---
8	95.22	CH	6.63 (1H, s)	C-6, C-7, C-4a
4a	104.6	C	---	---
8a	150.0	C	---	---
1′	122.2	C	---	---
2′	113.0	CH	7.67 (1H, d, J = 2.0)	C-2, C-1′, C-3′, C-4′, C-6′
3′	146.2	C	---	---
4′	150.0	C	---	---
5′	115.8	CH	6.99 (1H, d, J = 8.4)	C-1′, C-3′ C-4′, C-6′
6′	119.1	CH	7.58 (1H, dd, J = 8.4, 2.0)	C-2, C-2′, C-4′, C-5′
6-OCH_3	59.23	CH_3	3.87 (3H, s)	C-6
5-OH	---	H	12.0 (1H, br. s)	C-5

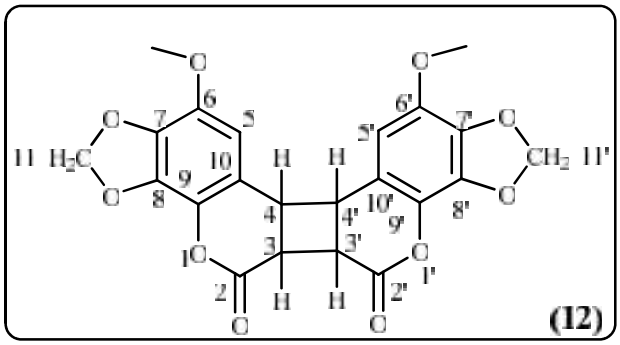
The proton at δ_H 7.58 (H-6′) showed correlations with the carbons resonating at δ_C 113.0 (C-2′), 115.8 (C-5′), 164.4 (C-2), 122.2 (C-1′) and 150.0 (C-4′). The proton at δ_H 6.99 (1H-5′) showed correlations with the carbons resonating at δ 119.1 (C-6′), 146.2 (C-3′), 150.0 (C-4′) and 122.2 (C-1′).

The above HMBC correlation confirmed the validity of 1D-NMR data and elucidates the compound **11** as nepetin,[116] which is hereby reported as a new source compound from *A. elegantissima*.

Compound **11** has been isolated from various plants species including; *Artemisia tridentata*,[51] *Eupatorium cuneifolium, Lippia nudiflora, Digitalis* species. and many other plants.[1,58]

In the literature, this compound was reported by synonyms: (a) nepetin, (b) eupafolin**,** (c) 6-methoxyluteolin (d) and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-methoxy-4*H*-1-benzopyran-4-one.[1,61]

2.6.8 *bis*-Dracunculin (12).



Compound **12** was isolated as chloroform soluble colorless needles, m.p. 138–139 °C (lit. m.p. 139 °C).[49]

The IR spectrum showed characteristic absorption at 1769 cm^{-1} attributed to the presence of benzo-*α*-lactone (coumarin) nucleus. The molecular formula of $C_{22}H_{16}O_{10}$ was obtained from the LR-EIMS which gave fragment ion peak at *m/z* 220.037 [½ M^+]; equivalent to (½) of the molecular ion peak.

The ^1H and ^{13}C-NMR spectra **(Table 2.12)** for the compound **12** suggested the structure to be a typical coumarin skeleton. The full structure assignment of the compound **12** was established by 2D-NMR experiments (HSQC, HMBC, COSY and NOESY)

In the ^1H-NMR spectrum of the compound **12**, the pair of equivalent aromatic protons were resonating at δ 5.93 (2H, s, H-5/H-5′). The two pair of equivalent aliphatic protons were resonating at δ 3.97 (2H, dd, *J* = 5.0, 4.5 Hz, H-3/H-3′) and 4.06 (2H, dd, *J* = 5.0, 4.5 Hz, H-4/H-4′). The nonequivalent dioxomethylene protons were resonating at δ 6.0 (2H s, OCH_2O-11) and 6.1 (2H, s, OCH_2O-11′). The two equivalent methoxy protons were resonating at δ 3.67 (6H, s, OCH_3-6/OCH_3-6′).

The ^{13}C-NMR spectrum of the compound **12** showed 12 carbons which were differentiated by DEPT-135 as one methyl, two methylenes, three methines and six quaternary carbons.

In the ^{13}C-NMR spectrum, the downfield resonance at δ 162.8 was assigned to the two equivalent carbonyls (C-2/C-2′). The resonances at δ 135.8, 140.5, 136.9, 111.2, 106.5 and 131.5 were assigned to the equivalent carbons C-6/C-6′, C-7/C-7′, C-8/C-8′, C-9/C-9′, C-5/C-5′ and C-10/C-10′ respectively. The resonances at δ 102.9 and 103.6 were assigned to the nonequivalent dioxomethylenes carbons (OCH_2O-11) and OCH_2O-11′) respectively. The resonances at δ 40.6 and 39.6 were assigned to the two pair of dimeric methines; C-3/C-3′ and C-4/C-4′ respectively. The resonance at δ 56.7 was assigned to the two equivalent methoxy carbons (CH_3O-6/CH_3O-6′).

From the information obtained from spectral data (IR, MS, ^{1}H and ^{13}C-NMR) and physicochemical constants, the structure of the compound **12** was established as a dimer of dracunculin known as *bis*-dracunculin or *bis*-4-methoxy-*8H*-[1,3]dioxolo[4,5-h]chromen-8-one.[49]

Table 2.12. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of *bis*-dracunculin **(12)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2/2′	162.8	2C	---	---
3/3′	40.6	2CH	3.97 (2H, dd, *J* = 5.0, 4.5)	C-1/C-1′, C-4/C-4′, C-10/C-10′
4/4′	39.6	2CH	4.06 (2H, dd, *J* = 5.0, 4.5)	C-1/C-1′, C-3/C-3′, C-9/C-9′
5/5′	106.5	2CH	5.93 (2H, s)	C-6/C-6′, C-7/C-7′, C-9/C-9′
6/6′	135.8	2C	---	---
7/7′	140.5	2C	---	---
8/8′	136.9	2C	---	---
9/9′	111.2	2C	---	---
10/10	131.5	2C	---	---
11	102.9	CH_2	6.00 (2H, s)	C-7, C-8
11′	103.6	CH_2	6.01 (2H, s)	C-7′, C-8′
6-OCH_3 6′-OCH_3	56.7	2CH_3	3.67 (6H, s)	C-6/C-6′

Furthermore, with the help of 2D-NMR experiments, the 1D-NMR assigned data was analyzed to finalize the structure of the compound **12**.

In the HMBC spectrum **(Table 2.12)**, the dimeric protons at δ_H 3.97 (H-3/H-3′) showed correlations with the carbons resonating at δ_C 162.8 (C-2/C-2′), 131.5 (C-10/C-10′), and 39.6 (C-4/C-4′). Similarly, the dimeric protons at δ_H 4.06 (H-4/H-4′) showed correlations with the carbons resonating at δ_C 162.8 (C-2/C-2′), 111.2 (C-9/C-9′) and 40.6 (C-3/C-3′). The aromatic proton at δ_H 5.93 (H-8/H-8′) showed correlations with the carbons resonating at δ_C 135.8 (C-6/C-6′), 111.2 (C9/C-9′) and 140.5 (C-7/C-7′). The correlations of methine protons with carbonyl, oxygenated and nonoxygenated aromatic carbons elucidated the benzo-*α*-lactone dimeric structure which was also supported by the HMBC correlations of nonequivalent dioxomethylene protons at δ_H 6.0 (H-11) and 6.01 (H-11′) with the carbons resonating at δ_C 140.5 (C-7/C-7′) and 136.9 (C-8/C-8′). The correlations of methoxy protons at δ_H 3.67 (CH_3O-6/CH_3O-6′) with the carbons resonating at δ_C 135.8 (C-6/C-6′), confirmed the orientation of the methoxy group adjacent to the dioxomethylene group on the benzene ring.

To demonstrate the compound **12** as benzo-*α*-lactone dimer, three spectral results were supporting this fact: (a) the molecular ion peak appeared at one-half of the molecular weight (*m/z* 220). (b) there were 12 instead of 11 carbons resonances in the ^{13}C-NMR (BB) spectrum, because of nonequivalent nature of the two dioxomethylene carbons and protons present on the two sides of the molecule. (c) the NOESY correlations between the aromatic proton at δ_H 5.93, the methoxy group at δ_H 3.67 and one of the methine proton at δ 4.1–4.6 (H-4/H-4′), confirmed one-half of a 6-methoxy-7,8-methylenedioxy benzo-α-lactone structure, while the presence of four dimeric methine protons instead of

two vinylic protons and their NOESY correlation with each other suggested the dimeric cyclobutane substation of 6-methoxy-7,8-methylenedioxy benzo-α-lactone. In addition, it was suggested that this dimer was a head-to-head [2+2] cycloadduct because the coupling constant of the spin interaction of the four dimeric protons (J = 5.0, 4.5 Hz) allow the (*cis/syn*) orientation along the four sides of the substituted cyclobutane ring.

Hence, in view of the information obtained from 1D and 2D-NMR spectra and physical data, the structure of the compound **12** was established as the [2+2] cycloadduct of 6-methoxy-7,8 methylenedioxy benzo-*α*-lactone which agreed with the literature.

Bis-dracunculin **(12)** has been reported from *Artemisia capillaris*,[1,49] however, hereby it is reported as a new source compound from *A. elegantissima*.

In the literature, compound **12** was reported by synonyms: (a) *bis*-dracunculin, (b) artemicapin D, (c) *bis*-6-methoxy-7,8-methylenedioxy benzo-*α*-lactone, (d) *bis*-4-methoxy-*8H*-[1,3]dioxolo[4,5-h]chromen-8-one.[1,117]

It is worth mentioning that in the literature, the dioxomethylene located on the two sides of the *bis*-dracunculin molecule has been assigned the same ^{1}H and ^{13}C-NMR values **(Table 2.12)** but we have experienced different values for these groups. Also, the dimeric methine protons were assigned as a multiplet but we have recorded the ^{1}H-NMR with doublet of doublet (J = 5.0, 4.5 Hz) for the methine pair of each monomer of the substituted cyclobutane nucleus due to difference in their dihedral angle. The molecule of *bis*-dracunculin was found to be very sensitive to light and cleaved to dracunculin by exposing to light.

The key NOESY correlations of *bis*-dracunculin **(12)** is given in **Figure 2.5.**

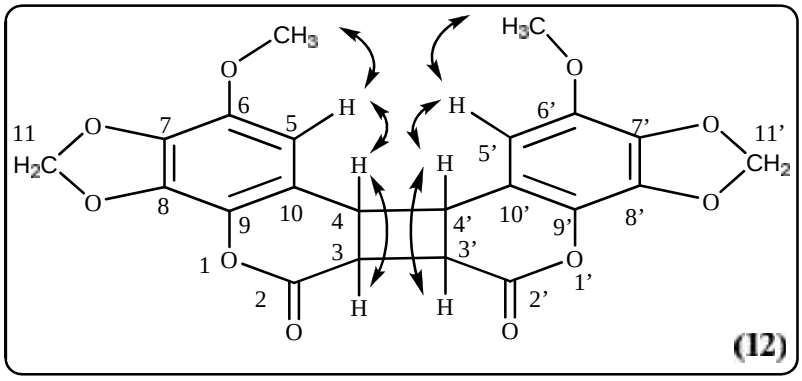
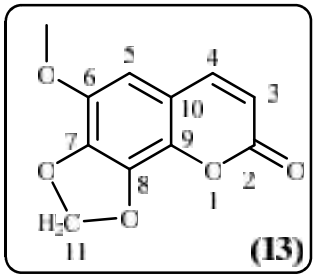


Figure 2.5. Key NOESY correlations of *bis*-dracunculin **(12**).

2.6.9 Dracunculin (13).



Compound **13** was obtained as colorless needles, m.p. 220–222 °C (lit. m.p. 219–221 °C).[49] In the IR spectrum, the absorption at 1769 cm^{-1} showed the presence of coumarin skeleton. The molecular formula of $C_{11}H_8O_5$ was obtained from the LR-EIMS which showed molecular ion peak at *m/z* 220.037 [M^+].

The ^{1}H-NMR and ^{13}C-NMR spectra of the compound **13** proposed the structure to be a coumarin skeleton.

In the ^{1}H-NMR spectrum of the compound **13**, the aromatic proton was resonating at δ 6.76 (1H, br. s, H-5). The olefinic protons were resonating at δ 6.34 (1H, d, *J* = 9.5 Hz,

H-3) and 7.67 (1H, d, *J* = 9.5 Hz, H-4). The dioxomethylene protons were resonating at δ 6.18 (2H, s, OCH_2O-11) and the methoxy protons were resonating at δ 3.77 (3H, s, OCH_3-6).

The ^{13}C-NMR spectrum of the compound **13 (Table 2.13)** showed eleven carbon atoms which were differentiated by DEPT-135 as one methyl, one methylene, three methines and six quaternary carbons. However, the full structure assignment of the compound **13** was made with the help of 2D-NMR (HSQC, HMBC, COSY and NOESY experiments) (**Table 2.13**).

In the ^{13}C-NMR spectrum, the carbonyl was resonating at δ 159.5 (C-2). The aromatic carbons were resonating at δ 141.1 (C-6), 140.0 (C-7), 133.9 (C-9), 134.6 (C-8), 106.0 (C-5) and 114.6 (C-10). The olefinic carbons were resonating at δ 113.9 (C-3) and 143.9 (C-4). The dioxomethylene and methoxy carbons were resonating at δ 103.7 (OCH_2O-11) and 56.8 (OCH_3-6).

From the information obtained from ^{1}H and ^{13}C-NMR **(Table 2.10)**, MS and IR spectra along with physical data, the structure of the compound **13** was assigned as dracunculin. Additionally, the 2D-NMR (HMBC and NOESY) correlation were applied, to analyze the data and finalize the proposed structure for the compound **13.**

Table 2.13. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of dracunculin **(13)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	159.5	C	---	---
3	113.9	CH	6.34 (1H, d, J = 9.5)	C-2, C-4, C-9
4	143.9	CH	7.67 (1H, d, J = 9.5)	C-2, C-3, C-9, C-10
5	106.0	CH	6.76 (1H, br. s)	C-6, C-7, C-9
6	141.1	C	---	---
7	140.0	C	---	---
8	134.6	C	---	---
9	133.9	C	---	---
10	114.6	C	---	---
11	103.7	CH_2	6.18 (2H, s)	C-7, C-8
6-OCH_3	56.8	CH_3	3.77 (3H, s)	C-6

In the HMBC spectrum **(Table 2.13)**, the olefinic proton at δ_H 6.34 (H-2) showed correlations with the carbons resonating at δ_C 159.5 (C-2), 143.9 (C-4) and 133.9 (C-9). Likewise, the olefinic proton at δ_H 7.67 (H-3) showed correlations with the carbons resonating at δ_C 159.5 (C-2), 113.9 (C-3) and 133.9 (C-9). These two correlations confirmed the benzo-α-lactone structure. The aromatic proton at δ_H 6.76 (br. s) showed correlations with the carbons resonating at δ_C 141.1 (C-6), 133.9 (C-9), and 140.0 (C-7), which confirmed the adjacent position of the methoxy and methylenedioxy groups. This is further supported by the correlation of dioxomethylene protons at δ_H 6.18 (2H) with the carbons resonating at δ_C 140.0 (C-7), 134.6 (C-8) and the correlations of methoxy protons at δ_H 3.77 (3H) with the carbon resonating at δ_C 141.1 (C-6).

To confirm the compound **13** as dracunculin, the NOESY correlations between the aromatic proton H-5, the methoxy protons OCH_3-6 and olefinic protons H-3 and H-4 confirmed a 6-methoxy-7,8-methylenedioxy benzo-*α*-lactone structure (dracunculin).[118]

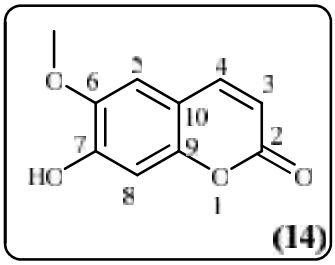
Hence, from the observation of physical data and the interpreted 1D and 2D-NMR spectra, the structure of the compound **13** was established to be 6-methoxy-7,8-methylenedioxy benzo-α-lactone (dracunculin), which was in full agreement with the literature.[49]

In the literature, the compound **13** was reported by synonyms: (a) dracunculin, (b) 4-methoxy-8*H*-1,3-dioxolo[4,5-*h*][1]benzopyran-8-one and (c) (6-methoxy-7,8-methylenedioxycoumarin).[1,49,117]

The compound **13** has been reported from a number of plant sources including; *Artemisia dracunculoides* (Russian tarragon), *Artemisia capillaris*, *Artemisia carruthii* and *Melochia tomentosa* roots.[1,49,117]

Hereby, it is reported as a new source compound from *A. elegantissima*.

2.6.10 Scopoletin (14).



(14)

Compound **14** (position isomer of isoscopoletin **(30)**) was obtained as greenish yellow crystals, m.p. 204–205 °C (lit. m.p. 205 °C).[119] The molecular formula of $C_{10}H_8O_4$ was determined from LR-EIMS which showed the molecular ion peak at *m/z* 192.042 [M^+].

The ^{1}H and ^{13}C-NMR spectra **(Table 2.14)**, suggested the structure of the compound **14** to be a typical coumarin skeleton.

In the ^{1}H-NMR spectrum **(Table 2.14)**, the methoxy and hydroxy protons were resonating at δ 3.77 (3H, br. s, OCH_3-6), δ 5.06 (1H, br. s, OH-7). The olefinic protons were resonating at δ 7.60 (1H, d, *J* = 9.5 Hz, H-4) and 6.27 (1H, d, *J* = 9.5 Hz, H-3). The aromatic protons were resonating at δ 6.94 (1H, br. s, H-5) and 6.83 (1H, s, H-8).

In the ^{13}C-NMR spectrum, there were ten carbon atoms which according to DEPT-135, were five quaternary, four methines and one methyl group.

In the ^{13}C-NMR spectrum, the carbonyl was resonating at δ 160.9 (C-2). The aromatic carbons were resonating at δ 149.8 (C-9), 153.1 (C-7), 145.3 (C-6), 102.4 (C-8), 106.5 (C-5) and 111.0 (C-10). The olefinic carbons were resonating at δ 112.8 (C-3) and 143.7 (C-4). The methoxy carbon was resonating at δ 56.3 (CH_3O-6).

From the spectral data (IR, MS, ^{1}H and ^{13}C-NMR) and physicochemical constants, the structure of the compound **14** was elucidated as scopoletin. The assigned structure was further analysed with the help of 2D-NMR experiments.

In the HMBC spectrum, the aromatic proton at δ_H 6.83, (H-5) showed correlations with the carbons resonating at δ_C 145.3 (C-6), 149.8 (C-9) and 153.1 (C-7). Similarly, the aromatic proton at δ_H 6.94 (H-8) showed correlations with the carbons resonating at δ 111.0 (C-10), 145.3 (C-6) and 153.1 (C-7). The olefinic proton at δ_H 6.27 (H-3) showed correlations with the carbons resonating at δ_C 143.7 (C-4), 111.0 (C-10) and 160.9 (C-2). Likewise, the olefinic proton at δ_H 7.60 (H-4) showed correlations with the carbons resonating at δ_C 112.8 (C-3), 149.8 (C-9) and 160.9 (C-2). These HMBC correlations verified the substitution of methoxy and hydroxy groups at C-6 and C-7 positions respectively.

The NOESY correlations between the aromatic protons H-8, the methoxy protons (OCH_3-6) and olefinic protons (H-3 and H-4) confirmed a 6-methoxy-7-hydroxy benzo-*α*-lactone structure which was also supported by the reported melting point.[119]

Table 2.14. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of scopoletin (**14**) in $CDCl_3$.

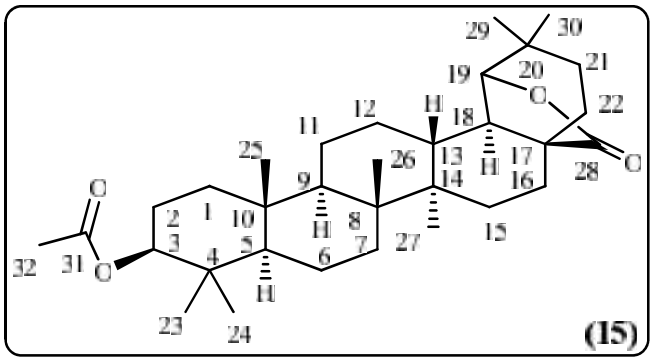
C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	160.9	C	--- ---	
3	112.8	CH	6.27 (1H, d, J = 9.5)	C-2, C-4, C-10
4	143.7	CH	7.60 (1H, d, J = 9.5)	C-2, C-3, C-9
5	106.5	CH	6.94 (1H, br. s)	C-6, C-7, C-9
6	145.3	C	---	---
7	153.1	C	---	---
8	102.4	CH	6.83 (1H, s)	C-6, C-7, C-10
9	149.8	C	---	---
10	111.0	C	---	---
6-OCH_3	56.3	CH_3	3.94 (3H, br. s)	C-6
7-OH	---	H	6.18 (1H, br. s)	C-7

To establish the compound **14** as 7-hydroxy-6-methoxycoumarin (scopoletin), the values of the two physicochemical constants were clarifying the fact (a) this compound **14** was less polar than (isoscopoletin) (**30).** (b) this compound; scopoletin (**14**) was soluble in acetone and ethyl acetate with m.p. 204–205 °C (lit. m.p. 205 °C)[120] as compared to 185 °C in case of (isoscopoletin) (**30**).[54] Thus, from the physical data and the spectra, the structure of the compound **14** was established as 7-hydroxy-6-methoxycoumarin (scopoletin) which is hereby reported as a new source compound from *A. elegantissima*.

In the literature, scopoletin **(14)** was reported by synonyms: (a) 7-hydroxy-6-methoxycoumarin, (b) scopoletin, (c) chrysatropic acid, (d) gelseminic acid, (e) esculetin 6-methyl ether, (f) 7-methoxy esculetin, (g) escopoletin, (h) scopoletol, (i) buxuletin, (j) baogongteng B and (k) murrayetin.[1,117]

Scopoletin **(14)** has been reported from various plants species including; the roots of *Gelsemium sempervirens, Convolvulus scammonia, Atropa belladonna, Ipomoea orizabensis, Fabiana imbricata, Prunus serotina* and also from the genus *Diospyros, Skimmia, Peucedanum, Heracleum* and *Artemisia*. Scopoletin is also an important constituent of the Chinese crude drug Toki (from *Angelica acutiloba*).[1,22]

2.6.11 Allograciolone acetate (15).



Compound **15** was obtained as white crystals, m.p. 307-311 °C (lit. m.p. 310 °C).[121] The molecular formula of $C_{32}H_{50}O_4$ was established from the HR-ESIMS (positive ion mode) which showed the quasimolecular ion peak at *m/z* 498.37 [M^++H].

The distinctive oxyallobetulin-type skeleton of triterpene for the compound **15** was discovered through ^{1}H, ^{13}C-NMR and HMBC spectra **(Table 2.15).**

In the ^{1}H-NMR spectrum, the methyl protons were resonating at δ 0.86 (3H, s, H-26), 1.02 (3H, s, H-27), 0.82 (3H, s, H-23), 0.83 (3H, s, H-24), 0.94 (3H, s, H-25), 2.03 (3H, s, H-32), 0.90 (3H, s, H-29) and 0.86 (3H, s, H-30). The methylenes protons were resonating at δ 1.93 (diastereotopic) (1H, dd, *J* = 14.8, 3.4 Hz, Ha-1); 1.64 (1H, m, Hb-1), 1.31 (2H, m, H-12), 1.63 (diastereotopic) (1H, m, Ha-15); 1.67 (1H, m, Hb-15), 1.02 (diastereotopic) (1H, m, Ha-2); 1.62 (1H, m, Hb-2), 1.36 (diastereotopic) (1H, m, Ha-6); 1.58 (1H, m, Hb-6), 0.99 (diastereotopic) (1H, m, Ha-7); 1.05 (1H, m, Hb-7), 1.44 (2H, m, H-11), 1.61 (diastereotopic) (1H, m, Ha-16); 1.65 (1H, m, Hb-16), 1.59 (2H, m, H-21); 1.33 (diastereotopic) (1H, m, Ha-22); 1.78 (1H, m, Hb-22).

Table 2.15. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of allograciolone acetate **(15)** in $CDCl_3$.

C.No	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.26	CH_2	1.93 (1H, dd, *J* = 14.8, 3.4, Ha); 1.64 (1H, m, Hb)	C-2, C-3, C-5, C-9
2	24.03	CH_2	1.62 (1H, m); 1.02 (1H, m)	---
3	80.92	CH	4.45 (1H, dd, *J* = 10.8, 6.4)	C-23, C-24, C-4, C-2
4	40.01	C	---	---
5	55.69	CH	0.88 (1H, m)	---
6	18.04	CH_2	1.36 (1H, m, Ha); 1.58 (1H, m, Hb)	---
7	35.14	CH_2	0.99 (1H, m, Ha); 1.05 (1H, m, Hb)	---
8	38.71	C	---	---
9	48.80	CH	0.94 (1H, m)	---
10	36.09	C	---	---
11	20.98	CH_2	1.44 (2H, m)	---
12	25.01	CH_2	1.31 (2H, m)	---
13	46.80	CH	1.53 (1H, m)	---
14	40.66	C	---	---
15	27.91	CH_2	1.63 (1H, m, Ha); 1.67 (1H, m, Hb)	---
16	32.02	CH_2	1.61 (1H, m, Ha); 1.65 (1H, m, Hb)	---
17	46.18	C	--- ---	

Continued on next page

C.No	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
18	28.83	CH	3.52 (1H, m)	---
19	86.09	CH	3.92 (1H, s)	---
20	37.88	C	---	---
21	33.75	CH_2	1.59 (2H, m)	---
22	32.40	CH_2	1.33 (2H, m)	---
23	23.73	CH_3	0.82 (3H, s)	C-3, C-4, C-5, C-24
24	13.71	CH_3	0.83 (3H, s)	C-3, C-4, C-5, C-23
25	15.60	CH_3	0.94 (3H, s)	C-1, C-2, C-5
26	26.58	CH_3	0.86 (3H, s)	C-14, C-9, C-7
27	25.62	CH_3	1.02 (3H, s)	C-8, C-14, C-15, C-16
28	179.95	C	---	---
29	33.63	CH_3	0.90 (3H, s)	C-20, C-21, C-22
30	16.68	CH_3	0.86 (3H, s)	C-20, C-21, C-22, C-18
31	171.14	C	---	---
32	21.39	CH_3	2.03 (3H, s)	C-31

The methine protons were resonating at δ 4.45 (1H, dd, *J* = 10.8, 6.4 Hz, H-3), 3.52 (1H, br. s, H-18), 2.04 (1H, s, H-19) (due to magic angle, COSE 90 # 0), 088 (1H, m, H-5), 0.94 (1H, m, H-9), 1.53 (1H, m, H-13).

The ^{13}C-NMR (BB) spectrum of the compound **15** showed thirty three carbons atoms, differentiated into eight quaternary, six methine, ten methylene and eight methyl carbons.

In the ^{13}C-NMR spectrum, the quaternary carbons were resonating at δ 40.01 (C-4), 38.71 (C-8), 36.09 (C-10), 40.66 (C-14), 46.18 (C-17), 37.88 (C-20), 171.14 (C-31), 179.95 (C-28). The methylene carbons were resonating at δ 35.14 (C-7), 20.98 (C-11), 25.01 (C-12), 27.91 (C-15), 32.02 (C-16), 37.26 (C-1), 24.03 (C-2), 18.04 (C-6), 18.6 (C-21), 33.75 (C-21) and 32.40 (C-22). The methine carbons were resonating at δ 46.80 (C-13), 28.83 (C-18), 55.69 (C-5), 80.92 (C-3), 86.09 (C-19) and 48.80 (C-9).

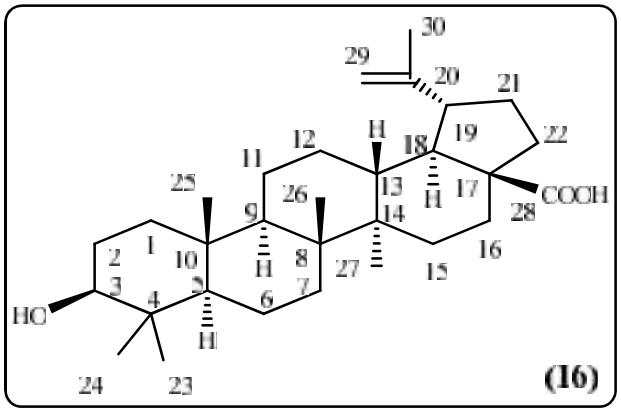
The compound **15** was also analysed with HMBC correlation which were the same as reported.[121]

The ^{1}H, ^{13}C-NMR, HMBC and MS spectra along with physical data of melting point and specific rotation of the compound **15** were compared to the reported values for oxyallobetulin acetate (allograciolone acetate).[1,121]

The compound **15** (allograciolone acetate) has been reported from some plants; *Diospyros montana*, *Diospyros lotus* and *Lemaireocereus stellatus.*[1,122]

It is hereby reported for the first time from the genus *Artemisia* as a new source compound.

2.6.12 Betulinic acid (16).



Compound **16** was obtained as white crystals, m.p. 275–276 °C (lit. m.p. 275–278 °C).[123] The molecular formula of $C_{30}H_{48}O_3$ was established from HR-ESIMS which showed the quasimolecular ion peak at *m/z* 456.36 [M^++H].

The distinctive triterpene type skeleton (ursane) of the compound **16** was established through ^{1}H and ^{13}C-NMR spectra **(Table 2.16)** comparison with the literature data for betulinic acid.[123]

In the ^{1}H-NMR spectrum, the methyl protons were resonating at δ 0.95 (3H, s, H-23), 1.01 (3H, s, H-24), 0.75 (3H, s, H-25), 0.98 (3H, s, H-26), 0.99 (3H, s, H-27) and 1.78 (3H, s, H-30). The methylene proton were resonating at δ 1.29 (diastereotopic) (2H, m, Ha-1), 1.46 (diastereotopic) (2H, m, Hb-1), 1.70 (diastereotopic) (1H, m, Ha-2); 1.80 (1H, m, Hb-2), 1.36 (diastereotopic) (1H, m, Ha-6); 1.52 (1H, m, Hb-6), 1.48 (diastereotopic) (1H, m, Ha-7); 1.79 (1H, m, Hb-7), 1.42 (2H, m, H-11), 1.31 (2H, m, H-12), 1.04 (diastereotopic) (1H, m, Ha-15); 1.06 (1H, m, Hb-15), 1.32 (diastereotopic) (1H, m, Ha-16); 2.05 (1H, d, *J* = 13.08 Hz, Hb-16), 1.36 (diastereotopic) (1H, m, Ha-21) and 1.73 (1H, m, Hb-21) and 1.41 (diastereotopic) (1H, m, Ha-22); 1.78 (1H, m, Hb-22), δ

4.72 (diastereotopic) (1H, s, Ha-29); 4.83 (1H, br. s, Hb-29). The methine proton were resonating at δ 3.94 (1H, m, H-3), 1.29 (1H, m, H-5), 1.76 (1H, m, H-9), 2.21 (1H, m, H-13), 1.07 (1H, m, H-18) and 2.60 (1H, dt, *J* = 10.4, 5.5 Hz, H-19).

The ^{13}C-NMR spectrum of the compound **16** showed thirty carbons, differentiated by DEPT-135 as seven quaternary, six methines, eleven methylenes and six methyl carbons.

The quaternary carbons were resonating at δ 150.33 (C-20), 180.53 (C-28), 38.82 (C-4), 40.75 (C-8), 37.23 (C-10), 42.48 (C-14) and 56.37 (C-17). The methylene carbons were resonating at δ 109.61 (C-29), 18.31 (C-6), 38.73 (C-1), 27.41 (C-2), 34.35 (C-7), 20.82 (C-11), 25.52 (C-12), 30.54 (C-15), 32.12 (C-16), 29.70 (C-21) and 37.06 (C-22). The signals at δ 78.90 (C-3), 55.34 (C-5), 50.59 (C-9), 38.49 (C-13), 46.86 (C-18) and 49.25 (C-19). The methyl carbons were resonating at δ 27.95 (C-23), 16.02 (C-25), 16.11 (C-26), 15.31 (C-24), 14.74 (C-27) and 19.42 (C-30).

From the physical and spectral (^{1}H, ^{13}C-NMR and ESIMS) data, the structure of the compound **16** was established as betulinic acid.[123]

Betulinic acid **(16)** has been isolated from *Betula Cornus*, *Zizyphus vulgaris*, *Arbutus menziesii* species[1,85,122] and it is hereby reported as new source compound from *A. elegantissima*.

Table 2.16. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of betulinic acid **(16)** in $CDCl_3$.

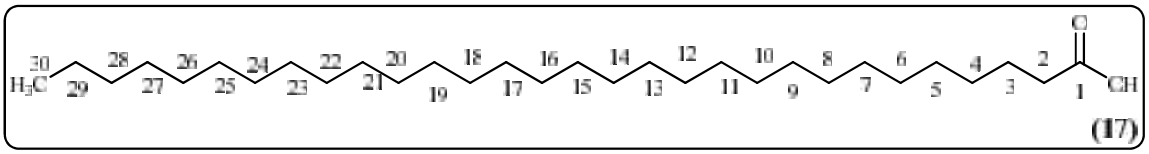
C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
1	38.73	CH_2	1.29 (1H, m, Ha); 1.46 (1H, m, Hb)
2	27.41	CH_2	1.70 (1H, m, Ha); 1.80 (1H, m, Hb)
3	78.90	CH	3.94 (1H, m)
4	38.82	C	---
5	55.34	CH	1.29 (1H, m)
6	18.31	CH_2	1.36 (1H, m, Ha); 1.52 (1H, m, Hb)
7	34.35	CH_2	1.48 (1H, m, Ha); 1.79 (1H, m, Hb)
8	40.75	C	---
9	50.5	CH	1.76 (1H, m)
10	37.23	C	---
11	20.82	CH_2	1.42 (2H, m)
12	25.52	CH_2	1.31 (2H, m)
13	38.49	CH	2.21 (1H, m)
14	42.48	C	---
15	30.54	CH_2	1.04 (1H, m, Ha); 1.06 (1H, m, Hb)
16	32.12	CH_2	1.32 (1H, m, Ha); 2.05 (1H, d, J =13.08, Hb)
17	56.37	C	---
18	46.86	CH	1.07 (1H, m)
19	49.25	CH	2.60 (1H, dt, J = 10.4, 5.5)
20	150.33	C	---
21	29.70	CH_2	1.36 (1H, m, Ha); 1.73 (1H, m, Hb)
22	37.06	CH_2	1.41 (1H, m, Ha); 1.78 (1H, m, Hb)
23	27.95	CH_3	0.95 (3H, s)
24	15.31	CH_3	1.01 (3H, s)

Continued on next page

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
25	16.02	CH_3	0.75 (3H, s)
26	16.11	CH_3	0.98 (3H, s)
27	14.74	CH_3	0.99 (3H, s)
28	180.53	C	---
29	109.61	CH_2	4.72 (1H, s, Ha); 4.83 (1H, br. s, Hb)
30	19.42	CH_3	1.78 (3H, s)

The different synonyms used for compound **16** in the literature are (a) betulinic acid, (b) melaleucin, (c) mairin, (d) gratiolone, (e) platanol, (f) platanolic acid and (g) betulic acid.[1,122]

2.6.13 Triacontanoic acid (17).



(17)

Compound **17** was obtained as a white powder, m.p. 92–93 °C (lit. m.p. 92–93 °C).[31] The molecular formula of $C_{30}H_{60}O_2$ was obtained from LR-EIMS which showed molecular ion peak at m/z 452.45 [M^+]. The IR spectrum showed absorption at $\bar{\upsilon}$ 1710 cm^{-1} attributed to the carboxyl group (COOH).

The ^1H and ^{13}C-NMR spectral data of the compound **17 (Table 2.17)** were compared and found to be in close agreement with the literature data for triacontanoic acid.[31]

This was further supported by the HMBC spectrum where the two methylenes appeared at δ 2.33 (2H, t, H-2) and 1.61 (2H, m, H-3) showed correlations with each other resonating at δ 24.69 (C-2), 33.80 (C-3) respectively and with carbonyl resonating at δ 178.11 (C-1).

From the information obtained from the spectral data (1D and 2D-NMR **(Table 2.17)**, IR and MS) revealed that the compound **17** was a long chain fatty acid consisting of thirty carbon atoms and known as triacontanoic acid.[31]

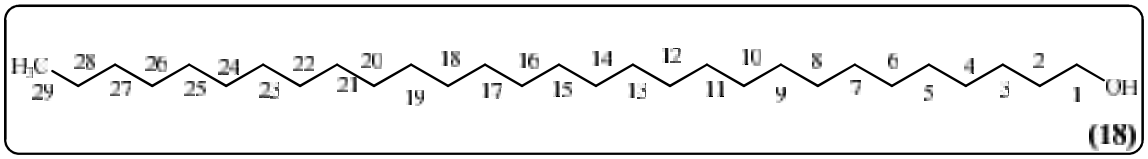
Table 2.17. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz and HMBC spectra of triacontanoic acid **(17)** in $CDCl_3$.

C. No.	^{13}CNMR (δ)	Multiplicity	^{1}HNMR (δ) (J_{HH} Hz)	HMBC Correlation
1	178.1	C	---	---
2	33.71	CH_2	2.33 (2H, t, J = 7.5)	C-1, C-3, C-4
3	24.70	CH_2	1.61 (2H, m)	---
29	22.68	CH_2	1.23 (2H, m)	---
28	31.92	CH_2	1.23 (2H, m)	---
4–27	29.06–29.70	$24CH_2$	1.23 (44H, m)	---
30	14.14	CH_3	0.86 (3H, t, J = 6.4)	C-29, C-28

Compound **17** was known by a common name of melissic acid A in the literature and have been isolated from some plant waxes, e.g. cotton, carnauba, various leaf waxes but hereby, it is reported as new source compound from *A. elegantissima*.

It was the main constituent of acid fraction of *Vitex divaricata* wood. It has also been reported from the genus *Artemisia*.[1,22,124]

2.6.14 Nonacosanol (18).



(18)

Compound **18** was obtained as a white powder with m.p. 85 °C (lit. m.p. 85 °C).[31] The molecular formula of $C_{29}H_{60}O$ was deduced from LR-EIMS which gave molecular ion peak at *m/z* 426.46 [M^+]. The IR spectrum of the compound **18** showed absorption at $\bar{\upsilon}$ 3280.33 cm^{-1} attributed to OH group.

The 1H and ^{13}C-NMR spectral data of the compound **18** were compared and found to be comparable to the literature data for nonacosanol.[31]

This was further supported by the HMBC spectrum which showed correlations of methylene protons at δ_H 3.6 (2H, t, J = 6.8 Hz, H-1), 1.54 (2H, m, H-2) and 1.56 (2H, m, H-3) with each other resonating at δ_C 64.41 (C-1), 32.81 (C-2) and 25.73 (C-3).

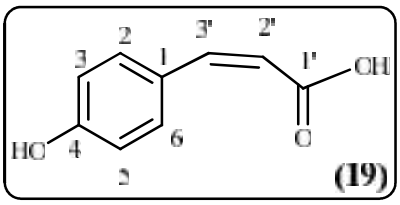
From the spectral data (1D and 2D-NM **(Table 2.18)**, IR and MS) and physicochemical constants, the structure of the compound **18** was elucidated as nonacosanol.[31]

Table 2.18. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of nonacosanol **(18)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	63.12	CH_2	3.62 (2H, t, J = 6.8)	C-1, C-3, C-4
2	32.81	CH_2	1.54 (2H, m)	---
3	25.73	CH_2	1.56 (2H, m)	---
28	22.69	CH_2	1.23 (2H, m)	---
27	31.92	CH_2	1.23 (2H, m)	---
4–26	29.36–29.70	CH_2	1.23 (46H, m)	---
29	14.13	CH_3	0.85 (3H, t, J = 6.4)	C-28, C-27

Nonacosanol **(18)** has been reported from *Agave sisalana, Citrullus colocynthis, Rhizophora apiculata* and *Artemisia*,[1,22,124] but hereby, it is reported as a new source compound from *A. elegantissima*.

2.6.15 (*Z*)-*p*-Hydroxycinnamic acid (19).



Compound **19** was isolated as white needles like crystals, m.p. 133–135 °C (lit. m.p. 134–134.5 °C).[125] The molecular formula of $C_9H_8O_3$ was deduced from LR-EIMS which showed the molecular ion peak at *m/z* 164.047 [M^+].

The 1H and ^{13}C-NMR data of the compound **19** were compared and found to be in agreement with the literature data for (*Z*)-*p*-hydroxy cinnamic acid.[125]

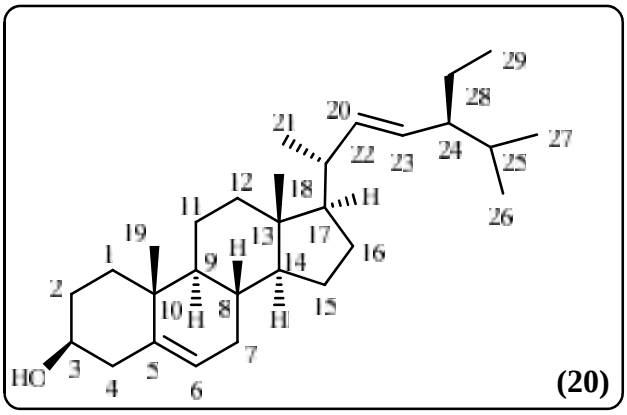
The literature showed that cinnamic acid has been isolated from the peel of black cherry (*Prunus serotina*), red clover (*Trifolium pratense*), *Daviesia latifolia*. According to Bate-Smith,[1] it has been obtained from previously investigated 55 % of monocotyledonous and 48 % dicotyledonous species.[1] However, the (*Z*)-*p*-hydroxycinnamic acid was particularly known to be isolated from *Larix sibirica* bark and *Miscanthus floridulus*,[1,22,46] but hereby, it is reported as new source compound from *A. elegantissima*.

The different synonyms used for compound **19** in the literature are; (a) (*Z*)-*p*-hydroxy cinnamic acid, (b) *p*-hydroxy phenylacrylic acid, (c) *p*-coumaric acid, (d) *p*-cumaric acid, (e) naringenic acid, (f) naringeninic acid.[1,15,46]

Table 2.19. ^{1}H-NMR (400 MHz) spectra of (*Z*)-*p*-hydroxycinnamic acid **(19)** in $CDCl_3$.

C. No.	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
2/6	2CH	6.83 (2H, d, *J* = 8.3)
3/5	2CH	7.42 (2H, d, *J* = 8.3)
2′	CH	6.30 (1H, d, *J* = 11.5)
3′	CH	7.62 (1H, d, *J* = 11.5)
4-OH	H	8.51 (1H, br. s)
1′-OH	H	11.5 (1H, br. s)

2.6.16 Stigmasterol (20).



(20)

Compound **20** was isolated as white crystals, m.p. 165–166 °C (lit. m.p. 165–167 °C).[64] The molecular formula of $C_{29}H_{48}O$ was established from LR-EIMS which showed the molecular ion peak [M^+] at *m/z* 412.37. The IR spectrum of the compound **20** showed absorption at $\bar{\upsilon}$ 3440, 1625 and 1599 cm^{-1} attributed to OH st. and C=C st. respectively.

The distinctive sterol type skeleton (cyclopentano perhydrophenathrene ring) was revealed through 2D spectra including; direct coupling of ^1H–^{13}C (one bond coupling) HSQC, the long range coupling of ^1H–^{13}C (HMBC) correlations and ^1H–^1H coupling (COSY).

All the spectral (IR, MS, 1D and 2D-NMR) **(Table 2.20)** and physical data of the compound **20** agreed with the reported data for stigmasterol.[64]

Stigmasterol has been isolated from *Artemisia* species along with several other plants,[1,21] but hereby, it is reported as new source compound from *A. elegantissima*.

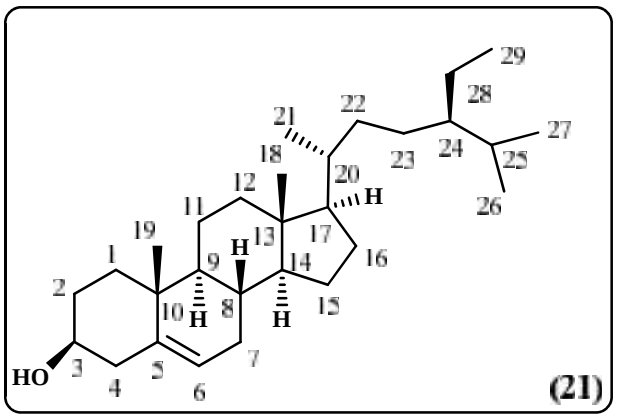
Table 2.20. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of stigmasterol **(20)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.31	CH_2	1.02 (1H, m, Ha);	C-3, C-5, C-19
			1.64 (1H, m, Hb)	
2	30.08	CH_2	1.19 (1H, m, Ha);	---
			1.84 (1H, m, Hb)	
3	78.82	CH	3.50 (1H, m)	---
4	39.79	CH_2	2.65 (1H, dd, *J* = 13.3, 2.1, Ha);	C-2, C-3, C-5
			2.39 (1H, t, *J* = 11.4, Hb)	C-6, C-10
5	140.77	C	---	---
6	121.72	CH	5.32 (1H, d, *J* = 1.74)	C-4, C-8, C-10
7	32.09	CH_2	1.64 (1H, m, Ha);	---
			1.90 (1H, m, Hb)	
8	31.88	CH	1.61 (1H, m)	---
9	50.17	CH	0.85 (1H, m)	---
10	36.77	C	---	---
11	21.12	CH_2	1.48 (1H, m, Ha);	---
			1.37 (1H, m, Hb)	
12	39.17	CH_2	1.91 (1H, dd, *J* = 12.1, 6.9, Ha)	---
			1.17 (1H, m, Hb)	
13	42.31	C	---	---
14	56.88	CH	1.04 (1H, m)	---
15	24.35	CH_2	1.48 (2H, m)	---
16	38.39	CH_2	1.21 (1H, m, Ha);	---
			1.85 (1H, m, Hb)	---

Continued on next page

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
17	56.08	CH	1.03 (1H, m)	---
18	11.81	CH_3	0.58 (3H, s)	C-12, C-13, C-14, C-17
19	19.27	CH_3	0.92 (3H, s)	C-1, C-9, C-10
20	40.48	CH	1.32 (1H, m)	---
21	19.81	CH_3	0.95 (3H, d, J = 6.5)	C-17, C-20, C-22
22	138.30	CH	5.01 (1H, dd, J = 15.5, 8.5)	C-23, C-24, C-20, C-21
23	129.31	CH	5.14 (1H, dd, J = 15.5, 8.5)	C-24, C-21, C-20, C-25, C-22
24	51.25	CH	2.13 (1H, m)	---
25	31.92	CH	1.84 (1H, m)	---
26	19.40	CH_3	0.80 (3H, d, J = 6.8)	C-24, C-25, C-26
27	19.83	CH_3	0.87 (3H, d, J = 7.0)	C-24, C-25, C-27
28	23.09	CH_2	1.43 (1H, m, Ha); 1.28 (1H, m, Hb)	C-4, C-5, C-6
29	12.23	CH_3	0.81 (3H, t, J = 7.3)	C-24, C-28

2.6.17 β-Sitosterol (21).



Compound **21** was isolated as white crystals, m.p. 135–136 °C (lit. m.p. 136 °C).[87] The molecular formula of $C_{29}H_{50}O$ was established from LR-EIMS which showed molecular ion peak at *m/z* 414.39 [M^+]. The IR spectrum of the compound **21** showed absorption at ῡ 3440 and 1625 cm^{-1} attributed to OH st. and C=C st. respectively.

The typical sterol skeleton (cyclopentano perhydrophenathrene ring) for the compound **21** was established through ^1H–^1H coupling (COSY), short range ^1H–^{13}C (HSQC) and long range ^1H–^{13}C (HMBC) correlations.

The spectral (1D and 2D-NMR **(Table 2.21)**, MS and IR) and physical data of the compound **21** were in close agreement with the reported data for β-sitosterol.[87]

β-Sitosterol has been reported from *Artemisia* species along with several other plants,[1,21] but it is reported as new source compound from *A. elegantissima.*

Table 2.21. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of β-sitosterol **(21)** in $CDCl_3$.

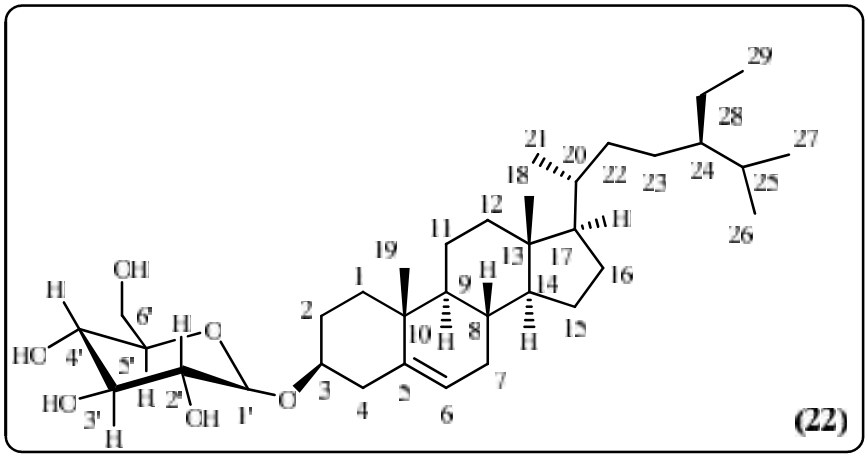
C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.02	CH_2	1.02 (1H, m, Ha); 1.64 (1H, m, Hb)	C-3, C-5, C-19
2	29.71	CH_2	1.20 (1H, m, Ha); 1.83 (1H, m, Hb)	---
3	71.82	CH	3.50 (1H, m)	---
4	39.78	CH_2	2.66 (1H, dd, *J* = 13.3, 2.1, Ha); 2.39 (1H, t, *J* = 11.5, Hb)	C-2, C-3, C-5, C-6, C-10
5	140.70	C	---	---
6	121.73	CH	5.29 (1H, d, *J* = 1.7)	C-4, C-8, C-10
7	31.91	CH_2	1.64 (1H, m, Ha); 1.89 (1H, m, Hb)	---
8	31.68	CH	1.62 (1H, m)	---
9	50.14	CH	0.84 (1H, m)	---
10	37.02	C	---	---
11	21.09	CH_2	1.47 (1H, m, Ha); 1.36 (1H, m, Hb)	---
12	39.17	CH_2	1.89 (1H, dd, *J* = 12.1, 6.9, Ha); 1.17 (1H, m, Hb)	---
13	42.32	C	---	---
14	56.87	CH	1.03 (1H, m,)	---
15	24.32	CH_2	1.47 (2H, m)	---
16	38.25	CH_2	1.20 (1H, m, Ha); 1.84 (1H, m, Hb)	---

Continued on next page

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
17	56.06	CH	1.02 (1H, m)	---
18	11.87	CH_3	0.58 (3H, s)	C-12, C-13, C-14, C-17
19	19.41	CH_3	0.99 (3H, s)	C-1, C-9, C-10
20	36.16	CH	1.32 (1H, m)	---
21	18.99	CH_3	0.91 (3H, d, J = 6.5)	C-17, C-20, C-22
22	33.95	CH_2	1.02 (1H, m, Ha); 1.28 (1H, m, Hb)	---
23	26.07	CH_2	1.17 (2H, m)	---
24	45.84	CH	0.92 (1H, m)	---
25	29.15	CH	1.66 (1H, m)	---
26	19.04	CH_3	0.78 (3H, d, J = 6.8)	C-24, C-25, C-26
27	19.83	CH_3	0.81 (3H, d, J = 7.0)	C-24, C-25, C-27
28	23.07	CH_2	1.30 (1H, m, Ha); 1.28 (1H, m, Hb)	C-4, C-5, C6
29	12.06	CH_3	0.82 (3H, t, J = 7.3)	C-24, C-28

2.7 New source compounds isolated from the roots of *Artemisia elegantissima*.

2.7.1 β-Sitosterol-3-*O*-β-D-glucopyranoside (22).



Compound **22** was obtained as a white powder, m.p. 231–234 °C (lit. m.p. 232–235 °C).[126] The exact molecular formula of the compound **22** was $C_{35}H_{50}O_5$ but it was determined from the LR-EIMS as the degraded molecular ion peak at *m/z* 414.44 [M^+] which corresponded to $C_{29}H_{30}O$. However, from the HR-ESIMS (positive ion mode) which showed the pseudo molecular ion peak at m/z 576.4386 [M^++H] corresponding to the molecular formula of $C_{35}H_{50}O_5$ for compound **22** which was confirmed and further supported by the ^{13}C-NMR (BB) spectrum showing characteristic signals for glucose moiety along with β-sitosterol nucleus.[87]

The IR spectrum of the compound **22** showed absorption for OH st. at ῡ 3450, 3452, C–O st. at ῡ 1379, 1065 and C=C st. at ῡ 1619 and 1596 cm.$^{-1}$

Table 2.22. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of β-sitosterol-3-*O*-β-D-glucopyranoside **(22)** in (C_5D_5N).

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.31	CH_2	1.02 (1H, m, Ha);	C-3, C-5, C-19
			1.64 (1H, m, Hb)	
2	30.09	CH_2	1.19 (1H, m, Ha);	---
			1.83 (1H, m, Hb)	
3	78.38	CH	3.94 (1H, m)	---
4	39.77	CH_2	2.66 (1H, dd, *J* = 13.3, 2.1, Ha);	C-2, C-3, C-5
			2.39 (1H, t, *J* = 11.4, Hb)	C-6, C-10
5	140.73	C	---	---
6	121.78	CH	5.29 (1H, d, *J* = 1.7)	C-4, C-8, C-10
7	32.09	CH_2	1.64 (1H, m, Ha);	---
			1.89 (1H, m, Hb)	
8	31.89	CH	1.62 (1H, m)	---
9	50.17	CH	0.84 (1H, m)	---
10	36.76	C	---	---
11	21.12	CH_2	1.47 (1H, m, Ha);	---
			1.36 (1H, m, Hb)	
12	39.17	CH_2	1.89 (1H, dd, *J* = 12.1, 6.9, Ha);	---
			1.17 (1H, m, Hb)	
13	42.31	C	---	---
14	56.65	CH	1.03 (1H, m)	---
15	24.35	CH_2	1.47 (2H, m)	---
16	38.39	CH_2	1.20 (1H, m, Ha);	---
			1.84 (1H, m, Hb)	

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C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
17	56.01	CH	1.02 (1H, m)	---
18	11.81	CH_3	0.58 (3H, s)	C-12, C-13, C-14, C-17
19	19.27	CH_3	0.98 (3H, s)	C-1, C-9, C-10
20	36.23	CH	1.32 (1H, m)	---
21	18.85	CH_3	0.91 (3H, d, *J* = 6.5)	C-17, C-20, C-22
22	34.03	CH_2	1.02 (1H, m, Ha); 1.28 (1H, m, Hb)	---
23	26.19	CH_2	1.17 (2H, m)	---
24	45.86	CH	0.92 (1H, m)	---
25	29.28	CH	1.66 (1H, m)	---
26	19.04	CH_3	0.78 (3H, d, *J* = 6.8)	C-24, C-25, C-26
27	19.83	CH_3	0.81 (3H, d, *J* = 7.0)	C-24, C-25, C-27
28	23.01	CH_2	1.30 (1H, m, Ha) 1.28 (1H, m, Hb)	C-4, C-5, C6
29	12.01	CH_3	0.82 (3H, t, *J* = 7.3)	C-24, C-28
1′	101.40	CH	5.0 (1H, d, *J* = 7.8)	C-3
2′	75.20	CH	4.01 (1H, dd, *J* = 7.8, 5.7)	---
3′	78.47	CH	4.26 (1H, t, *J* = 5.7)	---
4′	71.51	CH	4.25 (1H, t, *J* = 5.7)	---
5′	77.89	CH	3.94 (1H, m)	---
6′	62.66	CH_2	4.51 (2H, d, *J* =11.4)	---

The ^{1}H and ^{13}C-NMR values **(Table 2.22)** for the compound **22** were compared with the reported data of β-sitosterol glucoside.[126]

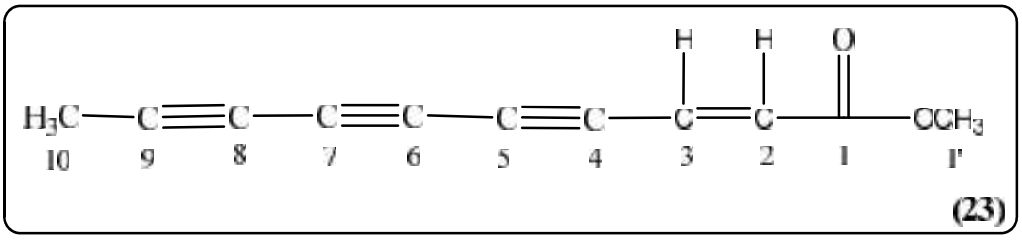
The large coupling constant value of H-3 and its NOESY correlation with H-9, characterizes that the *O*-glycoside moiety at C-3 was β-oriented.[127]

The anomeric configuration of glucose moiety was determined from H-1–H-2 splitting (*J* = 7.8 Hz) and the sugar moiety was confirmed to be glucose molecule from the characteristic ^{13}C-NMR data.[128]

The spectral (1D and 2D-NMR **(Table 2.22)**, IR and MS) and physical data of the compound **22** were similar to the reported data for β-sitosterol-3-*O*-β-D-glucopyranoside.[126,129]

Compound **22** has been reported from a number of plant species including; *A. annua*,[1,21] however, it is reported as new source compound from *A. elegantissima*.

2.7.2 *cis*-Dehydromatricaria ester (23).



(23)

Initially, compound **23** was isolated as orange yellow crystals, m.p. 112–113 °C (lit. m.p. 112 °C).[29] The color of the crystals was changed to dark brown with the passage of time (possibly due to drying; dehydration of crystals or light effect).

The IR spectrum of the compound **23** showed absorption for 2 x C≡C st. at ῡ 2217.11 and 2176.43, O-C=O st. at ῡ 1713.60, C=C st. at ῡ 1596.24 and C-C aliphatic and C-O ether st. at ῡ 1183.64, 995.45 and 815.58 $cm.^{-1}$ The molecular formula of $C_{11}H_8O_2$ was deduced from the LR-EIMS which gave molecular ion peak at *m/z* 172.05 [M^+].

In the ^{1}H-NMR spectrum **(Table 2.23)**, the two olefinic protons were resonating at δ 6.16 (1H, d, *J* = 11.5, H-2) and 6.27 (1H, d, *J* = 11.5, H-3). The methyoxy protons were resonating at δ 3.76 (3H, s, OCH_3-1′) while the terminal methyl protons were resonating at δ 1.99 (3H, s, H-10).

The ^{13}C-NMR (BB) spectrum **(Table 2.23)** of the compound **23** indicated a long chain dehydro polyacetylenic ester and showed eleven carbons differentiated by DEPT 135 experiments as seven quaternary, two methine and two methyl carbons.

Table 2.23. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) and HMBC spectra of *cis*-dehydromatricaria ester **(23)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	164.59	C	---	---
2	121.58	CH	6.16 (1H, d, J = 11.5)	C-1, C-3, C-4, C-5, C-6, C-7
3	132.56	CH	6.27 (1H, d, J = 11.5)	C-1, C-2, C-4, C-6, C-7
4	70.99	C	---	---
5	86.16	C	---	---
6	58.43	C	---	---
7	72.21	C	---	---
8	64.86	C	---	---
9	80.81	C	---	---
10	4.14	CH_3	1.99 (3H, s)	C-1, C-3, C-4, C-5, C-6, C-7 C-8, C-9
1′-OCH_3	51.77	CH_3	3.76 (3H, s)	C-1, C-2

In the ^{13}C-NMR spectrum **(Table 2.23)**, the olefinic carbons were resonating at δ 121.58 (C-2) and 132.56 (C-3). The methoxy carbon was resonating at δ 51.77 (OCH_3-1′) while the terminal methyl carbon was resonating at δ 4.14 (C-10). The acetylenic carbons were resonating at δ 58.43 (C-6), 64.86 (C-8), 70.99 (C-4), 72.21 (C-7), 80.81 (C-9) and 86.16 (C-5). The carbonyl was resonating at δ 164.59 (C-1).

The ^{1}H and ^{13}C-NMR data **(Table 2.23)** were further analysed by HMBC correlation.

In the HMBC spectrum, the olefinic protons at δ_H 6.16 (H-2) showed correlations with the carbons resonating at δ_C 132.56 (C-3), 164.59 (C-1), 70.99 (C4), 86.16 (C-5), 58.43 (C-6), 72.21 (C-7) and 64.86 (C-8). The olefinic proton at δ_H 6.27 (H-3) showed correlations with the carbons resonating at δ_C 121.58 (C-2), 164.59 (C-1), 70.99 (C-4) and 58.43 (C-6). The terminal methyl protons at δ_H 1.99 (H-10) showed correlation with the carbons resonating at δ_C 86.16 (C-5), 58.43 (C-6), 72.21 (C-7), 64.86 (C-8), 80.81 (C-9), 121.58 (C-2) and 132.56 (C-3). The methoxy protons at δ_H 3.76 (3H-1′) showed correlations with the carbons resonating at δ_C 164.59 (C-1) and 121.58 (C-2).

The HMBC correlation **(Table 2.23)** of the compound **23** were very interesting with respect to the fact that the olefinic protons as well as the terminal methyl group have shown more than four bonds away H → C correlations (normal HMBC correlation range is up to four bonds only). The terminal methyl group has shown H → C correlation up to nine bonds away which were possible due to highly conjugated system of the three linear acetylenic bonds attached to a *cis* double bond and an ester functionality. The key HMBC correlation of *cis*-dehydromatricaria ester **(23)** is given in **Figure 2.6.**

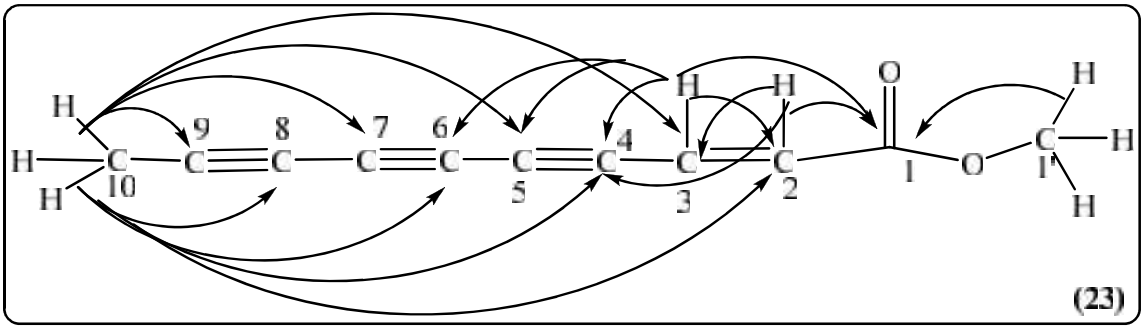
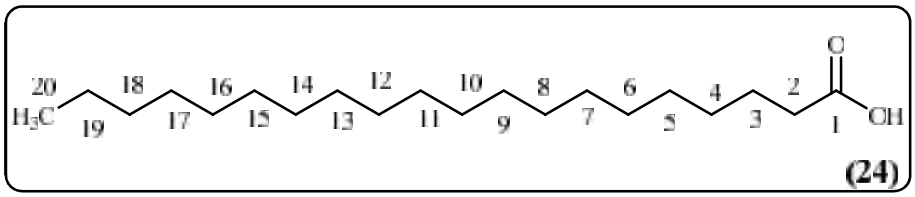


Figure 2.6. Key HMBC correlations of *cis*-dehydromatricaria ester **(23).**

All the spectral data (MS, IR, 1D and 2D-NMR) matched with the published data for *cis*-methyl deca-2-en-4,6,8-triynoate **(23),**[130] isolated from *Matricaria inodora* and *Matricaria oreades* and a number of Compositeae species including; *Artemisia.*[29] It has also been isolated from the culture medium of *Pleurotus ulmarius.*[1] However, from the roots of *A. elegantissima*, it is reported as a new source compound with full spectral (IR, MS, 1D and 2D-NMR) and physical data.

The synonyms used for the compound **23** in the literature are: (a) dehydromatricaria ester, (b) methyl-2-decene-4,6,8-triynoate.[1,29,130]

2.7.3 Eicosanoic acid (24).



Compound **24** was isolated as a white powder, m.p. 76–77 °C (lit. m.p. 77 °C).[26]

The LR-EIMS showed a characteristic molecular ion peak at *m/z* 312.30 [M^+] corresponding to the molecular formula of $C_{20}H_{40}O_2$.

Table 2.24. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of eicosanoic acid **(24)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	180.23	C	---	---
2	34.08	CH_2	2.33 (2H, t, J = 6.8)	C-1, C-3, C-4
3	24.69	CH_2	1.62 (2H, m)	---
19	22.70	CH_2	1.23 (2H, m)	---
18	31.94	CH_2	1.23 (2H, m)	---
4–17	29.07–30.37	$14CH_2$	1.23 (28H, m)	---
20	14.12	CH_3	0.90 (3H, t, J = 6.4)	C-20, C-19

The ^{1}H and ^{13}C-NMR spectral data of the compound **24** were found to be comparable to the reported data for eicosanoic acid (**24**).[26]

This was further supported by the HMBC spectrum **(Table 2.24)** where the methylene protons at δ_H 2.33 (2H, t, H-2) and 1.62 (2H, m, H-3) showed correlations with the carbons of each other resonating at δ_C 24.69 (C-2) and 34.08 (C-3) and 178.23 (C-1).

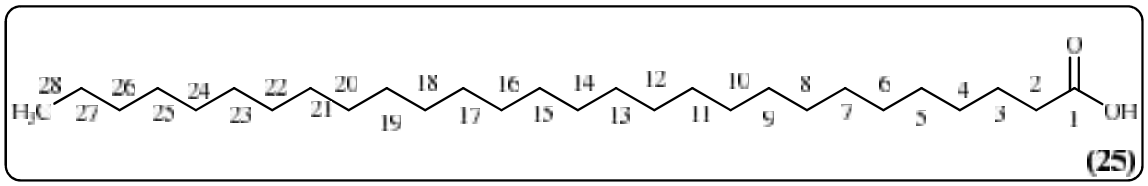
From spectral (1D and 2-NMR **(Table 2.24)**, IR and MS) and physical data, the structure of the compound **24** was established as a long chain fatty acid consists of twenty carbon atoms, known as eicosanoic acid.[131]

In the literature, the eicosanoic acid is known by synonyms: (a) arachidic acid (b) icosanoic acid and (c) arachic acid.[1,22,26,131]

It has been reported as a constituent of *Arachis hypogae* (ground-nut) oil glycerides. It has been widely distributed in other seed oils and in lipids of *Physalia physalis* (Portuguese-man-of-war).[1,26,124,131]

It has also been isolated from the genus *Artemisia*[26] but it is reported as a new source compound from *A. elegantissima*.

2.7.4 Octacosanoic acid (25).



(25)

Compound **25** was obtained as a white gummy solid, m.p. 90–91 °C (lit. m.p. 90.3–90.5 °C).[26] The LR-EIMS showed a molecular ion peak at *m*/*z* 424.42 [M^+] in accordance with the molecular formula of $C_{28}H_{56}O_2$.

The ^{1}H and ^{13}C-NMR spectral data of the compound **25** were compared and found to be in close agreement with the reported data for octacosanoic acid (**25**).

This was further supported by the HMBC spectrum **(Table 2.25)** where the methylenes at δ 2.33 (t, 2H-2) and 1.61 (m, 2H-3) showed HMBC correlations with each other at δ 24.69 (C-2) and 33.80 (C-3) and with quaternary carbonyl carbon at δ 178.72 (C-1).

From the spectral (1D and 2D-NMR **(Table 2.25)**, IR and MS) and physical data, the structure of the compound **25** was established as a long chain fatty acid consisting of twenty eight carbon atoms (octacosanoic acid).[26]

Table 2.25. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC correlation of octacosanoic acid **(25)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	178.72	C	---	---
2	33.80	CH_2	2.33 (2H, t, J = 6.8)	C-1, C-3, C-4
3	24.69	CH_2	1.61 (2H, m)	---
27	22.69	CH_2	1.23 (2H, m)	---
26	31.92	CH_2	1.23 (2H, m)	---
4–25	29.06–29.70	$22CH_2$	1.23 (44H, m)	---
28	14.14	CH_3	0.86 (3H, t, J = 6.4)	C-27, C-26

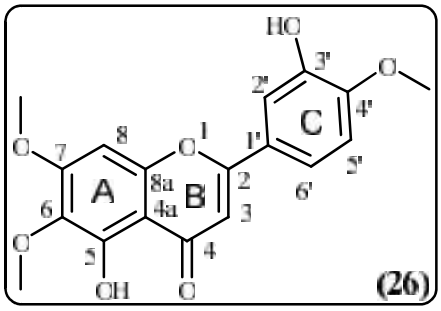
Octacosanoic acid (montanic acid) has been reported from carnauba wax, agave leaf wax, in wood of *Vitex divaricate,* essential oil of *Artemisia* and several other plants.[1,26,124] However, it is reported as a new source compound from *A. elegantissima*.

2.7.5 Other new source compounds (already discussed) isolated from the roots of *A. elegantissima*.

The result and discussion of the five compounds isolated from the roots of *A. elegantissima* including; scopoletin **(14)**,[50] allograciolone acetate **(15)**,[121] betulinic acid **(16)**,[123] (*Z*)-*p*-hydroxycinnamic acid **(19)**[125] and β-sitosterol (**21**)[87] have been already discussed in **Sections 2.6.10, 2.6.11, 2.6.12, 2.6.15 and 2.6.17 and Tables 2.14, 2.15, 2.16, 2.17, 2.19 and 2.21** respectively.

2.8 New source compounds isolated from the aerial parts (leaves and stems) of *Artemisia incisa*.

2.8.1 Eupatorin (26).



Compound **26** was obtained as yellow crystals, m.p. 195–196 °C (lit. m.p. 195–196 °C).[62] The molecular formula of $C_{18}H_{16}O_7$ was deduced from HR-ESIMS (positive ion mode) which exhibited a pseudo molecular ion peak at *m/z* 344.088 [M^++H]. This formula was further confirmed from ^{1}H and ^{13}C-NMR which established the structure of the compound **26** to be a typical flavone skeleton.

In the ^{1}H-NMR spectrum of the compound **26 (Table 2.26)**, the ring **A** proton was resonating at δ 6.64 (1H, s, H-8). The ring **B** proton was resonating at δ 6.85 (1H, s, H-3). The ring **C** protons were resonating at δ 7.92 (1H, dd, *J* = 8.5, 1.9 Hz, H-6′), 7.45 (1H, d, *J* = 8.5 Hz, H-5′) and 8.14 (1H, d, *J* = 1.9 Hz, H-2′). The three methoxy protons were resonating at δ 3.87 (3H, s, OCH_3-6), 3.93 (3H, s, OCH_3-7) and 3.83 (3H, s, OCH_3-4′). The hydroxyl protons were resonating at δ 12.0 (1H, br. s, OH-5) and 5.33 (1H, br. s, OH-3′).

Table 2.26. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-4H-chromen-4-one (eupatorin) **(26)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	`Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
2	163.99	C	---
3	102.83	CH	6.85 (1H, s)
4	182.37	C	---
5	152.76	C	---
6	137.98	C	---
7	140.85	C	---
8	94.91	CH	6.64 (1H, s)
4a	103.83	C	---
8a	149.55	C	---
1′	121.78	C	---
2′	112.69	CH	8.14 (1H, d, *J* = 1.9)
3′	140.91	C	---
4′	137.80	C	---
5′	114.54	CH	7.45 (1H, d, *J* = 8.5)
6′	119.73	CH	7.92 (1H, dd, *J* =8.5, 1.9)
6-OCH_3	59.21	CH_3	3.93 (3H, s)
7-OCH_3	60.18	CH_3	3.87 (3H, s)
4′-OCH_3	61.54	CH_3	3.83 (3H, s)
5-OH	---	H	12.3 (1H, s)
3′-OH	---	H	5.35 (1H, s)

The ^{13}C-NMR spectrum **(Table 2.26)** of the compound **26** showed eighteen carbons differentiated by (DEPTq) experiments as ten quaternary, five methine and three methyl carbons respectively.

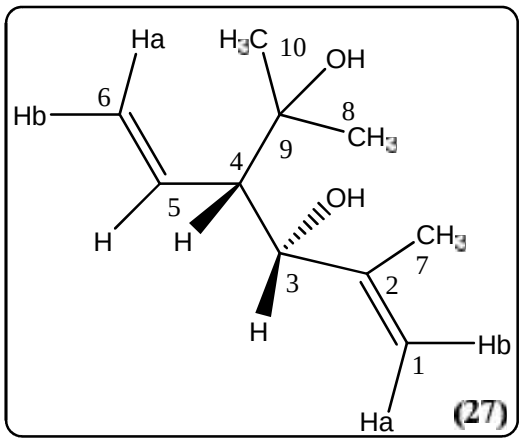
The ring **A** carbons were resonating at δ 94.91 (C-8), 149.55 (C-8a), 103.83 (C-4a), 152.76 (C-5), 130.98 (C-6) and 156.85 (C-7). The ring **B** carbons were resonating at δ 102.83 (C-3), 182.37 (C-4) and 163.99 (C-2). The ring **C** carbons were resonating at δ 140.91 (C-3′) and 137.80 (C-4′), 112.69 (C-2′), 121.78 (C-1′), 114.54 (C-5′), and 119.73 (C-6′). The methoxy carbons were resonating at δ 59.23 (OCH_3-6), 60.18 (OCH_3-7) and 61.54 (OCH_3-4′).

From the spectral (^{1}H, and ^{13}C-NMR **(Table 2.26)** and MS) and physical data, the structure of the compound **26** was established as 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-4H-chromen-4-one (eupatorin).[62]

Compound **26** has been reported from various plants species including; *Eupatorium semiserratum, Baccharis trimera, Centaurea aggregate, Sideritis altissimum, Artemisia annua*[63] and from the families of Asteraceae and Labiatae.[1,58] However, it is reported as new source compound from *A. incisa*.

In the literature, compound **26** is known by synonyms: (a) 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-6,7-dimethoxy-4H-1-benzopyran-4-one and (b) eupatorin.[1,61]

2.8.2 Santolinylol (27).



Compound **27** was isolated as an off white color gummy solid, with $^{20}[\alpha]_D$ -14.2 (*c* 1.0, $CHCl_3$) and UV(MeOH): λ_{max} : 228 nm.[67] The IR spectrum showed absorption for OH st. at $\bar{\upsilon}$ 3290 and C=C st. at $\bar{\upsilon}$ 1655 $cm.^{-1}$ The LR-EIMS showed molecular ion peak at *m/z* 170.12 [M^+] corresponding to the molecular formula of $C_{10}H_{18}O_2$.

Adjacent protons coupling; ^1H-^1H were established by COSY, the ^1H-^{13}C correlation were established by HSQC, while the long range ^1H-^{13}C correlations were established through the HMBC technique.

The ^1H and ^{13}C-NMR spectra of the compound **27** gave signals characteristic of an irregular monoterpene diol.

In the ^1H-NMR spectrum of the compound **27 (Table 2.27)**, the three methyl protons were resonating at δ 1.73 (3H, s, H-7), 0.91 (3H, s, H-8) and 0.98 (3H, s, H-10). The axial methylene protons were resonating at δ 4.92 (1H, br. s, Ha-1); 5.02 (1H, br. s, Hb-1) and δ 5.03 (1H, dd, *J* = 11.8, 1.9 Hz, Ha-6); 5.07 (1H, dd, *J* = 18.2, 2.8 Hz, Hb-6). The

methine protons were resonating at δ 4.36 (1H, d, *J* = 10.0 Hz, H-3), 2.37 (1H, t, *J* = 10.0 Hz, H-4) and 5.34 (1H, ddd, *J* =17.0, 7.0, 2.0 Hz, H-5).

In the ^{13}C-NMR spectrum **(Table 2.3)**, the quaternary carbons were resonating at δ 145.62 (C-2) and 73.93 (C-9). The three methyls were resonating at δ 16.24 (C-7), 24.35 (C-8) and 30.83 (C-10). The two axial methylenes were resonating at δ 114.20 (C-1) and 117.98 (C-6). The three methines were resonating at δ 78.52 (C-3), 56.71 (C-4) and 133.90 (C-5).

Compound **27**, also known as santolinylol have the hydroxyl group at position C-3 as compared to the compound **3** where there was an acetoxy group. This fact was supported by the up field shift of the methine proton by 1ppm, resonating at δ_H 4.35 (1H, d, *J* = 10 Hz, H-3) in the compound **27** as compared to the compound **3** (santolinylol-3-acetate) where it was resonating at δ_H 5.37 (1H, d, *J* = 9.6 Hz, H-3) **(Table 2.3.1)**. The signals position of the hydroxyl protons were not clear, however, their presence at the carbons; C-3 and C-9 were deduced from the characteristic downfield shift of ^{1}H and ^{13}C-NMR values **(Table 2.27)** of the methine resonating at δ_H 4.35 (H-3) and δ_C 78.52 (C-3) and hydroxylated quaternary carbon resonating at δ_C 73.93 (C-9) respectively.

In the COSY spectrum, the methine proton resonating at δ 4.35 (H-3) have correlation with the methine protons resonating at δ 2.38 (H-4) and 5.50 (H-5). The later have also correlation with the axial methylene (C-6) protons (diastereotopic) resonating at δ_H 5.07 (Ha-6) and 5.03 (Hb-6).

Table 2.27. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of santolinylol **(27)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	114.21	CH_2	4.93 (1H, br. s, Ha) 5.02 (1H, br. s, Hb)	C-2, C-3
2	145.6	C	---	---
3	78.52	CH	4.35 (1H, d, *J* = 10.0)	C-1, C-2, C-4
4	56.71	CH	2.37 (1H, dd, *J* = 10.0, 10.0)	C-3, C-5, C-6, C-9
5	133.9	CH	5.50 (1H, ddd, *J* = 15.6, 6.2, 2.0)	C-4, C-6, C-3, C-9
6	117.9	CH_2	5.07 (1H, dd, *J* = 19.2, 2.0, Ha) 5.03 (1H, dd, *J* = 12, 1.6, Hb)	C-4, C-5
7	16.24	CH_3	1.70 (3H, s)	C-1, C-2, C-3
8	24.35	CH_3	0.90 (3H, s)	C-4, C-9, C-10
9	73.93	C	---	---
10	30.83	CH_3	0.98 (3H, s)	C-4, C-8, C-9

These correlation confirmed the substitution of allylic and hydroxyl groups at the methine carbon (CH-3) while the substitution of vinylic and 2-hydroxy propanyl groups at the methine carbon (CH-4). The methines (CH-3) and (CH-4) are directly coupled to each other.

The HMBC correlations between the two methyl groups; CH_3-8 and CH_3-10 with each other and the carbons; C-9 and C-4 deduce the presence of 2-hydroxy propanol group substituted at the methine carbon (C-4).

The relative stereochemistry of the compound **27** was confirmed from the NOESY correlation between the methine protons; H-3 and H-4 which were the same as in case of the compound **3**. This was further supported by comparing the optical rotation of the compound **27** $[\alpha]^{20}_{D}$ -14.2 (*c* = 1.0, $CHCl_3$) with the literature $^{20}[\alpha]_{D}$ - 13.0 (*c* 0.873, $CHCl_3$) and thus the relative stereochemistry was consequently assigned as *3R* and *4S* configuration.[67]

By comparing the 1D and 2D-NMR, IR, UV and MS data with the literature, established the structure of the compound **27** as (*3R,4S*)-2,5-dimethyl-3-vinylhex-5-ene-2,4-diol (santolinylol).[67,109]

In the literature this compound is known by synonym of santolinylol, isolated from *Artemisia giraldii var. giraldi* and other *Artemisia* species.[1,67,109] Hereby, it is reported as a new source compound from *A. incisa*.

The key HMBC and COSY correlation of the compound **27** are given in **Figure 2.7a** while its NOESY correlation are given in **Figure 2.7b**.

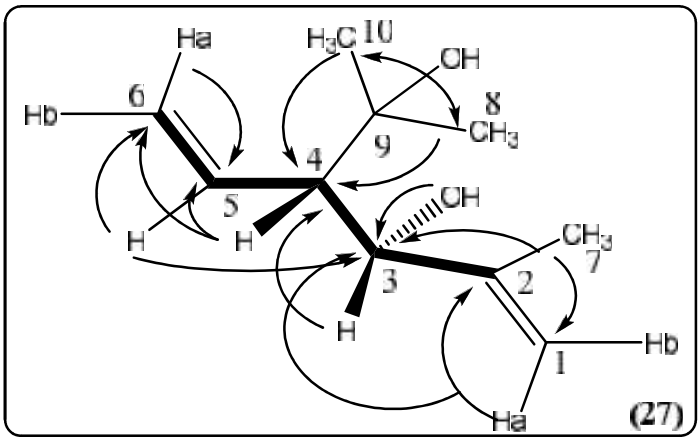


Figure 2.7a. Key HMBC (→) and COSY (—) correlations of santolinylol **(27).**

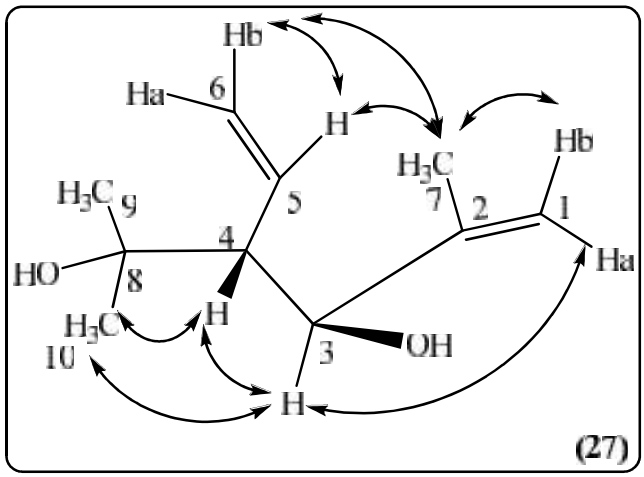
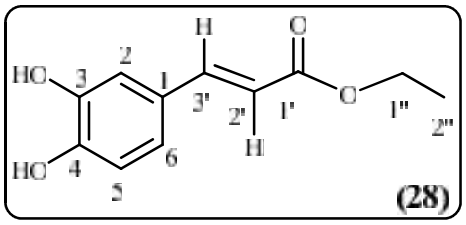


Figure 2.7b. Key NOESY (↔) correlations of santolinylol **(27).**

2.8.3 *trans*-Ethyl caffeate (28).



Compound **28** was obtained as white needles like crystals, m.p.148–150 °C (lit. m.p. 149–150 °C).[132] The molecular formula of $C_{11}H_{12}O_4$ was deduced from LR-EIMS spectrum showing molecular ion peak at *m/z* 208.073 [M^+].

In the ^{1}H-NMR spectrum **(Table 2.28)** the hydroxyl protons were resonating at δ 8.26 (1H, br. s, OH-3) and 8.51 (1H, br. s, OH-4). The methyl protons were resonating at δ 1.16 (3H, t, *J* = 7.0 Hz, H-2″). The methylene protons were resonating at δ 4.21 (2H, q, *J* = 7.0 Hz, H-1″). The methine protons were resonating at δ 7.60 (1H, d, *J* = 2.0 Hz, H-2), 7.21 (1H, d, *J* = 8.0 Hz, H-5), 7.17 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.58 (1H, d, *J* = 16.0 Hz, H-2′) and 7.96 (1H, d, *J* = 16.0 Hz, H-3′).

The ^{13}C-NMR **(Table 2.28)** spectrum of the compound **28** showed eleven carbons which according to DEPT-135, there were five methines, one methylene, one methyl and four quaternary carbons.

In the ^{13}C-NMR spectrum, the quaternary carbons were resonating at δ 167.0 (C-1′), 147.4 (C-3), 150.3 (C-4) and 126.6 (C-1). The methine carbons were resonating at δ 115.4 (C-2), 116.4 (C-5), 121.7 (C-6), 114.8 (C-2′) and 145.3 (C-3′). The methyl carbon was resonating at δ 14.1 (C-2″) while the methylene carbon was resonating at δ 59.8 (C-1″).

From 1D-NMR and the physical data, the structure of the compound **28** was established as *trans*-ethyl caffeate.[132] However, HMBC data was evaluated to finalize the 1D-NMR assigned structure.

In the HMBC spectrum **(Table 2.28)**, the proton at δ_H 7.60 (H-2) showed correlations with the carbons resonating at δ 145.3 (C-3′), 150.3 (C-4) and 121.7 (C-6). The proton at δ_H 7.17 (H-6) showed correlations with the carbons resonating at δ_C 116.4 (C-5), 115.4 (C-2) and 150.3 (C-4). The proton at δ_H 7.21 (H-5) showed correlations with the carbons resonating at δ_C 121.7 (C-6), 147.4 (C-3) and 126.6 (C-1). These correlations showed the 1,3,4-trisubstituted benzene ring with a side chain attached to the carbon; C-1. The proton at δ_H 6.58 (H-3′) showed correlation with the carbons resonating at δ_C 116.4 (C-3′), 150.3 (C-1) and 167.0 (C-1′). Similarly, the proton at δ_H 6.58 (H-2′) showed correlation with the carbon resonating at δ_C 114.8 (C-2′), 121.7 (C-6) and 167.0 (C-1′). The hydroxyl protons at δ_H 8.26 (OH-3) and 8.51 (OH-4) showed correlations with the carbon resonating at δ_C 147.4 (C-3) and 150.3 (C-4) respectively. These correlations confirmed the caffeic acid skeleton.

Table 2.28. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC correlation of *trans*-ethyl caffeate **(28)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	126.6	C	---	---
2	115.4	CH	7.60 (1H, d, *J* = 2.0)	C-3′, C-4, C-6
3	147.4	C	---	---
4	150.3	C	---	---
5	116.4	CH	7.21 (1H, d, *J* = 8.0)	C-1, C-3, C-6
6	121.7	CH	7.17 (1H, dd, *J* = 8.0, 2.0)	C-4, C-5, C-2
1′	167.0	C	---	---
2′	114.8	CH	6.58 (1H, d, *J* = 16.0)	C-1′, C-3′, C-1
3′	145.3	CH	7.96 (1H, d, *J* = 16.0)	C-1, C-6, C-2′
1″	59.8	CH_2	4.21 (2H, q, *J* = 7.0)	C-1′, C-2″
2″	14.1	CH_3	1.16 (3H, t, *J* = 7.0)	C-1″
3-OH	---	H	8.26 (1H, br. s)	C-3
4-OH	---	H	8.51 (1H, br. s)	C-4

However, the correlation of protons at δ_H 4.21 (H-1″) with the carbons resonating at δ_C 167.0 (C-1′), 14.1 (C-2″), 1.16 (t, 3H-2″) and 59.8 (C-1″) showed that the side chain was an ethyl propenoate moiety.

In the NOESY spectrum, the olefinic proton; H-2′ and the aromatic proton; H-6 were space correlated while H-2′ and H-3′ have no correlation with each other showing that the olefinic protons; H-2′ and H-3′ have anti configuration which was also evident from the large coupling constant (J = 16.0 Hz) of their protons doublets.

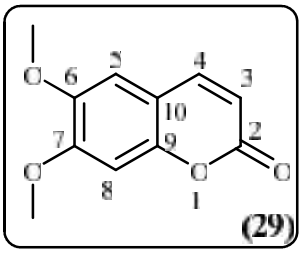
From the physicochemical constants and spectral data (IR, MS, 1D and 2D-NMR) **(Table 2.28)**, the compound **28** was elucidated as *trans*-ethyl caffeate which agreed with the literature.[132]

trans-Ethyl caffeate has been reported from *Solanum melongena, Solanum integrifolium, Salvia guarantica* and several other plants.[1,22,46,86,132]

trans-Ethyl caffeate has been reported by synonyms in the literature as; (a) ethyl-3,4-dihydroxycinnamate, (b) *trans*-ethyl caffeate.[1,15,22,49]

Hereby, it is reported as a new source compound from *A. incisa*.

2.8.4 Scoparone (29).



Compound **29** was isolated as greenish white needles, m.p. 143–144 °C (lit. m.p. 144 °C).[86] The molecular formula of $C_{11}H_{10}O_4$ was deduced from the LR-EIMS which showed the molecular ion peak at *m/z* 206.058 [M^+].

The spectral data of ^{1}H and ^{13}C-NMR **(Table 2.29)** proposed coumarin as a basic nucleus for the compound **29**.

In the ^{1}H-NMR spectrum **(Table 2.29)**, the methoxy protons were resonating at δ 3.94 (3H, br. s, OCH_3-6) and 3.91 (3H, br. s, OCH_3-7). The olefinic protons were resonating at δ 7.67 (1H, d, *J* = 9.5 Hz, H-4) and 6.28 (1H, d, *J* = 9.5 Hz, H-3). The aromatic protons were resonating at δ 6.86 (1H, br. s, H-8) and 6.93 (1H, s, H-5).

The ^{13}C-NMR spectrum (BB) **(Table 2.29)** showed eleven carbon atoms which according to DEPT-135, were two methyls, four methines and five quaternary carbons.

In the ^{13}C-NMR spectrum **(Table 2.29)**, the methoxy carbons were resonating at δ 60.1 (OCH_3-7) and 56.3 (OCH_3-6). The methine carbons were resonating at δ 105.2 (C-5) and 103.7 (C-8), 114.4 (C-3) and 143.4 (C-4). The quaternary carbons were resonating at δ 159.8 (C-2), 142.2 (C-7), 135.8 (C-9), 140.0 (C-6) and 114.5 (C-10).

Table 2.29. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of scoparone **(29)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	159.8	C	---	---
3	114.4	CH	6.28 (1H, d, J = 9.6)	C-2, C-4, C-10
4	143.4	CH	7.67 (1H, d, J = 9.6)	C-2, C-3, C-9
5	105.2	CH	6.93 (1H, br. s)	C-6, C-7, C-9
6	140.0	C	---	---
7	142.2	C	---	---
8	103.7	CH	6.86 (1H, s)	C-6, C-7, C-10
9	135.8	C	---	---
10	114.5	C	---	---
6-OCH_3	56.3	CH_3	3.94 (1H, br. s)	C-6
7-OCH_3	60.1	CH_3	3.91 (1H, br. s)	C-7

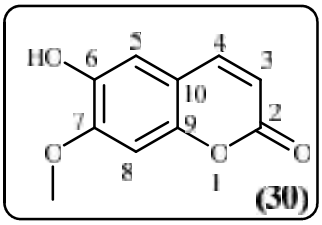
From the physical and spectral data of MS, ^{1}H and ^{13}C-NMR, the structure of the compound **29** was elucidated as scoparone. Furthermore, the structure elucidation and analysis of the compound **29** was made with the help of HMBC experiment.

The HMBC spectrum **(Table 2.29)** showed correlations of the proton at δ 6.28 (H-3) with the carbons resonating at δ 143.4 (C-4), 114.5 (C-10) and 159.8 (C-2). Likewise, the proton at δ 77.67 (H-4) showed correlations with carbons resonating at δ 114.4 (C-3), 135.8 (C-9) and 159.8 (C-2). The proton at δ 6.93 (H-5) showed correlations with the carbons resonating at δ 140.0 (C-6), 135.8 (C-10) and 142.2 (C-7). Similarly, the proton at δ 6.86 (H-8) showed correlations with the carbons resonating at δ 114.5 (C-10), 140.0 (C-6) and 142.2 (C-7). The protons of methoxy at δ 3.94 (OCH_3-6) and 3.91 (OCH_3-7) showed correlations with the carbons resonating at δ 140.0 (C-6) and 142.2 (C-7) respectively. These correlations established the structure of the compound **29** as 6,7-dimethoxy coumarin (scoparone).[54]

In the literature scoparone is known by synonyms: (a) 6,7-dimethoxy-2*H*-1-benzopyran-2-one, (b) 6,7-dimethoxycoumarin, (c) 6,7-dimethoxy-2*H*-chromen-2-one, (d) scoparone, (e) escoparone, (f) scoparin (g) scoparone.[1,46,54,86,124a]

Scoparone has been reported from citrus oils, *Artemisia scoparia*, *Zanthoxylum setosum*[1] and other plants,[1,49,53,86] but here it is reported as new source compound from *A. incisa*.

2.8.5 Isoscopoletin (30).



Compound **30** (position isomer of scopoletin **(14)**)[120] was obtained as white yellow crystals, m.p. 184–185 °C (lit. m.p. 185 °C).[54] The IR spectrum showed absorption at ῡ 1769 cm^{-1} which was attributed to the presence of benzo-*α*-lactone (coumarin) skeleton. The molecular formula of $C_{10}H_8O_4$ was obtained from the LR-EIMS which gave the molecular ion peak at *m/z* 192.042 [M^+].

The spectral data of ^{1}H and ^{13}C-NMR **(Table 2.30)**, established the structure of the compound **30** to be a coumarin skeleton.

In the ^{1}H-NMR spectrum **(Table 2.30)**, the methoxy protons were resonating at δ 3.77 (3H, br. s, OCH_3-7) and the hydroxyl proton was resonating at δ 5.06 (1H, br. s, OH-6). The methine protons were resonating at δ 6.29 (1H, d, *J* = 9.5 Hz, H-3) and 7.68 (1H, d, *J* = 9.5 Hz, H-4), 7.03 (1H, br. s, H-5) and 7.10 (1H, s, H-8).

The ^{13}C-NMR spectrum showed ten carbon atoms and from DEPT-135, these were one methyl, four methines and five quaternary carbons (**Table 2.30**).

Table 2.30. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC correlation of isoscopoletin **(30)** in C_5D_5N.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
2	161.5	C	---	---
3	112.4	CH	6.29 (1H, d, *J* = 9.5)	C-2, C-4, C-10
4	144.1	CH	7.68 (1H, d, *J* = 9.5)	C-2, C-3, C-9
5	109.6	CH	7.03 (1H, br. s)	C-6, C-7, C-9
6	153.1	C	---	---
7	144.6	C	---	---
8	104.2	CH	7.10 (1H, s)	C-6, C-7, C-10
9	151.1	C	---	---
10	111.2	C	---	---
7-OCH_3	56.3	CH_3	3.77 (1H, br. s)	C-7
6-OH	---	H	5.06 (1H, br. s)	C-6

The methoxy carbon was resonating at δ 56.3 (OCH_3-6). The methine carbons were resonating at δ 112.4 (C-3), 144.1 (C-4), 109.6 (C-5) and 104.2 (C-8). The quaternary carbons were resonating at δ 151.1 (C-9), 153.1 (C-6), 144.6 (C-7), 111.2 (C-10) and 159.5 (C-2).

From the spectral (^{1}H and ^{13}C-NMR **(Table 2.30)**, IR, MS) and physical data, the structure of the compound **30** was established as isoscopoletin. Additionally, with the help of HMBC experiments, further supported the structure of the compound **30.**

In the HMBC, the proton at δ_H 7.03 (H-5) showed correlation with the carbons resonating at δ_C 153.1 (C-6), 151.1 (C-9) and 144.6 (C-7). Similarly, the proton at δ_H 7.10 (H-8) showed correlations with the carbons resonating at δ_C 111.2 (C-10), 153.1 (C-6) and 144.6 (C-7). The proton at δ_H 6.29 (H-3) showed correlations with the carbons resonating at δ_C 144.1 (C-4), 111.2 (C-10) and 161.5 (C-2). Likewise, the proton at δ_H 7.68 (d, 1H-4) showed correlations with the carbons resonating at δ_C 112.4 (C-3), 151.1 (C-9) and 161.5 (C-2). These correlations established the protons of the methoxy and hydroxy groups at the carbons; C-7 and C-6 respectively.

The NOESY correlations between the proton at δ_H 7.03 (H-5), the methoxy protons at δ_H 3.77 (OCH_3) and the protons at δ_H 6.34 (H-3) and 7.67 (H-4) confirmed a 7-methoxy-6-hydroxy benzo-*α*-lactone structure (isoscopoletin).[54]

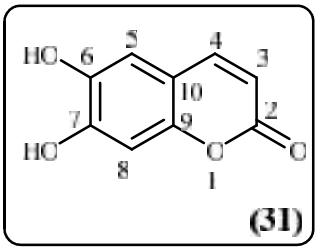
Compound **30** was confirmed to be isoscopoletin by comparison of its spectra and other data with the literature**.**[120]

Isoscopoletin was reported by synonyms: (a) 6-hydroxy-7-methoxycoumarin, (b) isoscopoletin and (c) 6-hydroxy-7-methoxy-2*H*-1-benzopyran-2-one.[1,49,117,124a]

Isoscopoletin (**30)** has been reported from a number of plant genera including; *Artemisia messerschmidiana, Ranunculus sceleratus* and *Bupleurum frutescens*.[1,46,53,63]

It is hereby reported as new source compound from *A. incisa*.

2.8.6 Esculetin (31).



(31)

Compound **31** was isolated as needles like crystals of white color, m.p. 269–271 °C (lit. m.p. 270 °C).[133] The molecular formula was of $C_9H_6O_4$ established from the LR-EIMS showing the molecular ion $[M^+]$ at *m/z* 178.027.

The ^{1}H and ^{13}C-NMR spectra were suggesting coumarin skeleton for the compound **31**.

In the ^{1}H-NMR spectrum **(Table 2.31),** the two hydroxyl protons were resonating at δ 5.33 (2H, br. s, OH-6/OH-7). The methine protons were resonating at δ 7.84 (1H, d, *J* = 9.5 Hz, H-4) and 6.17 (1H, d, *J* = 9.5 Hz, H-3), 7.19 (1H, br. s, H-5) and 6.79 (1H, s, H-8).

Table 2.31. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) spectra of esculetin **(31)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
2	161.9	C	---
3	113.4	CH	6.17 (1H, d, *J* = 9.5)
4	143.5	CH	7.84 (1H, d, *J* = 9.5)
5	112.8	CH	7.19 (1H, br. s)
6	144.5	C	---
7	148.1	C	---
8	103.6	CH	6.79 (1H, s)
9	150.1	C	---
10	112.2	C	---
6-OH	---	H	5.36 (1H, br. s)
7-OH	---	H	5.36 (1H, br. s)

The ^{13}C-NMR spectrum **(Table 2.31)** showed nine carbons; four methines and five quaternary carbons from DEPT-135.

In the ^{13}C-NMR spectrum, the quaternary carbons were resonating at δ 161.9 (C-2), 150.1 (C-10), 148.1 (C-9), 144.5 (C-7) and 112.2 (C-6). The methine carbons were resonating at δ 113.4 (C-3), 143.5 (C-4), 103.6 (C-8) and 112.8 (C-5).

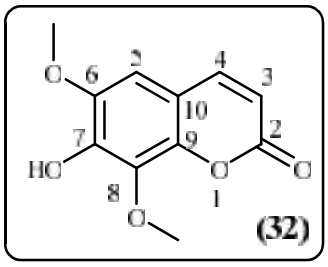
The structure of the compound **31** was elucidated as 6,7-dihydroxycoumarin (esculetin) and confirmed by comparison with the literature data.[133]

Esculetin is hereby reported as a new source compound from *A. incisa*.

In the literature, esculetin is known by synonyms: (a) 6,7-dihydroxycoumarin, (b) 6,7-dihydroxy-2*H*-1-benzopyran-2-one, (c) aesculetin, (4) esculetin, (d) cichorigenin, (e) esculetol, (f) 6,7-dihydroxy-2*H*-chromen-2-one.[1,56,124a,133]

Esculetin has been reported as metabolite of the infected sweet potato. It was also found in *Aesculus, Atropa, Datura, Digitalis* and in some ferns.[1,51,53,63,133]

2.8.7 Isofraxidin (32).



Compound **32** was obtained as colorless needles like crystals, m.p. 148–149 °C (lit. m.p. 148–149 °C).[134] The molecular formula of $C_{11}H_{10}O_5$ was obtained from HR-ESIMS (positive ion mode) which showed a pseudo molecular ion peak at *m/z* 223.053 [M^++H].

The ^{1}H and ^{13}C-NMR spectra **(Table 2.32)** suggested 6,7,8-oxygenated coumarin skeleton for the compound **32** which was obtained as a naturally degraded analogue of dracunculin **(13**) where the cleavage of dioxomethylene group resulted in methoxy and hydroxy groups.

In the ^{1}H-NMR spectrum **(Table 2.32)**, the methine protons were resonating at δ 6.68 (1H, br. s, H-5), 6.36 (1H, d, *J* = 9.5 Hz, H-3) and 7.63 (1H, d, *J* = 9.5 Hz, H-4). The methoxy protons were resonating at δ 3.91 (3H, s, OCH_3-6) and 3.86 (3H, s, OCH_3-8) while the hydroxy proton was resonating at δ 5.35 (1H, s, OH-7).

In the ^{13}C-NMR spectrum of the compound **32,** there were eleven carbons from DEPT-135, these were two methyls, three methines and six quaternary carbons.

The quaternary carbons were resonating at δ 160.6 (C-2), 141.8 (C-8), 150.5 (C-6), 145.1 (C-7), 143.0 (C-9) and 114.6 (C-10). The methine carbons were resonating at δ 103.9 (C-

5), 115.2 (C-3) and 143.5 (C-4). The methoxy carbons were resonating at δ 60.5 (OCH_3-8) and 56.3 (OCH_3-6).

From the spectral (^1H, ^{13}C-NMR, MS and IR) and physical data, the structure of the compound **32** was established as isofraxidin (6,8-methoxy-7-hydroxy coumarins).[51,117]

The structure of the compound **32** was confirmed HMBC and NOESY experiments (**Table 2.32**).

In the HMBC spectrum (**Table 2.32**) the proton at δ 6.36 (H-3) showed correlations with the carbons resonating at δ 160.6 (C-2), 103.9 (C-10) and 143.5 (C-4). The proton at δ 7.63 (H-4) showed correlations with the carbons resonating at δ 160.6 (C-2), 143.0 (C-9) and 115.2 (C-3), establishing *α*, ß-unsaturated benzo-α-lactone skeleton. The proton at δ 6.68 (1H) showed correlations with the carbons resonating at δ 150.5 (C-6), 145.1 (C-7) and 143.0 (C-9) which confirmed the position of the hydroxyl group at C-7 and the two methoxy groups at C-6 and C-8 respectively. This is further supported by the correlations of protons at δ 3.91 (OCH_3-6) and 3.86 (OCH_3-8) and 5.35 (OH-7) with the carbons resonating at δ 150.5 (C-6) 141.8 (C-7) and 145.1 (C-7) respectively.

Table 2.32. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) and HMBC spectra of isofraxidin **(32)** in $CDCl_3$.

C. No.	^{13}CNMR (δ)	Multiplicity	^{1}HNMR (δ) (J_{HH} Hz)	HMBC Correlation
2	160.6	C	---	---
3	115.2	CH	6.36 (1H, d, *J* = 9.5)	C-2, C-4, C-5a
4	143.5	CH	7.63 (1H, d, *J* = 9.5)	C-2, C-3, C-5, C-5a
5	103.9	CH	6.68 (1H, s)	C-6, C-7, C8a
6	150.5	C	---	---
7	145.1	C	---	---
8	141.8	C	---	---
9	143.0	C	---	---
10	114.6	C	---	---
6-OCH_3	56.3	CH_3	3.91 (3H, s)	C-6
8-OCH_3	60.5	CH_3	3.86 (3H, s)	C-8
7-OH	---	H	5.35 (1H, br. s)	C-7

The NOESY spectrum showed correlations between the proton H-5, the methoxy protons at δ 3.91 (OCH_3) and protons at δ 6.36 (H-3) and 7.63 (H-4) which confirmed the position of the two methoxy groups at carbons; C-6 and C-8 and the hydroxyl group at carbon; C-7 of benzene ring.

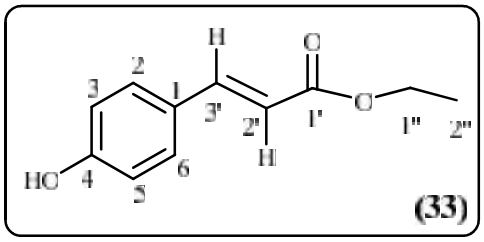
From the spectral (1D and 2D-NMR) and physical data, the structure of the compound **32** was established as 7-hydroxy-6,8-dimethoxy-2H-1-benzopyran-2-one (isofraxidin).[134b]

Isofraxidin (**32)** is reported by a number of synonyms: (a) 7-hydroxy-6,8-dimethoxy-2H-1-benzopyran-2-one, (b) 7-hydroxy-6,8-dimethoxycoumarin,(c) isofraxidin, (d) calycanthogenol and (f) phytodolor.[1,51,117,124a]

Isofraxidin has been reported from *Artemisia* species, *Fraxinus excelsior, Azadirachta indica* and *Pterocaulon redolens.*[1,53,117,134b]

It is reported as new source compound from *A. incisa*.

2.8.8 Ethyl-(*E*)-*p*-hydroxy cinnamate (33).



(33)

Compound **33** was obtained as white needles like crystals, m.p. 82–83 °C (lit. m.p. 83 °C).[15] The molecular formula of $C_{11}H_{12}O_3$ was deduced from LR-EIMS which showed molecular ion peak at *m/z*: 192.078 [M^+].

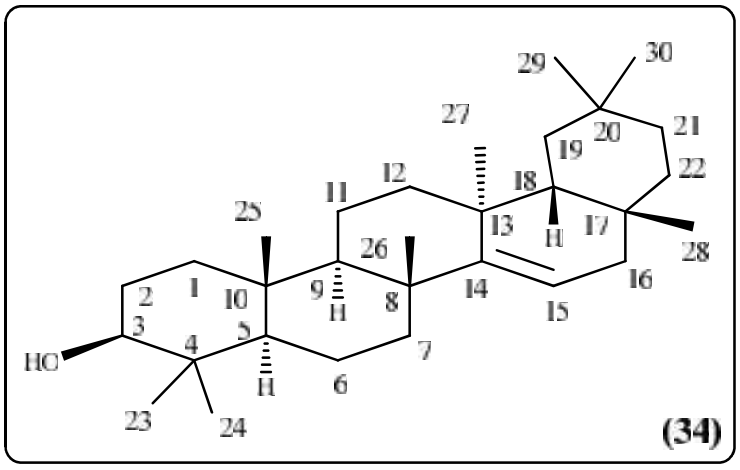
From the ^{1}H-NMR **(Table 2.33)** and physical data, the structure of the compound **33** was established as ethyl-(*E*)-*p*-hydroxy cinnamate and confirmed by comparison with the literature.[46,135]

Ethyl-(*E*)-*p*-hydroxy cinnamate is reported from *Artemisia* species,[135] and several other plants but here, it is reported as a new source compound from *A. incisa*.[1,22,49]

Table 2.33. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of ethyl-(*E*)-*p*-hydroxy cinnamate **(33)** in $CDCl_3$.

C. No.	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
2/6	2CH	6.82, (2H, d, *J* = 8.3)
3/5	2CH	7.41 (2H, d, *J* = 8.3)
2′	CH	6.29 (1H, d, *J* = 15.8)
3′	CH	7.63 (1H, d, *J* = 15.8)
1″	CH_2	4.20 (2H, q, *J* = 7.0)
2″	CH_3	1.17 (3H, t, *J* = 7.0)
4-OH	H	8.51 (1H, br. s)

2.8.9 Taraxerol (34).



Compound **34** was isolated as white crystals, m.p. 282–284 °C (lit. m.p. 282–283 °C) and $[\alpha]^{20}_{D}$ + 0.72 (*c* 0.974, $CHCl_3$).[21] The molecular formula of $C_{30}H_{50}O$ was established from LR-EIMS with *m/z* 426.38 [M^+].

The distinctive triterpene (*Taraxeranes* (*D-friedooleananes*) type skeleton)[122] was revealed through ^1H and ^{13}C-NMR spectra of the compound **(34)**.

The **Table 2.34** summarizes the ^1H, ^{13}C and HMBC assignment of the compound **34**.

Table 2.34. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of taraxerol **(34)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.72	CH_2	1.92 (1H, dd, *J* = 14.8, 3.2, Ha); 1.65 (1H, m, Hb)	C-2, C-3, C-5, C-9
2	27.15	CH_2	1.01 (1H, m, Ha); 1.60 (1H, m, Hb)	---
3	79.08	CH	3.15 (1H, dd, *J* = 10.8, 6.4)	C-2, C-4, C-23, C-24
4	38.98	C	---	---
5	55.54	CH	0.84 (1H, m)	---
6	18.80	CH_2	1.48 (1H, m, Ha); 1.51 (1H, m, Hb)	---
7	35.12	CH_2	0.97 (1H, m) 1.03 (1H, m)	---
8	38.76	C	---	---
9	48.76	CH	0.93 (1H, m)	---
10	35.79	C	---	---
11	17.50	CH_2	1.12 (2H, m)	---
12	36.68	CH_2	1.30 (2H, m)	---
13	38.00	C	---	---
14	158.10	C	---	---
15	116.88	CH	5.51 (1H, dd, *J* = 8.0, 3.2)	C-16, C-26
16	37.72	CH_2	1.61 (1H, m, Ha); 1.65 (1H, m, Hb)	C-18, C-15, C-14, C-17

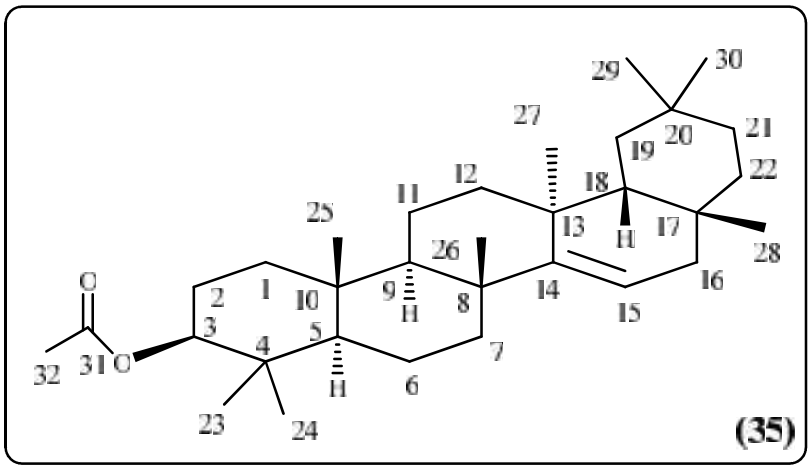
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C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
17	37.57	C	---	---
18	49.29	CH	1.39 (1H, m)	---
19	41.33	CH_2	2.01 (1H, m, Ha);	---
			1.39 (1H, m, Hb)	
20	28.93	C	---	---
21	33.70	CH_2	1.59 (2H, m)	---
22	33.10	CH_2	1.33 (2H, m)	---
23	28.00	CH_3	0.95 (3H, s)	C-3, C-4, C-5, C-24
24	15.45	CH_3	0.90 (3H, s)	C-3, C-4, C-5, C-23
25	15.42	CH_3	0.78 (3H, s)	C-1, C-2, C-5
26	29.92	CH_3	0.88 (3H, s)	C-14, C-9, C-7
27	25.91	CH_3	1.07 (3H, s)	C-8, C-14, C-18, C-19
28	29.83	CH_3	0.79 (3H, s)	C-16, C-17,
				C-18, C-22
29	33.35	CH_3	0.93 (3H, s)	C-20, C-21, C-22
30	21.31	CH_3	0.88 (3H, s)	C-20, C-21,
				C-22, C-18

The HMBC and COSY correlations **(Table 2.34)** established compound **34** as taraxerol[21] which has been reported from *Dandelion* and *Artemisia* species, *Taraxacum officinale, Alnusskimmia japonica, Rhododendron* species and several other plants.[1,85,122]

It is reported as a new source compound from *A. incisa*.

2.8.10 Taraxerol acetate (35).



(35)

Compound **35** was obtained as white crystals, m.p. 304–305 °C (lit. m.p. 304–305 °C).[21] The molecular formula of $C_{32}H_{52}O_2$ was established from LR-EIMS which showed the molecular ion peak at *m/z* 468.39 [M^+].

The distinctive triterpene (*Taraxeranes* (*D-friedooleananes*)) type skeleton[122] was determined through characteristic ^{1}H and ^{13}C-NMR data **(Table 2.35)** of the compound **35**.

By comparing the spectral (^{1}H and ^{13}C-NMR, HMBC and COSY) data and physicochemical constants of the compound **35 (Table 2.35)** with the compound **34** and literature, it was identified as taraxerol acetate.[21]

Table 2.35. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of taraxerol acetate **(35)** in $CDCl_3$.

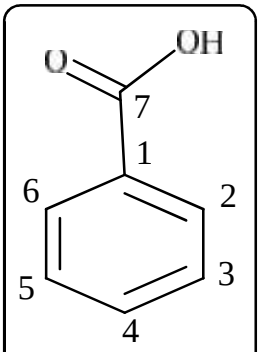
C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.72	CH_2	1.93 (1H, dd, *J* = 3.2, 14.8, Ha); 1.63 (1H, m, Hb)	C-2, C-3, C-5, C-9
2	24.87	CH_2	1.01 (1H, m, Ha); 1.61 (1H, m, Hb)	---
3	81.04	CH	4.45 (1H, dd, *J* = 10.8, 6.4)	C-23, C-24, C-4, C-2
4	37.90	C	---	---
5	55.66	CH	0.84 (1H, m, Ha)	---
6	18.70	CH_2	1.57 (2H, m)	---
7	35.13	CH_2	0.98 (1H, m, Ha); 1.04 (1H, m)	---
8	39.00	C	---	---
9	48.79	CH	0.93 (1H, m)	---
10	35.79	C	---	---
11	17.52	CH_2	1.43 (2H, m)	---
12	36.67	CH_2	1.30 (2H, m)	---
13	37.93	C	---	---
14	158.03	C	---	---
15	116.96	CH	5.52 (1H, dd, *J* = 8.0, 3.2)	C-16, C-26
16	37.56	CH_2	1.61 (1H, m, Ha); 1.65 (1H, m, Hb)	C-18, C-15, C-14, C-17
17	37.40	C	---	---
18	49.21	CH	1.31 (1H, m)	---

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C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)	HMBC Correlation
19	41.25	CH_2	2.01 (1H, m, Ha); 2.04 (1H, m, Hb)	---
20	28.80	C	--- --	
21	33.70	CH_2	1.59 (2H, m)	---
22	33.11	CH_2	1.33 (2H, m)	---
23	27.98	CH_3	0.84 (3H, m)	C-3, C-4, C-5, C-24
24	16.59	CH_3	0.86 (3H, m)	C-3, C-4, C-5, C-23
25	15.50	CH_3	0.93 (3H, m)	C-1, C-2, C-5
26	29.84	CH_3	0.89 (3H, m)	C-14, C-9, C-7
27	25.92	CH_3	1.07 (3H, m)	C-8, C-14, C-19
28	29.85	CH_3	0.80 (3H, m)	C-16, C-17, C-18, C-22
29	33.35	CH_3	0.93 (3H, s)	C-20, C-21, C-22
30	21.29	CH_3	0.88 (3H, s)	C-20, C-21, C-22, C-18
31	170.93	C	---	---
32	23.47	CH_3	2.02 (3H, d, J = 7.8)	C-31

Taraxerol acetate has been reported from *Alnus* species, *Lithocarpus* species, *Artemisia* species[20-21,122] and other plants[1] but here, it is reported from *Artemisia incisa* as new source compound.

2.8.11 Benzoic acid (36).



(36)

Compound **36** was obtained as colorless crystals having m. p. 122–124 °C (lit. m. p. 121–124 °C).[15] The molecular formula of $C_7H_6O_2$ was deduced from LR-EIMS at *m/z* 122.04 [M^+].

By comparing the spectral (^{1}H and ^{13}C-NMR) data and physicochemical constant of the compounds **36** with the literature, the structure was established as benzoic acid.[15]

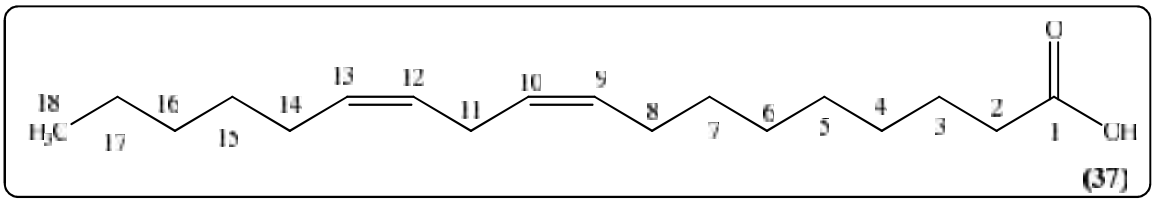
Benzoic acid is widespread in plants mostly in esterified form especially in essential oils. It has been isolated from a variety of biological sources like *Cucumis sativus*, S*tyrax streptomyces, Sasa Aspergillus, Lavendulae albomarginata, Fusarium oxysporum, Letharia vulpine, Rhizoctonia solani, odorus sporobolomyces, Lactobacillus plantarum* and *Artemisia annua*. It has also been isolated from the marine sponge *Halichondria okadai*.[1,22,82,136]

It is reported as a new source compound from *A. incisa*.

Table 2.36. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of benzoic acid **(36)** in $CDCl_3$.

C. No.	^{13}C-NMR (δ)	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
1	129.41	C	---
2/6	128.58	2CH	8.14 (2H, dd, *J* = 7.4, 1.2)
3/5	130.31	2CH	7.48 (2H, m)
4	133.92	CH	7.63 (1H, m)
7	172.5	C	---
7-OH	---	H	12.55 (1H, br. s)

2.8.12 Linoleic acid (37).



Compound **37** was obtained as white gummy solid, m.p. 29 °C (lit. m.p. 28–29 °C).[87] The IR spectrum showed absorption at ῡ 1710 and 1612 cm^{-1} attributed to COOH st. and C=C st. respectively. The LR-EIMS showed the molecular ion at m/z 280.24 [M^+] corresponding to $C_{18}H_{32}O_2$.

The spectral (^{1}H-NMR **(Table 2.37)**, IR and MS) and physical data of the compound **37** were compared with the literature and the structure was established as linoleic acid ((*9Z,12Z*)-octadeca-9,12-dienoic acid).[87]

Linoleic acid has been reported from the seeds of *Chilopsis linearis* and *Artemisia annua*.[1,22,87,136]

It is reported as a new source compound from *A. incisa*.

Table 2.37. ^{1}H-NMR (500 MHz), ^{13}C-NMR (100 MHz) and HMBC spectra of linoleic acid **(37)** in $CDCl_3$.

C. No.	Multiplicity	^{1}H-NMR (δ) (J_{HH} Hz)
1	C	---
2	CH_2	2.24 (2H, t, J = 7.5)
3	CH_2	1.58 (2H, m)
9, 13	CH	5.29 (1H, m)
10, 12	CH	5.32 (1H, m)
11	CH_2	2.75 (2H, m)
8, 14	$2CH_2$	2.02 (4H, m)
4–7, 15–17	$7CH_2$	1.24 (14H, m)
18	CH_3	0.86 (3H, t, J = 6.4)

2.8.13 Other compounds isolated (already discussed) from the aerial parts of *A. incisa*.

The structure of the other new source compounds; dracunculin **(13)** stigmasterol **(20)** and β-sitosterol **(21)** has been discussed already in **Sections 2.6.9,2.6.16** and **2.6.17** and **Tables 2.13, 2.20** and **2.21** respectively.

2.9 New source compound isolated from the roots of *Artemisia incisa*.

The new source compounds isolated from the roots of *Artemisia incisa* were including; stigmasterol **(20)**,[64] β-sitosterol **(21)**,[87] dracunculin **(13)**,[49] taraxerol acetate**(35)**,[21] taraxerol **(34)**,[21] ethyl-(*E*)-*p*-hydroxy cinnamate **(35)**,[15] scoparone **(29)**[86] and isoscopoletin **(30)**.[54] The results of the spectral data of these compounds have already been discussed in **sections 2.6.16, 2.6.17, 2.6.9, 2.8.10, 2.8.9, 2.8.8, 2.8.4, 2.8.5** and **Tables 2.20, 2.21, 2.13, 2.35, 2.34, 2.33, 2.29, 2.30** respectively.

Part A

Chapter 3

Experimental

3.1 General experimental conditions.

3.1.1 Melting point (m.p.).

Melting points were recorded on a Stuart digital melting point apparatus (SMP 10), Baby Scientific, UK and EZ-Melt automated melting point apparatus, Stanford Research Systems Product, California, USA and are uncorrected.

3.1.2 Optical rotations ($^{20}[\alpha]_D$).

Optical rotations were determined on Perkin Elmer 341 polarimeter at 20 ^oC using sodium D line (λ 589 nm).

3.1.3 UV-Vis spectra (UV-Vis).

Ultraviolet and visible (UV-Vis) spectra were recorded on Thermo Spectronic Unicam UV-300 spectrophotometer (USA) using vision 32 software (V1.25).

3.1.4 Infrared spectra (IR).

The IR spectra were taken as neat on JASCO FTIR-4200A (Japan) and Thermo Scientific FTIR Nicolet-380 (USA).

3.1.5 Mass spectra (MS).

Low resolution electron impact mass spectra (LR-EIMS) were recorded on JEOL (MS Route) instrument at HEJ, the University of Karachi, Pakistan. High resolution-electrospray ionization mass spectrometry (HR-ESIMS) was done with Thermo Scientific, LCQ Fleet instrument (USA) at the University of Strathclyde, Glasgow, UK.

The spectra were evaluated with the help of X-caliber software (Thermo scientific, USA).

3.1.6 Nuclear magnetic resonance spectra (NMR).

The 1D-NMR (^{1}H and ^{13}C-NMR) and 2D-NMR (NOESY, COSY, HSQC and HMBC) spectra were recorded in CD_3OD, C_5D_5N, $(CD_3)_2CO$ and $(CD_3)_2SO$ with JEOL FT (400, 500 MHz) NMR spectrophotometer at HEJ, the University of Karachi while with Bruker (Avance DRX-400, 500 and 600 MHz) and FT NMR spectrophotometer (400, 500 and 600 MHz) at SIPBS, the University of Strathclyde, Glasgow, UK. In ^{13}C-NMR, the types of carbon atom were assigned by using DEPT (135, 90). The 1D and 2-D NMR spectra acquired with JEOL were processed by JEOL DELTA (Japan) while that acquired with Bruker were processed with Top Spin S5 (Germany) softwares.

3.1.7 Solvents.

The solvents used were of analytical grade in case of prep. TLC, however, in case of extraction and column chromatography, commercial grade solvent were distilled before use.

3.2 Techniques employed for the purification of the compounds.

3.2.1 Column chromatography (CC) and sephadex LH20 column.

The fractionation and purification of the compounds from crude extract was done with column chromatography; glass column filled with silica gel 60 (0.063-0.200 mm Merck, Germany) as a stationary phase and organic solvents as the mobile phase. Sephadex LH20 (Amersham Pharmacia Biotech AB, Sweden) loaded on CC were also used for the purification of the compounds.

3.2.2 Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC).

All thin layer chromatographic (TLC) plates (20×20, 0.5 mm Merck, Germany) with precoated silica gel 60 F_{254} on aluminum sheets were used. The preparative thin layer chromatographic (PTLC) plates were prepared by smearing slurry of silica gel 60 F_{254} in distilled water on glass plates of 20×20 cm and activated at 100 ^{o}C.

3.3 Locating reagents for TLC.

The visualization of TLC plates were done by spraying with ceric sulphate or anisaldehyde solution.

3.3.1 Ceric sulphate solution.

The ceric sulphate 0.13 g were dissolved in 5 ml sulphuric acid and then diluted with water to 50 ml.

3.3.2 Anisaldehyde solution.

The anisaldehyde solution was made by mixing glacial acetic acid (>99 % pure), anisaldehyde and sulphuric acid (conc.) in the ratio of 98:1:1 (0.5 ml anisaldehyde, 0.5 ml H_2SO_4 and 49 ml of glacial acetic acid).

3.4 Plant materials.

Artemisia elegantissima and *Artemisia incisa* were collected from Kashmir (Pirchanasi, Muzaffarabad) in August, 2009 and Khyber Pakhtunkhwa (Samar Bagh, Lower Dir) in September, 2010 respectively. The plants were identified by Dr. Tanveer Akhtar,

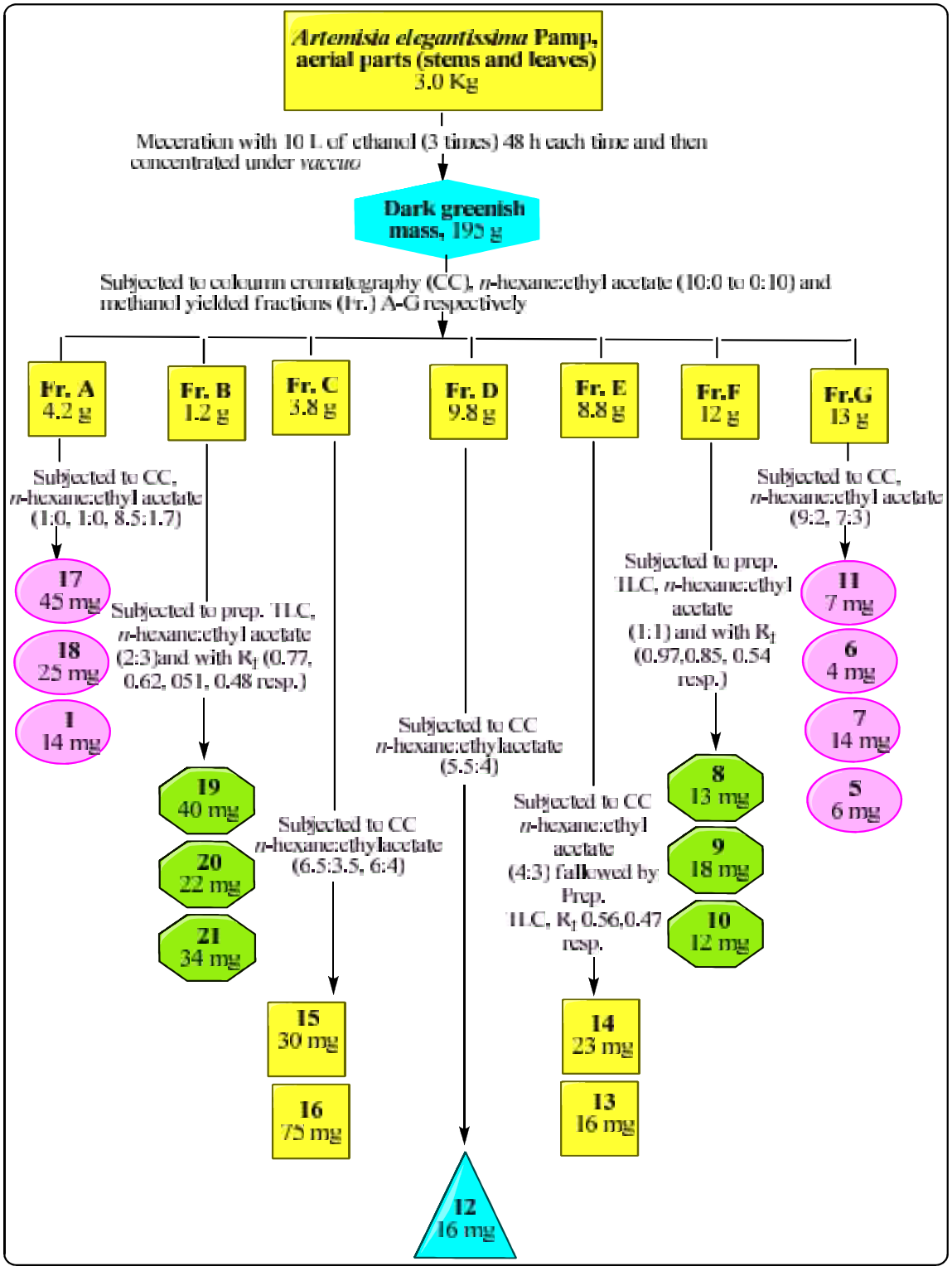
Department of Botany, University of Kashmir, Muzaffarabad, Kashmir. Voucher specimen number (Bot. 20021 (PUP and Bot. 20020 (PUP) respectively) were deposited in the Herbarium of the Botany Department, University of Peshawar, KPK, Pakistan.

3.4.1 General plan of extraction and isolation from the two *Artemisia* species.

3.4.2 Extraction, fractionation and isolation from the aerial parts (stems and leaves) of *Artemisia elegantissima*.

Air dried and pulverized aerial parts of *A. elegantissima* (3.0 kg) were extracted three times by maceration with 10 L of ethanol for 48 h each time. The extract was concentrated under *vacuuo,* to give a dark greenish mass (195 g) which was subjected to silica gel CC eluted with *n*-hexane:ethyl acetate (10:0 to 0:10) followed by methanol, yielded seven major fractions **A–G**. Fraction **A** (2.2 g) was subjected to CC eluting with *n*-hexane, yielded triacontanoic acid **(17) (**45 mg**)** and nonacosanol **(18) (**25 mg**)** while elution with *n*-hexane:ethyl acetate (8.5:1.7) yielded a new compound; artemglycerol (**1)** (16 mg). Fraction **B** (1.2 g) on CC eluting with *n*-hexane:ethyl acetate (8:1.8 to 7:3) yielded (*Z*)-*p*-hydroxycinnamic acid **(19) (**40 mg**)**, stigmasterol **(20) (**22 mg**)** and β-sitosterol **(21)** (34 mg**)**. Fraction **C** (3.8 g) on CC gave 105 sub-fractions, sub-fractions 22–36 and 44–87 eluted with *n*-hexane:ethyl acetate (6.5:3.5 and 6:4) yielded allograciolone acetate **(15)** (30 mg) and betulinic acid **(16)** (75 mg) respectively. Similarly, fraction **D (**9.8 g**)** on CC gave 20 sub-fractions, sub-fractions 9–16 eluted with *n*-hexane:ethyl acetate (4.5:2.0) yielded *bis*-dracunculin **(12)** (16 mg**)**, fraction **E** (8.8 g) gave 40 sub-fractions, sub-fractions 8–40 eluted with *n*-hexane:ethyl acetate (4:3) yielded a mixture of dracunculin **(13)** (16 mg) and scopoletin **(14)** (23 mg) which were purified

by prep. TLC eluting with *n*-hexane:ethyl acetate (4.5:3) (R_f 0.56 and 0.47 respectively). Fraction **F** (12 g) on prep. TLC eluting with *n*-hexane:ethyl acetate (1:1) yielded apigenin **(8) (**13 mg), dihydroluteolin **(9)** (18 mg) and scoparol **(10)** (12 mg) (R_f 0.97, 0.85 and 0.54 respectively). Fraction **G** (13.4 g) on prep. TLC eluting with *n*-hexane:ethyl acetate (2:3) yielded nepetin **(11) (**7 mg), bonanzin **(6) (**4 mg), 3′,4′-dihydroxybonanzin **(7)**, (14 mg) and 5,7,8,3',4'-pentahydroxy-6-methoxyflavone **(5)** (6 mg) (R_f 0.77, 0.62, 051 and 0.48 respectively). Apigenin (**8)** and dihydroluteolin **(9)** obtained above from prep. TLC were partially impure which were subjected to sephadex LH-20 column for further purification. The scheme of the above extraction, fractionation and isolation is given in **Scheme 3.1.**



Scheme 3.1. Scheme for extraction, fractionation and isolation from the aerial parts (stem and leaves) of *Artemisia elegantissima*.

3.4.3 Extraction, fractionation and isolation from the roots of *Artemisia elegantissima*.

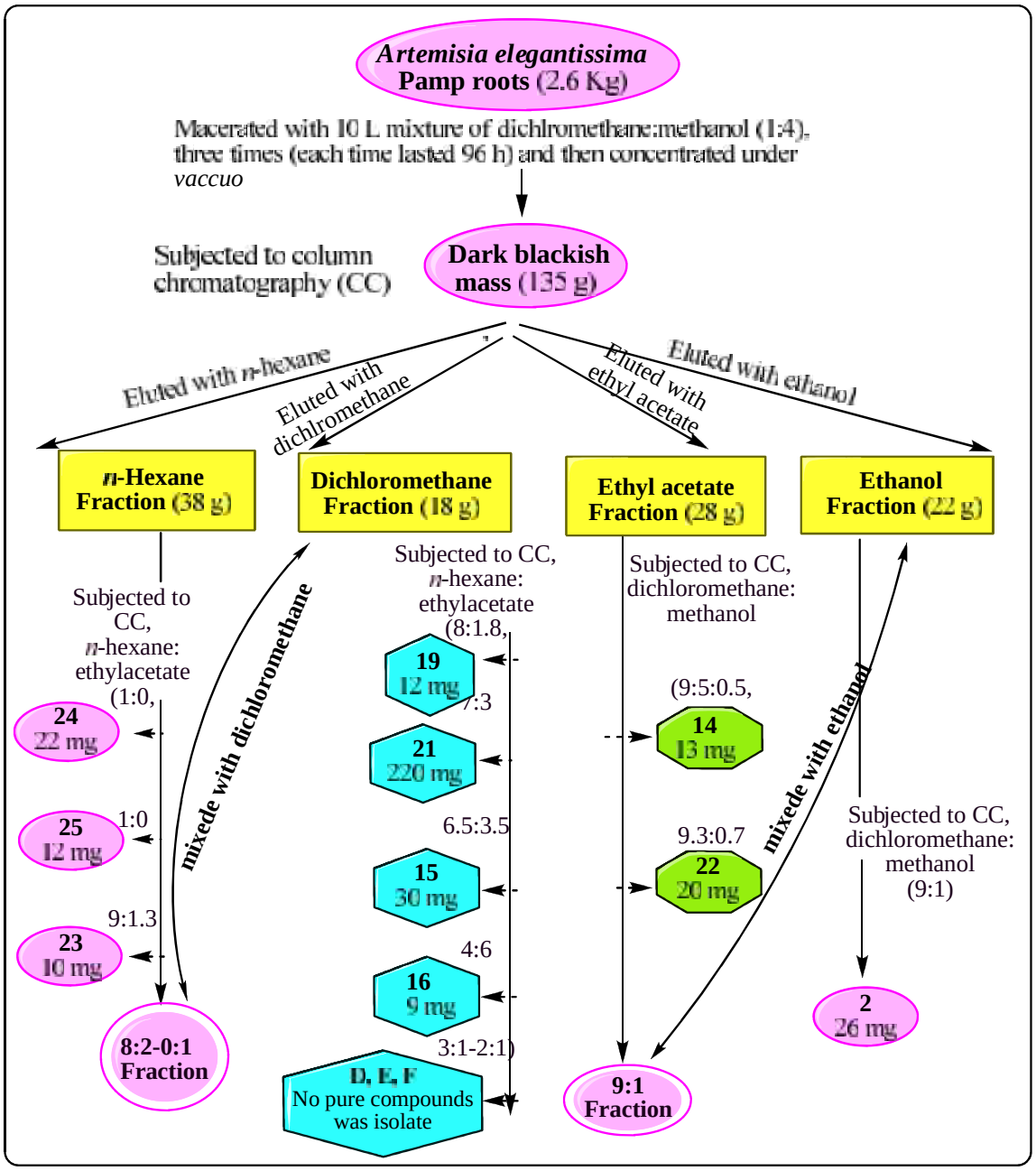
Air dried roots of *Artemisia elegantissima* (2.6 kg) were pulverized and extracted three times by maceration with 10 L mixture of dichloromethane:methanol (1:4) in a closed container (each time lasted 96 h). The extract obtained was concentrated under *vacuuo*, yielded a thick blackish, gummy mass (135 g) which was suspended in water and extracted successively with solvents; *n*-hexane, dichloromethane, ethyl acetate and ethanol yielding four major fractions; *n*-hexane (38 g), dichloromethane (18 g), ethyl acetate (28 g) and ethanol (22 g) respectively.

The *n*-hexane fraction (38 g) on CC eluted with *n*-hexane:ethyl acetate (10:1–1:10) yielded 90 sub-fractions in which the sub-fractions 1–9 and 10–23 eluted with *n*-hexane yielded eicosanoic acid (**25)** (22 mg) and octacosanoic acid (**24)** (12 mg) respectively. The sub-fractions 24-33 eluted with *n*-hexane:ethyl acetate (9:1.3) yielded dehydromatricaria ester **(23)** (10 mg) while the sub-fractions 34–90 were mixed with dichloromethane fraction due to similarity on TLC.

The dichloromethane fraction was subjected to CC eluted with *n*-hexane:ethyl acetate (9:1 7:2, 6:3, 4:1, 2:1, 0:1) yielded six fractions **A–F** respectively. Fraction **A** was subjected to CC eluted with *n*-hexane:ethyl acetate (8:1–8:2) yielded 33 sub-fractions in which the sub-fractions 24–29 eluted with *n*-hexane:ethyl acetate (8:1.8) yielded (*Z*)-*p*-hydroxycinnamic acid **(19)** (12 mg). Similarly, from fraction **B**, 80 sub-fractions were obtained with *n*-hexane:ethyl acetate (8:2–7:3) in which the sub-fractions 25–72 eluted with *n*-hexane:ethyl acetate (7:3) yielded β-sitosterol **(21)** (220 mg). While, from fraction

C, 72 sub-fractions were obtained on CC (eluted with *n*-hexane:ethyl acetate, 6.5:3.5–6:4). The sub-fractions 42–48 and 55–62 eluted with *n*-hexane:ethyl acetate (3.5:6.5 and 4:6) yielded allograciolone acetate **(15)** (30 mg) and betulinic acid **(16)** (9 mg) respectively. Fractions **D, E** and **F** were mixed and subjected to CC eluted with *n*-hexane:ethyl acetate (3:1–1:1) yielded 120 sub-fractions but no pure compound was isolated.

The ethyl acetate fraction was subjected to CC eluted with a mixture of dichloromethane:methanol (70:30–0:100). As a result 116 sub-fractions were obtained. The sub-fractions 18–40 eluted with dichloromethane:methanol (9.5:0.5 and 9.2:0.7), yielded scopoletin **(14)** (13 mg) and 3-O-β-D-sitosterol glucopyranoside **(22)** (20 mg). The sub-fractions 45–116 were mixed and again subjected to CC eluted with dichloromethane:methanol (9:1) yielded artemceramide-A **(2)** (26 mg). The scheme of the above extraction, fractionation and isolation is given in **Scheme 3.2.**



Scheme 3.2. Scheme for extraction, fractionation and isolation from the roots of *Artemisia elegantissima*.

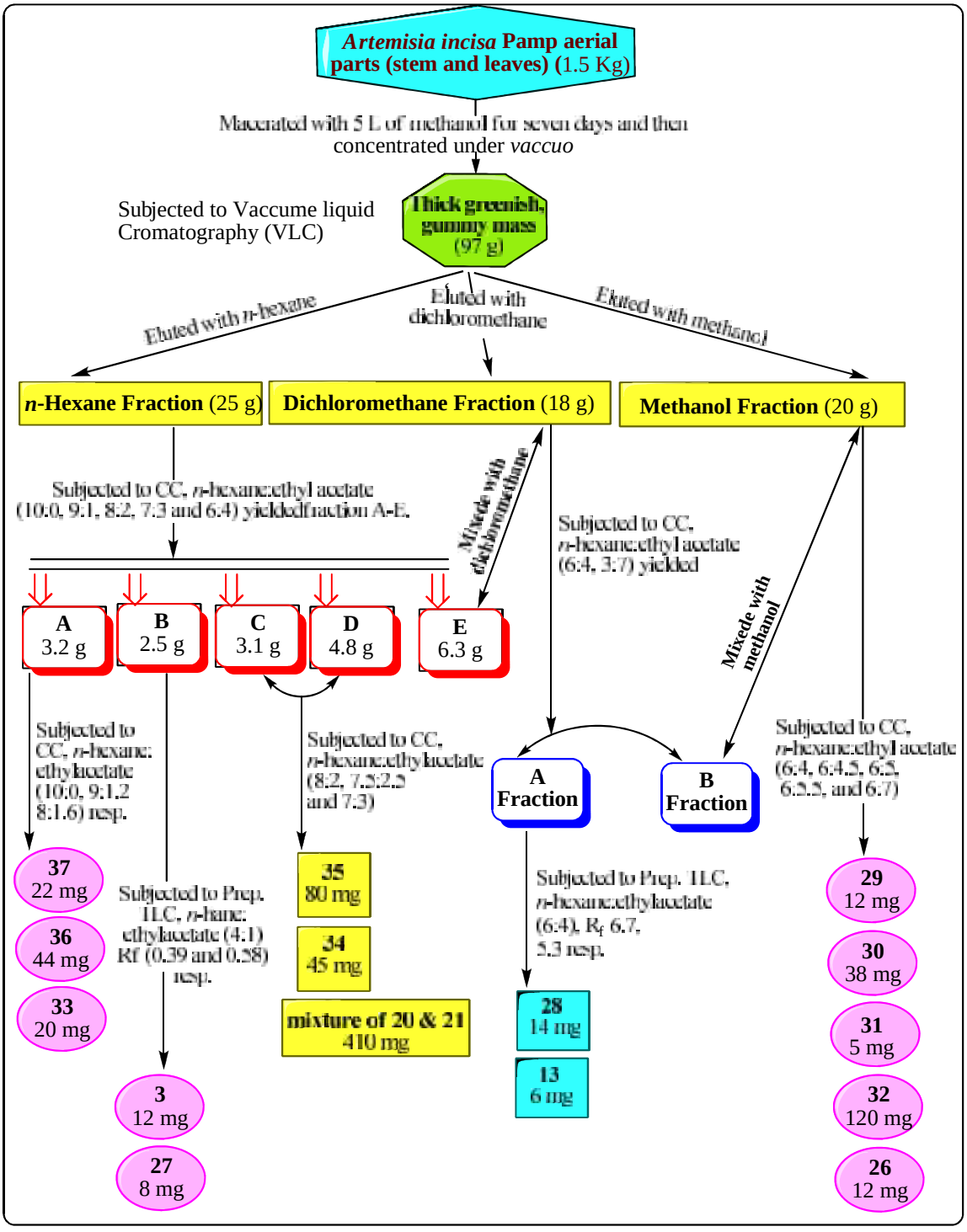
3.4.4 Extraction, fractionation and isolation from the aerial parts (stems and leaves) of *Artemisia incisa*.

The fresh aerial parts (stems and leaves) of *Artemisia incisa* (1.5 kg) were macerated with 5 L of methanol for seven days followed by concentration under *vacuuo*, yielding a greenish, gummy residue (97 g) which was subjected to vacuum liquid chromatography (VLC) eluted with *n*-hexane, dichloromethane and methanol successively, yielded three fractions; *n*-hexane (25 g), dichloromethane (18 g) and methanol (20 g).

The *n*-hexane fraction (25 g) was subjected to CC eluted with *n*-hexane:ethyl acetate (10:0, 9:1, 8:2, 7:3 and 6:4) yielded five fractions (**A**-**E)**. Fraction **A** (3.2 g) on CC eluted with *n*-hexane:ethyl acetate (10:0–5:5) yielded 60 sub-fractions. The sub-fractions 1–14, 15–21 and 35–44 eluted with *n*-hexane:ethyl acetate (10:0, 9:1.2 and 8:1.6) respectively, yielded linoleic acid **(37**, 22 mg), benzoic acid **(36)** (44 mg) and ethyl-(*E*)-*p*-hydroxy cinnamate **(33)** (20 mg). Fraction **B** (2.5 g) on prep. TLC eluted with *n*-hexane:ethyl acetate (2:8.5), R_f 0.39 and 0.58 yielded a new compound; santolinylol-3-acetate **(3)** (12 mg) and its known diol analogue; santolinylol **(27)** (8 mg) respectively. Fraction **C** (3.1 g) and **D** (4.8 g) were combined and were subjected to CC eluting with *n*-hexane:ethyl acetate (8:2, 7.5:2.5 and 7:3) yielded taraxerol acetate **(35)** (80 mg), taraxerol **(34)** (45 mg) and a mixture (410 mg) of stigmasterol **(20)** and β-sitosterol **(21)** respectively.

Fraction **E** (6.3 g) was mixed with dichloromethane fraction (18 g), subjected to CC eluted with *n*-hexane:ethyl acetate (6:4, 3:7) yielded sub-fraction **A** (5.8 g) and **B** (6.4 g) respectively. The sub-fraction **A** (5.8 g) was purified by prep. TLC eluted with

n-hexane:ethyl acetate (6:4) yielded *trans*-ethyl caffeate **(28)** (24 mg) and dracunculin **(13)** (6 mg), R_f 6.7, 5.3 respectively. The sub-fraction **B** (6.4 g) was mixed with methanol fraction (20 g) and then subjected to CC eluted with *n*-hexane:ethyl acetate (6:4, 6:4.5, 6:5, 6:5.5, and 6:7) yielded scoparone (**29)** (12 mg), isoscopoletin (**30)** (38 mg), esculetin **(31)** (5 mg), isofraxidin **(32)** (120 mg) and eupatorin **(26)** (12 mg) respectively. The scheme of the above extraction, fractionation and isolation is given in **Scheme 3.3.**

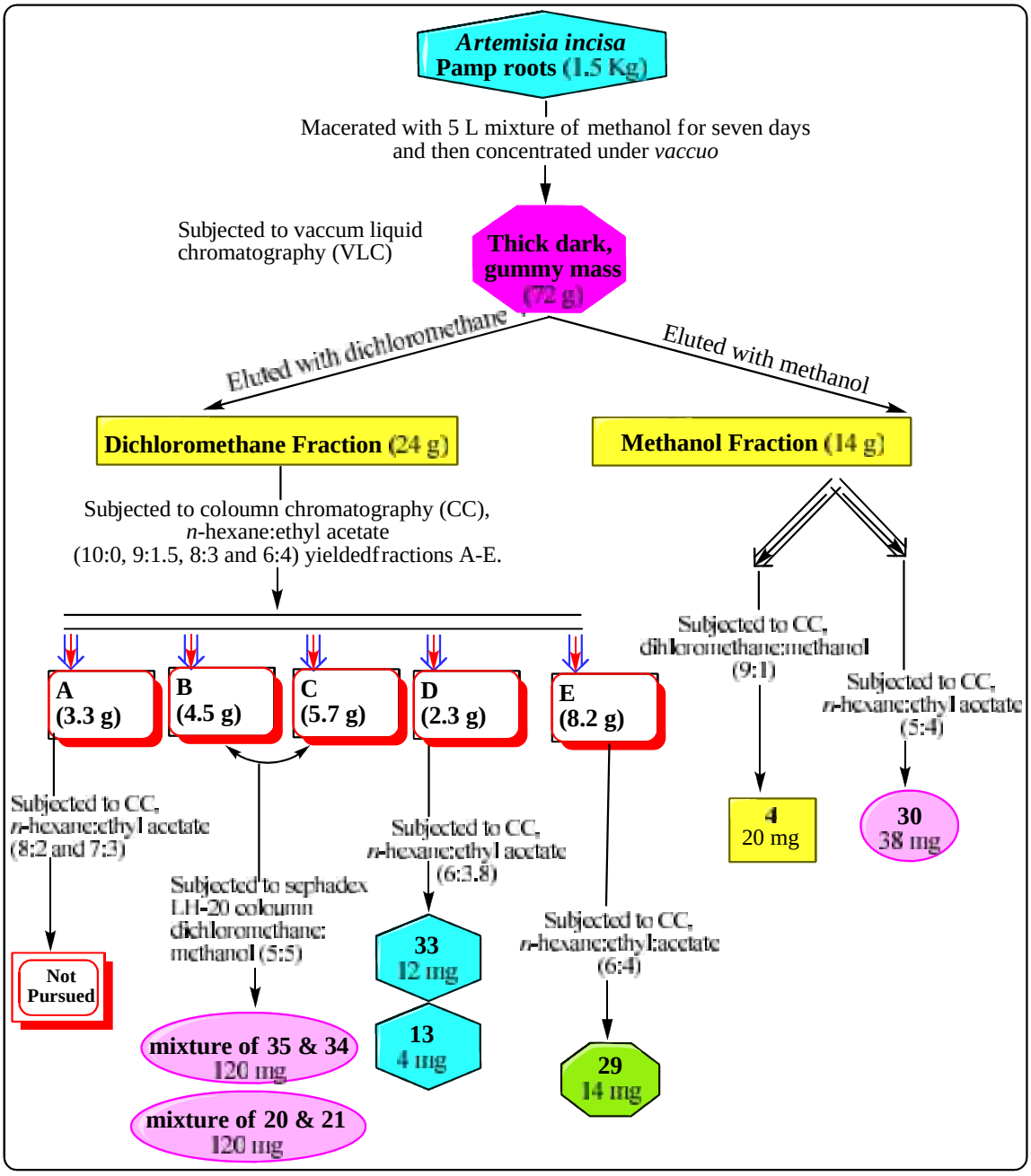


Scheme 3.3. Scheme for extraction, fractionation and isolation from the aerial parts (stems and leaves) of *Artemisia incisa*.

3.4.5 Extraction, fractionation and isolation from the roots of *Artemisia incisa*.

Air dried roots (1.5 kg) of *Artemisia incisa* were macerated with 5 L of methanol for seven days. The diluted extracts were concentrated under *vacuuo* yielding a thick gummy residue (72 g) which was subjected to vacuum liquid chromatography (VLC), eluted with dichloromethane followed by methanol and then concentrated under *vacuuo* to get two fractions of dichloromethane (24 g) and methanol (14 g) respectively.

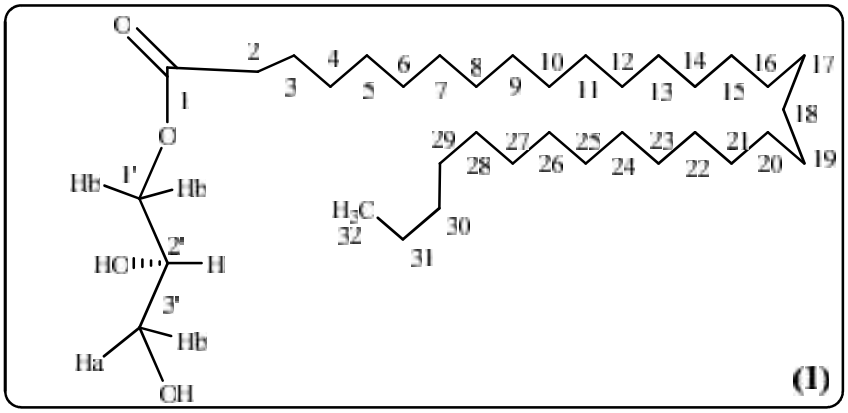
The dichloromethane fraction (24 g) on CC eluted with *n*-hexane:ethyl acetate (10:0, 9:1.5, 8:3 and 6:4) respectively, yielded five sub-fractions **(A-E)**. Sub-fraction **A** (0.8 g) was a mixture of oxygenated hydrocarbons (alcohols, fatty acids and esters) and not pursued further. The sub-fractions; **B** (4.5 g) and **C** (5.7 g) were mixed and subjected to CC eluted with *n*-hexane:ethyl acetate (8:2, 7:3) respectively, yielded two mixtures; a mixture (120 mg) of taraxerol acetate **(34)** and taraxerol **(35)** and a mixture (180 mg) of stigmasterol **(20)** and β-sitosterol (**21).** Fraction **D** (2.3) was passed through sephadex LH-20 column eluted with dichloromethane:methanol (5:5) yielded *trans*-ethyl caffeate **(28)** (12 mg) and dracunculin **(13)** (4 mg). Fraction **E** (8.2 g) on CC eluted with *n*-hexane:ethyl acetate (6:3.8) yielded scoparone **(29) (**14 mg). The methanolic fraction (14 g) was subjected to CC eluted with *n*-hexane:ethyl acetate (5:4) yielded isoscopoletin **(30)** (15 mg) while elution with dichloromethane:methanol (9:1) yielded a new compound; artemceramide-B **(4)** (20 mg**)**. The scheme of the above extraction, fractionation and isolation is given in **Scheme 3.4.**



Scheme 3.4. Scheme for extraction, fractionation and isolation from the roots of *Artemisia incisa*.

3.5 Experimental data of the new compound isolated from the aerial parts (leaves and stems) of *Artemisia elegantissima*.

3.5.1 (*R*)-2,3-Dihydroxypropyl dotriacontanoate (artemglycerol) (1).



(I)

White powder ($CHCl_3$).

Melting point: 81–82 °C.

Molecular formula: $C_{35}H_{70}O_4$.

HR-ESIMS: *m/z* 555.52 [M^++H] (Calcd. for $C_{35}H_{70}O_4$).

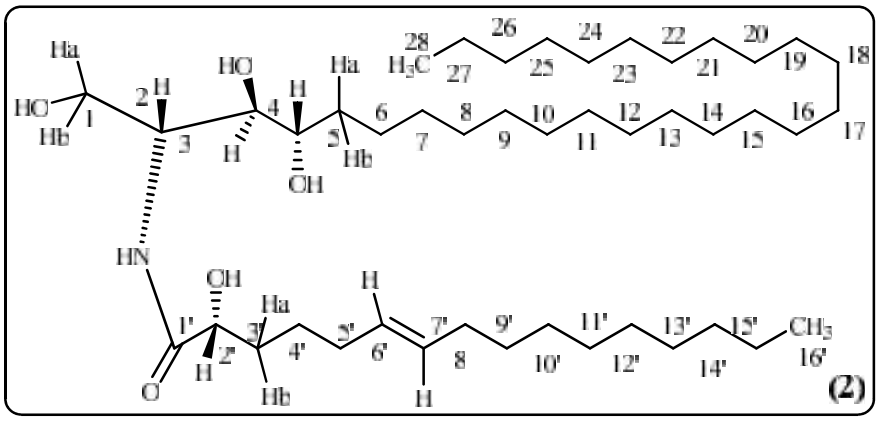
$^{20}[\alpha]_D$: - 0.011 (*c* 0.075, $CHCl_3$).

IR $\bar{\upsilon}$: 3460.11 (O-H st.), 2915.44, 2847.67 (C-H st.), 1732.60 (C=O st.), 1472, 1176, 730.28 (C-O and C-C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.1.**

3.6 Experimental data of the new compound isolated from the roots of *Artemisia elegantissima*.

3.6.1 (*R,E*)-2-Hydroxy-N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadec-6-enamide (artemceramide-A) (2).



White powder (C_5H_5N).

Melting point: 139–140 °C.

Molecular formula: $C_{44}H_{87}NO_5$.

HR-ESIMS: *m/z* 710.6662 [M^++H] (Calcd. for $C_{44}H_{87}NO_5$).

$^{20}[\alpha]_D$: + 8.88 (*c* 0.6, pyridine).

IR $\bar{\upsilon}$: 3328.5, 3246.6, 3218.6 (O-H and N-H st.), 2913.34, 2851.54 (C-H st.), 1621.8 (C=O st.), 1563.0, 1540.8 (C=C st.), 963.3, 720.3 (C-O and C-C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.2**.

3.6.1a Methanolysis of the compound 2.

Compound **2** (2.4 mg) was heated at 60 °C for 20 h in 5.0 mL of MeOH containing 3.0 ml of 0.9 N HCl under stirring to afford an *α*-hydroxy fatty acid methyl ester (FAME).

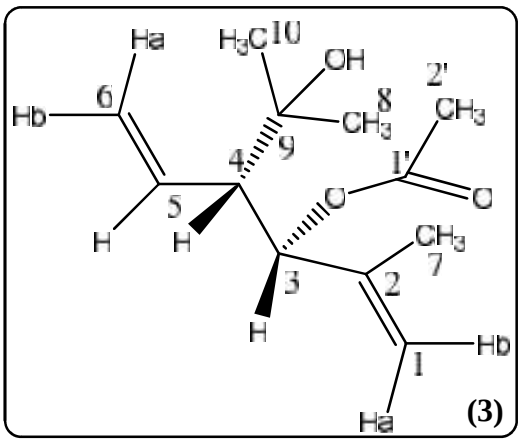
The mixture was neutralized with aqueous Na_2CO_3 solution and extracted with *n*-hexane. The FAME dissolved in *n*-hexane was carefully characterized by GC-MS analysis which gave the molecular ion peak [M^+] at *m*/*z* 284.23, Retention time (t_R): 4.87 min.

3.6.1b Oxidation of (*R*,*E*)-methyl 2-hydroxyhexadec-6-enoate.

(*R*,*E*)-Methyl 2-hydroxyhexadec-6-enoate (2 mg) was dissolved in CH_2Cl_2 (5 ml) to which a mixture of 5 ml of H_2SO_4 (10 %) and 100 mg of $KMNO_4$ was added and stirred for 12 h at room temperature.[112] The reaction was than quenched with aqueous $Na_2S_2O_3$ (5 %). The solvent was evaporated from the reaction mixture and then extracted with *n*-hexane. The organic layer was then concentrated, and subjected to GC/MS, t_R 12.06 min, EIMS; *m/z* 172 [M^+] (4), 155 [M-OH^+] (3), 143 (5), 129 (28), 87 (11), 73 (70), 60 [$CO_2H_2^+$] (100), which were characteristic of n-decanoic acid.

3.7 Experimental data of the new compounds isolated from the aerial parts (leaves and stems) of *Artemisia incisa.*

3.7.1 (*3R,4S*)-4-(2-Hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl-acetate) ((*3R,4S*)-santolinylol-3-acetate) (3).



(3)

Off white gummy oil ($CHCl_3$).

Molecular formula: $C_{10}H_{20}O_3$.

EIMS: *m/z* 212.14 [M^+] (calcd. for $C_{10}H_{20}O_3$).

EIMS: *m/z* (rel. int. %); 212 [M^+] (12.4), 195 [M^+–OH] (34.5), 169 (44.5), 153 [M–CH_3COO^+] (12), 121 (54.1), 95 (22), 73 (63), 59 (100), 55 (37.4), 43 (67.3).

UV (EtOH): λ_{max} (log ε); 242 (3.03) nm.

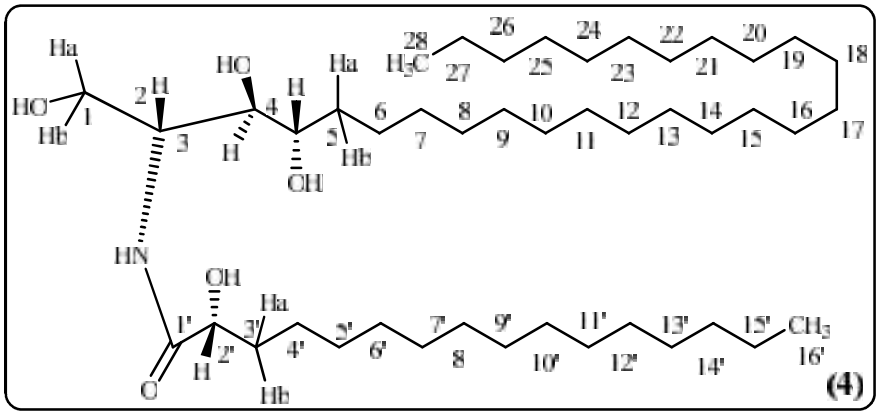
$^{20}[\alpha]_D$: - 9.1 (*c* 1.0, $CHCl_3$).

IR ῡ: 3335-3295 (O-H st.), 1737 (C=O st.), 1656, 1510 (C=C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.3.**

3.8 Experimental data of the new compound isolated from the roots of *Artemisia incisa*.

3.8.1 (*2′R*)-2′-Hydroxy-2N-((*2R,3R,4S*)-1,3,4-trihydroxyoctacosan-2-yl)hexadecanamide (artemceramide-B) (4).



White Powder (C_5H_5N).

Melting point: 141–142 °C.

$^{20}[\alpha]_D$: + 7.98 (*c* 0.07, pyridine).

IR $\bar{\upsilon}$: 3328.5, 3246.6, 3218.6 (O-H and N-H st.), 2916.32, 2849.14 (C-H st.). 1621.8 (C=O st.), 963.3, 720.3 (C-O and C-C st.) $cm.^{-1}$

Molecular formula: $C_{44}H_{89}NO_5$.

HR-ESIMS: *m/z* 712.6093 [M^++H] (Calcd. for $C_{44}H_{89}NO_5$).

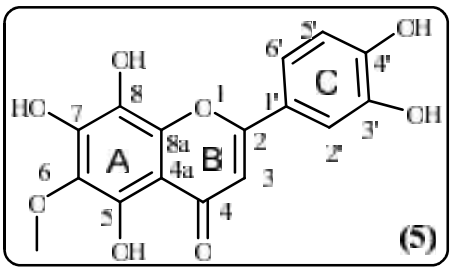
^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.4.**

3.8.1a Methanolysis of the compound 4.

Compound **4** (2.5 mg) was heated at 60 °C for 20 h in 5.0 mL of MeOH containing 3.0 mL of 0.9N HCl under stirring to afforded an *α*-hydroxy fatty acid methyl ester (FAME). The mixture was neutralized with aqueous Na_2CO_3 solution and extracted with *n*-hexane. The FAME was subjected to GC/MS analysis which gave the molecular ion [M^+] at *m/z* 286.23 (t_R: 4.87 min).

3.9 Experimental data of the new source compounds isolated from the aerial parts (leaves and stems) of *Artemisia elegantissima*.

3.9.1 5,7,8,3',4'-Pentahydroxy-6-methoxyflavone (5).[113]



Yellow prism like crystals (MeOH).

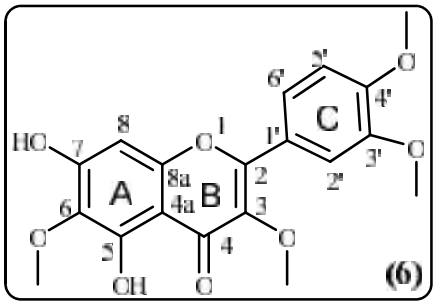
Melting point: 283–285 °C (lit. m.p. not reported).

Molecular formula: $C_{16}H_{12}O_8$.

HR-ESIMS: *m/z* 333.062 [M^++H] (calcd. for $C_{16}H_{12}O_8$.

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.5.**

3.9.2 Bonanzin (6).



Yellow crystals (MeOH).

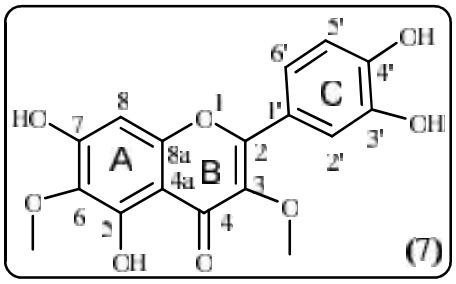
Melting point: 152–154 °C (lit. m.p. 152–153 °C).[64]

Molecular formula: $C_{19}H_{18}O_8$.

HR-ESIMS: *m/z* 375.100 [M^++H] (calcd. for $C_{19}H_{18}O_8$).

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.6.**

3.9.3 3′,4′-Dihydroxybonanzin (7).



Orange yellowish crystals (DMSO).

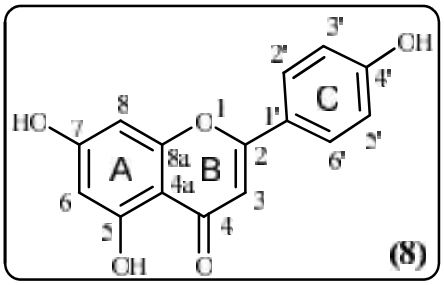
Melting point: 286–287 °C (lit. m.p. 284–288 °C).[64]

Molecular formula: $C_{17}H_{14}O_8$.

HR-ESIMS: 347.069 [M^++H] (calcd. for $C_{17}H_{14}O_8$).

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.7.**

3.9.4 Apigenin (8).



Yellow needles (MeOH).

Melting point: 351–352 °C (lit. m.p. 352 °C).[51]

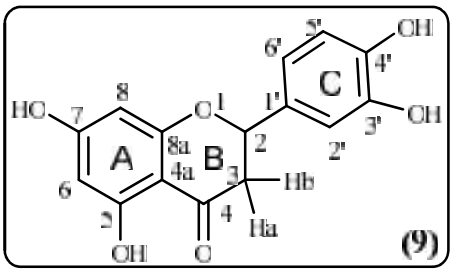
Molecular formula: $C_{15}H_{10}O_5$.

HR-ESIMS: *m/z* 271.052 [M^++H] (calcd. for $C_{10}H_{15}O_5$).

IR $\bar{\upsilon}$: 3422 (O-H st.), 1654 (C=O st.), 1612, 1520 (C=C st.), 1083 (C-O st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.8.**

3.9.5 (*S*)-Dihydroluteolin (9).



Yellow crystals (EtOAc).

Melting point: 265–267 °C (lit. m.p. 267 °C).[60]

Molecular formula: $C_{15}H_{12}O_6$.

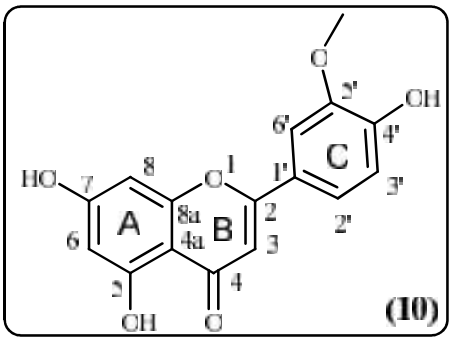
HR-ESIMS: *m/z* 289.063 [M^++H] (calcd. for $C_{15}H_{12}O_6$).

$^{20}[\alpha]_D$: - 47.1 (*c* 0.49, MeOH).

IR ῡ: 3400 (O-H st.), 1670 (C=O st.), 1620 (C=C st.) cm.$^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.9.**

3.9.6 Scoparol (10).



Yellow needles ($(CH_3)_2CO$).

Melting point: 330–332 °C (lit. m.p. 330–331 °C).[115]

Molecular formula: $C_{16}H_{12}O_6$.

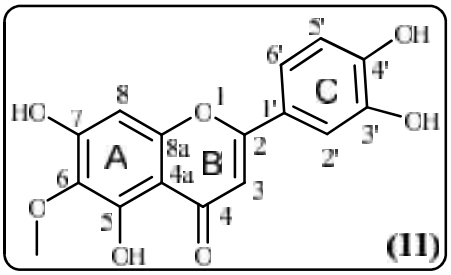
HR-ESIMS: 301.06 [M+H] (calcd. for $C_{16}H_{12}O_6$).

UV (EtOH): λ_{max} (log ε); 429 (3.1), 287 (2.2), 257 (3.9) nm.

IR ῡ: 3400 (O-H st.), 1670 (C=O st.), 1620 (C=C st.) cm.$^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.10.**

3.9.7 Nepetin (11).



Yellow prism like crystals ($(CH_3)_2CO$).

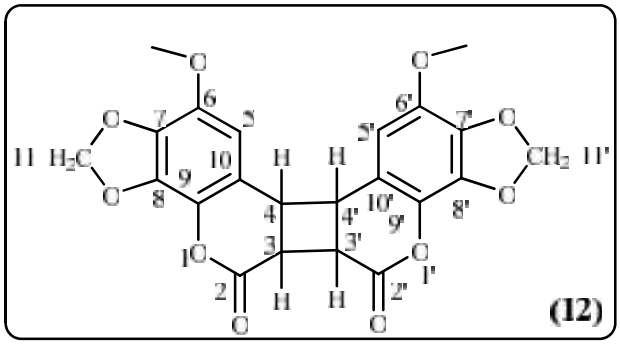
Melting point: 262–264 °C (lit. m.p. 261–263 °C).[116]

Molecular formula: $C_{16}H_{12}O_7$.

HR-ESIMS: *m/z* 317.067 [M+H] (calcd. for $C_{16}H_{12}O_7$).

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.11.**

3.9.8 *bis*-Dracunculin (12).



Colorless needles like crystals ($CHCl_3$).

Melting point: 138–139 °C (lit. m.p. 139 °C).[49]

Molecular formula: $C_{22}H_{16}O_{10}$.

EIMS: *m/z* 220.037 [½ M^+] (calcd. 240. for $C_{11}H_8O_5$).

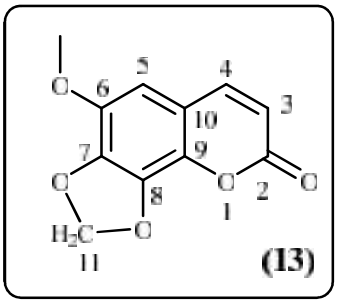
EIMS: *m/z* (rel. int. %); 220 (100), 205 (10.8), 192 (51.9), 177 (14.4), 147 (32.7), 121 (23.0), 107 (6.2), 91 (9.4), 79 (32.1), 76 (7.0), 75 (6.0), 74 (8.1), 63 (15.5), 51 (22.6).

HR-ESIMS: 441.0743 [M^++H] (calcd. for $C_{22}H_{16}O_{10}$).

IR ῡ: 1769 (C=O st.), 1514 (C=C st.), 937.4 (C-O st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.12.**

3.9.9 Dracunculin (13).



White yellowish crystals ($CHCl_3$).

Melting point: 220–222 °C (lit. m.p. 219–221 °C).[49]

Molecular formula: $C_{11}H_8O_5$.

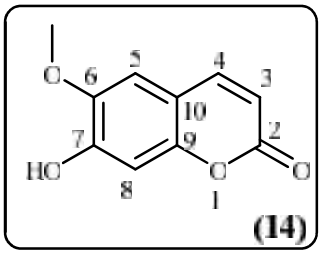
EIMS: *m/z* 220.037 [M^+] (calcd. for $C_{11}H_8O_5$).

EIMS: *m/z* (rel. int. %); 220 (100), 205 (11), 192 (22), 91 (19), 79 (21).

IR ῡ: 1769 (C=O st.), 1514 (C=C st.), 933.5 (C-O st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.13.**

3.9.10 Scopoletin (14).



Yellow greyish needles soluble in MeOH.

Melting point: 204–205 °C (lit. m.p. 205 °C).[119]

Molecular formula: $C_{10}H_8O_4$.

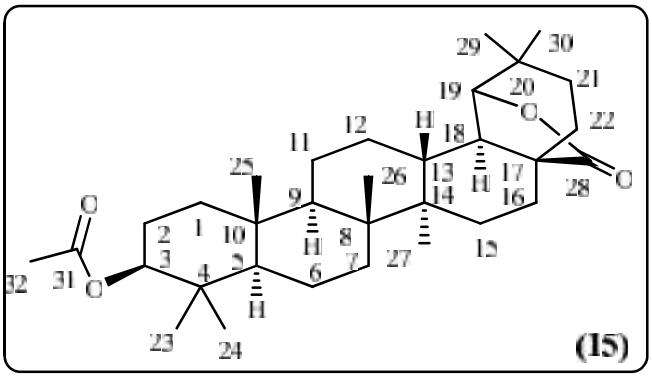
EIMS: *m/z* 192.042 [M^+] (calcd. for $C_{10}H_8O_4$).

UV (MeOH): λ_{max} (log ε); 344 (13500), 297 (9550), 260 (8710), 252 (9120), 229 (13800). By addition of sodium hydroxide solution bathochromic shift was observed from 340 to 390 nm.

IR $\bar{\upsilon}$: 3396 (O-H st.), 1713 (C=O st.), 1611, 1565, 1514 (C=C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.14.**

3.9.11 Allograciolone acetate (15).



White powder ($CHCl_3$).

Melting point: 307–311 °C (lit. m.p. 310 °C).[121]

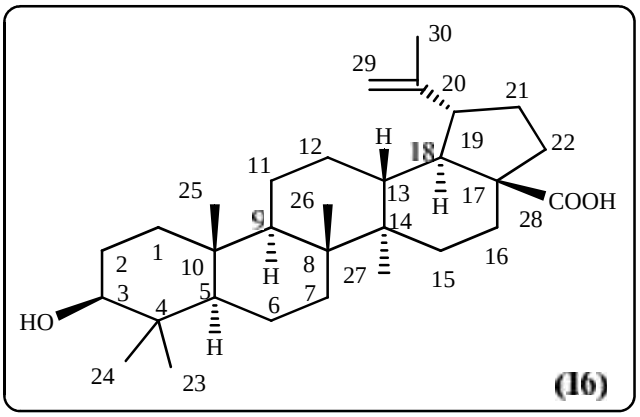
Molecular formula: $C_{32}H_{50}O_4$.

HR-ESIMS: *m/z* 499.37 [M+H] (calcd. for $C_{32}H_{50}O_4$).

$^{20}[\alpha]_D$: + 44 (*c* 0.8 in $CHCl_3$).

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.15.**

3.9.12 Betulinic acid (16).



White crystals ($CHCl_3$).

Melting point: 275–277 °C (lit. m.p. 275–278 °C).[123]

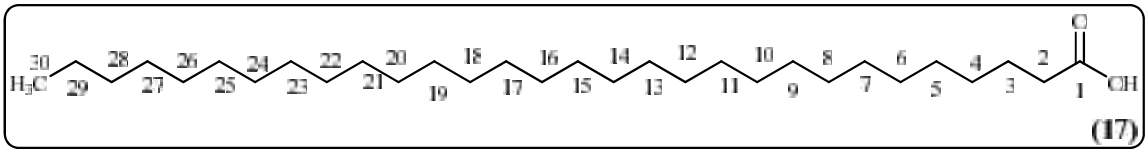
Molecular formula: $C_{30}H_{48}O_3$.

HR-ESIMS: *m/z* 456.36 [M^++H] (calcd. for $C_{30}H_{48}O_3$).

IR $\bar{\upsilon}$: 3449 (O-H st.), 1692 (C=O st.) $cm.^{-1}$

^{1}H-NMR and ^{13}C-NMR: given in **Table 2.16.**

3.9.13 Triacontanoic acid (17).



(17)

White powder ($CHCl_3$).

Melting point: 92–93 °C (lit. m.p. 92–93 °C).[31]

Molecular formula: $C_{30}H_{60}O_2$.

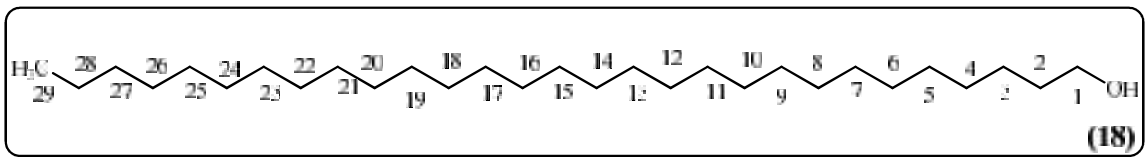
EIMS: *m/z* 452.06 [M^+] (calcd. for $C_{30}H_{60}O_2$).

EIMS: *m/z* (rel. int. %); 452 (4), 424 (22), 381 (32), 353 (2), 297(44), 269 (59), 241 (77), 185 (100), 129 (65), 97 (98).

IR $\bar{\upsilon}$: 3380–2620 (O-H st.), 1710 (C=O st.), 918 (C-O st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.17.**

3.9.14 Nonacosanol (18).



(18)

White powder (C_6H_{12}).

Melting point: 85 °C (lit. m.p. 85 °C).[31]

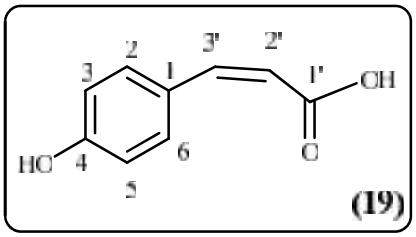
Molecular formula: $C_{29}H_{60}O$.

EIMS: *m/z* 426.46 [M^+] (calcd. 426.76 for $C_{29}H_{60}O$).

IR $\bar{\upsilon}$: 3280.33 (O-H st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.18.**

3.9.15 (*Z*)-*p*-Hydroxycinnamic acid (19).



(19)

White crystals ($CHCl_3$).

Melting point: 133–135 °C (lit. m.p. 134–134.5 °C).[132]

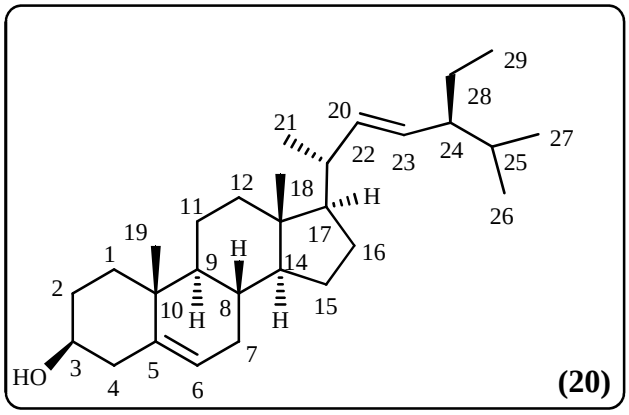
Molecular formula; $C_9H_8O_3$.

EIMS: *m/z* 164.047 [M^+] (calcd. for $C_9H_8O_3$).

IR $\bar{\upsilon}$: 3440, 3315 (O-H st.), 1625 (C=C st.), 1599 (C=C st.) $cm.^{-1}$

^{1}H-NMR and ^{13}C-NMR: given in **Table 2.19.**

3.9.16 Stigmasterol (20).



(20)

White powder ($CHCl_3$).

Melting point: 165–166 °C (lit. m.p. 165–167 °C).[64]

Molecular formula: $C_{29}H_{48}O$.

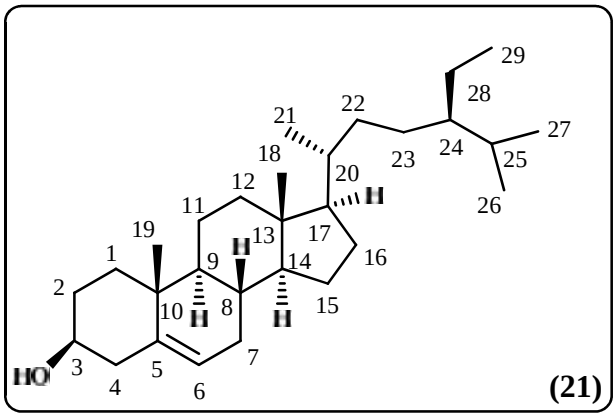
EIMS: *m/z* 412.37 [M^+] (calcd. for $C_{29}H_{48}O$).

EIMS: *m/z* (rel. int. %); 412.4 (21.2), 329.3 (15.7), 303.3 (15.7), 273.2 (17.2), 255.2 (29.9), 213.2 (28.0), 173.1 (16.0), 145.1 (42.2), 133.1 (34.1), 81.1(51.4), 55.1 (100).

IR ῡ: 3440 (O-H st.), 1622, 1599 (C=C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.20.**

3.9.17 β-Sitosterol (21).



(21)

White powder ($CHCl_3$).

Melting point: 135–136 °C (lit. m.p. 136 °C).[87]

Molecular formula: $C_{29}H_{50}O$.

EIMS: *m/z* 414.39 [M^+] (calcd. for $C_{29}H_{50}O$).

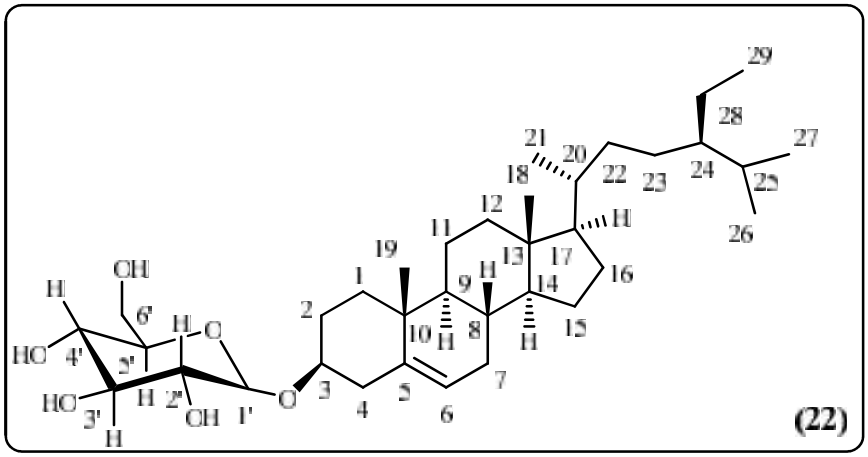
EIMS: *m/z* (rel. int. %); 414.4 (21.2), 381.4 (9.3), 329.3 (15.7), 303.3 (15.7), 273.2 (17.2), 255.2 (29.9), 213.2 (28.0), 173.1 (16.0), 145.1 (42.2), 133.1 (34.1), 81.1(51.4), 55.1 (100).

IR ῡ: 3440 (O-H st.), 1625, 1599 (C=C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.21.**

3.10 Experimental data of the new source compounds isolated from the roots of *Artemisia elegantissima*.

3.10.1 β-Sitosterol-3-*O*-D-glucopyranoside (22).



White powder (C_5H_5N).

Melting point: 232–236 °C (lit. m.p. 232–235 °C).[126]

Molecular formula: $C_{35}H_{50}O_5$.

EIMS: *m/z* 414.44 [M^+-glucose] (calcd. for $C_{35}H_{50}O_5$).

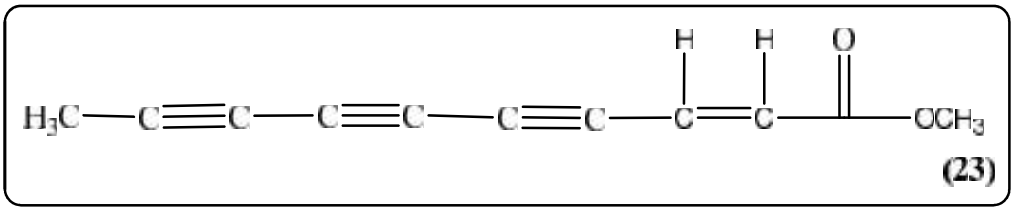
EIMS: *m/z* (rel. int. %); 414 (15), 399 (10), 396 (12), 381 (72), 329 (28), 275 (12), 273 (17), 255 (36).

HR-FABMS: *m/z* 576.4386 [M^+] (calcd. for $C_{35}H_{60}O_6$).

IR ῡ: 3450-3440 (OH st.), 1618, 1596 (C=C st.), 1379 and 1065, 1019 (C-O st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.22.**

3.10.2 *cis*-Dehydromatricaria ester (23).



(23)

Compound **23** was obtained as yellow crystals ($CHCl_3$). Initially, the color of the crystals was orange yellow, however, with the passage of time (drying; desolvation of crystals or due to light effect) the crystals color was changed to dark brown.

Melting point: 112–113 °C (lit. m.p. 112 °C).[29]

Molecular formula: $C_{11}H_8O_2$.

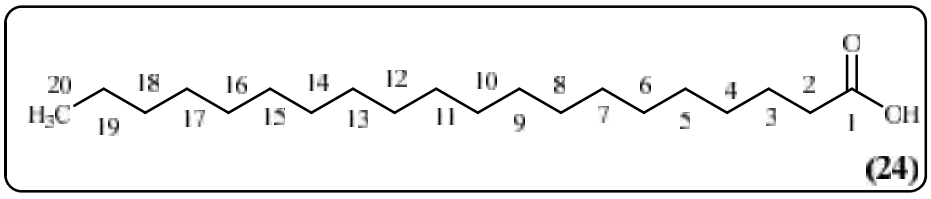
EIMS: *m/z* 172.05 [M^+] (calcd. for $C_{11}H_8O_2$).

EIMS *m/z* (rel. int. %); 172 (91.0), 157 (12.4), 144 (38.5), 129 (24.9), 113 (36.3), 101.1 (100), 87.1 (89.62), 75.0 (34.8), 63.0 (53.4), 51.0 (14.3).

IR $\bar{\upsilon}$: 2217.11, 2176.43 (C≡C st.), 1713.60 (C=O), 1596.24 (C=C st.), 995.45, 815.58 (C-O) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.23.**

3.10.3 Eicosanoic acid (24).



(24)

White powder ($CHCl_3$).

Melting point: 76–77 °C (lit. m.p. 76–76.5 °C).[26]

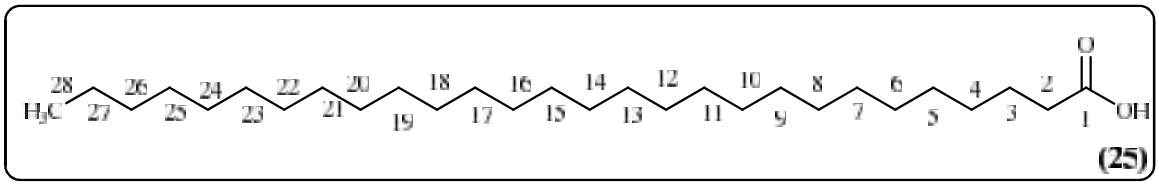
Molecular formula: $C_{20}H_{40}O_2$.

EIMS: *m/z* 254.34 [M^+] (calcd. 254 for $C_{20}H_{40}O_2$).

IR ῡ: 3419 (O-H st.), 1707 (C=O st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.24.**

3.10.4 Octacosanoic acid (25).



(25)

White powder ($CHCl_3$).

Melting point: 90–91 °C (lit. m.p. 90.3–90.5 °C).[26]

Molecular formula: $C_{28}H_{56}O_2$.

EIMS: *m/z* 424.38 [M^+] (calcd. 254 for $C_{28}H_{56}O_2$).

IR ῡ: 3419 (O-H st.), 1709 (C=O st.) $cm.^{-1}$

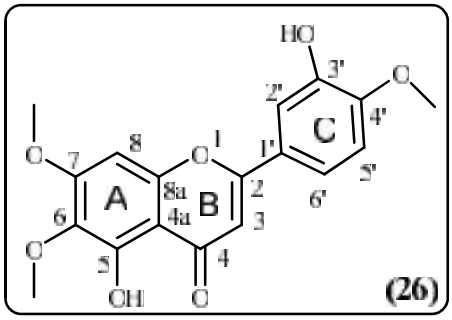
^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.25.**

3.10.5 Experimental data of the new source compounds (already discussed) isolated from the roots of *Artemisia elegantissima*.

The experimental data about the compounds; scopoletin **(14)**, allograciolone acetate **(15)** and betulinic acid **(16)**, (*Z*)-*p*-hydroxycinnamic acid **(19)**, and β-sitosterol **(21)** have been discussed in **Sections; 3.9.10, 3.9.11, 3.9.12, 3.9.15 and 3.9.17** respectively.

3.11 Experimental data of the new source compounds isolated from the aerial parts (leaves and stems) of *Artemisia incisa*.

3.11.1 Eupatorin (26).



Yellow crystals (MeOH).

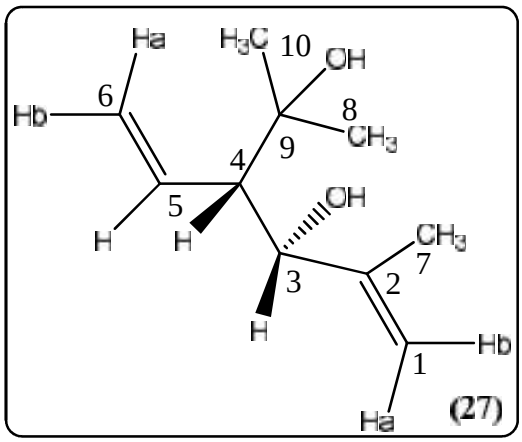
Melting point: 195–196 °C (lit. m.p. 195–196 °C).[62]

Molecular formula: $C_{18}H_{16}O_7$.

HR-ESIMS: *m/z* 345.088 [M^++H] (calcd. for $C_{18}H_{16}O_7$.

^{1}H-NMR and ^{13}C-NMR: given in **Table 2.26.**

3.11.2 Santolinylol (27).



Off white gummy solid ($CHCl_3$).[67]

Molecular formula: $C_{10}H_{18}O_2$.

EIMS: *m/z* 170.12 [M^+] (calcd. 170.14 for $C_{10}H_{18}O_2$).

EIMS: *m/z* (rel. int. %); 170 [M^+] (3), 152 [$M-H_2O^+$] (11), 55 (100).

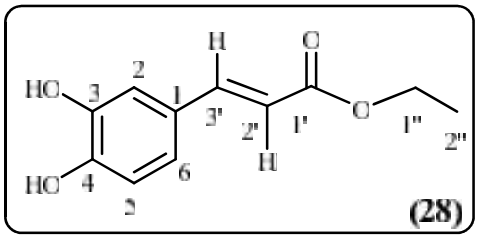
UV (MeOH): λ_{max} (log ε); 228 nm (2.30).

IR $\bar{\upsilon}$: 3290, 3190 (O-H st.), 1655, 1510 (C=C st.) $cm.^{-1}$

$^{20}[\alpha]_D$: - 14.2 (*c* 1.0, $CHCl_3$).[67]

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.27.**

3.11.3 *trans*-Ethyl caffeate (28).



White crystals soluble in $CHCl_3$.

Melting point: 148–150 °C (lit. m.p. 149–150 °C).[132]

Molecular formula: $C_{11}H_{12}O_4$.

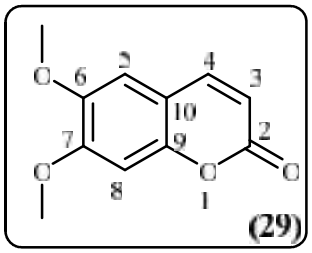
EIMS: *m/z* 208.1 [M^+] (calcd. for $C_{11}H_{12}O_4$).

EIMS: *m/z* (rel. int. %); 208 (92.0), 180 (19.9), 163 (100), 145 (11.9), 136 (37.4), 117 (10.8), 105 (3.9), 89 (18.5), 77 (9.1), 63 (8.2), 51 (6.8), 44 (5.6).

IR $\bar{\upsilon}$: 3478, 3315 (O-H st.), 1695 (C=O st.), 1658, 1603, 1518, 1465 (C=C st.), 1280 (C-O st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.28.**

3.11.4 Scoparone (29).



Greenish white needles like crystals (C_5H_5N).

Melting point: 145 °C (lit. m.p. 144–146 °C).[86]

Molecular formula: $C_{11}H_{10}O_4$.

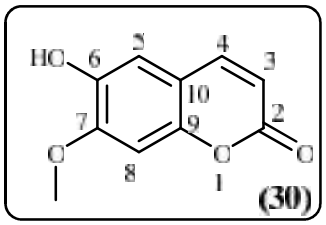
EIMS: *m/z* 206.058 [M^+] (calcd. 254 for $C_{11}H_{10}O_4$).

EIMS: *m/z* (rel. int. %); [M^+], 206 (100 %) [M - 15], 191 (50 %) [M - 28], 178 (18 %).

IR ῡ: 1713 (C=O st.), 1611, 1565, 1514 (C=C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.29.**

3.11.5 Isoscopoletin (30).



Grey white needles like crystals (C_5H_5N).

Melting point: 184–185 °C (lit. m.p. 185 °C).[54]

Molecular formula: $C_{10}H_8O_4$.

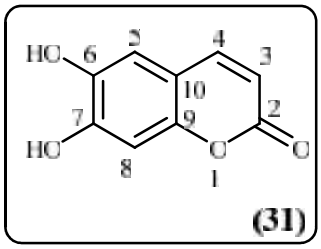
EIMS: *m/z* 192.042 [M^+] (calcd. for $C_{10}H_8O_4$).

EIMS: *m/z* (rel. int. %); 192 (100), 177 (70.3), 164 (34.4), 149 (63.4), 121 (24.7) 105 (2.6), 92 (2.9), 79 (13.4), 69 (22.0), 63.1 (3.4), 51 (9.5).

IR ῡ: 3396 (O-H st.), 1713 (C=O st.), 1611, 1565, 1514 (C=C st.) $cm.^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.30.**

3.11.6 Esculetin (31).



Greenish yellow crystals (C_5H_5N).

Melting point: 269–271 °C (lit. m.p. 270 °C).[133]

Molecular formula: $C_9H_6O_4$.

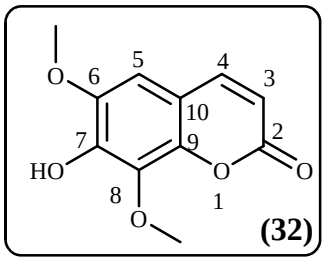
EIMS: *m/z* 178.027 [M^+] (calcd. for $C_9H_6O_4$).

EIMS: *m/z* (rel. int. %); 178 (100), 161 (38.8), 144 (17.9).

IR $\bar{\upsilon}$: 3400 (O-H st.), 1670 (C=O st.), 1620 (C=C st.) $cm.^{-1}$

^{1}H-NMR and ^{13}C-NMR: given in **Table 2.31.**

3.11.7 Isofraxidin (32).



Greenish yellow crystals (C_5H_5N).

Melting point: 148–149 °C (lit. m.p. 148–149 °C).[134a]

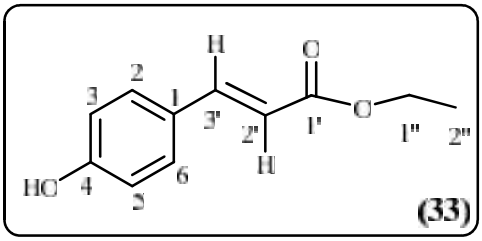
Molecular formula: $C_{11}H_{10}O_5$.

HR-ESIMS: *m/z* 223.053 [M+H] (calcd. for $C_{11}H_{10}O_5$).

IR ῡ: 3400 (O-H st.), 1670 (C=O st.), 1620 (C=C st.) cm.$^{-1}$

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.32.**

3.11.8 Ethyl-(*E*)-*p*-hydroxy cinnamate (33).



(33)

White needles ($CHCl_3$).

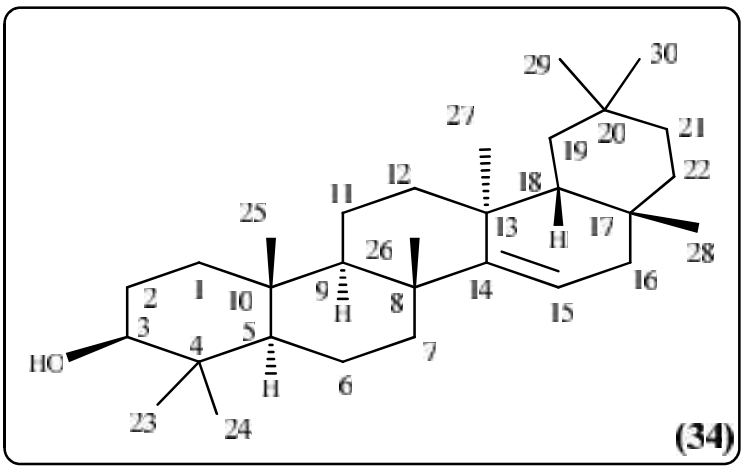
Melting point: 82–83 °C (lit. m.p. 83 °C).[15]

Molecular formula: $C_{11}H_{12}O_3$.

EI-MS: *m/z* 192.078 [M^+] (calcd. for $C_{11}H_{12}O_4$).

^{1}H-NMR: given in **Table 2.33.**

3.11.9 Taraxerol (34).



(34)

White crystals ($CHCl_3$).

Melting point: 282–284 °C (lit. m.p. 282–283 °C).[21]

$^{20}[\alpha]_D$: + 0.72 (*c* 0.974, $CHCl_3$).

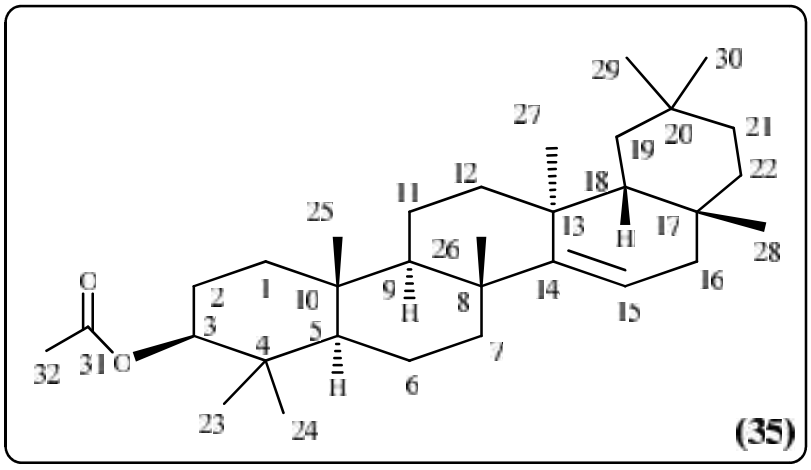
Molecular formula: $C_{30}H_{50}O$.

EIMS: *m/z* 426.38 [M^+] (calcd. for $C_{30}H_{50}O$).

EIMS: *m/z* (rel. int. %) 426 (59.4), 411 (45.1), 302 (100), 287 (84.7), 269 (43.6), 257 (29.9), 231 (20.7), 218 (43.4), 204 (100), 189 (46.9), 175 (30.2), 149 (37.6), 135 (77.1), 121 (56.3), 95 (51.9), 69 (52.2), 43 (33.8).

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.34.**

3.11.10 Taraxerol acetate (35).



(35)

White crystals ($CHCl_3$).

Melting point: 304–305 °C (lit. m.p. 304–305 °C).[21]

Molecular formula: $C_{32}H_{52}O_2$.

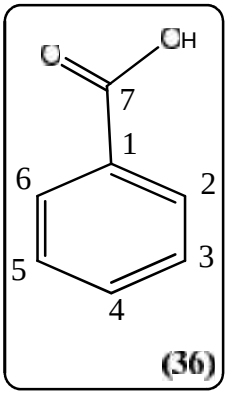
EIMS: *m/z* 468.39 [M^+] (calcd. for $C_{32}H_{52}O_2$).

EIMS: *m/z* (rel. int. %); 468 (63.4), 453 (44.1), 393 (14.0), 368 (13.9), 344 (90.9), 329 (59.3), 316 (7.9), 284 (19.2), 269 (49.6), 257 (24.1), 231 (16.0), 218 (28.3), 204 (100), 189 (40.7), 135 (46.4), 121 (34.1), 95 (29.3), 43 (38.8).

$^{20}[\alpha]_D$: + 9.0 (*c* 2.00, $CHCl_3$).

^{1}H-NMR, ^{13}C-NMR and HMBC: given in **Table 2.35.**

3.11.11 Benzoic acid (36).



(36)

Colorless crystals ($CHCl_3$).

Melting point: 122–124 °C (lit. m.p. 121–124 °C).[15]

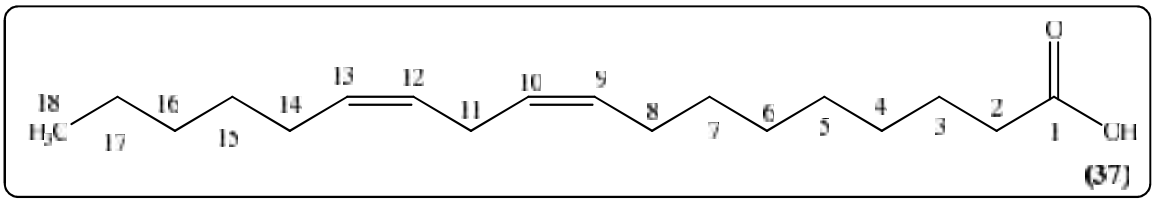
IR $\bar{\upsilon}$: 3250-2700 (O-H st.), 1704 (C=O st.), 1610 (C=C st.) $cm.^{-1}$

Molecular formula: $C_7H_6O_2$.

EIMS: *m/z* 122.04 [M^+] (calcd. for $C_7H_6O_2$).

^{1}H-NMR and ^{13}C-NMR: given in **Table 2.36.**

3.11.12 Linoleic acid (37).



(37)

Melting point: 29 °C (lit. m.p. 28–29 °C).[87]

Molecular formula: $C_{18}H_{32}O_2$.

EI-MS: *m/z* 280.24 [M^+] (calcd. for $C_{18}H_{32}O_2$).

IR $\tilde{\upsilon}$: 3433 (O-H st.), 1710 (C=O st.), 1612 (C=C st.) $cm.^{-1}$

^{1}H-NMR: given in **Table 2.37.**

3.11.13 Experimental data of the new source compounds (already discussed) isolated from the aerial parts of *Artemisia incisa*.

The experimental data about the compounds; dracunculin **(13)**, stigmasterol **(20)** and β-sitosterol **(21)** have been discussed in **Sections 3.9.9, 3.9.16** and **3.9.17** respectively.

3.12 Experimental data of the new source compounds (already discussed) isolated from the roots of *Artemisia incisa*.

The experimental data about the compounds; dracunculin **(13)**,[49] stigmasterol **(20)**,[64] β-sitosterol **(21)**,[87] scoparone **(29)**,[86] isoscopoletin **(30)**,[54] ethyl-(*E*)-*p*-hydroxy cinnamate **(33)**,[15] taraxerol acetate **(34)**[21] and taraxerol **(35)**[21] isolated from the roots of *A. incisa* have been discussed in **Sections 3.9.11, 3.9.16, 3.9.17, 3.11.4, 3.11.5, 3.11.8, 3.11.9** and **3.11.10** respectively.

Part B

Chapter 4

Introduction

4.1 General.

Diseases are responsible for almost 90 % deaths and mostly in the developing countries.[137] Out of the total 73 deadly diseases and infections documented worldwide, the six of them including; pneumonia, malaria, tuberculosis, measles, diarrheal diseases and HIV/AIDS account for almost half of all premature expiries, deaths of children and young adults. The multidrug resistance strains of malaria caused by *Plasmodium* species are infecting almost 300 million people per year in the world.[138]

It has been estimated that the number of flowering plants species in the world varied from 215,000 to 500,000. Only 15–18 % of these have been systematically explored in the discovery of biologically active substances. Hitherto, a large quantity of current drugs were derived from natural compounds, comprising semisynthetic and synthetic derivatives based on natural products prototypes.[139]

The genus *Artemisia* is morphologically unique (head-like inflorescences) and reflected to be one of the greatest evolutionary taxa in the dicotyledonous. The various species undergone through the process of taxonomical advancement may increase the chemical diversity and ecology as the advanced species may produce more complex (rearranged, cyclized and/or oxygenated) natural products.[111] This hypothesis has been protected by the reported phytochemical results relating the existence of varied constituents in *Artemisia* species. As the investigation carry on, this genus may demonstrate to be ironic source of lead compounds desired for the development of novel chemotherapeutic and/or agricultural agents.[22,49]

However, most of the biological assays reported on the constituents and extracts of *Artemisia* were carried out *in-vitro*. Meanwhile, a lot of products from the genus turn out to be failed when tested naturally. Therefore, biological and chemical concern on systematic basis is critically desired.[11,140]

4.2 Pharmacological importance of the genus *Artemisia*.

During the extensive exploration of the chemical constituents of *Artemisia*, much courtesy was paid to its bioactive components. According to the literature, some compounds from the genus were found to be antioxidant, antitumor, antipyretic, antihemorrhagic, antianginal, antiviral, anticoagulant, antihepatitis, antiulcerogenic, antispasmodic, antimalarial, interferon-inducing and anti-complementary.[111] The major bioactive constituents reported from the genus include; mono[141] and sesquiterpenoids,[142] α-tocopherol,[143] methyl jasmonate,[144] sterols,[145] phenyl propene,[146] isoprenylcoumaric acid derivatives,[147] caffeoylquinic acids,[148] phenoxychromene,[149] flavonoids,[149-150] coumarins[147, 151] and acetylenes.[152] Some plants of the genus *Artemisia* with active constituents and bioactivities are summarized in **Table 4.1**, and the active parts and fractions associated with particular biology are given in **Table 4.2.**

Table 4.1. Bioactive compounds from *Artemisia* species.

S. No.	Plant source.	Name of the compound.	Biological activity.
1	*A. abrotanum* L.	(*S*)-1-Hydroxy bisaboloxide acetate-A and isofraxidin.	Antimalarial.[153]
		3,5-Dimethoxy eupatolin.[153-154]	Spasmolytic.[155]
2	*A. absinthium* L.	Artemetin.[10,153]	Antitumor.[156]
		24-ξ-Ethylcholesta-7,22-dien-3β-ol.[154]	Antipyretic.[157]
3	*A. annua* L.	Artemisinin.	Antimalarial.[158]
		Arteannuin, artemetin chrysoplenetin, crisilineol.	Antimalarial.[159]
		Artemisic acid.	Antibacterial.[160]
		Scopoletin.	Anti-inflammatory.[160]
		Fisetin-3,7-dirhamnoside patuletin.	Angioterisin-converting enzyme inhibitory.[161]
		bis(1-Hydroxy-2-methylpropyl)phthalate.	Allelopathic.[162]
		Artemisinin, 6,7,3',4'-tetramethylether quercetagetin.	Antitumor.[85]
4	*A. argyi* Lev. et Van.	*seco*-Guaianolide.	Antitumor.[163]
		Eupatolin, β-sitosterol.	Antihemorrhagic.[164]
5	*A. austriaca* Jacq.	9-Hydroxy-1,8-cineole.	Aedes aegypti repellent.[165]
6	*A. barrelieri.*	Vulgarin, artemilin, berrelerin, barrelin.	Anti-inflammatory[166]
7	*A. borealis* Pall.	(*Z*)-3-Methylheptadeca-1,9,16-trien-4,6-diyn-8-ol, (*Z*)-3-methylheptadeca-1,9,16-trien-4,6-diyne.	Antimicrobial[167], larvicidal.[142b]

Continued on next page

S. No	**Plant source.**	**Name of the compound.**	**Biological activity.**
8	*A. caerulescens subsp. gallica.*	α-Santonin, β-santonin, arsubin.	Antipyretic.[167]
9	*A. capillaris* Thunb.	Crasimaritin, capillarisin.	Antitumor.[141a]
		Capillin.	Antiviral.[168]
		Eupatolitin, arcapillin, esculin, scopolin, isoscopoletin-*O*-glucoside.	Antihepatitis.[150]
		Scopoletin, scoparone.	Anticoagulant.[169]
		Scoparone.	Antianginal.[170]
		Scoparone, artepillin, capillartemisin B1, artepillin.	Choleretic.[171]
		Quercetin, α-tocopherol.	Antioxidant.[171-172]
		Scoparone.	Immunosuppressive.[173]
		Methyleugenol, capllarin, capillin.	Antifeedant.[174]
		Capillarol, capillin.	Plant growth regulatory.[175]
10	*A. cina* Berg. ex Pol.	α-Santonin.	Antibacterial (*Bacillus subtilis* and *B. cereus*).[176]
11	*A. douglasiana* Bess.	α-Hydroxycarvotagenone-3-ol, β-hydroxycarvotagenone-3-ol.	Hepatoprotective.[177]
12	*A. giraldi* Pamp.	6,2′-Dimethoxy flavone and 6,3′-dimethoxy flavone	Antimicrobial.[178]
13	*A. iwayomogi* Kitam.	Chlorogenic acid	Antioxidant.[179]

Continued on next page

S. No.	**Plant source.**	**Name of the compound.**	**Biological activity.**
14	*A. judaico.*	Cirsimaritin	Antispasmodic.[180]
15	*A. monosperma.*	7-Methyl ether eriodictyol, eupatolitin.	Antispasmodic.[181]
16	*A. motana* Pamp.	Caffeoyl chlorogenic acid, caffeoyl neochlorogenic acid.	Hemostatic.[182]
17	*A. myriantha* Wall. ex Bess.	Arglabin.	Antitumor.[183]
18	*A. nilagirica* Pamp.	Capillin.	Repellant to *Culex pipiens quinquefasciatus.*[184]
19	*A. pacifica.* Wall.	(*Z*)-3-Methylheptadeca-1,9,16-trien-4,6-diyn-8-ol.	Antimicrobial.[185]
20	*A. princeps* Pamp.	Chlorogenic acid, neochlorogenic acid.	Hemostatic.[182]
		Yomogin, genkwanin, jaceosidin, acacetin.	Cytotoxic.[147,185-186]
		Sulfated polysaccharides.	Anticoagulant.[185]
		Neutral polysaccharides.	Anticomplementary.[187]
21	*A. scoparia* Waldst. et Kit.	*p*-Hydroxyacetophenone.	Choleretic.[156]
22	*A. sieversiana* Ehrhart er Wild.	Artemetin, chrysoplenetin.	Antitumor.[156,188]

Continued on next page

S. No.	**Plant sources.**	**Name of the compound.**	**Biological activity.**
23	*A. stolinifera* Komar.	1-(2-Hydroxy-6-methoxyphenyl)ethanone-6-glucopyranoside.	Cytostatic.[189]
24	*A. subdigitata* Mattf.	Spathlenol	Antianginal.[190]
25	*A. sublessin-giana.*	Chryseriol, hispidulin, quercetin	Antimicrobial.[191]
26	*A. krash* ex Poijak.	Arsubin, hispidulin, jaceosidin, quercetin, 3-O-rutinoside isorhemnetin.	Enzyme regulator.[149,192]
27	*A. tridentata* Nutt.	Methyl jasmonate.	Plant growth regulator.[193]
28	*A. xanthochroa* Krasch.	7-Methyl ether eriodictyol.	Antitumor

Table 4.2. Bioactive fractions derived from *Artemisia* species.

S. No.	**Plant sources.**	**Bioactive fractions activities.**
1	*Artemisia* species.	Glycoproteins (interferon-inducing).[151]
2	*A. absinthium* L.	Aqueous MeOH extract (hepatoprotective).[145b]
3	*A. afra.*	Essential oils (antibiotic and antioxidant).[194]
4	*A. annua* L.	Extract (antipyretic).[160]
5	*A. apiacea* Hance.	Acidic polysaccharides (anticoagulant).[185]
6	*A. arborescens.*	Aqueous extract (antispasmodic).[195]
7	*A. asiatica* Nakai ex Pamp.	Essential oils (allelopathic).[196]
8	*A. asiatica* Nakai ex Pamp.	Essential oils (antibacterial).[147]
9	*A. Nakai* Pamp.	Hot-water extract (antibacterial).[147]
10	*A. capillaris* Thunb.	HPLC fractions (cytocidal).[141a]
		Ether, EtOAc and MeOH extracts (antioxidant).[172]
11	*A. cina* Berg. ex Poljak.	Total flavonoids (antitumor, antifungal).[197]
12	*A. dracunculus* L.	Essential oils (antifungal and antibacterial).[198]
13	*A. edgeworthil* Balakr.	Essential oils (antibacterial).[198]
14	*A. gmelini* Web. ex Stechm.	Essential oils (antifungal and antibacterial).[198]
15	*A. herba-alba.*	Essential oils (antifungal and antibacterial).[199]
16	*A. indica* Wild.	Extract (antifungal).[146]
17	*A. iwayomogi* Kitam.	Extract (hepatoprotective).[148a]
		Polysaccharide (immunomodulating and antitumor).[200]
18	*A. jacutica* Wild.	Essential oils (anti-inflammatory).[201]
		Essential oils (antifungal and antibacterial).[202]
19	*A. laciniata* Wild.	Essential oils (antibacterial).[198]

Continued on next page

S. No.	**Plant source.**	**Bioactive fractions activities.**
20	*A. macrocephaki jacq*. ex Bess.	Essential oils (anti-inflammatory).[201]
21	*A. maritima* L.	Essential oils (antifungal and antibacterial).[198]
22	*A. moorcroftiana* Wall. ex DC.	Essential oils (antifungal and antibacterial).[198]
23	*A. nilagrica* Pamp	Essential oils (antifungal and antibacterial).[198]
24	*A. parviflora* Buch.-Ham. ex Rox.	Essential oils (antifungal and antibacterial).[198]
25	*A. pontica* L.	Essential oils (anti-inflammatory).[201]
26	*A. princeps* Pamp.	Extract (antiaging).[152]
27	*A. princeps* Var. orientalis Hara.	Essential oils (allelopathic, antifungal and antibacterial).[203]
		MeOH extract (anti-inflammatory and analgesic).[204]
		Plant residues (allelopathic).[205]
28	*A. roxburghiana* Bess.	Essential oils (antibacterial and antifungal).[198]
29	*A. scoparia* Waldst. et Kirt.	Total flavonoids (antitumor).[197]
		Aqueous MeOH extract (hepatoprotective).[144]
		Essential oils (antifungal and antibacterial).[198]
30	*A. selengensis* Turcz. er Bess.	Flavonoids (antifungal).[148b]
		Polysaccharide parts (immunomodulating and antitumor).[200]
		Extract (hepatoprotective).[143]
31	*A. sieversiana* Wild.	Essential oils (anti-inflammatory).[201]
32	*A. stolonifera* Komar.	Flavonoids (antifungal).[148b]
33	*A. sublessingiana* Krasch.	Total flavonoids (antifungal).[197]
34	*A. tournefortiana* Reichb.	Essential oils (antifungal and antibacterial).[198]
35	*A. tridentata* Nutt.	Dry leaves (repellent to beetles).[206]

Part B

Chapter 5

Results and Discussion

5.1 Biological activities of *Artemisia elegantissima* and *Artemisia incisa*.

The selected fractions and compounds obtained from the above *Artemisia* species were studied for their anticancer, antidiabetic, antitrypanosomal, nematicidal and shpingokinase inhibition (anticancer) assays. The objective of our study was to assess these species for their bioactive constituents.

5.1.1 Anticancer activities.

The preliminary results obtained from the graphs shown as **Figures 5.1a, 5.1b and 5.1c** for the activities of L3.MR (apigenin; **8**), SB.MR (scopoletin; **14**), SC.MR (*trans*-ethyl caffeate; **28**) and SF.MR (taraxerol acetate; **35**) tested against the cancer cell lines; Human colon carcinoma cell line; HCT116, Adenocarcinomic human alveolar basal epithelial cells; A549 and Michigan cancer foundation-7; MCF7 have reported no effect in the MTT assay colorimetric assay based on tetrazolium reduction at the highest conc. of 100 μg/ml.

These samples are still under repeated testing and so should have results by the end of this year, and if there is any hint of an effect, dose response curves will be work out for these samples. But it should be noted that some of the compounds have been reported as anticancer in the literature but we have found negative results for these compounds.

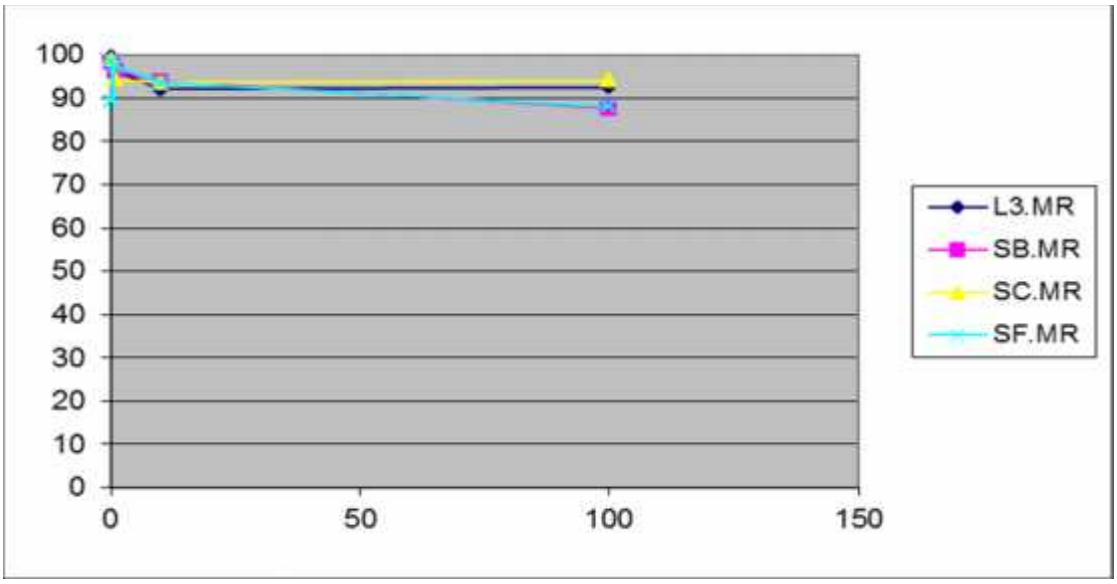


Figure 5.1a. Activities of L3.MR (apigenin) **(8)**, SB.MR (scopoletin) **(14)**, SC.MR (*trans*-ethyl caffeate) **(28)** and SF.MR (taraxerol acetate) **(35)** against HCT116 cell line.

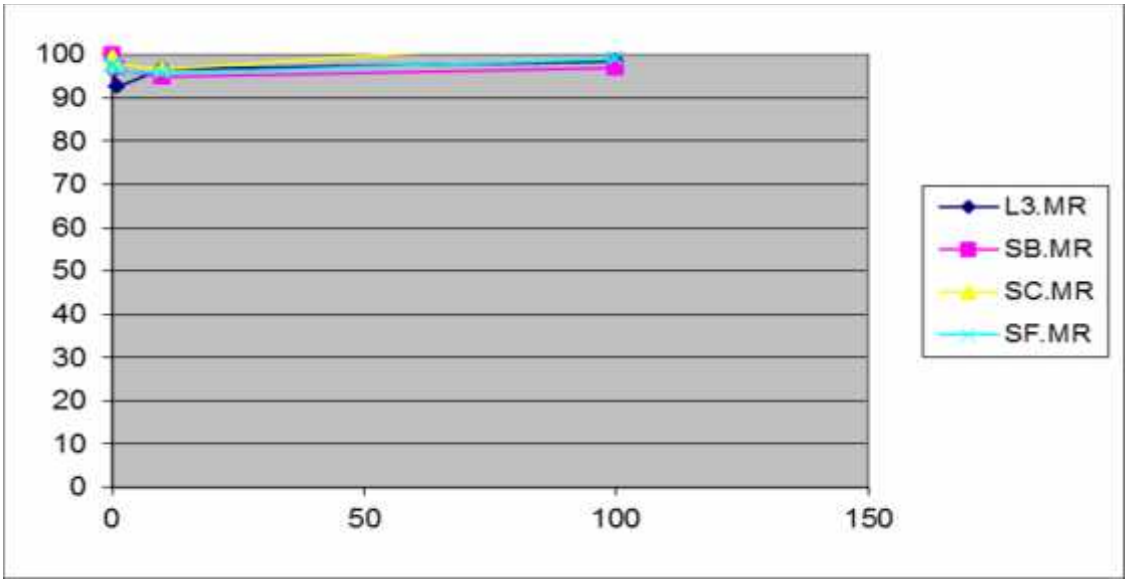


Figure 5.1b. Activities of L3.MR (apigenin) **(8)**, SB.MR (scopoletin) **(14)**, SC.MR (*trans*-ethyl caffeate) **(28)** and SF.MR (taraxerol acetate) **(35)** against A549 cell line.

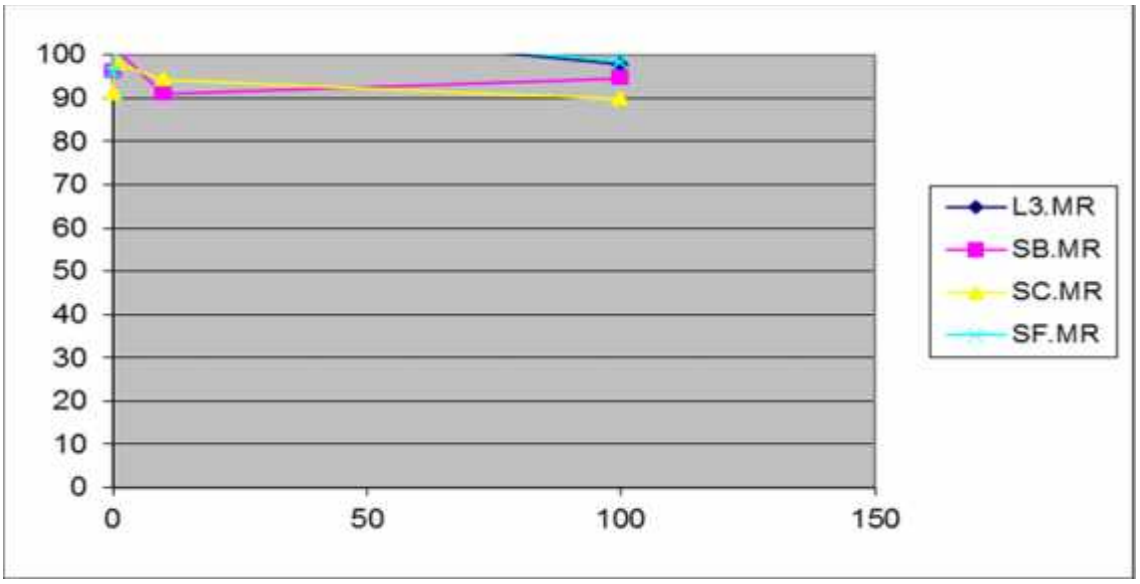


Figure 5.1c. Activities of L3.MR (apigenin) **(8)**, SB.MR (scopoletin) **(14)**, SC.MR (*trans*-ethyl caffeate) **(28)** and SF.MR (taraxerol acetate) **(35)** against MCF7 cell line.

5.1.2 Antidiabetic activities.

5.1.2a Antidiabetic activities of the fractions obtained from the aerial parts (stem and leaves) of *A. elegantissima*.

The screening of the samples was done at a concentration of 100 µg/ml. The fractions obtained from the aerial parts (stem and leaves) of *A. elegantissima* were tested against phospho-tyrosine protein phosphatase (PTP 1B). Samples which showed greater than 60 % inhibition (less than 40 % of the control) were selected to be probably active. Among the six fractions **(A–F)**, fractions **A** and **B** were inactive showing 88 % of the control and 76 % of the control respectively. The rest of the fractions; **C–F** were active, showed inhibition below 40 % of the control **(Table 5.1)**.

5.1.2b Antidiabetic activities of the compounds isolated from *A. elegantissima*.

The screening of the samples was done at a concentration of 100 µg/ml. The 13 compounds obtained from the six fractions **(A–F)** of the aerial parts (stem and leaves) of *A. elegantissima* were tested for their antidiabetic activity against PTP 1B. Among these compounds; bonanzin **(6)** and 3′,4′-dihdroxy bonanzin **(7)** were highly active which showed 22 % and 18 % of the control respectively. The other active compounds i.e., *bis*-dracunculin **(12)**, dracunculin **(13)**, scopoletin **(14)**, nepetin **(11)**, scoparol **(10)** and apigenin **(8)** also showed good inhibition (33, 35, 32, 30, 26, and 29 % of the control respectively). Among the flavonoids, the dihydroluteolin (**9**) (45 % of the control) was inactive and so were the sterols; stigmasterol **(20)** and β-sitosterol **(21)**.

Flavonoids **(6-11)** were more active than coumarins **(12-14)** and among flavonoids; flavones **(8-11)** were less active as compared to flavonols **(6-7)**. In case of flavonols, the substitution of methoxy groups with hydroxy groups increased the antidiabetic effect of the flavonols as depicted from the inhibition shown by bonanzin **(6)** and 3′,4′-dihydroxybonanzin **(7)** given in **Table 5.1.**

Table 5.1. Antidiabetic activities of the fractions and compounds isolated from *A. elegantissima*.

Fractions (100 µg/ml)	**Frc. A**	**Frc. B**	**Frc. C**	**Frc. D**	**Frc. E**	**Frc. F**	---	**Control**
% Control (100 µg/ml)	88	76	34	38	26	20	---	100
Compounds (100 µg/ml)	Cn	Bs	St	Bt	B-dr	Dr	Sc	**Control**
% Control (100 µg/ml)	39	86	80	56	33	35	32	100
Compounds (100 µg/ml)	Ap	Di	Sp	Np	Bn	Hbn	---	**Control**
% Control (100 µg/ml)	29	45	26	30	22	18	---	100

Frc. = Fraction, Cn = cinnamic acid, Bs = β-sitosterol, St = stigmasterol, Bt = betulinic acid, B-dr = bis-dracunculin, dr = dracunculin, Sc = scopoletin, Ap = apigenin, Di = dihydroluteolin, Sp = scoporol, NP = nepetin, Bn = bonanzin, Hbn = 3′,4′-dihydroxybonanzin.

5.1.3 Antitrypanosomal activities.

5.1.3a Antitrypanosomal activities of the fractions obtained from the aerial parts (stem and leaves) of *A. elegantissima*.

The six fractions **(A-E)** obtained from *A. elegantissima* were tested against *T.b. brucei.* In preliminary screening, the fractions whose values of % D control were less than 5 % were considered to be active. Fractions **A, B** and **C** were found to be inactive at all the three concentrations (5, 10 and 20 µg/ml). Fractions **D** and **E** were found to be active at the concentrations of 10 and 20 µg/ml, showed -6.7, -2.4 % D and -10.5, -6.0 % D respectively while these were found to be inactive at the concentrations of 5 µg/ml.

Fraction **F** was found to be active at all the three concentrations of 5, 10 and 20 µg/ml, showed -3.5, -5.7 and 2.1 % D respectively (**Table 5.2**).

Table 5.2. Antitrypanosomal activities of the fractions obtained from the aerial parts of *A. elegantissima*.

S. No	**Fractions**	***T.b. brucei*** **% of D control** **20 µg/ml**	***T.b. brucei*** **% of D control** **10 µg/ml**	***T.b. brucei*** **% of D control** **5 µg/ml**
1	Fraction **A**	22.9	82.5	91.5
2	Fraction **B**	7.4	1.8	11.4
3	Fraction **C**	5.7	0.3	2.4
4	Fraction **D**	-6.7	-10.5	81.4
5	Fraction **E**	-6.0	-2.4	86.3
6	Fraction **F**	-3.5	-5.7	2.1

T.b. brucei = Trypanosoma brucei brucei

5.1.3b Antitrypanosomal activities of the compounds isolated from the aerial parts (stem and leaves) of *A. elegantissima*.

Thirteen compounds **(6-14, 16, 19-21)** were obtained from the six fractions of the extract of *A. elegantissima* and were tested against *T.b. brucei.*

Out of the thirteen compounds **(6-14, 16, 19-21),** only three compounds **(6, 7** and **14)** were found to be active. Scopoletin **(14)** was found to be the most active compound (MIC: <0.19 µg/ml) and the flavonoids; 3′,4′-dihydroxybonanzin **(7)** (MIC: 6.25 µg/ml) and bonanzin **(6)** (MIC: 20 µg/ml) also showed good activities.

Table 5.3. Antitrypanosomal activities of the compounds isolated from the aerial parts of *A. elegantissima*.

S. No	**Name of the compound**	***T.b. brucei* % of D control 20 µg/ml**	***T.b. brucei* % of D control 10 µg/ml**	***T.b. brucei* MIC µg/ml**
1	(*Z*)-*p*-Hydroxycinnamic acid **(19)**	93.2	99.7	NC
2	ß-Sitosterol **(21)**	101.5	105.0	NC
3	Stigmasterol **(20)**	101.5	105.0	NC
4	Betulinic acid **(16)**	99.1	104.3	NC
5	*bis*-Dracunculin **(12)**	85.3	87.2	NC
6	Dracunculin **(13)**	89.0	106.2	NC
7	Scopoletin **(14)**	-0.4	-0.4	<0.19
8	Apigenin **(8)**	44.2	91.3	NC
9	Dihydroluteolin **(9)**	93.4	98.0	NC
10	Nepetin **(11)**	39.6	87.2	NC
11	Scoparol **(10)**	37.2	85.5	NC
12	Bonanzin **(6)**	-0.5	98.6	20
13	3′,4′-Dihydroxybonanzin **(7)**	-0.8	-1.2	6.25

NC = Not calculated

From the antitrypanosomal study of the six fractions **(A-F)** and thirteen compounds **(6-14, 16, 19-21)**, it was found that phenolics (coumarin and flavonoids) were the only active compounds against *T.b. brucei*. However, in case of phenolics, the molecular size, the number of active hydroxyl groups and substitution pattern of the hydroxyl group on coumarin and flavonoids skeleton played an important role in their antiparasitic potential against *T.b. brucei*.

The prominent activity of coumarin; scopoletin (**14**) (MIC: <0.19 µg/ml) showed that the phenolics with small molecular size and active hydroxyl group showed better antitrypanosomal activity. Among the flavonoids, the good activity of hexahydroxy flavonols; 3′,4′-dihydroxybonanzin (**7**) (MIC: 6.5 µg/ml) and bonanzin **(6)** (MIC: 20 µg/ml) showed that the flavonols with greater number of active hydroxyl groups showed high activity and methylation of hydroxyl group had a negative effect.

Literature has reported, *in-vitro* antiprotozoal activities of scopoletin (IC_{50}: >30, 31 and 90 µg/ml respectively) against *Leishmania donovani*, *T. brucei rodesiense* and *T. crusi* but it was not tested against *T.b. brucei*. Several compounds of coumarin class have showed antitrypanosomal (GAPDH inhibition) and anti-HIV (HIV protease inhibition) activities.[207] Furthermore, several inhibitors of the enzyme HIV integrase were structurally based on coumarins class of natural products.[187]

Among the flavonoids **(6-11**), the flavones **(8, 9** and **11**) were found to be less active than the flavonols **(6, 7** and **10)**. Also amongst the flavonoids **(6-11)**, the substitution of the hydroxy group with a methoxy group decreased the antitrypanosomal activity of the flavonoids at least two fold.

These results can help in directing the rational design of 3′,4′-dihydroxybonanzin **(7)** (flavonoid) and scopoletin **(14)** (coumarin), as other similar secondary metabolites or synthetic analogs as potent and effective antiprotozoal agents against *T.b. brucei*. These types of analogues could be further explored in *in-vivo* model studies against *T.b. brucei* as well as extended to other trypanosomal and protozoal parasites.

5.1.4 Antibacterial activities (*M. marinum, EMRSA* and *SMRSA*) of the compounds isolated from the aerial parts (stem and leaves) of *Artemisia elegantissima*.

Ten **(6-14)** out of thirteen **(6-14, 19-21)** compounds having different skeleton including; phenyl propanoids, triterpenes, coumarins and flavonoids were found to be active against *M. marinum* at a conc. of 100 µg/ml, *EMRSA* and *SMRSA* at conc. of 200 µg/ml respectively. The antibacterial screening data are given in **Table 5.4.**

Table 5.4. The antibacterial screening against *M. marinum* and *EMRSA*, *SMRSA* of the compounds isolated from *Artemisia elegantissima* and *Artemisia incisa*.

Compounds	***M. marinum*** **% of D control 100µg/ml**	***SMRSA*** **% of D control 200µg/ml**	***EMRSA*** **% of D control 200µg/ml**
(*Z*)-*p*-Hydroxycinnamic acid **(19)**	13.4	3.9	7.4
β-Sitosterol **(21)**	16.3	23.4	18.2
Stigmasterol **(20)**	14.7	20.1	16.9
Betulinic acid **(13)**	4.6	9.4	2.7
bis-Dracunculin **(12)**	-1.2	5.3	0.3
Dracunculin **(13)**	3.3	6.9	1.2
Scopoletin **(14)**	4.4	2.1	2.1
Apigenin **(8)**	3.6	1.9	-1.2
Dihydroluteolin **(9)**	4.6	4.5	2.9
Scoparol **(10)**	3.9	1.1	-3.2
Nepetin **(11)**	3.8	2.8	1.3
Bonanzin **(6)**	2.9	-1.0	-4.6
3′,4′-Dihydroxybonanzin **(7)**	0.3	-2.7	-10.8

The antibacterial screening against the three bacterial strains showed that the flavonone; 3′,4′-dihydroxybonanzin **(7)** was the most active compound at a conc. of 200 µg/ml against both *SMRSA* and *EMRSA* (-2.7 and -10.8 % D control respectively). Bonanzin **(6)** also showed good activity (-1.0 and -4.6 % D control respectively) followed by scoparol **(10)** (1.1 and -3.2 % D control respectively) and apigenin **(8)** (1.9 and -1.2 % D control respectively) against both *SMRSA* and *EMRSA* at a conc. of 200 µg/ml. Among the compounds; nepetin **(11)**, scopoletin **(14)** and (Z)-*p*-hydroxycinnamic acid **(19)** were

found to be active against *SMRSA* showing 2.8, 2.1 and 3.9 % of D control respectively. While at a conc. of 200 µg/ml, nepetin **(11)**, dihydroluteolin **(9)**, scopoletin **(14)**, dracunculin **(13)**, *bis*-dracunculin **(12)** and betulinic acid **(13)** were found to be active against *EMRSA* showing 1.3, 2.9, 2.1, 1.2, 0.3 and 2.7 % of D control respectively as shown in **Table 5.3.**

bis-Dracunculin **(12)** (-1.2 % of D control) was found to be the most active and selective compound against *M. marinun* at conc. of 100 µg/ml, while dracunculin **(13),** apigenin **(8),** scoparol **(10),** nepetin **(11),** bonanzin **(6)** and 3′,4′-dihydroxybonanzin **(7)** were also found to be active against *M. marinun* showing 3.3, 3.6, 3.9, 3.8, 0.3 % of D control respectively at a conc. of 100 µg/ml. The other compounds were found to be inactive against *M. marinun* as shown in **Table 5.3.**

5.1.5 Nematicidal activities of the compounds isolated from *Artemisia elegantissima* and *Artemisia incisa*.

The 16 compounds isolated from the methanolic extract of the two *Artemisia* species were divided into four classes on the basis of their structure including; phenyl propanoids, coumarins, flavonoids and triterpenes. The four classes of *Artemisia* phytochemicals with their unique SAR is bidden to be established and linked with targeted nematicidal activity, through this study.

The nematicidal activity was performed at three conc. of 100, 200 and 300 µg ml^{-1} and LC_{50} µg ml^{-1} values were calculated for almost all the tested compounds. Esculetin **(31)** was found to be the most active nematicidal (LC_{50}: 90.4 µg ml^{-1}) followed by isofraxidin **(32)** (LC_{50}: 93.0 µg ml^{-1}) and ethyl caffeate **(28)** (LC_{50}: 99.5 µg ml^{-1}). Scopoletin **(14)** and

isoscopoletin **(30)** were also found to be active (LC_{50}: 144.5 and 146.8 µg ml^{-1} respectively). The SAR showed that the high activity of coumarins was due to the small molecular size and the presence of active hydroxyl or methoxy groups in the phenyl group. The other compounds were found to be less active which are given in **Table 5.4.**

Literature has reported many classes of natural products which have been evaluated for their antagonistic applications against nematodes; however, due to non-systematic approach *i.e.*, avoiding the structure activity relationship (SAR) of the different classes of natural products, the development of new nematicidal has become a difficult task.

Once the SAR of these nematicidal is established, the compounds could be produced on commercial basis either by the cultivation of plants or the alternatives methods of synthesis or genetic engineering. These compounds could also be produced as by-products from the biomass of *Artemisia annua* as the plant is already cultivated for the production of artemisinin on commercial basis.[22]

Table 5.5. Nematicidal activities of the compounds isolated from *Artemisia elegantissima* and *Artemisia incisa*.

Compound Name	**100 µg ml^{-1}**	**200 µg ml^{-1}**	**300 µg ml^{-1}**	**LC_{50} µg ml^{-1}**
Esculetin **(31)**	56.6	78	100	90.4
Scopoletin **(14)**	36.3	69	95.67	144.5
Isoscopoletin **(30)**	38.3	62.3	90.33	146.8
Dracunculin **(13)**	43	70.67	98	138.5
bis-Dracunculin **(12)**	31.3	56.3	82	189.9
Apigenin **(8)**	30.7	48.67	74.67	205.7
Nepetin **(11)**	39	56.3	82.77	198.6
Bonanzin **(6)**	41. 67	59	82	197.9
trans-Ethyl caffeate **(28)**	51.3	72	94.33	99.5
Taraxerol **(34)**	40.7	67	90.33	161.9
Betulinic acid **(16)**	50	69.3	93	110.6
Allograciolone acetate **(15)**	40.3	65.33	86.67	171.6
Artemglycerol **(1)**	42.3	72.67	92.67	173.9
Linoleic acid **(37)**	33.7	51.3	79	190.6
Taraxerol acetate **(33)**	43.3	57.67	71	168.9
Isofraxidin **(32)**	53.3	73.67	89	93.0
Water/Avermectin	18.7	19.3	20	

5.1.6 Anticancer studies (Sphingosine Kinase 1 and 2 inhibition) of artemceramide-A (2) and artemceramide-B (4).

There is substantial evidence that the two mammalian isotypes, SPHK1 and SPHK2 are promising anti-cancer therapeutic targets including; proliferation, cell death and autophagy. Sphingosine kinase (SPHK), which catalyzes the phosphorylation of sphingosine to breed sphingosine 1-phosphate have involve positive selection of cancer cells and sphingosine up regulation. The enzyme SPHK1 and SPHK2 are potential target in cancer chemotherapy.

The present results revealed the novel anti-cancer potential of a sphingosine analogue (ceramides) that acts as a SPHK2 inhibitor, induces apoptosis via phosphorylation of ceramides through SPHK2.

The new compounds; artemceramide-A **(2)** and artemceramide-B **(4)** at a conc. of 4 mM were found to be SPHK2-specific inhibitor showed inhibition of 43 % and 49 % respectively **(Table 5.5)**. It accelerates fast-mediated cell death progression in the murine B lymphoma-derived cell line A20/2J. The artemceramides-A **(2)** was more active than the artemceramides-B **(4)** which was possibly due to unsaturation in the first one as compared to saturation in the second one**.** In the present study, it is demonstrated that artemceramides-A **(2)** and artemceramides-B **(4)** induce apoptosis via phosphorylation by SPHK2 and these are considered to be SPHK2 specific inhibitor, can exert its effect on cell death independently. While against SPHK1, the compounds; artemceramides-A **(2)** and artemceramides-B **(4)** showed minimum inhibition. The preliminary data of

inhibition of SPHK1 and SPHK2 with artemceramide-A **(2)** and artemceramides-B **(4)** at the concentration of 4mM is given in **Figure 5.2a** and **5.2b** respectively.

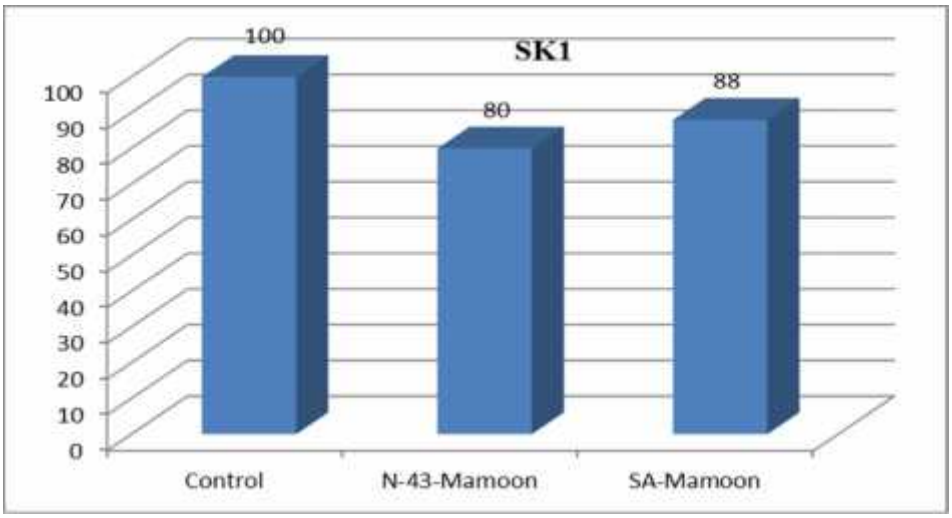


Figure 5.2a. Preliminary data of inhibition of SPHK1 with artemceramide-A and artemceramide-B at the concentration of 4 mM.

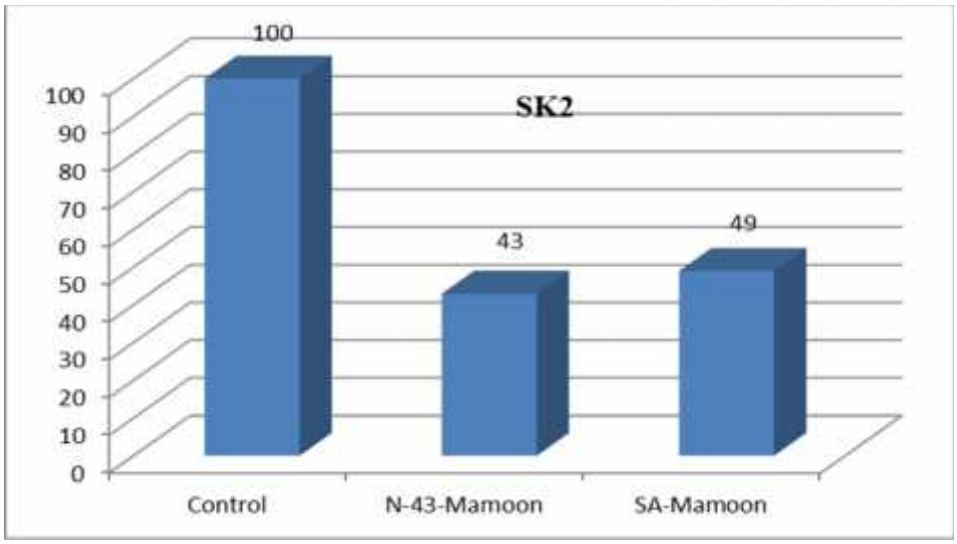


Figure 5.2b. Preliminary data of inhibition of SPHK2 with artemceramides-A and artemceramide-B at the concentration of 4 mM.

Part B

Chapter 6

Experimental

6.1 Plant materials.

Artemisia elegantissima and *Artemisia incisa* have been collected from Kashmir and Lower Dir, Pakistan respectively. The details are given in **Chapter 3 (3.4 plant materials)**.

6.2 Preparation of the extracts, fractionation and isolation of the compounds.

Besides the phytochemical studies, the extracts, fractions and the selected compounds isolated from the two species of *Artemisia* were subjected to biological activities. The details of extraction and isolation are given in **Schemes 3.1-3.4** and **Sections 3.4.2-3.4.6 (Chapter 3).**

6.3 Anticancer assay.

The fractions and the selected compounds were screened for their anticancer activity by using the human cancer cell lines; A549 (lung), HCT116 (colon) and MCF7 (breast) which were obtained from the Cell Production Laboratories of Cancer Research UK and maintained in culture medium supplemented with 10 % (v/v) foetal bovine serum (FBS), penicillin/streptomycin (basal culture medium) and 2 mM L-glutamine. All cell lines were incubated in a humidified atmosphere with 5 % CO_2 in air at 37 °C and wasted when the passage number exceeded than 15.

To inspect the effect of the compounds on cell growth, cells growing exponentially were added to 96-well plates at a density of 5x104 per well. Drugs were then added to the wells, ensuring an equal volume of 200 µl across the plate. Cell number was calculated at 72 h. using the methylthiazoletetrazolium (MTT) assay and the conc. of each drug

obligatory to reduce cell capability by 50 % [IC_{50}] was measured using the sigmoid Emax model.[208]

6.4 Antidiabetic Assay.

6.4a Buffer preparation.

A buffer solution was prepared, consisting of 5.95 g of Hepes (50 mM), 2.922 g of sodium chloride (100 mM), 0.64 g of dithiothreitol (4.0 mM), 0.73 g of ethylene diamine tetra acetic acid (EDTA, 5.0 mM) and 10 mg of bovine serum albumin (BSA 0.02 mg/ml) were dissolved in 1000 ml of distilled water and the pH was adjusted to 7.2.

6.4b Samples preparation.

The stock solution concentration for each sample was 10 mg/ml. The screening of samples was done at concentration of 100 µg/ml by mixing 1.2 µl of each sample with 98 µl of the buffer solution.

6.4c Enzyme preparation.

The enzyme, Protein Tyrosine Phosphatase 1B (PTP 1B) was prepared by adding 100 µl to 25 ml of the buffer, then aliquoted into 250 ml (enough for 1 plate) and stored at low temperature (-80 ^{o}C).

6.4d Substrate preparation.

The substrate material used was 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) (Invitrogen D6567) dissolved in DMSO. A solution of 7 µl of the stock (10 mM) was added to 1.75 ml of buffer and was stored at low temperature (-20 ^{o}C).

6.4e Inhibitor preparation.

The inhibiting material of bis(4-trifluoromethylsulfonamidophenyl)-1,4-diisopropylbenzine (TFMS, calbiochem 540211, 10 mg) was dissolved in DMSO (10 mg in 1.64 ml) and shift curve was accomplished for the TFMS (concentration range 0.0003–3 µM).

6.4f Assay assembly method.

The well plate (Costar®) assembly method was used in which 10 µl of standard (TFMS) and samples were poured into the plate wells, then 20 µl of PTP1B enzyme were added to each well of the plate and incubated at 37 °C in 5 % CO_2 air composition for 30 min. After incubation, 10 µl of the substrate was added to each well of the plate and then again incubated for 10 min. The plate was screened on the Wallac Victor2 microplate reader using umbelliferone at UV range of 355 nm/460 nm.[209]

6.5 Antibacterial assay.

Alamar blue96™ well microplate assays was used to determine the antibacterial potential of the fractions and the isolated compounds at the concentrations of 100, 200 and 500 µg/ml. The stock solution of the samples was prepared by dissolving 10 mg/ml of DMSO and then diluted to the concentration of 1 mg/ml by using HMI-9 medium. Samples of various concentration i.e., 100, 200 and 500 µg/ml were prepared from the stock solution.

The well plate was properly assigned by adding DMSO as a negative control in column 1, suramin as a positive control in column 12 and the samples in column 2–11. The testing microbes used were diluted up to 3×104 microbes/ml and counted through a

haemocytometer. The microbial suspension of 100 µl was added to each well of the assay plate except the column 1, having the control. The plate incubation was done at 37 ºC and 5 % CO_2 composition of humidified atmosphere for 24 h. The florescence from the well was determined at a particular wavelength (emission 590 nm, excitation 530 nm), through Wallac Victor microplate reader.

The activity on the fractions and the compounds was performed using well established Alamar blue 96^{TM} well microplate assays. The samples activity was done at a concentration of 100, 200 and 500 µg/ml for the extracts and the compounds.

In DMSO, 10 mg/ml of samples were dissolved to prepare the stock solution and then diluted to a concentration of 1 mg/ml by using HMI-9 medium. Samples of 100, 200 and 500 µg/ml concentrations were prepared from the stock solution.

The wells on the plate were arranged by having DMSO as a negative control in column 1, samples in column 2-11 and positive control (suramin) in column 12. The testing microbes used were counted through a haemocytometer and were diluted up to $3x10^4$ microbes/ml. The 100 µl microbial suspension was transferred to each well of the assay plate except column 1; the control. The plate was incubated at 37 ºC in 5 % CO_2 for 24 h. in a humidified atmosphere.

Wallac Victor microplate reader (emission 590 nm, excitation 530 nm) was used to determine the florescence of the well. Percentage of the DMSO control values were regarded as % D control.[209]

6.6 Antitrypanosomal assay.

The method was followed as established by Clark.[210] Chemicals and solvents were of reagent or anhydrous grade and used as obtained from commercial sources without further purification. The *T.b. brucei* bloodstream form strain 427 isolated from a sheep in Uganda were cultured in HMI-9 medium containing 20 % (v/v) heat activated (Foetal Calf Serum) FCS, in a 5 % CO_2 atmosphere at 37 °C.

Minimum inhibitory concentrations (MICs) for the fractions and the compounds were carried out in duplicate. The assay was conducted in 96-wells microplates. Stock solutions (10 mg/ml) of the test agents were prepared in dimethylsulfoxide (DMSO) and then diluted to a concentration of 1 mg/ml using HMI-9 medium. From the stock solution, various concentrations i.e., 20, 10 and 5 µg/ml of the samples were prepared.

The plate was arranged by adding DMSO as a negative control in column 1, suramin as a positive control in column 12 and the samples to be tested in columns 2–11. The trypanosomes were diluted to 3×10^4 microbes/ml and added in suspension (100 µl) to each well of the assay plate. AlamarBlue® (Trek Diagnostic Systems, Inc.) was added to all the wells of the plate to give a final concentration of 10 % (v/v) and a total well volume of 200 µl. The microplates were incubated at 37 °C in a 5 % CO_2 humidified atmosphere for 24 h. The level of fluorescence was measured using a Perkin Elmer Victor 2 microplate reader in fluorescence mode (Excitation 560 nm, Emission 590 nm. The percentage (%) of the DMSO control values were recorded as % D control.[211]

6.7 Nematicidal assay.

6.7a Maintenance of *Meloidogyne javanica*.

The root-knot nematode *Meloidogyne javanica*, was obtained from okra roots. Pure culture was preserved on tomato cultivar; Riogrande under greenhouse conditions (temperature adjusted to 25 °C) at the Plant Pathology Department, The University of Agriculture, Peshawar. After inoculation for sixty days, eggs from the galled roots were mined according to the methods established by Naz.[212] Freshly mined eggs and the second stage juveniles (J2) that emerged from the eggs after 48 h (at 30 °C) were used for nematicidal bioassay.

6.7b Nematicidal action.

The nematicidal activity of the 16 compounds **1, 6, 8, 11-16, 28, 30-34** and **37** at the concentration of 100, 200 and 300 µg ml^{-1} was done in a microwell plate (Multiwell,™ 24 well, Becton Dickinson, USA). Stock solution for each sample (1 mg ml^{-1}) was prepared by dissolving compounds/oil in DMSO (<1 % v/v). The test nematode dilution (I ml containing 200 J2s and 1000 ± 50 eggs) were added to each well at a final concentration of 100, 200 and 300 µg ml^{-1} in two different simultaneous experiments. Mixture of distilled water and DMSO (< 1 % v/v) served as a control. The experiment was performed twice at room temperature (~ 25 °C) in a completely randomized (CR) design with five replication. The plates were incubated in the dark at room temperature for 48 h. The total number of unhatched eggs or active/inactive J2s in each well was counted after 48 h of incubation. Percentages of unhatched eggs and the J2 mortality were calculated for each well. Juveniles were defined as dead if their body was straight and

they did not move, even after mechanical prodding.[213] Eggs and J2 solutions were removed and hatching or mobility was assessed in water for three days in each treatment. Nematode recovery was not observed in the dead nematodes. To further determine the LC_{50} values of these compounds, 1 % DMSO solutions of each sample at 100, 200 and 300 $\mu g/ml^{-1}$ were assayed for nematicidal activity by the described method. Avermectin B1 was used as the positive control with the purity of 97.5 %. It was a mixture of avermectin B1a and avermectin B1b in the ratio of 9.5 to 0.5 (w/w).[212]

6.7c Statistical analysis.

Data were subjected to analysis of variance (ANOVA) using statistics (NH Analytical Software, Roseville, MN, USA). The mortality (%) values were represented by their means (%). To describe the nematicidal effects of the compounds against the nematodes, the median lethal concentration (LC_{50}) values were calculated using the linear relation between the inhibitory probability and concentration logarithm according to the method of Sakuma.[212]

6.8 Sphingokinase inhibition assay.

The murine B lymphoma-derived cell line A20/2J was maintained in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO), supplemented with 8 % heat-inactivated fetal bovine serum (F6178, Sigma-Aldrich, St Louis, MO), 2-mercaptoethanol (50 µM), penicillin (50 U/ml), and streptomycin (50 µg/ml), were incubated in a humidified atmosphere at 37 °C with 5 % CO_2.

The samples of the compounds; artemceramide-A **(2)** and artemceramide-B **(4)** were prepared by dissolved in ethanol at 4 mM concentrations. The anti-mouse Fas monoclonal antibody (Jo2) and FITC-annexin V were purchased from BD Pharmingen (San Diego, CA). Z-VAD-fmk was obtained from the Strathclyde Institute of Pharmacy and Biomedical Sciences.

Mouse SPHK1 and SPHK2 were tagged with a triple FLAG epitope at their N-terminus. Site-directed mutagenesis (R293E/R294E, G213D, L219A) was performed with pCEpuro-m SPHK2, using a Prime STAR Mutagenesis Basal Kit (Takara, Japan). Transfections were performed using the Nucleofector technology (Amaxa), and puromycin-resistant cells were selected. The expression levels of mRNA of SPHK1 and SPHK2 was evaluated by PCR using primers.

Cell death was evaluated based on flow cytometric analyses and the release of lactose dehydrogenase (LDH). Cells were stained with FITC-annexin V and propidium iodide (PI), and analyzed by flow cytometry using a FACS Calibur (BD Bioscience, Mountain View, CA). Cells were classified as live (Annexin V^- PI^-), apoptotic (Annexin V^+ PI^-), or dead (Annexin V^+ PI^+ PI^-). For the LDH release assay, cells (1 x 10^6 cells/ml) were incubated in the presence or absence of the compounds; artemceramide-A **(2)** and artemceramide-B **(4)** and the culture media were centrifuged at 390 g for 5 min. The LDH activities in 20 µl aliquots of the supernatants were measured with a LDH Cytotoxicity Detection Kit (TaKaRa MK401, Japan), according to the manufacturer's instructions. To measure the total LDH activity, the cells were analysed with a final conc. of 1 % Triton X-100, and 5 µl aliquots were used for the assay.

To examine the phosphorylation of the compounds; artemceramide-A **(2)** and artemceramide-B **(4)**, homogenates from mouse SPHK2 or SPHK1-transfectants were prepared by disruption of the cells in buffer A (20 mM Tris-HCl, pH 7.5, 0.25 mM EDTA, 1 mM sodium pyrophosphate, 5 mM sodium fluoride, 5 % glycerol), using an ultrasonic homogenizer (Brson Sonifier 250).

Protein concentrations were measured with a BCA protein assay kit (Pierce), using bovine serum albumin as the standard. The reaction mixture contained 2 mM dithiothreitol, 200 mM KCl, 5 mM sodium orthovanadate, 0.5 mM 4-deoxypyridoxine, 1 mM phenylmethylsulfonyl fluoride, 1mM [γ-^{32}P]ATP (100 µCi/ml), and either ceramides; artemceramide-A **(2)** and artemceramide-B **(4)** (dissolved in ethanol), or solvents in buffer A. Reactions were initiated by the addition of the homogenates (75 µg protein to 100 µl of the reaction mixture), and were quenched by the addition of 375 µl chloroform/methanol/concentrated HCl (100:200:1, v/v). The reactions were performed at 30 °C. The radio-labeled lipids in the organic phase were extracted as described previously, resolved by TLC on silica gel 60 high performance TLC plates (Merck) with 1-butanol/acetic acid/water (3:1:1. v/v/v), and visualized by autoradiography (BAS3000, Fuji, Japan). For transmission electron microscopy, cells were collected by centrifugation and then fixed in 2.5 % glutaraldehyde in 100 mM phosphate buffer (pH 7.4) overnight at 4 °C. After exchanging the fixative to 100 mM phosphate buffer (pH 7.4), the samples were post-fixed with 2 % osmium tetroxide for 2 h, and then dehydrated. The samples were finally embedded in Epon mixture. Ultra-thin sections (80 nm thick) were prepared with a HERZ ultramicrotome. The specimens were stained with lead citrate and uranyl acetate and examined with a HITACHI H-7100 transmission electron microscope, at an

accelerating voltage of 75 kV. Differences between mean values were assessed by the Student's t test. A p-value of <0.05 or <0.01 was considered statistically significant.[214]

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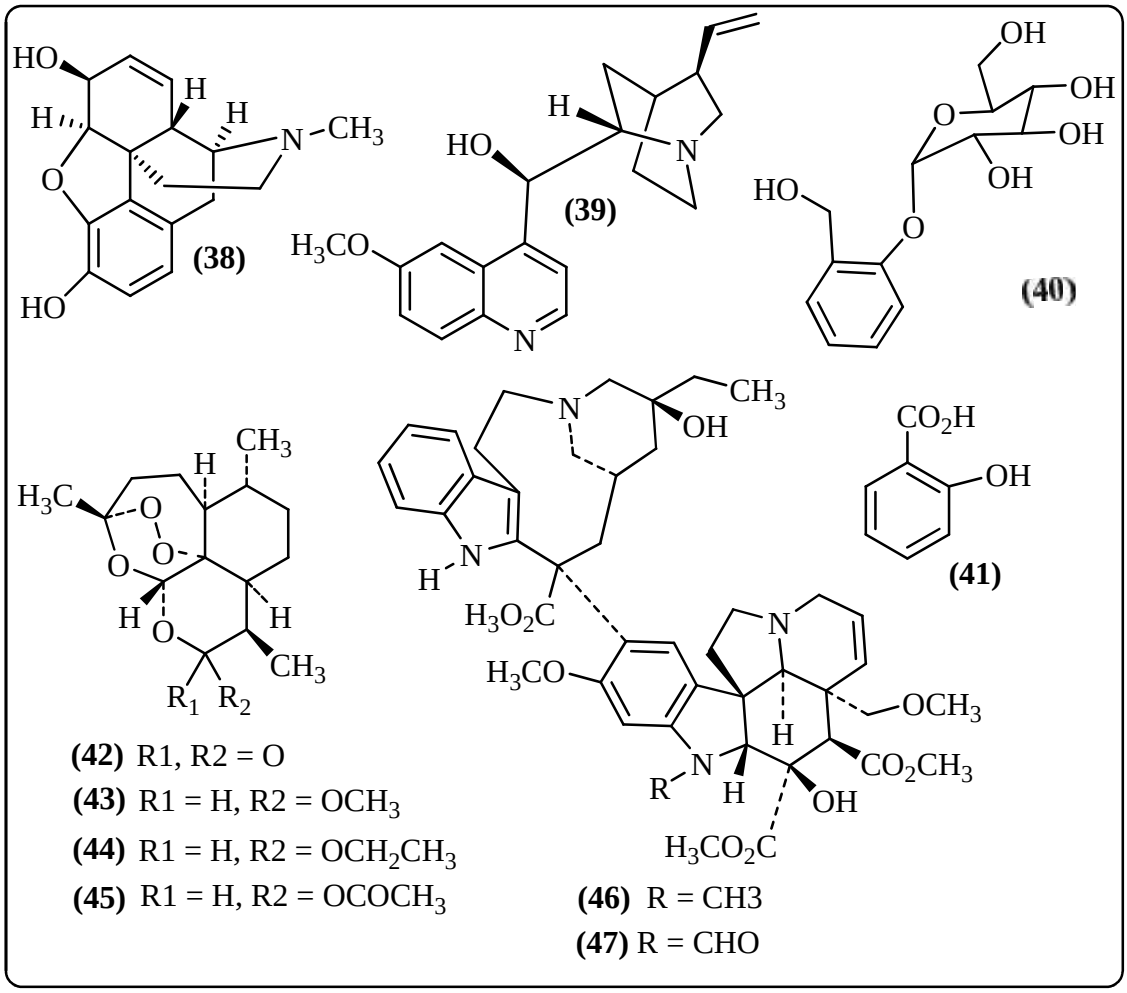
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Appendices

Appendix-A (Figure 1.1a, 1.2a, 1.3a, 1.4a, 1.5a,1.5b.1.6a,1.6b, 1.6c and **1.7a)**

Figure 1.1a Some of the important natural products and their depravities of plant origin used as a drug.



(42) R1, R2 = O
(43) R1 = H, R2 = OCH_3
(44) R1 = H, R2 = OCH_2CH_3
(45) R1 = H, R2 = $OCOCH_3$

(46) R = CH3
(47) R = CHO

Figure 1.2a acetylenes, cyclic hydrocarbon and furan derivatives.

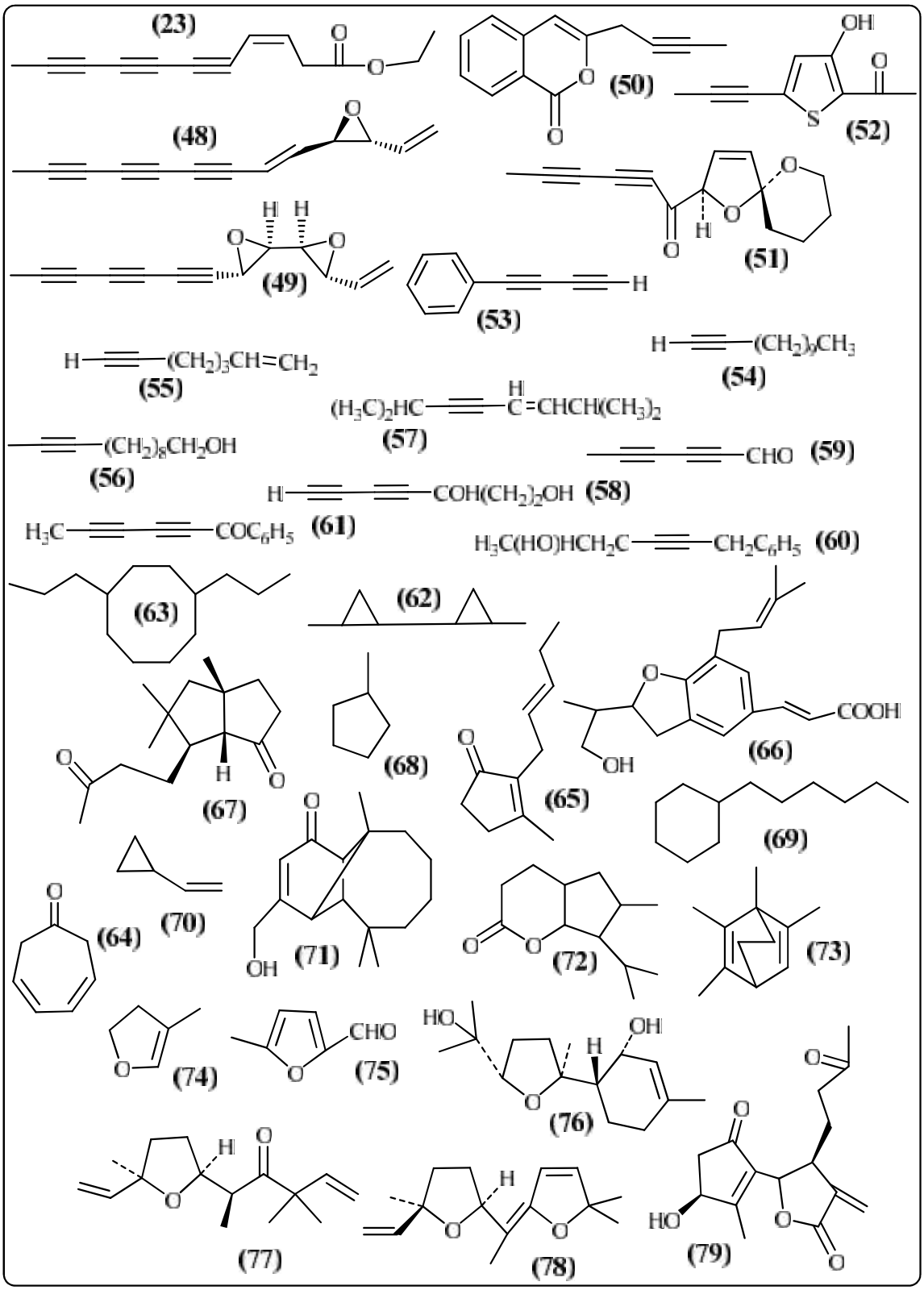


Figure 1.3a Phenolic, benzylic, phenyl propanoids, chalcones and polyaromatic derivtives.

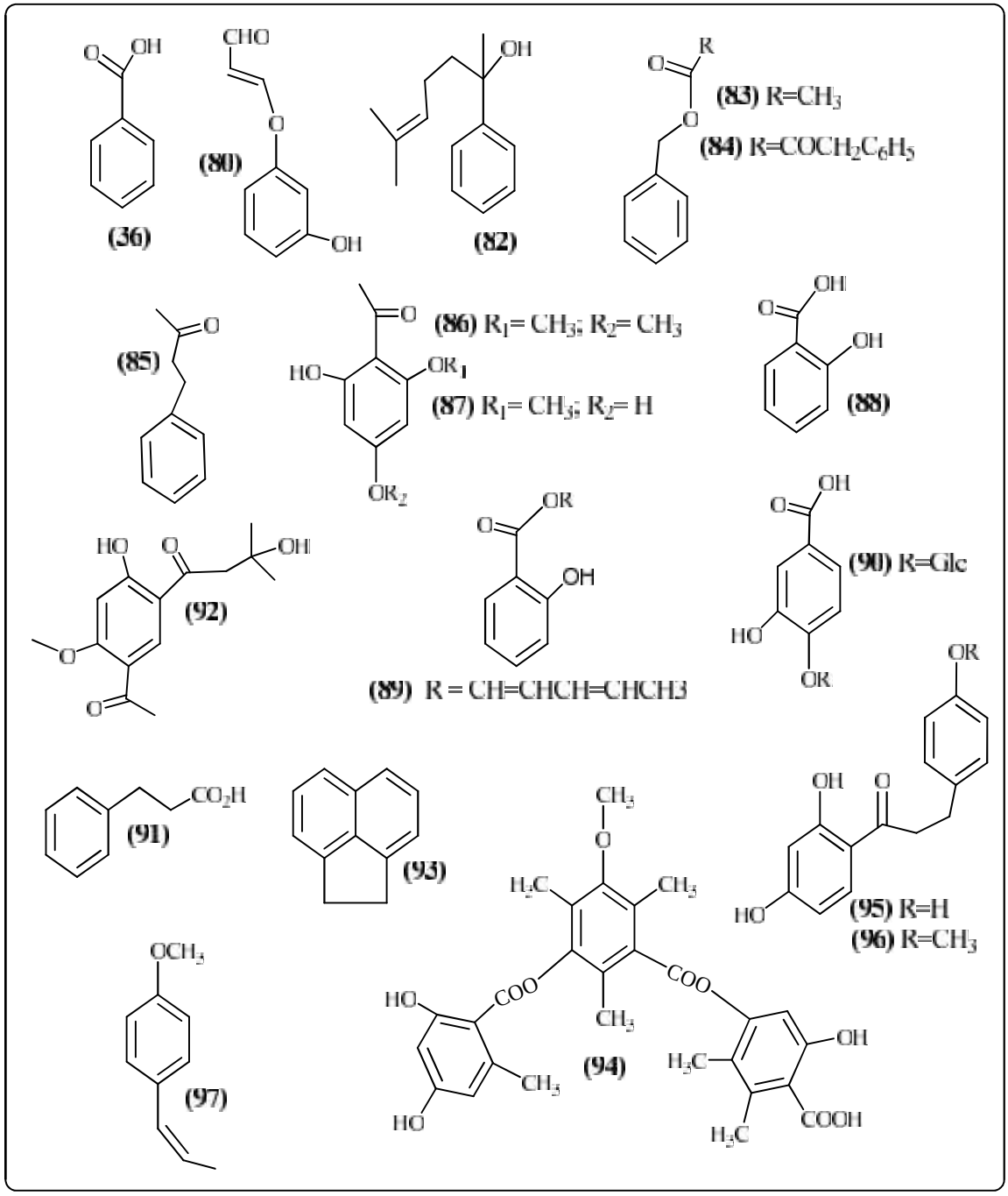


Figure 1.4a Cinnamyl, caffeoyl, ferruloyl, coumarin, isocoumarin and bicoumarin derivatives.

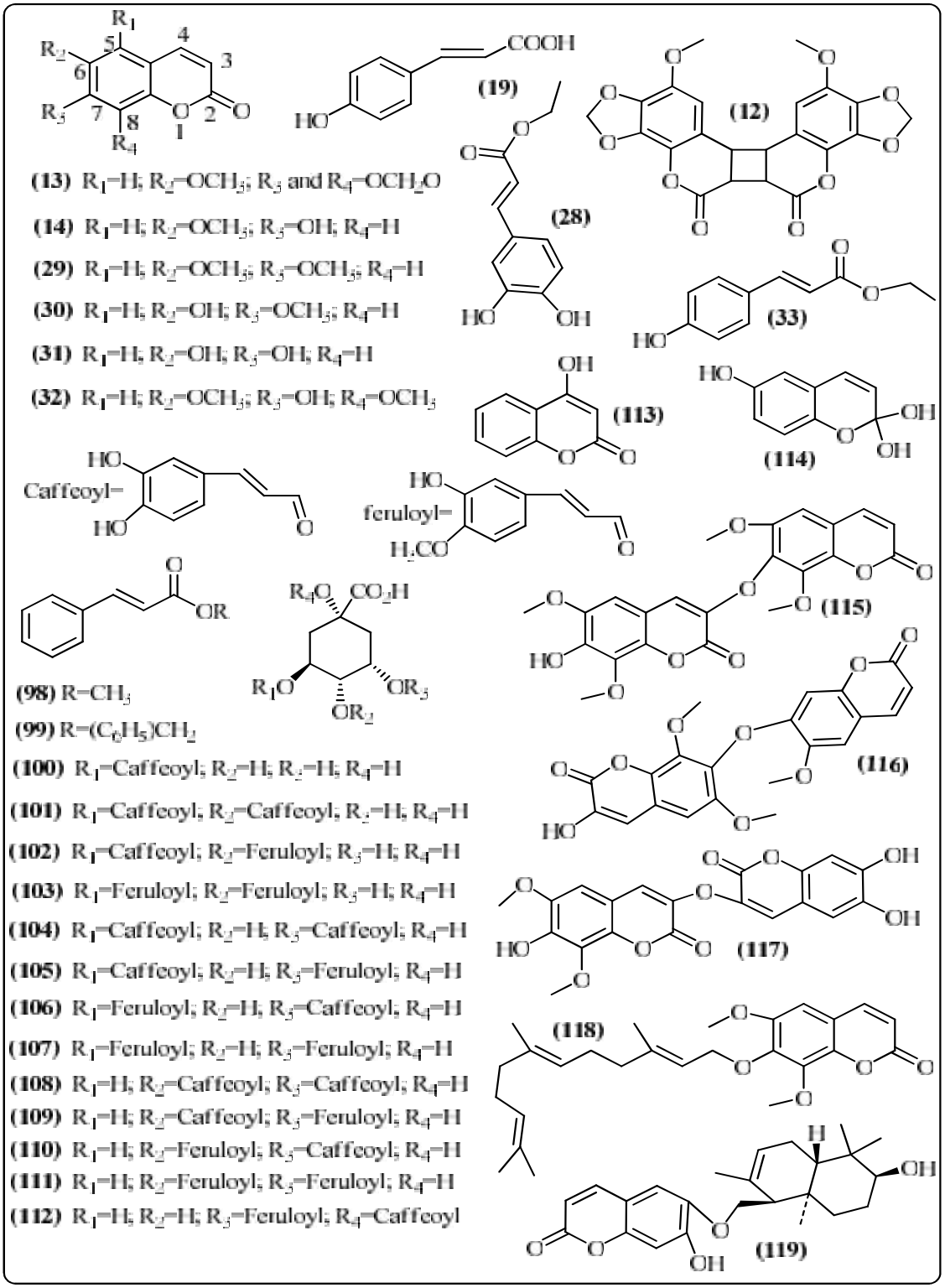


Figure 1.5a Tetra, penta and hexahydroxy flavone, flavonol and flavonone derivatives.

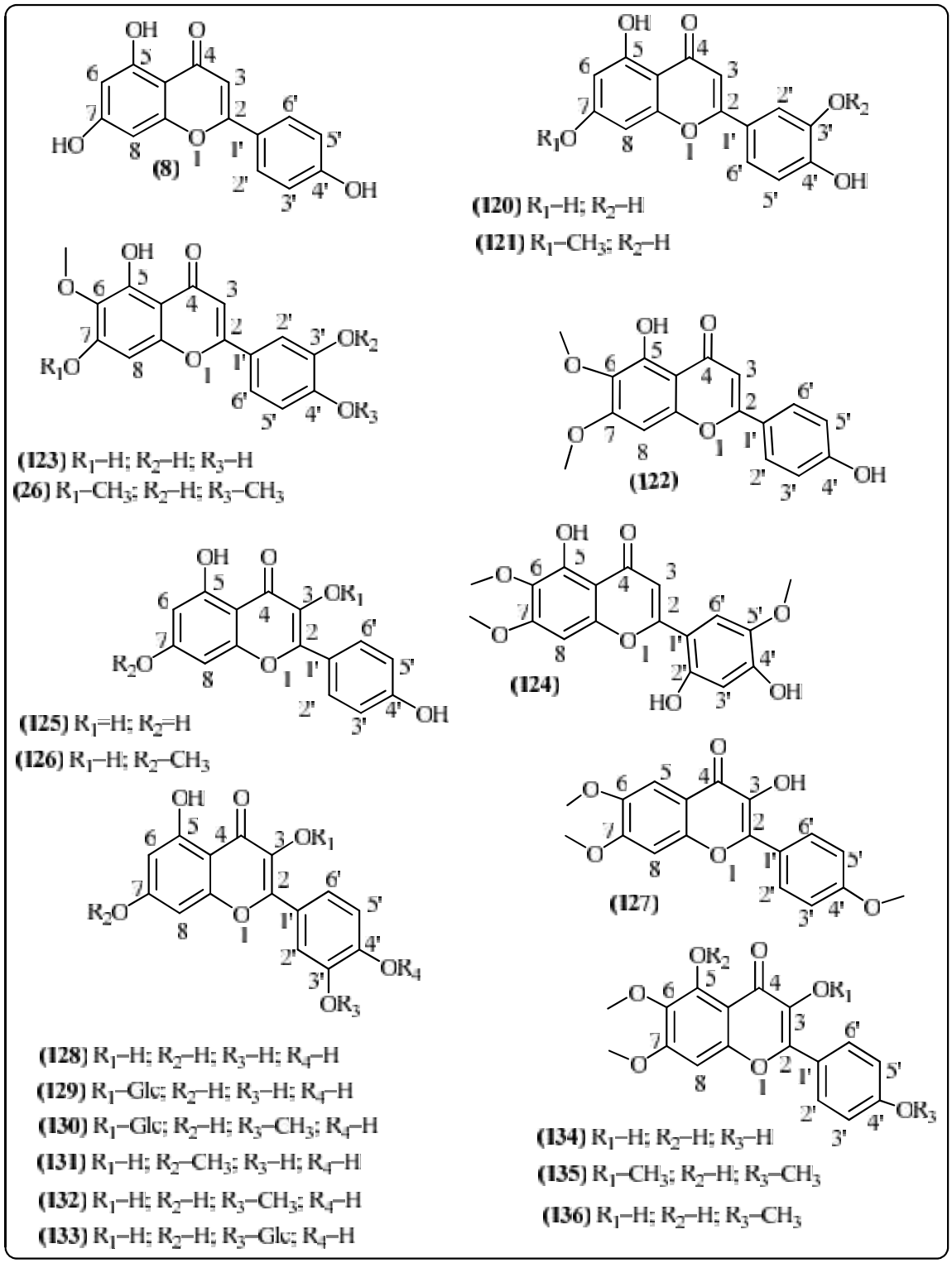


Figure 1.5b Tetra, penta and hexahydroxy flavove, flavonol and flavonone derivatives.

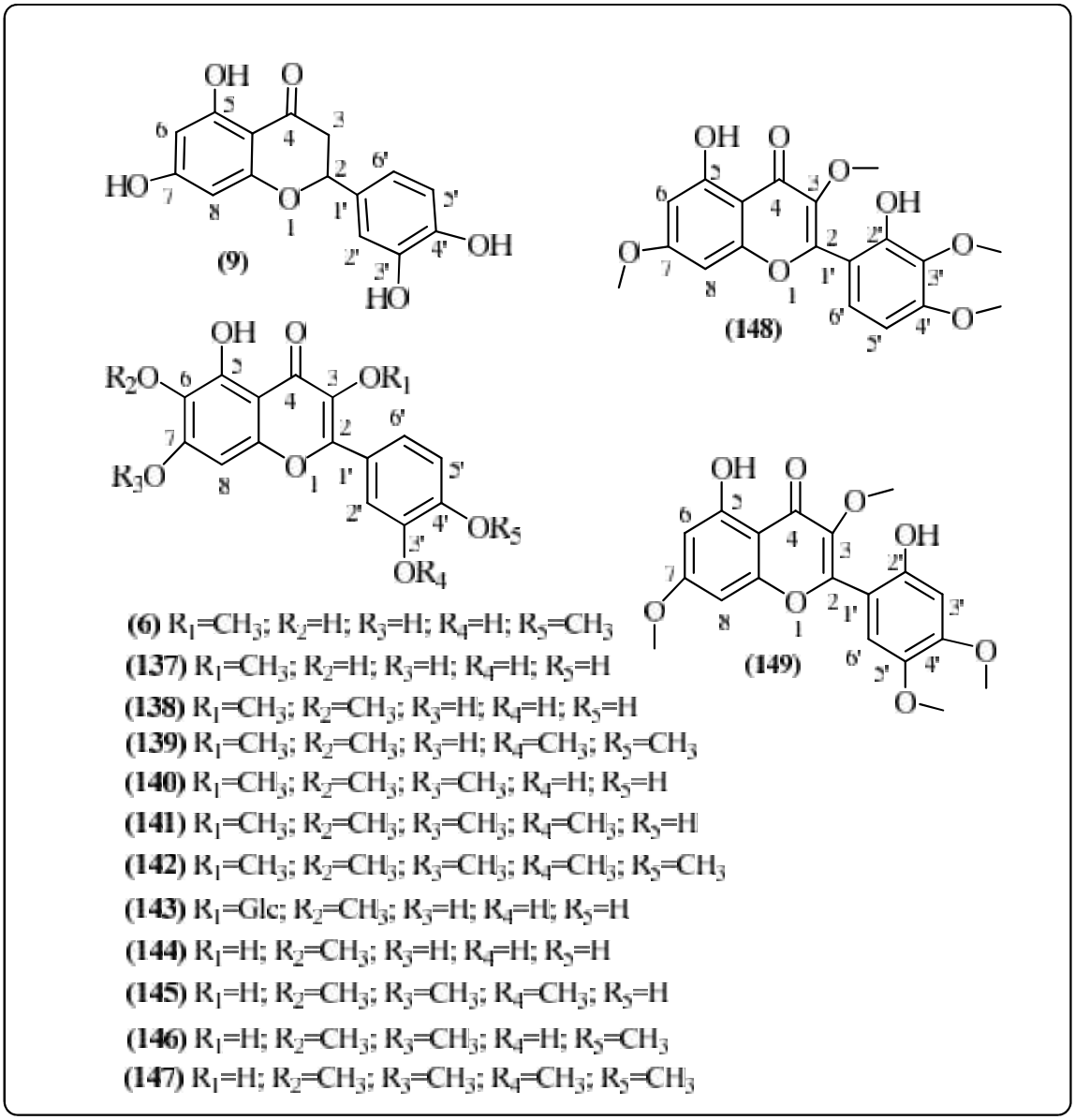


Figure 1.6a Acyclic, monocyclic, bicyclic and tricylic monoterpenes, sesquiterpenes and diterpenes.

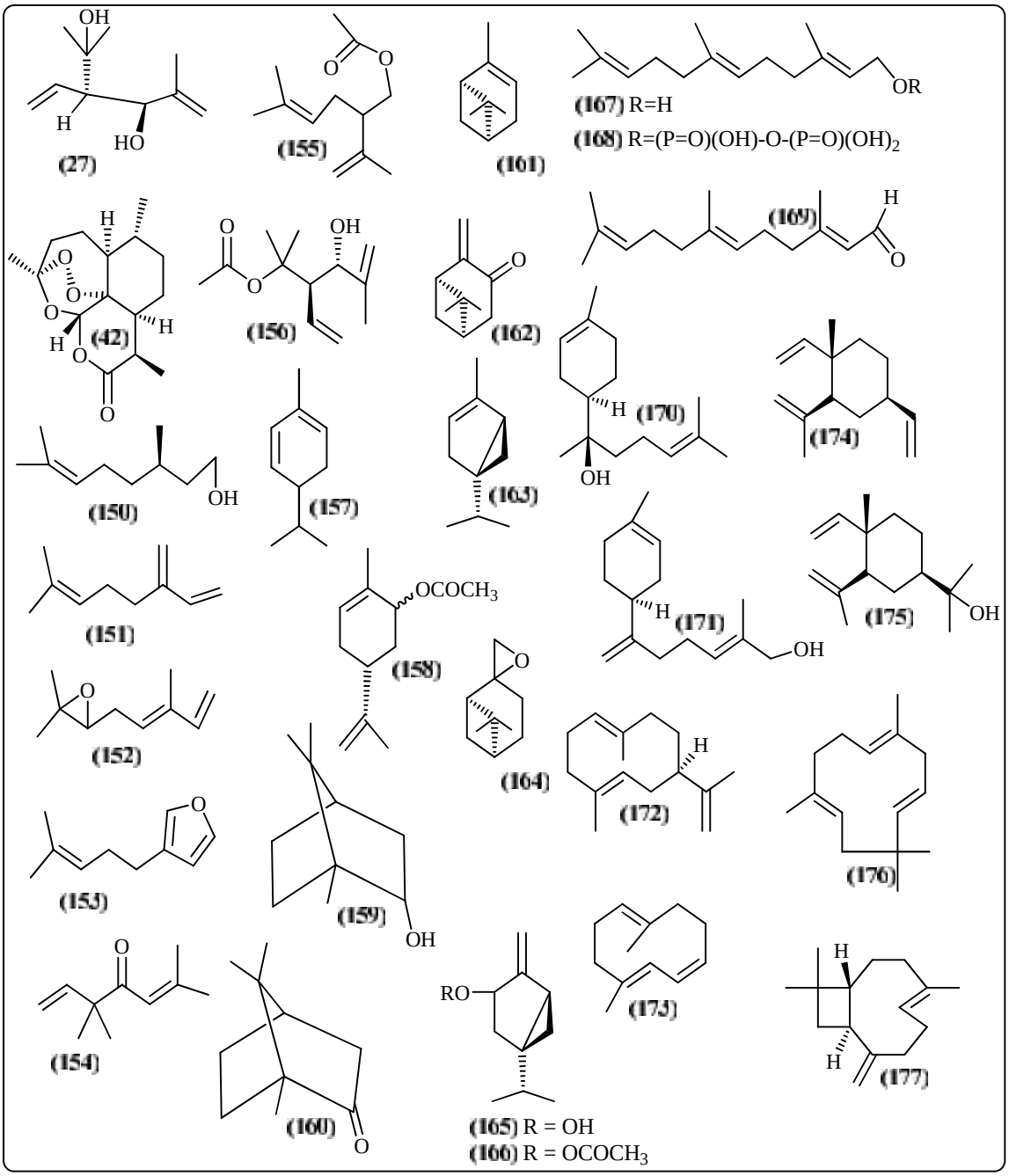


Figure 1.6b Acyclic, monocyclic, bicyclic and tricylic monoterpenes, sesquiterpenes and diterpenes.

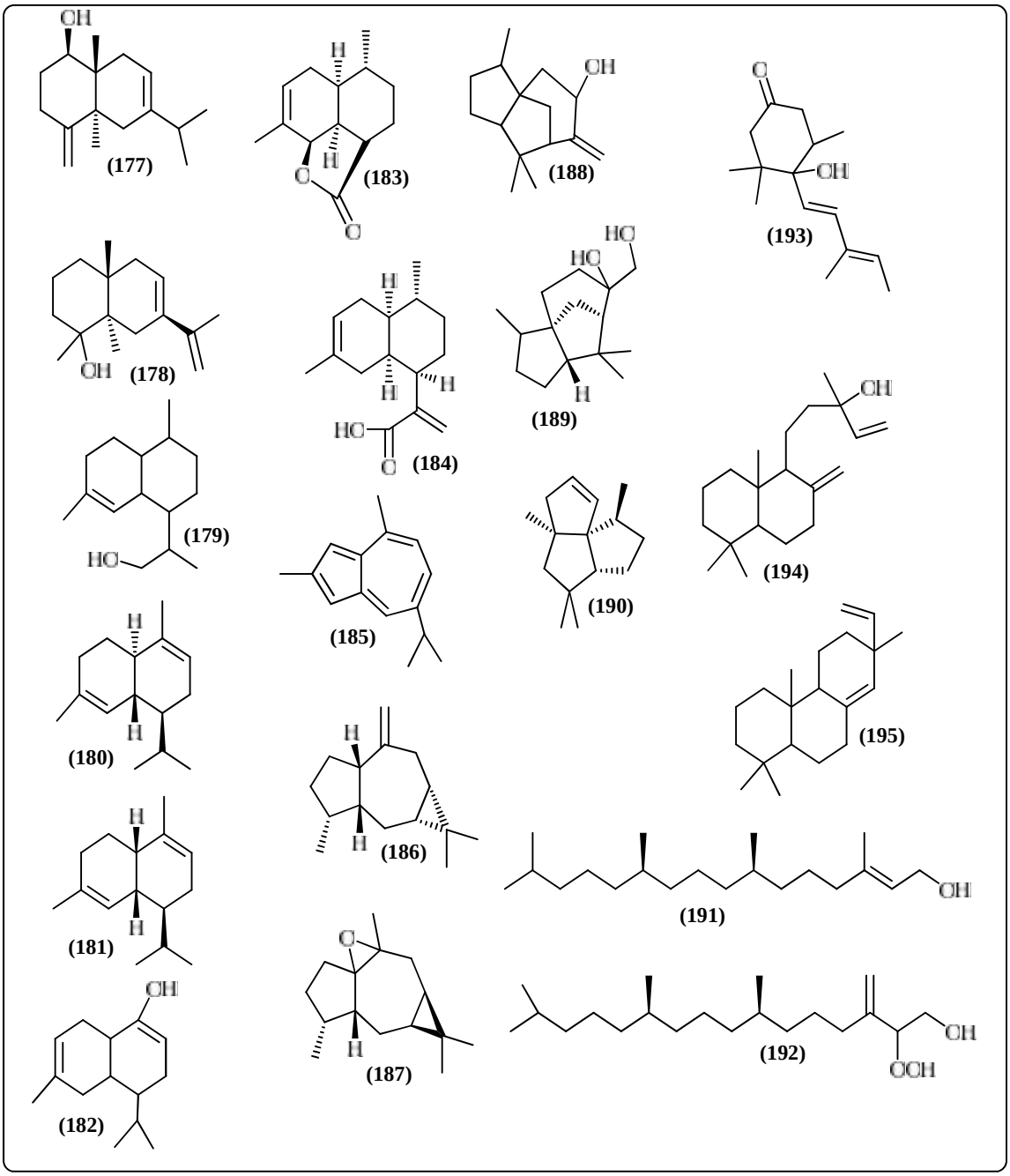


Figure 1.6c Triterpenes and sterols.

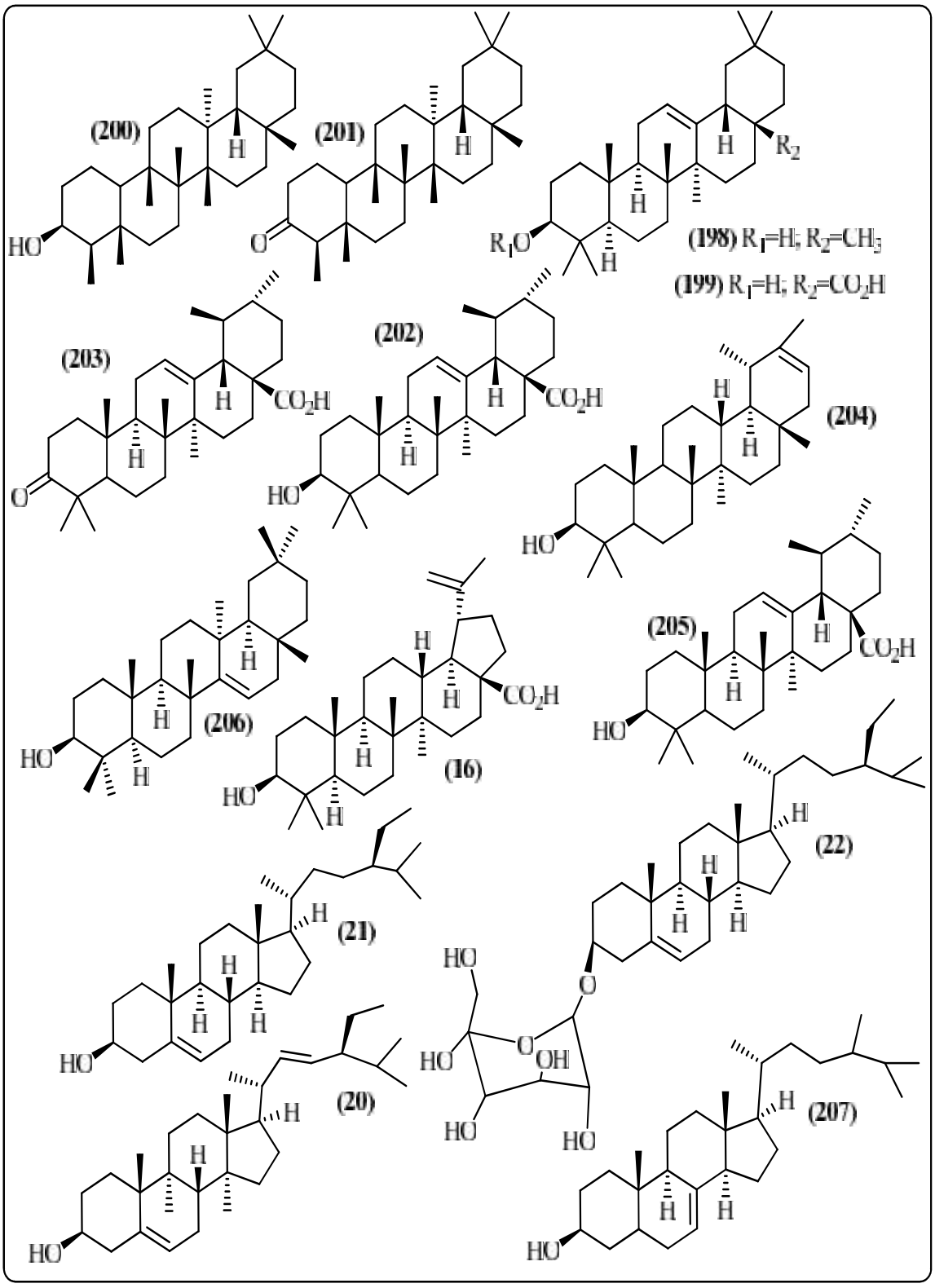
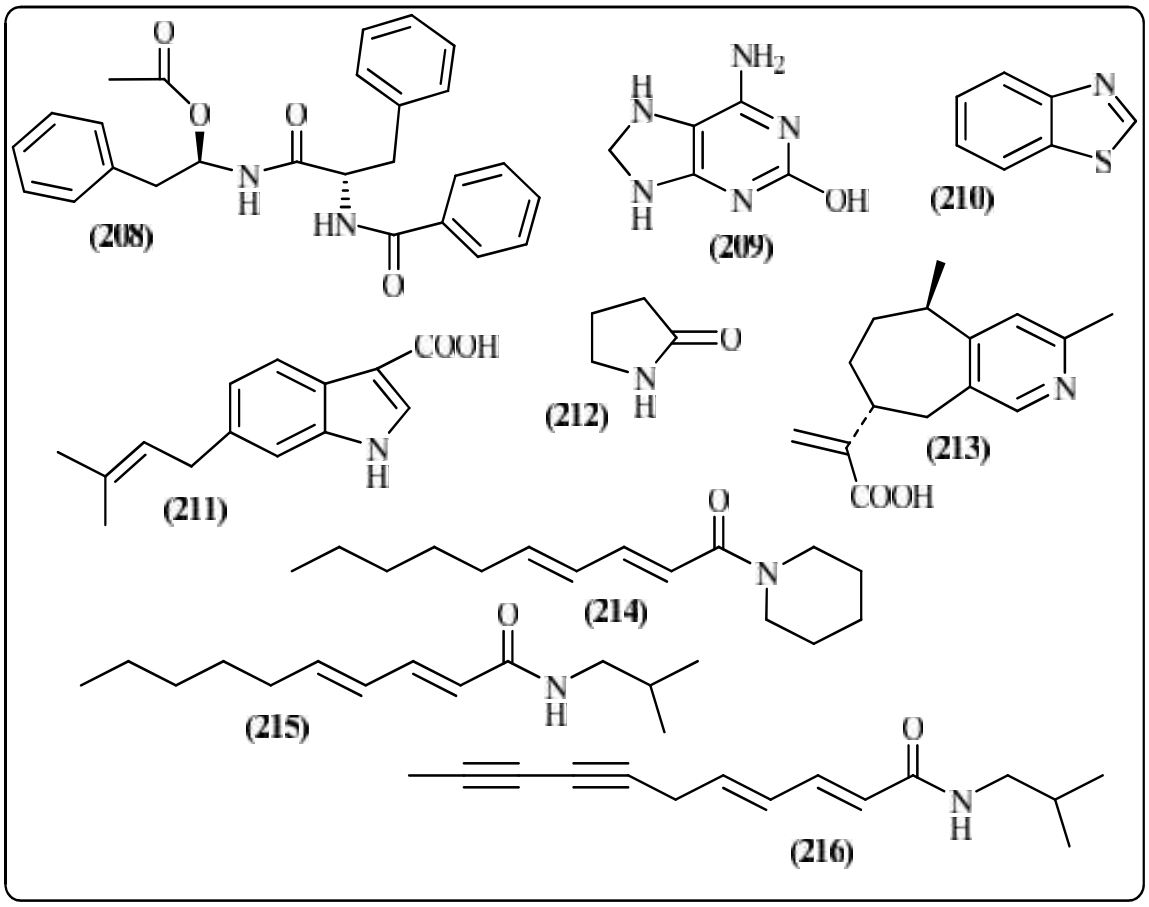


Figure 1.7a Nitrogen containing compounds.



Appendix B

List of Publications

Published (05)

1. **Mamoon-Ur-Rashid[1],** M. Rafiullah Khan*[1], Saqib Ali[1], Muhammad Alamzeb[1], , Alexander Irvine Gray[2] and Valeri A Ferro[2]. Phytochemical and antitrypanosomal investigation of thecompounds isolated from *Artemisia elegantissima* Pamp (Astereceae). *Pharm. biol.* doi: 10.3109/13880209.2013.874534. (**Accepted 2013**).

2. Muhammad Alamzeb[1], M. Rafiullah Khan[1], Saqib Ali[1], Syed Qaiser Shah[2] and **Mamoon-Ur-Rashid[1].** Bioassay guided isolation and characterization of anti-microbial and anti-trypanosomal agents from *Berberis glaucocarpa* Stapf. *Afr. J. Pharm. Pharmacol*. **2013**, *7*, 2065-2071. DOI 10.5897/AJPP2013.3444.

3. Muhammad Alamzeb[1], M. Rafiullah Khan[1], Saqib Ali[1], Syed Qaiser Shah[2] and **Mamoon-Ur-Rashid**[1] Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*. *Bangladesh J Pharmacol.* ***2013****; 8:* 107-109. DOI: 10.3329/bjp.v8i2.13551.

4. Ghias Uddin, Ashfaq Ahmad Khan*, Muhammad Alamzeb, Saqib Ali, **Mamoon-Ur-Rashid**, Anwar Sadat, Muhammad Alam, Abdul Rauf and Wali Ullah. Biological screening of ethyl acetate extract of Hedera nepalensis stem. *African J of Pharm. Pharmacol*. **2012**, 6, 2934-2937. DOI: 10.5897/AJPP12.828.

5. Saqib Ali[1], John Igoli[2], Carol Clements[2], Dima Semaan[2], Muhammad Alamzeb[1], **Mamoon-Ur-Rashid[1]**, Syed Qaisar Shah[3], Valerie A. Ferro[2], Alexander I. Gray[2],

M. Rafiullah Khan[1,*] Antidiabetic and antimicrobial activities of fractions and compounds isolated from *Berberis brevissima* Jafri and *Berberis parkeriana* Schneid. *Bangladesh J Pharmacol*. **2013**; 8: 336-342. DOI: 10.3329/bjp.v8i3.13888.

Submitted (04)

1. **Mamoon-Ur-Rashid[1],** Saqib Ali1, Muhammad Alamzeb[1], John Igoli[2], Valerie A Ferro[2], Alexander Irvine Gray[2], Syed Qaiser Shah[3] and Mohammad Rafiullah Khan[1]*. A New Ceramide Along With Eight Known Compounds from the Roots of *Artemisia incisa* Pamp (Astereceae). *Records Nat. Prod.* **(Online, 2014).**

2. **Mamoon Ur Rashid[1]** • Ishrat Naz[2] • Saqib Ali[1] • Muhammad Alamzeb[1] • Syed Qaiser Shah[3]• Muhammad Rafiullah Khan[1]*. Phytochemical and nematicidal investigations on the aerial parts of *Artemisia elegantissima* Pamp and *Artemisia incisa* Pamp (Asteraceae). *J Pest sci.* **(Submitted 2013).**

3. Muhammad Alamzeb[1], M. Rafiullah Khan[1], **Mamoon-Ur-Rashid[1]**, Saqib Ali[1] and Syed Qaiser Shah[2] A new bisbenzoyl isoquinoline alkaloid from *Berberis jaeschkeana schneid* var. *Records Nat prod* **(Submitted 2013).**

4. Saqib Ali[1], Muhammad Alamzeb[1], **Mamoon-Ur-Rashid[1]**, Syed Qaiser Shah[3], Valerie A. Ferro[2], Alexander I. Gray[2], M. Rafiullah Khan[1,*] Antinematicidal activities of fractions and compounds isolated from *Berberis brevissima* Jafri and *Berberis parkeriana* Schneid. *Pak. J Pharmacol.* **(Submitted 2013).**