PHYTOCHEMISTRY AND BIOACTIVITY OF SELECTED HIGH ALTITUDE PLANTS BELONGING TO *LIGULARIA, SENECIO* AND *VERNONIA* GENERA

Ph.D Thesis

By

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UNIVERSITY OF PESHAWAR

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DECLARATIONS

It is recommended that this dissertation prepared by **Mr. Syed Hamid Hussain** entitled **"phytochemistry and bioactivity of selected high altitude plants belonging to *Ligularia, Senecio* and *Vernonia* genera"** be accepted as fulfilling this part of the requirements for the degree of **"DOCTOR OF PHILOSOPHY IN ORGANIC CHEMISTRY"**.

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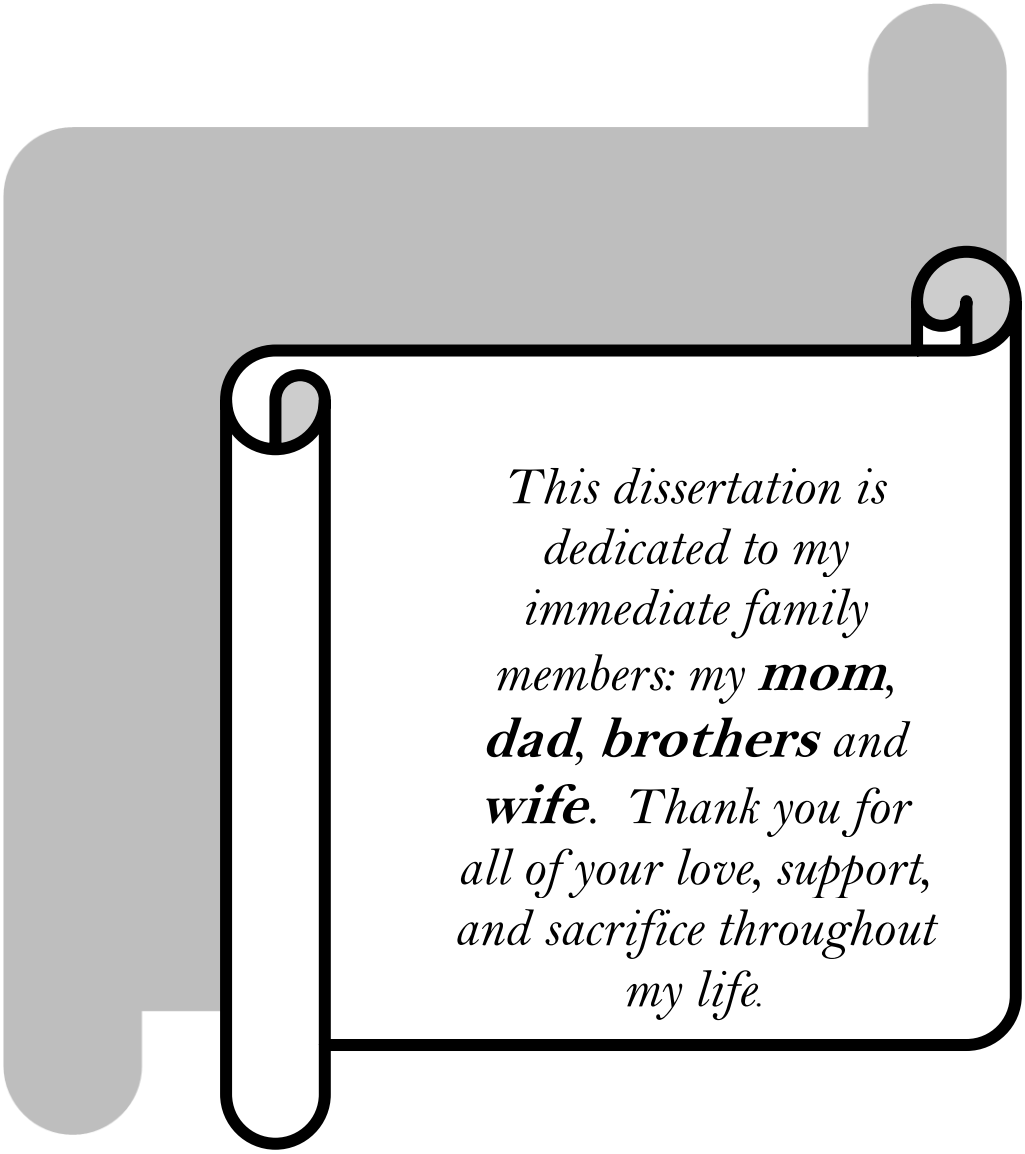
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This dissertation is dedicated to my immediate family members: my ***mom***, ***dad***, ***brothers*** *and* ***wife***. *Thank you for all of your love, support, and sacrifice throughout my life.*

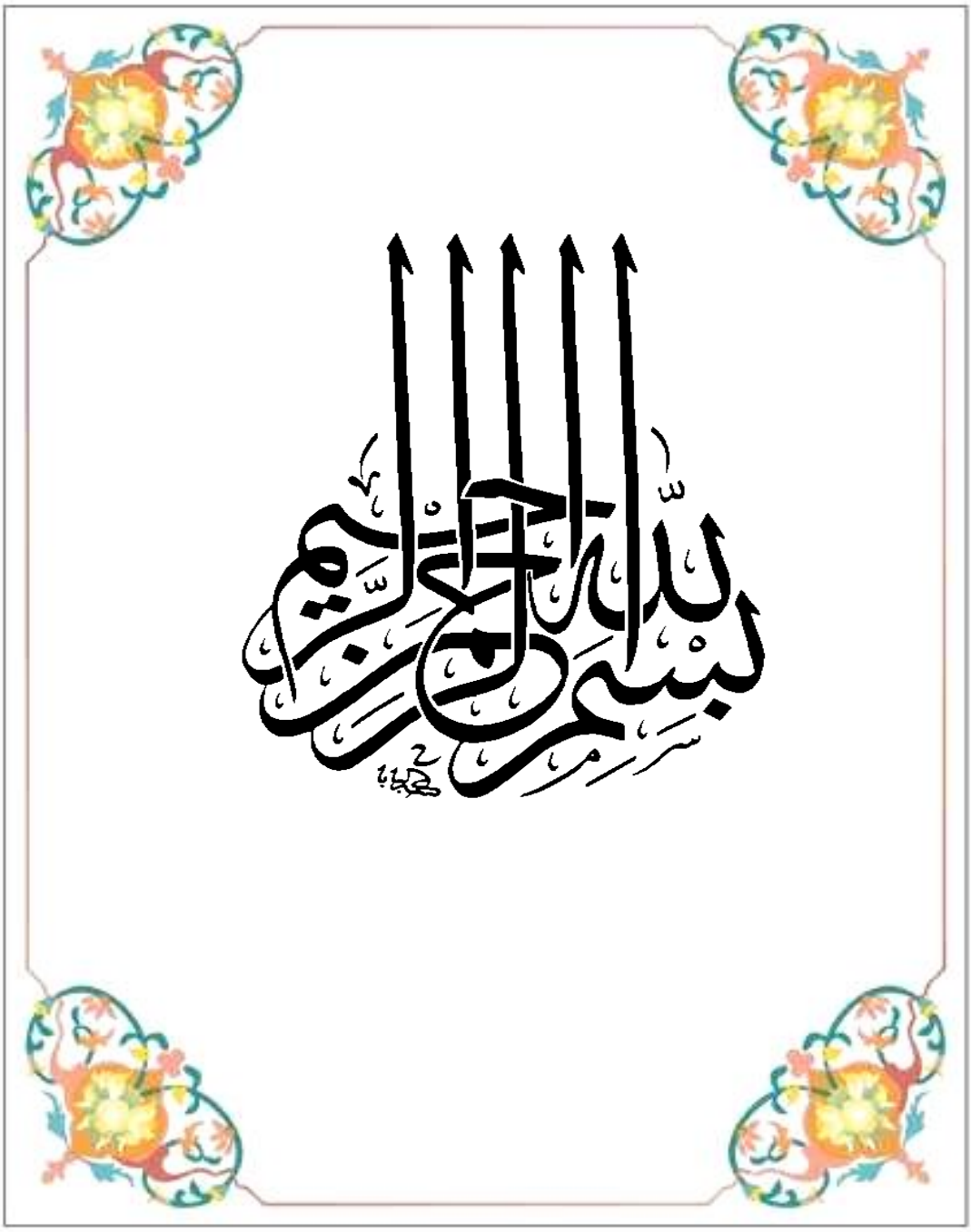

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ABSTRACT

The research work included in the present dissertation is mainly focused on isolation and structure elucidation of pure chemical constituents from the selected high altitude plants belong to *Ligularia*, *Senecio* and *Vernonia* genera. Moreover, biological activities have also been incorporated for various crude fractions and the purified compounds. There are three main parts of the dissertation:

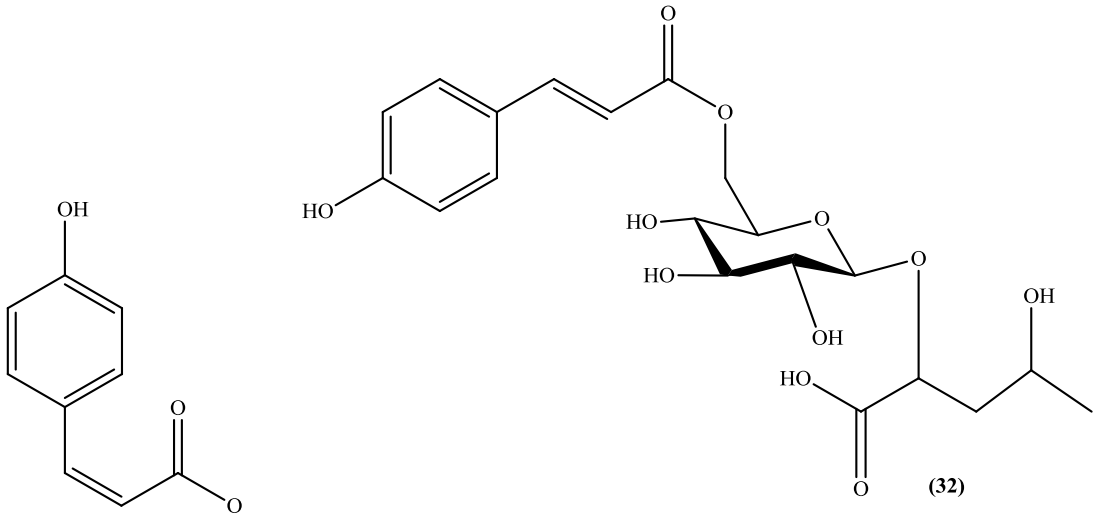
Part-A: Phytochemistry and bioactivities of *Ligularia thomsonii*

Part-B: Phytochemistry and bioactivities of *Senecio desfontainei* and *Senecio chrysanthemoides*

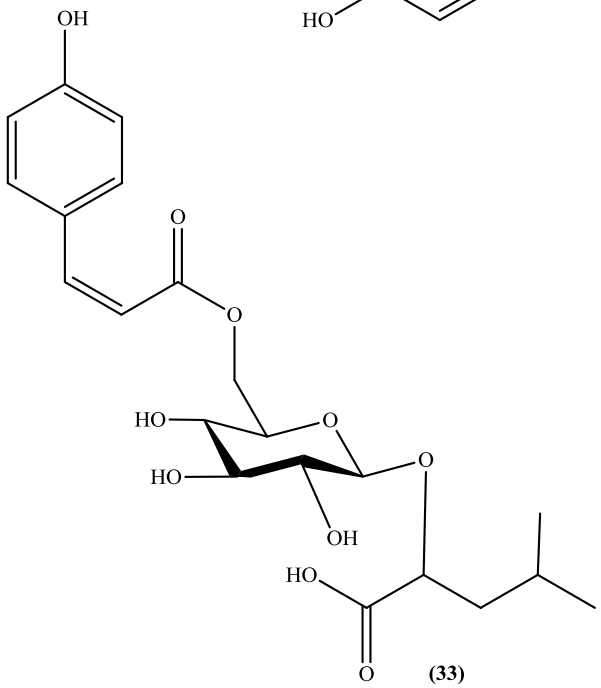
Part-C: Phytochemistry and bioactivities of *Vernonia anthelmintica*

Part-A

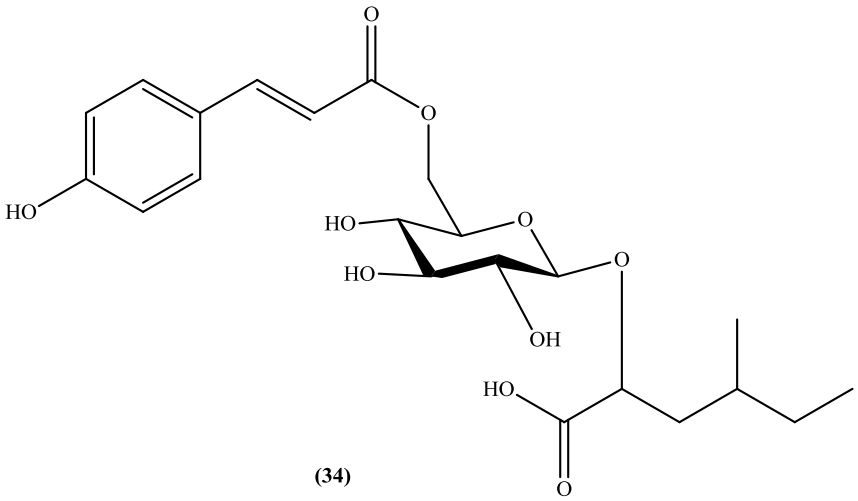
Part A describes the phytochemical investigation and bioactivities of *L. thomsonii* (Compositae). Three new (**32-34**) along with nine known (**35-43**) compounds were isolated from the plant. Various spectroscopic techniques were used to characterize their structures. Compounds (**32-36** and **39-43)** were also evaluated for their radical scavenging activities in the diphenylpicrylhydrazyl (DPPH) assay using ascorbic acid as standard.



(32)



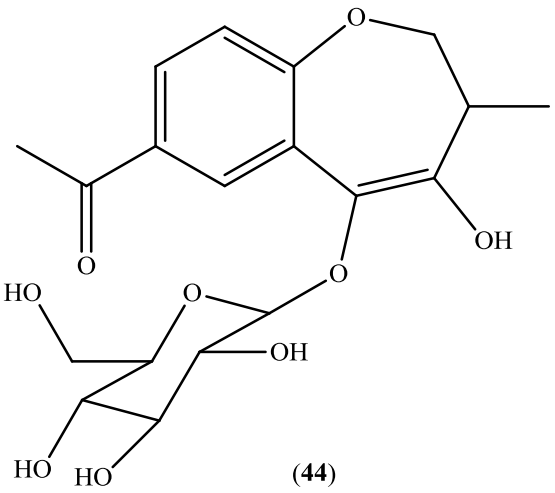
(33)



(34)

Part-B

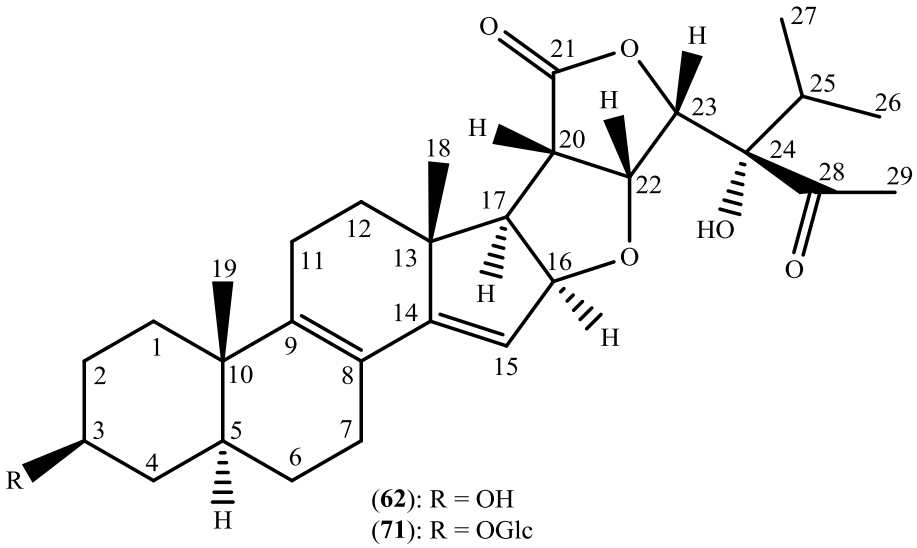
Part B deals with the phytochemistry and bioactivities of *S. desfontainei* and *S. chrysanthemoides* (Compositae). One new (**44**) and eleven known compounds (**45-55**) were isolated from *S. desfontainei* while six known compounds (**56-61**) obtained from *S. chrysanthemoides*. Physical characteristics and extensive spectroscopic studies were used for characterization of the isolated and purified compounds which were also evaluated for their antioxidant activities.



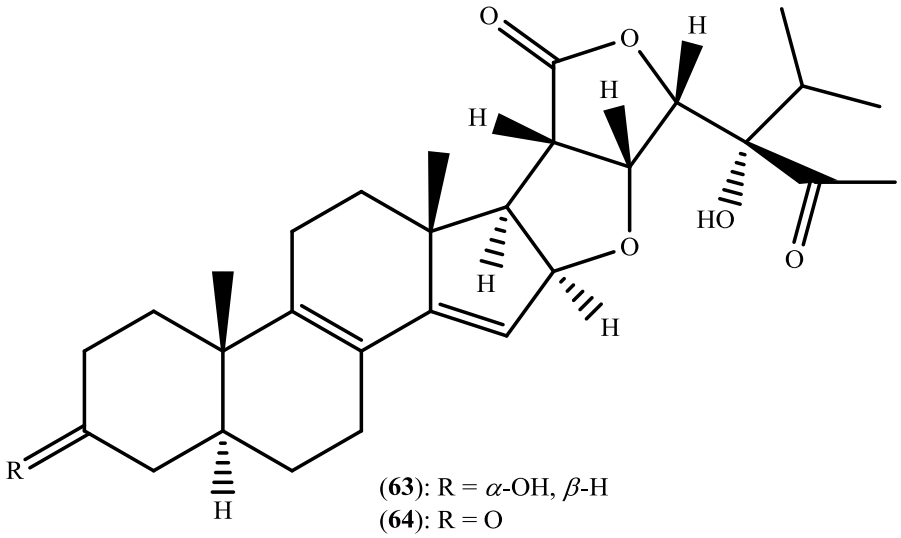
(**44**)

Part-C

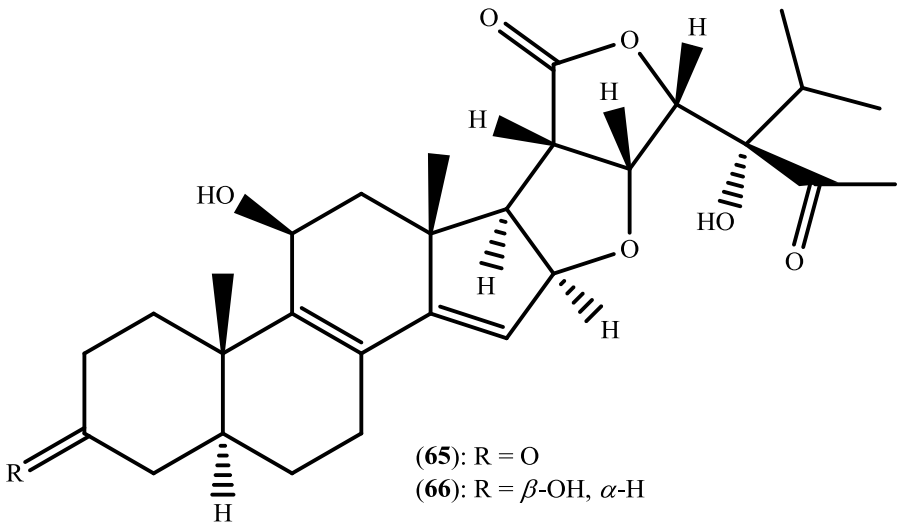
Part C of the dissertation, describing the phytochemistry and bioactivities of *V. anthelmintica* (Compositae). Eleven new compounds (**62-72**) were isolated and their structures have been characterized by physical characteristics and advanced spectroscopic techniques.



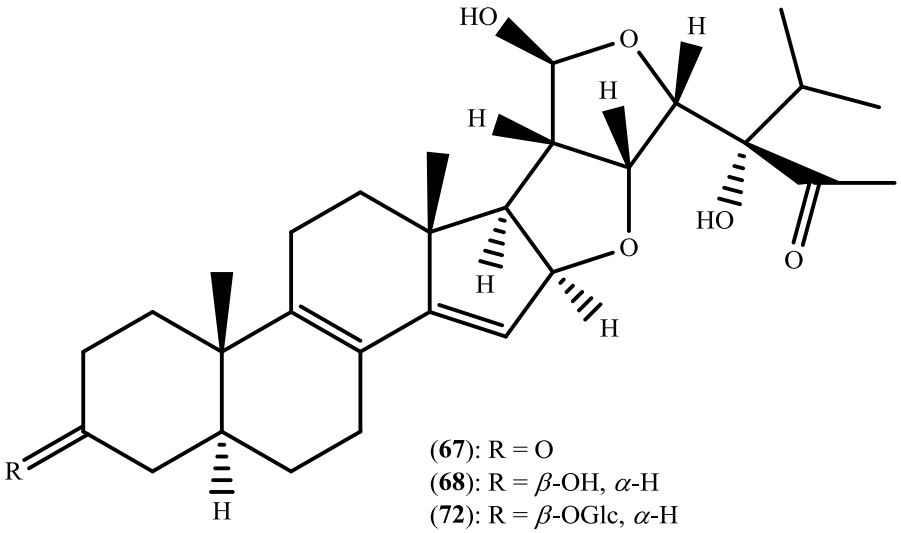
(**62**): R = OH
(**71**): R = OGlc



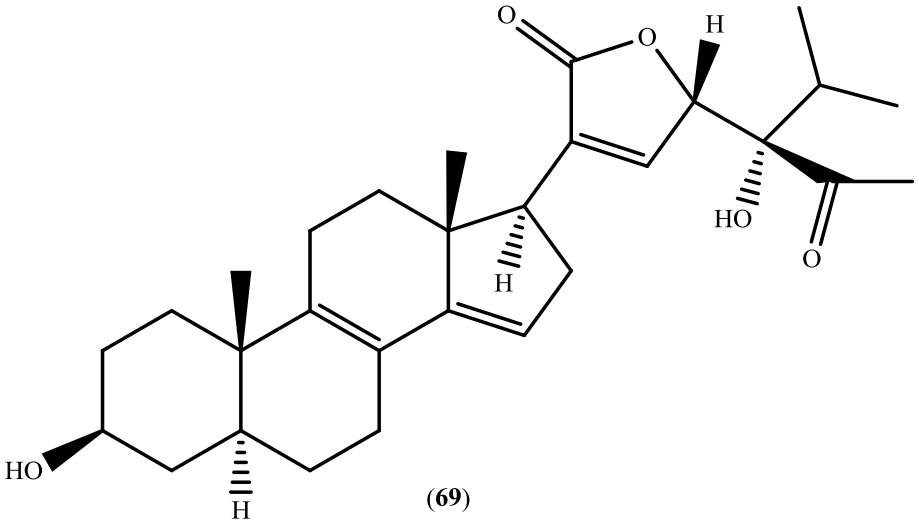
(**63**): R = α-OH, β-H
(**64**): R = O



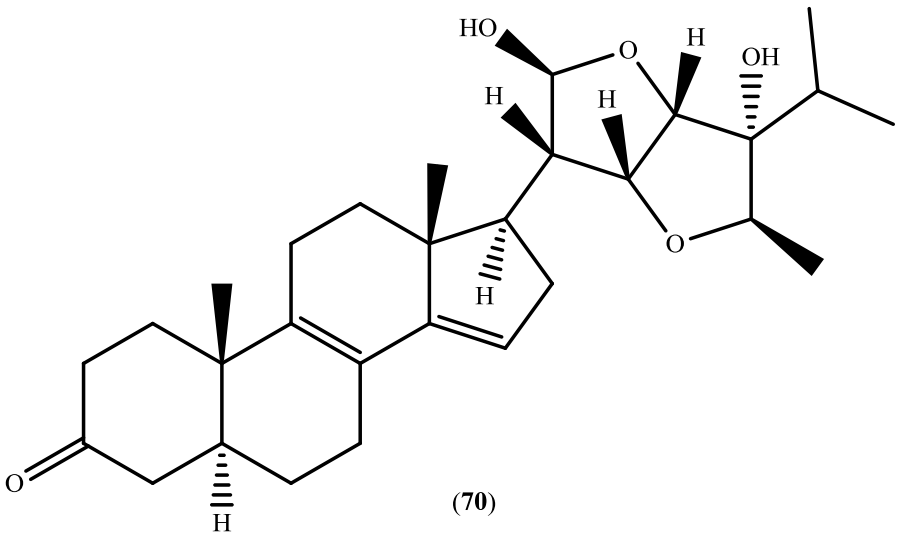
(**65**): R = O
(**66**): R = β-OH, α-H



(**67**): R = O
(**68**): R = β-OH, α-H
(**72**): R = β-OGlc, α-H



(**69**)



(**70**)

LIST OF ABBREVIATIONS

Ar	Aryl
AcO	Acetate
BB	Broad Band
CD	Circular dichorism
COSY	Correlation Spectroscopy
d	Doublet
D	Deuterium
DCM	Dichloromethane
DMSO	Dimethyl sulphoxide
DPPH	Diphenylpicrylhydrazyl
EIMS	Electron impact mass spectrometry
Glc	Glucopyranosyl
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HSQC	Heteronuclear single quantum coherence
HRESIMS	High resolution electrospray ionization mass spectrometry
Hz	Hertz
^{1}H-NMR	Hydrogen-1-nuclear magnetic resonance
^{13}C-NMR	Carbon-13-nuclear magnetic resonance
IC_{50}	Inhibitory concentration 50 percent
IR	Infrared
J	Coupling constant
m	Multiplet
mM	Millimole
m/z	Mass to charge ratio

Me	Methyl
MeO	Methoxy
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect spectroscopy
p	Probability
s	Singlet
t	Triplet
υ	Wave number

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Products of natural origins can be called "natural products" which include an entire organism such as plants, animals, or microorganisms that has not been subjected to any kind of processing or treatment other than a simple process of preservation (e.g. drying), an extract of an organism, exudates and isolated secondary metabolites such as alkaloids, coumarins, flavonoids, glycosides, lignans, steroids, sugars, terpenoids, etc. from the plants, animals, or microorganisms.[1] Concepts of secondary metabolism include products of overflow metabolism as a result of nutrient limitation, shunt metabolism produced during idiophase, defense mechanism regulator molecules, etc.[2]

Natural products especially plants are used as universal and prehistoric remedies for healing. From the beginning man had three basic requirements: to have something to eat, to have place to live and to remain fit. Natural products had played an important role to fulfill all these requirements. One of the oldest activities of man has been the study of plants and animals, particularly as a source of food and a cure of various human ailments. Most of the plants were found appropriate as food, but other were either fatal or medicinally helpful. This acquaintance of plants remedies was transferred to generations as a folk medicine by their skills.

The drug's history can be classified generally into various periods. The early period expanded over the Indian, Chinese, Sumerian, Egyptian and Assyrian civilization followed by the Greco-Roman, Arabian, Medieval and modern periods.

The earliest mentioned of the medicinal use of plants are derived from *Rig Vida* (4500-1600 B.C) and *Ayurveda* (2500-1600 B.C).[3] Egyptians (1550 B.C.) in the *Ebers Papyrus* employed medicinal plants in the curing of various human diseases.

The history of Greco-Roman period began from Hippocrates (460 B.C.) and Theopharastus (370-267 B.C.) who presented many medicinal substances in their writings followed by Dioscorides[4] who did substantial pharmacological assemblage of the Greeks. Furthermore Pliny the Elder (23-79 A.D.) wrote "*Natural History*" in 37 volumes.

The Chinese traditional system of medicines has been comprehensively documented more than the centuries and according to accessible historical records, Shen Nung of China and Sekhet Enanach of Egypt (around 3,000 B.C) were the earlier physicians. Obtainable references have further brought to light the contributions of the Babylonian civilization. On the other hand, it is exhilarating to learn that Shen Nung around 5000 years ago, was deeply fascinated in the field of medicines.[5]

Muslim scientists also showed significant contribution in the field of medicines. Among the famous scientists of this period are Rhazes (Abu Bakar Mohammad Bin Zakaria Al-Razi, 932 A.D.), his famous work is "*Alhavi Kabeer*" (Continents of Rhazes) and Kitab-al-Mansoori. Bu-Ali Ibn Sina (908-1037 A.D.) an eminent physician, philosopher, astronomer and mathematician of that time has mentioned about 760 herbal drugs obtained from natural products in his book known as "The Cannon of Medicine". Zia-ud-din ibn al-Baitar (1248 A.D.), a famous botanist and pharmacist described the use and importance of medicinal plants in his books *Kitab al-Jami fial Adwiya al-Mufrada, Kitab al-Mughni fial-Adwiya al-Mufrada*. Al-Idrisi (1099-1166 A.D.) also documented the importance of medicinal plants in his famous book *Kitab al-Jamili-sifat, Ashtat-al-Nabatat*.[6]

The idea of isolation of pure compounds as drugs from the plants and herbs began after 1806, when F. W. Serturner (1783-1814) separated morphine from the dried latex of *Papaver somniferum* L. (opium). In the next fifteen years strychnine, brucine, quinine, cinchonine and caffeine were isolated by Pelleties and Cavantou followed by the isolation of morphine (E. *Merck* in 1826) and aspirin, (Bayer in 1899). Coniine was identified and characterized by Schiff for the first time in 1870 which was also synthesized by Ladenburg in 1889. These compounds are considered to be the first typical natural products isolated as pure compounds by mankind.[7-8]

The revolutionary studies on the isolation and characterization of a wide range of alkaloids from various species of *Rauwolfia,* out of which reserpine gained a global importance in the treatment of hypertension and mental ailments whereas ajmaline hydrochloride was accepted as a drug of choice in the treatment of cardiac arrhythmias of various origins, served to motivate a wide interest in the isolation of natural products.

In recent years, a momentous resumption of interest in natural products as a promising source for new medicines has been developed. Several modern drugs have been developed from natural products.

The maturity of modern scientific techniques of isolation and structural studies also greatly improved the world interest in the exploration of medicinal plants. It is on this account that University of Peshawar has established the Centre for Phytomedicine and Medicinal Organic Chemistry which is equipped with advanced instruments needed for the natural products isolation and their characterization.

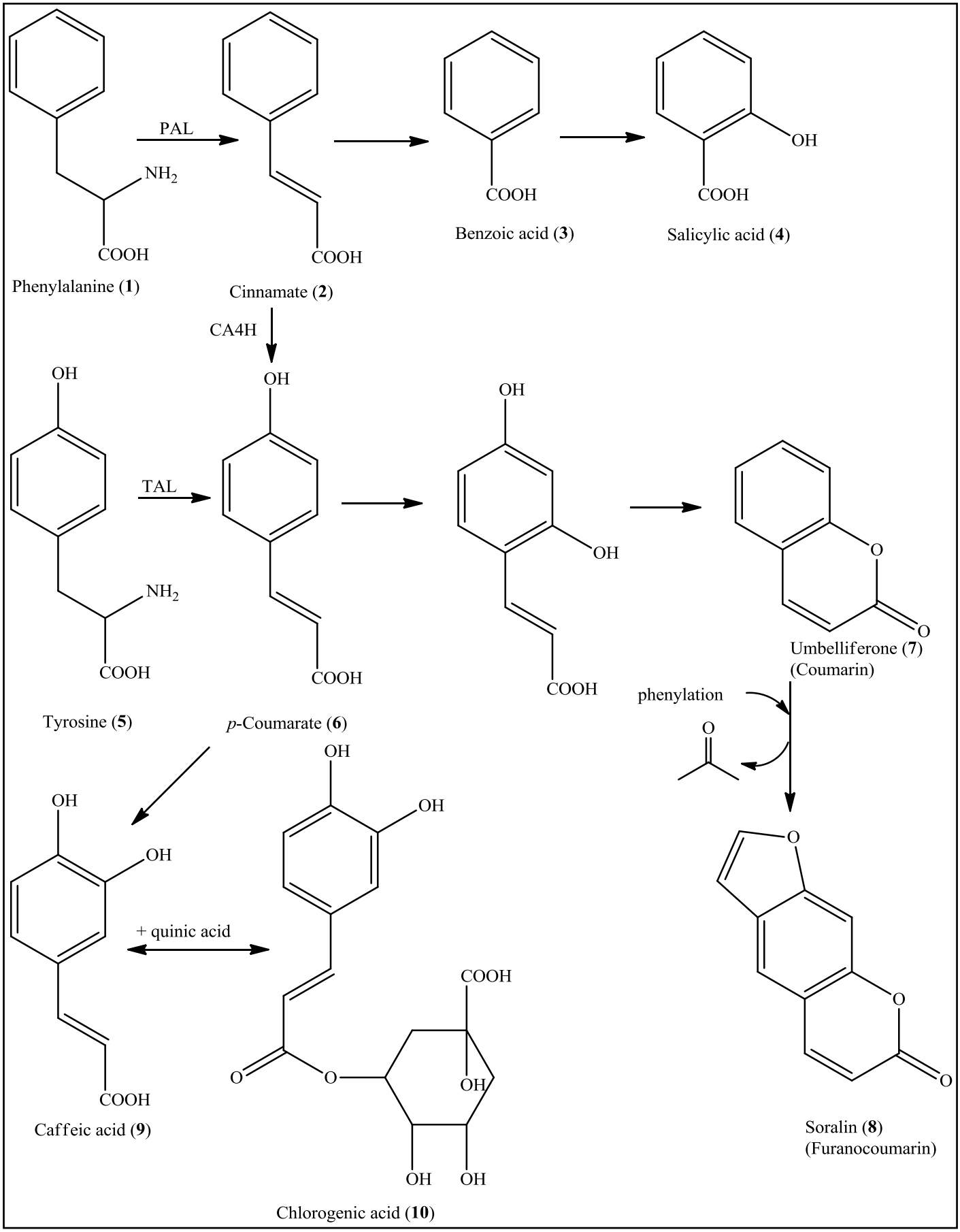
The work undertaken for the present doctoral dissertation is focused on pytochemistry and bioactivity of the selected high altitude plants belonging to *Ligularia, Senecio and Vernonia* genera.

2.1. Biosynthesis of phenylpropanoids

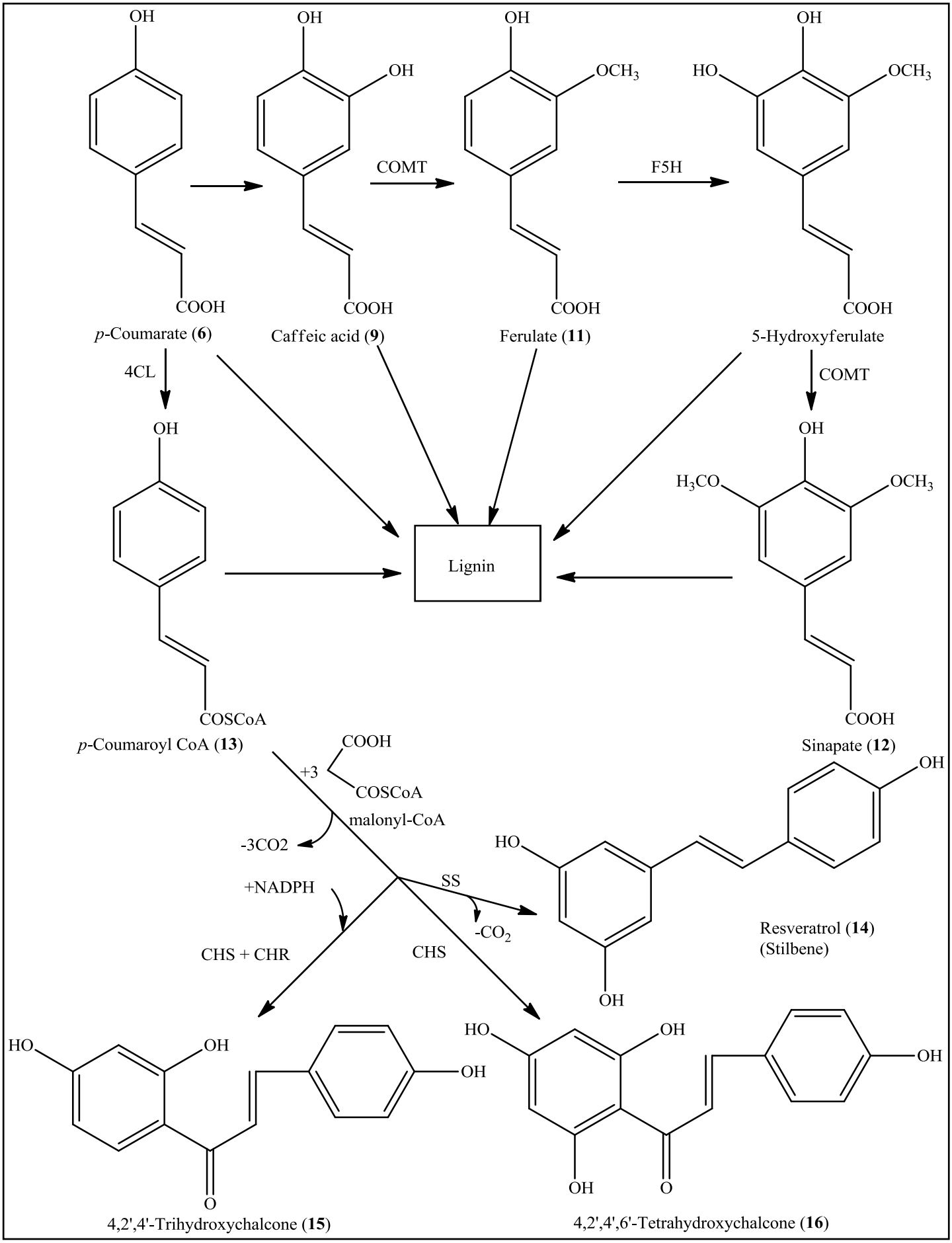
Almost all the phenylpropanoids are biosynthesized from cinnamic acid, a product obtained by the action of phenylalanine ammonia–lyase (PAL), the interconnecting enzyme of primary (shikimate pathway)[9] and secondary (phenylpropanoid) metabolism.[10-13] The simple phenylpropanoids having C6-C3 skeleton of phenylalanine (**1**) are produced from cinnamate (**2**) through a series of hydroxylation, methylation and dehydration reactions, which include *p*-coumaric (**6**), caffeic (**9**), ferulic (**11**) and sinapic (**12**) acids and simple coumarins (**7**, **8**) as shown in **Schemes-2.1** & **2.2**. Benzoic (**3**) and salicylic (**4**) acids (**Scheme-2.1**), although not strictly phenylpropanoids themselves because they lack the three carbon side chain, originate from the phenylpropanoids cinnamate and *p*-coumarate.[14-16] Lignins like complex polymers are derived from a mixture of simple phenylpropanoids although their composition may vary from species to species[12,17,18] (**Scheme-2.2**).

A large number of stress-induced phenylpropanoids are products obtained from the C-15 flavonoid skeleton, which is synthesized by the condensation of *p*-coumaroyl-coenzyme A (CoA) (**13**) and three molecules of malonyl-COA,[10] a reaction catalysed by the chalcone synthase (CHS) enzyme (**Scheme-2.2**). Initia1ly CHS produces a tetrahydroxychalcone (**16**) (**Scheme-2.2**), which is further converted to other flavonoid classes such as flavones (**20**), flavanones (**19**), flavanols (**27**), anthocyanins (**31**)[19] and 3-deoxyanthocyanidins (**29**) (**Schemes-2.3**, **2.4** & **2.5**). In leguminous plants, which possess chalcone reductase (CHR) as well as CHS, a trihydroxychalcone (**15**) may also be formed[20] (**Scheme-2.2**). In a number of species, including pine, grapevine and peanut, the condensation of *p*-coumaroyl-COA or cinnamoyl-COA (**13**) with three malonyl-COA molecules can also give rise to

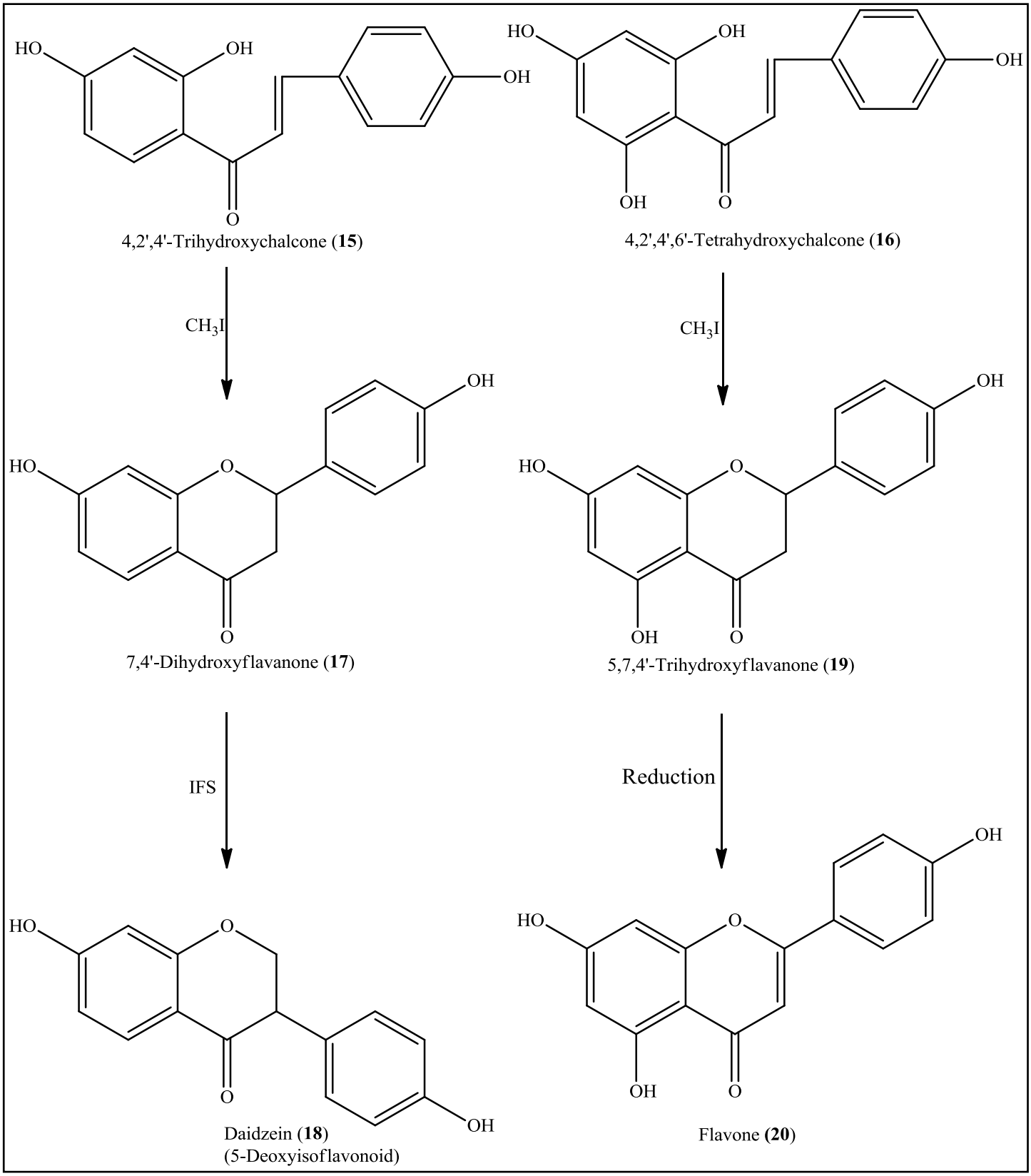
stilbenes (**14**) by the action of stilbene synthase (SS)[21] (**Scheme-2.2**). In legumes, the flavonoid skeleton changes in the presence of isoflavone synthase (IFS), giving rise to the formation of a wide range of simple isoflavonoids, coumestans (**23**), pterocarpans (**22**), and isoflavones (**24**) (**Scheme-2.4** & **2.6**). Structural variety amongst the phenylpropanoids is brought about by a number of modifications, including region-specific hydroxylation, glycosylation, acylation, prenylation, sulfation and methylation.



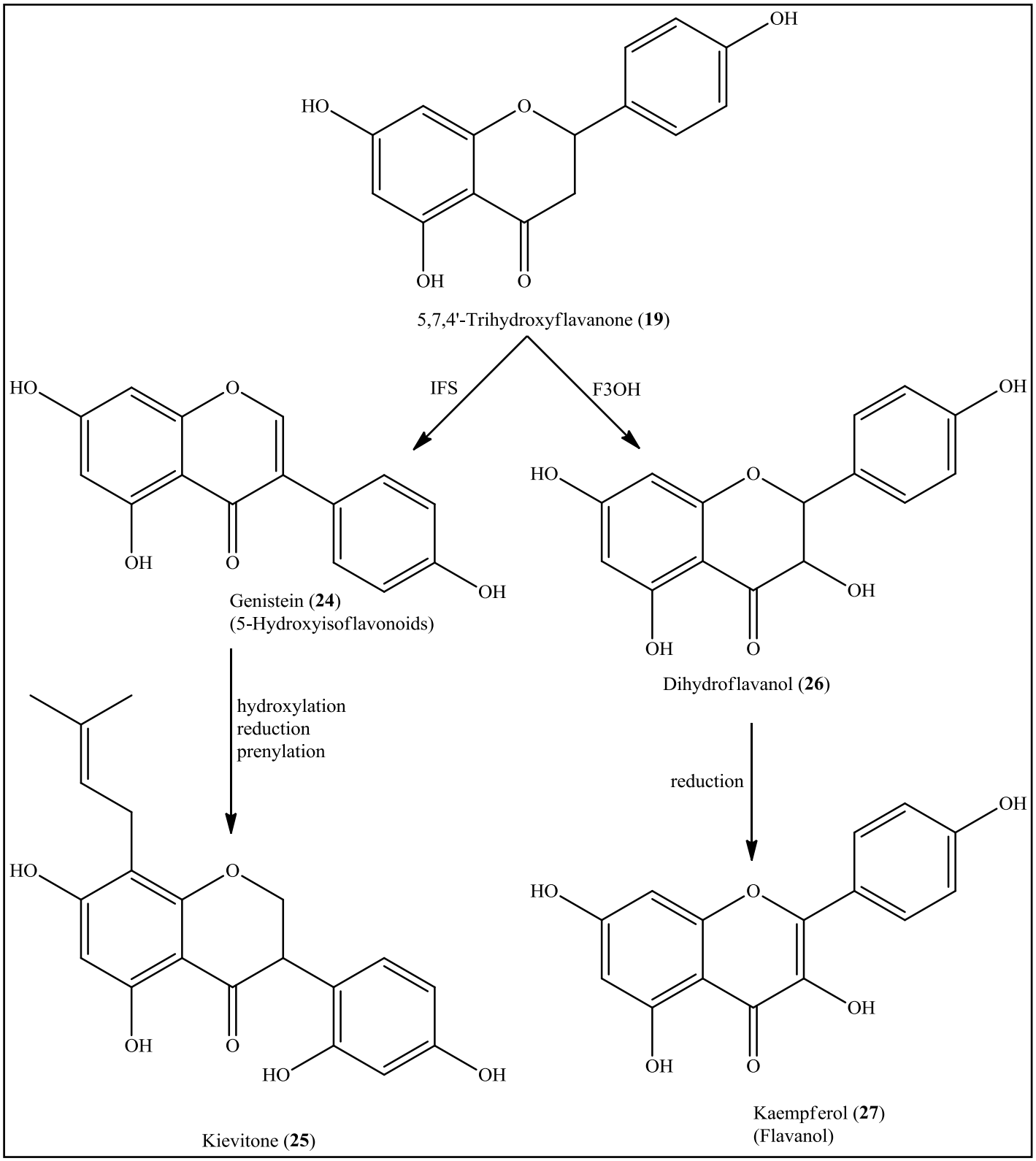
Scheme-2.1: Biosynthesis of benzoic acid, salicylic acid, *p*-coumaric acid, caffeic acid, chlorogenic acid, umebelliferone and soralin



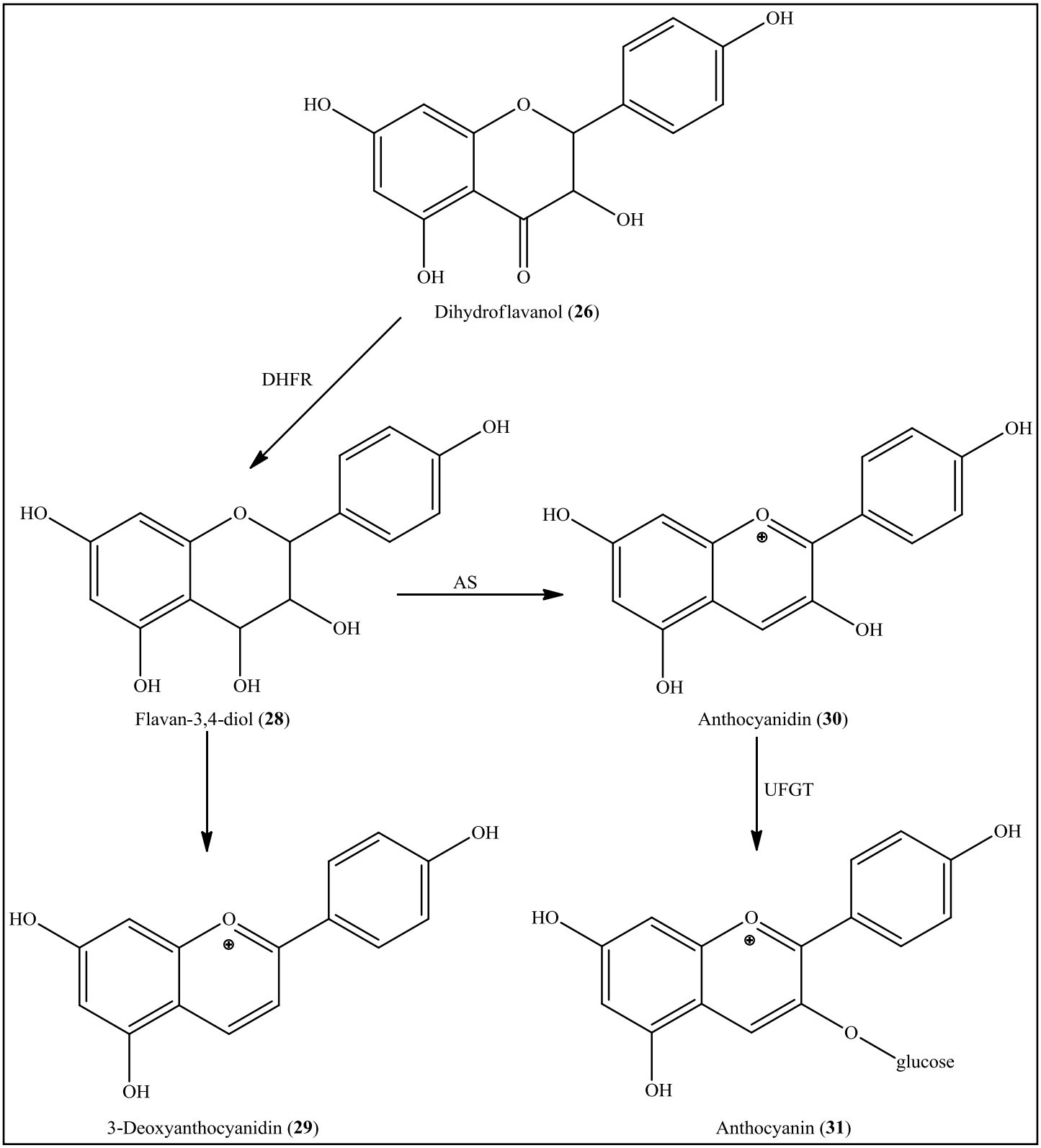
Scheme-2.2: Biosynthesis of C15 flavonoid skeleton



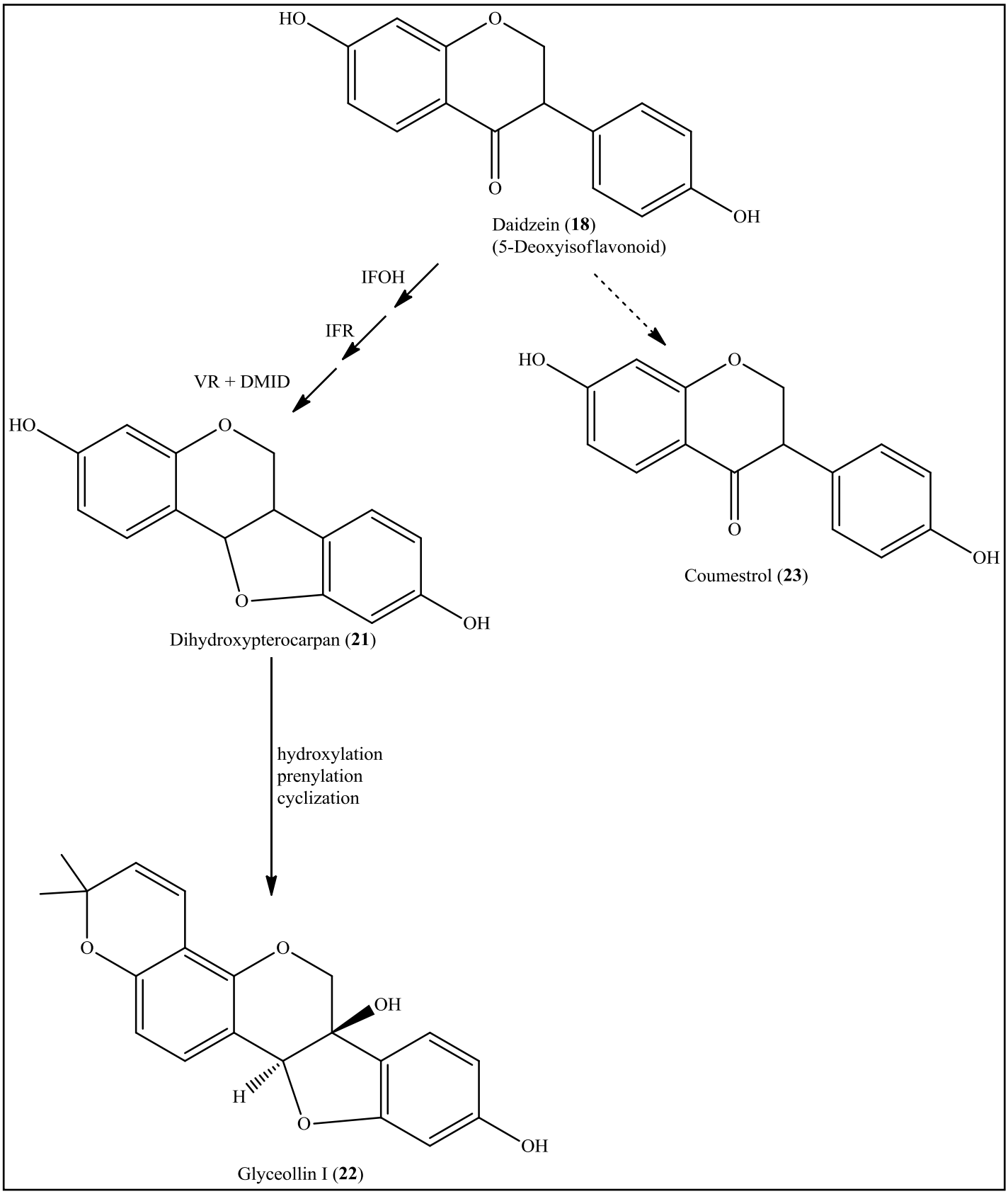
Scheme-2.3: Biosynthesis of flavanones and flavones



Scheme-2.4: Biosynthesis of isoflavanoids and flavanols



Scheme-2.5: Biosynthesis of anthocyanidins and anthocyanins

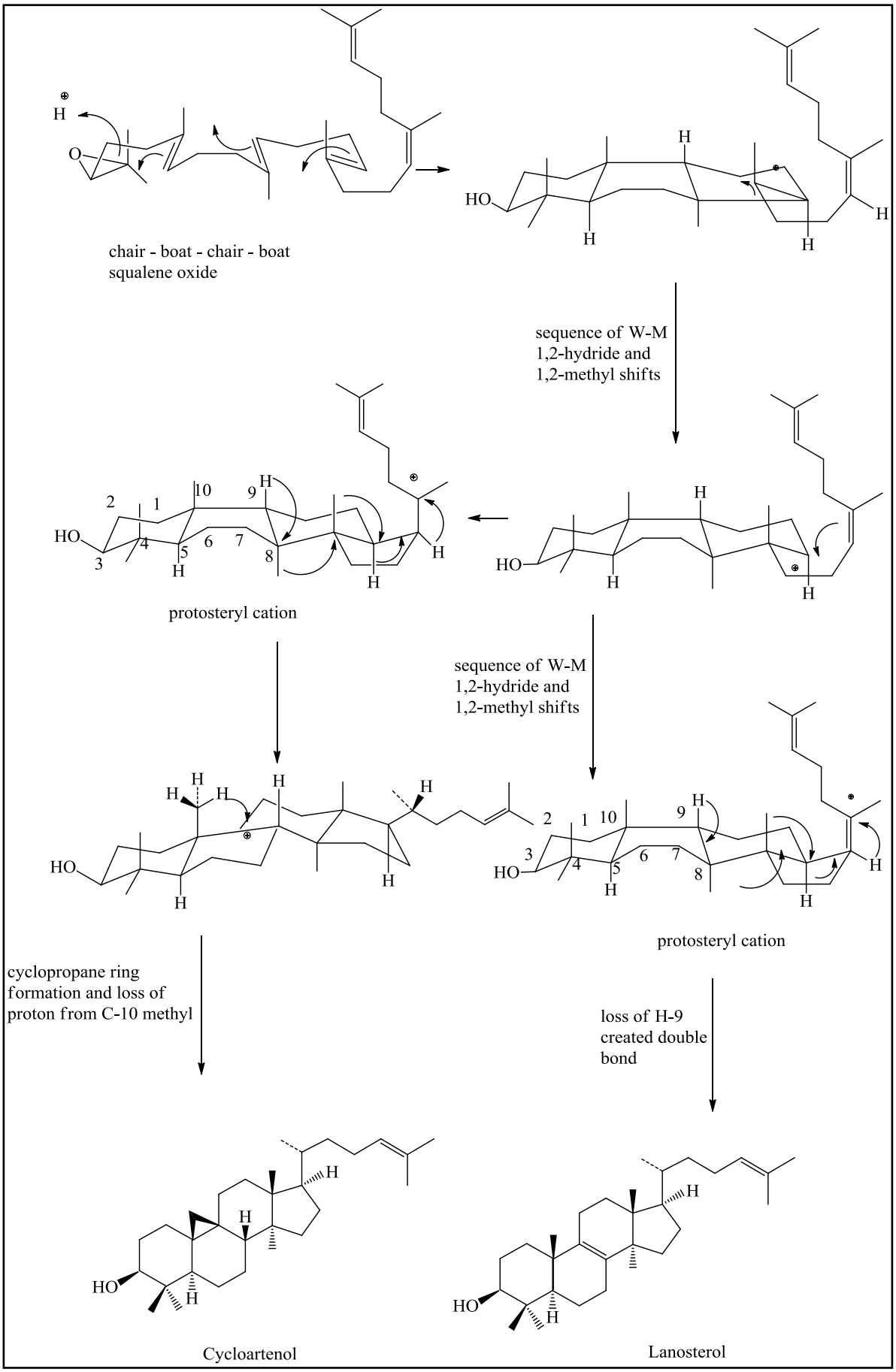


Scheme-2.6: Biosynthesis of pterocarpans

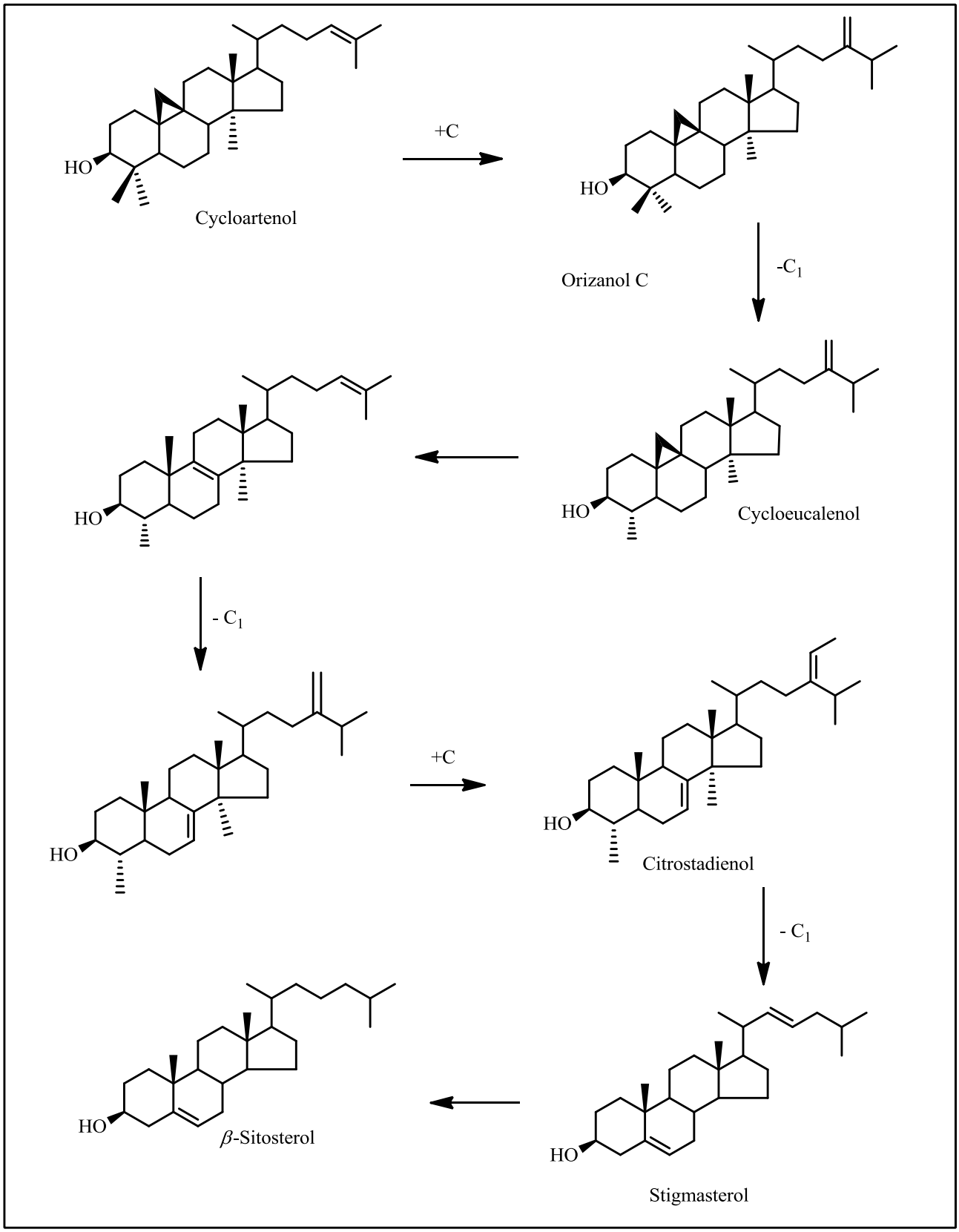
2.2. Biosynthesis of steroids

Naturally, steroids are produced from two precursors i.e. lanosterol and cycloartenol. The initial steps in the biosynthesis of steroids involve the conversion of acetic acid to lanosterol and cycloartenol through mevalonic acid (MVA) and squalene. Steroids of animal origin are thought to be derived mostly from lanosterol, while steroids of vegetable origin evolved from cycloartenol. Both cycloartenol and lanosterol are based upon the chair-boat-chair-boat conformation of squalene oxide *via* transitory protosteryl cation. The cation undergoes a sequence of Wagner-Meerwein 1,2-shift, thus generating a new cation by migrating two hydride ions followed by a methyl group to form lanosterol. Cycloartenol on the other hand acts like lanosterol in plants. It has a cyclopropane ring formed by addition of methyl at C-10. H-9 migrates to C-8 and carbocation is formed, followed by loss of proton from methyl group[22] (**Scheme-2.7**).

Sterols are steroidal metabolites having either of the stigmastane, cholestane or ergostane type of skeletons. Stigmasterol is one of the common phytosterols biosynthesized from cycloartenol. Here, ring opening occurs on the cyclopropane ring of cycloartenol subsequent to the loss of the 4α-methyl group before the loss of the 14β-methyl group just like in the case of biosynthesis of cholesterol. Series changing in timing of the process shown in **Scheme-2.8** is responsible for the occurrence of different sterols in plants. That is why, compound like 24-norcycloartenol and stigmasta-8,14-(*Z*)-24(28)-trien-3β-ol were obtained from different plants.[22]



Scheme-2.7: Biosynthesis of cycloartenol and lanosterol



Scheme-2.8: Biosynthesis of citrostadienol, stigmasterol and *β*-sitosterol

3.1 Plant introduction

Ligularia belongs to the family of Compositae (Asteraceae), tribe Senecioneae, is a highly diversified genus, containing six sections and about 129 species,[23] seven species of which are distributed in Pakistan including three being cultivated i.e. *L. amplexicalis, L. arnicoides, L. macrophylla, L. nana, L. persica, L. sibirica, L. thomsonii.*[24] Many of *Ligularia* species have been used as folk remedies such as anti-inflammatory agents, having the ablity to reduce phlegm, help blood circulation, relieve coughs and pain, as anti-biotic, anti-phlogistic, anti-tussive, anti-asthmatic, anti-cancer, anti-viral,[25] cytotoxic,[26] anti-HIV,[27] anti-diabetic,[28] analgesic, anti-pyretic,[29] for the treatment of hepatitis and arthirites,[28] pulmonary tuberculosis, haemoptysis, urinary tract blockages, rheumatism, common cold, pharyngitis, laryngitis and bronchitis.[30,31]

3.2 Literature survey of genus *Ligularia*

The list of compounds in **Table-3.1** (chronological order) showed diversity of constituents in genus *Ligularia*.

Table-3.1:	Chemical Constituents of the Genus *Ligularia*	
S. No.	**Compounds**	**Species**
1	Ligularenolide	*L. sibirica*[32]
2	Cyperene	*L. veitchiana*[33]
3	Ogarukaya ether	*L. veitchiana*[33]
4	Liguloxide	*L. veitchiana*[33]
5	*p*-Cymene	*L. veitchiana*[33]
6	Limonene	*L. veitchiana*[33]
7	Furoeremophilan-14*β*-6*α*-olide	*L. thomsonii*[34]
8	*β*-Sitosterol	*L. thomsonii*[34]
9	Liguhodgsonal	*L. dentata*[35]
10	Ligularinon A	*L. dentata*[35]
11	8*α*-Angeloyloxyligularinone A	*L. dentata*[35]
12	8*β*-Angeloyloxyligularinone A	*L. dentata*[35]
13	Ligularinon B	*L. dentata*[35]
14	Ligujapone	*L. dentata*[35]
15	Ligucalthaefolin	*L. dentata*[35]
16	3*β*-Angeloyloxyeremophilenolide	*L.tussilaginea*[36]
17	3*β*-Angeloyloxy-8*β*-hydroxyeremophilenolide	*L.tussilaginea*[36]
18	3*β*-Angeloyloxy-6*β*-hydroxyeremophilenolide	*L.tussilaginea*[36]
19	Bakkenolide	*L. hiberniflora*[37]
20	Palmitic acid	*L. hiberniflora*[37]
21	Linoleic acid	*L. hiberniflora*[37]
22	Linolenic acid	*L. hiberniflora*[37]
23	Tsuwabukinonol	*L. tussilaginea*[38]
24	3*β*-Angeloyloxy-6*β*-hydroxy-8-epieremophilenolide	*L. tussilaginea*[39]
25	3*β*-Angeloyloxy-8-epieremophilenolide	*L. tussilaginea*[39]
26	3*β*-Angeloyloxy-6*β*-hydroxyeremophilenolide	*L. tussilaginea*[39]
27	Bakkenolide A	*L. tussilaginea*[39]
28	*α*-Amyrin	*L. tussilaginea*[39]
29	Brein	*L. tussilaginea*[39]
30	Ligularidine	*L. dentata*[40]
31	2-Isopropenyl-5,6-dimethoxycumaran	*L. intermedia*[41]
32	2-Hydroxyplatyphyllid	*L. intermedia*[41]
33	3*β*-Isovaleryloxy-6*β*-angeloyloxy-10*β*-*H*-furanoeremo-philan-15-saure	*L. schmidtii*[41]
34	6*α*-[2-Hydroxymethylacryloyloxy]-1*β*,10*β*-epoxyfurano-eremophilane	*L. intermedia*[41]
35	2-Isopropenyl-5,6-dimethoxycumaran	*L. intermedia*[41]
36	2-Hydroxyplatyphyllid	*L. schmidtii*[41]
37	3*β*-Isovaleryloxy-6*β*-angeloyloxy-10*β*-*H*-furanoeremo-	*L. schmidtii*[41]

	philan-15-saure	
38	6*α*-[2-Hydroxymethylacryloyloxy]-1*β*,10*β*-epoxyfurano-eremophilan	*L. intermedia*[41]
39	3*β*-Angeloyloxy-9-en-8-*epi*-eremophilenolide	*L. tussilaginea*[42]
40	3*β*-Angeloyloxy-8-*epi*-eremophilenolide	*L. tussilaginea*[42]
41	lsofukinone	*L. speciosa*[43]
42	6,15-Epoxy-10β-*H*-furanoeremophilane	*L. thyrsoidea*[44]
43	6*β*-Angeloyloxy-10*β*-furanoeremophilane-15-acid	*L. thyrsoidea*[44]
44	*α*,*α*-bis(3*β*-Angeloyloxyfuranoeremophilane)	*L. tussilaginea*[45]
45	3*β*-Angeloyloxy-8*β*-hydroxy-9*β*-senecioyloxyeremophilen-olide	*L. tussilaginea*[45]
46	Campesterol	*L. tussilaginea*[45]
47	Stigmasterol	*L. tussilaginea*[45]
48	Yantianmasu	*L. dictyoneura*[46]
49	Neoligularidine	*L. dentata*[47]
50	Ligularizine	*L. dentata*[47]
51	Ligularinine	*L. dentata*[47]
52	Clivorine	*L. dentata*[47]
53	Yantianmasu A	*L. dictyoneura*[48]
54	Yantianmasu B	*L. dictyoneura*[48]
55	Yantianmasu C	*L. dictyoneura*[48]
56	Liganolide	*L. thomsonii*[49]
57	2-Methylpentanae-2,4-diol	*L. veitchiana*[50]
58	2-(3′-Pentenyl)-3,7-dimethylbenzofuran-1,4-dione	*L. virgaurea*[51]
59	1-Hydroxy-2-(3′-pentenyl)-3,7-dimethylbenzufuran	*L. virgaurea*[51]
60	1-Methoxy-2-(3′-pentenyl)-3,7-dimethylbenzofuran	*L. virgaurea*[51]
61	Cacalol	*L. virgaurea*[51]
62	4*α*-*H*-9-Methoxyfuranoeremophilane	*L. virgaurea*[51]
63	8-*O*-Tigloyltovarol	*L. persica*[52]
64	8-*O*-Senecioylshiromodiol	*L. persica*[52]
65	6-*O*-Acetyl-8-*O*-senecioylshiromodiol	*L. persica*[52]
66	8-*O*-Tigloylshiromodiol	*L. persica*[52]
67	6-*O*-Acetyl-8-*O*-tigloyfshiromodiol	*L. persica*[52]
68	Fukinone	*L. persica*[52]
69	*α*-Eudesmol	*L. persica*[52]
70	*β*-Eudesmol	*L. persica*[52]
71	1*β*,10*β*-Epoxy-6*β*-(2'-hydroxymethylacryloyloxy)-8*β*-hydroxyeremophila-7(11)-en-12,8*α*-olide	*L. veitchiana*[53]
72	1*β*,10*β*-Epoxy-6*β*-(2'-hydroxymethylacryloyloxy)-8*β*-ethoxyeremophila-7(11)-en-12,8*α*-olide	*L. veitchiana*[53]
73	Taraxol	*L. veitchiana*[53]
74	1*β*,10*β*-Epoxy-6*β*-(2'-semialdehydeacetalhydroxymethyl-acryloyloxy)-8*β*-ethoxyeremophil-7(11)-en-12,8*α*-olide	*L. veitchiana*[54]
75	1*β*-Hydroxy-6,9-diene-8-oxo-eremophil-(12)-oic acid	*L. veitchiana*[54]
76	1*β*-Hydroxy-6,9-diene-8-oxo-11-nor-11-hydroxyeremo-philane	*L. veitchiana*[54]
77	Myricadiol acetate	*L. nelumbifolia*[55]
78	Pentadecanoic acid	*L. nelumbifolia*[55]

79	Caffeic acid ethyl ester	*L. nelumbifolia*[55]
80	Ferulic acid *n*-docosyl ester	*L. nelumbifolia*[55]
81	2-Isopropylidene-3-oxo-5-acetal-6-hydroxybenzodihydro-furan	*L. nelumbifolia*[55]
82	4-*O*-Geranyl-sinapyl alcohol	*L. nelumbifolia*[55]
83	4-*O*-Geranyl-sinapyl aldehyde	*L. nelumbifolia*[55]
84	Linoleic acid 4-*O*-geranyl sinapyl ester	*L. nelumbifolia*[55]
85	1*β*-Acetoxy-11(*R*)-8-oxoeremophil-6,9-dien-12-al	*L. sagitta*[56]
86	1*β*-Acetoxy-11(*S*)-8-oxoeremophil-6,9-dien-12-al	*L. sagitta*[56]
87	1*β*-Diacetoxy-6,9,12*E*-triene-8-oxoeremophilane	*L. sagitta*[56]
88	1*β*-Acetoxy-6,9-dien-8-oxoeremophil-11-nor-11-ketone	*L. sagitta*[56]
89	1*β*-Hydroxy-6,9-dien-8-oxoeremophil-11-nor-11-ketone	*L. veitchiana*[56]
90	1*β*-Acetoxy-6*α*,7*α*-epoxy-9-en-8-oxoeremophil-11-nor-11-ketone	*L. veitchiana*[56]
91	2-Acetyl-5,6-dimethoxybenzofuran	*L. przewalskii*[57,58]
92	2-Propenyl-5-acetyl-7-hydroxy-2,3-dihydroxybenzofuran	*L. przewalskii*[57,58]
93	5-Acetyl-7-methoxybenzofuran	*L. przewalskii*[57,58]
94	1,3-Dimethoxy-4,6,11-trimethylnaphthofuran	*L. przewalskii*[57,58]
95	8*β*-Methoxyermophil-7(11)-en-6*α*,l5;8*α*,12-diolide	*L. przewalskii*[58]
96	Eremophil-8(9)7,(11)-dien-6α,15;8,12-diolide	*L. przewalskii*[58]
97	10*β*-Hydroxyeremophil-8(9)7,(11)-dien-6*α*,15;8,12-diolide	*L. przewalskii*[58]
98	10*β*-Hydroxy-8*β*,9*β*-epoxyermophil-7(1l)-en-6*α*,15;8*α*,12-diolide	*L. przewalskii*[58]
99	8*β*-Hydroxy-6*β*-angeloyloxyeremophil-7(11)-en-8*α*,12-olide-15-oic acid	*L. przewalskii*[58]
100	2-Acetyl-3*α*,*β*-methyl-3*α*,4,5,6,7,7*α*-hexahydroinden-4*β*-carboxylic methyl ester	*L. przewalskii*[58]
101	11(*R*)-8-Oxo-eremophil-6(7)-en-dimethyl-12,15-dioate	*L. przewalskii*[58]
102	11(*S*)-8-Oxo-eremophil-6(7)-en-dimethyl-12,15-dioate	*L. przewalskii*[58]
103	Benzofuranoeremophil-1-ene	*L. sagitta*[59]
104	Ligulaverin A	*L. veitchiana*[60]
105	Ligulaverin B	*L. veitchiana*[60]
106	Ligulaverin C	*L. veitchiana*[60]
107	Ligulaverin D	*L. veitchiana*[60]
108	Ligulaverin E	*L. veitchiana*[60]
109	2,5-Diacetyl-6-methoxybenzofuran	*L. intermedia*[61]
110	2-Isoprepenyl-6-acetyl-8-methoxy-1,3-benzodioxin-4-one	*L. intermedia*[61]
111	(*E*)-4-(6-Hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)-syringenin	*L. intermedia*[61]
112	(*E*,*E*)-4-(7-Hydroperoxy-3,7-dimethylocta-2,5-dienyloxy)-syringenin	*L. intermedia*[61]
113	6*α*,9-Dihydroxy-14*β*-carboxyfuranoeremophil-9(10)-ene	*L. intermedia*[62]
114	7*α*,8*α*-Epoxyeremophilan-l2*β*,8*β*(l4*β*,6*α*)-diolide	*L. intermedia*[62]
115	Eremophil-7(11),8(9)-dien-12,8(14*β*,6*α*)-diolide	*L. intermedia*[62]
116	Eremophil-7(8)-en-12,8(14*β*,6*α*)-diolide	*L. intermedia*[62]
117	4-*O*-[6-Hydroxy-7(9)-dehydro-6,7-dihydrogeranyl]-coniferyl alcohol	*L. duciformis*[63]
118	4-*O*-[7-Hydroxy-5,6E-dehydro-6,7-dihydrogeranyl]-coniferyl alcohol	*L. duciformis*[63]

119	4-*O*-[6-Hydroperoxy-7(9)-dehydro-6,7-dihydrogeranyl]-coniferyl alcohol	*L. duciformis*[63]
120	4-*O*-[7-Hydroperoxy-5,6*E*-dehydro-6,7-dihydrogeranyl]-coniferyl alcohol	*L. duciformis*[63]
121	4-*O*-[6-Hydroxy-7(9)-dehydro-6,7-dihydrogeranyl]-sinapyl alcohol	*L. duciformis*[63]
122	4-*O*-[7-Hydroxy-5,6*E*-dehydro-6,7-dihydrogeranyl]-sinapyl alcohol	*L. duciformis*[63]
123	Ligularine	*L. hodgsonii*[64]
124	Ligudentatin A	*L. dentata*[65]
125	Ligudentatin B	*L. dentata*[65]
126	3-(4′-Formylphenoxy)-4-methoxybenzaldehyde	*L. dentata*[65]
127	9*α*-Hydroxypinoresinol	*L. kanaitizensis*[66]
128	11-Hydroxyeremophil-6,9-dien-8-one	*L. kanaitizensis*[66]
129	Emodin	*L. kanaitizensis*[66]
130	Physcion	*L. kanaitizensis*[66]
131	Physcion-8-*β*-D-monoglucoside	*L. kanaitizensis*[66]
132	Scopoletin	*L. kanaitizensis*[66]
133	Daucosterol	*L. kanaitizensis*[66]
134	4-Hydroxyacetophenone	*L. vellerea*[67]
135	8*α*-Hydroxy-7(11)-eremophilen-12,8*β*-olide	*L. vellerea*[67]
136	Umbelliferone	*L. vellerea*[67]
137	Tiglic acid	*L. vellerea*[67]
138	6*β*-Hydroxy-7(11)-eremophilen-12,8*α*-olide	*L. vellerea*[67]
139	Daucosterin	*L. vellerea*[67]
140	11-Hydroperoxyeremophil-6,9-dien-8-one	*L. kanaitzensis*[68]
141	Oleanolic acid	*L. kanaitzensis*[68]
142	*β*-Amyrin	*L. kanaitzensis*[68]
143	*β*-Amyrin acetate	*L. kanaitzensis*[68]
144	Lupeol	*L. kanaitzensis*[68]
145	7(11)-Eremophilen-8-one	*L. dictyoneura*[69]
146	6*β*-Hydroxyeremophil-7(11)-en-12,8*α*-olide	*L. dictyoneura*[69]
147	6*β*,8-Hydroxyeremophil-7(11)-en-12,8*α*-olide	*L. dictyoneura*[69]
148	6*β*,8*β*-Dimethoxy-10*β*-hydroxyeremophil-7(11)-en-12,8*α*-olide	*L. sagitta*[70]
149	6*β*-Angeloyloxy-10*β*-hydroxy-8*β*-methoxyeremophil-7(11)-en-12,8*α*-olide	*L. sagitta*[70]
150	6*β*-(2'-Methylbutanoyloxy)-10*β*-hydroxy-8*β*-methoxyeremophil-7(11)-en-12,8*α*-olide	*L. sagitta*[70]
151	6*β*-Angeloyloxy-10*β*-hydroxy-8*α*-methoxyeremophil-7(11)-en-12,8*β*-olide	*L. sagitta*[70]
152	6*β*-(2'-Methylbutanoyloxy)-10*β*-hydroxy-8*α*-methoxyeremophil-7(11)-en-12,8*β*-olide	*L. sagitta*[70]
153	8*β*,10*β*-Dihydroxy-6*β*-methoxyeremophil-7(11)-en-12,8*α*-olide	*L. sagitta*[70]
154	(3*R*,4*R*,6*S*)-3,6-Dihydroxy-1-menthene	*L. sagitta*[70]
155	Ursolic acid	*L. sagitta*[70]
156	Ligudicin A	*L. dictyoneura*[71]
157	Ligudicin C	*L. dictyoneura*[71]

158	Ligudicin D	*L. dictyoneura*[71]
159	Isopetacin	*L. dictyoneura*[71]
160	1*β*,10*β*-Epoxy-6*β*-acetoxy-3*β*-angeloyloxy-8*β*-hydroxy-eremo-phil-7(11)-en-8,12*α*-olide	*L. sagitta*[72]
161	Esculetin	*L. sagitta*[72]
162	3*α*-Hydroxy-8-oxo-eremophila-6,9-dien-12-oic acid	*L. pleurocaulis*[73]
163	Furannoligularenone	*L. pleurocaulis*[73]
164	3-Oxo-eremophila-1,7(11)-dien-12,8*β*-olide	*L. pleurocaulis*[73]
165	3-Oxo-8*α*-hydroxyl-10*α*-*H*-eremophila-1,7(11)-dien-12,8*β*-olide	*L. pleurocaulis*[73]
166	3-Oxo-8*α*-methoxyl-10*α*-*H*-eremophila-1,7(11)-dien-12,8*β*-olide	*L. pleurocaulis*[73]
167	7*α*,8*α*-Epoxy-eremophila-12*β*,8*β*-(15*β*,6*α*)-diolide	*L. pleurocaulis*[73]
168	8*β*-Ethoxy-eremophila-7(11)-en-12,8*α*-(15*β*,6*α*)-diolide	*L. pleurocaulis*[73]
169	8*β*-Hydroxy-7,11-eremophila-12,8*α*-olide	*L. pleurocaulis*[73]
170	8*β*-Hydroxyeremophil-7(11)-en-12,8*α*-olide	*L. dictyoneura*[74]
171	3,3',4', 7-Tetramethoxyflavone	*L. dictyoneura*[74]
172	3,3',7-Trihydroxy-4'-methoxyflavone	*L. dictyoneura*[74]
173	Ligulolide A	*L. virgaurea*[75]
174	6*β*,8*α*-Dihydroxy-1-oxo-eremophila-7(11),9(10)-diene-12,8-olide	*L. virgaurea*[75]
175	Bieremoligularolide	*L. muliensis*[76]
176	Eremoligularin	*L. muliensis*[76]
177	15*β*-Formic ether-6-oxo-furanoeremophilane	*L. macrophylla*[77]
178	6*α*,15*β*-Epoxyeremophila-7(11)-en-8*α*,12-olide	*L. macrophylla*[77]
179	3*β*-(2'-Methylbutanoyloxy)-8β-*H*-eremophil-7(11)-ene-12,8*α*-(14,6*α*)-iolide	*L. tongolensis*[30]
180	8*β*-*H*-Eremophil-3,7(11)-diene-12,8*α*-(14,6*α*)-diolide	*L. tongolensis*[30]
181	2*α*,3*β*,19*α*-Trihydroxy-28-norurs-12-ene	*L. tongolensis*[30]
182	2*α*,3*α*,19*α*-Trihydroxy-28-norurs-12-ene	*L. tongolensis*[30]
183	Eremophil-1(10),7(11),8(9)-trien-12,8-olide	*L. platyglossa*[78]
184	8*β*-Hydroxyeremophil- 7(11),9(10)-dien-12,8*α*-olide	*L. platyglossa*[78]
185	2-Oxo-eremophil-1(10),7(11),8(9)-trien-12,8-olide	*L. platyglossa*[78]
186	Fisetin	*L. platyglossa*[78]
187	Isovanillic acid	*L. platyglossa*[78]
188	7-Angelylheliotridine	*L. platyglossa*[78]
189	Friedelinol	*L. stenocephala*[79]
190	Friedelin	*L. stenocephala*[79]
191	5-Acetyl-6-hydroxy-2-isopropenylbenzofuran	*L. stenocephala*[79]
192	5,6-Dimethoxy-2-isopropenylbenzofuran	*L. stenocephala*[79]
193	Rhamnazin-3-*O*-*β*-D-rutinoside	*L. macrophlla*[80]
194	Rhamnetin-3-*O*-*β*-*O*-rutinoside	*L. macrophlla*[80]
195	Rutin	*L. macrophlla*[80]
196	Afzelin	*L. macrophlla*[80]
197	Betulalbuside A	*L. macrophlla*[80]
198	3,7-Dimethyloct-1-en-3,8-diol-8-*O*-*β*-D-glucopyranoside	*L. macrophlla*[80]
199	Squalene	*L. stenocephala*[81]
200	Cycloartanol	*L. stenocephala*[81]

201	24-Methylenecycloartanol	*L. stenocephala*[81]
202	Dammara-20,24-dien-3*β*-ol	*L. stenocephala*[81]
203	24-Methylenedammar-20-en-3*β*-ol	*L. stenocephala*[81]
204	19*α*-*H*-Lupeol	*L. stenocephala*[81]
205	Isoarborinol	*L. stenocephala*[81]
206	6-Angeloyloxy-furanoligularenone	*L. pleurocaulis*[82]
207	2-Oxo-3-hydroxyeremophila-1(10),3,7(11),8-tetraen-8,12-olide	*L. pleurocaulis*[82]
208	Tiglic acid	*L. pleurocaulis*[82]
209	Caffeic acid	*L. pleurocaulis*[82]
210	Emodin	*L. pleurocaulis*[82]
211	7-Methoxy-coumarin	*L. pleurocaulis*[82]
212	Ferulic acid	*L. pleurocaulis*[82]
213	4-Hydroxy-2,5-dimethoxybenzaldehyde	*L. pleurocaulis*[82]
214	(*E*)-Docosyl-3,4-dihydroxycinnamate	*L. duciformis*[83]
215	(*E*)-Docosyl 3-methoxy-4-hydroxyferulate	*L. duciformis*[83]
216	*β*-Amyrone	*L. duciformis*[83]
217	Aurantiamide benzoate	*L. narynensis*[84]
218	Aurantiamide acetate	*L. narynensis*[84]
219	1-*O*-Octacosanoylgerol	*L. narynensis*[84]
220	Virgaurenone A	*L. virgaurea*[85]
221	Virgaurenone B	*L. virgaurea*[85]
222	Virgaurenone C	*L. virgaurea*[85]
223	Virgaurenone D	*L. virgaurea*[85]
224	Virgaurenolide A	*L. virgaurea*[85]
225	3*β*,6*β*-Bis(3-methylpentanoyloxy)furlanoeremophilan-15-oic acid	*L. tongolensis*[86]
226	3*β*-(3-Methylpentanoyloxy)furanoeremophilan-15,6*α*-olide	*L. cymbulifera*[86]
227	3*β*-Angeloyloxyfuranoeremophilan-15,6*α*-olide	*L. cymbulifera*[86]
228	Furanoeremophil-3-en-15,6*α*-olide	*L. cymbulifera*[86]
229	Furanoeremophilan-10*β*-ol	*L. cymbulifera*[86]
230	3-(Methacryloyloxy)furanoeremophilan-15,6*α*-olide	*L. cymbulifera*[86]
231	Eremophil-1(10)-en-11-ol	*L. cymbulifera*[86]
232	3*β*,6*β*-Bis(angeloyloxy)furanoeremophilan-15-oic acid	*L. atroviolacea*[86]
233	3*β*-Angeloyloxy-6*β*-(3-methylbutyryloxy)-furanoeremophilan-15-oic acid	*L. atroviolacea*[86]
234	6*β*,10*α*-Dihydroxy-1-oxo-eremophila-7(11),8(9)-dien-12,8-olide	*L. virgaurea*[87]
235	6*β*-Acetyl-2-oxo-eremophila-1(10),7(11), 8(9)-trien-12,8-olide	*L. virgaurea*[87]
236	6*β*,8*β*-Diacetyl-2-oxo-eremophila-1(10),7(11)-dien-12,8-olide	*L. virgaurea*[87]
237	Ligulolide B	*L. virgaurea*[87]
238	6*β*,8*α*-Diacetyleremophila-1(10),7(11)-dien-12,8-olide	*L. virgaurea*[87]
239	(6*β*,8*α*)-6-(Acetyloxy)-8-hydroxyeremophil-7(11)-en-12,8-olide	*L. muliensis*[88]
240	(6*α*,8*α*)-6-Hydroxyeremophil-7(11)-en-12,8-olide	*L. muliensis*[88]
241	(6*α*,8*α*)-6-Acetyloxyeremophil-7(11)-en-12,8-olide	*L. muliensis*[88]
242	((8*α*)-Eremophil-7(11)-en-12,8-olide=(4*αR*,5*S*,8*αR*,9*αS*)-	*L. muliensis*[88]

	4*α*,5,6,7,8,8*α*,9,9*α*-octahydro-3,4*α*,5-trimethylnaphtho[2,3-*β*]furan-2(4*H*)-one)	
243	Eremoligularin	*L. muliensis*[88]
244	Bieremoligularolide	*L. muliensis*[88]
245	*rel*-(1*R*,2*R*,3*R*,4*S*,5*S*)-*p*-Menthane-1,2,3,5-tetrol	*L. muliensis*[88]
246	2-(1,2-Dihydroxyisopropyl)-5,6-dimethoxybenzofuran	*L. przewalskii*[89]
247	2-(1-*O*-Feruloyl-2-hydroxyisopropyl)-5,6-dimethyoxybenzo-furan	*L. przewalskii*[89]
248	(2*E*,6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-ol	*L. stenocephala*[90]
249	(3*E*, 6*E*, 10*E*, 14*E*, 18*E*)-2,6,10,15,19,23-Hexamethyl-3,6,10,14,18,22-tetracosahexaen-2-ol	*L. stenocephala*[90]
250	Neophytadiene	*L. stenocephala*[90]
251	5-Acetyl-6-hydroxy-2*α*-isopropenyl-3*β*-methoxy-2,3*H*-benzofuran	*L. stenocephala*[90]
252	3*β*-Acetoxy-6-acetyl-5-hydroxy-2*α*-isopropenyl-2,3*H*-benzo-furan	*L. stenocephala*[90]
253	*p*-Hydroxybenzaldehyde	*L. stenocephala*[90]
254	Vanillin	*L. stenocephala*[90]
255	4-Hydroxyacetophenone	*L. stenocephala*[90]
256	4-Hydroxy-3-methoxyacetophenone	*L. stenocephala*[90]
257	6-Hydroxy-3*α*-methoxytremetone	*L. stenocephala*[91]
258	2,2'-(1",1"-Dimethyl-3"-methoxy-3"-methyl-1",3"-propan-diyl)-bis(5,6-dimethoxybenzofuran)	*L. stenocephala*[91]
259	7,8-Dimethoxy-1,4-dimethyldibenzofuran	*L. stenocephala*[91]
260	24-Chlorocycloart-25-en-*3β*-ol	*L. stenocephala*[91]
261	(2,4-Dimethoxy-5-al)phenyl 2,2-dimethyl-4-(5,6-dimethoxy-2-benzofuranyl)-4-pentenate	*L. stenocephala*[92]
262	1*β*,6*α*-Dihydroxy-4(15)-eudesmene	*L. stenocephala*[92]
263	5-Acetyl-6-hydroxy-2-isopropenylbenzofuran	*L. stenocephala*[92]
264	7*α*-Hydroxy-9(10)-ene-1,8-dioxo- 6,7-dihydrofuranoeremo-philane	*L. sagitta*[93]
265	1*β*,10*β*-Epoxy-6*β*,8*β*-dihydroxy- eremophil- 7(11)-en-12,8*α*-olide	*L. sagitta*[93]
266	1-Oxo-9-desoxycacalol	*L. sagitta*[93]
267	Benzofuranoeremophil-1-ene	*L. sagitta*[93]
268	2*α*,8-Diangeloyloxy-3*β*,4*β*,10,11-diepoxy-1*α*-hydroxy-bisabol-7(14)-ene	*L. cymbulifera*[94]
269	2*β*,8-Diangeloyloxy-3*α*,4*α*,10,11-diepoxy-1*α*-hydroxy-bisabol-7(14)-ene	*L. cymbulifera*[94]
270	3*β*,4*β*,10,11-Diepoxy-1*β*,2*β*,8-triangeloyloxybisabol-7(14)-ene	*L. cymbulifera*[94]
271	1*β*,8-Diangeloyloxy-3*β*,4*β*,10,11-diepoxybisabol-7(14)-ene	*L. cymbulifera*[94]
272	1*β*,8-Diangeloyloxy-3*β*,4*β*-epoxy-2*β*,10,11-trihydroxybisabol-7(14)-ene	*L. cymbulifera*[94]
273	1*α*,8-Diangeloyloxy-10,11-dihydroxy-3*β*,4*β*-epoxybisabol-7(14)-en-2-one	*L. cymbulifera*[94]
274	10,11-Epoxy-1*β*-hydroxy-2*β*,4*α*,8-triangeloyloxybisabol-7(14)-ene	*L. cymbulifera*[94]

275	4*α*-Chloro-1*β*,8-diangeloyloxy-10,11-epoxy-2*β*-hydroxy-bisabol-7(14)-ene	*L. cymbulifera*[94]
276	6*β*-Angeloyloxy-furanoeremophil-1(10)-ene	*L. pleurocaulis*[95]
277	6*β*-Tigloyloxy-furanoeremophil-1(10)-ene	*L. pleurocaulis*[95]
278	6*β*-Angeloyloxy-furanoeremophil-1(10)-en-3*β*-ol	*L. pleurocaulis*[95]
279	6*β*-Tigloyloxy-furanoeremophil-1(10)-en-3*β*-ol	*L. pleurocaulis*[95]
280	3*β*-Angeloyloxyfuranoeremophil-1(10)-en-6*β*-ol	*L. pleurocaulis*[95]
281	Furanoligularenone	*L. pleurocaulis*[95]
282	(*R*)-(+)-Ligulaodonin A	*L. odontomanes*[96]
283	3-Oxo-16*β*-Hydroxy-olean-12-en-28-al	*L. odontomanes*[96]
284	Euparin	*L. odontomanes*[96]
285	2-Acetyl-5,6-dimethoxybenzofuran	*L. odontomanes*[96]
286	Gummosogenin	*L. odontomanes*[96]
287	1*β*,6*α* -Dihydroxy-4(14)-eudesmene	*L. odontomanes*[96]
288	2-Hydroxy-4,5-dimethoxybenzaldehyde	*L. odontomanes*[96]
289	Butyrospermol	*L. dentata*[97]
290	Stigmast-4-en-3-one	*L. dentata*[97]
291	Stigmast-4-ene-3,6-dione	*L. dentata*[97]
292	(+)-Methyl abscisate	*L. dentata*[97]
293	Methyl Indole-3-carboxylate	*L. dentata*[97]
294	Methyl-(*E*)-caffeate	*L. dentata*[97]
295	Methyl-(*E*)-ferulate	*L. dentata*[97]
296	Methyl-(*E*)-feruloylglycolate	*L. dentata*[97]
297	Methyl-7,8-dihydro-(*S*)-7-methoxyferulate	*L. dentata*[97]
298	3-Methoxy-4-hydroxyphenylpropyl caffeate	*L. duciformis*[98]
299	6*β*,8*β*-Dihydroxyeremophil-7(11)-en-12,8*β*-olide	*L. duciformis*[98]
300	6*β*-Hydroxyeremophil-7(11)-en-12,8*β*-olid	*L. duciformis*[98]
301	8*β*-Hydroxyeremophil-7-ene-12,8*α*(4*β*,6*α*)-diolide	*L. intermedia*[99]
302	8*β*-Methoxyeremophil-7(11)-ene-12,8*α*(4*β*,6*α*)-diolide	*L. intermedia*[99]
303	Petasin	*L. intermedia*[99]
304	Isopetasin	*L. intermedia*[99]
305	Igudentatol	*L. intermedia*[99]
306	Lupeol palmitate	*L. intermedia*[99]
307	2-Isopropenyl-6-acetyl-8-methoxy-1,3-benzodioxin-4-one	*L. stenocephala*[100]
308	Phthalic acid dibutyl ester	*L. stenocephala*[100]
309	*N*-phenyl-2-naphthylamine	*L. stenocephala*[100]
310	5,6-Dimethoxy-2-isopropenylbenzofuran	*L. stenocephala*[100]
311	Liguladentanorol	*L. dentata Hara*[101]
312	8*α*-Hydroxyligudentatol	*L. dentata Hara*[101]
313	8*α*-Hydroxyligujapone	*L. dentata hara*[101]
314	8*β*-Hydroxyeremophil-7(11)-en-12,8*α*(14*β*,6*α*)-dioxide	*L. veitchiana*[102]
315	5,6-Dimethoxy-2-(1-methylethenyl)-1-benzofuran	*L. villosa*[103]
316	1-[(2*R**,3*S**)-3-Ethoxy-2,3-dihydro-6-hydroxy-2-(1-methyl-ethenyl)-1-benzofuran-5-yl]ethanone	*L. latihastata*[103]
317	(2*R**,3*S**)-5-Acetyl-2,3-dihydro-6-hydroxy-2-(1-methyl-ethenyl)-1-benzofuran-3-yl (2*Z*)-2-[(acetoxy)methyl]but-2-enoate	*L. latihastata*[103]
318	6*β*-Sarracinoyloxy-1*β*,10*β*-epoxy-furanoeremophilane	*L. macrophylla*[104]

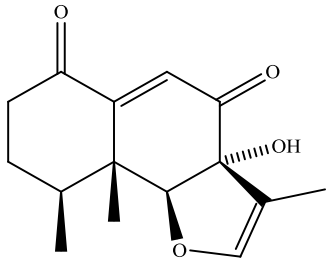
319	6*α*-Angeloyloxy-10*βH*-furanoeremophil-1-one	*L. macrophylla*[104]
320	1*α*-Hydroxy-9-deoxycacalol	*L. macrophylla*[104]
321	1*β*-hydroxy-11(*R,S*)-8-oxoeremophil-6,9-dien-12-al	*L. macrophylla*[104]
322	1,10-Epoxy-6-hydroxyeuryopsin	*L. macrophylla*[104]
323	6-Angeloyloxy-1,10-epoxyeuryopsin	*L. macrophylla*[104]
324	1-Oxo-9-desoxycacalol	*L. macrophylla*[104]
325	Farfugin A	*L. macrophylla*[104]
326	*γ*,3,5-Trimethyl-6-benzofuranbutanal	*L. macrophylla*[104]
327	8*β*-Hydroxyeremophil-3,7(11)-diene-8*α*,12(6*α*,15)-diolide	*L. lapathifolia*[105]
328	8*β* -Methoxyeremophil-3,7(11)-diene-8*α*,12(6*α*,15)-diolide	*L. lapathifolia*[105]
329	8*β* -Ethoxyeremophil-3,7(11)-diene-8*α*,12(6*α*,15)-diolide	*L. lapathifolia*[105]
330	6*β*-(2*ξ*-Methylbutyryloxy)eremophil-3,7(11),8-trien-8,12-olide-15-oic acid methyl ester	*L. lapathifolia*[105]
331	3*β*-Angeloyloxy-8-oxoeremophil-6(7)-ene-12,15-dioic acid methyl ester	*L. lapathifolia*[105]
332	2-Acetyl-3a*β*-methyl-3a,6,7,7a*β*-tetrahydro-1*H*-inden-4-oic acidmethyl ester	*L. lapathifolia*[105]
333	Ligulasagitin A	*L. sagitta*[106]
334	Ligulasagitin B	*L. sagitta*[106]
335	Ligulasagitin C	*L. sagitta*[106]
336	Ligulasagitin D	*L. sagitta*[106]
337	Ligulasagitin E	*L. sagitta*[106]
338	8*β*-Hydroxy-eremophil-3,7 (11)-dien-12,8*α*(14,6*α*)-diolide	*L. atroviolacea*[107]
339	8*β*-Methoxy-eremophil-3,7(11)-dien-12,8*α*(14,6*α*)-diolide	*L. atroviolacea*[107]
340	8*α*-Hydroxy-eremophil-3,7(11)-dien-12,8*β*(14,6*α*)-diolide	*L. atroviolacea*[107]
341	Eremophil-3,7(11),8-trien-12,8 (14,6*α*)-diolide	*L. atroviolacea*[107]
342	7-Hydroxy-9(10)-ene-1,8-diox-6,7-dihydrofuranoeremo-philane	*L. sagitta*[108]
343	6*β*-Angeloyloxyfuranoeremophilan-15-oic acid	*L.vellerea*[109]
344	6*β*-Angeloyloxyfuranoeremophilan-15-oate	*L.vellerea*[109]
345	6*α*,15-Epoxyfuranoeremophilane	*L.vellerea*[109]
346	Furanoeremophilan-15,6*α*-olide	*L.vellerea*[109]
347	8*β*-Hydroxyeremophil-7(11)-ene-15,6*α*:12,8-diolide	*L.vellerea*[109]
348	Eremophila-1(10),11-diene	*L.vellerea*[109]
349	1*α*-Angeloyloxyfuranoeremophilan-6*β*-ol	*L.vellerea*[109]
350	Petasalbin	*L.vellerea*[109]
351	Ligularol ethyl	*L.vellerea*[109]
352	Narynenol	*L. narynensis*[110]
353	1*β*,5*α*,8-Trisangeloyloxy-3*β*,4*β*,10,11-bisepoxybisabol-7(14)-en-2*β*-ol	*L. lankongensis*[111]
354	1*β*,8-Bisangeloyloxy-3*β*,4*β*,10,11-bisepoxybisabol-7(14)-en-2*β*-ol	*L. lankongensis*[111]
355	8-Angeloyloxy-3*β*,4*β*,10,11-bisepoxy-1*β*-(2-methylbutyryl-oxy)-bisabol-7(14)-en-2*β*-ol	*L. lankongensis*[111]
356	2*β*,8-Bisangeloyloxy-3*β*,4*β*,10,11-bisepoxybisabol-7(14)-en-1*β*-ol	*L. lankongensis*[111]
357	2*β*,8-Bisangeloyloxy-4*α*-chloro-10,11-epoxybisabol-7(14)-ene-1*β*,3*β*-diol	*L. lankongensis*[111]
358	3,28-Dihydroxy-lupeol	*L. xanthotricha*[112]

359	Betulinic acid	*L. xanthotricha*[112]
360	Taraxasterol	*L. xanthotricha*[112]
361	Taraxasteryl palmitate	*L. xanthotricha*[112]
362	Taraxasteryl acetate	*L. xanthotricha*[112]
363	1*α*-Chloro-6*β*-isobutyroxy-9-oxo-10*β*-hydroxy-furanoeremo-philane	*L. atroviolacea*[113]
364	1*α*-Hydroxy-6*β*-isobutyroxy-9-oxo-10*β*-H-furanoeremo-philane	*L. atroviolacea*[113]
365	1*α*-Hydroxy-6*β*-isobutyroxy-9-oxo-10*α*-H-furanoeremo-philane	*L. atroviolacea*[113]
366	1*α*,10*β*-Dihydroxy-6*α*-angeloyloxy-9-oxo-furanoeremo-philane	*L. atroviolacea*[113]
367	Furanoeremophil-15*β*,6*α*-olide	*L. atroviolacea*[26]
368	1,2,4-Trimethyl-7,8-dimethoxy-dibenzofuran	*L. caloxantha*[114]
369	2,5-Diacetyl-6-hydroxy-benzofuran	*L. caloxantha*[114]
370	(*E*)-2,5-dihydroxy-cinnamic acid	*L. caloxantha*[114]
371	Isoscopoletin	*L. duciforms*[115]
372	Isoline	*L. duciforms*[115]
373	Duciformine	*L. duciforms*[115]
374	(2*S*,3*S*,4*R*)-Sphinganine-Δ5',6'(*E*)-2'-hydroxytetracosanoyl-amino	*L. duciforms*[115]
375	Tetracosanoic acid	*L. duciforms*[115]
376	Tetracosanoicacid glycerin ester	*L. duciforms*[115]
377	(*E*)-Docosyl ferulate	*L. duciforms*[115]
378	Docosyl caffeate	*L. duciforms*[115]
379	Franchetianonies A	*L. frachetiana*[116]
380	Franchetianonies B	*L. frachetiana*[116]
381	Furanoeremophilan-14*β*,6*α*-olide	*L. macrophylla*[117]
382	6*β*-Angeloyloxy-10*β*-hydroxyfuranoeremophilane	*L. macrophylla*[117]
383	Eremophil-7(11)-ene-12,8*α*,14*β*,6*α*-diolide	*L. macrophylla*[117]
384	3*α*-Angeloyloxybakkenolide A	*L. macrophylla*[117]
385	(1*R*,2*R*,3*S*,5*S*,6*R*)-2,8-Diangeloyloxy-1,3,5-trihydroxy-bisabola-7(14),10-dien-4-one	*L. dentata hara*[118]
386	Biligulaplenolide	*L. platyglossa*[119]
387	8*β*-Hydroxy-1-oxo-(14*α*,15*α*-eremophil-7(11),9(10)-dien-12,8*α*-olide	*L. platyglossa*[119]
388	1-Hydroxy-2-oxo-(14*α*,15*α*-eremophil-1(10),7(11),8(9)-trien-12,8-olide	*L. platyglossa*[119]
389	4*α*,8*α*,9*α*-Trihydroxy-5*α*-*H*-7(11)-eudesmen-12,8*α*-olide	*L. platyglossa*[119]
390	10*α*-Hydroxy-1-oxo-eremophil-7(11),8(9)-dien-12,8-olide	*L. platyglossa*[119]
391	Furanoeremophil-1(10)-ene-2,9-dione	*L. platyglossa*[119]
392	Kanaitzensol A	*L. kanaitzensis*[120]
393	Eremofarfugin C	*L. kanaitzensis*[120]
394	8*β*,10*β*-Dihydroxy-6*β*-isobutyryloxyeremophil-7(11)-en-(12,8)-olide	*L. kanaitzensis*[120]
395	3*α*-Tigloyloxyeremophila-9,11-dien-8-one	*L. kanaitzensis*[120]
396	3*α*-Propionyloxy-7*β*-*H*-eremophila-9,11-dien-8-one	*L. kanaitzensis*[120]
397	6*β*-Acetoxyfuranoeremophilan-10*β*-ol	*L. kanaitzensis*[120]
398	6*β*-Ethoxyfranoeremophilan-10*β*-ol	*L. kanaitzensis*[120]

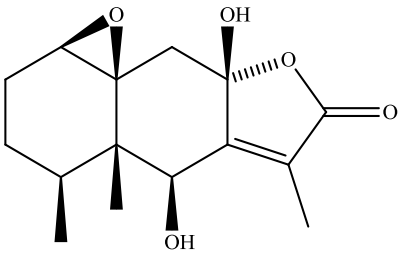
399	6*β*-((20*S*)-2-Methylbutyryloxy)furanoeremophilan-10*β*-ol ((20*S*)-7)	*L. kanaitzensis*[120]
400	8-Oxo-11*S*-eremophil-6-en-12-oic acid	*L. kanaitzensis*[120]
401	8-Oxo-11*R*-eremophil-6-en-12-oic acid	*L. kanaitzensis*[120]
402	Subspicatin A	*L. subspicata*[121]
403	Subspicatin B	*L. subspicata*[121]
404	Subspicatin C	*L. subspicata*[121]
405	Subspicatin D	*L. subspicata*[121]
406	Ubspicatolide	*L. subspicata*[121]
407	1*α*-Chloro-6*β*-isobutyroxy-9-oxo-10*β*-hydroxy-furanoeremophilane	*L. atroviolacea*[122]
408	8*β*-[Eremophil-3′,7′(11′)-dien-12′,8′*α*;15′,6′*α*diolide]-eremophil-3,7(11)-dien-12,8*α*;15,6*α*-diolide	*L. atroviolacea*[122]
409	Ligulatrovine A	*L. atroviolacea*[122]
410	Furoeremophilanolide	*L. atroviolacea*[122]
411	(1*R*,2*R*,3*S*,5*S*,6*R*)-5-Acetoxy-2,8-diangeloyloxy-1,3-dihydroxybisabola-7(14),10-dien-4-one	*L. altaica*[123]
412	3*β*,16*β*-Dihydroxy-12-oleanen-28-al	*L. altaica*[123]
413	Ergosterol peroxide	*L. altaica*[123]
414	Stigmasta-7-en-ol	*L. altaica*[123]
415	11-Hydroxy-10,11-dihydro-euparin	*L. altaica*[123]
416	5-Hydroxybenzofuran-2(3*H*)-one	*L. altaica*[123]
417	Hyperoside	*L. fischeri*[124]
418	2"-Acetylhyperoside	*L. fischeri*[124]
419	2-Hydroxyplatyphyllide	*L. fischeri*[125]
420	6'-Linoleic-*β*-daucosterol	*L. fischeri*[125]
421	6,7-Dihydroxy-coumarin	*L. fischeri*[125]
422	(2*S*,3*S*,4*R*,12*E*)-*N*-[2'-(*R*)-Hydroxydocosanoate]-1,3,4-trihydroxy-2-amino-eicosanoate-12-ene	*L. fischeri*[125]
423	Monostearin	*L. fischeri*[125]
424	*α*-Hydroxylignoceric acid	*L. fischeri*[125]
425	1,3-Dipalmitin	*L. fischeri*[125]
426	Heptacosanol	*L. fischeri*[125]
427	Quercetin	*L. hodgsonii*[126]
428	(*E*)-3-(2-Hydroxy-4-methoxyphenyl)-2-acrylic acid	*L. hodgsonii*[126]
429	(*E*)-3-(2,4-Dihydroxyphenyl)-2-acrylic acid	*L. hodgsonii*[126]
430	(*S*)-3-Hydroxy-3-phenylpropanoic acid	*L. hodgsonii*[126]
431	(1*R*,4*S*,5*S*,6*R*,8*S*,10*R*)-1-Acetoxyeremophil-7(11)-en-6,15;8,12-diolide	*L. hodgsonii*[127]
432	(1*R*,4*S*,5*S*,6*R*,8*S*,10*R*)-1-Acetoxy-8*β*-hydroxyeremophil-7(11)-en-6,15;8,12-diolide	*L. hodgsonii*[127]
433	(4*S*,5*S*,6*R*,8*R*,9*S*,10*S*)-8-Hydroxy-9-(angeloyloxy)eremophil-7(11)-en-6,15;8,12-diolide	*L. hodgsonii*[127]
434	(4*S*,5*S*,6*R*,10*R*)-10-Hydroxyeremophil-7(11),8(9)-diene-6,15;8,12-diolide	*L. hodgsonii*[127]
435	(4*S*,5*S*,6*R*,8*R*,10*R*)-6-(Angeloyloxy)-8-hydroxyeremophil-7(11)-en-8,12-olide-15-carboxylic acid methyl ester	*L. hodgsonii*[127]
436	(4*S*,5*S*,6*R*,8*R*,10*R*)-6-(Angeloyloxy)-8-ethoxyeremophil-7(11)-en-8,12-olid-15-oic acid	*L. hodgsonii*[127]

437	(4*S*,5*S*,6*S*,8*R*,10*R*)-6-(Angeloyloxy)-8-ethoxyeremophil-7(11)-en-8,12-olid-15-oic acid	*L. hodgsonii*[127]
438	Kaempferol	*L. macrophylla*[128]
439	2,4'-Dihydroxy-5-methoxychalcone	*L. macrophylla*[128]
440	5-Hydroxy-3,4',7-trimethoxyflavone	*L. macrophylla*[128]
441	*Iso*-Butyl ester terephthalic acid	*L. macrophylla*[128]
442	4-Hydroxybenzaldehyde	*L. macrophylla*[128]
443	Mono-(2-ethylhexyl)-terephthalate	*L. macrophylla*[128]
444	Ligulactone A	*L. fischeri*[129]
445	Ligulactone B	*L. fischeri*[129]
446	1*β*,10*β*-Epoxy-6*β*-isobutyryloxy-9-oxo-furanoeremophilane	*L. fischeri*[129]
447	Altaicalarin A	*L. altaica*[130]
448	Altaicalarin B	*L. altaica*[130]
449	Altaicalarin C	*L. altaica*[130]
450	Altaicalarin D	*L. altaica*[130]
451	1*β*,8-Diangloyloxy-3*β*,4*β*,10,11-diepoxybisabol-7(14)-ene	*L. altaica*[130]
452	1*β*-Acetoxy-1*β*,8-diangloyloxy-10,11-dihydroxy-3,4-epoxy-bisabol-7(14)-ene	*L. altaica*[130]
453	Bisabolone	*L. altaica*[130]
454	2,5-Diacetyl-6-methoxybenzofuran	*L. nanchuanica*[131]
455	Ligudentatin	*L. nanchuanica*[131]
456	Ergosta-6,22-dien-3*β*-5*α*,8*α*-triol	*L. nanchuanica*[131]
457	Ergosta-6-en-3*α*,5*α*,8*α*-triol-22-one	*L. nanchuanica*[131]
458	6-Methoxyeuparin	*L. nanchuanica*[131]
459	5,6-Dimethoxy-2-acetylbenzofuran	*L. nanchuanica*[131]
460	7,22-Dien-ergosta-3*β*-ol	*L. nanchuanica*[131]
461	Palmitoleic acid-1-monoglycerate	*L. nanchuanica*[131]
462	7,8-Dimethoxy-4-methyl-dibenzo-1-carbaldehyde	*L. nanchuanica*[131]
463	12,29(30)-Dien-3*β*-lupeol	*L. nanchuanica*[131]
464	(+)-4-Hydroxypinoresinol-4-*O*-*β*-*D*-glucopyranoside	*L. nanchuanica*[131]
465	Sucrose	*L. nanchuanica*[131]

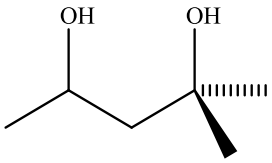
3.3 Structures of some compounds reported from genus *Ligularia*



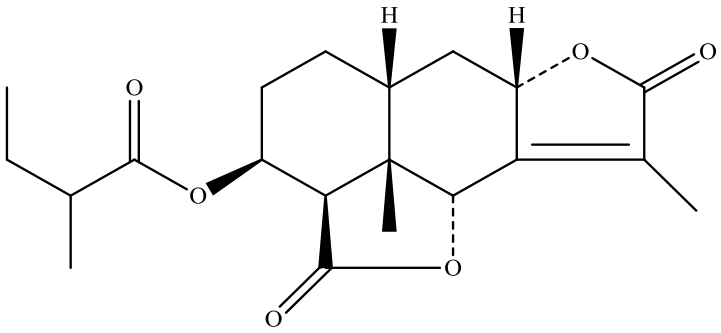
7*α*-Hydroxy-Δ^9-1,8-dioxo-6,7-dihydrofuranomemophilane[50]



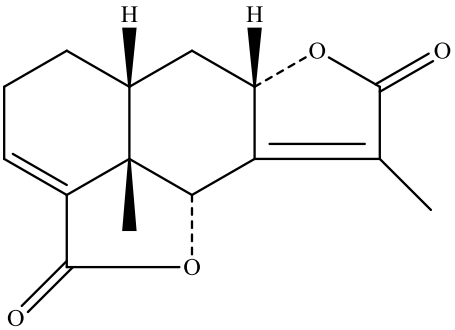
1*β*,10*β*-Epoxy-6*β*,8*β*-dihydroxy-erwophil-7(11)-en-12,8*α*-olide[50]



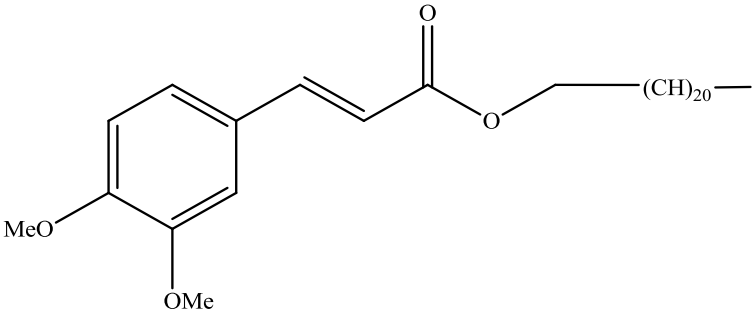
2-Methyl-pentan-2,4-diol[50]



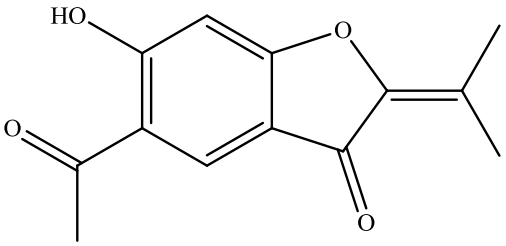
3*β*-(2′-Methylbutanoyloxy)-8*β*-*H*-eremophil-7(11)-en-12,8*α*(14,6*α*)-diolide[30]



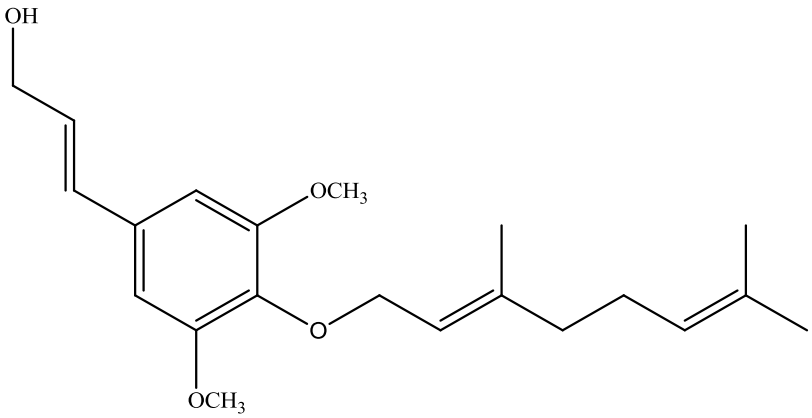
8*β*-*H*-Eremophil-3,7(11)-dien-12,8*α*(14,6*α*)-diolide[30]



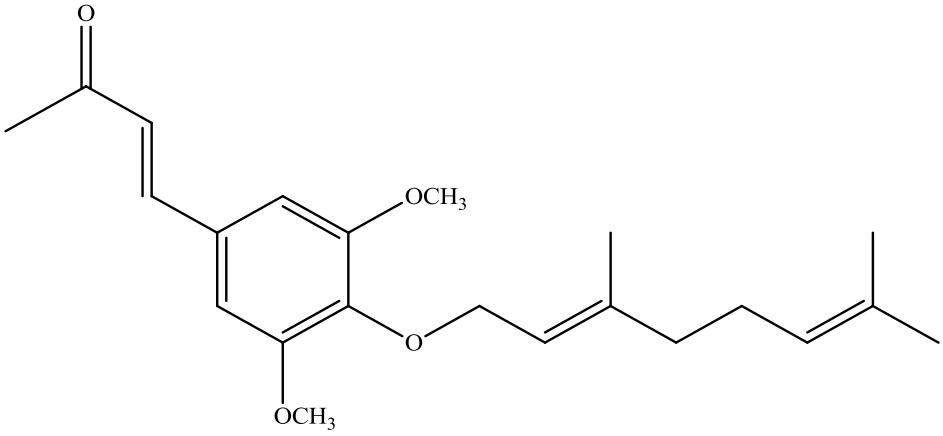
Ferulic acid *n*-docosyl ester[55]



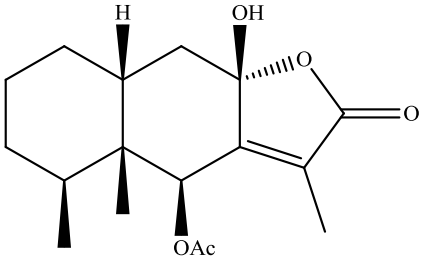
2-Isopropylidene-3-oxo-5-acetal-6-hydroxy-benzodihydrofuran[55]



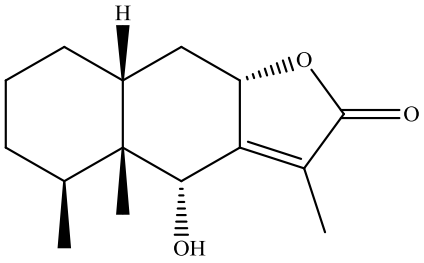
4-*O*-Geranyl-sinapyl alcohol[55]



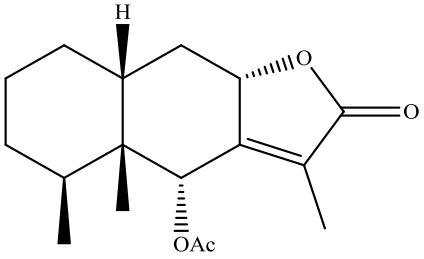
4-*O*-Geranyl-sinapyl aldehyde[55]



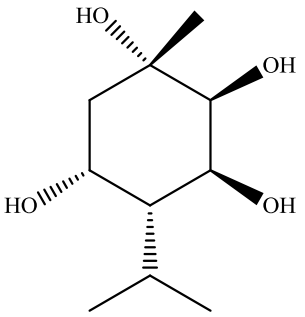
(6*β*,8*α*)-6-(Acetyloxy)-8-hydroxyeremophil-7(11)-en-12,8-olide[88]



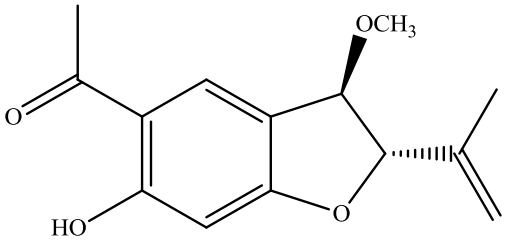
(6*α*,8*α*)-6-Hydroxyeremophil-7(11)-en-12,8-olide[88]



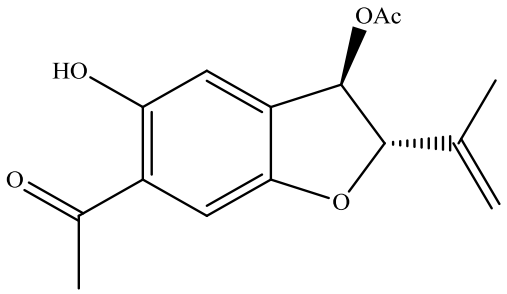
(6*α*,8*α*)-6-(Acetyloxy)eremophil-7(11)-en-12,8-olide[88]



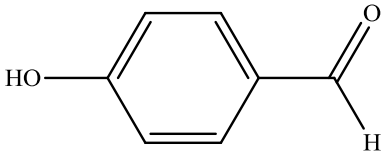
rel-(1*R*,2*R*,3*R*,4*S*,5*S*)-*p*-Menthane-1,2,3,5-tetrol[88]



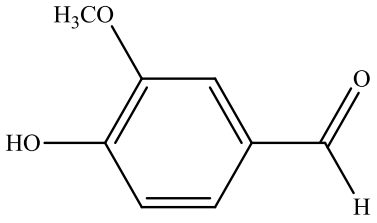
5-Acetyl-6-hydroxy-2α-isopropenyl-3β-methoxy-2,3*H*-benzofuran[90]



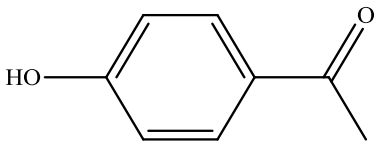
3β-Acetoxy-6-acetyl-5-hydroxy-2α-isopropenyl-2,3*H*-benzofuran[90]



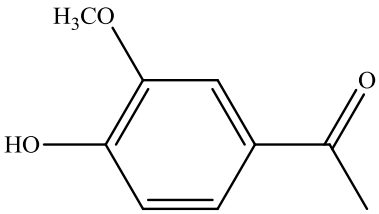
p-Hydroxybenzaldehyde[90]



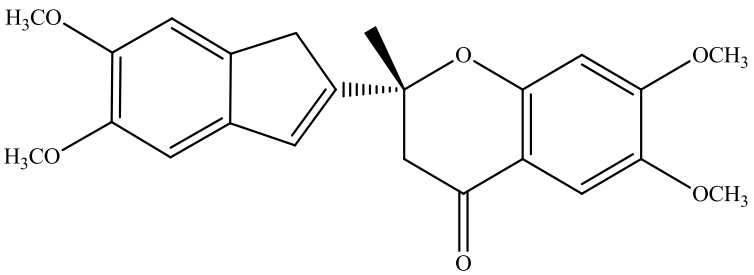
Vanillin[90]



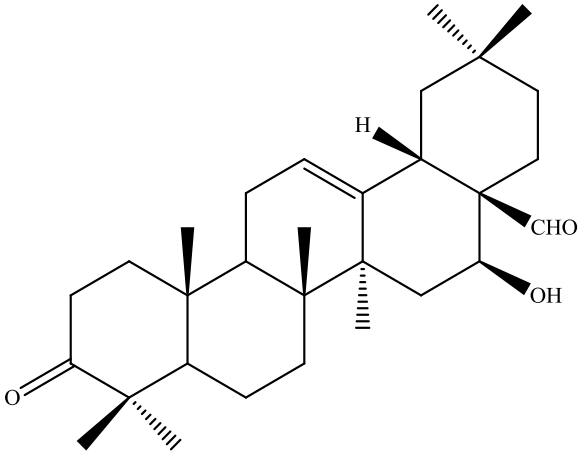
4-Hydroxyacetophenone[90]



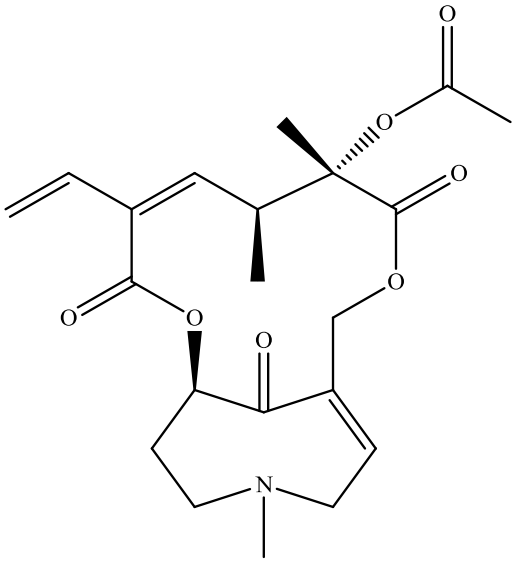
4-Hydroxy-3-methoxyacetophenone[90]



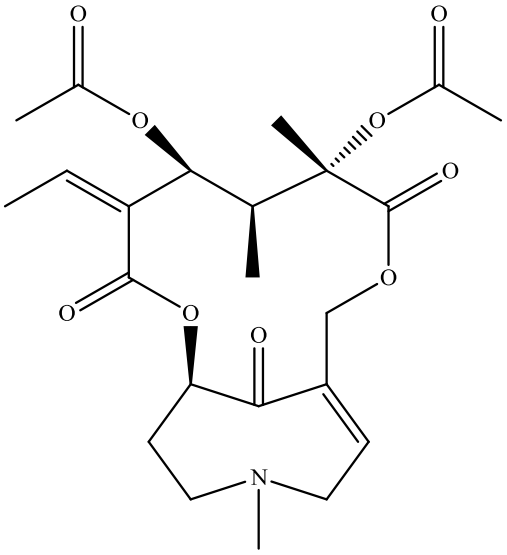
(*R*)-(+)-Ligulaodonin A[96]



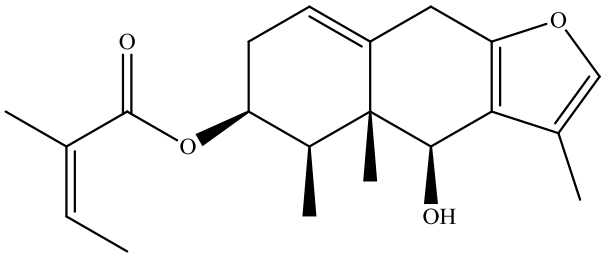
3-Oxo-16*β*-hydroxy-olean-12-en-28-al[96]



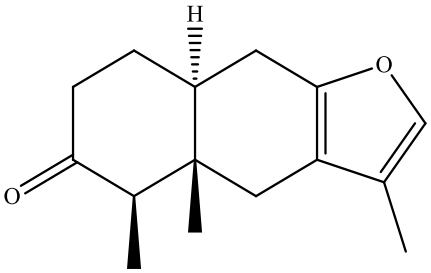
Clivorine[64]



Ligularine[64]



3*β*-Angeloyloxyfuranoeremophil-1(10)-en-6*β*-ol[95]



Furanoligularenone[95]

3.4 Medicinal and pharmacological properties of genus *Ligularia*

Ligularia species have been used as folk remedies.[132] *L. fischeri* has long been used traditionally for its antiphlogistic, antitussive and hemostatic properties.[133] Many *Ligulaira* species are valuable anti-inflammatory agents and are used as folk medicines for easing breathing, stimulating blood flow, reducing inflammation, stopping cough and eliminating phlegm.[96,97,134] The roots of *L. caloxantha* are used to cure injuries and have an antiascarid activity.[114] *L. nanchuanica* is clinically utilized because of its antitussive and expectorant actions.[131] *L. stenocephala* has been used in the treatment of edema and scrofula in China.[90] *L. virgaurea* has been used to treat stomachache and nausea.[135] Several *Ligularia* species have been used to cure asthma, hemoptysis, hepatitis, urinary track blockages, common cold, apoplexy, pharyngitis and pulmonary tuberculosis.[30,55,58] The rhizomes and roots of *L. macrophylla* have been used for the treatment of asthma, cough, phthisis, tracheitis and hemoptysis. Some species of *Liglaria* have been shown to possess anti-bacterial activity. Eremophilane sesquiterpenes, isolated from *L. macrophylla* showed significant activities against *Pasteurella multocida.*[104] Eremophilenolides isolated from *L. atroviolacea* have significant antiviral activity and cytotoxic activity against HL, CNE, KB, A549, BEL-7404 and Hela human cancer cell lines.[26] *L. macrophylla* has also been reported for their insecticidal, fungicidal and herbicidal activities.[136] The leaves of *L. fischeri* have been used to cure scarlet-fever, jaundice, hepatic diseases and rheumatoidal arthritis.[124]

4.1 *Ligularia thomsonii*

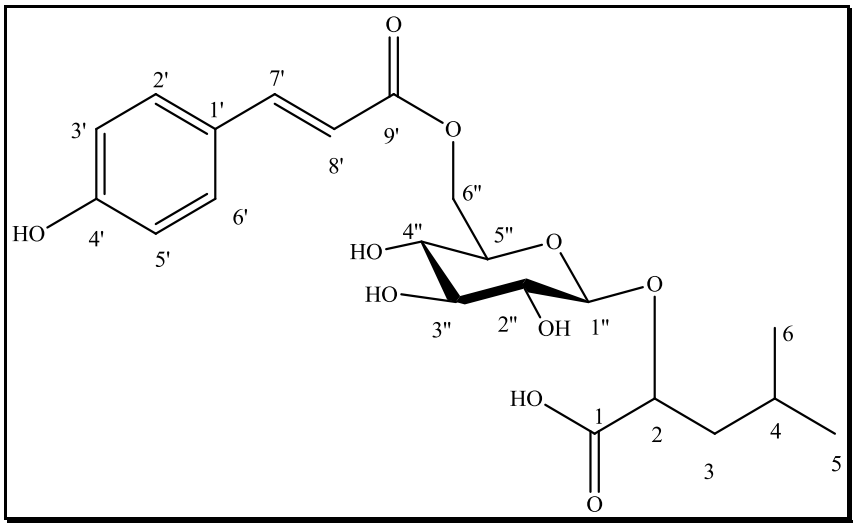
The present studies describe the phytochemical investigation and bioactivities of the aerial parts of *L. thomsonii* which resulted in the isolation and structure elucidation of three new (**32-34**) along with nine known (**35-43**) compounds, hitherto unreported from this plant. The structures of these compounds were elucidated through advanced spectroscopic techniques which were also evaluated for their antioxidant activity in the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay.



Ligulria thomsonii

4.1.1 Structure elucidation of new compounds isolated from *L. thomsonii*

4.1.1.1 Ligularoside I (32)



Compound **32** is a brownish solid which showed a molecular formula $C_{21}H_{28}O_{10}$ (eight degrees of unsaturation) having a molecular mass of 440 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 463.1583, calcd. for $C_{21}H_{28}O_{10}Na$ $[M + Na]^+$ 463.1574). Maximum absorption at 295 nm in the UV spectrum indicated the presence of conjugating double bond. The IR spectrum revealed the absorption bands of hydroxyl group (3336 cm^{-1}), double bonds (1603 cm^{-1}) and carbonyl groups (1688 cm^{-1}).

Three structural moieties were present in **32**, as evidenced from ^{1}H-NMR (**Table-4.1**) signals [δ_H 6.81 x 2 (1H each, d, *J* = 8.7 Hz; H-3′,5′), 7.46 x 2 (1H each, d, *J* = 8.7 Hz; H-2′,6′), 7.63 (1H, d, *J* = 15.9 Hz; H-7′) and 6.34 (1H,d, *J* = 15.9 Hz; H-8′)] in the combination with ^{13}C-NMR (**Table-4.1**) signals [δ_C 127.1 (C-1′), 131.6 x 2 (C-2′,6′), 116.8 x 2 (C-3′,5′), 161.2 (C-4′), 146.7 (C-7′), 114.9 (C-8′) and 169.0 (C-9′)].[137] A pair of trans-configured of olefinic H-atoms (δ_H 7.63 and 6.34; *J* = 15.9 Hz) was confirmed by the NOESY spectra of **32** (**Figure-4.1**). These signals were typical

of a *p*-coumaroyl moiety.[137] The anomeric H-atom signal appeared at δ_H 4.37 (1H, d, J = 7.7Hz; H-1″), corresponding ^{13}C-NMR signal at δ_C 103.4, together with the multiple signals at the region of δ_H 3.27-4.51 and relevant ^{13}C-NMR resonances and coupling constant, indicated the presence of a *β*-glucopyranosyl (Glc) moiety. Apart from *p*-coumaroyl and *β*-glucopyranosyl moieties, the ^{13}C-NMR (BB and DEPT) data of **32** displayed 6 carbons signals: two CH_3, two CH, a CH_2, and a quaternary C-atom. The downfield C-atoms signals at δ_C 177.3 and 76.8 were assigned to carboxylic carbonyl (C-1) and oxygenated CH (C-2) respectively, while the ^{1}H-NMR spectrum showed two germinal methyl doublets at δ_H 0.90 (3H, d, J = 6.6 Hz, 5-CH_3) and 0.89 (3H, d, J = 8.7 Hz, 6- CH_3) together with multiplets at δ_H 1.53 (1H, H_a-3), 1.67 (1H, H_b-3), 1.85 (1H, H-4) and 4.39 (1H, H-2) demonstrated the presence of a 2-hydroxy-4-methylpentanoic acid moiety.[138] The attachment of the *p*-coumaroyl and 2-hydroxy-4-methylpentanoic acid moieties at C-6″ and C-1″ respectively of Glc and the position of hydroxyl group were confirmed through 2D-NMR (HSQC, HMBC and NOESY) analysis. The substitution pattern of *p*-coumaroyloxy (**Figure-4.1**) and the location of *β*-glucopyranose moiety were established by the HMBC (**Figure-4.1**) cross-peaks H-1″/C-2 and H-2/C-1″.[137]

From the spectral data, the structure of **32** was characterized as 2-{{6″-*O*-[(2*E*)-3-(4-hydroxyphenyl)-1-oxoprop-2-en-1-yl]-*β*-D-glucopyranosyl}-oxy}-4-methyl pentanoic acid.

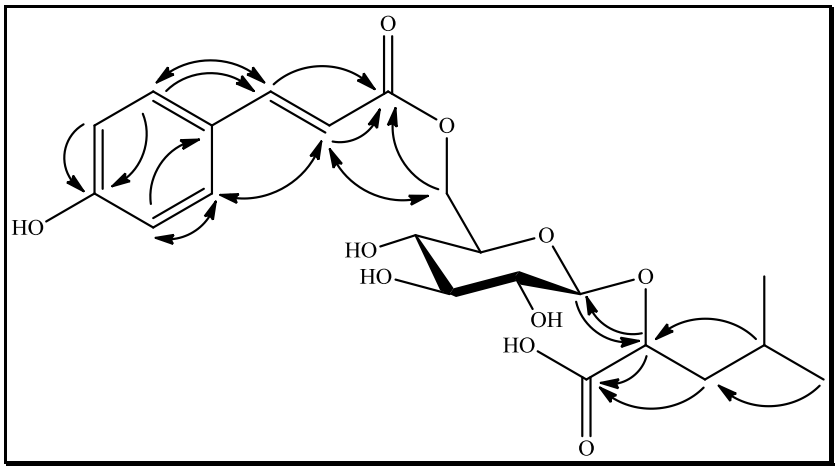
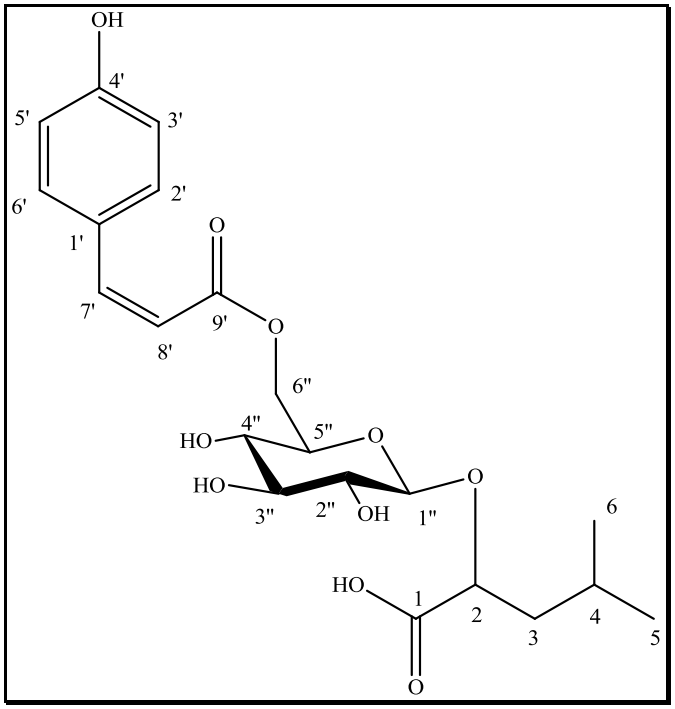


Figure-4.1: Selected HMBC (H→C) and NOESY (H↔H) for ligularoside I (**32**)

Table-4.1:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of ligularoside I (**32**) in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H**, (multiplicity,** ***J*** **in Hz)**
1	177.0	C	--
2	76.5	CH	4.39, m
3	42.8	CH_2	1.53, m
			1.67, m
4	25.3	CH	1.85, m
5	21.7	CH_3	0.90, (d, 6.6)
6	23.5	CH_3	0.89, (d, 6.7)
1′	127.1	C	--
2′	131.6	CH	7.46, (d, 8.7)
3′	116.8	CH	6.81, (d, 8.7)
4′	161.2	C	--
5′	116.8	CH	6.81, (d, 8.7)
6′	131.6	CH	7.46, (d, 8.7)
7′	146.7	CH	7.63, (d, 15.9)
8′	114.9	CH	6.34, (d, 15.9)
9′	169.0	C	--
1″	103.4	CH	4.37, (d, 7.7)
2″	75.1	CH	3.27, m
3″	77.7	CH	3.40, m
4″	71.7	CH	3.37, m
5″	75.4	CH	3.54, m
6″	64.6	CH_2	4.31, m
			4.51, (dd, 11.8, 2.1)

4.1.1.2 Ligularoside II (33)



Compound **33** was isolated as a brownish solid. Its molecular formula ($C_{21}H_{28}O_{10}$) corresponding to a molecular mass of 440 a.m.u. as deduced from positive-mode HRESIMS (*m/z* 463.1589, calcd. for $C_{21}H_{28}O_{10}Na$ $[M + Na]^+$ 463.1574). In UV spectrum maximum absorption at 307 nm, indicating that conjugated double bond is present in **33**. The IR spectrum showed the absorption bands for hydroxyl (3329 cm^{-1}), alkene (1603 cm^{-1}) and carbonyl groups (1701 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra (**Table-4.2**) indicated three structural moieties in **33**. Two doublets at δ_H (ppm): 6.76 (2H, d, J = 8.7 Hz, H-3′,5′) and 7.66 (2H, d, J = 8.7 Hz, H-2′,6′), two olefinic hydrogens at 6.88 (1H, d, J = 12.8 Hz, H-7′) and 5.79 (1H,d, J = 12.8 Hz, H-8′), together with six aromatic carbons δ_C(ppm): 127.6 (C-1′); 133.8 (C-2′,6′); 115.8 (C-3′,5′); 160.1 (C-4′), two olefinic carbons at 145.4 (C-7′); 116.2 (C-8′) as well as one carboxy carbonyl carbon at 167.9 (C-9′) indicated that a *p*-coumaroyl (3-(4-hydroxyphenyl)-1-oxoprop-2-en-1-yl) group is present in **33**.[137] The

^{1}H-NMR chemical shifts and coupling constants of the two olefinic hydrogens at δ_H 6.88 (1H, d, J = 12.8 Hz, H-7′) and 5.79 (1H, d, J = 12.8 Hz, H-8′), HMBC and NOESY spectra of **33** (**Figure-4.2**) revealed that both the H-atoms are *cis* to each other. Two methyl doublets at δ_H 0.90 (3H, d, J = 6.8 Hz, 5-CH_3) and 0.89 (3H, d, J = 6.8 Hz, 6-CH_3), the two multiplets at 1.52 (1H) and 1.64 (1H) correspond to a CH_2 group at (C-3), two further hydrogens at 4.33 (1H, m, H-2) and 1.84 (1H, m,H-4) in combination with a carbonyl resonance at δ_C 177.3 (C-1) demonstrated the presence of a 2-hydroxy-4-methylpentanoic acid moiety.[138] In the ^{1}H- and ^{13}C-NMR spectra of **33**, the signals at δ_H 4.32 (1H, d, J = 7.7 Hz, 1H, C-1″), δ_H 3.27-4.47 and δ_C 103.5, 75.1, 77.8, 71.6, 75.4, 64.6 were ascribable to a β-glucopyranosyl (Glc) unit. The ^{1}H- and ^{13}C-NMR signals were confirmed though HSQC and HMBC data (**Figure-4.2**). The HMBC correlation of C-9′ with CH_2-6″ indicated that the *p*-coumaroyloxy group was connected to C-6″ of the Glc moiety. The HMBC correlations between H-1″ of the glucose and C-2 of the aglycone indicated that the β-glucopyranose moiety was attached to the C-2 position of the aglycone.[137]

Finally compound **33** was assigned as 2-{{6″-*O*-[(2*Z*)-3-(4-Hydroxyphenyl)-1-oxoprop-2-en-1-yl]-β-D-glucopyranosyl}-oxy}-4-methyl pentanoic acid, a new phenyl propanoid.

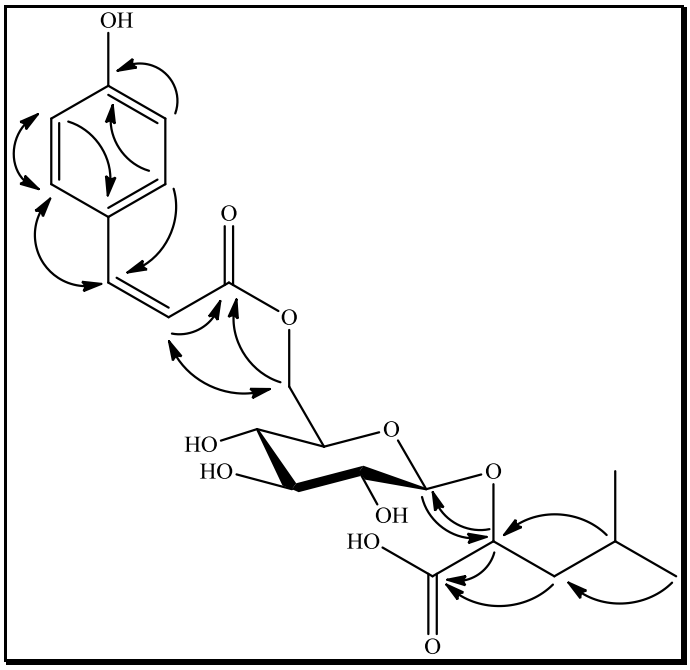
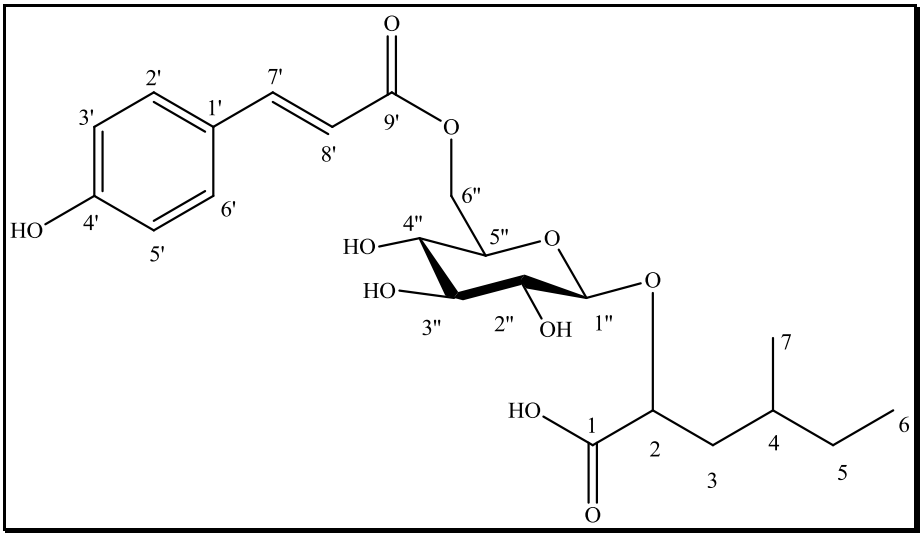


Figure-4.2: Selected HMBC (H → C) and NOESY (H ↔ H) for ligularoside II (**33**)

Table-4.2:	^{1}H- (400 MHz) and ^{13}C- (125 MHz) NMR data of ligularoside II (**33**) in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	177.3	C	--
2	76.8	CH	4.33, m
3	42.9	CH_2	1.52, m
			1.64, m
4	25.3	CH	1.84, m
5	21.7	CH_3	0.90, (d, 6.8)
6	23.6	CH_3	0.89, (d, 6.8)
1′	127.6	C	--
2′	133.8	CH	7.66, (d, 8.7)
3′	115.8	CH	6.76, (d, 8.7)
4′	160.1	C	--
5′	115.8	CH	6.76, (d, 8.7)
6′	133.8	CH	7.66, (d, 8.7)
7′	145.4	CH	6.88, (d, 12.8)
8′	116.2	CH	5.79, (d, 12.8)
9′	167.9	C	--
1″	103.5	CH	4.32, (d, 7.7)
2″	75.1	CH	3.27, m
3″	77.8	CH	3.38, m
4″	71.6	CH	3.29, m
5″	75.4	CH	3.47, m
6″	64.6	CH_2	4.26, m
			4.47, (dd, 12.0, 2.2)

4.1.1.3 Ligularoside III (34)



Compound **34** a brownish solid was obtained from ethyl acetate fraction, showed a molecular formula $C_{22}H_{30}O_{10}$ (eight degrees of unsaturation) having a molecular mass of 454 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 477.1751, calcd. for $C_{22}H_{30}O_{10}Na$ $[M + Na]^+$ 477.1731). The UV spectrum of **34** showed maximum absorption at 313 nm, indicating the presence of a conjugated double bond.

Three structural moieties were present in **34** as evidenced from ^{1}H-NMR (**Table-4.3**) signals [δ_H 6.81 x 2 (1H each, d, J = 8.6 Hz; H-3′,5′), 7.46 x 2 (1H each, d, J = 8.7 Hz; H-2′,6′), 7.64 (1H, d, J = 15.9 Hz; H-7′) and 6.35 (1H,d, J = 15.9 Hz; H-8′)] in the combination with ^{13}C-NMR (**Table-4.3**) signals [δ_C 127.1 (C-1′), 131.1 x 2 (C-2′,6′), 116.8 x 2 (C-3′,5′), 161.3 (C-4′), 146.7 (C-7′), 114.9 (C-8′) and 169.0 (C-9′)].[137] A pair of trans-configured of olefinic H-atoms (δ_H 7.64 and 6.35; J = 15.9 Hz) was confirmed by the NOESY spectra of **34** (**Figure-4.3**). These signals were typical of a *p*-coumaroyl moiety.[137] Two methyl groups, one at δ_H 0.82 (3H, t, J = 7.4 Hz, CH_3-6) and other at 0.88 (3H, d, J = 6.4 Hz, CH_3-7), the multiplets at δ_H 1.11-1.69

correspond to two CH_2 groups (C-3 and C-5), two H-atoms one at δ_H 4.39 (m, H-2) and the other at 1.65 (m, H-4), in combination with a C=O signal at δ_C 177.4 (1) demonstrated the presence of a 2-hydroxy-4-methylhexanoic acid moiety.[139,140] In the ^{1}H- and ^{13}C-NMR spectra of **34**, the signals at δ_H 4.36 (1H, d, J = 7.8 Hz, 1H, C-1″), δ_H 3.27-4.49 and δ_C 103.5, 75.2, 77.8, 71.7, 75.4, 64.7 were ascribable to a *β*-glucopyranosyl (Glc) unit. All the ^{1}H- and ^{13}C-NMR signals were assigned with the help of HSQC and HMBC data of **34** (**Figure-4.3**). The HMBC correlation of C-9′ with CH_2-6″ indicated that the *p*-coumaroyloxy group was connected to C-6″ of the Glc moiety. The HMBC correlations between H-1″ of the glucose and C-2 of the aglycone indicated that the *β*-glucopyranose moiety was attached to the C-2 position of the aglycone.[137]

Thus the structure of **34** was confirmed as a new 2-{{6″-*O*-[(2*E*)-3-(4-Hydroxyphenyl)-1-oxoprop-2-en-1-yl]-*β*-D-glucopyranosyl}-oxy}-4-methylhexan-oic acid on the basis of 1D (^{1}H and ^{13}C-NMR) as well as 2D (HSQC, COSY and HMBC) techniques.

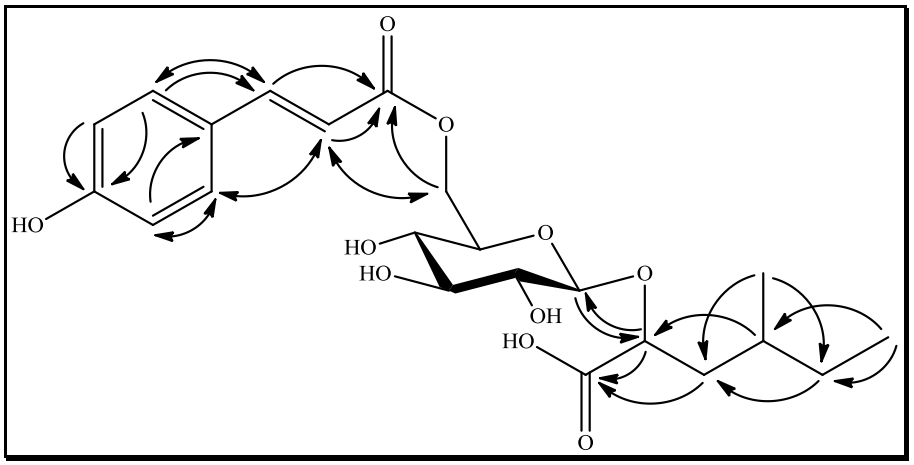
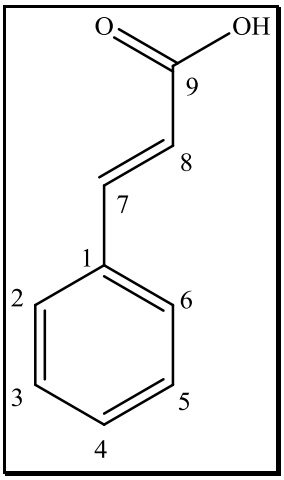


Figure-4.3: Selected HMBC (H → C) and NOESY (H ↔ H) for ligularoside III (**34**)

Table-4.3:	^{1}H- (400 MHz) and ^{13}C- (125 MHz) NMR data of ligularoside III (**34**) in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H, **(multiplicity, *J* in Hz)**
1	177.4	C	--
2	76.8	CH	4.39, m
3	40.9	CH_2	1.60, m
			1.69, m
4	31.6	CH	1.65, m
5	28.9	CH_2	1.11, m
			1.50, m
6	11.2	CH_3	0.82, (d, 7.4)
7	19.9	CH_3	0.88, (d, 6.4)
1′	127.1	C	--
2′	131.1	CH	7.46, (d, 8.7)
3′	116.8	CH	6.81, (d, 8.6)
4′	161.3	C	--
5′	116.8	CH	6.81, (d, 8.6)
6′	131.1	CH	7.46, (d, 8.7)
7′	146.7	CH	7.64, (d, 15.9)
8′	114.9	CH	6.35, (d, 15.9)
9′	169.0	C	--
1″	103.5	CH	4.36, (d, 7.7)
2″	75.2	CH	3.27, m
3″	77.8	CH	3.39, m
4″	71.7	CH	3.36, m
5″	75.4	CH	3.53, m
6″	64.7	CH_2	4.30, m
			4.49, (dd, 11.8, 2.1)

4.1.2 Structure elucidation of compounds isolated for the first time from *L. thomsonii*

4.1.2.1 Cinnamic acid (35)



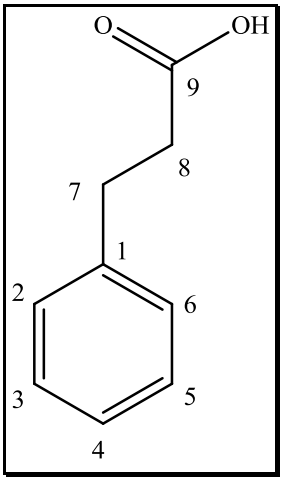
Compound **35** was isolated in white crystalline form. The IR spectrum exhibited absorption bands at 3315 cm^{-1} and 1692 cm^{-1} corresponding to O–H (carboxylic acid) and C=O respectively. Its molecular formula ($C_9H_8O_2$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 149 and $[M + Na]^+$ at 171 in ESI-positive mode and $[M – H]^-$ at *m/z* 147 in ESI-negative mode, suggesting a molecular mass of 148 a.m.u.

The ^{1}H-NMR spectrum (**Table-4.4**) of compound **35** showed aromatic signals at δ_H 7.42 (3H, m, H-3,4,5) and 7.57 (2H, m, H-2,6), indicating a mono-substituted benzene ring. Olefinic signal at δ_H 7.81 (1H, d, J = 15.9 Hz, H-7) and 6.47 (1H, d, J = 15.9 Hz, H-8) with the corresponding carbon signals of these protons in ^{13}C-NMR spectrum at δ_C 147.2 and 117.4 along with 172.4 for carboxyl group confirmed the presence of 2-propenoic acid moiety having trans configuration in the molecule.

All the NMR assignments were in complete agreement to that of cinnamic acid.[141]

Table-4.4:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of cinnamic acid **(35)** in $CDCl_3$		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	134.1	C	--
2	129.1	CH	7.57, m
3	128.5	CH	7.42, m
4	130.9	CH	7.42, m
5	128.5	CH	7.42, m
6	129.1	CH	7.57, m
7	147.2	CH	7.81, (d, 15.9)
8	117.4	CH	6.47, (d, 15.9)
9	172.4	C	--

4.1.2.2 3-Phenylpropanoic acid (36)



Compound **36** was isolated as white powder. The IR spectrum exhibited absorption bands at 3300 cm^{-1}, 1703 cm^{-1} and 1610 cm^{-1} corresponding to O–H (carboxylic acid), C=O and C=C (aromatic), respectively. Its molecular formula ($C_9H_{10}O_2$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 151 and $[M + Na]^+$ at 173 in ESI-

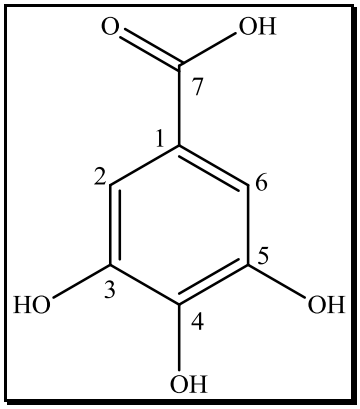
positive mode and $[M - H]^-$ at *m/z* 149 in ESI-negative mode, suggesting a molecular mass of 150 a.m.u.

The ^{1}H-NMR spectrum of compound **36** showed aromatic signals at δ_H 7.25 (3H, m, H-2,4,6) and 7.33 (2H, m, H-3,5), thus indicating a mono-substituted benzene ring. In the ^{1}H- and ^{13}C-NMR spectra of **36**, the signals at δ_H 2.99 (2H, t, *J* = 7.5 Hz, H-7), 2.72 (2H, t, *J* = 7.5 Hz, H-8) and δ_C 30.6, 35.7, along with 179.6 for carboxyl group were ascribable to 2-propanoic acid moiety.

The spectral data (**Table-4.5**) of **36** was in complete agreement to that reported in literature for 3-phenylpropanoic acid.[142]

Table-4.5:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of 3-phenylpropanoic acid **(36)** in $CDCl_3$		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	140.2	C	--
2	128.3	CH	7.25, m
3	128.6	CH	7.33, m
4	126.5	CH	7.25, m
5	128.6	CH	7.33, m
6	128.3	CH	7.25, m
7	30.6	CH_2	2.99, (t, 7.5)
8	35.7	CH_2	2.72, (t, 7.5)
9	179.6	C	--

4.1.2.3 Gallic acid (37)

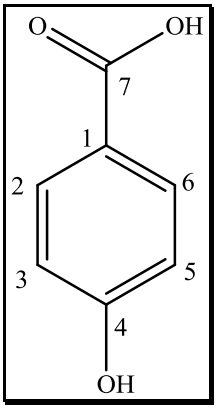


Compound **37** was obtained as white crystals. The IR spectrum revealed the absorption bands of hydroxyl (3432 cm^{-1}) and carbonyl (1706 cm^{-1}) groups. Its molecular formula ($C_7H_6O_5$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 171 and $[M + Na]^+$ at 193 in ESI-positive mode and $[M – H]^-$ at *m/z* 169 in ESI-negative mode, suggesting a molecular mass of 170 a.m.u.

The ^{1}H-NMR spectrum of **37** showed the aromatic signal at δ_H 7.12 (2H, s, H-2, 6). The ^{13}C-NMR spectrum of **37** indicated a carbonyl carbon at δ_C 171.2 (C-7), four quaternary carbons at 122.7 (C-1), 147.2 (C-3,5), 140.5 (C-4), and two methine carbons at 111.4 (C-2,6). The ^{1}H- and ^{13}C-NMR spectral data (**Table-4.6**) of **37** were found identical to that reported in literature for gallic acid.[143]

Table-4.6:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of gallic acid **(37)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	122.7	C	--
2	111.4	CH	7.12, s
3	147.2	C	--
4	140.5	C	--
5	147.2	C	--
6	111.4	CH	7.12, s
7	171.2	C	--

4.1.2.4 *p*-Hydroxybenzoic acid (38)

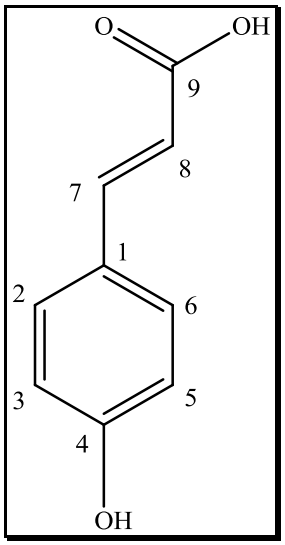


The Ethyl acetate fraction afforded compound **38** as white powder. The IR spectrum suggested the presence of hydroxyl (3450 cm^{-1}) and carbonyl (1705 cm^{-1}) groups. Its molecular formula ($C_7H_6O_3$) was determined by NMR data interpretation and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 139 and $[M + Na]^+$ at 161 in ESI-positive mode and $[M – H]^-$ at *m/z* 137 in ESI-negative mode, suggesting a molecular mass of 138 a.m.u.

^{1}H-NMR spectrum of compound **38** revealed the presence of *para*-substituted aromatic ring proton signals at δ_H 7.80 (2H, d, J = 8.9 Hz, H-2,6) and 6.76 (2H, d, J = 8.9 Hz, H-3,5). In ^{13}C-NMR spectrum signals for a carbonyl carbon at δ_C 170.8, two quaternary carbons at 123.7 and 164.1 along with four methine carbons at 134.1 (C-2,6) and 117.3 (C-3,5) were observed. These data indicated that compound **38** is *p*-hydroxybenzoic acid by comparison of NMR spectral data (**Table-4.7**) with that isolated in the earlier literature.[143]

Table-4.7:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of 4-hydroxybenzoic acid **(38)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	123.7	C	--
2	134.1	CH	7.80, (d, 8.9)
3	117.3	CH	6.76, (d, 8.9)
4	164.1	C	--
5	117.3	CH	6.76, (d, 8.9)
6	134.1	CH	7.80, (d, 8.9)
7	170.8	C	--

4.1.2.5 Coumaric acid (39)



Compound **39** was isolated as white powder. The presence of hydroxyl and carbonyl groups were revealed by the bands at 3446 cm^{-1} and 1694 cm^{-1}, respectively, in the IR spectrum. Its molecular formula ($C_9H_8O_3$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 165 and $[M + Na]^+$ at 187 in ESI-positive mode and $[M – H]^-$ at *m/z* 163 in ESI-negative mode, suggesting a molecular mass of 164 a.m.u.

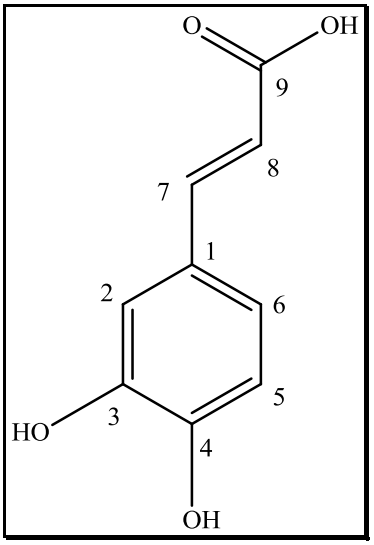
In ^{1}H-NMR spectrum of **39** aromatic signals at δ_H 7.49 (2H, d, *J* = 7.4 Hz, H-2,6) and 6.86 (2H, d, *J* = 7.4 Hz, H-3,5) indicated a di-substituted benzene ring. Olefinic signal at δ_H 7.65 (1H, d, *J* = 15.8 Hz, H-7) and 6.34 (1H, d, *J* = 15.8 Hz, H-

8) with the corresponding carbon signals of these protons in ^{13}C-NMR spectrum at δ_C 146.2 and 116.4 along with 171.8 for carboxyl group confirmed the presence of 2-propenoic acid moiety having trans configuration in the molecule.

The NMR spectral data (**Table-4.8**) of **39** were in agreement to coumaric acid.[144,145]

Table-4.8:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of coumaric acid **(39)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	127.8	C	--
2	134.3	CH	7.49, (d, 7.4)
3	116.5	CH	6.86, (d, 7.4)
4	156.1	C	--
5	116.5	CH	6.86, (d, 7.4)
6	134.3	CH	7.49, (d, 7.4)
7	146.2	CH	7.65, (d, 15.8)
8	116.4	CH	6.34, (d, 15.8)
9	171.8	C	--

4.1.2.6 Caffeic acid (40)



Compound **40** was isolated from ethyl acetate fraction in white powder form. IR absorption bands for hydroxyl (3445 cm^{-1}) and carbonyl functions (1710 cm^{-1})

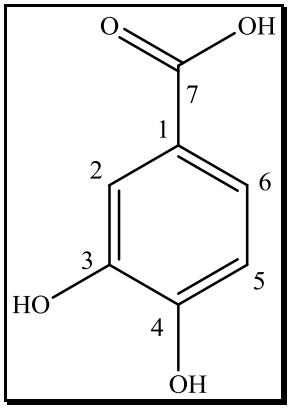
were observed. A molecular formula of $C_9H_8O_4$ was assigned to **40** on the basis of NMR analysis and LC-ESIMS data, where molecular ion adducts $[M + H]^+$ at *m/z* 181 and $[M + Na]^+$ at 203 in ESI-positive mode and $[M – H]^-$ at *m/z* 179 in ESI-negative mode were observed.

In ^{1}H-NMR spectra of **40** aromatic signals at δ_H 7.03 (1H, d, *J* = 2.1 Hz, H-2), 6.78 (1H, d, *J* = 8.2 Hz, H-5) and 6.93 (1H, dd, *J* = 2.1, 8.3 Hz, H-6) indicated a tri-substituted benzene ring. The signals at δ_H 7.51 (1H, d, *J* = 15.8 Hz, H-7) and 6.22 (1H, d, *J* = 15.8 Hz, H-8) attached to carbons at δ_C 146.5 and 116.1 along with 171.4 for carboxyl group confirmed the presence of 2-propenoic acid moiety having *trans* configuration in the molecule.

The ^{1}H-NMR and ^{13}C-NMR spectral data (**Table-4.9**) of **40** were identical with the reported compound, caffeic acid.[146]

Table-4.9:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of caffeic acid **(40)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	127.9	C	--
2	115.0	CH	7.03, (d, 2.1)
3	146.7	C	--
4	149.3	C	--
5	116.4	CH	6.78, (d, 8.2)
6	122.7	CH	6.93, (d, 2.1, 8.3)
7	146.5	CH	7.51, (d, 15.8)
8	116.1	CH	6.22, (d, 15.8)
9	171.4	C	--

4.1.2.7 3,4-Dihydroxybenzoic acid (41)

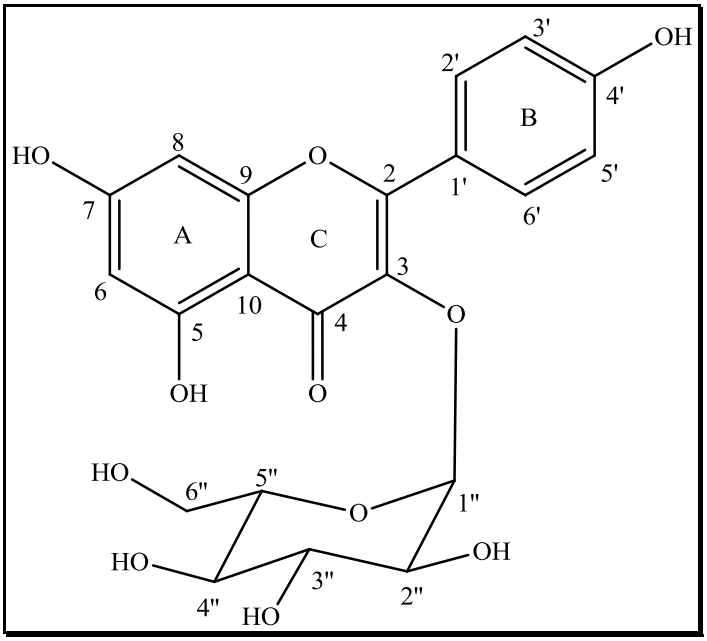


Compound **41** was purified in white powder state. The IR spectrum showed absorption bands at 3450 cm^{-1}, 3300 cm^{-1}, 1700 cm^{-1} and 1615 cm^{-1} corresponding to O–H, C–H (aromatic), C=O and C=C (aromatic), respectively. Its molecular formula ($C_7H_6O_4$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 155 and $[M + Na]^+$ at 177 in ESI-positive mode and $[M – H]^-$ at *m/z* 153 in ESI-negative mode, suggesting a molecular mass of 154 a.m.u.

In comparison of NMR spectral data (**Table-4.10**), **41** was identified to be similar to the reported compound, 3,4-dihydroxybenzoic acid.[143]

Table-4.10:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of 3,4-dihydroxybenzoic acid **(41)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H**, (multiplicity,** ***J*** **in Hz)**
1	123.7	C	--
2	117.7	CH	7.43, m
3	145.9	C	--
4	151.2	C	--
5	115.6	CH	6.79, (d, 8.1)
6	123.7	CH	7.80, m
7	170.7	C	--

4.1.2.8 Kaempferol 3-*O*-*β*-D-glucopyranoside (42)



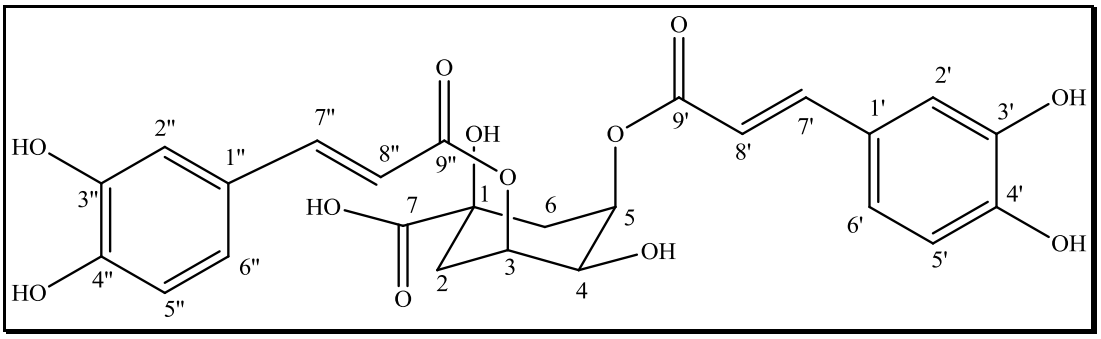
Compound **42** was isolated as yellowish powder. The IR spectrum showed the presence of hydroxyl (3398 cm^{-1}) and carbonyl (1702 cm^{-1}) groups. Its molecular formula ($C_{21}H_{20}O_{11}$) was estimated from ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which exhibited molecular ion adducts $[M + H]^+$ at *m/z* 449 and $[M + Na]^+$ at 471 in ESI-positive mode and $[M – H]^-$ at *m/z* 447 in ESI-negative mode, suggesting a molecular mass of 448 a.m.u.

The NMR spectral data (**Table-4.11**) of compound **42** indicated its flavonoidic nature, and in particular 15 carbon atoms ascribable to the aglycone and 6 to the sugar moieties. In ^{1}H-NMR spectrum the chemical shifts and the coupling constants of protons indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled doublet at δ_H 6.21 (1H, d, J = 1.9 Hz, H-6) and 6.41 (1H, d, J = 1.9 Hz, H-8)) and a 4′-hydroxylation for ring B (two *ortho*-coupled doublet signals at δ_H 8.05 (2H, d, J = 9.0 Hz, H-2′,6′) and 6.89 (2H, d, J = 9.0 Hz, H-3′,5′), permitting to recognize the aglycone as kaemferol. An anomeric proton was easily identified in the ^{1}H-NMR

spectrum which resonated at δ_H 5.25 (1H, d, J =7.4 Hz, H-1″) and correlated to δ_C 104.0 in HSQC spectra. Chemical shifts, multiplicity of the signal, absolute values of the coupling constant and the magnitude in the ^{1}H-NMR spectrum as well as ^{13}C-NMR data indicated the presence of a glucopyranosyl moiety with *β*-configuration at the anomeric carbon. The ring protons of the glucopyranosyl moiety were assigned by means of COSY, HSQC and HMBC experiments. A long range correlation, observed in the HMBC experiment between the anomaric proton at δ_H 5.25 and the carbon signal at δ_C 135.4 indicated that glucopyranosyl moiety was linked at the C-3 of the aglycone. Thus the structure of **42** was established as kaempferol 3-*O*-*β*-D-glucopyranoside. The NMR spectral data (**Table-4.11**) of compound **42** completely agreed to the reported values for kaempferol 3-*O*-*β*-D-glucopyranoside.[143]

Table-4.11:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of kaempferol 3-*O*-*β*-D-glucopyranoside **(42)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
2	159.0	C	--
3	135.4	C	--
4	179.5	C	--
5	163.1	C	--
6	99.8	CH	6.21, (d, 1.9)
7	165.9	C	--
8	94.7	CH	6.41, (d, 1.9)
9	158.5	C	--
10	105.7	C	--
1′	122.8	C	--
2′	132.2	CH	8.05, (d, 9.0)
3′	116.0	CH	6.89, (d, 9.0)
4′	161.5	C	--
5′	116.0	CH	6.89, (d, 9.0)
6′	132.2	CH	8.05, (d, 9.0)
1″	104.0	CH	5.25, (d, 7.4)
2″	75.7	CH	3.44, m
3″	78.0	CH	3.42, m
4″	71.3	CH	3.31, m
5″	78.4	CH	3.20, m
6″	62.6	CH_2	3.54, m
			3.69, m

4.1.2.9 3,5-Di-*O*-caffeoylquinic acid (43)



Compound **43** was isolated as brownish yellow solid. The IR spectrum showed absorption bands for hydroxyl group (3395 cm^{-1}), carbonyl of –COOH (1710 cm^{-1}), and α,β-unsaturated ester carbonyl (1685). Its molecular formula ($C_{25}H_{24}O_{12}$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, showing molecular ion adducts $[M + H]^+$ at *m/z* 517 and $[M + Na]^+$ at 539 in ESI-positive mode and $[M – H]^-$ at *m/z* 515 in ESI-negative mode. The ion at *m/z* 499 was formed by a neutral loss of NaOH from the [M + Na] ion, or by cleavage of water from the protonated molecule (*m/z* 517). The ion at *m/z* 163 indicated the presence of a caffeoyl group. These results suggesting a molecular mass of 516 a.m.u. for compound **43**.

One- and two-dimensional NMR spectra of compound **43** confirmed the presence of a quinic acid moiety along with two caffeic acid moieties in the molecule. Four doublets appearing at δ_H 7.59 (1H, d, *J* = 15.9 Hz, H-7′), 7.61 (1H, d, *J* = 15.9 Hz, H-7″), 6.31 (1H, d, *J* = 15.9 Hz, H-8′) and 6.41 (1H, d, *J* = 15.9 Hz, H-8″) indicaed that the olefinic protons H-7′ (H-7″) and H-8′ (H-8″) are *trans* to each other. In ^{1}H-NMR spectra (**Table-4.12**) of **43** the presence of 1,3,4-trisubstituted benzenes were confirmed by the characteristic coupling pattern of the three aromatic protons. ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra also confirmed the presence of a quinic acid moiety. In the C^{13}-NMR (BB and DEPT) spectra, signals at δ_C 37.6 indicated the

presence of two methylene carbons at C-2 and C-6, while signals at δ_C 74.3, 73.1, 72.3 were ascribable to three methine carbons at C-3, C-4 and C-5 respectively. Furthermore, signals at δ_C 76.4 and 181.3 were ascribed to C-1 and C-7, repectively. HMBC correlations of H-3/C-9′ and H-5/C-9″ of **43** indicated that the two caffeoyl groups were linked to C-3 and C-5 respectively.

In comparison of NMR data, compound **43** was found similar to the reported compound, 3,5-di-*O*-caffeoylquinic acid.[147,148]

Table-4.12:	^{1}H- (400 MHz) and ^{13}C- (125 MHz) NMR data of 3,5-di-*O*-caffeoylquinic acid **(43)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	76.4	C	--
2	37.6	CH_2	2.11, m
			2.27, m
3	74.3	CH	5.40, m
4	73.1	CH	3.91, (dd, 3.5, 9.8)
5	72.3	CH	5.54, m
6	37.6	CH_2	2.11, m
			2.27, m
7	181.3	C	--
1′	127.8	C	--
2′	115.1	CH	7.06, (d, 1.93)
3′	146.7	C	--
4′	149.3	C	--
5′	116.4	CH	6.78, (d, 8.05)
6′	122.9	CH	6.96, m
7′	146.8	CH	7.59, (d, 15.9)
8′	115.5	CH	6.31, (d, 15.9)
9′	168.9	C	--
1″	128.0	C	--
2″	115.1	CH	7.08, (d, 1.93)
3″	146.7	C	--
4″	149.4	C	--
5″	116.4	CH	6.78, (d, 8.05)
6″	122.9	CH	6.96, m
7″	146.8	CH	7.61, (d, 15.9)
8″	116.0	CH	6.41, (d, 15.9)
9″	169.3	C	--

4.2 Biological studies

4.2.1 Anti-bacterial assay

n-Hexane, dichloromethane and methanol fractions of the aerial parts of *L. thomsonii* were subjected for anti-bacterial assay. All the three fractions showed no activity against six selected bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* at the tested concentration (03 μg/mL) (**Table-4.13**).

Table-4.13:	Anti-bacterial assay of various fractions from the aerial parts of *L. thomsonii*					
Aerial parts						
Fractions	***E. c***	***B. s***	***S. f***	***S. a***	***P. a***	***S. t***
F1	x	x	x	x	x	x
F2	x	x	x	x	x	x
F3	x	x	x	x	x	x
DMSO (–)	x	x	x	x	x	x
Imipenum 10μg/Disc (+)	35	36	36	43	32	40
Keywords:	well size= 6mm, F1 = *n*-hexane, F2 = DCM, F3 = MeOH *E. c = Escherichia coli, B. s = Bacillus subtilis, S. f = Shigella flexenari, S. a = Staphylococcus aureus, P. a = Pseudomonas aeruginosa, S. t = Salmonella typhi*					

4.2.2 Anti-fungal assay

n-Hexane, dichloromethane and methanol fractions of the aerial parts of *L. thomsonii* were subjected for anti-fungal activity. All the three fractions showed non-significant activity against five selected fungal strains, *Microsporum canis*, *Candida albicans*, *Aspergillus flavus*, *candida glabrata* and *Fusarium solani* (**Table-4.14**).

Table-4.14:	Anti-fungal assay of various fractions from the aerial parts of *L. thomsonii*		
Fractions	**Name of Fungus**	**% inhibition**	**Std. Drug Mic (*μ*g/mL)**
F1	*C. a*	0	Miconazole (110.8)
	A. f	10	Amphotericin B (20.20)
	M. c	20	Miconazole (98.4)
	F. s	20	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F2	*C. a*	0	Miconazole (110.8)
	A. f	05	Amphotericin B (20.20)
	M. c	15	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F3	*C. a*	0	Miconazole (110.8)
	A. f	0	Amphotericin B (20.20)
	M. c	25	Miconazole (98.4)
	F. s	20	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = MeOH, *C. a = Candida albicans, A. f = Aspergillus flavus, M. c =Microsporum canis, F. s = Fusarium solani, C. g= Candida glabrata*		

4.2.3 Phytotoxicity

n-Hexane, dichloromethane and methanol fractions of the aerial parts of *L. thomsonii* were tested for their *in vitro* phytotoxic bioassay. They were tested on *Lemna minor* plant. *n*-hexane and dichloromethane fractions showed good activity at highest dose while methanol fraction showed low activity (**Table-4.15**).

Table-4.15:	Phytotoxicity of various fractions from the aerial parts of *L. thomsonii*				
Extract/ Fraction	**Compound Conc. (*µ*g/mL)**	**No. of fronds**		**% growth regulation**	**Std. Drug Conc. (*µ*g/mL)**
		Sample	**Control**		
F1	1000	10	20	50	0.015
	100	16		20	
	10	18		10	
F2	1000	10	20	50	0.015
	100	14		30	
	10	16		20	
F3	1000	14	20	30	0.015
	100	17		15	
	10	18		10	
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = MeOH				

4.2.4 Insecticidal assay

Insecticidal assay of the *n*-hexane, dichloromethane and methanol fractions was carried out through contact toxicity method. Dichloromethane and methanol fractions showed moderate activity against insect *Rhyzopertha dominica* and *Callosbruchus analis* respectively, while *n*-hexane fraction shown non-significant (**Table-4.16**).

Table-4.16:	Insecticidal assay of various fractions from the aerial parts of *L. thomsonii*	
Fractions	**Name of Insects**	**% Mortality**
F1	*Tribolium castaneum*	0
	Rhyzopertha dominica	20
	Callosbruchus analis	0
F2	*Tribolium castaneum*	20
	Rhyzopertha dominica	40
	Callosbruchus analis	20
F3	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	40
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = MeOH	

4.2.5 Brine shrimp (*Artemia salina*) lethality bioassay

n-Hexane, dichloromethane and methanol fractions of the aerial parts of *L. thomsonii* were subjected for brine shrimp (*Artemia salina*) lethality bioassay. *n*-hexane and dichloromethane fractions showed no cytotoxicity while methanol fraction showed cytotoxicity at highest dose **(Table-4.17).**

Table-4.17:	Brine Shrimp (*Artemia salina*) Lethality bioassay of various fractions from the aerial parts of *L. thomsonii*					
Fractions	**Dose (*μ*g/mL)**	**No. of shrimps**	**No. of survivors**	**LD50 (*μ*g/mL)**	**STD. Drug**	**LD50 (*μ*g/mL)**
F1	1000	29	23	15760	Etoposide	7.4625
	100	29	26			
	10	29	29			
F2	1000	29	20	3727.87	Etoposide	7.4625
	100	29	24			
	10	29	29			
F3	1000	29	14	846.705	Etoposide	7.4625
	100	29	21			
	10	29	26			
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = MeOH					

4.2.6 *α,α*-Diphenyl-*β*-picrylhydrazyl (DPPH) radical scavenging activity

Free radicals play a vital role in carcinogenesis through their participation in breaking of DNA strands.[149] Several other pathological actions are also associated with the generation of reactive oxygen species (ROS) constituting a key mechanism of tissue injury. They are known to involve in inflammation process, cardiovascular disease[150-152] rheumatoid arthritis, neurodegenerative disease and aging process.[153,154] The harmful action of free radicals can be blocked by antioxidants. There has been growing demand for antioxidants due to their defensive role against the free radical toxicity in our living system. The proton-radical scavenging action is known as an important mechanism of anti-oxidation. The DPPH radical scavenging assay is one of

the familiar methods to assess the antioxidant potential. It is an organic free radical with purple colour and absorbs maximum UV radiations in the range of 515-528 nm. The absorption intensity is reduced when the radical accepts an electron or another free radical, thus changing colour from purple to yellow. The radical scavenging powers (**Figure-4.4** & **Table -4.18**) of the pure compounds (**32-36, 39-43**) isolated from the aerial parts of *L. thomsonii* and ascorbic acid taken as standard were studied against DPPH radical. Compounds **40, 41** and **43** have a significant potent radical-scavenging effect comparable to that of ascorbic acid, that is, almost complete scavenging at a concentration of 250-500 μM (86-97% scavenging for compounds **40, 41** and **43**, as compared to 98.2% scavenging for ascorbic acid) and > 45% scavenging was still observed at 5 μM while compounds **32-36, 39** and **42** showed activities significantly less than ascorbic acid at higher concentration but still comparable to that of ascorbic acid at lower concentrations (5-30 μM). The radical scavenging activities of these compounds are due to their phenolic nature.

Table-4.18	Antioxidant activities of pure compounds isolated from the aerial parts of *L. thomsonii* showing % scavenging at different concentrations and 50% inhibitory concentrations (IC_{50}) in the DPPH radical scavenging assay[a].							
Compound	**% Scavenging[b]**							
	5[c]	**15**	**30**	**60**	**125**	**250**	**500**	**IC_{50}**
32	39.7 ± 1.5	38.5 ± 0.9	40.9 ± 0.9	41.6 ± 1.2	44.3 ± 0.9	47.5 ± 1.2	55.0 ± 1.2	332.8
33	39.5 ± 2.2	40.4 ± 2.0	42.4 ± 1.1	43.6 ± 1.0	44.5 ± 2.2	48.1 ± 1.8	51.1 ± 0.8	403.7
34	37 ± 0.8	39.5 ± 1.3	38.1 ± 1.1	40.0 ± 1.8	42.3 ± 0.8	42.6 ± 0.7	49.1 ± 0.8	N.C[f]
35	38.2 ± 1.0	38.3 ± 0.8	37.8 ± 1.7	39.6 ± 1.2	38.5 ± 1.2	40.3 ± 2.8	38.5 ± 0.9	N.C
36	39.8 ± 2.8	38.3 ± 1.0	37.4 ± 0.9	39.8 ± 1.0	39.3 ± 1.3	40.3 ± 2.0	39.8 ± 2.1	N.C
39	37.5 ± 1.3	38.1 ± 1.2	38.6 ± 0.8	39.3 ± 1.8	39.7 ± 1.2	38.6 ± 1.5	39.2 ± 1.4	N.C
40	47.4 ± 0.9	47.8 ± 1.0	53.8 ± 2.1	57.4 ± 1.0	67.5 ± 1.0	91.3 ± 1.1	96.6 ± 1.2	19.6
41	49.0 ± 0.6	48.0 ± 0.5	51.3 ± 0.9	53.9 ± 1.7	64.9 ± 0.6	83.8 ± 1.7	95.2 ± 0.5	23.3
42	45.8 ± 1.2	46.1 ± 0.6	46.7 ± 1.1	47.2 ± 0.4	49.6 ± 2.0	53.4 ± 4.8	61.1 ± 3.2	141.0
43	48.1 ± 1.7	52.5 ± 1.4	57.0 ± 0.8	64.5 ± 1.2	85.0 ± 1.8	96.3 ± 1.2	96.6 ± 0.9	8.9
Asorbic acid (AA)[d]	43.0 ± 0.8	45.7 ± 1.1	46.7 ± 2.4	57.5 ± 0.9	73.6 ± 2.2	98.1 ± 0.5	98.2 ± 0.7	33.8 ± 0.9

[a]Values are mean ± SD (n = 3). [b]Scavenging % = 100-(A sample[e] x 100/A control[e]). [c]Concentrations in μmol/L. [d]Ascorbic acid used as a standard (AA). [e]Absorbance of sample and control measured at 517 nm. [f]N.C = Not calculated

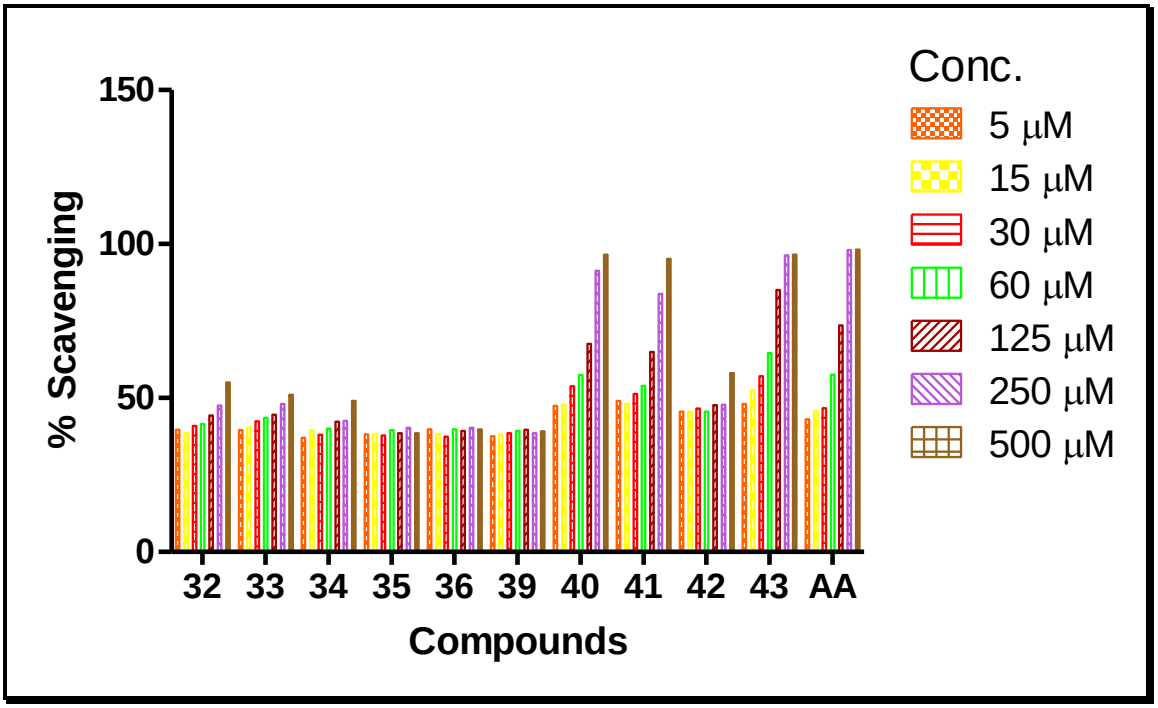


Figure-4.4: DPPH radical scavenging activities of compounds (**32-36**, **39-43**)

5.1 General experimental

Optical rotations were measured using an ADP 220 polarimeter at 589 nm. IR spectra were obtained using a Perkin-Elmer FTIR instrument for compounds mounted directly on the diamond cell. NMR spectra were recorded on Varian VNMRS-400 and Varian VNMRS-500 spectrometers with TMS as internal standard while CD_3OD and $CDCl_3$ as solvent. HRESIMS were carried out on an APEX IV Fourier-transform Ion Cyclotron resonance instrument (Bruker Daltonics, Coventry, UK) using an Apollo ESI source. The nebulizer and drying gas was nitrogen at 180 ^oC. Samples were infused from solution (50% MeOH/DCM) by syringe pump at 100 microlitres per hour. Accurate masses to within 5 ppm were obtained using a broad range external calibration using a mixture of PEG polymer grades. Reverse phase HPLC was conducted using a Waters mass-directed auto-purification system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex LUNA column (5μ, C_{18}, 100 Å, 10 x 250 mm) equipped with a Phenomenex Security Guard precolumn (Luna C_5 300 Å) eluting at 4 mL/min. Solvent A, HPLC grade H_2O + 0.05% formic acid; Solvent B, HPLC grade CH_3CN + 0.045% formic acid or HPLC grade CH_3OH + 0.045% formic acid. The post-column flow was split (100:1) and the minority flow was made up with MeOH + 0.045% formic acid to 1 mL/min for simultaneous analysis by diode array (Waters 2998), evaporative light scattering (Waters 2424) and ESI mass spectrometry in positive and negative modes (Waters Quatro Micro). Silica gel (220-440 mesh) was used for column chromatography and Merck keiselgel 60 PF_{254} pre-coated silica gel glass plates were used for analytical and preparative TLC. TLCs were visualized by UV light (λ = 254 nm, 366 nm) and by heating after spraying with 15% aqueous sulfuric acid saturated with ceric sulfate.

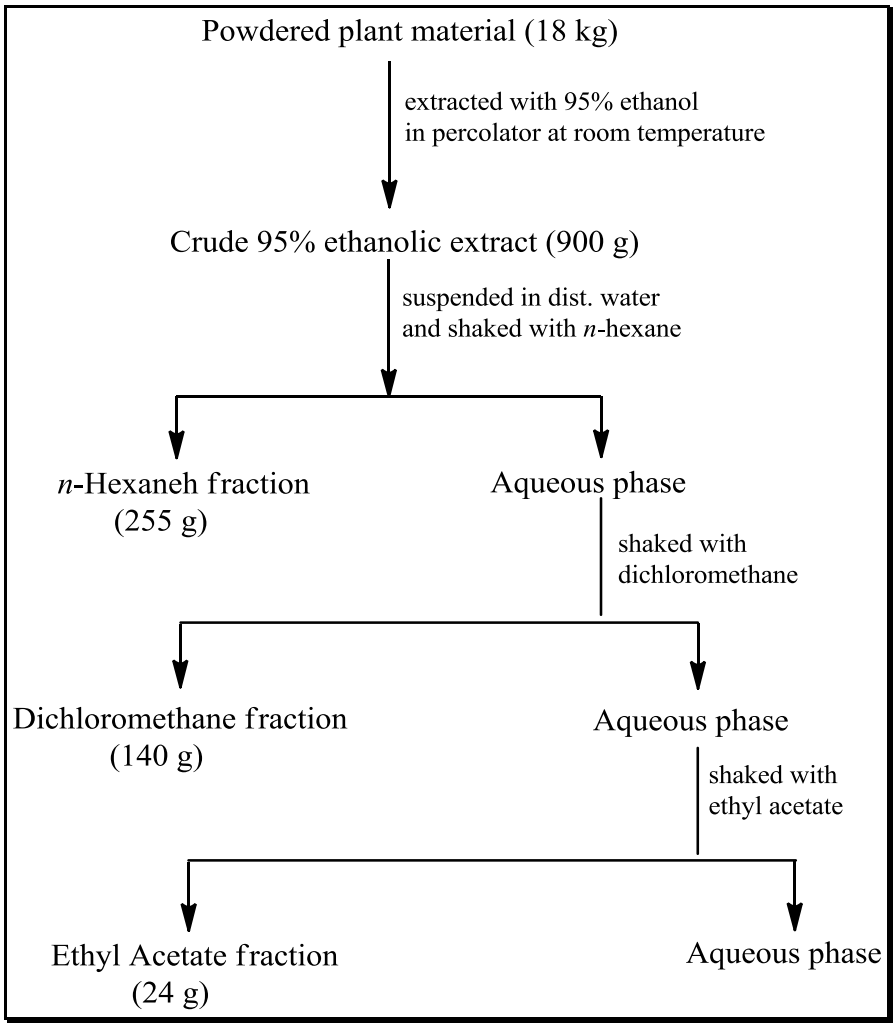
5.2 *Ligularia thomsonii*

5.2.1 Plant material

The plant (*L. thomsonii*) was collected from Naltar (Gilgit), Baltistan, Pakistan, in August 2007. It was identified and authenticated by Dr. Sher Wali Khan, (Curator) Botany Department, University of Karachi, Pakistan. A voucher specimen (No. 348) has been deposited in the herbarium of the Botany Department, University of Karachi Pakistan.

5.2.2 Extraction and isolation

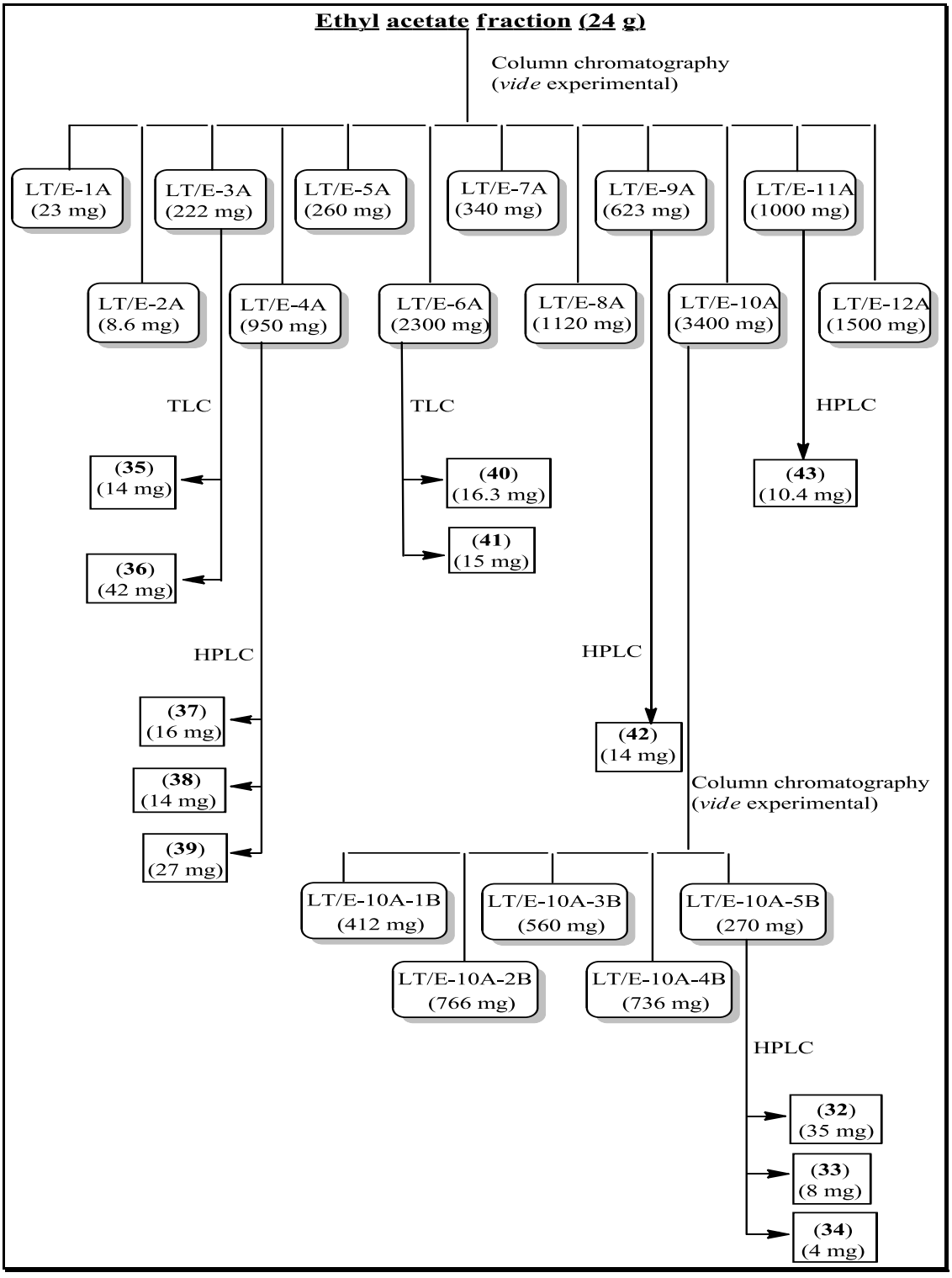
The air dried and powdered aerial parts (18 kg) of the plant (*L. thomsonii)* were extracted at room temperature with 95% ethanol (x3; 4 days). The combined EtOH extracts was evaporated in vacuum to yield 900 g of the residue which was then partitioned using H_2O and *n*-hexane, DCM, EtOAc and MeOH, successively to get *n*-hexane (255 g), DCM (140 g), EtOAc (24 g), and aqueous MeOH (127 g) extracts **(Scheme-5.1)**.



Scheme-5.1: Extraction and fractionation of the aerial parts of *Ligularia thomsonii.*

The EtOAc fraction (24 g) was chromatographed on silica gel (220-440 mesh, 300 g, 6 x 100 cm) column, eluting successively with a gradient of DCM-MeOH (100:00, 98:02, 92:08, 88:12, 84:16 & 80:20). According to the differences in composition indicated by TLC, twelve crude fractions (LT/E-1A to LT/E-12A) were collected. Fraction LT/E-3A (222 mg) was purified by preparative TLC [DCM-MeOH (99:01) plus few drops of acetic acid, 4 developments] to afford 18 mg of **35** and 42 mg of **36**. LT/E-4A (950 mg) was subjected to HPLC mass-directed preparative purification using H_2O-MeOH as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 25% B; 13 min, 95% B; 15

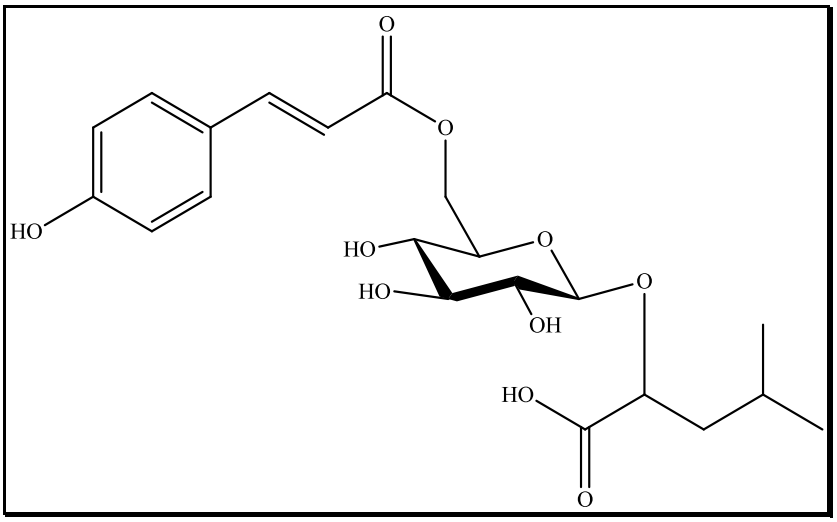
min, 95% B; 17 min, 25% B; 20 min, 25% B). Evaporation of the eluted compounds under a stream of dry nitrogen yielded 16 mg of **37**, 14 mg of **38** and 27 mg of **39**. Fraction LT/E-6A (2300 mg) was purified by preparative TLC [DCM-MeOH (95:05) plus few drops of acetic acid, 7 developments] to afford 16.3 mg of **40** and 15 mg of **41**. LT/E-9A (623 mg) was subjected to HPLC mass-directed preparative purification using H_2O-CH_3CN as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 25% B; 0.01 min, 25% B; 15 min, 35% B; 16 min, 95% B; 18.50 min, 95% B; 19 min, 25% B; 20 min, 25% B). Evaporation of the eluted compound under a stream of dry nitrogen yielded 14 mg of **42**. LT/E-11A (1000 mg) was subjected to HPLC mass-directed preparative purification using H_2O-MeOH as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 40% B; 13 min, 95% B; 17 min, 95% B; 19.50 min, 40% B; 20 min, 40% B). Evaporation of the eluted compound under a stream of dry nitrogen yielded 10.4 mg of **43**. Fraction LT/E-10A (3400 mg) was chromatographed on silica gel (220-440 mesh, 250 g, 4 x 90 cm) column, eluting successively with a gradient of DCM-MeOH (96:04, 94:06, 92:08, 90:10 & 00:100). According to the differences in composition indicated by TLC, five crude fractions (LT/E-10A-1B to LT/E-10A-5B) were collected. LT/E-10A-5B (270 mg) was subjected to HPLC mass-directed preparative purification using H_2O-CH_3CN as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 05% B; 1.00 min, 50% B; 15 min, 60% B; 15.50 min, 95% B; 16.50 min, 95% B; 17 min, 05% B; 20 min, 05% B). Evaporation of the eluted compounds under a stream of dry nitrogen yielded 35 mg of **32**, 8 mg of **33** and 4 mg of **34 (Scheme-5.2)**.



Scheme-5.2: Isolation of pure compounds (**32-43**) from *L. thomsonii*

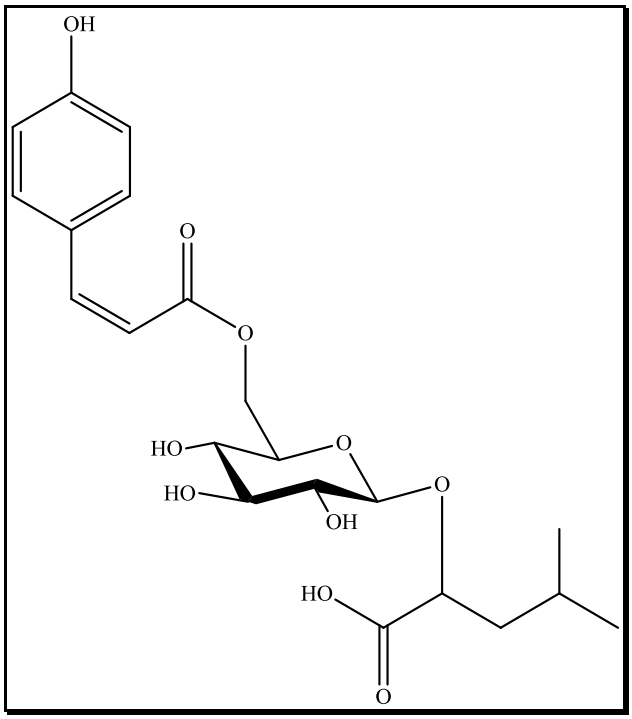
5.2.3 Characterization of the new compounds isolated from *L. thomsonii*

5.2.3.1 Ligularoside I (32)



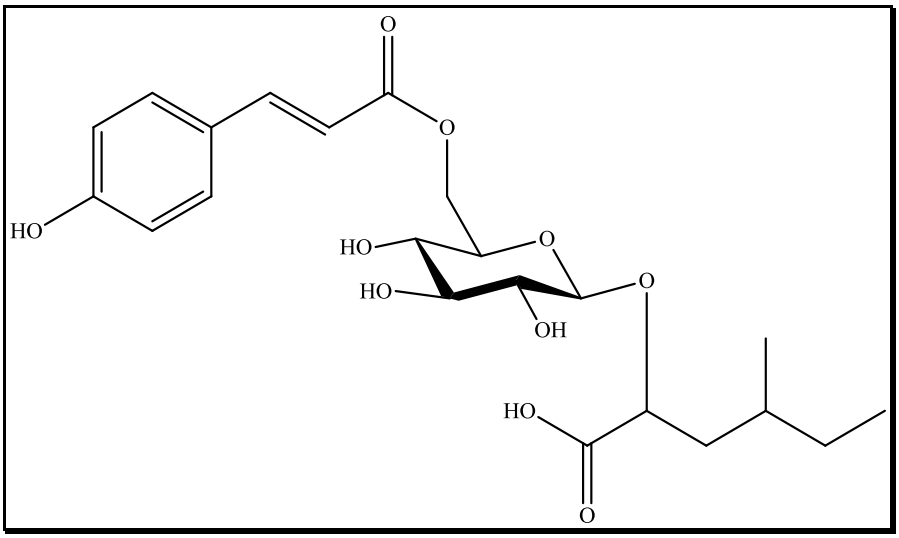
Physical State:	Brownish amorphous solid
Yield:	35 mg
$[\alpha]_D^{20}$:	$-37.5°$ (c 0.93, MeOH)
UV (MeOH) λ_{max}:	295 and 228 nm.
IR ν_{max} (MeOH) cm^{-1}:	3336, 1688 and 1603
HR-ESI-MS (*m/z*):	463.1583 (calculated for $C_{21}H_{28}O_{10}Na$ $[M + Na]^+$ 463.1574)
ESI(+)-MS (*m/z*):	463.16, 413.27, 391.29, 324.18 and 154.40
^1H- and ^{13}C-NMR:	Table-4.1

5.2.3.2 Ligularoside II (33)



Physical State:	Brownish amorphous solid
Yield:	8 mg
$[\alpha]_D^{20}$:	$-28.5°$ (c 0.283, MeOH)
UV (MeOH) λ_{max}:	307 and 211 nm.
IR ν_{max} (MeOH) cm^{-1}:	3329, 1701 and1603
HR-ESI-MS (*m/z*):	463.1589 (calculated for $C_{21}H_{28}O_{10}Na$ $[M + Na]^+$ 463.1574)
ESI(+)-MS (*m/z*):	463.1589, 413.2675, 349.0905, 331.0799 and 154.3970
^1H- and ^{13}C-NMR:	Table-4.2

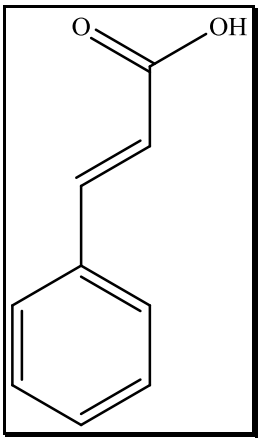
5.2.3.3 Ligularoside III (34)



Physical State:	Brownish amorphous solid
Yield:	4 mg
$[\alpha]_D^{20}$:	$-30.0°$ (c 0.13, MeOH)
UV (MeOH) λ_{max}:	313 and 226 nm.
HR-ESI-MS (*m/z*):	477.1751 (calculated for $C_{22}H_{30}O_{10}Na$ $[M + Na]^+$ 477.1731)
ESI(+)-MS (*m/z*):	477.1751, 447.2949, 429.2421, 413.2680, 349.0909, 301.1423 and 137.7647
^{1}H- and ^{13}C-NMR:	Table-4.3

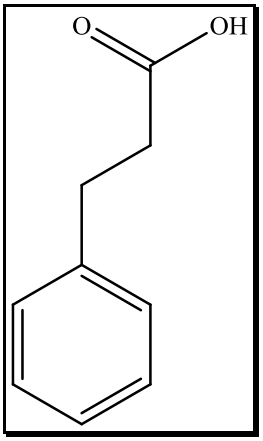
5.2.4 Characterization of the new source isolated compounds from *L. thomsonii*

5.2.4.1 Cinnamic acid (35)



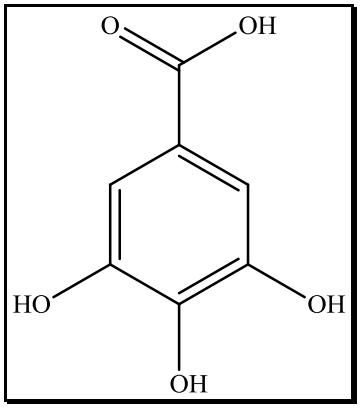
Physical State:	White crystalline solid
Yield:	14 mg
Melting Point:	133°C
IR ν_{max} (MeOH) cm^{-1}:	3315 and 1692
LC-ESI(+)-MS (*m/z*):	149 and 171
LC-ESI (–)-MS (*m/z*):	147
^{1}H- and ^{13}C-NMR:	Table-4.4

5.2.4.2 3-Phenylpropanoic acid (36)



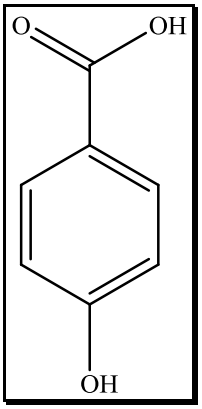
Physical State:	White powder
Yield:	42 mg
Melting Point:	49°C
IR ν_{max} (MeOH) cm^{-1}:	3300, 1703 and 1610
LC-ESI(+)-MS (*m/z*):	151 and 173
LC-ESI (–)-MS (*m/z*):	149
^{1}H- and ^{13}C-NMR:	Table-4.5

5.2.4.3 Gallic acid (37)



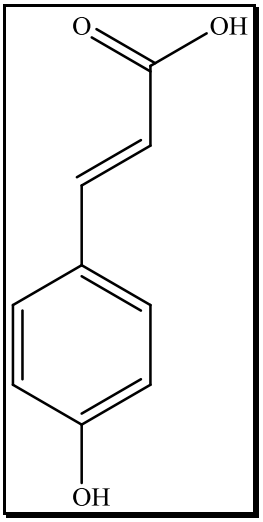
Physical State:	White crystalline solid
Yield:	16 mg
Melting Point:	250°C
IR ν_{max} (MeOH) cm^{-1}:	3432 and 1706
LC-ESI(+)-MS (*m/z*):	171 and 193
LC-ESI (–)-MS (*m/z*):	169
^{1}H- and ^{13}C-NMR:	Table-4.6

5.2.4.4 *p*-Hydroxybenzoic acid (38)



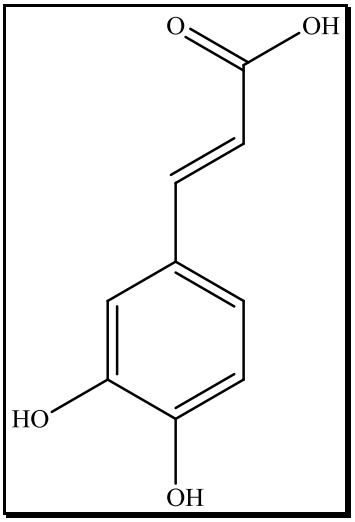
Physical State:	White powder
Yield:	14 mg
Melting Point:	216°C
IR ν_{max} (MeOH) cm^{-1}:	3450 and 1705
LC-ESI(+)-MS (*m/z*):	139 and 161
LC-ESI (–)-MS (*m/z*):	137
^{1}H- and ^{13}C-NMR:	Table-4.7

5.2.4.5 Coumaric acid (39)



Physical State:	White powder
Yield:	27 mg
Melting Point:	212°C
IR ν_{max} (MeOH) cm^{-1}:	3446 and 1694
LC-ESI(+)-MS (*m/z*):	165 and 187
LC-ESI (–)-MS (*m/z*):	163
^{1}H- and ^{13}C-NMR:	Table-4.8

5.2.4.6 Caffeic acid (40)



Physical State: White powder

Yield: 16.3 mg

Melting Point: 225°C

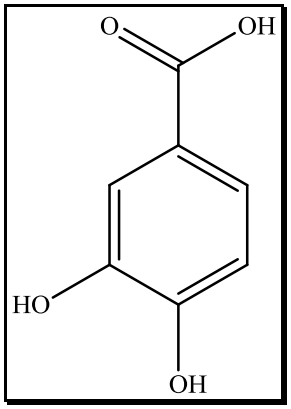
IR ν_{max} (MeOH) cm^{-1}: 3445 and 1710

LC-ESI(+)-MS (*m/z*): 181 and 203

LC-ESI (–)-MS (*m/z*): 179

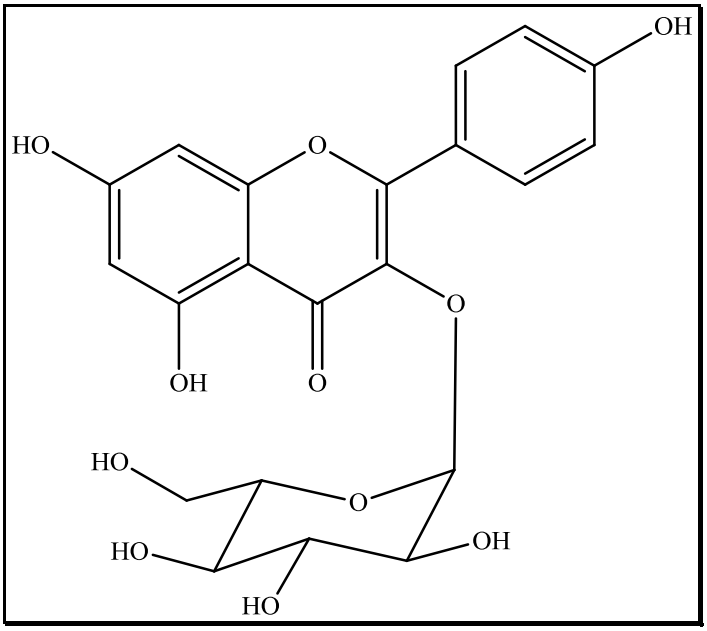
^{1}H- and ^{13}C-NMR: Table-4.9

5.2.4.7 3,4-Dihydroxybenzoic acid (41)



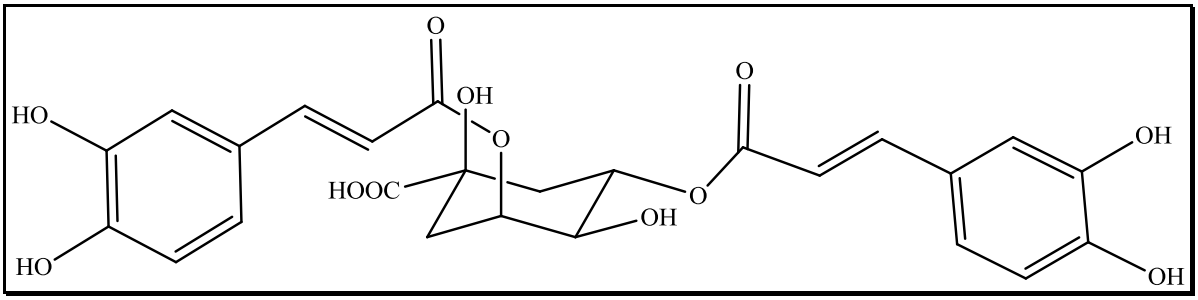
Physical State:	White powder
Yield:	15 mg
Melting Point:	202°C
Melting point:	200-201 ^{o}C
IR v_{max} (MeOH) cm^{-1}:	3450, 3300, 1700 and 1615
LC-ESI(+)-MS (*m/z*):	155 and 177
LC-ESI (–)-MS (*m/z*):	153
^{1}H- and ^{13}C-NMR:	Table-4.10

5.2.4.8 Kaempferol 3-*O*-*β*-D-glucopyranoside (42)



Physical State:	Yellowish powder
Yield:	14 mg
IR ν_{max} (MeOH) cm^{-1}:	3398 and 1702
LC-ESI(+)-MS (*m/z*):	449 and 471
LC-ESI (–)-MS (*m/z*):	447
^{1}H- and ^{13}C-NMR:	Table-4.11

5.2.4.9 3,5-di-*O*-Caffeoylquinic acid (43)



Physical State:	Yellowish solid
Yield:	10.4 mg
Melting point:	150-152 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3395, 1710 and 1685
LC-ESI(+)-MS (*m/z*):	163, 499, 517 and 539
LC-ESI (–)-MS (*m/z*):	515
^{1}H- and ^{13}C-NMR:	Table-4.12

5.3 Biological studies

5.3.1 Anti-bacterial assay

Agar tube diffusion method[155] was employed for determination of anti-bacterial activity which involved nutrient agar (solid medium), nutrient broth (liquid medium) and soft agar (semi-solid medium). The fractions were dissolved in DMSO (3 mg/mL) serving as stock solution. Bacterial culture (single colony) was inoculated in liquid medium and incubated for 24 hours at 37°C. 100 μL of freshly prepared bacterial culture was added to soft agar tube and then poured it on to the solid medium containing plate and allowed it to solidify. Sterile borer (6mm-diameter) was used for making wells and 100 μL of sample were added in respective agar well plate. DMSO and standard drug (Imipenum) were also added to other wells. The plates were incubated at 37°C for 24 hours with continuous observation of inhibition.

5.3.2 Anti-fungal assay

Anti-fungal assay was determined through agar tube dilution method[156,157] In this method miconazole and amphotericin B were used as standard drugs. The fractions were dissolved in sterile DMSO (24 mg/mL) serving as stock solution. Sabouraud's dextrose agar (SDA) medium (4 mL) was placed in screw caps tubes and inoculated with sample solution (400 μg/mL). Tubes then allowed solidifying at room temperature in slanting position, which were then inoculated with a piece (4 mm diameter) of fungus (seven-day-old culture). The samples were then incubated at 28°C for 7 days with continuous observation of inhibition. Percent growth inhibition was calculated by using the following formula.[158]

$$\% \text{ Inhibition} = 100 - \frac{\text{Linear growth and test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

5.3.3 Phytotoxicity

Phytotoxicity of the various fractions was evaluated against the *Lemna minor* L.[159] E-medium was prepared by mixing different constituents in distilled water (1000 mL, pH = 5.5-6.0). Working E-medium was prepared by mixing of E-medium (100 mL) and of distilled water (900 mL). Stock solution was prepared by dissolving 30 mg of each fraction in 1.5 mL suitable organic solvent. Three flasks with 1000, 100 10 μL of solution pipette from the stock solution (30 mg/1.5 mL organic solvent) for 1000, 100 and 10 μg/mL were inoculated and the solvent was evaporated. Working E-medium (20 mL) and *Lemna minor* (containing a rosette of three fronds) was added to each flask which were then incubated for seven days in the growth cabinet. In this assay paraquat was used as reference growth inhibitor. The percentage growth regulation was calculated as follows.

$$\% \text{ Regulation} = 100 - \frac{\text{No. of fronds in test}}{\text{No. of frond in negative control}} \times 100$$

5.3.4 Insecticidal assay

Insecticidal assay was determined by contact toxicity method[160] in which various fractions were tested against selected insects; *Callosbruchus analis*, *Rhyzopertha dominica* and *Tribolium castaneum.* The test samples were prepared by dissolving 20 mg fractions in 2 mL acetone and loaded in a petri plate covered with filter paper for 24 hours and the solvent was completely evaporated. Next day 10 insects of each species were placed in each plate, incubated for next 24 hours at 27 °C with 50 % relative humidity in growth chamber. Permethrin was taken as a

standard drug in this assay. Survival of the insects was assessed in terms of percent inhibition, calculated as follows.

$$\% \text{ Inhibition} = 100 - \frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

5.3.5 Brine shrimp (*Artemia salina*) lethality bioassay

Cytotoxicity of the solvent extracted fractions was evaluated against *Artemia salina* (Leach) shrimp larvae. The eggs of brine shrimp were sprinkled (50 mg) to a tray containing filtered brine solution (half filled). The test samples were prepared by dissolving 20 mg different solvent extracted fractions in 2 mL acetone from which we prepared final concentrations of 10, 100 and 1000 μg/mL. After 2-days of hatching and maturation as nauplii, 10 larvae/vials were placed with the help of a Pasteur pipette and seawater was added to make the volume upto 5 mL. Vials were then incubated for 24 hours at 25-27ºC under illumination. Solvent and Standard drug (Etoposide) was also added to other vials. The data was analyzed with Finny computer program to determine LD_{50} values with 95% confidence intervals.

5.3.6 *α,α*-Diphenyl-*β*-picrylhydrazyl (DPPH) radical-scavenging effects

Antioxidant assay was performed in flat bottom polystyrene 96-well micro-titer plates. The DPPH radical-scavenging effects were measured using a modified previously established methodology.[161,162] To 100 μL of each sample at different concentrations in MeOH were added 150 μL of DPPH (0.25 mM) in MeOH. The resultant mixture was briefly shaken and maintained at room temperature in the dark for 30 min. The absorbance was measured at 517 nm by using a Spectra Max 190 96-well plate reader and data was processed on SOFTmax pro 4.0 software. The

percentage of scavenging of DPPH radical from a sample at a given concentration was calculated from the absorbance readings as shown below.

$$\%\ scavenging = 100 - \frac{\left(A_{sample} - A_{sample\ blank}\right)\ x\ 100}{A_{control} - A_{blank}}$$

6.1 Plant introduction

The genus *Senecio* comprises nearly 3000 species,[163] of which about 37 occur in Pakistan including *S. amplexicaules, S. analogus, S. auriculata, S. chenopodifolius, S. chrysanthemoides, S. coronopifolius, S. decaisnei, S. desfontanei, S. dubius, S. flavus, S. goringensis, S. jacobea and S. tibeticus.*[164] The importance of genus *Senecio* is based on its botanical, pharmacological and toxicological properties.[163] The genus *Seinecio* is widely known for its pyrrolizidine alkaloids and some of these reveal a potentially helpful level and spectrum of antitumor activity. However, use of these *Senecio* alkaloids has been restricted because of hepatotoxicity and mutagenic properties.[165] Many species of this genus are widely used in Chinese traditional medicine and as folk remedy for inflammatory and infectious diseases.[166] They are also used as blood regulator, for relieving stomach pain, in curing of ulcer, to counteract mountain sickness and are also used as emenagogue, digestive and cough suppressant.[167]

6.2 Literature survey of genus *Senecio*

The list of compounds in **Table-6.1** (chronological order) showed diversity of constituents in genus *Senecio*.

Table-6.1:	Chemical Constituents of the Genus *Senecio*	
S. No.	**Compounds**	**Species**
1	Senecionine	*S. integerrimus*[168,169]
2	Longilobine	*S. longilobus*[168]
3	Seneciphylline	*S. spartioides*[168]
4	Riddelliine	*S. riddellii*[168,169]
5	Jacobine	*S. jacobaea*[170]
6	Jacoline	*S. jacobaea*[170]
7	Jaconine	*S. jacobaea*[170]
8	Retrorsine	*S. discolor*[171]
9	Isatidine	*S. discolor*[171]
10	Isovaleric acid	*S. eriophyton*[172]
11	Othosenine	*S. desfontainei*[169] *S. aegyptius*[169]
12	Fumaric acid	*S. desfontainei*[169] *S. aegyptius*[169]
13	Choline	*S. desfontainei*[173] *S. aegyptius*[173]
14	*β*-Sterol	*S. tournefortii*[174]
15	Isobauerenol	*S. tournefortii*[174]
16	*Ψ*-Taraxasterol	*S. tournefortii*[174]
17	Deisobutyryladenostilone	*S. tournefortii*[174]
18	8,8*α*-Epoxyfuranoligularan	*S. silvaticus*[175]
19	Swazine	*S. swaziensis*[176]
20	Abrotanifolone	*S. abrotanifolius*[177]
21	3,4:10,11-Diepoxy-1,8-dihydroxy-7(14)-bisabolen-2-one, diangeloyl	*S. abrotanifolius*[177]
22	6*β*-Hydroxy-9,10-didehydrofuranoeremophilan-1-one	*S. polyanthemoides*[178]
23	6*β*-Isobutryloxy-9,10-didehydrofuraneremo-philan-1-one	*S. polyanthemoides*[178]
24	6*β*-Angeloyloxy-9,10-didehydrofuraneremo-philan-1-one	*S. polyanthemoides*[178]
25	9,10-Dehydrofuranoeremophilane	*S. arten*[179]
26	9,10-Dehydrofuranoeremophil-1-one	*S. arten*[179]
27	10*α-H*-Furanoeremophil-9-one	*S. teretifolius*[180]
28	Germacrene D	*S. scytophyllus*[180]
29	Oleanol	*S. nemorensis*[181]
30	Lupeol	*S. nemorensis*[181]
31	Bauerenol	*S. nemorensis*[181]
32	*β*-Sitosterol	*S. nemorensis*[181]

33	Furanoligularenone	*S. nemorensis*[181]
34	3-Oxo-8*α*,10*α*-eremophila-1,7-dien-8,12-olide	*S. nemorensis*[181]
35	3-Oxo-8*α*-hydroxy-10*α*-eremophila-1,7-dien-8,12-olide	*S. nemorensis*[181]
36	3-Oxo-*δα*-methoxy-10*α*-eremophila-1,7-dien-8,12-olide	*S. nemorensis*[181]
37	3-Oxo-8*α*-ethoxy-10*α*-eremophila-1,7-dien-8,12-olide	*S. nemorensis*[181]
38	*α*-Methoxy-(3)-oxoeremophil-1,7-dien-8,12-olide	*S. nemorensis*[182]
39	10*β*-Furanoeremophilane	*S. medley-woodii*[183]
40	3*β*-Acetoxy-6*β*-(2-methylbutyryloxy)-10*β*-furanoeremophilane	*S. medley-woodii*[183]
41	3*β*-Acetoxy-6*β*-(2-methylbutyryloxy)-10*β*-*H*-furanoeremophil-9-one	*S. medley-woodii*[183]
42	6*β*-Angeloyloxy-9,10-dehydrofuranoeremophil-1-one	*S. halimifolius*[183]
43	12-Hydroxy-2,3-dihydroeuparine	*S. halimifolius*[183]
44	14-Isovaleryloxy-*O*-methyl-1,2-dehydrocacalol	*S. inaequidens*[183]
45	4-Hydroxy-3-methoxy-1-oxo-*O*-methyl-2,3-dehydrocacalol	*S. inaequidens*[183]
46	14-Hydroxy-3-methoxy-1-oxo-*O*-methyl-2,3-dehydrocacalol	*S. inaequidens*[183]
47	Calcalolpropionate	*S. inornatus*[183]
48	14-Acetoxy-cacalolpropionate	*S. inornatus*[183]
49	14-Hydroxy-cacalolpropionate	*S. inornatus*[183]
50	1,2-Dihydrosenedigital-2-one	*S. digitalifolius*[184]
51	5,6-Dehydrosenedigitalene	*S. digitalifolius*[184]
52	Senedigitalene	*S. digitalifolius*[184]
53	Pentaynene	*S. oxyodontus*[185]
54	3-*O*-Methyl-2,5-dehydrosenecioodantol	*S. oxyodontus*[185]
55	3-*O*-Methyl senecioodantol	*S. oxyodontus*[185]
56	2,5-Di-*O*-methyl senecioodantol	*S. oxyodontus*[185]
57	3-*O*-Angeloyl senecioodontol	*S. oxyodontus*[185]
58	8-Angeloyloxy-3,4-dihydro-3,4-epoxy-*β*-bisabolen-5-one	*S. oxyodontus*[185]
59	1*β*-Isobutyryloxy-5*β*-hydroxy-bisabolol	*S. pubigerus*[186]
60	1*β*-[2-Methylbutyryloxy]-5*β*-hydroxybisabolol	*S. pubigerus*[186]
61	Senecioodontol-2-*O*-acetate	*S. longifolius*[186]
62	Senecioodontol-2,5-*O*-diacetate	*S. longifolius*[186]
63	6-Methoxy-9-acetoxyisoeugenolgeranylether	*S. longifolius*[186]
64	4-*O*-Angeloyl-8-desangeloyloxy-8-[2-methyl-2,3-epoxybutyryloxy]-dehydrosenecioodontol	*S. longifolius*[186]
65	Octadeca-9*c*,16*t*-dien-12,14-diin-1-ol	*S. brevilorus*[186]
66	Octadeca-2*t*,9*c*,16*t*-trien-12,14-diin-1-ol	*S. brevilorus*[186]
67	1,2,6-Trimethoxy-8,9-diangeloyloxy-cumol	*S. macroglossus*[186]
68	Furanoligularenone	*S. nemorensis*[187]
69	3-Oxo-8*α*-eremophila-1,7-dien-8,12-olide	*S. nemorensis*[187]
70	3-Oxo-8*α*-hydroxyeremophila-1,7-dien-8,12-olide	*S. nemorensis*[187]
71	3*β*-Acetoxy-6*β*-angeloyloxy-10*β*-*H*-furanoeremo-	*S. pyramidatus*[188]

	philane	
72	3*β*-Acetoxy-6*β*-tiglinoyloxy-10*β*-*H*-furanoeremo-philane	*S. pyramidatus*[188]
73	3*β*-Acetoxy-6*β*-senecioyloxy-10*β*-*H*-furanoeremo-philane	*S. pyramidatus*[188]
74	3*β*-Hydroxy-6*β*-angeloyloxy-10*β*-*H*-furanoeremo-philane	*S. pyramidatus*[188]
75	3*β*-Acetoxy-6*β*-tiglinoyloxy-10*β*-*H*-furanoeremo-phil-9-one	*S. pyramidatus*[188]
76	6*β*-Tiglinoyloxy-9*β*-hydroxy-10*β*-*H*-furanoeremo-philane	*S. pyramidatus*[188]
77	3*β*-Hydroxy-6*β*-angeloyloxy-7,8-epoxyeremo-philenolide	*S. pyramidatus*[188]
78	14-Angeloyloxy-cacalol-propionate	*S. affinis*[188]
79	13,14-Diacetoxy-2*β*-hydroxycacalolpropionate	*S. affinis*[188]
80	14-Angeloyloxy-2*β*-hydroxycacalolpropionate	*S. affinis*[188]
81	2*β*,13,14-Triacetoxycacalolpropionate	*S. affinis*[188]
82	2*β*,14-Diacetoxycacalolisobutyrate	*S. affinis*[188]
83	3*β*-Acetoxy-cacalol-methylether	*S. affinis*[188]
84	14-Angeloyloxy-3*β*-hydroxycacalolpropionate	*S. affinis*[188]
85	14-Angeloyloxycacalohastinpropionate	*S. affinis*[188]
86	14-Acetoxycacalohastinpropionate	*S. affinis*[188]
87	14-Angeloyloxy-dehydrocacalohastin-propionate	*S. affinis*[188]
88	14-Acetoxy-dehydrocacalohastinpropionate	*S. affinis*[188]
89	7,8-*H*-6,7-Dehydroblodiol-1-*O*-angelicate	*S. serpens*[188]
90	14,15-*trans*-Senaetnine	*S. aucheri*[188]
91	Senampelin E	*S. mikanoides*[188]
92	Senampelin F	*S. mikanoides*[188]
93	Senampelin G	*S. mikanoides*[188]
94	3,4-Dimethoxy-zimtalkohol-angelicat	*S. colaminus*[189]
95	Sencolaminsaure-*N*-phenylethylamide	*S. colaminus*[189]
96	Sencolaminsaure-*N*-*iso*-amylamide	*S. colaminus*[189]
97	6*β*-Propionyloxy-furanoeremophil-9,10-en-1-one	*S. pampse*[189]
98	6*β*-Acetoxy-1*α*-hydroxy-10*α*-*H*-furanoeremo-phil-9-one	*S. pampse*[189]
99	1*α*,6*β*-Diacetoxy-10*α*-*H*-furanoeremophil-9-one	*S. pampse*[189]
100	1-Oxo-9-desoxycacalol	*S. serratifolius*[189]
101	Isopterophorin	*S. pulviniformis*[189]
102	Hydroquinone,	*S. scandens*[190]
103	*p*-Hydroxyphenylacetic acid	*S. scandens*[190]
104	Vanillic acid	*S. scandens*[190]
105	Salicylic acid	*S. scandens*[190]
106	Pyromucic acid	*S. scandens*[190]
107	Ethyl-*α*-[4-(4-hydroxy-cyclohexa-2,5-dien-1-one)]-acetate	*S. fendleri*[191]
108	6*α*-Isobutyryloxy-furanoeremophil-9-en-1-one	*S. vitalis*[192]
109	8*β*-Hydroxy-8*α*-methoxy-3-oxo-eremophil-1,7(11)-dien-(12)-oic acid	*S. vitalis*[192]
110	6*β*-Acetoxy-4,5-epoxy-3*β*-senecioyloxygermacr-	*S. vitalis*[192]

	1(10)-ene	
111	*β*-Farnesene	*S. vitalis*[192]
112	Germacrene C	*S. vitalis*[192]
113	Bicycloermacrene	*S. vitalis*[192]
114	9-Gernayl-*α*-terpinene	*S. vitalis*[192]
115	Oleanoic aldehyde	*S. vitalis*[192]
116	Oleanoic acid	*S. vitalis*[192]
117	Lupenone	*S. vitalis*[192]
118	Lutin-5(6)-en-3*β*-ol	*S. vitalis*[192]
119	Furanoeremophil-1(10)-ene	*S. vitalis*[192]
120	6*β*-Isobutyryloxyfuranoeremophil-1(10)-en-9-one	*S. vitalis*[192]
121	Furanoeremophil-1(2)-en-3-one	*S. vitalis*[192]
122	6*α*-Angeloyloxyfuranoeremophil-9-ene-1-one	*S. vitalis*[192]
123	6*β*-Hydroxyfuranoeremophil-9-ene-1-one	*S. vitalis*[192]
124	6*β*-Isobutyryloxyfuranoeremophil-9-ene-1-one	*S. vitalis*[192]
125	8*β*-Hydroxy-8*α*-methoxy-3-oxo-eremophil-1,7(11)-dien-12-oic acid lactone	*S. vitalis*[192]
126	6*β*-Acetoxy-3*β*-angeloyloxy-4,5-epoxygermacr-1(10)-ene	*S. vitalis*[192]
127	3*β*-Angeloyloxy-4,5-epoxy-6*β*-hydroxygermacr-1(10)-ene	*S. vitalis*[192]
128	4,5-Epoxy-6*β*-hydroxy-3*β*-senecioyloxygermacr-1(10)-ene	*S. vitalis*[192]
129	4,5-Epoxy-6*β*-hydroxy-3*β*-tigloyloxygermacr-1(10)-ene	*S. vitalis*[192]
130	10*β*-Isovaleryloxy-furanoeremophil-9-one	*S. inaequidens*[193]
131	Isoacacalone	*S. inaequidens*[193]
132	2*α*-Angeloyloxy-1-oxocacalol	*S. inaequidens*[193]
133	3*α*-Hydroxy-14-senecioyloxy-1,2-dehydrocacalol methyl ether	*S. trichopterygius*[193]
134	14-Acetoxy-1,2-dehydrocacalol methyl ether	*S. trichopterygius*[193]
135	12-(Dehydrocacalohastin-14-yl)-cacalol	*S. trichopterygius*[193]
136	Senaequidolide	*S. inaequidens*[193]
137	4-Hydroxy-10*α*-*H*-eremophil-11(12)ene	*S. amplexicaulis*[194]
138	2*β*-Angeloyloxy-10*β*-*H*-furanoeremophilane	*S. alatus*[194]
139	2*β*-[5′-Hydroxyangeloyloxy]-10*β*-*H*-furanoeremo-philane	*S. alatus*[194]
140	2*β*-[5′-Hydroxyangeloyloxy]-10*β*-*H*-eremophilan-olide	*S. alatus*[194]
141	2*β*-[5′-Hydroxyangeloyloxy]-8*β*-hydroxy-10*β*-*H*-eremophilanolide	*S. alatus*[194]
142	2*β*-Angeloyloxy-8*β*-hydroxy-10*β*-*H*-eremophilan-olide	*S. alatus*[194]
143	Integerrimine	*S. alpinus*[195]
144	Jacozine	*S. alpinus*[195]
145	Cleviolide	*S. clevelandii*[196]
146	4-*trans*-4,5-Dihydrocleviolide	*S. clevelandii*[196]
147	4-*cis*-4,5-Dihydrocleviolide	*S. clevelandii*[196]
148	4-[Carbomethoxymethyl]-4-hydroxycyclohexanone	*S. clevelandii*[196]

149	14-Angeloyloxy-tetradeca-5*c*-10*t*-12*t*-trien-8-yn-1-al	*S. clevelandii*[196]
150	Oleanolic acid	*S. yegua*[197]
151	Ursolic acid acetate	*S. yegua*[197]
152	Ursolic acid methyl ester	*S. yegua*[197]
153	Rutin	*S. yegua*[197]
154	Quercitrin	*S. yegua*[197]
155	Quercitin-3-*O*-galactoside	*S. yegua*[197]
156	Euryopsonol senecioate	*S. smithii*[198]
157	1*α*-Hydroxyeuryopsonol senicioate	*S. smithii*[198]
158	1*α*-Hydroxyeuryopsonol angelate	*S. smithii*[198]
159	1*α*-Hydroxyeuryopsonol isobutyrate	*S. smithii*[198]
160	6*β*-Hydroxy-1*β*,10*β*-epoxyfuranoerempohil-9-one	*S. smithii*[198]
161	6,9-Dioxo-1*β*,10*β*-epoxyfuranoerempohilane	*S. smithii*[198]
162	12-Acetoxybisabol-1-one	*S. smithii*[198]
163	1-Oxo-bisabol-12-al	*S. smithii*[198]
164	14-Acetoxy-7*β*-[3′-ethyl crotonoyloxy]-notonipetranone	*S. kleinia*[199]
165	7*β*- [3'-Ethyl crotonoyloxy]-14-hydroxy-l*α*-[2'-methylbutyryloxy]-notonipetranone	*S. kleinia*[199]
166	14-Acetoxy-7*β*-[3'-ethyl crotonoyloxy]-1*α*-[2'-methylbutyryloxy]-notonipetranone	*S. kleinia*[199]
167	7*β*-[3'-Ethyl crotonoyloxy]-l*α*-[2'-methylbutyryl-oxy]-3,14-dehydro-*E*-notonipetranone	*S. kleinia*[199]
168	7*β*-[3'-Ethyl crotonoyloxy]-1*α*-[2'-methylbutyryl-oxy]-3,14-dehydro-*Z*-notonipetranone	*S. kleinia*[199]
169	6*β*-Angeloyloxysecomacrolide acetate	*S. mactrotis*[200]
170	8-*epi*-6*β*-Angeloyloxysecomacrotolide acetate	*S. mactrotis*[200]
171	6*β*-Tiglinoyloxysecomacrotolide acetate	*S. mactrotis*[200]
172	8-*epi*-6*β*-Tiglinoyloxysecomacrotolide acetate	*S. mactrotis*[200]
173	6*β*-*iso*-Valeryloxysecomacrotolide acetate	*S. mactrotis*[200]
174	l*α*,8-Diangeloyloxy-3*β*,4*β*,10,1l-diepoxy-bisabol-7(14)en-2-one	*S. fulgens*[201]
175	8-Acetoxy-1*β*,2*β*(-diangeloyloxy-3*β*,4*β*-epoxy-bisobola-7(14),10-diene	*S. fulgens*[201]
176	2-Desoxyliguhodgonal	*S. nebrodensis*[201]
177	Senecrassidiol	*S. crassissimus*[201]
178	All-*cis*-1,7,10,13-hexadecatetreaene	*S.isatideus*[202]
179	Isocomene	*S.isatideus*[202]
180	Eicosyl alcohol	*S. otites*[203]
181	Arachidic acid	*S. otites*[203]
182	Daucosterine	*S. otites*[203]
183	Retronecine	*S. otites*[203]
184	Senecicannabine	*S. cannabifolius*[204]
185	Jacaranone ethyl ester	*S. anonymus*[205]
186	Ethyl oleate	*S. anonymus*[205]
187	14-Oxo-1,2-dehydrocacalol methyl ether	*S. anonymus*[205]
188	4′-Angeloyloxysenspeciosone	*S. glanduloso-pilosus*[206]
189	l*β*-Peroxygermacra-4(15),5,10(14)-triene	*S. glanduloso-pilosus*[206]

190	Senkirkine	*S. desfontainei*[207]
191	7-Angelylretronecine	*S. desfontainei*[207]
192	Retrosine	*S. desfontainei*[207]
193	Eremophila-7(11),9-dien-8,1 2-olide	*S. hieracioides*[208]
194	8*β*-Hydroxyeremophil-7(11),9-dien-9,12-olide	*S. hieracioides*[208]
195	7-(Senecioloxy)retronecine	*S. cacaliaster*[209]
196	13,14-Diacetoxy-cacalolpropionate	*S. lydenburgensis*[210]
197	14-Angeloyloxy-2*α*-hydroxycacalolpropionate	*S. lydenburgensis*[210]
198	14-Senecioyloxy-2*α*-hydroxycacalolpropionate	*S. lydenburgensis*[210]
199	14-Propionyloxy-2*α*-hydroxycacalolpropionate	*S. lydenburgensis*[210]
200	14-Isovaleryloxy-2*α*-hydroxycacalolpropionate	*S. lydenburgensis*[210]
201	14-[2-Methylbutyryloxy]-2*α*-hydroxycacalol propionate	*S. lydenburgensis*[210]
202	3*β*-Angeloyloxy-14-acetoxycacalohastin	*S. inaequidens*[210]
203	13-Hydroxydehydrocacalohastin	*S. heliopsis*[210]
204	14-Methoxydehydrocacalohastin	*S. coronatus*[210]
205	14-Hexadecyloxy-dehydrocacalohastin	*S. coronatus*[210]
206	14-Octadecyloxy-dehydrocacalohastin	*S. coronatus*[210]
207	6*β*-Isobutyryloxy-10*α*-*H*-furoeremophil-1-one	*S. heliopsis*[210]
208	6*β*-Methacryloyloxy-8,8-dihydroxy-1*β*,10*β*-epoxyeremophil-7(11)-en-8,12-olide	*S. isatideus*[210]
209	6*β*-Senecioyloxy-8,8-dihydroxy-1*β*,10*β*-epoxyeremophil-7(11)-en-8,12-olide	*S. isatideus*[210]
210	6*β*-Angeloyloxy-8,8-dihydroxy-1*β*,10*β*-epoxyeremophil-7(11)-en-8,12-olide	*S. isatideus*[210]
211	6*β*-Methacryloyloxy-1*β*,8,8-trihydroxy-eremophila-9,7(11)-dien-8,12-olide	*S. isatideus*[210]
212	6*β*-Senecioyloxy-1*β*,8,8-trihydroxy-eremophila-9,7(11)-dien-8,12-olide	*S. isatideus*[210]
213	3*α*-[5-Methyldodeca-2*E*,4*E*,6*E*-trienolyoxy]-9*α*,13-dihydroxy-10*α*-*H*-eremophil-8-one-semi acetal	*S. speciosus*[210]
214	3*α*-[5-Methyldodeca-2*E*,4*E*,6*E*-trienolyoxy]-9*α*,13-dihydro-10*α*-*H*-eremophil-8-one-8-*O*-methylacetal	*S. speciosus*[210]
215	3*α*-[5-Methyldodeca-2*E*,4*E*,6*E*-trienolyoxy]-8,9*α*-dihydroxy-8-methoxy-10*α*-*H*-eremophil-8,12-olide	*S. erubescens*[210]
216	3*α*-Senecioyloxy-9*α*-[2-Methylbutyryloxy]-7*β*,10*α*-*H*-eremophil-11-en-8-one	*S. speciosus*[210]
217	3*α*-[4-Pent-2*E*-enolyoxy-pent-2*E*-enolyoxy]-9*α*-hydroxy-7*β*,10*α*-*H*-eremophil-11-en-8-one	*S. speciosus*[210]
218	3*α*-[5-Methyldodeca-2*E*,4*E*,6*E*-trienolyoxy]-9*α*-hydroxy-10*α*-*H*-eremophil-7(11),en-8one	*S. speciosus*[210]
219	3*α*-[4-Pent-2*E*-enolyoxy-pent-2*E*-enolyoxy]-9*α*-hydroxy-10*αH*-eremophil-7(11)-en-8-one	*S. erubescens*[210]
220	3*α*-[5-Methyldodeca-2*E*,4*E*,6*E*-trienolyoxy]-9*α*,13-dihydroxy-10*α*-*H*-eremophil-7(11),en-8one	*S. erubescens*[210]
221	3*α*-[4-Pent-2*E*-enolyoxy-pent-2*E*-enolyoxy]-8*α*,9α-dihydroxy-7*β*,10*α*-*H*-eremophil-11-ene	*S. erubescens*[210]
222	3*α*-[4-Angeloyloxy-pent-2*E*-enolyoxy]-8*α*,9α-dihydroxy-7*β*,10*α*-*H*-eremophil-11-ene	*S. erubescens*[210]

223	3*α*,9*α*-dipent-2*E*,enolyoxy-8*α*-hydroxy-7*β*,10*α*-*H*-eremophil-11-ene	*S. erubescens*[210]
224	3*α*-Hydroxy-7*β*,10*α*-*H*-eremophil-9-one	*S. isatideus*[210]
225	Bisabol-2,7,(14),10-trien-4-one	*S. macroglossus*[210]
226	15-Hydroxy-nor-bisabol-2,11-dien-1-one	*S. affinis*[210]
227	5-Acetoxy-8-pent-2*E*-enolyoxy-2,3-epoxybisabol-7(14),10-dien-4-one	*S. erubescens*[210]
228	8-Angeloyloxy-2-hydroxy-5-desoxy-7,14-dehydro-perezone	*S. longifolius*[210]
229	Menth-2-en-1,7-diol	*S. affinis*[210]
230	3-*O*-[3-Acetoxypalmitoyl]-shikimic acid-4,5-*O*-acetate	*S. erubescens*[210]
231	3,5-bis-[3,3-Dimethyl allyl]-*p*-hydroxybenz-aldehyde	*S. erubescens*[210]
232	3,4,5-Trimethoxy-8,9-dihydroxycumolditiglate	*S. macroglossus*[210]
233	Phytol	*S. desfontainei*[211]
234	Isopetasol	*S. desfontainei*[211]
235	Dehydrofukinone	*S. glaucus*[212]
236	Platyphyllide	*S. gilliesiano*[213]
237	1-Hydroxyplatyphyllide	*S. gilliesiano*[213]
238	Coumarine	*S. gilliesiano*[213]
239	Isocoumarine	*S. gilliesiano*[213]
240	*α*-Curcumene	*S. multivenius*[214]
241	6*β*-Isovaleryloxyfuroeremophhil-9-one	*S. discolor*[214]
242	6*β*-Senecioyloxyfuroeremophhil-9-one	*S. discolor*[214]
243	6*β*-Acetoxyfuroeremophhil-9-one	*S. discolor*[214]
244	6*β*-Isovaleryloxy-4*α*-hydroxyfuroeremophhil-9-one	*S. discolor*[214]
245	6*β*-Isobutyryloxy-4*α*-hydroxyfuroeremophhil-9-one	*S. discolor*[214]
246	6*β*-Angeloyloxy-4*α*-hydroxyfuroeremophhil-9-one	*S. discolor*[214]
247	6*β*-Senecioyloxy-4*α*-hydroxyfuroeremophhil-9-one	*S. discolor*[214]
248	*α*-Humulene	*S. richii*[214]
249	Senaetnin	*S. richii*[214]
250	Isosenaetnin	*S. richii*[214]
251	Cacalol methyl ether	*S. coahuilensis*[214]
252	Dehydrocacalohastin	*S. coahuilensis*[214]
253	Florisenin	*S. quebradensis*[214]
254	7-Angeloyl heliotridin	*S. salignus*[214]
255	1*β*-Angeloyloxy-4*β*-hydroxyeudesm-7-ene	*S. mandralicae*[214]
256	14*E*-Senaetnin	*S. mandralicae*[214]
257	Neoserkirkine	*S. grandifolius*[214]
258	Spathulenol	*S. grandifolius*[214]
259	*γ*-Cadinene	*S. grandifolius*[214]
260	*δ*-Cadinene	*S. grandifolius*[214]
261	Caryophyllene	*S. dolichodoryius*[214]
262	6*β*-Angeloyloxy-1(10)-dehydrofuroeremophil-9-one	*S. dolichodoryius*[214]
263	6*β*-Propionyloxy-1(10)-dehydrofuroeremophil-9-one	*S. dolichodoryius*[214]
264	18-Hydroxy senkirkine	*S. laricifolius*[214]

265	18-Acetoxy senkirkine	*S. laricifolius*[214]
266	5*α*-Hydroxyeudesm-4(15)-ene	*S. variabilis*[214]
267	6*α*-Tigloyloxyeudesm-5(15)-ene	*S. variabilis*[214]
268	Triphenyl acetate	*S. variabilis*[214]
269	Uspallatine	*S. argentino*[215]
270	Senecivernine	*S. seratophiloides*[215]
271	Usaramine	*S. seratophiloides*[215]
272	1,2-Dihydroretrorsine	*S. subulatus*[215]
273	Euparin	*S. desfontainei*[216]
274	9,10-Dehydrofukinone	*S. desfontainei*[216]
275	3-*O*,5-*O*-Di-[2-methylbutyryl]-4-*O*-decanoyl-shikimic acid	*S. lividus*[217]
276	3-*O*,5-*O*-Di-[2-methylbutyryl]-4-*O*-octanoyl-shikimic acid	*S. lividus*[217]
277	3-*O*,5-*O*-Diisobutyryl-4-*O*-decanoylshikimic acid methyl ester	*S. lividus*[217]
278	3-*O*-[2-methylbutyryl-5-*O*-isobutyryl]-4-*O*-decanoylshikimic acid methyl ester	*S. lividus*[217]
279	9*β*-Hydroxy-3*β*-[4′-angeloyloxyangeloyloxy]-10*α*-*H*-eremophil-7(11)-en-8-one	*S. lividus*[217]
280	9*β*-Hydroxy-3*β*-[5′-angeloyloxy-senecioyloxy]-10*α*-*H*-eremophil-7(11)-en-8-one	*S. lividus*[217]
281	9-Hydroxy-3*β*-[4′-angeloyloxy-angeloyloxy]-eremophil-7(11)-9-dien-8-one	*S. lividus*[217]
282	9-Hydroxy-3*β*-[4′-angeloyloxy-senecioyloxy]-eremophil-7(11)-9-dien-8-one	*S. lividus*[217]
283	2*α*-[Angeloyloxy]-8-acetoxy-4*β*,5*β*-epoxybisabola-7(11),10-dien-3-one	*S. lividus*[217]
284	2*α*-[Senecioyloxy]-8-acetoxy-4*β*,5*β*-epoxybisabola-7(11),10-dien-3-one	*S. lividus*[217]
285	2*β*-Acetoxy-6*β*-angeloyloxy-1*β*,10*β*-epoxyfuro-eremophilane	*S. pyrenaicus*[217]
286	1,10*β*-Epoxy-6*β*-angeloyloxy-9*β*-hydroxyfurano-eremophilane	*S. arnicoides*[218]
287	1,10*β*-Epoxy-6*β*-(2-methylacryloyl)-9*β*-hydroxy-furanoeremophilane	*S. arnicoides*[218]
288	1,10*β*-epoxy-8*β*-angeloyloxyfuranoeremophil-9-one	*S. arnicoides*[218]
289	1,10*β*-epoxy-8*β*-angeloyloxyfuranoeremophil-9-one	*S. arnicoides*[218]
290	1,10*β*-epoxy-6*β*-(2-methylacryloylfuranoeremo-phil-9-one	*S. arnicoides*[218]
291	Stigmasterol	*S. arnicoides*[218]
292	Neosenkirkine	*S. anonymus*[219]
293	Otosenine	*S. anonymus*[219]
294	Hydroxysenkirkine	*S. anonymus*[219]
295	Anonamine	*S. anonymus*[219]
296	Hydroxyneosenkirkine	*S. anonymus*[219]
297	3a*β*-Hydroxy-4-ethoxy-2,6-perhydroindoledione	*S. anonymus*[219]

298	Desacetyldoronine	*S. inaequidens*[220]
299	6*β*-Hydroxy-3*β*-[4-methylsenecioyloxy]-euryopsin	*S. pericalia*[221]
300	6*β*-Hydroxy-3*β*-[4-methylsenecioyloxy]-eremo-phila-1(10),7(11)-dien-12,8*β*-olide	*S. pericalia*[221]
301	6*β*,8*α*-Dihydroxy-3*β*-[4-methylsenecioyloxy]-eremophila-1(10),7(11)-dien-12,8*β*-olide	*S. pericalia*[221]
302	6*β*-Hydroxy-3*β*-[4-methylsenecioyloxy]-8*α*-methoxyeremophila-1(10),7(11)-dien-12,8*β*-olide	*S. pericalia*[221]
303	6*β*-Hydroxy-8*α*-methoxyeremophila-1(10),7(11)-dien-12,8*β*-olide	*S. pericalia*[221]
304	6*β*-Hydroxy-3*β*-[angeloyloxy]-ligularenolide	*S. pericalia*[221]
305	6*β*-Hydroxy-3*β*-[tigloyloxy]-ligularenolide	*S. pericalia*[221]
306	6*β*-Hydroxy-3*β*-[senecioyloxy]-ligularenolide	*S. pericalia*[221]
307	6*β*-[2-Methyl-2-vinylacetoxy]-furoeremophil-9-one	*S. pericalia*[221]
308	6*β*-[2-Methylbutyryloxy]-furoeremophil-9-one	*S. pericalia*[221]
309	6*β*-[2-Methyl-2-vinylacetoxy]-euryopsin-9-one	*S. pericalia*[221]
310	14-Acetoxy-2-hydroxycalamene	*S. tomentosus*[221]
311	4*β*-Methoxycadina-1,9-dien-3-one	*S. tomentosus*[221]
312	1*α*,10-Epoxyfuranoeremophilane	*S. leptolobus*[222]
313	Senglutinosin	*S. glutinosus*[223]
314	3*α*-Hydroxy-10*β*-*H*-eremophil-11(13)-en-9-one	*S. glutinosus*[223]
315	*nor*-*seco*-Glutinosone	*S. glutinosus*[223]
316	14-*nor*-Dehydrocacalohastine	*S. linifolius*[224]
317	8-Hydroxy-6-methoxy-3,4,5-trimethylnaphtho[2,3-b]-furan-9(4*H*)-one	*S. linifolius*[224]
318	4,8-Dihydroxy-6-methoxy-3,4,5-trimethylnaphtho-[2,3-b]-furan-9(4*H*)-one	*S. linifolius*[224]
319	8-Hydroxy-6-methoxy-3, 5-dimethylnaphtho[2,3-b]-furan-4,9-dione	*S. linifolius*[224]
320	Desacetyl doronine	*S. inaequidens*[220]
321	Valeranone	*S. linearifolius*[225]
322	*α*-Gurjunene	*S. linearifolius*[225]
323	Desacetylsenaetin	*S. magnifcus*[225]
324	Acetylsenecionin	*S. magnifcus*[225]
325	Bicyclogermacrene	*S. magnifcus*[225]
326	Sesamin	*S. magnifcus*[225]
327	Senaetnin	*S. magnifcus*[225]
328	Angelic acid	*S. coronopifolius*[226]
329	6-Hydroxytremetone	*S. coronopifolius*[226]
330	6-Hydroxyeuparin	*S. coronopifolius*[226]
331	6*β*-Propionyloxy-10*α*-*H*-furoeremophil-9-one	*S. pachyphyllos*[227]
332	6*β*-lsobutyryloxy-12*α*-methoxy-8,12-epoxyeremo-phila-1(10),7(11),8-triene	*S. pachyphyllos*[227]
333	6*β*-lsobutyryloxy-12*β*-methoxy-8,12-epoxyeremophila-1(10),7(11),8-triene	*S. pachyphyllos*[227]
334	6*β*-Propionyloxy-12*α*-methoxy-8,12-epoxyeremo-phila-1(10),7(11),8-triene	*S. pachyphyllos*[227]
335	6*β*-Propionyloxy-12*β*-methoxy-8,12-epoxyeremo-phila-1(10),7(11),8-triene	*S. pachyphyllos*[227]

336	3*β*-Acetoxy-6*β*-propionyloxyeuryopsin	*S. subumbellatus*[228]
337	3*β*-Acetoxy-6*β*-isovaleryloxyeuryopsin	*S. subumbellatus*[228]
338	3*β*-Acetoxy-6*β*-isobutyryloxyeuryopsin	*S. subumbellatus*[228]
339	3*β*-Hydroxy-6*β*- isovaleryloxyeuryopsin	*S. subumbellatus*[228]
340	8*α*-Tigloyloxy-1*β*,10*β*-epoxyeremophil-11(13)-ene	*S. olivaceobracteatus*[228]
341	8*α*-Angeloyloxy-1*β*,10*β*-epoxyeremophil-11(13)-ene	*S. olivaceobracteatus*[228]
342	1*α*-Acetoxy-8*α*-angeloyloxy-10*α*-*H*-eremophil-11(13)-en-9-one	*S. olivaceobracteatus*[228]
343	1*α*-Acetoxy-8*α*-tigloyloxy-10*α*-*H*-eremophil-11(13)-en-9-one	*S. olivaceobracteatus*[228]
344	7-*epi*-Desacetylsenaetnin	*S. olivaceobracteatus*[228]
345	1*β*-Hydroxygermacra-4(15),5*E*,9*Z*-triene	*S. philippicus*[228]
346	1*α*-Angeloxy-6*β*-isobutyroxy-9-oxo-10*α*-*H*-furano-eremophilane	*S. chilensis*[229]
347	6*β*-lsobutyryloxy-9*β*-hydroxy-1*β*,10*β*-epoxyfuro-eremophilane	*S. candollii*[230]
348	1*α*-Acetoxy-6*β*-angeloyloxy-10*β*-hydroxyfuro-eremophil-9-one	*S. dryophyllus*[230]
349	6*β*,8*α*-Dihydroxyeremophil-1(10),7(11)-dien-12,8*β*-olide	*S. glaber*[230]
350	6*β*-Hydroxyeremophil-1(10),7(11)-dien-12,8*β*-olide	*S. glaber*[230]
351	6*β*-Hydroxyeremophil-1(10),7(11)-dien-12,8*α*-olide	*S. glaber*[230]
352	6*β*-Hydroxy-11*β*-*H*-eremophil-1(10),7-dien-12,8-olide	*S. glaber*[230]
253	1*α*-Angeloxy-8*β*-methoxyeremophil-7(11)-en-12,8*α*-olide	*S. cachinalensis*[230]
354	1*α*-Tigloyloxy-8*β*-hydroxyeremophil-7(11)-en-12,8*α*-olide	*S. almeydae*[230]
355	3*α*,8*α*-Dihydroxy-7*β*,10*α*-*H*-eremophil-11(13)-ene	*S. almeydae*[230]
356	3*α*,9*α*-Dihydroxy-7*β*,10*α*-*H*-eremophil-11(13)-en-8-one	*S. almeydae*[230]
357	3*α*,9*α*-Dihydroxy-10*α*-*H*-eremophil-11(13)- en-8-one	*S. almeydae*[230]
358	3*α*,8*α*-Dihydroxy-7*α*,10*β*-*H*-eremophil-11(13)- en-9-one	*S. almeydae*[230]
359	Serratifolide A	*S. serratifolius*[230]
360	Serratifolide B	*S. serratifolius*[230]
361	13-Acetoxyeremophil-7(11),9-dien-8-one	*S. serratifolius*[230]
362	12-Acetoxyeremophil-7(11),9-dien-8-one	*S. serratifolius*[230]
363	11-Hydroxyeremophil-6,9-dien-8-one	*S. reicheanus*[230]
364	11-Hydroperoxyeremophil-6,9-dien-8-one	*S. reicheanus*[230]
365	3-*O*-Acetyl-4,5-*O*-[2-methylbutyryl]-propionyl-shikimic acid	*S. reicheanus*[230]
366	3-*O*-Acetyl-4,5-*O*-[3-methylvaleryl]-propionyl-shikimic acid	*S. reicheanus*[230]
367	3-*O*-Acetyl-4,5-*O*-di-[2-methylbutyryl]-propionyl-shikimic acid	*S. reicheanus*[230]

368	3-*O*-Acetyl-4,5-*O*-[3-methylvaleryl]-[2-methyl-butyryl]-shikimic acid	*S. reicheanus*[230]
369	3-*O*-Acetyl-4,5-*O*-di-[3-methylvaleryl]-shikimic acid	*S. reicheanus*[230]
370	*α*-Cadinol methyl ether	*S. adenophyllus*[230]
371	3*β*,4*β*-Dihydroxygermacra-1(10)*E*,5*E*-diene	*S. adenophyllus*[230]
372	1*β*,3*β*,4*β*-Triihydroxygermacra-5*E*,10(14)-diene	*S. adenophyllus*[230]
373	1*β*-Hydroperoxy-3*β*,4*β*-dihydroxygermacra-5*E*,10(14)-diene	*S. adenophyllus*[230]
374	1*β*,4*β*-Dihydroxy-3*β*-acetoxygermacra-5*E*,10(14)-diene	*S. adenophyllus*[230]
375	1*β*,4*β*-Dihydroxygermacra-5*E*,10(14)-diene	*S. adenophyllus*[230]
376	3*α*-Acetoxyoplopanone	*S. adenophyllus*[230]
377	11-Hydroxyeremophil-6(7),9(10)-dien-8-one	*S. desfontainei*[231]
378	Rosmarinine	*S. hadiensis*[232]
379	Hadiensine	*S. hadiensis*[232]
380	1-*O*-Acetylhadiensine	*S. hadiensis*[232]
381	12-*O*-Acetylhadiensine	*S. hadiensis*[232]
382	1,12-Di-*O*-acetylhadiensine	*S. hadiensis*[232]
383	Neorosmarinine	*S. hadiensis*[232]
384	12-*O*-Acetylneohadiensine	*S. hadiensis*[232]
385	2-*O*-Acetylrosmarinine	*S. hadiensis*[232]
386	12-*O*-Acetylrosmarinine	*S. hadiensis*[232]
387	2,12-Di-*O*- acetylrosmarinine	*S. hadiensis*[232]
388	Petitianine	*S. hadiensis*[232]
389	1*α*-Acetoxy-4*α*-hydroxy-5*α*,6*β*,7*α*,11*β*-*H*-eudesm-2-en-12,6-olide	*S. chrysanthemoides*[233]
390	1*α*-Acetoxy-3*α*-hydroxy-5*α*,6*β*,7*α*,11*β*-*H*-eudesm-4(15)-en-12,6-olide	*S. chrysanthemoides*[233]
391	1*α*,10*β*-Dihydroxy-6*β*-angeloyloxy-9-oxo-furano-eremophylane	*S. nemorensis*[234]
392	Ruwenine	*S. ruwenzoriensis*[235]
393	Ruzorine	*S. ruwenzoriensis*[235]
394	6-Hydroperoxy-6-desoxycacalonol	*S. canescens*[236]
395	14-Angeloyloxy-12-(cacalohastin-14-yl)cacalo-hastine	*S. canescens*[236]
396	13-Hydroxy-3,4-dehydrocacalohastine	*S. canescens*[236]
397	Senecioracenine	*S. racemosus*[237]
398	Sarracine	*S. racemosus*[237]
399	9-Angelylplatynecine	*S. racemosus*[237]
400	12,13-Dihydroxyeuparin	*S. desfontainei*[238]
401	Sitosterol-3-*O*-*β*-D-glucoronopyranoside	*S. bonariensis*[239]
402	6*β*-Isobutyryloxy-9-oxo-10*α*-*H*-furanoeremo-philane	*S. bonariensis*[239]
403	1*α*,6*β*-Diacetoxy-9-oxo-10*α*-*H*-furanoeremophilane	*S. bonariensis*[239]
404	6*β*-Angeloyloxy-9-oxo-10*α*-*H*-furanoeremophilane	*S. bonariensis*[239]
405	1*α*-Acetoxy-6*β*-angeloyloxy-9-oxo-10*α*-*H*-furano-eremophilane	*S. bonariensis*[239]
406	Campesterol	*S. bonariensis*[239]

407	Sitosterol	*S. bonariensis*[239]
408	5,6-Dihydrositosterol	*S. bonariensis*[239]
409	Sitosterol-3-*O*-*β*-D-glucopyranoside	*S. bonariensis*[239]
410	Stigmasterol-3-*O*-*β*-D-glucopyranoside	*S. bonariensis*[239]
411	9-(Angeloyloxy) platynecine	*S. chrysocoma*[240]
412	7*α*-(Angeloyloxy)-1-methylidene-8*α*-pyrrolizidine	*S. chrysocoma*[241]
413	7*α*-(Angeloyloxy)-1-methylidene-8*α*-pyrrolizidine-*N*-oxide	*S. chrysocoma*[241]
414	Piceol	*S. hollermayerii*[242]
415	Scopoletin	*S. hollermayerii*[242]
416	Scopolin	*S. hollermayerii*[242]
417	Picein	*S. hollermayerii*[242]
418	Chrysoeriol-7-*β*-*O*-D-glucoside	*S. hollermayerii*[242]
419	Cosmosin	*S. hollermayerii*[242]
420	*α*-Amyrin	*S. racemosus*[243]
421	*β*-Amyrin	*S. racemosus*[243]
422	*α*-Taraxerol	*S. racemosus*[243]
423	*β*-sitosterol	*S. racemosus*[243]
424	Dihydrositosterol	*S. crassiflorus*[244]
425	*Δ*7-Sitosterol	*S. crassiflorus*[244]
426	Dihydrocampesterol	*S. crassiflorus*[244]
427	Dihydrostigmasterol	*S. crassiflorus*[244]
428	Cholesterol	*S. crassiflorus*[244]
429	*Δ*7-Cholesterol	*S. crassiflorus*[244]
430	Stigmast-4-en-3-one	*S. crassiflorus*[244]
431	Stigmast-4,6-dien-3-one	*S. crassiflorus*[244]
432	Stigmast-3,5-dien-7-one	*S. crassiflorus*[244]
433	Stigmast-3,5-diene	*S. crassiflorus*[244]
434	Stigmast-1,3,5-triene	*S. crassiflorus*[244]
435	Sitosterone	*S. crassiflorus*[244]
436	Senecionine-*N*-oxide	*S. crassiflorus*[244]
437	Seneciophylline-*N*-oxide	*S. crassiflorus*[244]
438	Chrysin	*S. crassiflorus*[244]
439	Tricin	*S. crassiflorus*[244]
440	Luteolin	*S. crassiflorus*[244]
441	Quercetin-3-*O*-*α*-L-arabinopoyranoside	*S. crassiflorus*[244]
442	*α*-Amyrin acetate	*S. crassiflorus*[244]
443	6-Hydroxy-*p*-menth-4(5)-en-3-one	*S. chrysanthemoides*[245]
444	7*β*-Hydroxy-1-methylidene-8*α*-pyrrolizidine	*S. schweinfurthii*[246]
445	7*β*-Hydroxy-1-methylidene-8*β*-pyrrolizidine	*S. schweinfurthii*[246]
446	7*β*-Hydroxy-1-methylidene-8*α*-pyrrolizidine-*N*-oxide	*S. schweinfurthii*[246]
447	6β-Methacroyloxy-secomacrotolide acetate	*S. macedonicus*[247]
448	8-epi-6β-Methacroyloxy-secomacrotolide acetate	*S. macedonicus*[247]
449	(13*R*)-13-Hydroxyretroisosenine	*S. roseus*[248]
450	(12*S*)-12-Hydroxyretroisosenine	*S. roseus*[248]
445	6*β*-Isobutiroyloxy-10*α*-*H*-9-oxofuranoeremo-philane	*S. bonariensis*[249]

446	1*α*,6*β*-Diacetoxy-10*β*-*H*-9-oxofuranoeremophilane	*S. bonariensis*[249]
447	1*α*-Acetoxy-6*β*-angeloyloxy-10*α*-*H*-9-oxofurano-eremophilane	*S. bonariensis*[249]
448	6*β*-Angeloyloxy-10*α*-*H*-9-oxofurano-eremophilane	*S. bonariensis*[249]
449	Flammein-A	*S. flammeus*[250]
450	Callosine	*S. callosus*[251]
451	11-*O*-Acetyl bulgarsenine hydrochloride	*S. callosus*[251]
452	11-*O*-Acetyl bulgarsenine-*N*-oxide	*S. callosus*[251]
453	Bulgarsenine hydrocholride	*S. callosus*[251]
454	*N*-Chloromethyl bulgarsenine chloride	*S. callosus*[251]
455	6*β*-(2-Methylbutyryloxy)-10*α*-*H*-furanoeremo-philan-1-one	*S. auricula*[252]
456	6*β*-Isobutyryloxysenberginone	*S. auricula*[252]
457	6*β*-(2-Methylbutytyloxy)-senberginone	*S. auricula*[252]
458	Senauricolide	*S. auricula*[252]
459	2*α*,3*β*,23-Trihydroxy-*urs*-12,19(29)-dien-28-oic acid	*S. pseudotites*[253]
460	2*α*,3*β*,23-Trihydroxy-*urs*-12,19(29)-dien-28-oic acid-23-acetyl ester	*S. pseudotites*[253]
461	23(*trans*-*p*-Coumaroyloxy)-2*α*,3*β*-dihydroxy-*urs*-12,19(29)-dien-28-oic acid	*S. pseudotites*[253]
462	*α*-Pinene	*S. cineraria*[254]
463	Camphene	*S. cineraria*[254]
464	Borneol	*S. cineraria*[254]
465	Dihydroquercetin	*S. cineraria*[254]
466	Apigenin 7-*O*-glucoside	*S. cineraria*[254]
467	Luteolin 7-*O*-glucoside	*S. cineraria*[254]
468	Quercetin 3-*O*-rhamnoside	*S. cineraria*[254]
469	Xanthotoxin	*S. cineraria*[254]
470	Isopimpinellin	*S. cineraria*[254]
471	Marmesin	*S. cineraria*[254]
472	Aesculetin	*S. cineraria*[254]
473	3*β*-Methylbutyryloxyeuryopsin	*S. flavus*[255]
474	3*β*-Abgeloyloxyeuryopsin	*S. flavus*[255]
475	3*β*-Senecioyloxyeuryopsin	*S. flavus*[255]
476	3*β*-Hydroxyeuryopsin	*S. flavus*[255]
477	Euryopsin-3-one	*S. flavus*[255]
478	Furanoligularenone	*S. flavus*[255]
479	3-Oxo-8*α*,12*α*-dimethoxy-8,12-dihydro-10*α*-*H*-furanoeremophil-1-ene	*S. flavus*[255]
480	3-Oxo-8*α*-methoxy-10*α*-*H*-eremophila-1,7(11)-dien-12,8*β*-olide	*S. flavus*[255]
481	3-Oxo-8*α*-hydroxy-10*α*-*H*-eremophila-1,7(11)-dien-12,8*β*-olide	*S. flavus*[255]
482	3-Oxoeremophila-1,7(11)-dien-12,8*β*-olide	*S. flavus*[255]
483	3-Oxo-8*α*-methoxy-10*α*-*H*-eremophila-1,7(11)-dien-12,8β-lactam	*S. flavus*[255]
484	1*β*,10*β*-Epoxy-8*α*-methoxyeremophil-7(11)-en-12,8*β*-olide	*S. flavus*[255]

485	Iodanthine	*S. iodanthus*[256]
486	Retroisosenine	*S. iodanthus*[256]
487	Bulgarsenine	*S. iodanthus*[256]
488	Mulgediifoliine	*S. iodanthus*[256]
489	Neosarracine	*S. doratophyllus*[257]
490	7*β*-Angelyl-1-methylene-8*α*-pyrrolizidine	*S. doratophyllus*[257]
491	7-Tiglylplatynecine	*S. doratophyllus*[257]
492	Sarracine-*N*-oxide	*S. deformis*[257]
493	Jacaranone	*S. minutus*[258]
494	Methyl-1′-hydroxy-2′,6′-dimethoxy-4′-oxo-cyclo-hexanacetate	*S. minutus*[258]
495	Friedelin	*S. minutus*[258]
496	3-Epifriedelinol	*S. minutus*[258]
497	Faradiol	*S. minutus*[258]
498	Maniladiol	*S. minutus*[258]
499	Arnidiol	*S. minutus*[258]
500	Isohumulene	*S. boissieri*[258]
501	Lupanone	*S. boissieri*[258]
502	6*β*-Isobutyryloxy-1(10)-furanoeremophilene	*S. boissieri*[258]
503	6*β*-Isobutyryloxy-1-oxo-10*α*-*H*-furanoeremo-philene	*S. boissieri*[258]
504	14-Isovaleryloxy-*O*-methyl-1,2-dehydrocacalol	*S. madagascariensis*[259]
505	Cycloart-23-ene-3,25-diol	*S. madagascariensis*[259]
506	1-Hydroxy-2-methoxy-1,2,3,4-dehydro-6-dehydroxycacalone	*S. madagascariensis*[259]
507	1-Hydroxy-2-methoxy-1,2,3,4-dehydrocacalone	*S. madagascariensis*[259]
508	1,2-Dimethoxy-1,2,3,4-dehydro-6-dehydroxy-cacalone	*S. madagascariensis*[259]
509	1,2-Dimethoxy-1,2,3,4-dehydrocacalone	*S. madagascariensis*[259]
510	2-Methoxy-*O*-methyl-1-oxo-2,3-dehydrocacalol	*S. madagascariensis*[259]
511	1*α*-Acetoxy-8*β*-methoxy-10*β*-*H*-eremophil-7(11)-en-8*α*,12-olide	*S. miser*[260]
512	1*α*-Angeloyloxy-6*β*-hydroxy-8*β*-methoxy -10*β*-*H*-eremophil-7(11)-en-8*α*,12-olide	*S. miser*[260]
513	1*α*-Angeloyloxy-8*βH*,10*β*-*H*-eremophil-7(11)-en-8*α*,12-olide	*S. miser*[260]
514	5*α*-Senecioyloxysilphinen-3-one	*S. palmensis*[261]
515	5*α*-Tigloyloxysilphinen-3-one	*S. palmensis*[261]
516	3*β*-Hydroxy-5*α*-angeloyloxysilphinene	*S. palmensis*[261]
517	(6*S*)-2,10-Bisaboladien-1-one	*S. palmensis*[261]
518	6,7-Epoxy-3(15)-caryophyllene	*S. palmensis*[261]
519	6,7-Epoxy-2,9-humuladiene	*S. palmensis*[261]
520	5*α*-Angeloyloxysilphinen-3-one	*S. palmensis*[261]
521	5*α*-Acetoxysilphinen-3-one	*S. palmensis*[261]
522	Erucifoline	*S. aquaticus*[262]
523	9-Angeloylhastanecine	*S. aquaticus*[262]
524	7-Angelylheliotridine	*S. elodes*
525	Platyphylline	*S. granatensis*[263]
526	Platyphylline-*N*-oxide	*S. granatensis*[263]

527	Asirensane-a	*S. asirensis*[264]
528	Asirensane-b	*S. asirensis*[264]
529	Asirensane-c	*S. asirensis*[264]
530	14-*nor*-dehydrocalohastine	*S. asirensis*[264]
531	Maturinone	*S. asirensis*[264]
532	Tsoongianolides A	*S. tsoongianus*[265]
533	Tsoongianolides B	*S. tsoongianus*[265]
534	Tsoongianolides C	*S. tsoongianus*[265]
535	Tsoongianolides D	*S. tsoongianus*[265]
536	Tsoongianolides E	*S. tsoongianus*[265]
537	Tsoongianolides F	*S. tsoongianus*[265]
538	(1*S*,4*S*,5*R*,10*R*)-1-Hydroxy-6-isobutyryloxy-10*H*-9-oxofuranoeremophilane	*S. chionophilus*[266]
539	1*α*-Hydroxy-6*β*-(2*ξ*-methylbutyryloxy)-10*α*-*H*-9-oxofuranoeremophilane	*S. chionophilus*[266]
540	6*β*-Angeloyloxy-1*α*-hydroxy-10*α*-*H*-9-oxofuranoeremophilane	*S. chionophilus*[266]
541	4'-Hydroxyacetophenone	*S. chionophilus*[266]
542	*p*-Hydroxybenzeneacetic acid	*S. cannabifolius*[267]
543	1,4-Benzenediol	*S. cannabifolius*[267]
544	Methyl-*p*-Hydroxy benzeneacetate	*S. cannabifolius*[267]
545	3,4-Dihydroxy benzoic acid	*S. cannabifolius*[267]
546	2,5-Dihydroxy benzeneacetic acid	*S. cannabifolius*[267]
547	2,5-Dihydroxy benzeneacetic acid methyl ester	*S. cannabifolius*[267]
548	3-Hydroxy-4-methoxy benzoic acid	*S. cannabifolius*[267]
549	*p*-Hydroxy benzoic acid	*S. cannabifolius*[267]
550	*o*-Hydroxy benzoic acid	*S. cannabifolius*[267]
551	1*β*-Hydroxy-8*α*-*H*-eremophil-7(11),9-dien-8*β*,12-olide	*S. aegyptius*[268]
552	1*β*,8*α*-Dihydroxyeremophil-7(11),9-dien-8*β*,12-olide	*S. aegyptius*[268]
553	1*β*-Hydroxy-8*α*-methoxyeremophil-7(11),9-dien-8*β*,12-olide	*S. aegyptius*[268]
554	1-Oxo-8*α*-methoxy-10α*H*-eremophil-7(11)-en-8*β*,12-lactam	*S. aegyptius*[268]
555	1*β*,10*β*-Epoxy-8*α*-hydroxyeremophil-7(11)-en-8*β*,12-olide	*S. aegyptius*[268]
556	1*β*,10*β*-Epoxy-8*α*-methoxyeremophil-7(11)-en-8*β*,12-olide	*S. aegyptius*[268]
557	Benzyl 6-*O*-*α*-L-arabinopyranosyl-*β*-D-gluco-pyranoside	*S. cannabifolius*[269]
558	(6*S*,9*S*)-Roseoside	*S. cannabifolius*[269]
559	(6*S*,9*R*)-Roseoside	*S. cannabifolius*[269]
560	Methyl-5-hydroxy-2-pyridinecarboxylate	*S. cannabifolius*[270]
561	6-Hydroxy-7,7a-dihydro-2 (6*H*)-benzofuranone	*S. cannabifolius*[270]
562	2-(1,4-Dihydroxycyclohexanyl)-acetic acid	*S. cannabifolius*[270]
563	3-Hydroxycyclohexanecarboxylic acid	*S. cannabifolius*[270]
564	4-Hydroxy benzaldehyde	*S. cannabifolius*[270]
565	Senecivernine-*N*-oxide	*S. procumbens*[271]

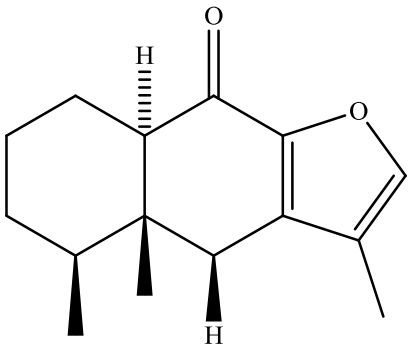
566	Retrorsine-*N*-oxide	*S. procumbens*[271]
567	132-Hydroxyphaeophytin A	*S. procumbens*[271]
568	Caffeic acid methyl ester	*S. procumbens*[271]
569	Cacticin	*S. procumbens*[271]
570	Cannabiside B	*S. cannabifolius*[272]
571	Cannabiside C	*S. cannabifolius*[272]
572	Cannabiloid B	*S. cannabifolius*[272]
573	Cannabilactone A	*S. cannabifolius*[272]
574	Oleanane	*S. scandens*[273]
575	Daucosterol	*S. scandens*[273]
576	Adonifoline	*S. scandens*[273]
577	*p*-Hydroxybenzene acetic acid	*S. scandens*[273]
578	Hyperoside	*S. scandens*[273]
579	Linarin	*S. scandens*[273]
580	Mairetolide A	*S. mairetianus*[274]
581	Mairetolide B	*S. mairetianus*[274]
582	Mairetolide C	*S. mairetianus*[274]
583	Mairetolide D	*S. mairetianus*[274]
584	Mairetolide E	*S. mairetianus*[274]
585	Mairetolide F	*S. mairetianus*[274]
586	Mairetolide G	*S. mairetianus*[274]
587	Mairetolide H	*S. mairetianus*[274]
588	Mairetin	*S. mairetianus*[274]
589	4*α*-[2′-Hydroxymethylacryloxy]-1*β*-hydroxy-14-(5→6) abeo eremophilan-12,8-olide	*S. burtonii*[275]
590	(3′*E*)-(1*α*)-3-Hydroxymethyl-4*β*,5*α*-dimethoxy-cyclohex-2-enyloctadec-3′-enoate	*S. burtonii*[275]
591	Octacosan-1-ol	*S. burtonii*[275]
592	3*β*-Hydroxyolean-12-en-28-oic acid	*S. burtonii*[275]
593	3*β*-Acetoxyolean-12-en-28-oic acid	*S. burtonii*[275]
594	Florosenine	*S. ambiguus*[276]
595	Ergosterol peroxide	*S. laetus*[277]
596	Cycloart-23-ene-3*β*,25-diol	*S. laetus*[277]
597	Quercetin	*S. scandens*[278]
598	4-(Pyrryl-2-carbonyl0-5-methoxy-phenyl acetic acid	*S. scandens*[278]
599	*dl*-Syringaresinol	*S. scandens*[278]
600	Frangulic emodin	*S. scandens*[278]
601	3*β*-Angeloyloxy-6*β*-hydroxyeremophil-1(10)-en-8*β*,12-olide	*S. sinuatus*[279]
602	3*β*-Angeloyloxy-6*β*-hydroxyeremophil-1(10)-ene	*S. sinuatus*[279]
603	3*β*-Senecioyloxy-6*β*-hydroxyeremophil-1(10)-ene	*S. sinuatus*[279]
604	3*β*-Angeloyloxy-6*β*,8*α*-dihydroxyeremophil-1(10)-en-8*β*,12-olide	*S. sinuatus*[279]
605	(*E*)-Seneciolactone	*S. scandens*[280]
606	(E)-Cannabifolactone A	*S. scandens*[280]
607	4-Methoxyphenylacetic acid	*S. scandens*[280]
608	Ethyl 2-(1-hydroxy-4-oxocyclohexa-2,5-dienyl)	*S. scandens*[280]

	acetate	
609	Methyl 2-(1-hydroxy-4-oxocyclohexyl) acetate	*S. scandens*[280]
610	Ethyl 2-(1-hydroxy-4-oxocyclohexyl) acetate	*S. scandens*[280]
611	5-Methoxybenzofuran-2(3*H*)-one	*S. scandens*[280]
612	Methyl-2-(1,4-dihydroxycyclohexyl)-acetate	*S. scandens*[280]
613	1*β*,10*β*(8,12)-Diepoxy-7*β*,11*β*-dihydroxy-8*β*,12*α*-dimethoxyeremophilane	*S. nemorensis*[281]
614	1*β*,10*β*-Epoxy-7*β*-hydroxy-8*α*-methoxyeremophil-11*α*-*H*-12,8*β*-olide	*S. nemorensis*[281]
615	6*β*-Isobutyryloxy-8*α*-hydroxy-1-oxoeremophil-7(11),9-dien-12,8*β*-olide	*S. nemorensis*[281]
616	8,8''-Methylenebis(3, 3', 4', 5, 7-pentahydroxy-flavone)	*S. argunensis*[282]
617	Isorhamnetin	*S. argunensis*[282]
618	Succinic acid	*S. argunensis*[282]
619	Quercetin-5-*O*-*β*-D-glucoside	*S. argunensis*[282]
620	Senecine	*S. cannabifolius*[283]
621	Protocatechuic acid	*S. cannabifolius*[283]
622	3,4-Dihydroxybenzeneacetic acid	*S. cannabifolius*[283]
623	2-Furoic acid	*S. cannabifolius*[283]
624	1,2,4,5-Tetrahydrojacaranone	*S. cannabifolius*[283]
625	4-(Pyrrolidin-2-one)-phenylacetic acid	*S. cannabifolius*[283]
626	Isorhamnetin-3-*O*-*β*-D-galactoside	*S. laetus*[284]
627	Fisetin	*S. laetus*[284]
628	Dictamnoside	*S. laetus*[284]
629	2-(4'-Hydroxy-3',5'-dimethoxyphenyl)-1,3-propanediol	*S. laetus*[284]
630	Sugiol	*S. cannabilodius*[285]
631	Zhebeiresinol	*S. cannabilodius*[285]
632	Lirriodendrin	*S. cannabilodius*[285]
633	1,4-Benzene diol	*S. cannabilodius*[285]
634	Ethyl-2,5-dihydroxybenzeneacetic acid	*S. cannabilodius*[285]
635	*p*-Hydroxyphenylacetylamide	*S. cannabilodius*[285]
636	Tetrahydrojacaranone	*S. scandens*[286]
637	2,3-Dihydro-3-hydroxyljacaranone ethyl ester	*S. scandens*[286]
638	Jacaranone ethyl ester 4-*O*-glucoside	*S. scandens*[286]
639	Jacaranone glycose ester	*S. scandens*[286]
640	Neotriangularine	*S. stabianus*[287]
641	3a-Hydroxy-3,3a,7,7a-tetrahydrobenzofuran-2,6-dione	*S. giganteus*[288]
642	Chlorogenic acid	*S. giganteus*[288]
643	Quercetin 3-*O*-*β*-D-robinobioside	*S. giganteus*[288]
644	Isorhamnetin-3-*O*-*β*-D-glucuronide	*S. giganteus*[288]
645	Quercetin-3-*O*-*β*-D-glucuronide	*S. giganteus*[288]
646	Isorhamnetin-3-O-*β*-*D*-glucuronide-6''-methyl ester	*S. giganteus*[288]
647	Isodauc-7(14)-en-6*α*,10*β*-diol	*S. argunensis*[289]
648	10*β* -Hydroxyisodauc-6-en-14-al	*S. argunensis*[289]
649	(7*S**)-Opposit-4(15)-en-1*β*,7-diol	*S. argunensis*[289]

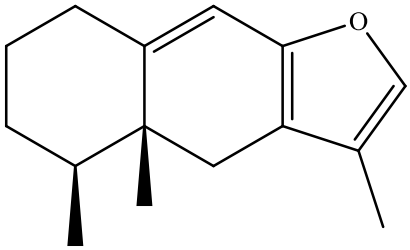
650	Artabotrol	*S. argunensis*[289]
651	(7*R**)-Opposit-4(15)-en-1*β*,7-diol	*S. argunensis*[289]
652	Opposit-4(15)-en-1*β*,11-diol	*S. argunensis*[289]
653	Ioliolide	*S. argunensis*[289]
654	5*α*,6*α*-Epoxy-3*β*-hydroxymegastigm-7-en-9-one	*S. argunensis*[289]
655	Chromolaevane dione	*S. argunensis*[289]
656	Pregn-4-en-3,20-dione	*S. argunensis*[289]
657	12*β*-Hydroxypregn-4-en-3,20-dione	*S. argunensis*[289]
658	Ergost-6,22-dien-3*β*,5*α*,8*α*-triol	*S. argunensis*[289]
659	(23*Z*)-Cycloart-23-en-3*β*,25-diol	*S. argunensis*[289]
660	Vulgarine	*S. vulgaris*[290]
661	7*R**,10*S**-Epoxy-3*E*,5*E*-farnesadien-1,2,11-triol	*S. cannabilolius*[291]
662	Ethyl chlorogenate	*S. cannabifolius*[292]
663	Ethyl-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetate	*S. cannabifolius*[292]
664	8*α*-Hydroxy-6*β*-(isobutanoyloxy)-eremophila-7(11),1(10)-dieno-12,8*β*-lactone	*S. nemorensis*[293]
665	1,2-Dihydrocacalohastine	*S. chrysanthemoides*[294]
666	Eudesm-4(15)-ene-1*β*,6*α*-diol	*S. chrysanthemoides*[294]
667	7,11,15-Trimethyl-3-methylidenehexadecane-1,2-diol	*S. chrysanthemoides*[294]
668	Faradiol 3-*O*-palmitate	*S. chrysanthemoides*[294]
669	Maniladiol 3-*O*-palmitate	*S. chrysanthemoides*[294]
670	Faradiol	*S. chrysanthemoides*[294]
671	Betulin	*S. chrysanthemoides*[294]
672	Loranthol	*S. chrysanthemoides*[294]
673	(+)-Syringaresinol	*S. chrysanthemoides*[294]
674	1-Hydroxy-4-oxo-cyclohexane-1-acetate	*S. chrysanthemoides*[294]
675	2,6-Dimethoxy-*p*-benzoquinone	*S. chrysanthemoides*[294]
676	Stigmasta-5,22-dien-3*β*-hydroxy-7-one	*S. chrysanthemoides*[294]
677	Stigmasta-5,22-dien-7-one	*S. chrysanthemoides*[294]
678	Stigmasta-4-en-3-one	*S. chrysanthemoides*[294]
679	Stigmasta-4,22-dien-3-one	*S. chrysanthemoides*[294]
680	Glycerol-1-hendecanoate	*S. chrysanthemoides*[294]
681	Methyl hendecan	*S. chrysanthemoides*[294]
682	1*β*,5*α*-Diangeloyloxy-eudesm-(15)-ene	*S. faberii*[295]
683	1*β*,6*α*-Dihydroxyeudesm-4(15)-ene	*S. faberii*[295]
684	10*α*-Hydroxyoplopan-4-one	*S. faberii*[295]
685	Friedelinol	*S. faberii*[295]
686	4-Hydroxy-3-methoxycinmamaldehyde	*S. faberii*[295]
687	Loliolide	*S. faberii*[295]
688	Cycloart-23*Z*-ene-3*β*,25-diol	*S. vulgaris*[296]
689	1*β*,6*α*-Dihydroxyeudesm-4(15)-ene	*S. vulgaris*[296]
690	1*β*,5*α*-Diangeloyloxy-eudesm-(15)-ene	*S. vulgaris*[296]
691	1*β*,7*α*-Dihydroxyeudesm-4(15)-ene	*S. vulgaris*[296]
692	Oplopanone	*S. vulgaris*[296]
693	1'-Hydroxy-2'-methoxy-4'-oxocyclohexanacetate methyl	*S. vulgaris*[296]

694	4-Carbomethoxymethyl-4-hydroxycyclohexanone	*S. vulgaris*[296]
695	1-Hydroxy-2,6-dimethoxy-4-oxocyclohexanacetate methyl	*S. vulgaris*[296]
696	2-[2,2-Dimethyl-6-oxo-7-dihydro-1,3-benzodioxol-3(6*H*)-yl] acetate methyl	*S. vulgaris*[296]
697	5-Acetyl-2-(2-hydroxyisopropyl)-7-(3,3-dimethyl-allyl)-2,3-dihydrobenzofuran	*S. vulgaris*[297]
698	7-Angeloylturneforcidine	*S. scandens*[298]
699	Hordenine	*S. scandens*[298]
700	1,3,6,6-Tetramethyl-5,6,7,8-tetrahydro-isoquinolin-8-one	*S. scandens*[298]
701	4-(Pyrrolidin-2-one)-phenyl acetic acid	*S. scandens*[298]
702	(4-Pyrrolidinophenyl) acetic acid	*S. scandens*[298]
703	Niacinamide	*S. nemorensis*[299]
704	Syringic acid	*S. nemorensis*[299]
705	Syringaldehyde	*S. nemorensis*[299]
706	3-Acetyl-4-hydroxybenzoic acid	*S. nemorensis*[299]
707	4, 4-Dimethyl-1,7-heptanedioic acid	*S. nemorensis*[299]
708	1*H*-Indole-3-carboxaldehyde	*S. nemorensis*[299]
709	Ethyl caffeate	*S. nemorensis*[299]
710	1-*O*-(*E*)-*p*-Methoxycinnamoyl-*β*-Dglucopyranoside	*S. nemorensis*[299]
711	(6*S*, 7*E*)-6-Hydroxy-4,7-megastigmadien-3, 9-dione	*S. nemorensis*[299]
712	Annuionone D	*S. nemorensis*[299]
713	(1'*S*, 6'*R*)-Abscisic acid	*S. nemorensis*[299]
714	Pentacosanoic acid	*S. scandens*[300]
715	19*α*-*H*-Lupeone	*S. scandens*[300]
716	Sucrose	*S. scandens*[300]
717	Senecainin	*S. scandens*[301]
718	3-Methoxyisonicotinic acid	*S. scandens*[301]

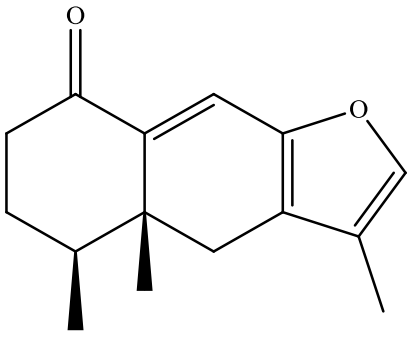
6.3 Structures of some compounds reported from genus *Senecio*



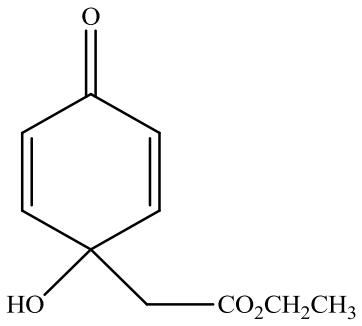
10*α*-*H*-Furanoeremophil-9-one[180]



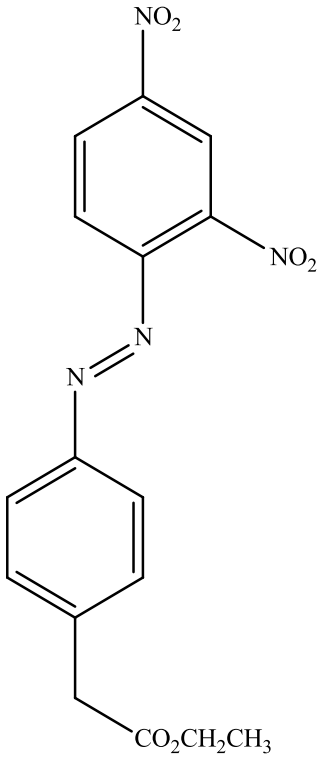
9,10-Dehydrofuranoeremophilane[180]



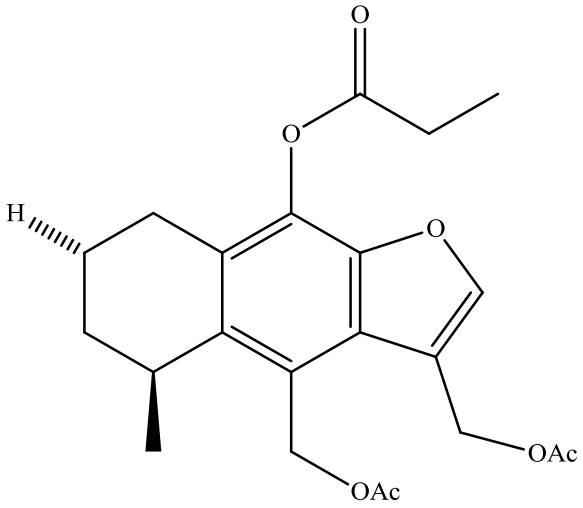
9,10-Dehydrofuranoeremophil-1-one[180]



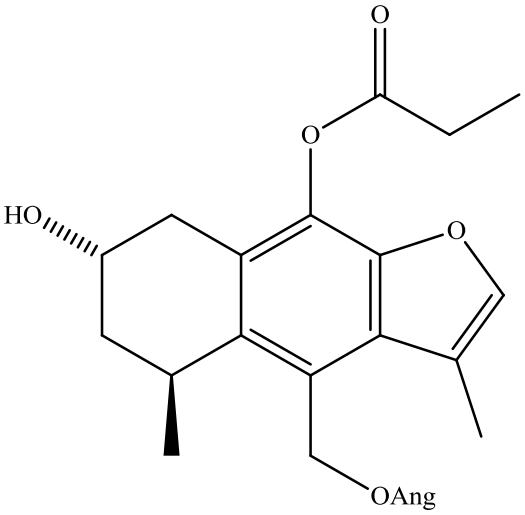
Ethyl-*α*-[4-(4-hydroxy-cyclohexa-2,5-dien-1-one)]-acetate[165]



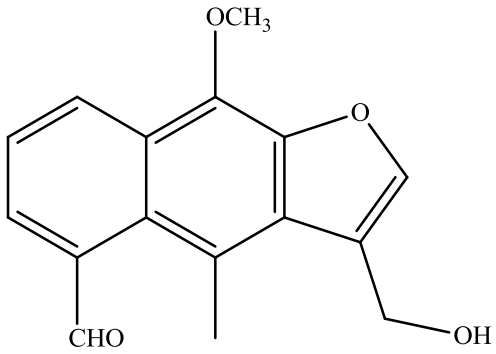
Azobenzene[165]



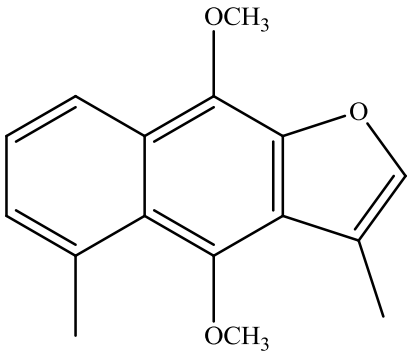
13,14-Diacetoxy-cacalolpropionate[302]



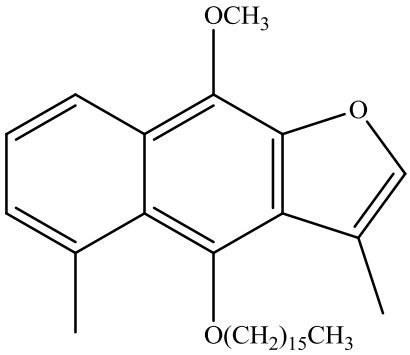
14-Angeloyloxy-2α-hydroxy-cacalolpropionate[302]



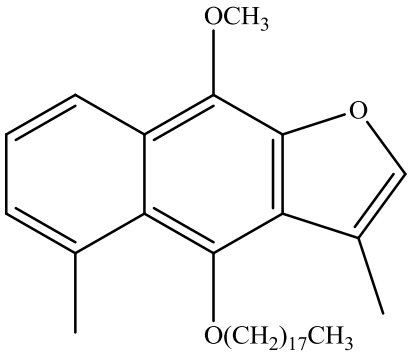
13-Hydroxydehydrocacalohastin-15-al[302]



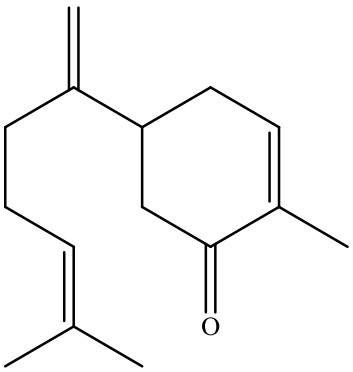
14-Methoxydehydrocacalohastin[302]



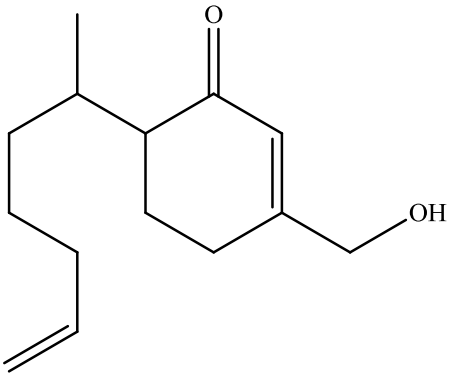
14-Hexadecyloxydehydrocacalohastin[302]



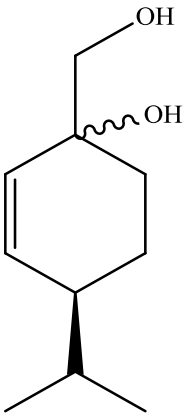
14-Octadecyloxydehydrocacalohastin[302]



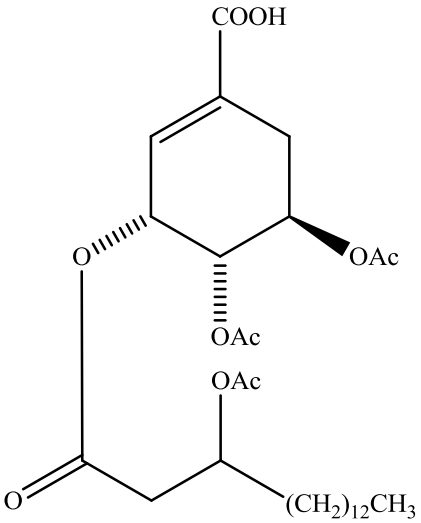
Bisabol-2,7,(14),10-triene-4-one[302]



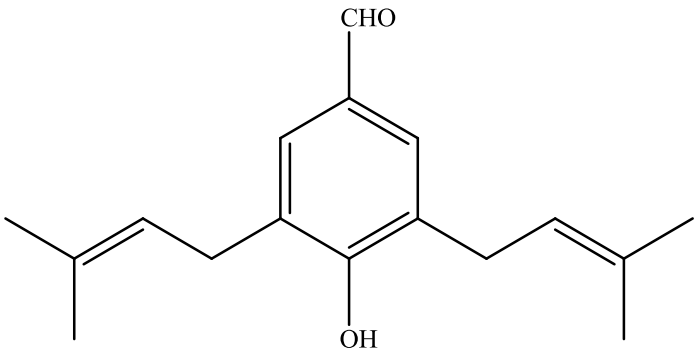
15-Hydroxy-*nor*-bisabol-2,11-diene-1-one[302]



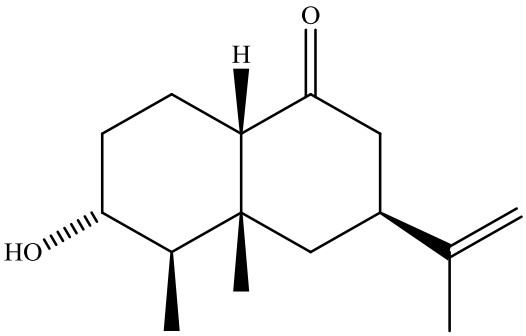
Menth-2-ene-1,7-diol[302]



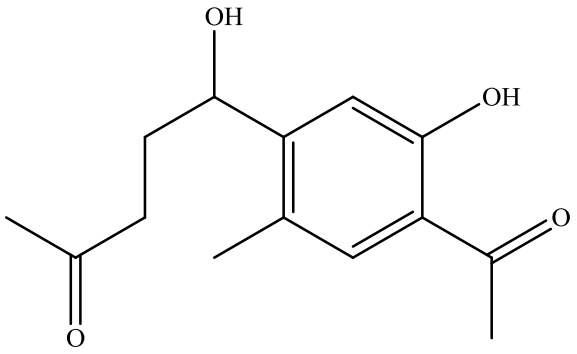
3-*O*-[3-Acetoxypalmitoyl]-shikimic acid-4,5-*O*-acetate[302]



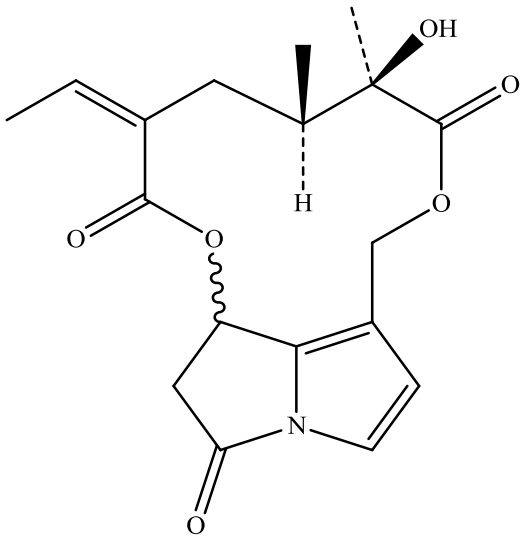
3,5-*Bis*-[3,3-dimethylallyl]-*p*-hydroxybenzaldehyde[302]



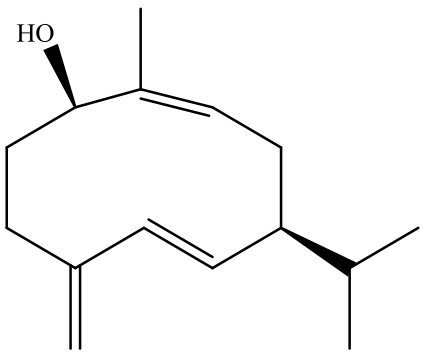
3*α*-Hydroxy-10*β*-*H*-eremophil-11(13)-en-9-one[223]



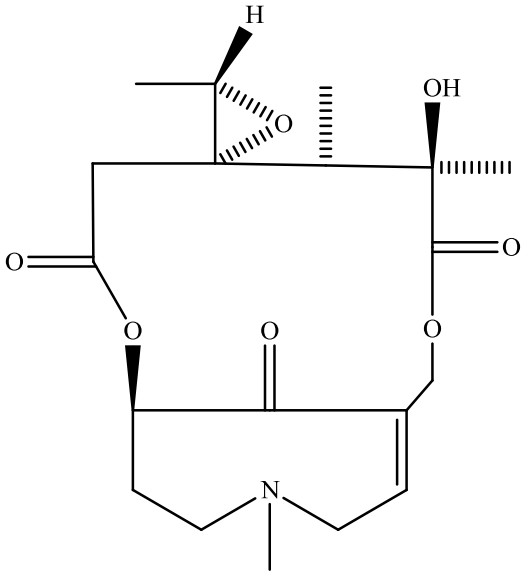
nor-seco-Glutinosone[223]



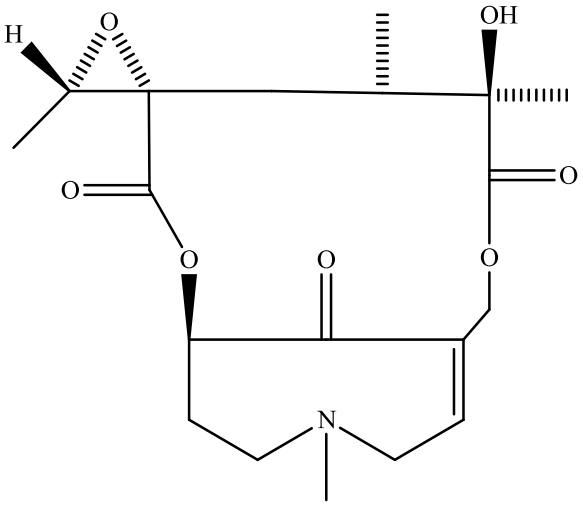
Desacetylsenaetin[225]



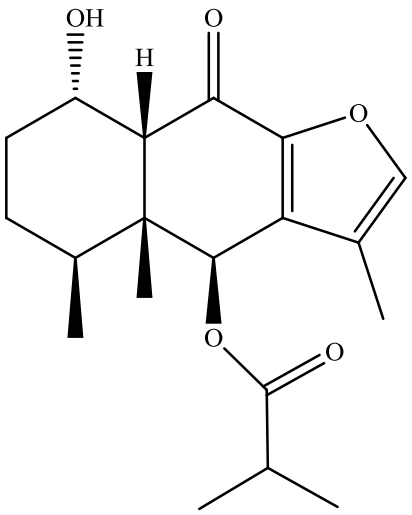
1β-Hydroxygermacra-4(15),5*E*,9*Z*-triene[228]



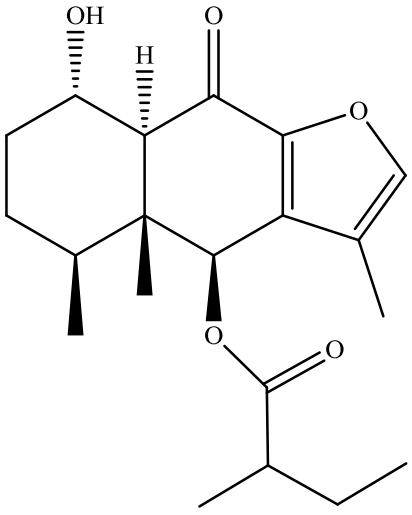
Senecioracenine[303]



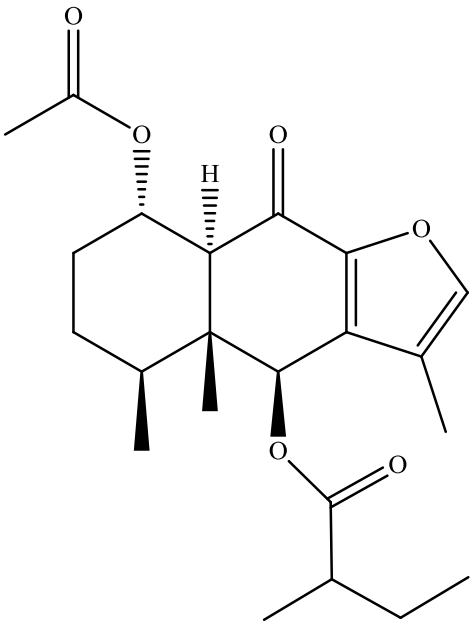
Sarracine[303]



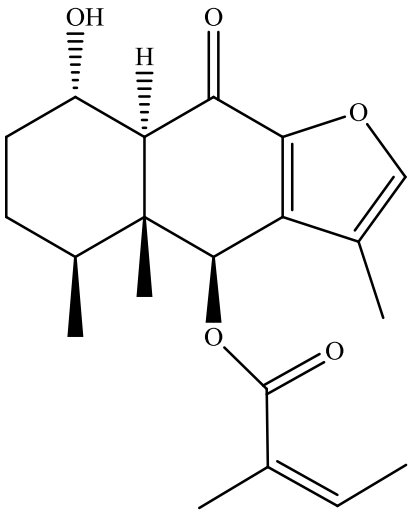
(1*S*,4*S*,5*R*,10*R*)-1-Hydroxy-6-isobutyryloxy-10*H*-9-oxofuranoeremophilane[266]



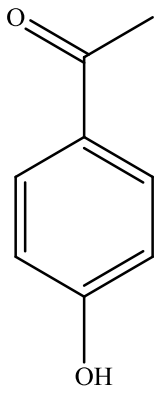
1*α*-Hydroxy-6*β*-(2*ξ*-methylbutyryloxy)-10*α*-*H*-9-oxofuranoeremophilane[266]



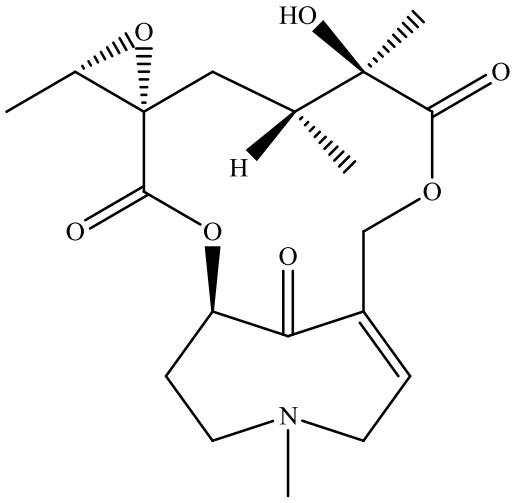
1*α*-Acetoxy-6*β*-(2*ξ*-methylbutyryloxy)-10*α*-*H*-9-oxofuranoeremophilane[266]



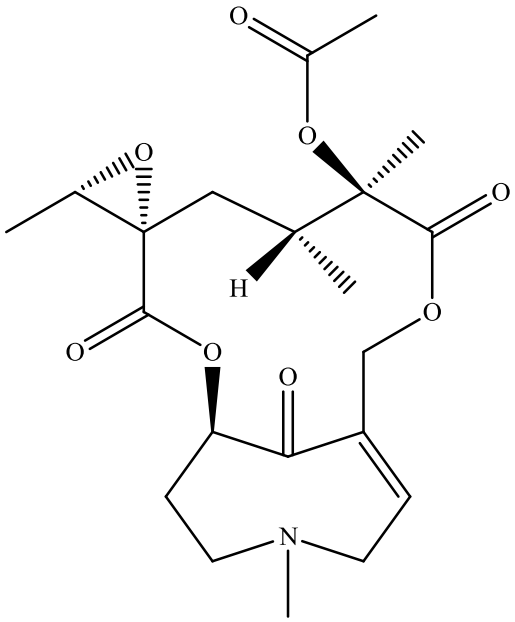
6*β*-Angeloyloxy-1*α*-hydroxy-10*α*-*H*-9-oxofuranoeremophilane[266]



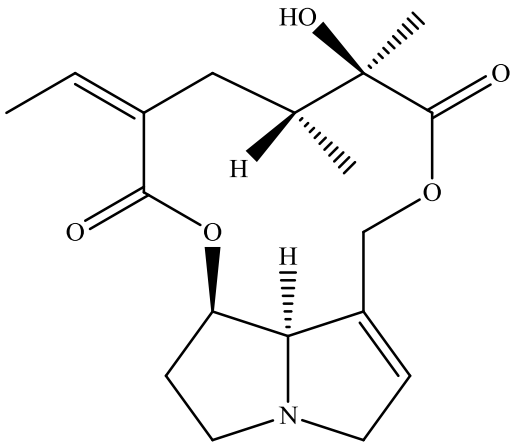
4′-Hydroxyacetophenone[266]



Otosenine[276]



Florosenine[276]



Senecionine[276]

6.4 Medicinal and pharmacological properties of genus *Senecio*

The *Senecio* is the largest genus of the family Compositae and well known for its pyrrolizidine alkaloids,[165] having toxic, carcinogenic and mutagenic effects[251] and are generally called hepatotoxic pyrrolizidine alkaloids (HPAs). Many *Senecio* species have been used in traditional or folk medicine as single or as an ingredient in over hundreds finished products due to their anti-inflammatory, antipyretic and detoxification effects. *S. vulgaris* played a vital role as emmenagogue and in case of functional amenorrhoea.[304] Some *Senecio* species have been described as herbivorous insect antifeedant, active against some phytopathogenic fungi and as cytotoxic.[279] Many species of the genus *Senecio* were used as antiemetic, anti-inflammatory, vasodilatory preparations, for the treatment of coughs, bronchitis, asthma, eczema and for wound healing. *Senecio* genus also have antimicrobial, antibacterial, antitubercular, molluscicidal and cytotoxic activities.[305,306] The leaves of *S. nutans* are used as gastric antispasmodic, in the treatment of gastric ulcers and headache.[307] *S. scandens* has been used for influenza, infections of the upper repiratory tract and infections of the urinary tract.[300] Many *Senecio* species has also been used for the treatment of heatstroke, malaria, inflammation, scalding, heavy colds and runny noses.[266,301] *S. cannabifolius* and *S. argunensis* have been used to treat virus influenza, enteritis pneumonia, cure sore throat, dysentery, trauma and fracture. [272,289]

7.1 *Senecio desfontainei*

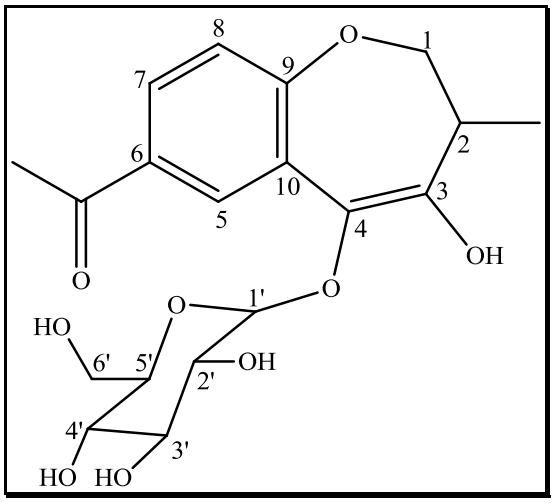
Phytochemical investigations and biological activities of the aerial parts of *S. desfontainei* resulted in the isolation and structure elucidation of one new (**44**) along with eleven known **(45-55)** compounds, hitherto unreported from this plant. The structures of these compounds were elucidated through advanced spectroscopic techniques which were also evaluated for their antioxidant activity in the *α,α*-diphenyl-*β*-picrylhydrazyl (DPPH) radical scavenging assay.



Senecio desfontainei

7.1.1 Structure elucidation of new compound isolated from *S. desfontainei*

7.1.1.1 Senecioside (44)



Compound **44** is a brownish yellow solid having a molecular formula $C_{19}H_{24}O_9$ (eight degrees of unsaturation) as deduced from its positive-mode HRESIMS (*m/z* 419.1306, calcd. for $C_{19}H_{24}O_9Na$ $[M + Na]^+$ 419.1313). The IR spectrum revealed absorption bands of hydroxyl group (3329 cm^{-1}), double bonds (1661 cm^{-1}) and carbonyl groups (1714 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-7.1**) of **44** showed an ABX-type of proton signals resonated at δ_H 7.46 (1H, dd, *J* = 0.6,8.7 Hz, H-8), 7.92 (1H, dd, *J* = 1.9,8.7 Hz, H-7) and 8.46 (1H, dd, *J* = 0.6,1.9 Hz, H-5), at 1.32 (1H, d, *J* = 7.0 Hz) for methyl and a singlet at 2.68 for the methyl of acetyl group. In ^{1}H- and ^{13}C-NMR spectra of **44** the signals appeared at δ_H 4.75 (1H, d, *J* = 7.6 Hz), δ_H 3.45-3.89 and δ_C 62.4, 71.2, 75.1, 77.9, 78.0, 106.4 suggested the presence of a glucose moiety. From the HMBC correlation spectrum of **44** (**Figure-7.1**), four carbons at δ_C 35.1, 65.9, 138.2 and 152.1 observed in the ^{13}C-NMR spectrum were assigned to the C1, C-2, C-3 and C-4 respectively. The HMBC correlations of CH_3 at δ_H 1.32 with δ_C 65.9 and 152.1

suggest that the methyl group is located at C-2. The position of the double bond in oxepin ring was decided on the basis of HMBC correlation of H-11 to C-3 and H-5 to C-4. Furthermore the HMBC correlations of acetyl proton at δ_H 2.68 with δ_C 133.5 and 200.4 suggest that acetyl group is located at C-6. The glucose moiety was also decided to be linked to the C-4 position (**Fig-7.1**). The configuration of the anomeric centre was confirmed to be β from the coupling constant (J = 7.6 Hz) of the H-1′ signal.

Thus the structure of **44** was characterized as a new 1-(4-hydroxy-3-methyl-5-(((3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-2*H*-pyran-2-yl)oxy)-2,3-dihydrobenzo[b]oxepin-7-yl)ethanone (senecioside).

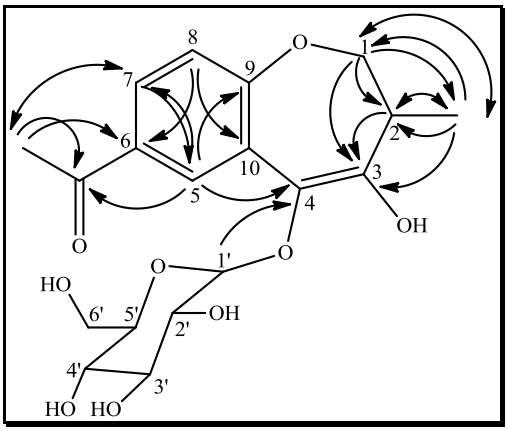
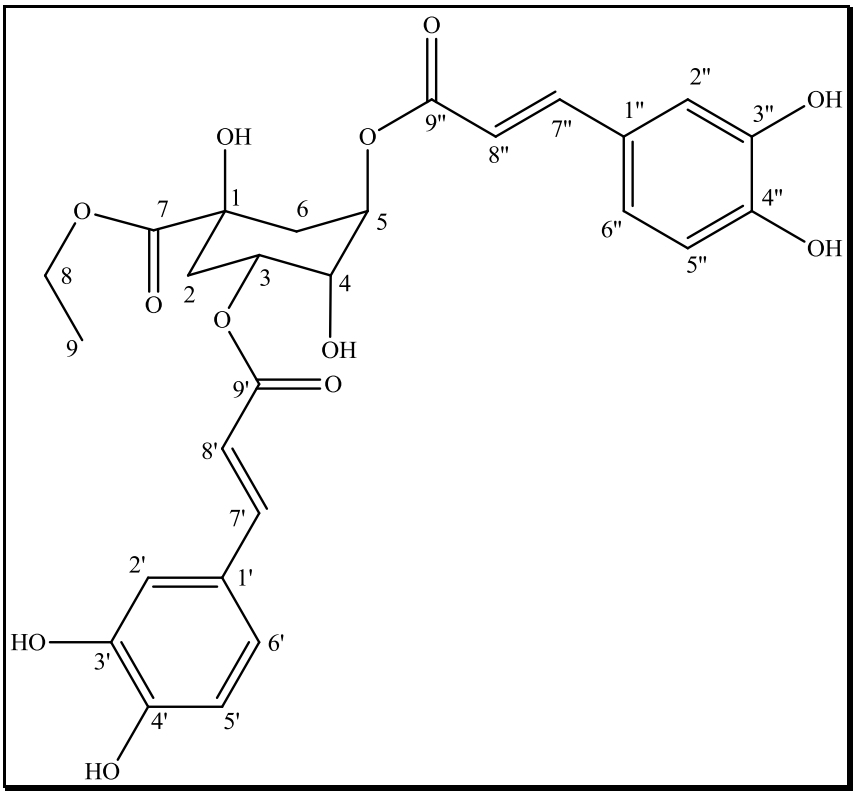


Figure-7.1: Selected HMBC (H→C) & NOESY (H↔H) for senecioside (**44**)

Table-7.1:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of senecioside (**44**) in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H, **(multiplicity, *J* in Hz)**
1	65.9	CH_2	3.75, m
			3.82, m
2	35.1	CH	3.53, m
3	152.1	C	--
4	138.2	C	--
5	122.2	CH	8.46, (dd, 0.6, 1.9)
6	133.5	C	--
7	125.4	CH	7.92, (dd, 1.9, 8.7)
8	112.3	CH	7.46, (dd, 0.6, 8.7)
9	156.3	C	--
10	125.5	C	--
2-Me	15.2	CH_3	1.32, (d, 7.0)
6-CO	200.4	C	--
6-CO-Me	26.9	CH_3	2.68, s
1′	106.4	CH	4.75, (d, 7.6)
2′	75.1	CH	3.51, m
3′	77.9	CH	3.45, m
4′	71.2	CH	3.46, m
5′	78.0	CH	3.30, m
6′	62.4	CH_2	3.77, m
			3.89, m

7.1.2 Structure elucidation of compounds isolated for the first time from *S. desfontainei*

7.1.2.1 Ethyl 3,5-di-*O*-caffeoylquinic acid (45)

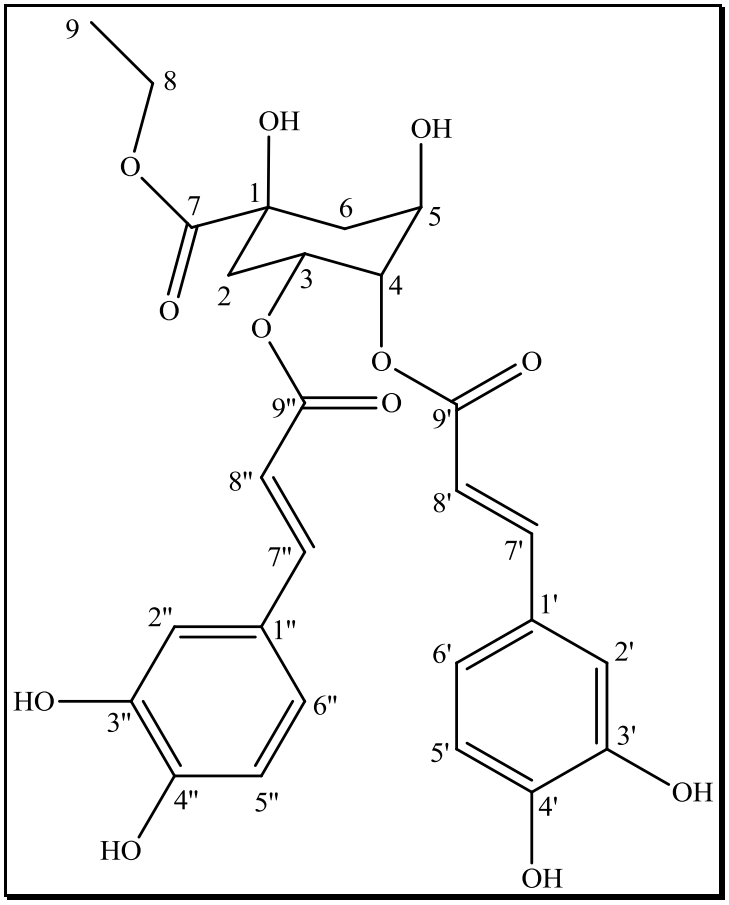


Compound **45** was isolated as yellow solid. The IR spectrum exhibited hydroxyl (3332 cm^{-1}), ester carbonyl (1687 cm^{-1}) and double bonds (1598 cm^{-1}) absorption bands. Its molecular formula ($C_{27}H_{28}O_{12}$) was determined by ^{1}H- and ^{13}C-NMR analysis and LCESI-MS, which gave molecular ion adducts $[M - H]^-$ at *m/z* 543, $[M - \text{caffeoyl} - H]^-$ at *m/z* 381, $[\text{caffeoyl} - H]^-$ at *m/z* 207 in ESI-negative mode. In the ESI-positive mode $[M + H]^+$ at *m/z* 545, the formation of sodium adduct ions $[M + Na]^+$ at *m/z* 567 and $[M + H - 208]^+$ at *m/z* 337 were observed. The ion at *m/z* 527 was formed by a neutral loss of a NaOH from the $[M + Na]^+$ ion, or by cleavage of water from the protonated molecule at *m/z* 545. The ion at *m/z* 163 indicated the presence of a caffeoyl group.

^{1}H- and ^{13}C-NMR (BB and DEPT) spectra (**Table-7.2**) of compound **45** confirmed the presence of a quinic acid moiety along with an ethyl group and two caffeic acid moieties in the molecule. In comparison of NMR spectral data, **45** was characterized as ethyl 3,5-di-*O*-caffeoylquinic acid.[308-310]

Table-7.2:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of ethyl 3,5-di-*O*-caffeoylquinic acid **(45)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	74.5	C	--
2	35.5	CH_2	2.17, m
			2.35, m
3	72.2	CH	5.32, m
4	69.7	CH	3.98, (dd, 3.2, 6.5)
5	71.9	CH	5.42, m
6	35.5	CH_2	2.17, m
			2.35, m
7	175.1	C	--
8	62.5	CH_2	4.12, (q, 7.2)
9	14.2	CH_3	1.25, (t, 7.2)
1′	127.6	C	--
2′	115.1	CH	7.06, (d, 1.8)
3′	146.7	C	--
4′	149.5	C	--
5′	116.4	CH	6.79, (d, 8.1)
6′	122.9	CH	6.97, (dd, 1.8, 8.1)
7′	147.3	CH	7.56, (d, 15.8)
8′	114.8	CH	6.23, (d, 15.8)
9′	167.9	C	--
1″	127.8	C	--
2″	115.1	CH	7.08, (d, 1.8)
3″	146.8	C	--
4″	149.7	C	--
5″	116.5	CH	6.80, (d, 8.1)
6″	123.0	CH	6.97, (dd, 1.8, 8.1)
7″	147.1	CH	7.63, (d, 15.8)
8″	115.4	CH	6.35, (d, 15.8)
9″	168.7	C	--

7.1.2.2 Ethyl 3,4-di-*O*-caffeoylquinic acid (46)

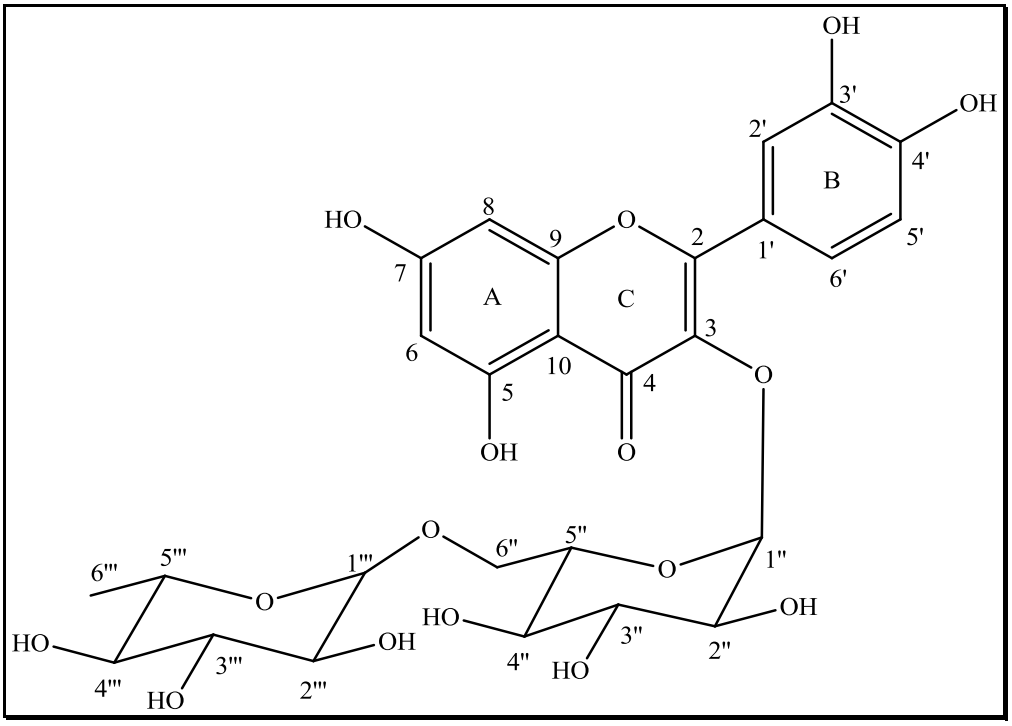


Compound **46** was obtained from ethyl acetate fraction as yellow solid. The IR spectrum exhibited hydroxyl (3339 cm^{-1}), ester carbonyl (1699 cm^{-1}) and double bonds (1598 cm^{-1}) absorption bands. Its molecular formula was found to be $C_{27}H_{28}O_{12}$ from ^{1}H- and ^{13}C-NMR analyses and LCESI-MS, which gave molecular ion adducts $[M - H]^-$ at *m/z* 543, $[M$ – caffeoyl – $H]^-$ at *m/z* 381, [caffeoyl – $H]^-$ at *m/z* 207 in ESI-negative mode. In the ESI-positive mode $[M + H]^+$ at *m/z* 545, the formation of sodium adduct ions $[M + Na]^+$ at *m/z* 567 and $[M + H - 208]^+$ at *m/z* 337 were observed. The ion at *m/z* 527 was formed by a neutral loss of a NaOH from the $[M + Na]^+$ ion, or by cleavage of water from the protonated molecule at *m/z* 545. The ion at *m/z* 163 indicated the presence of a caffeoyl group. ^{1}H- and ^{13}C-NMR (BB and DEPT) spectra (**Table-7.3**) showed the presence of quinic acid moiety along with an

ethyl group and two caffeic acid moieties in **46**. The NMR spectral data of **46** well matched with the reported compound, ethyl 3,4-di-*O*-caffeoylquinic acid.[308-310]

Table-7.3:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of ethyl 3,4-di-*O*-caffeoylquinic acid **(46)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	75.6	C	--
2	38.3	CH_2	2.09, m
			2.34, m
3	69.1	CH	5.55, m
4	74.9	CH	5.12, (dd, 2.8, 8.1)
5	68.5	CH	4.35, m
6	38.5	CH_2	2.09, m
			2.34, m
7	174.3	C	--
8	62.6	CH_2	4.17, (q, 7.1)
9	14.3	CH_3	1.27, (t, 7.1)
1′	127.6	C	--
2′	115.1	CH	7.01, (d, 1.5)
3′	146.8	C	--
4′	149.7	C	--
5′	116.5	CH	6.76, (d, 8.2)
6′	123.1	CH	6.93, (dd, 1.6, 8.2)
7′	147.7	CH	7.61, (d, 15.9)
8′	114.7	CH	6.31, (d, 15.9)
9′	168.4	C	--
1″	127.5	C	--
2″	115.1	CH	7.01, (d, 1.5)
3″	146.8	C	--
4″	149.7	C	--
5″	116.5	CH	6.76, (d, 8.2)
6″	123.1	CH	6.93, (dd, 1.6, 8.2)
7″	147.6	CH	7.51, (d, 15.9)
8″	114.5	CH	6.18, (d, 15.9)
9″	167.9	C	--

7.1.2.3 Quercetin-3-*O*-rutinoside (Rutin) (47)



Compound **47** was isolated as brownish yellow crystals. The IR spectrum exhibited hydroxyl (3395 cm^{-1}), ester (1710 cm^{-1}), conjugated carbonyl (1660 cm^{-1}) and aromatic (1610 cm^{-1}) absorption bands. The molecular formula ($C_{27}H_{30}O_{16}$) of **47** was determined by NMR spectral interpretation and LC-ESIMS, which gave molecular ion adducts $[M - H]^-$ at *m/z* 609 in ESI-negative mode and $[M + H]^+$ at *m/z* 611, $[M + Na]^+$ at 633, $[M - Rha]^+$ at 465 and $[M - Rha - Glu]^+$ at 303 in ESI-positive mode, suggesting a molecular mass of 610 a.m.u. These fragments are characteristic of aglycone residues after loss of their rhamnosyl and rhamnoglucosyl moieties, repectively.

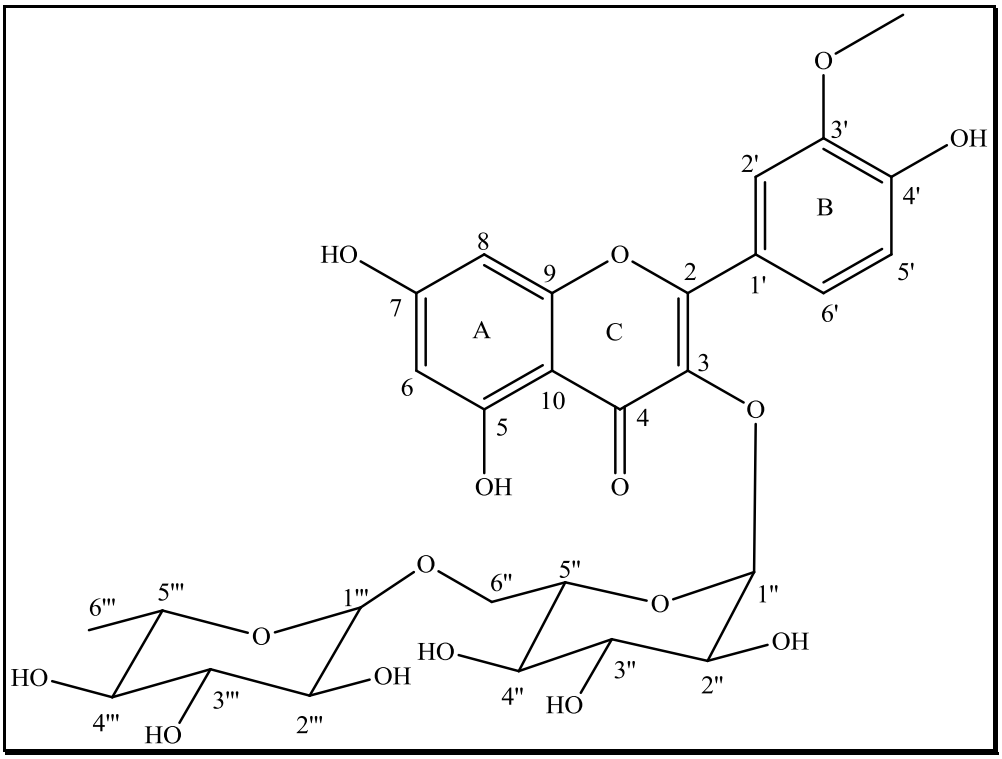
The ^{1}H-NMR spectrum of compounds **47** showed the presence of three aromatic proton signals, which were characteristic of H-5′, H-6′, and H-2′ of the flavonoid B ring with 3′, 4′-substitution (**Table-7.4**). Two weakly coupled doublet signals were assigned to two protons on C-6 and C-8 in the A ring. The carbonyl signal at δ_C 179.2

in the ^{13}C-NMR spectrum (**Table-7.4)** suggesting a flavonol skeleton in compound **47**. Two anomeric proton signals at δ_H 4.52 and 5.12 attached to carbons at δ_C 102.4 and 104.6, respectively, confirmed the presence of disaccharide in the nucleus. Furthermore, HMBC from the H^1 signal at δ_H 5.12 to carbon at δ_C 135.6 suggested that the C-3 position of flavonol was glycosylated in compound **47**. A proton signal at δ_H 1.12 (3H, d, J = 6.2 Hz) and carbon signal at δ_C 17.8 indicated that one of the sugars had a terminal methyl group. The ^{1}H-, ^{13}C-, and 2D-NMR spectra indicated that the disaccharide is rhamnopyranosyl-(α-1→6)-glucopyranose (rutinose), of which linkage was verified by HMBC from H-6″ to C-1‴ and from H-1‴ to C-6″. Thus, compound **47** was identified as quercetin-3-*O*-rutinoside (rutin). The chemical shifts of this compound well matched to the reported values.[311,312]

Table-7.4:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of quercetin-3-*O*-rutinoside (Rutin) **(47)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
2	159.3	C	--
3	135.6	C	--
4	179.2	C	--
5	162.9	C	--
6	99.9	CH	6.41, (d, 1.7)
7	166.0	C	
8	94.8	CH	6.22, (d, 1.9)
9	158.5	C	--
10	105.6	C	--
1′	123.1	C	--
2′	117.6	CH	7.67, (d, 2.0)
3′	145.8	C	--
4′	149.8	C	--
5′	116.0	CH	6.88, (d, 8.4)
6′	123.5	CH	7.63, (dd, 2.1, 8.4)
1″	104.6	CH	5.12, (d, 7.7)
2″	75.7	CH	3.48, m
3″	77.2	CH	3.33, m
4″	71.4	CH	3.27, m
5″	78.1	CH	3.43, m
6″	68.5	CH_2	3.39, m
			3.81, (dd, 1.3, 10.9)
1‴	102.4	CH	4.52, (d, 1.3)
2‴	72.1	CH	3.63, m

3‴	72.2	CH	3.54, (dd, 3.3, 9.5)
4‴	73.9	CH	3.28, m
5‴	69.7	CH	3.45, m
6‴	17.8	CH_3	1.12, (d, 6.2)

7.1.2.4 Isorhamentin 3-*O*-*β*-D-rutinoside (48)



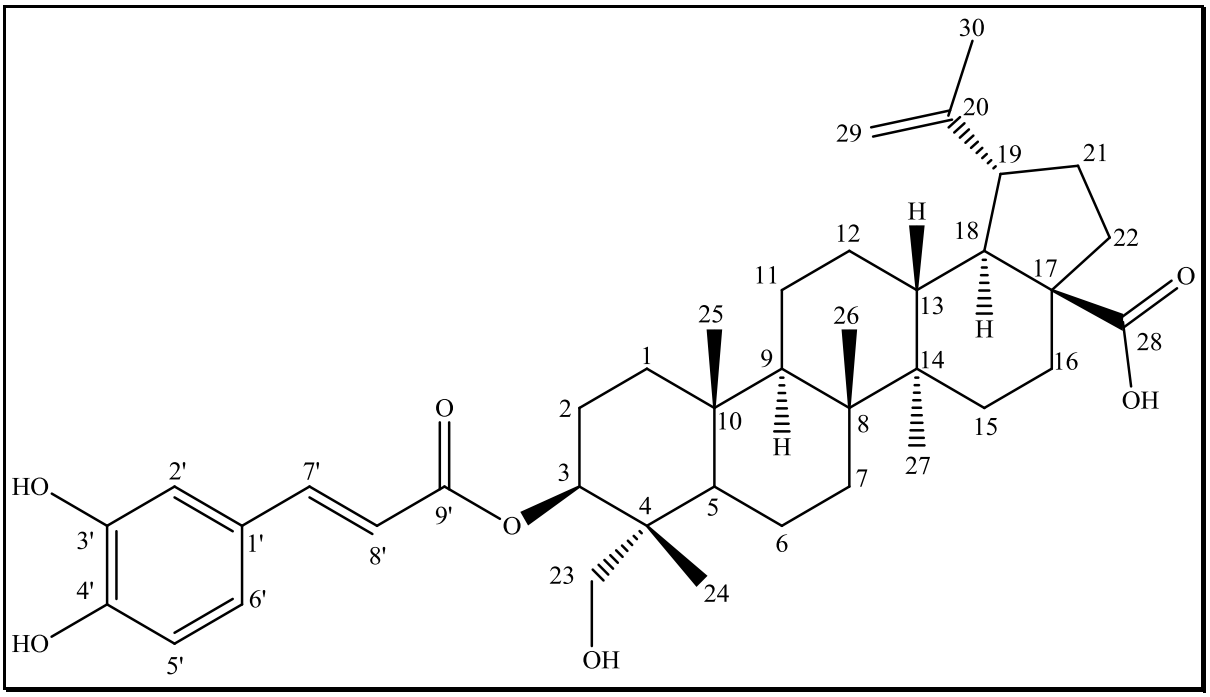
Compound **48** was isolated as yellow amorphous powder. The IR spectrum exhibited hydroxyl (3390 cm^{-1}), ester (1715 cm^{-1}), conjugated carbonyl (1650 cm^{-1}) and aromatic (1615 cm^{-1}) absorption bands. Its molecular formula ($C_{28}H_{32}O_{16}$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS (which gave molecular ion adducts $[M-H]^-$ at *m/z* 623 in ESI-negative mode and $[M + H]^+$ at *m/z* 625, $[M + Na]^+$ at 647, $[M – Rha]^+$ at 479 and $[M – Rha – Glu]^+$ at 317 in ESI-positive mode, suggesting a molecular mass of 624 a.m.u.). These fragments indicated the loss of rhamnosyl and rhamnoglucosyl moieties from the aglycone part. In the ^{1}H-NMR spectrum (**Table-7.5)**, five aromatic signals corresponding to δ_H 6.17 (1H, s, H-6) and 6.35 (1H, s, H-8)] on the A ring, and to an ABM spin system [δ_H 7.92 (1H, d, *J* = 1.7

Hz)], 6.88 (1H, m) and 7.59 (1H, m) on the B ring of the aglycone were found. A methoxy group signal at δ_H 3.94 (3H, s) was observed in ^{1}H-NMR spectrum. On the basis of NMR analyses, the aglycone was identified as isorhamnetin, which was further confirmed by correlation between the methoxy group and C-3′ in the HMBC spectrum. The presence of carbonyl signal at δ_C 179.7 in ^{13}C-NMR spectrum (**Table-7.5)** indicated a flavonol skeleton in **48**. The ^{1}H- and ^{13}C-NMR data also showed signals for rhamnosyl and glucosyl moieties. HMBC correlations of H-1″ with C-3, and H-1‴ with C-6″ indicated that glucosyl and rhamnosyl moieties were poresent in C-3 and C-6″, respectively. The spectral data of **48** was found similar to the reported compound; isorhamentin 3-*O*-*β*-D-rutinoside.[311-314]

Table-7.5:	^{1}H- (500 MHz) and ^{13}C- (100 MHz) NMR data of isorhamentin 3-*O*-*β*-D-rutinoside **(48)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
2	158.7	C	--
3	135.4	C	--
4	179.7	C	--
5	162.8	C	--
6	99.9	CH	6.17, s
7	165.8	C	
8	94.9	CH	6.35, s
9	158.5	C	--
10	105.5	C	--
1′	122.8	C	--
2′	114.4	CH	7.92, (d, 1.7)
3′	148.1	C	--
4′	150.7	C	--
5′	116.0	CH	6.88, m
6′	123.9	CH	7.59, m
3′-OCH_3	56.7	CH_3	3.94, s
1″	104.4	CH	5.20, m
2″	75.8	CH	3.47, m
3″	77.2	CH	3.39, m
4″	71.5	CH	3.27, m
5″	78.8	CH	3.48, m
6″	68.4	CH_2	3.40, m
			3.80, (dd, 1.2, 10.5)
1‴	102.4	CH	4.52, (d, 1.2)
2‴	71.9	CH	3.62, m

3‴	72.2	CH	3.49, m
4‴	73.8	CH	3.28, m
5‴	69.7	CH	3.41, m
6‴	17.8	CH_3	1.11, (d, 6.0)

7.1.2.5 3*β*,23-Dihydroxy-lup-20(29)-en-28-oic acid-3*β*-caffeate (49)



Compound **49** was isolated as white amorphous powder. Its molecular formula ($C_{39}H_{54}O_7$) was established by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M+H]^+$ at m/z 635 in ESI-positive and $[M - H]^-$ at m/z 633 in ESI-negative mode, suggesting a molecular mass of 634 a.m.u.

The ^{1}H-NMR spectrum **(Table-7.6)** of **49** exhibited the presence of five tertiary methyl groups at δ_H 0.80(3H, s), 0.97(3H, s), 0.98(3H, s), 1.06(3H, s) and 1.72(3H, s) at C-24, C-25, C-26, C-27, and C-30 positions respectively. The characteristic coupling pattern of three aromatic protons at δ_H (ppm): 7.04 (1H, d, J = 2.0 Hz, H-2′), 6.76 (1H, d, J = 8.5 Hz, H-5′) and 6.94 (1H, dd, J = 2.0,8.5 Hz, H-6′) indicated the presence of 1,3,4-trisubstituted benzene. Two signals appeared at δ_H

(ppm): 7.55 (1H, d, *J* = 15.7 Hz, H-7′) and 6.24 (1H, d, *J* = 15.7 Hz, H-8′) supported the *trans* relationship between olefinic protons H-7′ and H-8′ of the caffeic acid moiety. Which was attached at C-3 position as confirmed by the HMBC correlation.[308,310,315]

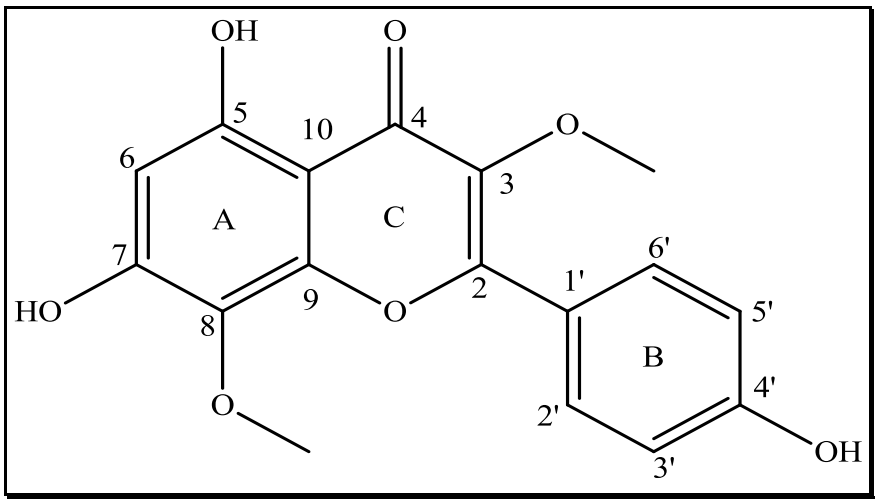
The ^{13}C-NMR spectrum (**Table-7.6**) of **49** showed an ester carbonyl, eight olefinic carbons, five tertiary methyl groups, a carboxylic acid carbon and a terminal double bond. In addition to these signals, twenty three other carbon resonances were found in ^{13}C-NMR spectrum.

The ^{1}H-NMR and ^{13}C-NMR spectral data (**Table-7.6**) of compound **49** were in complete agreement to those reported in literature for 3*β*,23-dihydroxy-lup-20(29)ene-28-oic acid-3*β*-caffeate).[315]

Table-7.6:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of 3*β*,23-dihydroxy-lup-20(29)ene-28-oic acid-3*β*-caffeate) **(49)** in CD_3COCD_3		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	39.5	CH_2	1.09, m
			2.40, m
2	24.3	CH_2	1.75, m
3	75.8	CH	4.86, m
4	43.0	C	--
5	48.4	CH	1.42, m
6	19.0	CH_2	1.53, m
			1.43, m
7	35.2	CH_2	1.29, m
			1.52, m
8	42.1	C	--
9	52.4	CH	1.39, m
10	38.3	C	--
11	22.5	CH_2	1.21, m
			1.55, m
12	27.3	CH_2	1.13, m
			1.77, m
13	39.4	CH	2.30, m
14	44.0	C	--
15	31.0	CH_2	1.23, m
			1.35, m

16	33.5	CH_2	1.47, m
			2.31, m
17	57.7	C	--
18	50.6	CH	1.66, m
19	48.5	CH	3.05, m
20	152.0	C	--
21	32.0	CH_2	1.36, m
			1.91, m
22	38.3	CH_2	1.41, m
			1.88, m
23	64.9	CH_2	3.15, (d, 11.6)
			3.38, (d, 11.6)
24	14.0	CH_3	0.80, s
25	17.3	CH_3	0.97, s
26	16.7	CH_3	0.98, s
27	15.4	CH_3	1.06, s
28	180.0	C	-
29	110.0	CH_2	4.63, (dd, 1.3, 2.1)
			4.76, (d, 2.1)
30	19.8	CH_3	1.72, s
1′	128.0	C	--
2′	115.3	CH	7.04, (d, 2.0)
3′	146.6	C	--
4′	149.8	C	--
5′	116.8	CH	6.76, (d, 8.5)
6′	123.1	CH	6.94, (dd, 2.0, 8.5)
7′	147.0	CH	7.55, (d, 15.7)
8′	116.0	CH	6.24, (d, 15.7)
9′	169.5	C	--

7.1.2.6 5,7,4′-Trihydroxy-3,8-dimethoxyflavone (50)



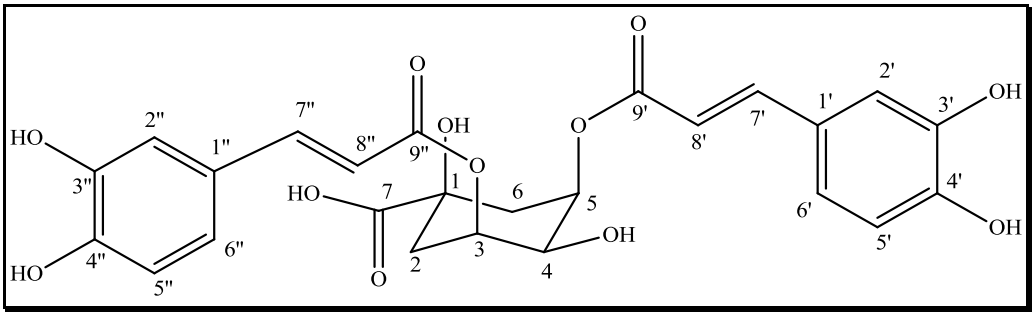
Compound **50** was obtained as yellow needles. The IR spectrum exhibited hydroxyl (3430 cm^{-1}), conjugated carbonyl (1660 cm^{-1}) and aromatic (1617 cm^{-1}) absorption bands. NMR (^{1}H and ^{13}C) and ESIMS data ($[M - H]^-$ at *m/z* 329 in ESI-negative mode and $[M + H]^+$ at *m/z* 331 and $[M + Na]^+$ at 353 in ESI-positive mode, suggesting a molecular mass of 330 a.m.u. indicated a molecular formula of $C_{17}H_{14}O_7$.

In the ^{1}H-NMR spectrum (**Table-7.7)**, two aromatic signals at δ_H 7.94 (2H, d, J = 8.9 Hz) and 6.92 (2H, d, J = 8.9 Hz) showed an AA′BB′ spin system on the B ring. An aromatic signal at δ_H 6.54 (1H, s) along with a low field signal at 12.7 (1H, s) of a chelated hydroxyl guop on the A ring indicated 5,7,8-substituted pattern.

The ^{1}H-NMR and ^{13}C-NMR spectral data (**Table-7.7**) of compound **50** were in complete agreement to those reported in literature for 5,7,4′-trihydroxy-3,8-dimethoxyflavone.[316-318]

Table-7.7:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of 5,7,4′-trihydroxy-3,8-dimethoxyflavone **(50)** in DMSO		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
2	155.6	C	--
3	137.2	C	--
4	178.1	C	--
5	151.5	C	12.7 (OH), s
6	93.9	CH	6.54, s
7	157.3	C	--
8	131.1	C	--
9	152.3	C	--
10	104.5	C	--
C-3 (-OCH_3)	59.6	CH_3	3.77, s
C-8 (-OCH_3)	59.9	CH_3	3.75, s
1′	120.6	C	--
2′	130.1	CH	7.94, (d, 8.9)
3′	115.6	CH	6.92, (d, 8.9)
4′	160.1	C	--
5′	115.6	CH	6.92, (d, 8.9)
6′	130.1	CH	7.94, (d, 8.9)

7.1.2.7 3,5-Di-*O*-caffeoylquinic acid (51)

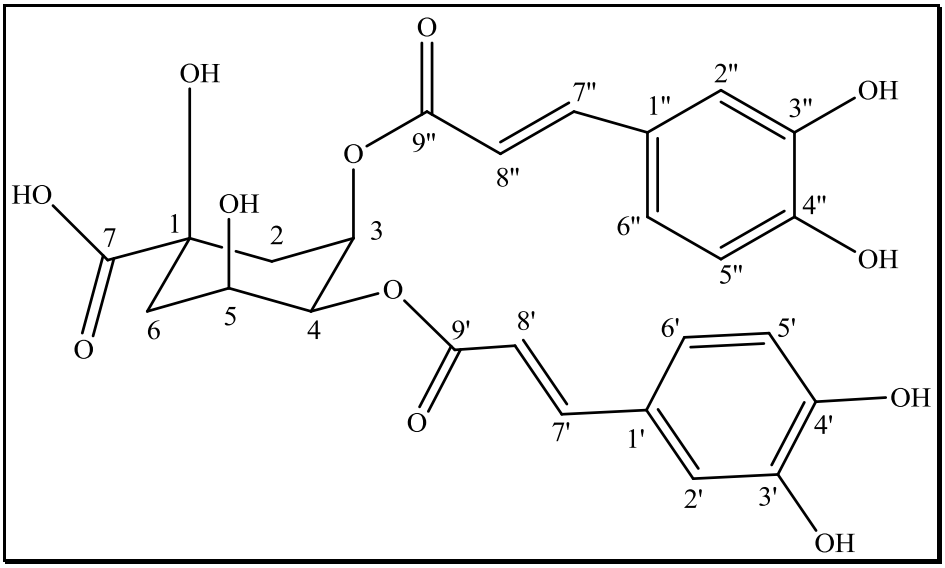


Compound **51** was purified from ethyl acetate fraction as light yellow amorphous powder. The IR spectrum exhibited hydroxyl (3380 cm^{-1}), –COOH carbonyl (1710 cm^{-1}) ester carbonyl (1690 cm^{-1}) and double bonds (1608 cm^{-1}) absorption bands. A molecular formula of $C_{25}H_{24}O_{12}$ was assigned to **51** by ^{1}H- and ^{13}C-NMR analyses and LC-ESIMS, which gave molecular ion adducts $[M - H]^-$ at *m/z* 515, $[M - \text{caffeoyl} - H]^-$ at *m/z* 353, [M – 2caffeoyl – H] (or [quinic acid – H]) at *m/z* 191, [caffeoyl – H] at *m/z* 179 in ESI-negative mode, corresponded to the successive losses of two caffeoyl groups and a quinic acid moiety, respectively. In the ESI-positive mode $[M + H]^+$ at *m/z* 517 and the formation of sodium adduct ions $[M + Na]^+$ at *m/z* 539 was observed. The ion at *m/z* 499 was formed by a loss of NaOH from the $[M + Na]^+$ ion, or by cleavage of water from the protonated molecule at *m/z* 517. The ion at *m/z* 163 indicated the presence of a caffeoyl group. These results suggested a molecular mass of **51** as 516 a.m.u.

By comparing the ^{1}H- and ^{13}C-NMR spectral data (**Table-7.8**) to the reported literature, compound **51** was characterized as 3,5-di-*O*-caffeoylquinic acid.[308,310]

Table-7.8:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of 3,5-di-*O*-caffeoylquinic acid **(51)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	76.0	C	--
2	37.5	CH_2	2.08, m
			2.27, m
3	74.0	CH	5.38, m
4	73.0	CH	3.90, (dd, 3.3, 10.0)
5	72.0	CH	5.56, m
6	37.5	CH_2	2.08, m
			2.27, m
7	174.0	C	--
1′	128.0	C	--
2′	114.7	CH	7.07, s
3′	147.0	C	--
4′	151.0	C	--
5′	116.2	CH	6.78, (d, 8.0)
6′	122.5	CH	6.96, m
7′	148.0	CH	7.62, (d, 15.7)
8′	116.0	CH	6.41, (d, 15.7)
9′	169.0	C	--
1″	128.0	C	--
2″	114.7	CH	7.07, s
3″	147.0	C	--
4″	151.0	C	--
5″	116.2	CH	6.78, (d, 8.0)
6″	122.5	CH	6.96, m
7″	148.0	CH	7.51, (d, 15.7)
8″	116.0	CH	6.20, (d, 15.7)
9″	169.0	C	--

7.1.2.8 4,5-Di-*O*-caffeoylquinic acid (52)

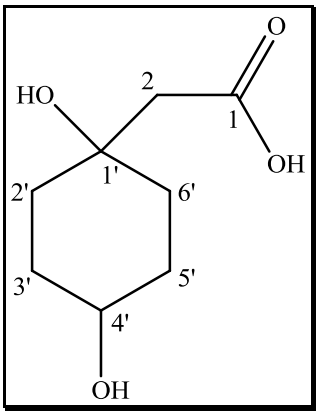


Compound **52** was isolated as light yellow amorphous powder. The IR spectrum exhibited hydroxyl (3390 cm^{-1}), –COOH carbonyl (1720 cm^{-1}) ester carbonyl (1698 cm^{-1}) and double bonds (1610 cm^{-1}) absorption bands. The molecular formula ($C_{25}H_{24}O_{12}$) was estimated by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M - H]^-$ at *m/z* 515, [M – caffeoyl – H]$^-$ at *m/z* 353, [M – 2caffeoyl – H] (or [quinic acid – H]) at *m/z* 191, [caffeoyl – H] at *m/z* 179 in ESI-negative mode, corresponded to the successive losses of two caffeoyl groups and a quinic acid moiety, respectively. In the ESI-positive mode $[M + H]^+$ at *m/z* 517 and the formation of sodium adduct ions $[M + Na]^+$ at *m/z* 539 was observed. The ion at *m/z* 499 was formed by a neutral loss of a NaOH from the $[M + Na]^+$ ion, or by cleavage of water from the protonated molecule at *m/z* 517.

In comparison of NMR spectral data (**Table-7.9**), **52** was identified as similar to the reported compound, 4,5-di-*O*-caffeoylquinic acid.[308,310]

Table-7.9:	^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of 4,5-di-*O*-caffeoylquinic acid **(52)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	76.0	C	--
2	39.8	CH_2	2.06, m
			2.28, m
3	71.5	CH	4.34, m
4	77.7	CH	5.12, (dd, 2.5, 90.8)
5	70.3	CH	5.69, m
6	39.8	CH_2	2.06, m
			2.28, m
7	174.0	C	--
1′	128.7	C	--
2′	116.2	CH	6.99, s
3′	147.8	C	--
4′	151.0	C	--
5′	117.4	CH	6.73, (d, 8.6)
6′	124.2	CH	6.89, m
7′	148.4	CH	7.59, (d, 15.8)
8′	116.0	CH	6.28, (d, 15.8)
9′	169.0	C	--
1″	128.7	C	--
2″	116.2	CH	7.01, s
3″	147.8	C	--
4″	151.0	C	--
5″	117.4	CH	6.73, (d, 8.6)
6″	124.2	CH	6.89, m
7″	148.7	CH	7.50, (d, 15.8)
8″	116.0	CH	6.18, (d, 15.8)
9″	169.0	C	--

7.1.2.9 2-(1,4-dihydroxy cyclohexanyl)-acetic acid (53)



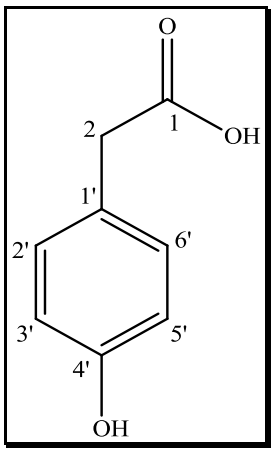
Compound **53** was isolated as white crystals.[319] The IR spectrum exhibited hydroxyl (3224 cm^{-1}), CH_2 (2942 cm^{-1}), -COOH (2500-3000 cm^{-1}) and carbonyl (1695 cm^{-1}) absorption bands. Its molecular formula ($C_8H_{14}O_4$) was determined by ^{1}H- and ^{13}C-NMR, IR and EI-MS analysis, which gave molecular ion adducts [M^+ – H_2O] at *m/z* 156.1, [M^+ – OH] at *m/z* 157.1 and [M^+ – 60] (MacLafferty Rearrangement)[320] at *m/z* 96.1, suggesting a molecular mass as 174 a.m.u.

The ^{13}C-NMR (BB and DEPT) spectra **(Table-7.10)** showed eight carbon signals. The carbon signal at δ_C 175.2, 35.7, 31.1 were assigned to the carbonyl carbon (C-1), two pairs of symmetrical carbons (C-3′,5′) and (C-2′,6′), respectively. This suggested that compound **53** has symmetrical structure. The carbon signals at δ_C 70.5 and 69.9 were indicated the two oxygen-containing carbons.

The NMR spectral data (**Table-7.10**) of compound **53** were in complete agreement to those reported in literature for 2-(1,4-dihydroxy cyclohexanyl)-acetic acid.[320]

Table-7.10:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of 2-(1,4-dihydroxy cyclohexanyl)-acetic acid **(53)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1′	70.5	C	--
2′	35.7	CH_2	1.53, m
			1.80, m
3′	31.1	CH_2	1.52, m
			1.70, m
4	69.9	C	3.53 (OH), m
5	31.1	CH_2	1.52, m
			1.70, m
6	35.7	CH_2	1.53, m
			1.80, m
1	175.2	C	--
2	47.6	CH_2	2.42, s

7.1.2.10 *p*-Hydroxyphenylacetic acid (54)



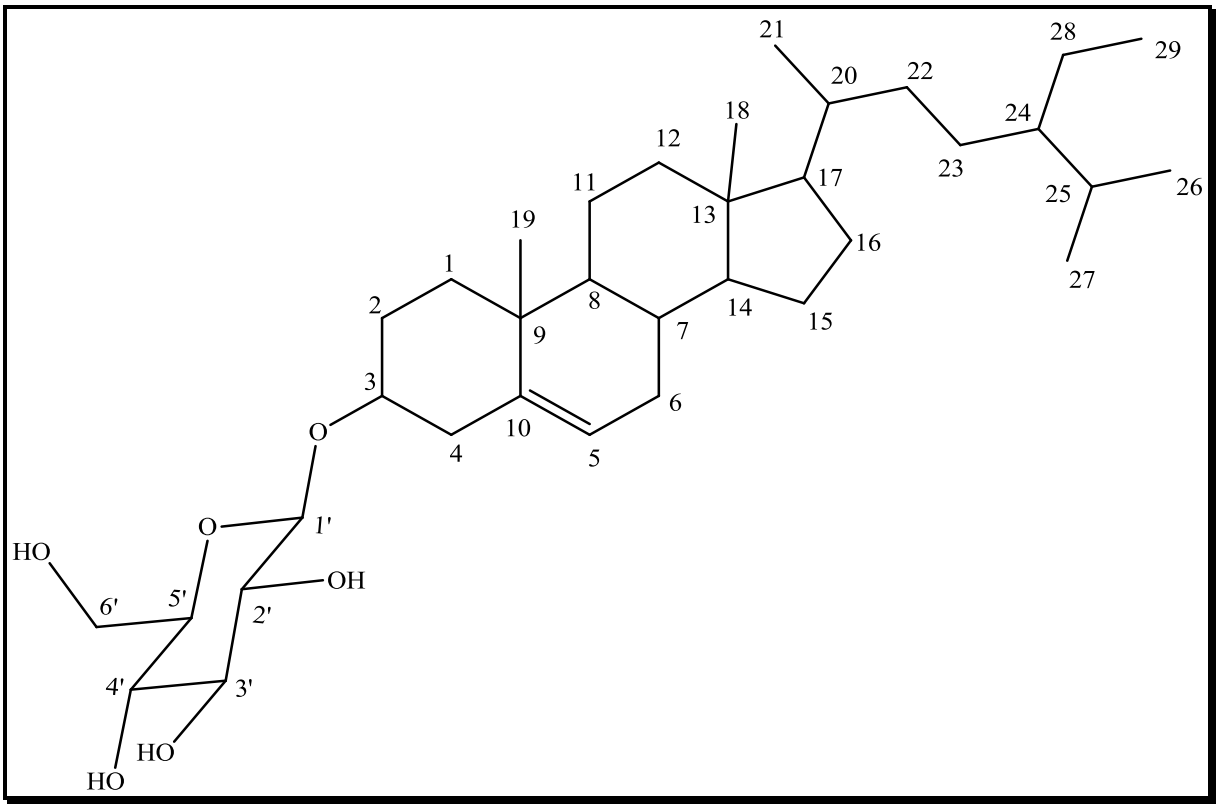
Compound **54** was obtained as colourless crystalline solid. The IR spectrum suggested the presence of hydroxyl (3390 cm^{-1}) and carbonyl (1715 cm^{-1}) groups. The molecular formula ($C_8H_8O_3$) was determined by NMR spectral analysis and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 153 in ESI-positive mode and $[M – H]^-$ at *m/z* 151 in ESI-negative mode.

^{1}H-NMR spectrum of compound **54** revealed the presence of *para*-substituted aromatic ring proton signals at δ_H 7.09 (2H, d, *J* = 8.6 Hz, H-2′,6′) and 6.72 (2H, d, *J*

= 8.6 Hz, H-3′,5′). ^{13}C-NMR (BB and DEPT) spectra showed the presence of a carbonyl carbon at δ_C 176.3, a metylene carbon at at δ_C 41.2, two quaternary carbons at 126.9 and 157.3 along with four methine carbons at 131.3 (C-2′,6′) and 116.1 (C-3′,5′). These data indicated that compound **54** was *p*-hydroxyphenylacetic acid by comparison of NMR spectral data (**Table-7.11**) with that reported in the earlier literature.[321]

Table-7.11:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of *p*-hydroxyphenylacetic acid **(54)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	176.3	C	--
2	41.2	CH_2	3.48, s
1′	126.9	C	--
2′	131.3	CH	7.09, (d, 8.6)
3′	116.1	CH	6.72, (d, 8.6)
4′	157.3	C	--
5′	116.1	CH	6.72, (d, 8.6)
6′	131.3	CH	7.09, (d, 8.6

7.1.2.11 *β*-Sitosterol-3-*O*-*β*-D-glucopyranoside (55)



Compound **55** was purified from dichloromethane fraction as white amorphous powder. The molecular mass was established at *m/z* 576.1023 from the FAB-MS showing the molecular formula $C_{60}H_{35}O_6$. The IR spectrum exhibited hydroxyl (3460 cm^{-1}) and trisubstituted double bonds (3050, 1650 and 815 cm^{-1}) absorption peaks. The EI-MS of **55** gave fragment ions *m/z* 396.1, 255.1, 175.1 and 145.1.[322,323]

The spectral data **(Table-7.12)** of **55** were identical to the reported values for *β*-sitosterol-3-*O*-*β*-D-glucopyranoside.[324,325]

Table-7.12:	^{1}H- (500 MHz) and ^{13}C- (100 MHz) NMR data of *β*-sitosterol-3-*O*-*β*-D-glucopyranoside **(55)** in pyridine		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	37.5	CH_2	1.63, m
			1.77, m
2	28.6	CH_2	1.27, m
			1.86, m
3	78.1	CH	3.93, m
4	39.2	CH_2	2.46, m
			2.72, m
5	140.9	C	--
6	121.8	CH	5.34, m
7	32.4	CH_2	1.34, m
			1.88, m
8	32.2	CH	1.51, m
9	50.3	CH	0.91, m
10	36.9	C	--
11	21.2	CH_2	1.36, m
			1.42, m
12	40.0	CH_2	1.16, m
			1.94, m
13	42.5	C	--
14	56.9	CH	1.05, m
15	24.6	CH_2	1.20, m
			1.55, m
16	26.5	CH_2	1.22, m
			1.24, m
17	56.2	CH	0.92, m
18	11.9	CH_3	0.66, s
19	19.4	CH_3	1.06, s
20	36.4	CH	1.47, m
21	19.0	CH_3	1.02, (d, 6.2)
22	34.1	CH_2	1.08, m
			1.38, m
23	30.2	CH_2	1.69, m
24	46.0	CH	0.98, m
25	29.2	CH	1.76, m
26	19.2	CH_3	0.83, (d, 6.5)
27	19.9	CH_3	0.99, (d, 6.5)
28	23.5	CH_2	1.27, m
29	12.1	CH_3	0.88, (t, 6.8)
1′	102.5	CH	5.05, (d, 7.5)
2′	75.4	CH	4.06, m
3′	78.2	CH	4.29, m
4′	71.7	CH	4.27, m
5′	78.6	CH	3.98, m
6′	62.9	CH_2	4.42, (dd, 2.5, 8.3)
			4.55, (dd, 1.3, 8.3)

7.1.3 Biological studies

7.1.3.1 Anti-bacterial assay

n-Hexane, dichloromethane, ethyl acetate and methanol fractions of the aerial parts of *S. desfontainei* were tested for their anti-bacterial assay. All the fractions showed no activity against six selected bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* at the tested concentration (03 μg/mL) (**Table-7.13**). Concentration of stock solution 3mg/mL and 100 μL was used for assay.

Table-7.13:	Anti-bacterial assay of various fractions from the aerial parts of *S. desfontainei*					
Aerial parts						
Fractions	***E. c***	***B. s***	***S. f***	***S. a***	***P. a***	***S. t***
F1	x	x	x	x	x	x
F2	x	x	x	x	x	x
F3	x	x	x	x	x	x
F4	x	x	x	x	x	x
DMSO (–)	x	x	x	x	x	x
Imipenum 10μg/Disc (+)	35	36	36	43	32	40
Keywords:	well size = 6mm, F1=*n*-hexane, F2=DCM, F3= EtOAc, F4 = MeOH, *E. c = Escherichia coli, B. s = Bacillus subtilis, S. f = Shigella flexenari, S. a = Staphylococcus aureus, P. a = Pseudomonas aeruginosa, S. t = Salmonella typhi*					

7.1.3.2 Anti-fungal assay

All the fractions (*n*-hexane, dichloromethane, ethyl acetate and methanol) isolated from the methanol extract of the aerial parts of *S. desfontainei* showed non-significant activity against five selected fungal strains, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *candida glabrata* **(Table-7.14)**.

Table-7.14:	Anti-fungal assay of various fractions from the aerial parts of *S. desfontainei*		
Fractions	**Name of Fungus**	**% inhibition**	**Std. Drug Mic (μg/mL)**
F1	*C. a*	0	Miconazole (110.8)
	A. f	10	Amphotericin B (20.20)
	M. c	20	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F2	*C. a*	0	Miconazole (110.8)
	A. f	0	Amphotericin B (20.20)
	M. c	15	Miconazole (98.4)
	F. s	10	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F3	*C. a*	0	Miconazole (110.8)
	A. f	10	Amphotericin B (20.20)
	M. c	20	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F4	*C. a*	0	Miconazole (110.8)
	A. f	05	Amphotericin B (20.20)
	M. c	15	Miconazole (98.4)
	F. s	20	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH, *C. a = Candida albicans, A. f = Aspergillus flavus, M. c =Microsporum canis,F. s = Fusarium solani, C. g= Candida glabrata*		

7.1.3.3 Phytotoxicity

Various fractions (*n*-hexane, dichloromethane, ethyl acetate and methanol) obtained from the methanolic extract of the aerial parts of *S. desfontainei* were tested on *Lemna minor* plant for their *in vitro* phytotoxic bioassay. *n*-Hexane fraction showed significant activity while EtOAc fraction showed moderate activity at highest doses. The dichloromethane and methanol fractions showed low activity (**Table-7.15**).

Table-7.15:	Phytotoxicity of the various fractions from the aerial parts of *S. desfontainei*				
Extract/ Fraction	**Compound Conc. (*µ*g/mL)**	**No. of fronds**		**% growth regulation**	**Std. Drug Conc. (*µ*g/mL)**
		Sample	**Control**		
F1	1000	04	20	80	0.015
	100	16		20	
	10	18		10	
F2	1000	13	20	35	0.015
	100	15		25	
	10	19		05	
F3	1000	12	20	40	0.015
	100	17		15	
	10	19		05	
F4	1000	14	20	32	0.015
	100	16		20	
	10	18		10	
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH				

7.1.3.4 Insecticidal assay

n-Hexane, dichloromethane, ethyl acetate and methanol fractions of the aerial parts of *S. desfontainei* were tested for insecticidal activity through contact toxicity method. *n*-Hexane and methanol fractions showed moderate activity against insect *Rhyzopertha dominica*, while dichloromethane and EtOAc fractions were shown non-significant (**Table-7.16**).

Table-7.16:	Insecticidal assay of the various fractions from the aerial parts of *S. desfontainei*	
Fractions	**Name of Insects**	**% Mortality**
F1	*Tribolium castaneum*	0
	Rhyzopertha dominica	40
	Callosbruchus analis	0
F2	*Tribolium castaneum*	0
	Rhyzopertha dominica	20
	Callosbruchus analis	0
F3	*Tribolium castaneum*	0
	Rhyzopertha dominica	20
	Callosbruchus analis	0
F4	*Tribolium castaneum*	20
	Rhyzopertha dominica	40
	Callosbruchus analis	0
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH	

7.1.3.5 Brine shrimp (*Artemia salina*) lethality bioassay

All the fractions (*n*-hexane, dichloromethane, ethyl acetate and methanol) isolated from the methanolic extract of the aerial parts of *S. desfontainei* showed no cytotoxicity **(Table-7.17)**.

Table-7.17:	Brine Shrimp (*Artemia salina*) Lethality bioassay of the various fractions from the aerial parts of *S. desfontainei*					
Fractions	**Dose (*μ*g/mL)**	**No. of shrimps**	**No. of survivors**	**LD50 (*μ*g/mL)**	**STD. Drug**	**LD50 (*μ*g/mL)**
F1	1000	29	21	9132.20	Etoposide	7.4625
	100	29	27			
	10	29	28			
F2	1000	29	22	357277	Etoposide	7.4625
	100	29	25			
	10	29	26			
F3	1000	29	21	6170.64	Etoposide	7.4625
	100	29	25			
	10	29	29			
F4	1000	29	29	0.6903	Etoposide	7.4625
	100	29	28			
	10	29	24			
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH					

7.1.3.6 *α,α*-Diphenyl-*β*-picrylhydrazyl (DPPH) radical scavenging activity

Compounds (**44-54**) isolated from the aerial parts of *S. desfontainei* and ascorbic acid taken as standard were tested for antioxidant acitvity. Compounds **45, 46, 47, 49** and **52** have a significant potent radical-scavenging effect comparable to that of ascorbic acid, which is almost complete scavenging at a concentration of 250-500 μM (95- 97% scavenging for compounds **45, 46, 47, 49** and **52**, as compared to 98.2% scavenging for ascorbic acid) and > 48% scavenging was still observed at 5 μM (**Figure-7.2** & **Table -7.18**). Compounds **44, 48, 50,** and **51** were more reactive at lower concentrations and less reactive at higher concentrations while compounds **53** and **54** showed activities significantly less than ascorbic acid at higher concentration but still comparable to that of ascorbic acid at lower concentrations (5-30 μM).

Table-7.18 Antioxidant activities of pure compounds isolated from the aerial parts of *S. desfontanii* showing % scavenging at different concentrations and 50% inhibitory concentrations (IC_{50}) in the DPPH radical scavenging assay[a].

Compound	% Scavenging[b]							
	5[c]	15	30	60	125	250	500	IC_{50}
44	49.2 ± 1.6	51.6 ± 3.1	52.6 ± 2.1	53.5 ± 2.1	57.3 ± 2.1	61.3 ± 2.9	71.4 ± 1.5	13.8
45	52.9 ± 2.8	57.5 ± 3.0	66.2 ± 4.1	77.7 ± 2.1	95.5 ± 1.0	95.5 ± 2.1	96.1 ± 0.7	N.C[f]
46	51.2 ± 2.7	57.0 ± 3.5	64.3 ± 2.0	78.4 ± 2.1	93.9 ± 1.8	94.1 ± 1.8	95.3 ± 1.0	N.C
47	48.2 ± 1.7	51.3 ± 1.7	57.3 ± 2.1	66.4 ± 2.9	83.5 ± 2.2	93.2 ± 2.7	95.3 ± 1.1	8.3
48	46.4 ± 2.9	46.4 ± 2.1	48.2 ± 1.7	49.2 ± 0.4	51.3 ± 2.7	59.4 ± 3.1	77.5 ± 2.1	75.5
49	46.5 ± 0.8	51.5 ± 2.0	54.6 ± 3.1	62.3 ± 1.9	75.4 ± 1.1	96.1 ± 0.7	96.4 ± 1.6	7.7
50	46.3 ± 1.6	46.4 ± 1.2	47.4 ± 2.8	49.9 ± 1.7	47.8 ± 3.0	52.0 ± 1.9	56.6 ± 2.1	166.5
51	46.1 ± 2.4	46.6 ± 0.9	46.6 ± 2.1	48.3 ± 1.7	52.3 ± 3.9	54.2 ± 3.1	61.6 ± 2.9	114.6
52	50.3 ± 2.7	54.3 ± 3.2	65.3 ± 2.2	78.6 ± 3.0	96.4 ± 1.2	96.6 ± 0.9	96.6 ± 0.8	N.C
53	43.6 ± 1.7	43.4 ± 1.8	43.7 ± 1.2	44.4 ± 2.6	44.5 ± 2.1	44.8 ± 3.1	45.4 ± 1.3	N.C
54	46.1 ± 2.8	46.3 ± 1.9	47.3 ± 3.1	47.8 ± 1.2	48.4 ± 1.4	49.1 ± 2.8	49.4 ± 1.9	N.C
Asorbic acid (AA)[d]	43.0 ± 0.8	45.7 ± 1.1	46.7 ± 2.4	57.5 ± 0.9	73.6 ± 2.2	98.1 ± 0.5	98.2 ± 0.7	33.8 ± 0.9

[a]Values are mean ± SD (n = 3). [b]Scavenging % = 100-(A sample[e] x 100/A control[e]). [c]Concentrations in μmol/L. [d]Ascorbic acid used as a standard (AA). [e]Absorbance of sample and control measured at 517 nm. [f]N.C = Not calculated

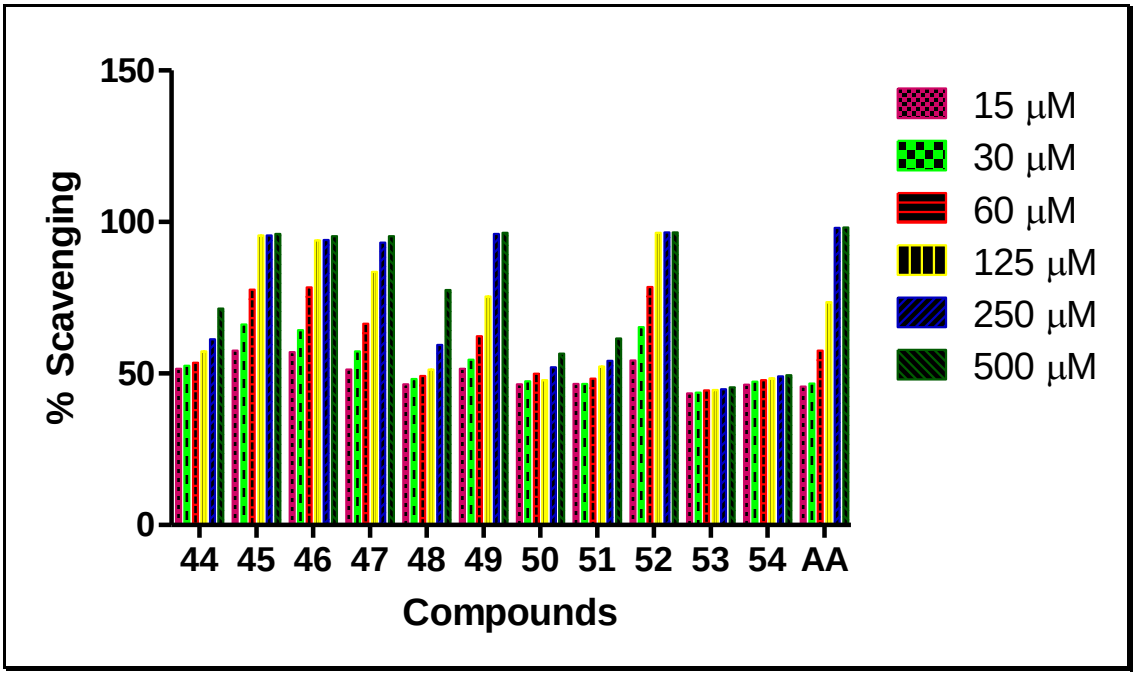


Figure-7.2: DPPH radical scavenging activities of compounds (**44-54**)

7.2 *Senecio chrysanthemoides*

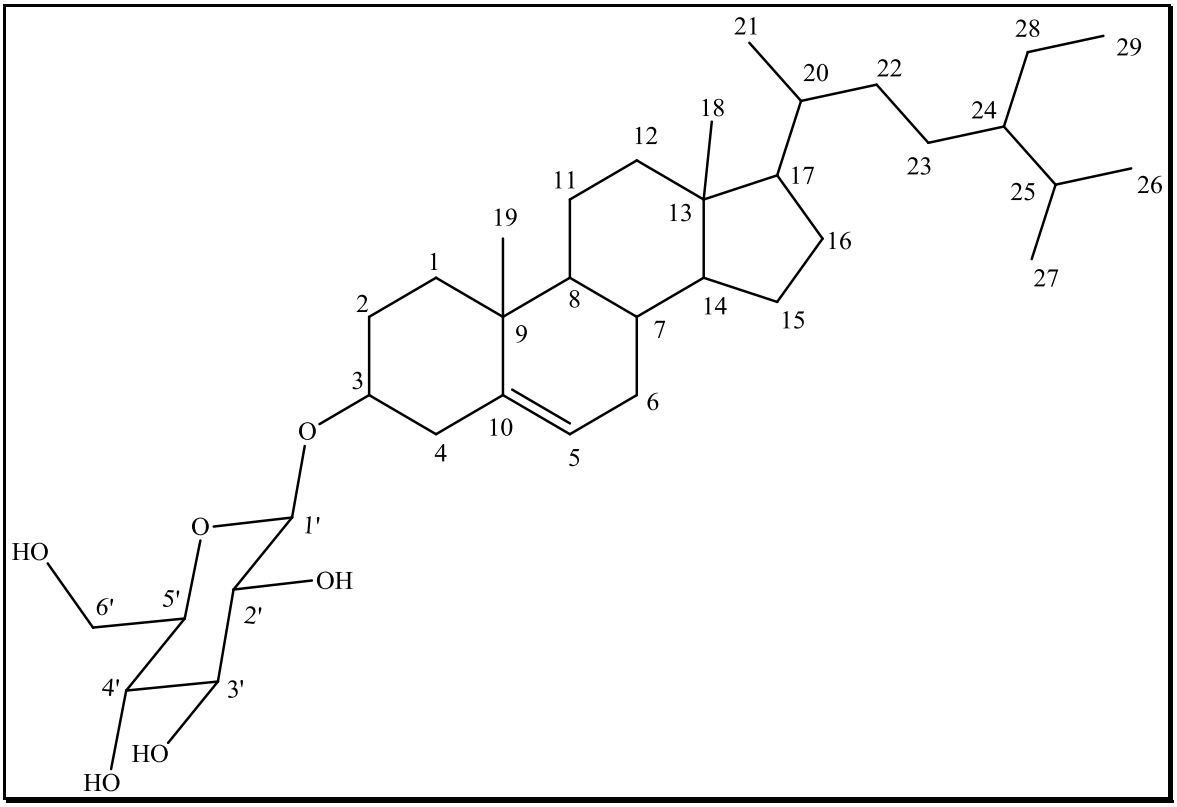
Phytochemical investigations on the aerial parts of *S. chrysanthemoides* have resulted in the isolation and structure elucidation of six known compounds **(56-61)**. All these compounds were characterized by advanced spectroscopic techniques.



Senecio chrysanthemoides

7.2.1 Structure elucidation of compounds isolated for the first time from *S. chrysanthemoides*

7.2.1.1 *β*-Sitosterol-3-*O*-*β*-D-glucopyranoside (56)

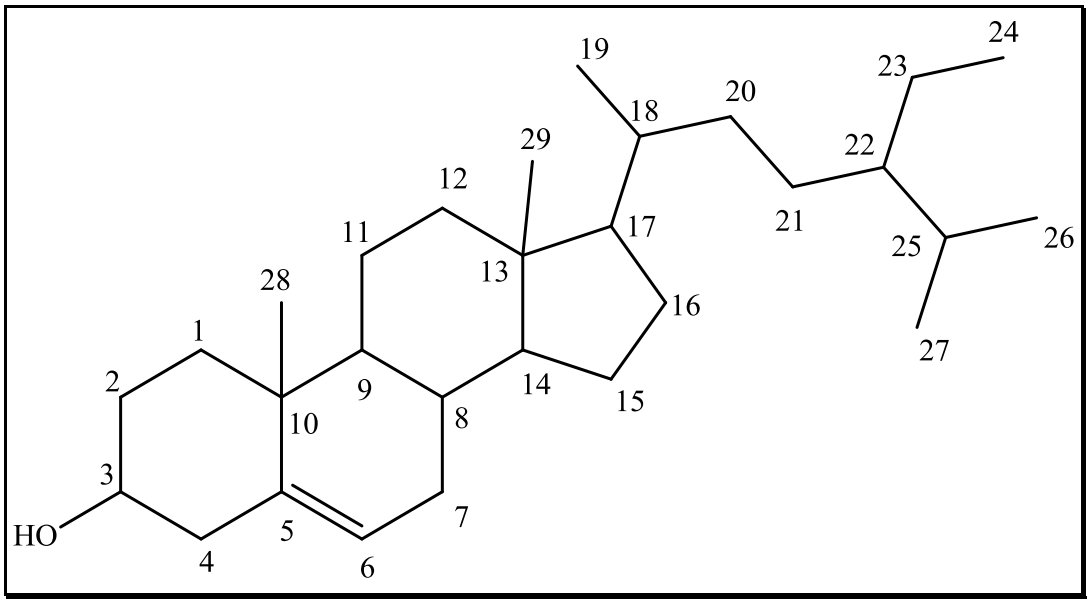


Compound **56** was isolated as white amorphous powder. The molecular mass was established at *m/z* 576.1029 from the FAB-MS showing the molecular formula $C_{60}H_{35}O_6$. The IR spectrum exhibited hydroxyl (3432 cm^{-1}) and trisubstituted double bonds (3046, 1665 and 815 cm^{-1}) absorption bands. The EI-MS of **56** gave characteristic mass fragment ions at *m/z* 396.1, 255.1, 175.1, and 145.1.[322,323]

The NMR data (**Table-7.19**) of compound **56** were in agreement to the repoerted values for *β*-sitosterol-3-*O*-*β*-D-glucopyranoside.[324,325]

Table-7.19:	^{1}H- (500 MHz) and ^{13}C- (100 MHz) NMR data of *β*-sitosterol-3-*O*-*β*-D-glucopyranoside **(56)** in pyridine		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	39.3	CH_2	1.56, m
			1.83, m
2	27.2	CH_2	1.30, m
			1.81, m
3	77.0	CH	3.94, m
4	39.8	CH_2	2.38, m
			2.78, m
5	141.4	C	--
6	119.0	CH	5.41, m
7	34.1	CH_2	1.25, m
			1.97, m
8	32.8	CH	1.44, m
9	52.1	CH	0.95, m
10	35.6	C	--
11	23.1	CH_2	1.46, m
12	40.7	CH_2	1.23, m
			1.90, m
13	41.2	C	--
14	55.6	CH	1.15, m
15	24.6	CH_2	1.10, m
			1.62, m
16	26.2	CH_2	1.30, m
17	56.8	CH	0.94, m
18	12.3	CH_3	0.68, s
19	19.8	CH_3	1.16, s
20	38.0	CH	1.40, m
21	20.4	CH_3	1.07, (d, 6.5)
22	35.3	CH_2	1.14, m
			1.33, m
23	30.7	CH_2	1.62, m
24	46.2	CH	1.03, m
25	29.9	CH	1.72, m
26	19.1	CH_3	0.87, (d, 6.0)
27	19.5	CH_3	0.92, (d, 6.0)
28	23.0	CH_2	1.37, m
29	12.6	CH_3	0.81, (t, 6.8)
1′	103.6	CH	5.11, (d, 7.5)
2′	74.3	CH	4.13, m
3′	77.7	CH	4.25, m
4′	72.4	CH	4.21, m
5′	78.1	CH	3.94, m
6′	62.5	CH_2	4.48, (dd, 2.2, 8.6)
			4.45, (dd, 1.5, 8.6)

7.2.1.2 *β*-Sitosterol (57)

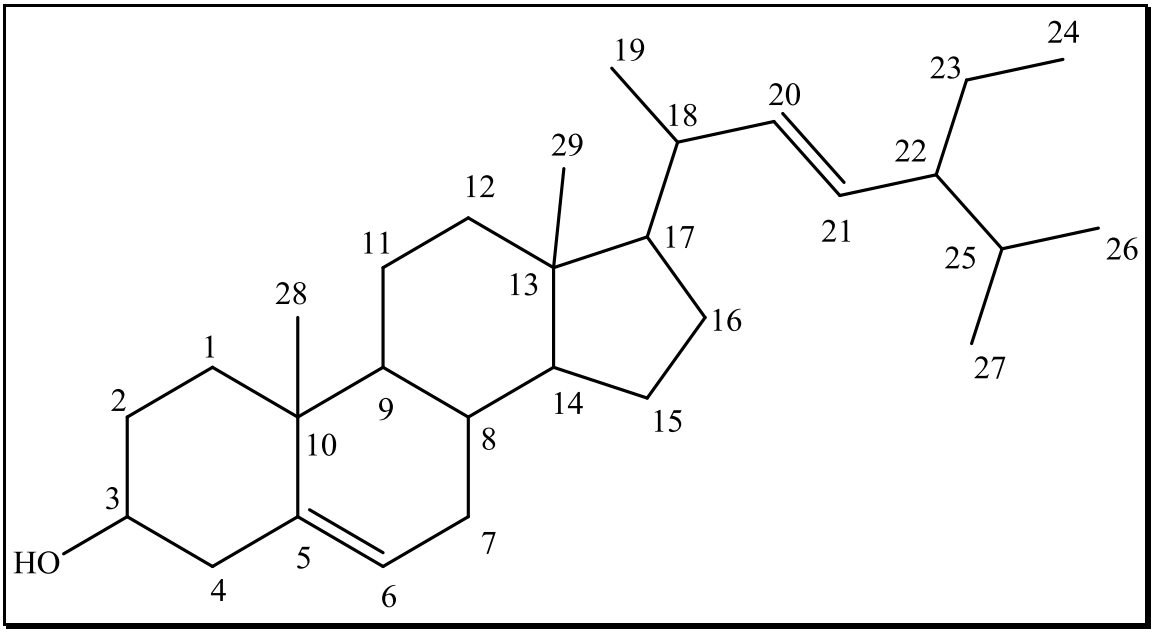


Compound **57** was obtained as colourless crystals from the ethyl acetate fraction. Its molecular mass was established at *m/z* 414.1 from the EI-MS showing the molecular formula $C_{29}H_{50}O$. Characteristic mass fragments were also observed at *m/z* = 367.1, 255.1, 175.1, 133.1 and 105.1. The IR spectrum exhibited hydroxyl (3445 cm^{-1}), olefinic CH stretching (3060 cm^{-1}) and double bond (1650 cm^{-1}) absorption bands.

The chemical shifts (**Table-7.20**) of compound **57** were similar to the reported values for *β*-sitosterol.[326]

Table-7.20:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of *β*-sitosterol **(57)** in $CDCl_3$		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	337.4	CH_2	1.56, m
			1.62, m
2	28.2	CH_2	1.41, m
			1.73, m
3	78.4	CH	3.30, m
4	38.1	CH_2	2.38, m
			2.42, m
5	141.9	C	--
6	122.6	CH	5.11, m
7	33.2	CH_2	1.31, m
			1.76, m
8	33.8	CH	1.52, m
9	51.6	CH	0.97, m
10	37.5	C	--
11	20.2	CH_2	1.28, m
			1.49, m
12	42.1	CH_2	1.36, m
			1.84, m
13	39.0	C	--
14	56.7	CH	1.12, m
15	24.2	CH_2	1.29, m
			1.58, m
16	26.2	CH_2	1.21, m
17	55.8	CH	0.98, m
18	38.4	CH	1.58, m
19	19.8	CH_3	1.15, (d, 6.3)
20	34.8	CH_2	1.12, m
			1.24, m
21	29.5	CH_2	1.73, m
22	40.2	CH	0.96, m
23	24.1	CH_2	1.25, m
24	12.6	CH_3	0.85, (t, 6.5)
25	29.2	CH	1.65, m
26	20.2	CH_3	0.90, (d, 6.0)
27	19.5	CH_3	0.92, (d, 6.0)
28	20.8	CH_3	1.17, s
29	11.8	CH_3	0.67, s

7.2.1.3 Stigmasterol (58)



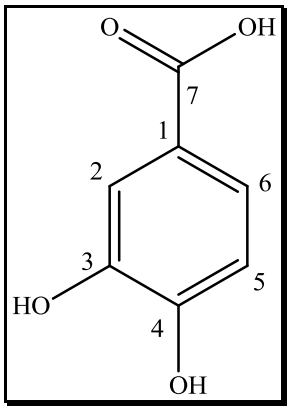
Compound **58** was isolated from ethyl acetate fraction as colourless crystals. Its molecular mass was established as 412.1 from the EI-MS indicating the molecular formula $C_{29}H_{48}O$. The IR spectrum exhibited hydroxyl (3380 cm^{-1}), olefinic CH stretching (3075 cm^{-1}) and double bond (1635 cm^{-1}) absorptions bands.

The NMR data (**Table-7.21**) of compound **58** well matched with the reported compound, stigmasterol.[326]

Table-7.21:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of stigmasterol **(58)** in $CDCl_3$		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	37.4	CH_2	1.56, m
			1.62, m
2	27.8	CH_2	1.40, m
			1.82, m
3	27.8	CH	3.22, m
4	39.5	CH_2	2.44, m
			2.53, m
5	142.7	C	--
6	120.2	CH	5.26, m
7	33.6	CH_2	1.37, m

			1.80, m
8	32.4	CH	1.60, m
9	51.2	CH	0.96, m
10	36.6	C	--
11	21.5	CH_2	1.33, m
			1.38, m
12	41.3	CH_2	1.28, m
			1.77, m
13	43.2	C	--
14	56.5	CH	1.15, m
15	25.6	CH_2	1.22, m
			1.48, m
16	26.7	CH_2	1.29, m
			1.33, m
17	55.3	CH	0.94, m
18	39.7	CH	2.25, m
19	20.2	CH_3	1.10, (d, 6.4)
20	138.2	CH	4.81, m
21	127.6	CH	5.03, m
22	45.8	CH	1.05, m
23	24.1	CH_2	1.25, m
24	13.3	CH_3	0.88, (t, 6.5)
25	28.7	CH	1.70, m
26	20.2	CH_3	0.90, (d, 6.3)
27	19.5	CH_3	0.94, (d, 6.3)
28	20.1	CH_3	1.20, s
29	12.5	CH_3	0.72, s

7.2.1.4 3,4-Dihydroxybenzoic acid (59)



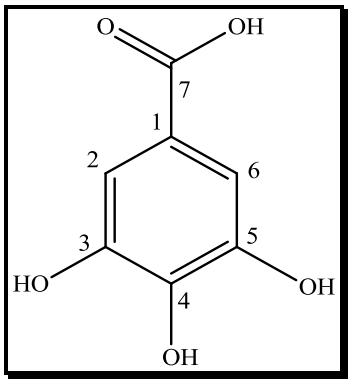
The ethyl acetate fraction afforded compound **59** as white powder. The IR spectrum exhibited hydroxyl (3390 cm^{-1}), C–H aromatic (3315 cm^{-1}), carbonyl (1710

cm^{-1}) and C=C aromatic (1627 cm^{-1}) absorption bands. Its molecular formula ($C_7H_6O_4$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS, which gave molecular ion adducts $[M + H]^+$ at *m/z* 155 and $[M + Na]^+$ at 177 in ESI-positive mode and $[M – H]^-$ at *m/z* 153 in ESI-negative mode, suggesting a molecular mass 154 a.m.u.

The NMR spectral data (**Table-7.22**) of **59** was quite identical to 3,4-dihydroxybenzoic acid.[143]

Table-7.22:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of 3,4-dihydroxybenzoic acid **(59)** in CD_3OD		
C. No.	δ_C	**Multiplicity**	δ_H**, (multiplicity,** ***J*** **in Hz)**
1	123.6	C	--
2	118.2	CH	7.43, (d, 1.8)
3	146.8	C	--
4	152.1	C	--
5	116.5	CH	6.74, (d, 8.2)
6	124.7	CH	7.48, (dd, 1.8, 8.2)
7	171.5	C	--

7.2.1.5 Gallic acid (60)



Compound **60** was obtained as white crystals. The IR spectrum exhibited hydroxyl (3410 cm^{-1}) and carbonyl (1700 cm^{-1}) absorption bands. Its molecular formula ($C_7H_6O_5$) was determined by ^{1}H- and ^{13}C-NMR analysis and LC-ESIMS,

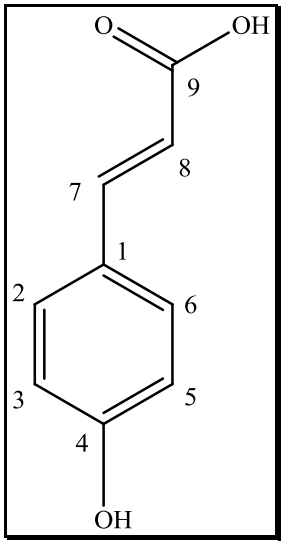
which gave molecular ion adducts $[M + H]^+$ at *m/z* 171 and $[M + Na]^+$ at 193 in ESI-positive mode and $[M – H]^-$ at *m/z* 169 in ESI-negative mode, suggesting a molecular mass of 170 a.m.u.

The ^{1}H-NMR spectrum of **60** showed the aromatic signal at δ_H 7.21 (2H, s, H-2,6). The ^{13}C-NMR (BB and DEPT) spectra of **60** showed a carbonyl carbon at δ_C 172.0 (C-7), four quaternary carbons at δ_C 121.5 (C-1), 146.8 (C-3,5), 141.7 (C-4), and two methine carbons at δ_C 112.2 (C-2,6).

The NMR data (**Table-7.23**) of **60** matched the reported data of gallic acid.[143]

Table-7.23:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of gallic acid **(60)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	121.5	C	--
2	112.2	CH	7.21, s
3	146.8	C	--
4	141.7	C	--
5	146.8	C	--
6	112.2	CH	7.21, s
7	172.0	C	--

7.2.1.6 *p*-Coumaric acid (61)



Compound **61** was isolated as white powder. The IR spectrum exhibited hydroxyl (3433 cm^{-1}) and carbonyl (1718 cm^{-1}) absorption bands. Its molecular formula ($C_9H_8O_3$) was determined by ^{1}H- and ^{13}C-NMR analysis and ESIMS with molecular ion adducts $[M + H]^+$ at *m/z* 165.1 and $[M + Na]^+$ at 187.1 in ESI-positive mode and $[M – H]^-$ at *m/z* 163.1 in ESI-negative mode.

The ^{1}H-NMR spectrum of **61** showed aromatic signals at δ_H 7.56 (2H, d, *J* = 7.8 Hz, H-2,6) and at δ_H 6.93 (2H, d, *J* = 7.8 Hz, H-3,5) which indicating a di-substituted benzene ring. Olefinic signal at δ_H 7.58 (1H, d, *J* = 15.5 Hz, H-7) and δ_H 6.23 (1H, d, *J* = 15.5 Hz, H-8) with the corresponding carbon signals of these protons in ^{13}C-NMR spectrum at δ_C 146.2 and 117.7 along with δ_C 172.3 for carboxyl group confirmed the presence of 2-propenoic acid moiety having *trans* configuration in the molecule.

The chemical shifts (**Table-7.24**) of **61** were found similar to the reported values for coumaric acid.[144,145]

Table-7.24:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of coumaric acid **(61)** in CD_3OD		
C. No.	**δ_C**	**Multiplicity**	**δ_H, (multiplicity, *J* in Hz)**
1	128.6	C	--
2	133.8	CH	7.56, (d, 7.8)
3	115.4	CH	6.93, (d, 7.8)
4	157.4	C	--
5	115.4	CH	6.93, (d, 7.8)
6	133.8	CH	7.56, (d, 7.8)
7	147.5	CH	7.58, (d, 15.5)
8	117.7	CH	6.23, (d, 15.5)
9	172.3	C	--

7.2.2 Biological studies

7.2.2.1 Anti-bacterial assay

All the fractions (*n*-hexane, dichloromethane, ethyl acetate and methanol) isolated from the methanolic extract of the aerial parts of *S. chrysanthemoides* showed no activity against six selected bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* at the tested concentration (03 μg/mL) (**Table-7.25**). Concentration of stock solution 3mg/mL and 100 μL was used for assay.

Table-7.25:	Anti-bacterial assay of various fractions from the aerial parts of *S. chrysanthemoides*					
Aerial parts						
Fractions	***E. c***	***B. s***	***S. f***	***S. a***	***P. a***	***S. t***
F1	x	x	x	x	x	x
F2	x	x	x	x	x	x
F3	x	x	x	x	x	x
F4	x	x	x	x	x	x
DMSO (–)	x	x	x	x	x	x
Imipenum 10μg/Disc (+)	35	36	36	43	32	40
Keywords:	well size = 6mm, F1=*n*-hexane, F2=DCM, F3= EtOAc, F4 = MeOH, *E. c = Escherichia coli*, *B. s = Bacillus subtilis*, *S. f = Shigella flexenari*, *S. a = Staphylococcus aureus*, *P. a = Pseudomonas aeruginosa*, *S. t = Salmonella typhi*					

7.2.2.2 Anti-fungal assay

n-Hexane, dichloromethane, ethyl acetate and methanol fractions of the aerial parts of *S. chrysanthemoides* were tested for anti-fungal assay. All of them showed non-significant activity against five selected fungal strains, *Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *candida glabrata* (**Table-7.26)**

Table-7.26:	Anti-fungal assay of various fractions from the aerial parts of *S. chrysanthemoides*		
Fractions	**Name of Fungus**	**% inhibition**	**Std. Drug Mic (μg/mL)**
F1	*C. a*	0	Miconazole (110.8)
	A. f	0	Amphotericin B (20.20)
	M. c	20	Miconazole (98.4)
	F. s	20	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F2	*C. a*	0	Miconazole (110.8)
	A. f	10	Amphotericin B (20.20)
	M. c	15	Miconazole (98.4)
	F. s	25	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F3	*C. a*	0	Miconazole (110.8)
	A. f	10	Amphotericin B (20.20)
	M. c	20	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F4	*C. a*	0	Miconazole (110.8)
	A. f	0	Amphotericin B (20.20)
	M. c	20	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH, *C. a = Candida albicans, A. f = Aspergillus flavus, M. c =Microsporum canis, F. s = Fusarium solani, C. g= Candida glabrata*		

7.2.2.3 Phytotoxicity

Various fractions obtained from the methanolic extract of the aerial parts of *S. chrysanthemoides* were tested for their *in vitro* phytotoxic bioassay, applied on *Lemna minor* plant. *n*-Hexane and methanol fractions showed significant activity while dichloromethane and EtOAc fractions showed moderate activity at highest doses (**Table-7.27**).

Table-7.27:	Phytotoxicity of the various fractions from the aerial parts of *S. chrysanthemoides*				
Extract/ Fraction	**Compound Conc. (*μ*g/mL)**	**No. of fronds**		**% growth regulation**	**Std. Drug Conc. (*μ*g/mL)**
		Sample	**Control**		
F1	1000	06	20	70	0.015
	100	17		15	
	10	19		05	
F2	1000	12	20	40	0.015
	100	14		30	
	10	17		15	
F3	1000	11	20	45	0.015
	100	15		25	
	10	19		05	
F4	1000	08	20	60	0.015
	100	16		20	
	10	18		10	
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH				

7.2.2.4 Insecticidal assay

Insecticidal assay of the *n*-hexane, dichloromethane, ethyl acetate and methanol fractions of the aerial parts of *S. chrysanthemoides* was carried out through contact toxicity method. All fractions were non-significant (**Table-7.28**).

Table-7.28:	Insecticidal assay of the various fractions from the aerial parts of *S. chrysanthemoides*	
Fractions	**Name of Insects**	**% Mortality**
F1	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	0
F2	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	0
F3	*Tribolium castaneum*	20
	Rhyzopertha dominica	20
	Callosbruchus analis	0
F4	*Tribolium castaneum*	0
	Rhyzopertha dominica	20

	Callosbruchus analis	20
Keywords:	F1 = *n*-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH	

7.2.2.5 Brine Shrimp (*Artemia salina*) Lethality bioassay

n-Hexane, dichloromethane, ethyl acetate and methanol fractions of the aerial parts of *S. chrysanthemoides* showed cytotoxicity at highest doses **(Table-7.29).**

Table-7.29:	Brine Shrimp (*Artemia salina*) Lethality bioassay from the aerial parts of *S. chrysanthemoides*					
Fractions	**Dose (µg/mL)**	**No. of shrimps**	**No. of survivors**	**LD50 (µg/mL)**	**STD. Drug**	**LD50 (µg/mL)**
F1	1000	29	17	0.0428	Etoposide	7.4625
	100	29	21			
	10	29	23			
F2	1000	29	17	2924.28	Etoposide	7.4625
	100	29	21			
	10	29	25			
F3	1000	29	14	732.35	Etoposide	7.4625
	100	29	22			
	10	29	28			
F4	1000	29	15	1080.69	Etoposide	7.4625
	100	29	22			
	10	29	26			
Keywords:	F1 = n-hexane, F2 = DCM, F3 = EtOAc, F4 = MeOH					

8.1 General experimental

Optical rotations were measured using an ADP 220 polarimeter at 589 nm. IR spectra were obtained by a Perkin-Elmer FTIR instrument for compounds mounted directly on the diamond cell. NMR spectra were recorded on Varian VNMRS-400 and Varian VNMRS-500 spectrometers with TMS as internal standard while CD_3OD and $CDCl_3$ as solvent. HRESIMS were carried out on an APEX IV Fourier-transform Ion Cyclotron resonance instrument (Bruker Daltonics, Coventry, UK) using an Apollo ESI source. The nebulizer and drying gas was nitrogen at 180 ^oC. Samples were infused from solution (50% MeOH/DCM) by syringe pump at 100 microlitres per hour. Accurate masses to within 5 ppm were obtained by a broad range external calibration using a mixture of PEG polymer grades. Reverse phase HPLC was conducted employing a Waters mass-directed auto-purification system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex LUNA column (5μ, C_{18}, 100 Å, 10 x 250 mm) equipped with a Phenomenex Security Guard precolumn (Luna C_5 300 Å) eluting at 4 mL/min. Solvent A, HPLC grade H_2O + 0.05% formic acid; Solvent B, HPLC grade CH_3CN + 0.045% formic acid or HPLC grade CH_3OH + 0.045% formic acid. The post-column flow was split (100:1) and the minority flow was made up with MeOH + 0.045% formic acid to 1 mL/min for simultaneous analysis by diode array (Waters 2998), evaporative light scattering (Waters 2424) and ESI mass spectrometry in positive and negative modes (Waters Quatro Micro). Silica gel (70-230 mesh and 220-440 mesh) was used for column chromatography and Merck keiselgel 60 PF_{254} pre-coated silica gel glass plates were used for analytical and preparative TLC. TLCs were visualized by UV light (λ = 254 nm, 366 nm) and by heating after spraying with 15% aqueous sulfuric acid saturated with ceric sulfate.

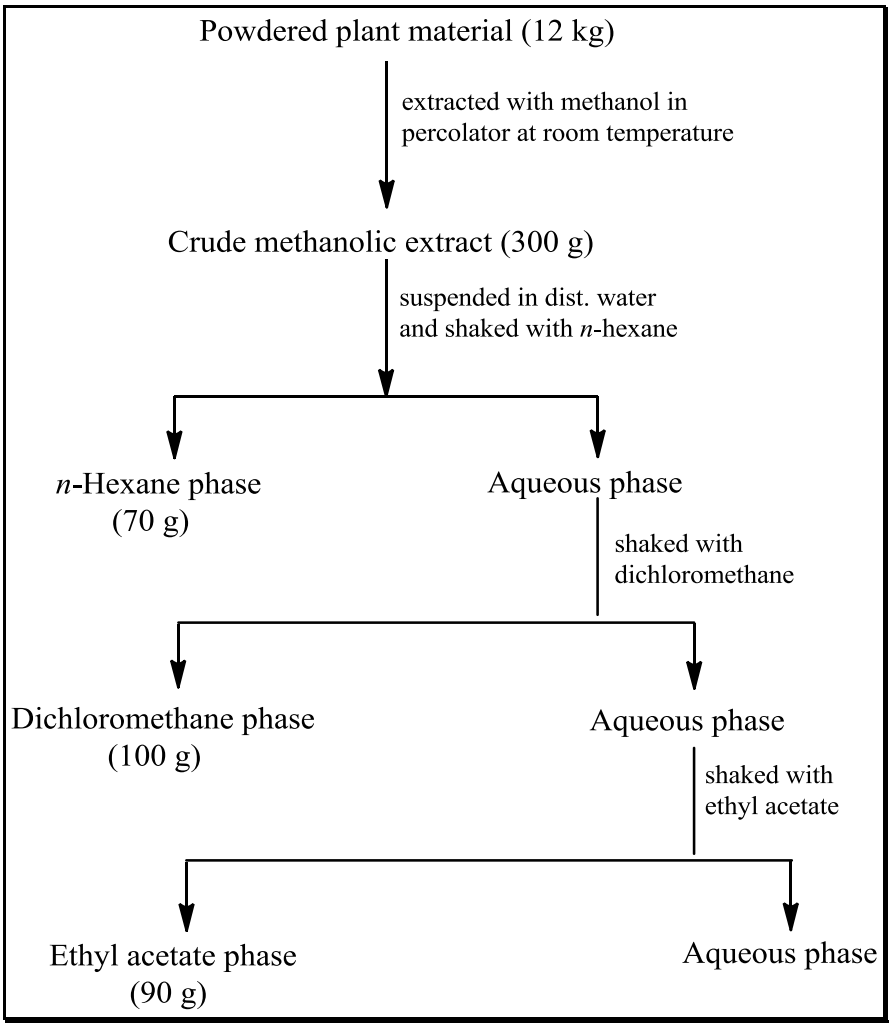
8.2 *Senecio desfontainei*

8.2.1 Plant material

The plant (*S. desfontainei*) was collected from Kaghan, K.P.K., Pakistan, in August 2008, which was identified and authenticated by Prof. Dr. Habib Ahmad, Chairman, Department of Genetics, Hazara University, Pakistan. A voucher specimen (No. 123) has been deposited in the herbarium of the Botany Department, Hazara University, Pakistan.

8.2.2 Extraction and isolation

The air dried aerial parts of *S. desfontainei* (12 kg) were extracted at room temperature with methanol (x3; 4 days). The combined methanolic extract was evaporated under vacuum pressure to yield 300 g of the residue, which was then partitioned with organic solvent (*n*-hexane, DCM, EtOAc and MeOH) by addition of H_2O to obtained *n*-hexane (70 g), dichloromethane (100 g), ethyl acetate (90 g), and aqueous methanolic (40 g) fractions **(Scheme-8.1)**.



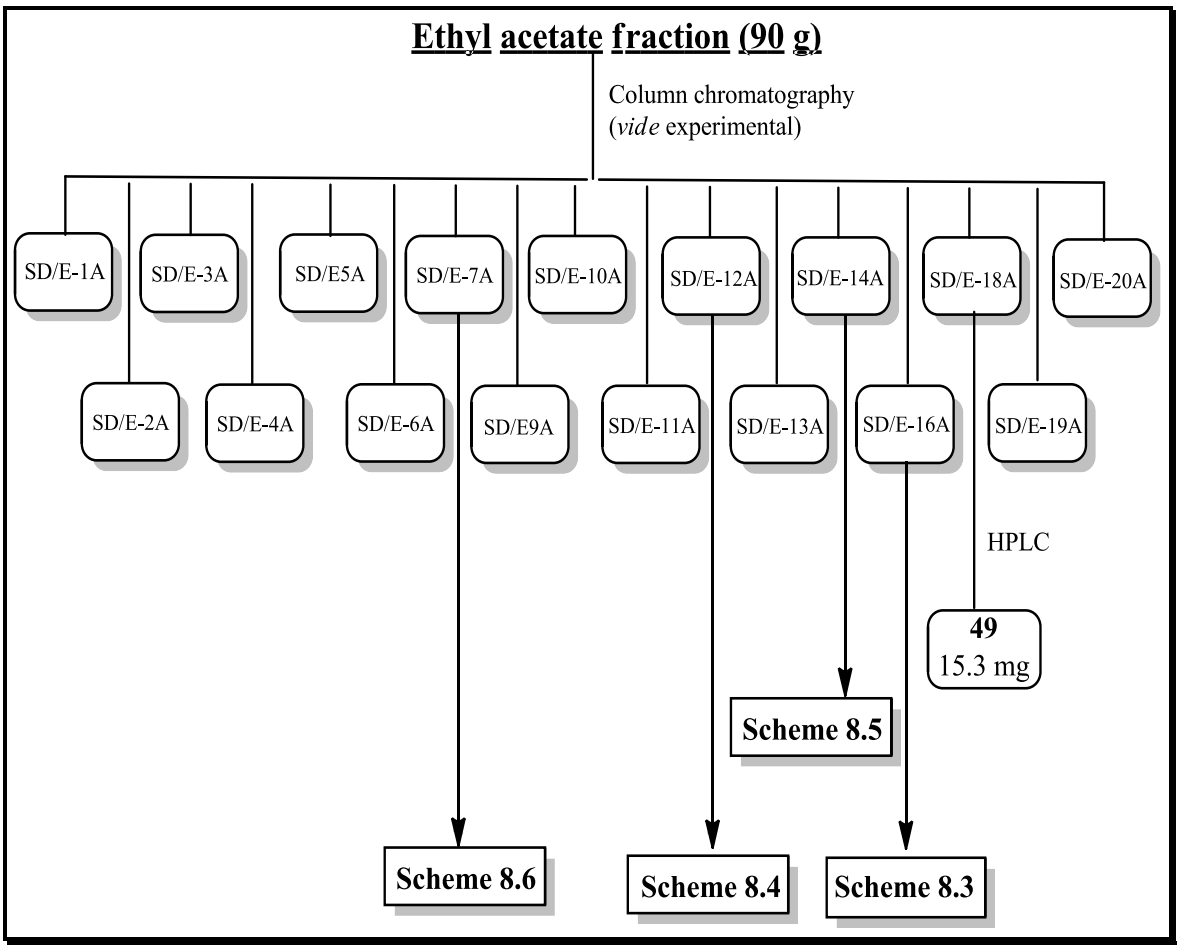
Scheme-8.1: Extraction and fractionation of the aerial parts of *S. desfontainei.*

The EtOAc fraction (90 g) was chromatographed on silica gel (70-230 mesh, 1500 g, 9 x 120 cm) column, eluting with n-hexane, ethyl acetate and methanol, in order to increase the polarity of solvent to methanol 100%. As a result twenty major semi pure fractions (SD/E-1A to SD/E-20A) were obtained. These fractions were analyzed by TLC and reunited in 17 new fractions **(Scheme-8.2)**. Fraction SD/E-16A (10 g) was chromatographed on silica gel (70-230 mesh, 300 g, 6 x 100 cm) column, eluting successively with a gradient of *n*-hexane, ethyl acetate and methanol increasing the polarity to methanol 100%. Seventeen sub-fractions (SD/E-16A-1B to SD/E-16A-17B) were collected. These fractions were analyzed by TLC and reunited

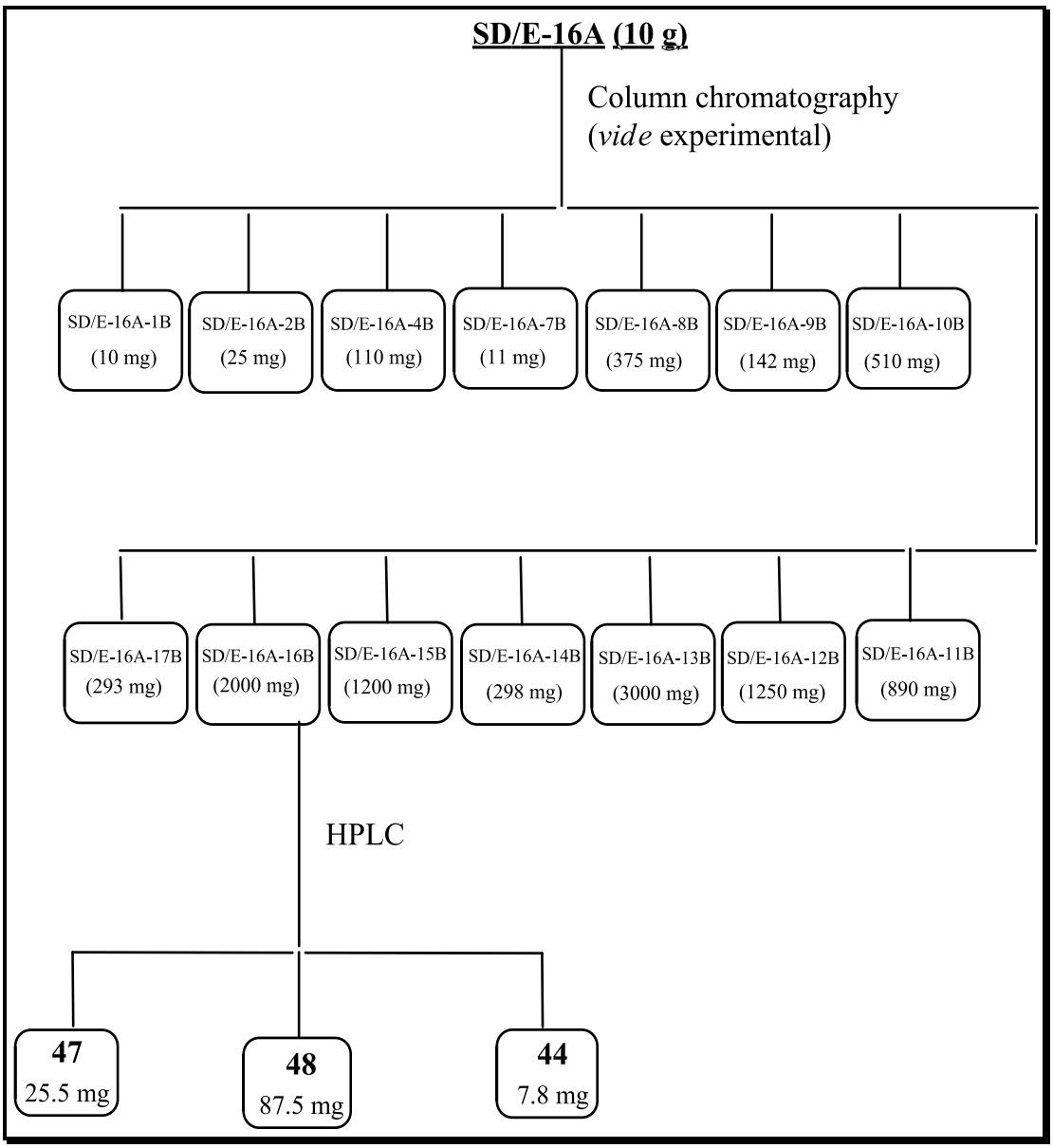
in 14 new fractions. SD/E-16A-16B (2 g) was subjected to HPLC mass-directed preparative purification using H_2O- CH_3CN as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 25% B; 0.01 min, 25% B; 15 min, 35% B; 16 min, 95% B; 18.50 min, 95% B; 19 min, 25% B; 20 min, 25% B). Evaporation of the collected fractions under a stream of dry nitrogen yielded 7.8 mg of **44**, 25.5 mg of **47** and 87.5 mg of **48 (Scheme-8.3)**. Fraction SD/E-12A (5 g) was chromatographed on silica gel (220-440 mesh, 200 g, 4 x 90 cm) column, eluting successively with a gradient of DCM-MeOH (100:00, 98:02, 96:04, 94:06, 90:10, 85:15 & 80:20). As a result eleven sub-fractions SD/E-12A-1B to SD/E-12A-11B) were collected based on TLC profile. SD/E-12A-8B (825 mg) was subjected to HPLC mass-directed preparative purification using deionized H_2O and MeOH as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 40% B; 13.00 min, 95% B; 17 min, 95% B; 19.50 min, 40% B; 20 min, 40% B). Evaporation of the collected fractions under high vacuum yielded 12 mg of **45** and 5.5 mg of **46**. SD/E-12A-5B (617 mg) yielded a crystalline compound containing minor impurities. The impurities were washed off with dichloromethane. Transparent needles of the compound **53** (212 mg) were obtained by re-crystarlization using a mixture of EtOAc:MeOH (85:15). **(Scheme-8.4)**. SD/E-18A (1 g) was subjected to HPLC mass-directed preparative purification using H_2O- MeOH as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 40% B; 13.00 min, 95% B; 17 min, 95% B; 19.50 min, 40% B; 20 min, 40% B), yielded 15.3 mg of **49**. Fraction SD/E-14A (11 g) was chromatographed on silica gel (70-230 mesh, 300 g, 6 x 100 cm) column, eluting successively with a gradient of *n*-hexane, ethyl acetate and methanol increasing the polarity to methanol 100%. Twenty five sub-fractions SD/D-14A-1B to SD/D-14A-25B were collected based on TLC profile.

SD/E-14A-17B (51 mg) was subjected to HPLC mass-directed preparative purification using H_2O-MeOH as a mobile phase. 200 μL (0 min, 25% B; 13.00 min, 95% B; 15 min, 95% B; 17.00 min, 25% B; 20 min, 25% B), furnished 23.7 mg of **50**. SD/E-14A-16B (315 mg) was also subjected to HPLC (injected volume 200 μL; H_2O-MeOH mobile phase; 0 min, 25% B; 13.00 min, 95% B; 15 min, 95% B; 17.00 min, 25% B; 20 min, 25% B), to afford compounds **51** (1.2 mg) and **52** (3.5 mg) **(Scheme-8.5)**. Fraction SD/E-7A (2 g) was chromatographed on silica gel (70-230 mesh, 300 g, 4 x 90 cm) column, eluting successively with a gradient of *n*-hexane, ethyl acetate and methanol increasing the polarity to methanol 100%. Twenty sub-fractions (SD/E-7A-1B to SD/E-7A-20B) were collected, which were analyzed by TLC and reunited in 11 major fractions. SD/E-7A-10B (55 mg) was subjected to HPLC (injected volume 200 μL; H_2O-MeOH mobile phase; 0.00 min, 20% B; 0.01 min, 20% B; 6.00 min, 30% B; 13.50 min, 60% B; 14.50 min, 95% B; 18.50 min, 95% B; 19.00 min, 20% B; 20.00 min, 20% B), to obtained **54** (16.6 mg) **(Scheme-8.6)**.

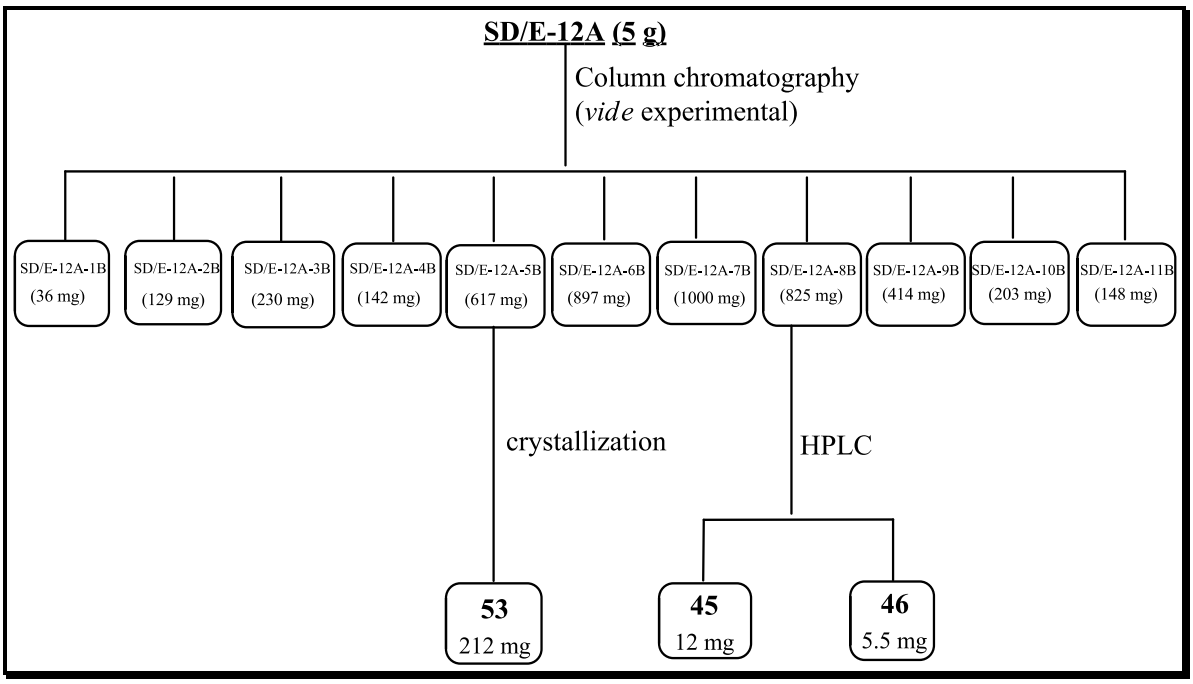
The DCM fraction (100 g) was chromatographed on silica gel (70-230 mesh, 1500 g, 9 x 120 cm) column, eluting with *n*-hexane, dichloromethane, ethyl acetate and methanol, increasing the polarity to methanol 100%. Thirteen sub-fractions SD/D-1A to SD/D-13A were collected based on TLC profile. Fraction SD/D-9A (*n*-hexane:EtOAc (2:8)-EtOAc:MeOH (0.3:9.7)) was precipitated which was purified by washing with acetone and methanol, yielded **55** (100 mg) **(Scheme-8.7)**.



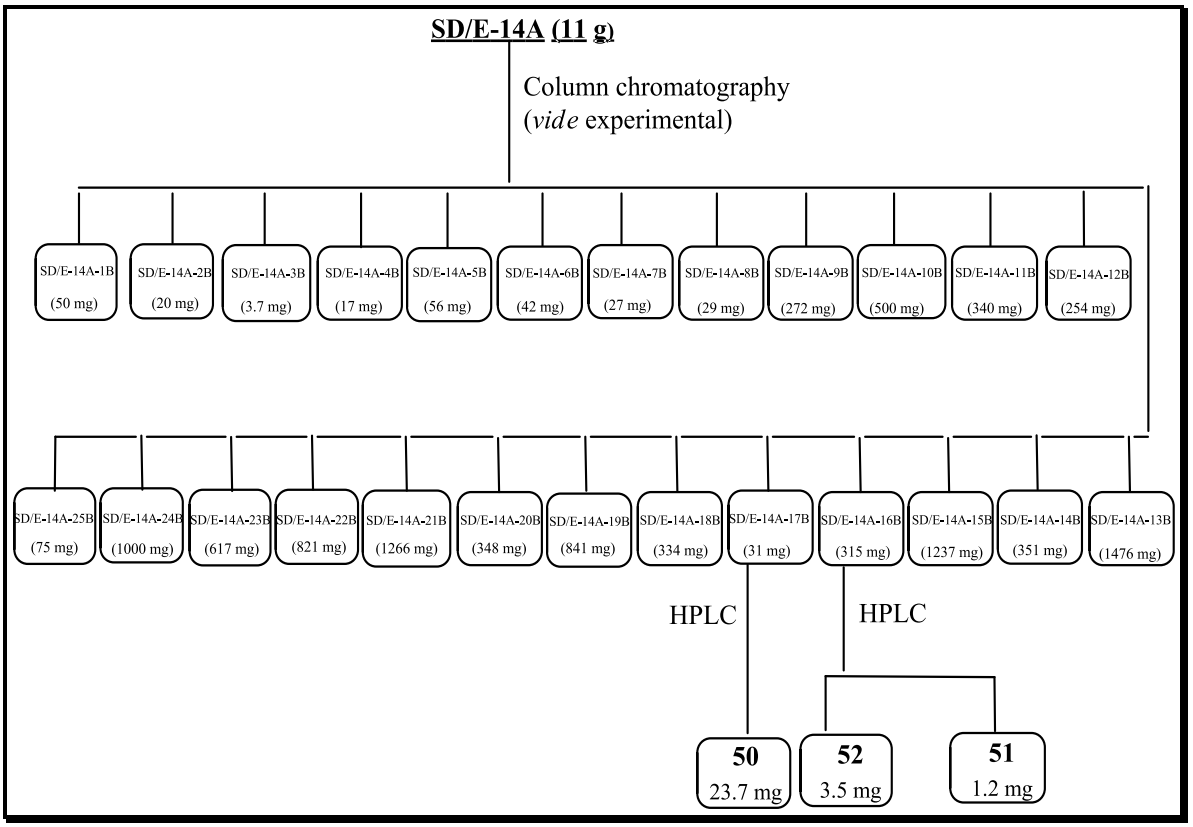
Scheme-8.2: Isolation of pure compound (**49**) from *S. desfontainei.*



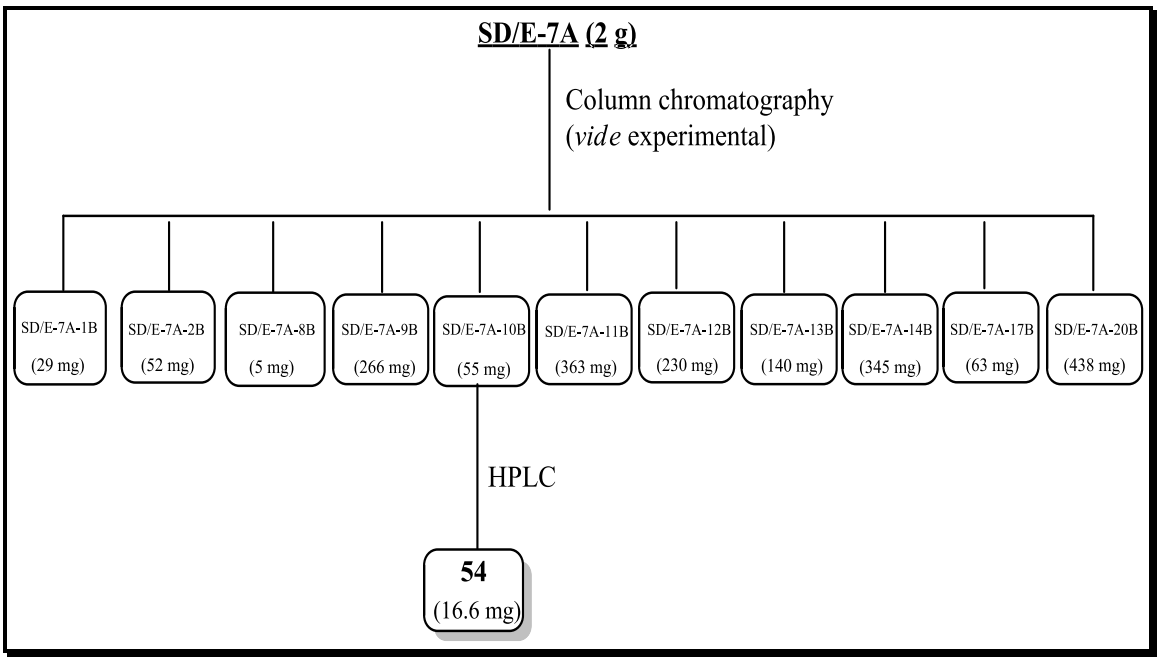
Scheme-8.3: Isolation of pure compounds (**44**, **47** and **48**) from *S. desfontainei.*



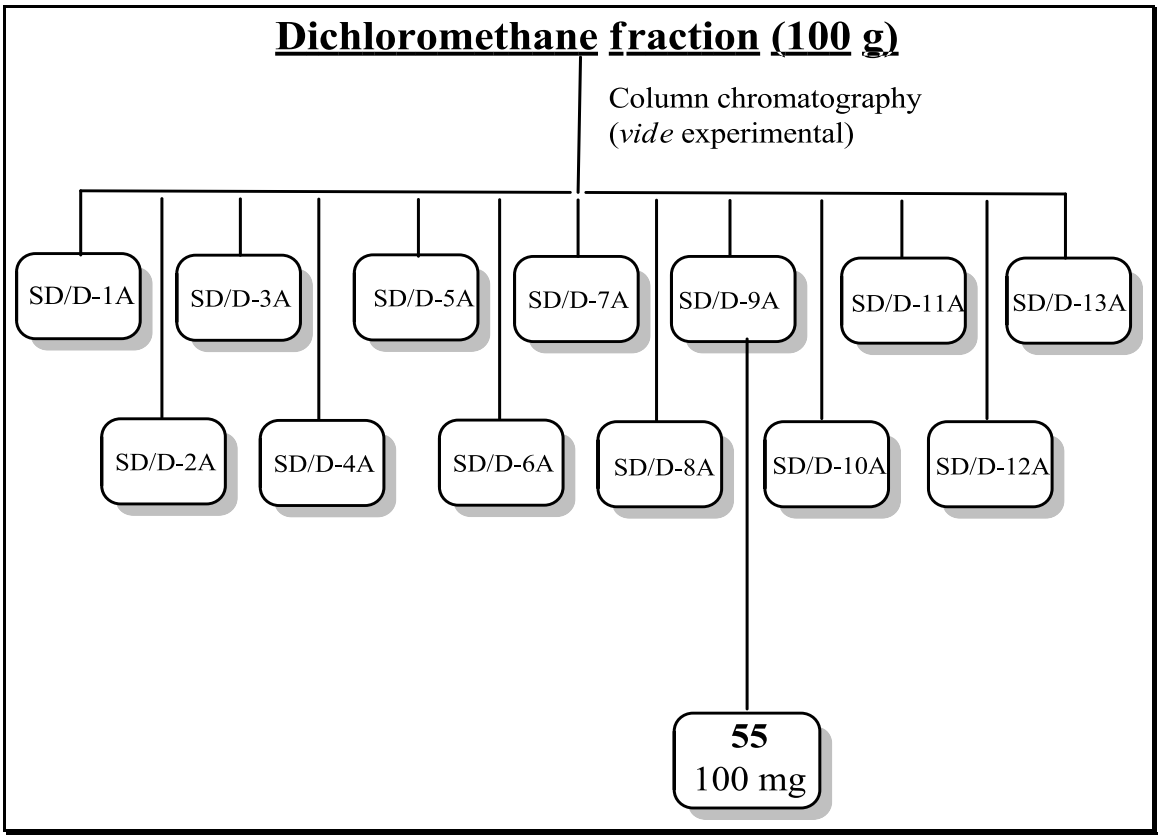
Scheme-8.4: Isolation of pure compounds (**45**, **46** and **53**) from *S. desfontainei.*



Scheme-8.5: Isolation of pure compounds (**50**-**52**) from *S. desfontainei.*



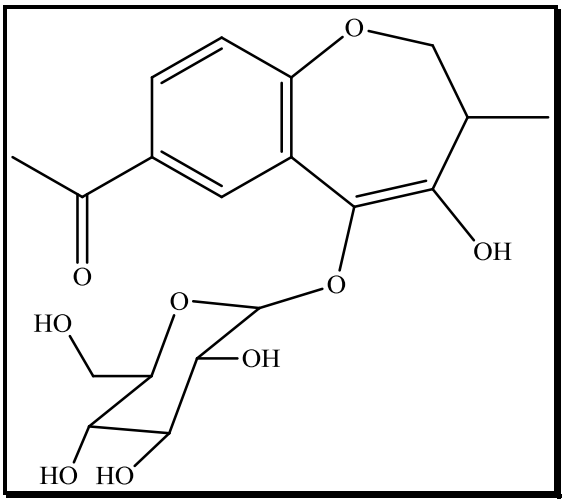
Scheme-8.6: Isolation of pure compound (**54**) from *S. desfontainei.*



Scheme-8.7: Isolation of pure compound (**55**) from *S. desfontainei.*

8.2.3 Characterization of the new compound isolated from *S. desfontainei*

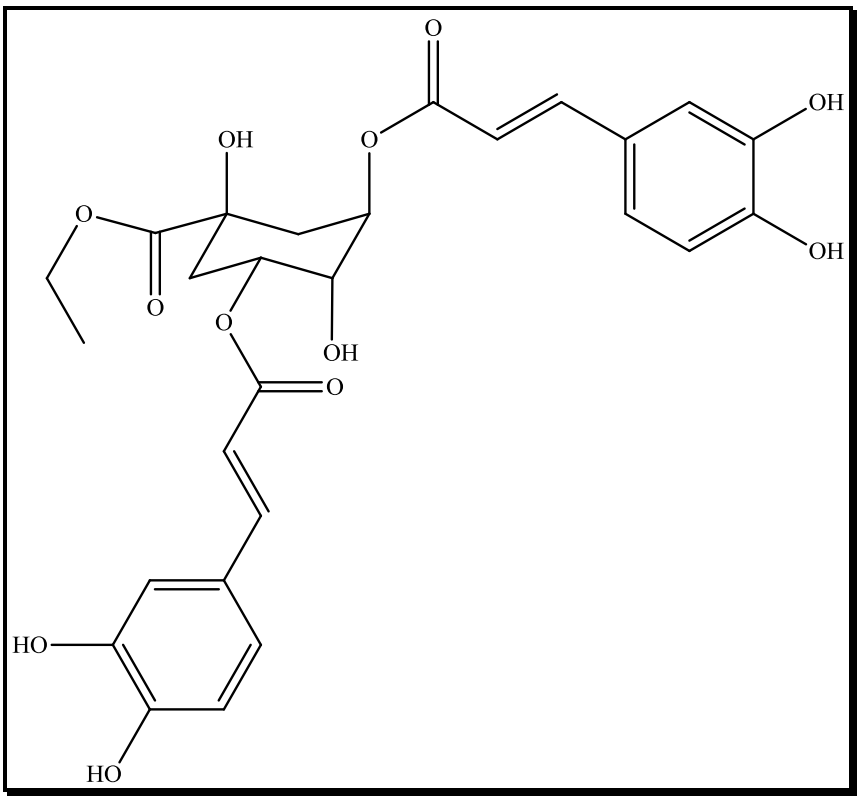
8.2.3.1 Senecioside (44)



Physical State:	Brownish yellow solid
Yield:	7.8 mg
$[\alpha]_D^{20}$:	$-12.9°$ (c 0.15, MeOH)
UV (MeOH) λ_{max}:	240 nm.
IR v_{max} (MeOH) cm^{-1}:	3329, 1714 and 1661
HR-ESI-MS (*m/z*):	419.1306 (calculated for $C_{19}H_{24}O_9Na$ $[M + Na]^+$ 419.1313)
ESI(+)-MS (*m/z*):	419.13 and 235.1
^{1}H- and ^{13}C-NMR:	Table-7.1

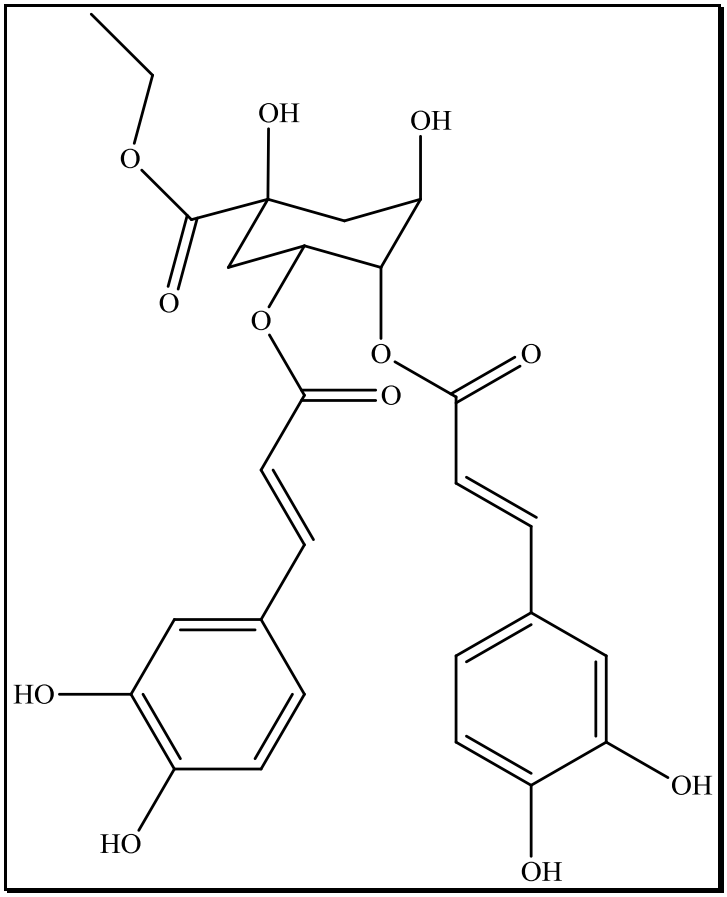
8.2.4 Characterization of the new source isolated compounds from *S. desfontainei*

8.2.4.1 Ethyl 3,5-di-*O*-caffeoylquinic acid (45)



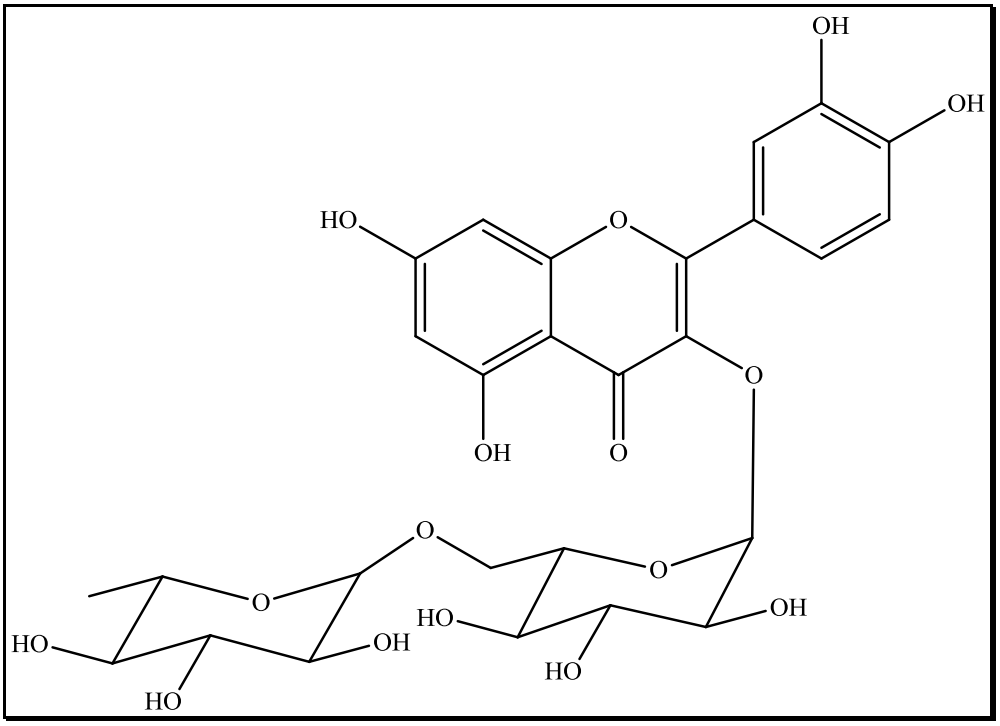
Physical State:	Yellow solid
Yield:	12 mg
Melting point:	120-122 ^{o}C
IR v_{max} (MeOH) cm^{-1}:	3332, 1687 and 1598
LC-ESI(+)-MS (*m/z*):	545, 567, 527, 337 and 163
LC-ESI (–)-MS (*m/z*):	543, 381 and 207
^{1}H- and ^{13}C-NMR:	Table-7.2

8.2.4.2 Ethyl 3,4-di-*O*-caffeoylquinic acid (46)



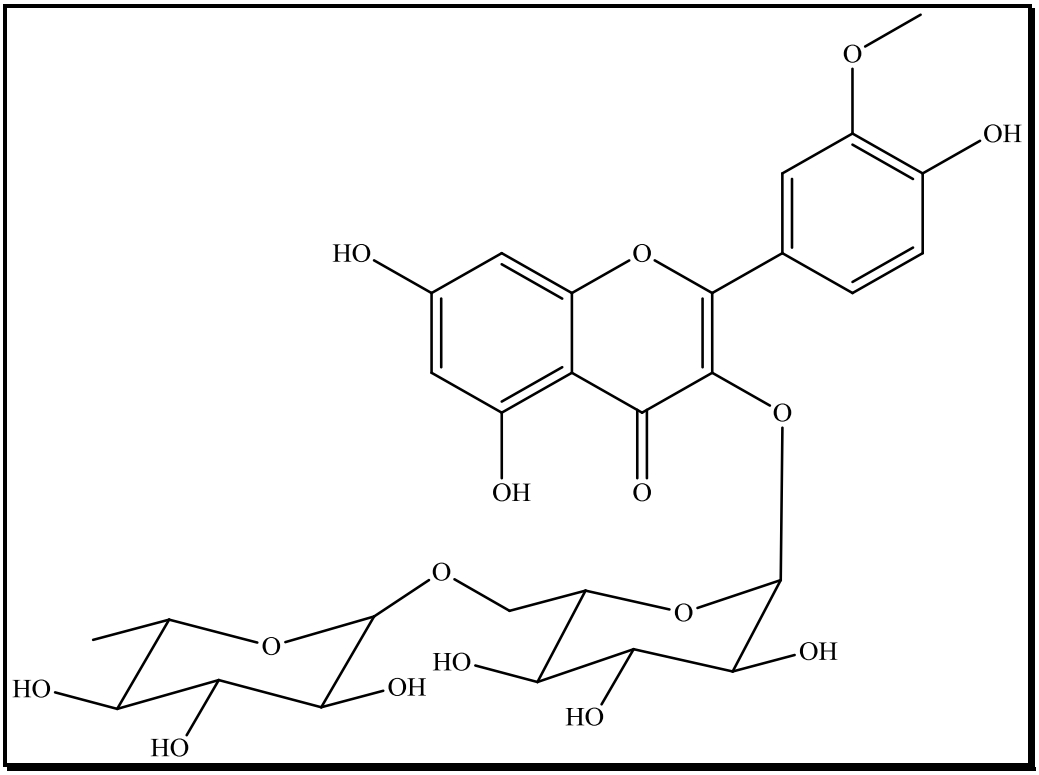
Physical State:	Yellow solid
Yield:	5.5 mg
Melting point:	114-116 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3339, 1699 and 1598
LC-ESI(+)-MS (*m/z*):	545, 567, 527, 337 and 163
LC-ESI (–)-MS (*m/z*):	543, 381 and 207
^{1}H- and ^{13}C-NMR:	Table-7.3

8.2.4.3 Quercetin-3-*O*-rutinoside (Rutin) (47)



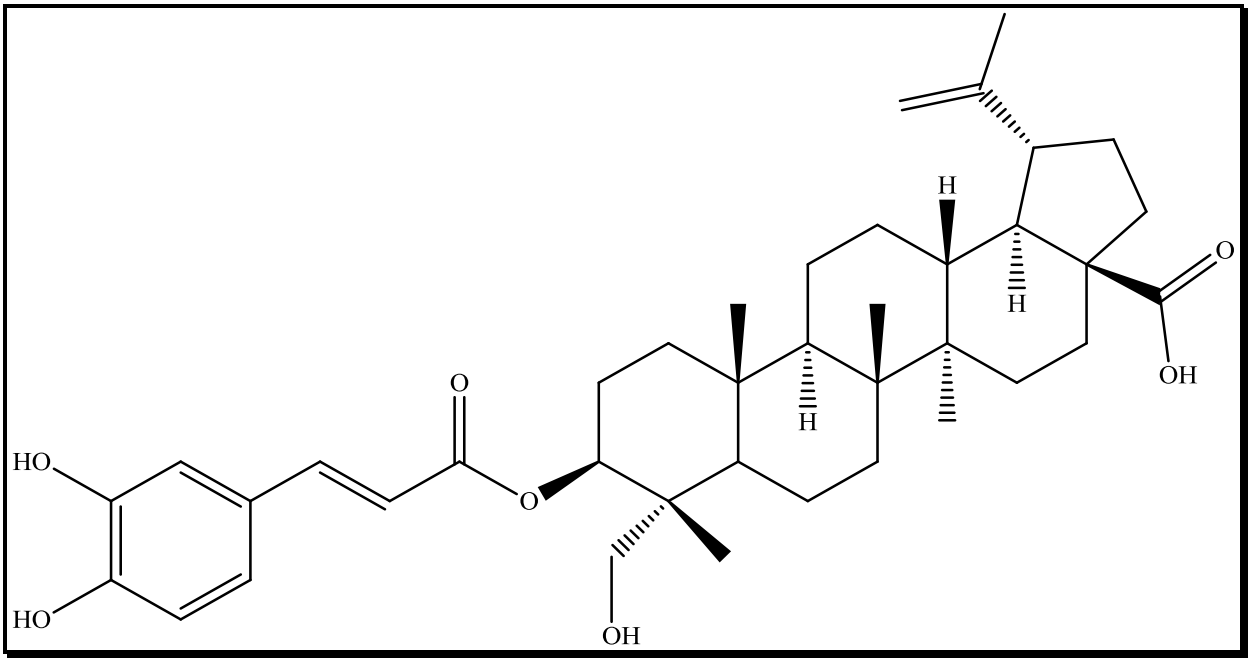
Physical State:	Brownish yellow crystals
Yield:	25.5 mg
Melting point:	213-215 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3395, 1710, 1660 and 1610
LC-ESI(+)-MS (*m/z*):	611, 633, 465 and 303
LC-ESI (–)-MS (*m/z*):	609
^{1}H- and ^{13}C-NMR:	Table-7.4

8.2.4.4 Isorhamentin 3-*O*-*β*-D-rutinoside (48)



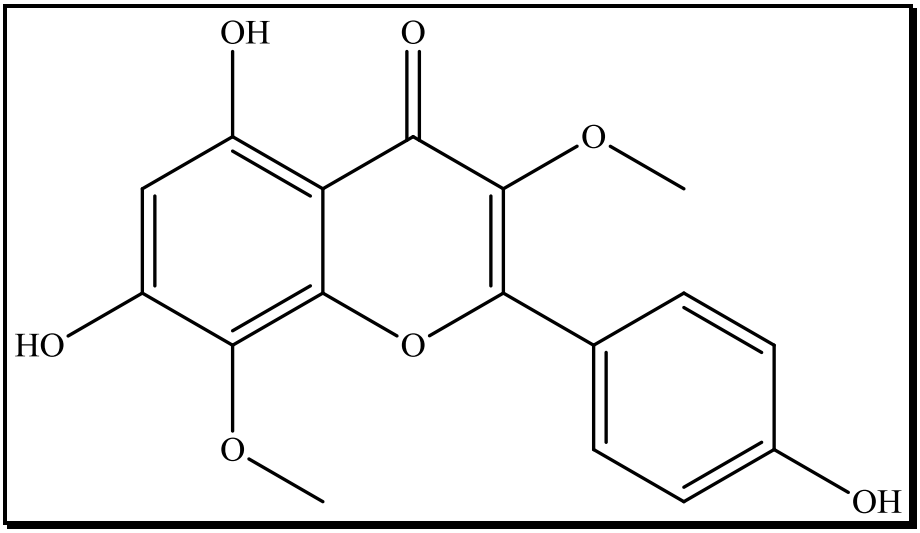
Physical State:	Yellow amorphous powder
Yield:	87.5 mg
IR ν_{max} (MeOH) cm^{-1}:	3390, 1715, 1650 and 1615
LC-ESI(+)-MS (*m/z*):	625, 647, 479 and 317
LC-ESI (–)-MS (*m/z*):	623
^{1}H- and ^{13}C-NMR:	Table-7.5

8.2.4.5 3*β*,23-Dihydroxy-lup-20(29)-en-28-oic acid-3*β*-caffeate (49)



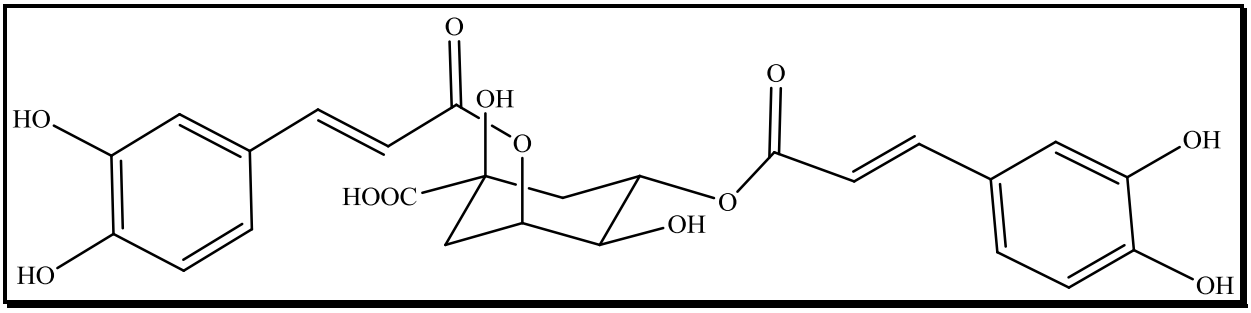
Physical State:	White amorphous powder
Yield:	15.3 mg
LC-ESI(+)-MS (*m/z*):	635
LC-ESI (–)-MS (*m/z*):	633
^{1}H- and ^{13}C-NMR:	Table-7.6

8.2.4.6 5,7,4′-Trihydroxy-3,8-dimethoxyflavone (50)



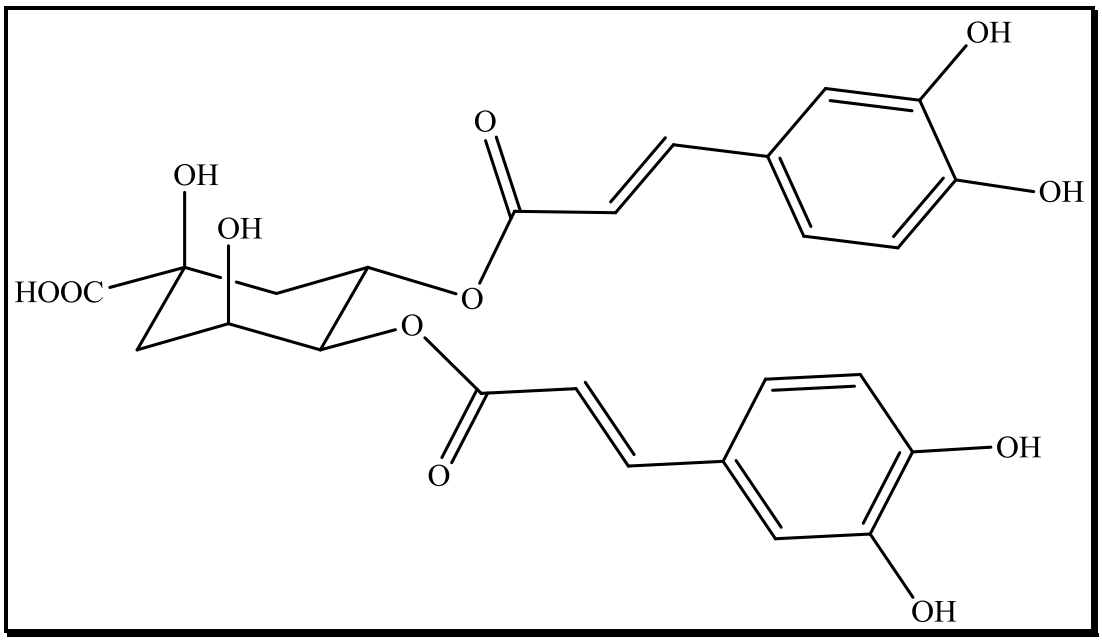
Physical State:	Yellow needles
Yield:	23.7 mg
Melting point:	257-259 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3430, 1660 and 1617
LC-ESI(+)-MS (*m/z*):	331 and 353
LC-ESI (–)-MS (*m/z*):	329
^{1}H- and ^{13}C-NMR:	Table-7.7

8.2.4.7 3,5-Di-*O*-caffeoylquinic acid (51)



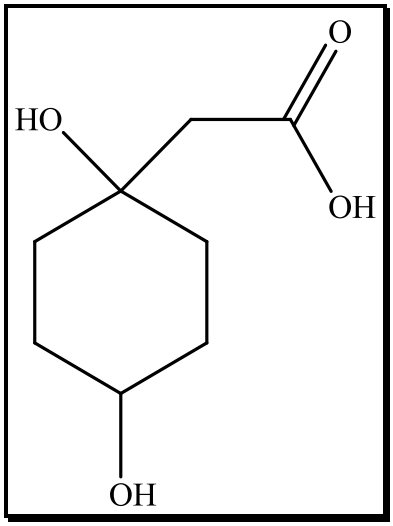
Physical State:	Light yellow amorphous powder
Yield:	1.2 mg
Melting point:	150-152 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3380, 1710, 1690 and 1608
LC-ESI(+)-MS (*m/z*):	517, 539, 499 and 163
LC-ESI (–)-MS (*m/z*):	515, 353, 191 and 179
^{1}H- and ^{13}C-NMR:	Table-7.8

8.2.4.8 4,5-Di-*O*-caffeoylquinic acid (52)



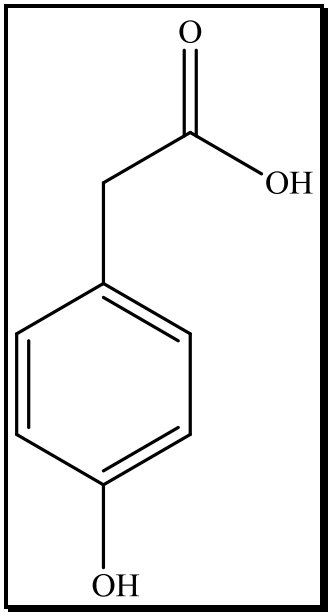
Physical State:	Light yellow amorphous powder
Yield:	3.5 mg
Melting point:	191-193 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3390, 1720, 1698 and 1610
LC-ESI(+)-MS (*m/z*):	517, 539, 499 and 163
LC-ESI (–)-MS (*m/z*):	515, 353, 191 and 179
^{1}H- and ^{13}C-NMR:	Table-7.9

8.2.4.9 2-(1,4-dihydroxy cyclohexanyl)-acetic acid (53)



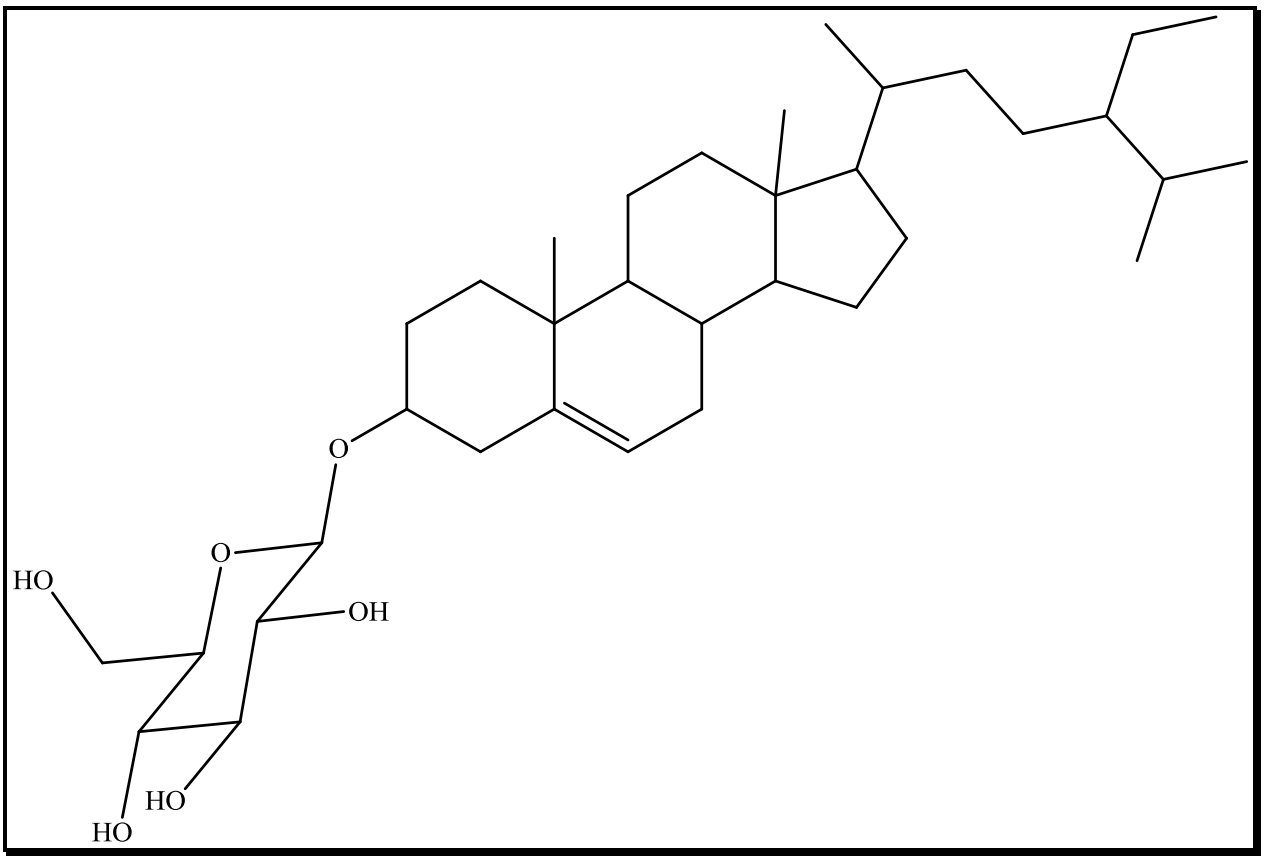
Physical State:	White crystals
Yield:	212 mg
Melting point:	162-163 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3224, 2942, 2500-3000 and 1695
EI(+)-MS (*m/z*):	156.1, 157.1 and 96.1
^{1}H- and ^{13}C-NMR:	Table-7.10

8.2.4.10 *p*-Hydroxyphenylacetic acid (54)



Physical State:	Colourless crystals
Yield:	16.6 mg
Melting Point:	149°C
IR ν_{max} (MeOH) cm^{-1}:	3390 and 1715
LC-ESI(+)-MS (*m/z*):	153
LC-ESI (–)-MS (*m/z*):	151
^{1}H- and ^{13}C-NMR:	Table-7.11

8.2.4.11 *β*-Sitosterol-3-*O*-*β*-D-glucopyranoside (55)



Physical State:	White amorphous powder
Yield:	100 mg
IR ν_{max} (MeOH) cm^{-1}:	3460, 3050, 1650 and 815
EI(+)-MS (*m/z*):	396.1, 255.1, 175 and 145.1
^{1}H- and ^{13}C-NMR:	Table-7.12

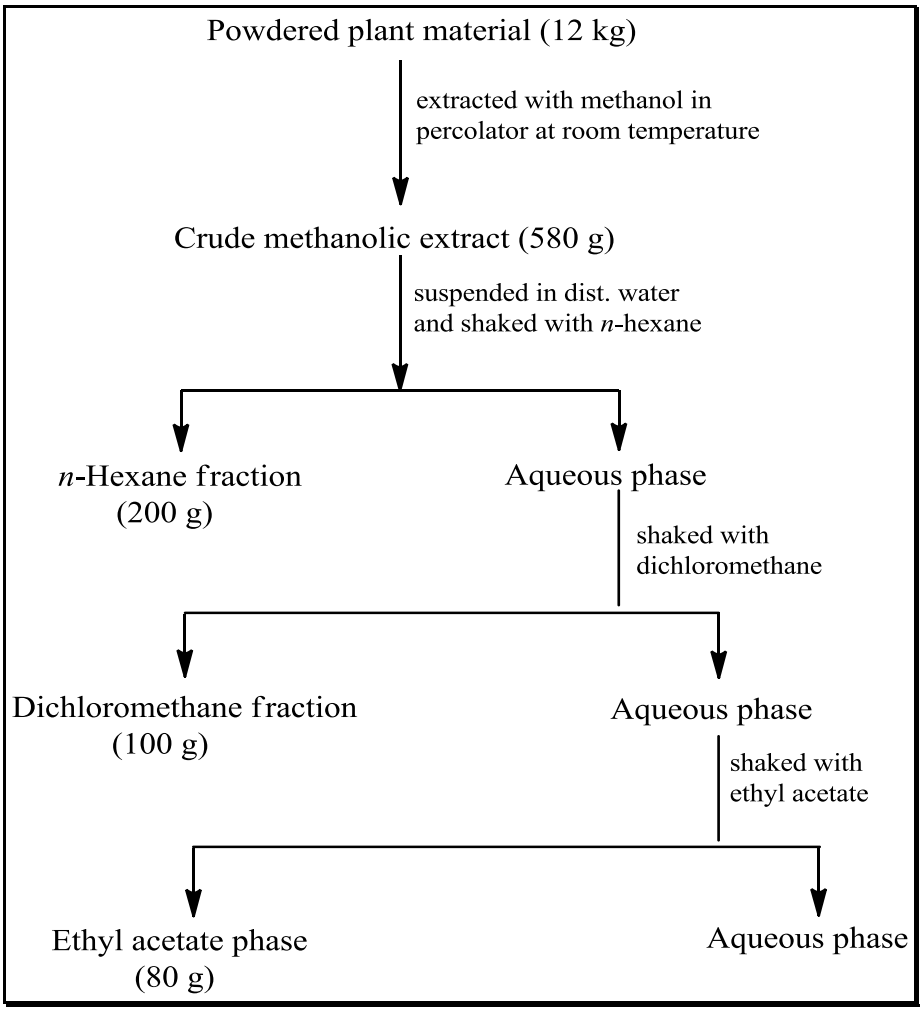
8.3 *Senecio chrysanthemoides*

8.3.1 Plant material

The plant (*S. chrysanthemoides*) was collected from Barra Gali, K.P.K., Pakistan, in August 2008. It was identified by Prof. Dr. Habib Ahmad, Chairman, Department of Genetics, Hazara University, Pakistan. A voucher specimen (No. 60) has been deposited in the herbarium of the Botany Department, Hazara University, Pakistan.

8.3.2 Extraction and isolation

The air dried aerial parts of *S. chrysanthemoides* (12 kg) were extracted with methanol (x3; 4 days) at room temperature. The combined methanolic extract was evaporated under vacuum to yield 580 g of the residue, which was then partitioned by H_2O and *n*-hexane, DCM, EtOAc and MeOH, successively to get *n*-hexane (200 g), dichloromethane (100 g), ethyl acetate (80 g) and aqueous MeOH (200 g) fractions **(Scheme-8.8)**.

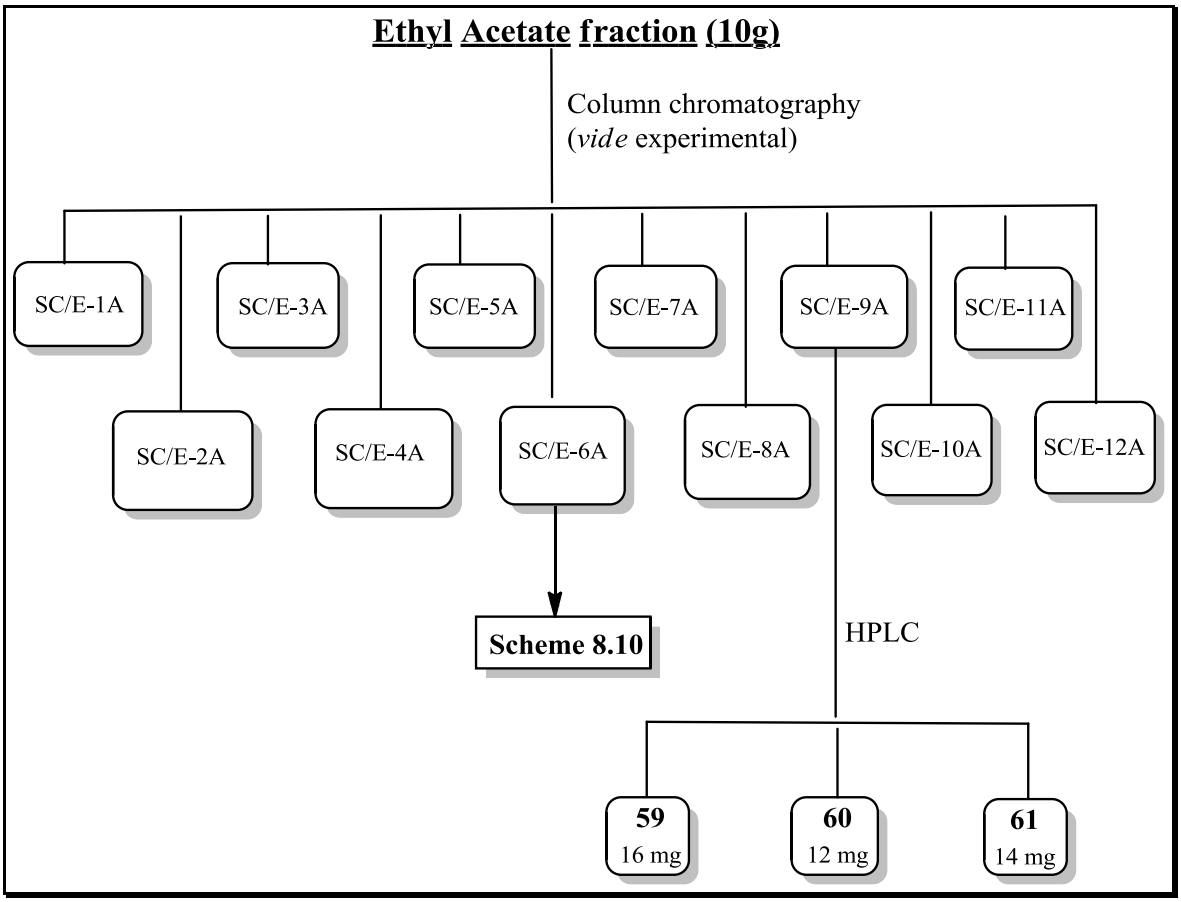


Scheme-8.8: Extraction and fractionation of the aerial parts of *S. chrysanthemoides*

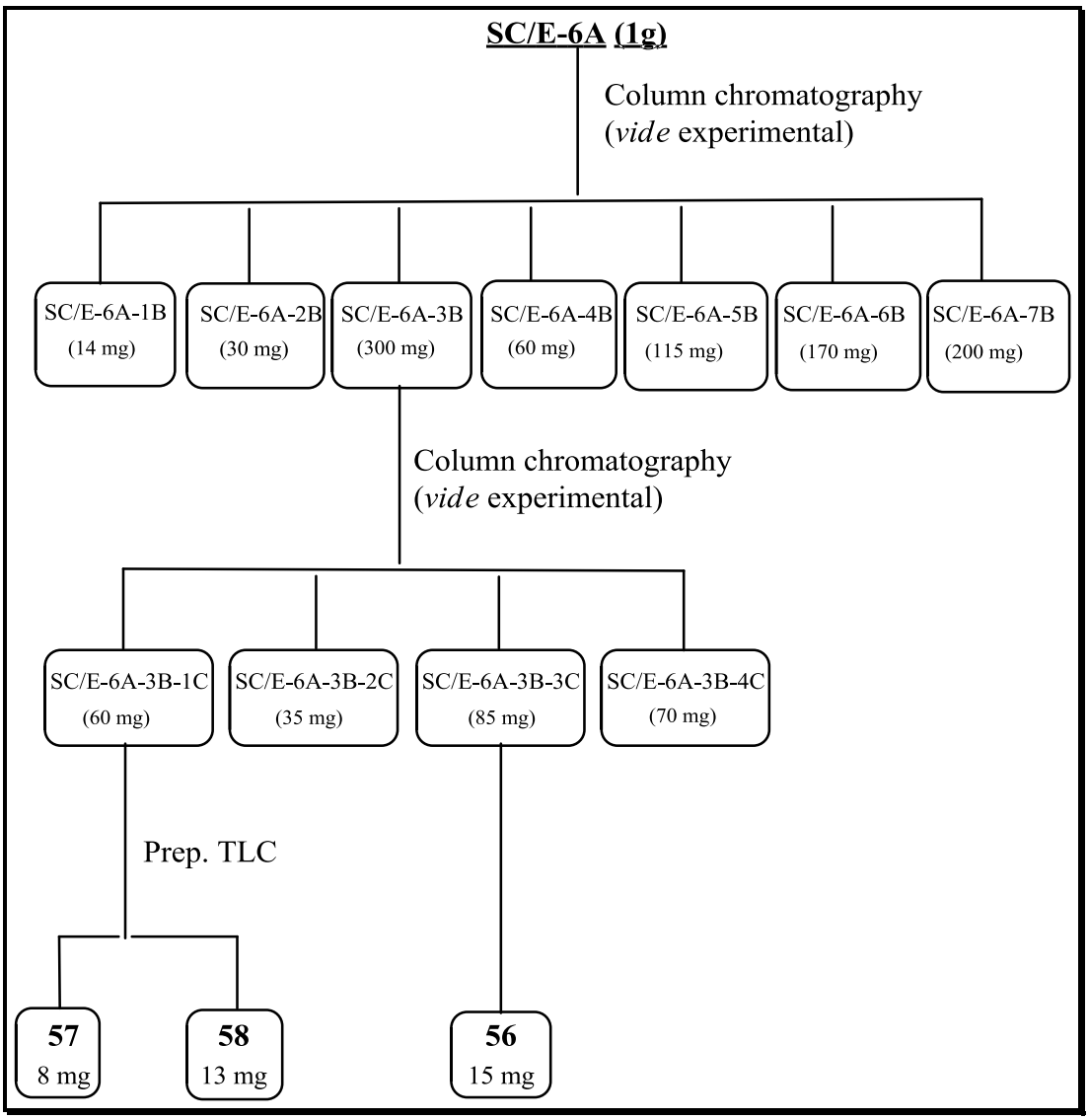
The EtOAc fraction (10 g) was chromatographed on silica gel (220-440 mesh, 300 g, 6 x 100 cm) column, eluting with n-hexane, ethyl acetate and methanol, increasing the polarity to methanol 100%. Twelve sub-fractions SC/E-1A to SC/E-12A) were collected based on TLC profile **(Scheme-8.9)**. Fraction SC/E-6A (1 g) was chromatographed on silica gel (220-440 mesh, 250 g, 4 x 90 cm) column, eluting successively with a gradient of *n*-hexane, ethyl acetate and methanol increasing the polarity to methanol 100%. As a result seven sub-fractions SC/E-6A-1B to SC/E-6A-7B) were collected based on TLC profile. SC/E-6A-3B (300 mg) was chromatographed on silica gel (220-440 mesh, 60 g, 2 x 90 cm) column, eluting

successively with a gradient of *n*-hexane and ethyl acetate increasing the polarity to ethyl acetate 100%. Four sub-fractions SC/E-6A-3B-1C to SC/E-6A-3B-4C) were collected based on TLC profile. A white amorphous substance which settled in fraction SC/E-6A-3B-3C [EtOAc:*n*-hexane (8:2)], was filtered and washed with methanol to give a pure compound **56** (15 mg). Fraction SC/E-6A-3B-1C (60 mg) was purified by preparative TLC [*n*-hexane-EtOAc (80:20) plus few drops of acetic acid, 3 developments] to afford 8 mg of **57** and 13 mg of **58 (Scheme-8.10)**.

SC/E-9A (400 mg) was subjected to HPLC (injected volume 200 μL; H_2O- MeOH mobile phase, 0 min, 40% B; 13.00 min, 95% B; 17 min, 95% B; 19.50 min, 40% B; 20 min, 40% B), yielded 16 mg of **59**, 12 mg of **60** and 14 mg of **61**. **(Scheme-8.9)**.



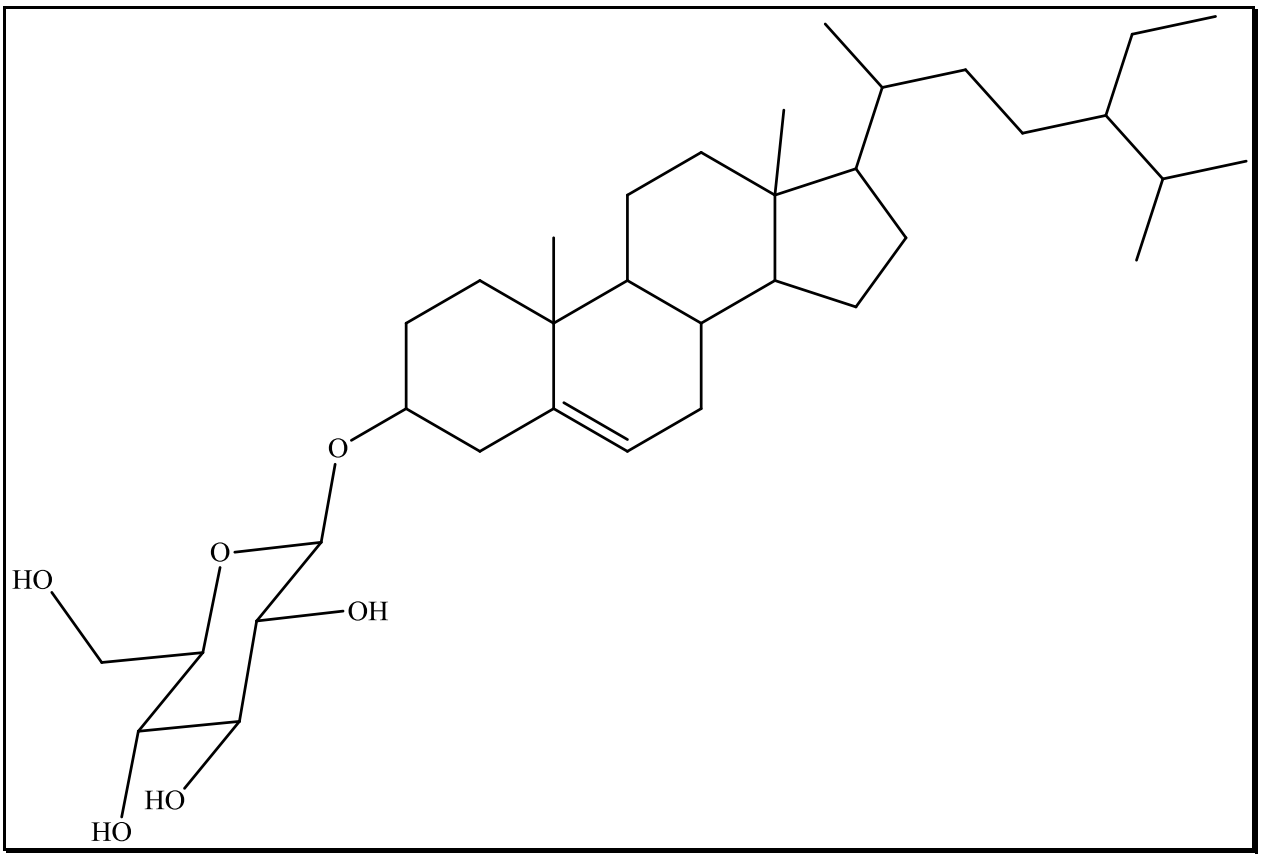
Scheme-8.9: Isolation of pure compounds (**59-61**) from *S. chrysanthemoides*



Scheme-8.10: Isolation of pure compounds (**56**-**58**) from *S. chrysanthemoides*

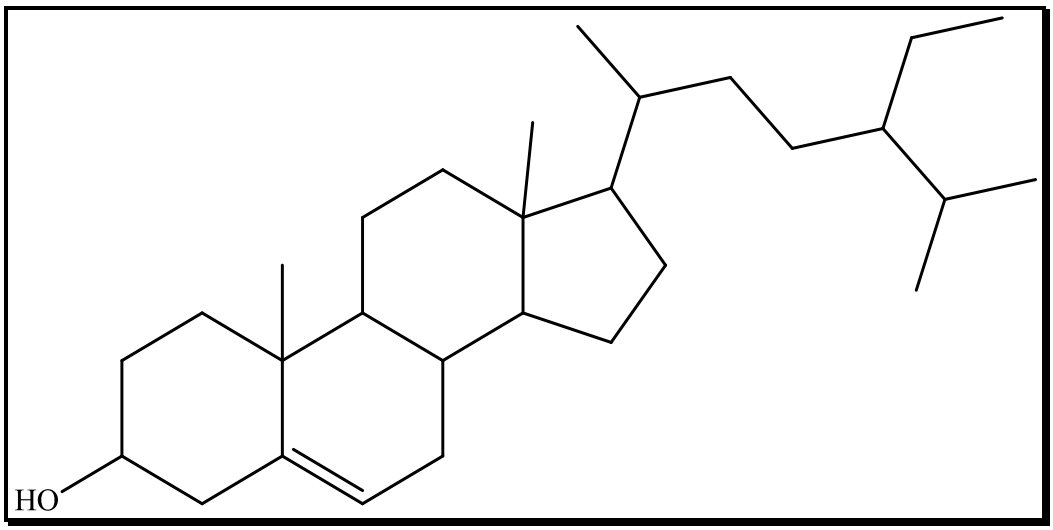
8.3.3 Characterization of the new source isolated compounds from *S. chrysanthemoides*

8.3.3.1 *β*-Sitosterol-3-*O*-*β*-D-glucopyranoside (56)



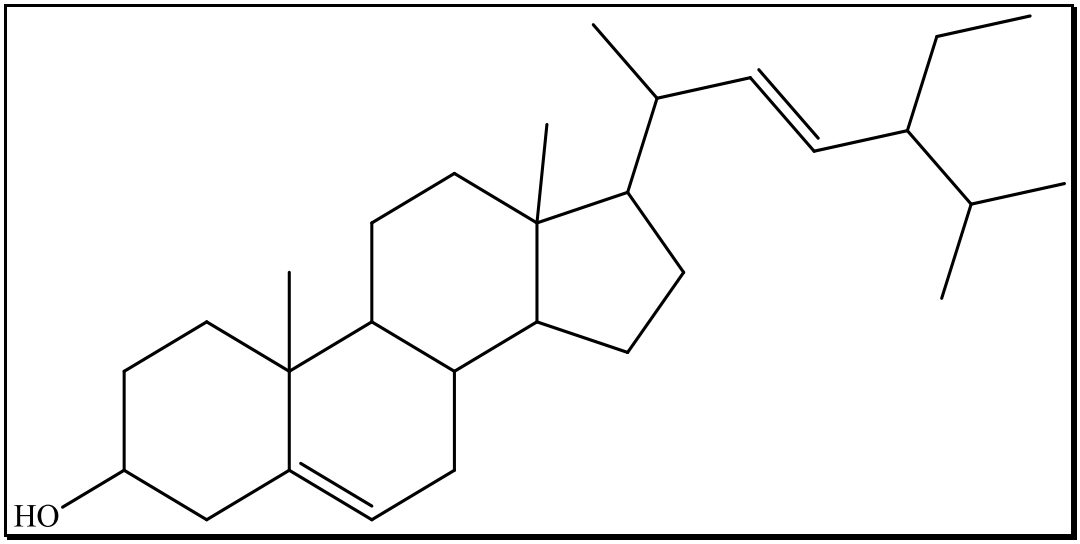
Physical State:	White amorphous powder
Yield:	15 mg
IR ν_{max} (MeOH) cm^{-1}:	3432, 3046, 1665 and 815
EI(+)-MS (*m/z*):	396.1, 255.1, 175.1 and 145.1
^{1}H- and ^{13}C-NMR:	Table-7.19

8.3.3.2 β-Sitosterol (57)



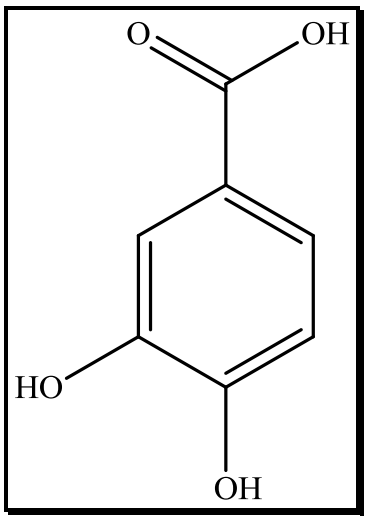
Physical State:	White amorphous powder
Yield:	8 mg
IR ν_{max} (MeOH) cm^{-1}:	3445, 3060 and 1650
EI(+)-MS (*m/z*):	414.1, 367.1, 255.1, 175.1, 133.1 and 105.1
^{1}H- and ^{13}C-NMR:	Table-7.20

8.3.3.3 Stigmasterol (58)



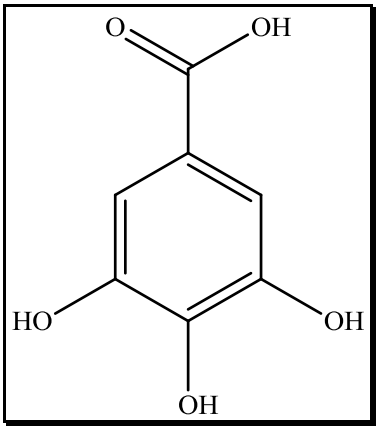
Physical State:	White amorphous powder
Yield:	13 mg
IR ν_{max} (MeOH) cm^{-1}:	3380, 3075 and 1635
EI(+)-MS (*m/z*):	412.1, 365.1 and 253.1
^{1}H- and ^{13}C-NMR:	Table-7.21

8.3.3.4 3,4-Dihydroxybenzoic acid (59)



Physical State:	White amorphous powder
Yield:	16 mg
Melting point:	200-201 ^{o}C
IR ν_{max} (MeOH) cm^{-1}:	3390, 3315, 1710 and 1627
LC-ESI(+)-MS (*m/z*):	155 and 177
LC-ESI (–)-MS (*m/z*):	153
^{1}H- and ^{13}C-NMR:	Table-7.22

8.3.3.5 Gallic acid (60)



Physical State: White crystalline solid

Yield: 12 mg

Melting Point: 250°C

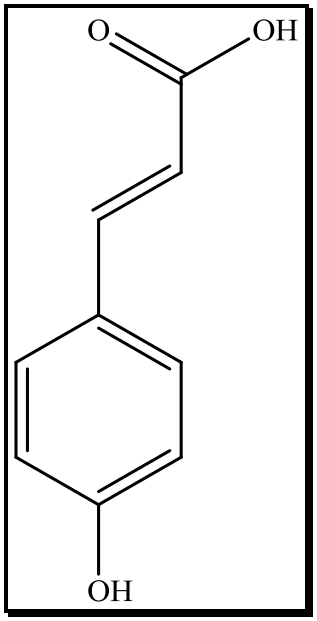
IR ν_{max} (MeOH) cm^{-1}: 3410 and 1700

LC-ESI(+)-MS (*m/z*): 171 and 193

LC-ESI (–)-MS (*m/z*): 169

^{1}H- and ^{13}C-NMR: Table-7.23

8.3.3.6 Coumaric acid (61)



Physical State:	White powder
Yield:	14 mg
Melting Point:	212°C
IR ν_{max} (MeOH) cm^{-1}:	3433 and 1718
LC-ESI(+)-MS (*m/z*):	165 and 187
LC-ESI (–)-MS (*m/z*):	163
^{1}H- and ^{13}C-NMR:	Table-7.24

8.4 Biological studies

8.4.1 Anti-bacterial assay

Agar Tube diffusion method[155] was employed for determination of anti-bacterial activity as discussed in **section-5.3.1.**

8.4.2 Anti-fungal assay

Anti-fungal assay was determined through Agar Tube Dilution Method[156,157] as discussed in **section-5.3.2.**

.8.4.3 Phytotoxicity

Phytotoxicity of the various fractions was evaluated against the *Lemna minor* L.[159] as discussed in **section-5.3.3.**

8.4.4 Insecticidal assay

Insecticidal assay was determined by Contact Toxicity Method[160] as discussed in **section-5.3.4.**

8.4.5 Brine shrimp (*Artemia salina*) lethality bioassay

Cytotoxicity of the solvent extracted fractions was evaluated against *Artemia salina* (Leach) shrimp larvae as discussed in **section-5.3.5.**

8.4.6 *α,α*-Diphenyl-*β*-picrylhydrazyl (DPPH) radical-scavenging effects

The DPPH radical-scavenging effects were measured using a modified previously established methodology as discussed in **section-5.3.6.**

9.1 Plant introduction

Vernonia genus, belongs to Asteraceae family, consists of about 1000 species of forbs and shrubs. *Vernonia* genus have five species in Pakistan, including *V. anthelmintica, V. arborea, V. cinera, V. cinerascens* and *V. javanica*. Some species are known by their common names as iron-weeds, bitter leaf, ewuro and ndole.[327,328] Throughout the world *Vernonia* species are generally used in native cultures for various therapeutic purposes such as anthelmintic, stomachic, diuretic, sedative, antiflatulent, and for the treatment of various diseases such as hepatitis, diabetes, malaria, skin infections, hiccups, kidney problems, eczema, wounds, venereal diseases.[329-337] Some *Vernonia* species have been used to cure sore throat, cold, tooth aches, rheumatism aches,[338] tridosa, consumption, asthma and bronchitis.[339]

9.2 Literature survey of genus *Vernonia*

The list of compounds in **Table-9.1** (chronological order) showed diversity of constituents in genus *Vernonia*.

Table-9.1:	Chemical Constituents of the Genus *Vernonia*	
S. No.	**Compounds**	**Species**
1	Shomerajin	*V. anthelmintica*[340]
2	Vernolic acid	*V. anthelmintica*[341]
3	*β*-Amyrin acetate	*V. cinerea*[342]
4	*β*-Amyrin benzoate	*V. cinerea*[342]
5	*β*-Sitosterol	*V. cinerea*[342]
6	Stigmasterol	*V. cinerea*[342]
7	*α*-Spinasterol	*V. cinerea*[342]
8	Trivernolin	*V. anthelmintica*[343]
9	1,3-Divernolin	*V. anthelmintica*[343]
10	Vernolepin	*V. hymenolepis*[344]
11	Vernomenin	*V. hymenolepis*[344]
12	Vernodalin	*V. amygdalina*[345]
13	Vernomygdin	*V. amygdalina*[345]
14	Confertolide	*V. conferta*[346]
15	Glaucolide-A	*V. glauca*[347]
16	Glaucolide-B	*V. baldwinii*[347]
17	Vernudifloride	*V. nudiflora*[348]
18	8*α*-Senicioyloxydehydrocostus lactone	*V. nudiflora*[348]
19	Trifloculoside	*V. trifloculosa*[348]
20	Vernodalol	*V. anthelmintica*[349]
21	Lupeol palmitate	*V. westiniana*[350]
22	Lupeol acetate	*V. westiniana*[350]
23	3*β*-*H*-Zaluzanin	*V. anisochaetoides*[351]
24	15-Senecioyloxycostunolide	*V. hirsute*[351]
25	15-Isovaleryloxycostunolide	*V. hirsute*[351]
26	1,9-Dioxo-8-hydroxybisabolene	*V. hirsute*[351]
27	8*β*-(2-Methylacryloyloxy)-hirsutinolide-13(*O*)-acetate	*V. hirsute*[351]
28	8*β*-(2-Methyl-2,3-epoxypropionyloxy)-hirsutinolide-13(*O*)-acetate	*V. hirsute*[351]
29	8*β*-(2-Hydroxymethylacryloyloxy)-hirsutinolide-13(*O*)-acetate	*V. hirsute*[351]
30	8*β*-(2-Methylacryloyloxy)-hirsutinolide	*V. angulifolia*[351]
31	8*β*-(2-Methyl-2,3-epoxypropionyloxy)-hirsutinolide	*V. angulifolia*[351]
32	8*β*-(2-Methylacryloyloxy)-15-hydroxyhirsutinolide	*V. oligocephala*[351]
33	8*α*-Senecioyloxyvanillosmin	*V. oligocephala*[351]
34	8*β*-(2-Methylacryloyloxy)isohirsutinolide	*V. novebaracensis*[351]
35	8*α*-Hydroxy-11*β*,13-dihydrodehydrozaluzanin	*V. novebaracensis*[351]
36	4-Vinylcatechol	*V. novebaracensis*[351]
37	Vernopolyanthone	*V. polyanthes*[352]

38	Vernopolyanthofuran	*V. polyanthes*[352]
39	8-*O*-Desacetyl-8-*O*-glaucolide B propionate	*V. lanuginosa*[352]
40	4*α*-5*β*-Epoxy-4,5-dehydrovernonallenolide	*V. lilacina*[352]
41	4*β*-Hydroxy-4,5-dihydro-5,6-dehydrovernonallenolide	*V. lilacina*[352]
42	Lupeyl acetate	*V. lilacina*[352]
43	Germacrene D	*V. lilacina*[352]
44	*α*-Humulene	*V. lilacina*[352]
45	Caryophyllene	*V. lilacina*[352]
46	8*α*-Tiglinoyloxy-1*β*,5*β*-*H*-4*α*,7*α*-epoxybourbon-11(13)-en-6,12-olide	*V. arkansana*[352]
47	8*α*-(2-Methylacryloyloxy)-1*β*,5*β*H-4*α*,7*α*-epoxy-bourbon-11(13)-en-6,12-olide	*V. arkansana*[352]
48	Tridecapentaynene	*V. arkansana*[352]
49	Germacrene A	*V. arkansana*[352]
50	Sitosterol	*V. arkansana*[352]
51	8-*O*-Desacylglaucolide E acetate	*V. lilacina*[352]
52	13-Hydroxy-4*β*,15-*H*-*α*-selinen-2-one	*V. lanuginosa*[352]
53	13-Hydroxy-4*β*,15,11,13-*H*-*α*-selinen-2-one	*V. lanuginosa*[352]
54	Lupeol	*V. lanuginosa*[352]
55	*γ*-Cadinine	*V. fagifolia*[352]
56	*σ*-Cadinine	*V. fagifolia*[352]
57	5-Methoxyeudesmin	*V. echitifolia*[353]
58	11,13*β*-Dihydrodehydrozaluzanin	*V. echitifolia*[353]
59	1(*O*)-Acetyl-8*α*-acetoxy-10*β*-hydroxy-hirsutinolide-1(*O*)-isovalerate	*V. venosissima*[353]
60	8*α*-Acetoxy-10*β*-hydroxy-isohirsutinolide	*V. venosissima*[353]
61	8*α*-Hydroxydehydrozaluzanin C	*V. profuga*[354]
62	8*α*-Hydroxy-4*β*,15,11*β*,13-tetrahydrozaluzanin C	*V. profuga*[354]
63	8-Deacyl marginatinmethacrylate	*V. arkansana*[354]
64	8*α*,13-Diacetoxy-4*β*-hydroxybourbon-7(11)-en-6,12-olide	*V. arkansana*[354]
65	13-Acetoxy-8*α*-tiglinoyloxy-4*β*-hydroxy-bourbon-7(11)-en-6,12-olide	*V. arkansana*[354]
66	13-Acetoxy-8*α*-methacryloyloxy-4*β*-hydroxy-bourbon-7(11)-en-6,12-olide	*V. arkansana*[354]
67	8*α*-Acetoxy-4*α*-hydroxy-10*α*-methoxyguai-6,7,11(13)-dien-6,12-olide	*V. arkansana*[354]
68	3*β*-Hydroxystilpnotomentolide-8-*O*-(5-acetoxy-senecioate)	*V. staehelinoides*[355]
69	Preethulia coumarin	*V. cinarescens*[356]
70	Dauca-2,11(13)-Diene	*V. galpinii*[356]
71	Trideca-3,5,7,9-tetrayne-1,11-diene	*V. galpinii*[356]
72	*β*-Bergamotene	*V. galpinii*[356]
73	Bisabolene	*V. galpinii*[356]
74	1,10-Desoxidoglaucolide	*V. natalensis*[356]
75	Vernonataloide	*V. natalensis*[356]
76	Natalensolide	*V. natalensis*[356]
77	Lupenone	*V. natalensis*[356]
78	Vernostipulal A	*V. stipulacea*[356]

79	Vernostipulal B	*V. stipulacea*[356]
80	Linolenic acid	*V. stipulacea*[356]
81	2*α*-8*α*-13-Triacetoxygermacra-1(10),5,7(11)-trien-6,12-olide	*V. cotoneaster*[357]
82	8*β*-Caproyloxycompactifloride	*V. compactiflora*[357]
83	8*β*-Caproyloxy-10*β*-hydroxy-1-desoxyhirsutinolide	*V. compactiflora*[357]
84	4*α*-Hydroxyeudesm-11-en-12-al	*V. glabra*[358]
85	8*α*-(4′-Hydroxysenecioyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate	*V. poskeana*[359]
86	8*α*-(4′-Hydroxysenecioyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide	*V. poskeana*[359]
87	8*α*-(4′-Hydroxysenecioyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate	*V. poskeana*[359]
88	8*α*-(2-Metylacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate	*V. poskeana*[359]
89	8*α*-(5′-Hydroxysenecioyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate	*V. poskeana*[359]
90	8*α*-(5′-Acetoxysenecioyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate	*V. poskeana*[359]
91	11,13-Dihydrovernodalin	*V. amygdalina*[359]
92	*δ*-Amyrin acetate	*V. cinerea*[339]
93	*α*-Amyrin acetate	*V. cinerea*[339]
94	3*β*-Acetoxyurs-13(18)-ene	*V. cinerea*[339]
95	*β*-Amyrin	*V. cinerea*[339]
96	*α*-Amyrin	*V. cinerea*[339]
97	Campesterol	*V. cinerea*[360]
98	24-Hydroxytaraxer-14-ene	*V. cinerea*[360]
99	17,18-Epoxyvernonataloide	*V. oligocephala*[361]
100	2-Oxo-2-desacetoxyglaucolide	*V. sutherlandii*[361]
101	12-Acetoxy-*α*-bergamotene	*V. sutherlandii*[361]
102	12-Acetoxy-*α*-santalene	*V. sutherlandii*[361]
103	14-Hydroxy-8-desacyl-2,3-dehydrovernonatoloide	*V. adoensis*[361]
104	14-Hydroxy-8-desacyl-2,3-dehydrovernonatoloide-8-*O*-[2-methyl butyrate]	*V. adoensis*[361]
105	Vernofultanin	*V. fulta*[362]
106	Isovernofultanin	*V. fulta*[362]
107	8*S*,13-Diacetoxy-1*R*,(10*R*)-4*R*,5*R*-diepoxygermacra-7(11)-en-6*S*,12-olide	*V. fulta*[362]
108	Friedelin	*V. volkameriaefolia*[363]
109	Epifriedelinol	*V. volkameriaefolia*[363]
110	Poskeanolide	*V. poskeana*[364]
111	3-Oxo-10*α*-*H*-stilpnotomentolide-8-*O*-(5-acetoxy-senecioate)	*V. poskeana*[364]
112	8*α*-Tigloyloxyhirsutinolide-13-*O*-acetate	*V. cinerea*[364]
113	8*α*-[4-Hydroxytigloyloxy]-hirsutinolide-13-*O*-acetate	*V. cinerea*[364]
114	8*α*-[4-Hydroxymethacryloyloxy]-10*α*-hydroxy-hirsutinolide-13-*O*-acetate	*V. cinerea*[364]
115	8*α*-[4-Hydroxytigloyloxy]-10*α*-hydroxyhirsutinolide-13-*O*-acetate	*V. cinerea*[364]

116	Vernocinerolide-8-*O*-[4-hydroxymethacrylate]	*V. cinerea*[364]
117	19-Hydroxyglaucolide	*V. cinerea*[364]
118	Diacetylpiptocarphol	*V. mollissima*[365]
119	8-Acetyl-13-ethoxypiptocarphol	*V. mollissima*[365]
120	Piptocarphin D	*V. mollissima*[365]
121	Scopoletin	*V. mollissima*[365]
122	Glaucolide G	*V. squamulosa*[365]
123	13-Deacetylmarginatin	*V. marginata*[366]
124	8,13-Bis-deacylvernonataloide-8-*O*-tiglate	*V. marginata*[366]
125	8-Deacylvernonataloide-8-*O*-tiglate	*V. marginata*[366]
126	1*α*-Hydroxy-13-deacetyl-9,10*Z*-dehydro-1,10-dihydromarginatin	*V. marginata*[366]
127	1*α*-Hydroxy-9,10*Z*-dehydro-1,10-dihydromarginatin	*V. marginata*[366]
128	Pseudoelephantopide-8-*O*-tiglate	*V. marginata*[366]
129	1*α*-Hydroxy-9*α*,5*β*-epoxy-4,10-dimethyl-6-oxo-7(1-carbomethoxy-ethyliden)-cyclodeca-7*Z*,9*Z*-diene	*V. marginata*[366]
130	8*α*-Tigloyloxy-4*α*,5*α*-epoxyjalcaguaianolide	*V. marginata*[366]
131	10,14-Dehydrostilpnotomentolide-8-*O*-tiglate	*V. marginata*[366]
132	4*α*-Hydroxy-8*α*-tigloyloxy-1*β*-*H*-jalcaguaianolide-13-*O*-acetate	*V. marginata*[366]
133	8*α*-Tigloyloxy-vernomargolide	*V. marginata*[366]
134	8*α*-Tigloyloxy-2-*epi*-vernomargolide-1,4-cyclosemi-acetal	*V. marginata*[366]
135	8*α*-Hydroxy-2-*epi*-vernomargolide-1,4-cyclosemi-acetal	*V. marginata*[366]
136	8*α*-Tigloyloxy-2-*epi*-vernomargolide-1,5-cyclosemi-acetal	*V. marginata*[366]
137	Tricin	*V. remotiflora*[367]
138	Taraxasterol	*V. elaegnifolia*[368]
139	Taraxasterol acetate	*V. elaegnifolia*[368]
140	Desacetylglaucolide A	*V. chamaedrys*[369]
141	(4*R**,5*R**,6*S**,8*S**)-1-Oxo-4,5-epoxy-8-methacryloxy-13-acetoxy-germacra-7(11),10(14)-dien-6,12-olide	*V. chamaedrys*[369]
142	(1*S**,4*R**,8*S**,10*R**)-1,4-Epoxy-8-methacryloxy-10-hydroxy-13-acetoxygermacra-5*E*,7(11)-dien-6,12-olide	*V. chamaedrys*[369]
143	4(*R**,8*S**,10*R* *)-1-Oxo-4-hydroxy-8-methacryloxy-13-ethoxygermacra-5*Z*,7(11)-dien-6,12-o1ide	*V. chamaedrys*[369]
144	(2*R**,4*R**,5*R**,6*S**,8*S**, 10*R**)-1-Oxo-2-hydroxy-4,5-epoxy-8-methacryloxy-13-acetoxygermacr-7(11)-en-6,12-o1ide	*V. chamaedrys*[369]
145	(4*R**,5*R**,6*R**,7*S**,8*S**,10*R**)-1-Oxo-4,5,7,10-diepoxy-8-methacryloxy-11(13)-en-6,12-olide	*V. chamaedrys*[369]
146	(l*S**,4*R**,7*S**,8*S**,10*R**)-1-Hydroxy-I,4,7,10-diepoxy-8-methacryloxygermacra-S*Z*,11(13)-dien-6,12-o1ide	*V. chamaedrys*[369]
147	(1*S*,3*S*,5*R*,6*R*,7*R*,8*S*)-8-Angelyloxy-3-hydroxyguai-3(15),10(14),11(13)-trien-6,12-elide	*V. nitidula*[370]
148	(1*S*,3*S*,5*R*,6*R*,7*R*,8*S*)-3-Hydroxy-8-tiglyloxyguai-3(15),10(14),11(13)-trien-6,12-elide	*V. nitidula*[370]

149	(1*S*,3*S*,5*R*,6*R*,7*R*,8*S*)-3-Hydroxy-8-[2-(1-hydroxy-ethyl)-propenoyloxyguai-3(15),10(14),11(13)-trien-6,12-elide	*V. nitidula*[370]
150	(1S,3S,5R,6R,7R,8S)-8-[2-(1-Acetoxyethyl)-propen-oyloxyl-3-Hydroxyguai-3(15),10(14),11(13)-trien-6,12-elide	*V. nitidula*[370]
151	(1*S*,3*S*,5*R*,6*R*,7*R*,8*S*)-3-Acetoxy-8-methacrylyloxy-guai-4(15),10(14),11(13)-trien-6,12-elide	*V. nitidula*[370]
152	(1*S*,3*S*,5*R*,6*R*,7*R*,8*S*)-3-Acetoxy-8-angelyloxyguai-4(15),10(14),11(13)-trien-6,12-elide	*V. nitidula*[370]
153	(1*S*,3*S*,5*R*,6*R*,7*R*,8*S*)-3-Acetoxy-8-tiglyloxyguai-4(15),10(14),11(13)-trien-6,12-elide	*V. nitidula*[370]
154	(3*S*,5*R*,6*R*,7*R*,8*S*)-8-angelyloxy-3,14-dihydroxyguai-1(10),4(15),11(13)-trien-6,12-elide	*V. nitidula*[370]
155	(1*R**,4*R**,8*S**,10*R**)-8,13-Diacetoxy-1,4-epoxy-10-hydroxygermacra-5*E*,7(11)-dien-6,12-olide	*V. cognata*[371]
156	Uniflorolide	*V. uniflora*[372]
157	2-Desacylglaucolide E	*V. uniflora*[372]
158	8-Desacylglaucolide E	*V. uniflora*[372]
159	Mespilifoliolide	*V. mespilifolia*[372]
160	11*β*,13-Dihydrovernolepin	*V. mespilifolia*[372]
161	Glaucogalamensolide-*iso*-valerate	*V. galamensis*[373]
162	Glaucogalemensolide-*iso*-butyrate	*V. galamensis*[373]
163	(4*R**,5*R**,6*S**,8*S**,10*R**)-1-Oxo-4,5-epoxy-8-senecioyloxy-10,13-diacetoxygermacr-7(11)-en-6,12-oIide	*V. incana*[374]
164	(4*R*,5*R**,6*S**,8*S**,10*R**)-1(10),4,5-Diepoxy-8-senecioyloxy-13-acetoxygermacr-7(11)-en-6,12-oIide	*V. incana*[374]
165	(1*R**,4*R**,5*R**,6*S**,8*S**,10*R**)-1-Oxo-4,5-epoxy-8-senecioyloxy-10,13-diacetoxygermacr-7(11)-en-6,12-oIide	*V. incana*[374]
166	3,7-Dimethoxy-5,3′,4′-trihydroxyflavone	*V. eremophila*[375]
167	3-Oxo-10*α*-*H*-stilpnotomentolide-8-*O*-[5-hydroxy-senecioate]	*V. steetziana*[376]
168	8*α*-(4-Hydroxymethacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide	*V. jugalis*[377]
169	8*α*-(4-Hydroxymethacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-methyl ether	*V. jugalis*[377]
170	8*α*-(4-Hydroxymethacryloyloxy)-3-oxo-1-desoxy-1,2-dehydrohirsutinolide-13-*O*-acetate	*V. jugalis*[377]
171	Jugalinone	*V. jugalis*[377]
172	*p*-Hydroxybenzoylvernovan	*V. anthelmintica*[378]
173	Vernovan	*V. anthelmintica*[378]
174	Butein	*V. anthelmintica*[378]
175	3,4,2′,4′,5′-Pentahydroxy-6′-methoxy-2-methyl-chalcone	*V. anthelmintica*[378]
176	Daucosterol	*V. anthelmintica*[378]
177	13-Acetoxy-8*α*-angeloyloxy-14-hydroxy-2*α*,3*α*,4*α*,5*β*-bisepoxygermacra-1(10)*E*,7(11)-dien-12,6*α*-olide	*V. holstii*[379]

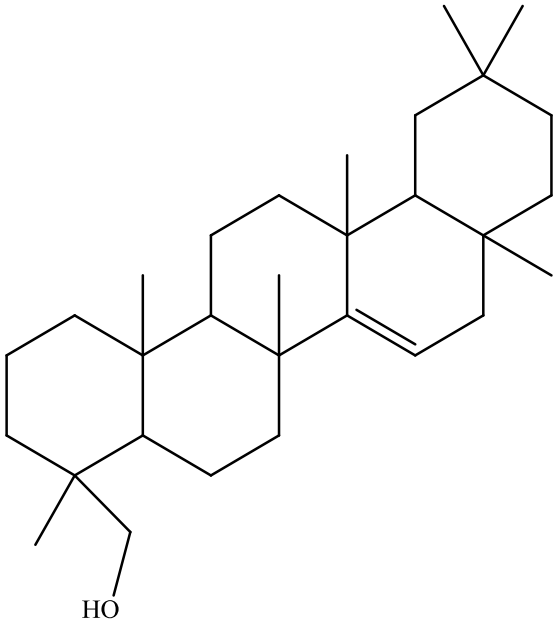
178	13-Acetoxy-8*α*-methacryloyloxy-14-hydroxy-2*α*,3*α*,4*α*,5*β*-bisepoxygermacra-1(10)*E*,7(11)-dien-12,6*α*-olide	*V. holstii*[379]
179	13-Acetoxy-8*α*-angeloyloxy-14-oxo-2*α*,3*α*,4*α*,5*β*-bisepoxygermacra-1(10)*E*,7(11)-dien-12,6*α*-olide	*V. holstii*[379]
180	13-Acetoxy-8*α*-angeloyloxy-4*α*,14-dihydroxy-germacra-1(10)*E*,5*E*,7(11)-trien-12,6*α*-olide	*V. holstii*[379]
181	2*α*,13-Diacetoxy-8*α*-angeloyloxy-4*α*,14-dihydroxy-germacra-1(10)*E*,5*E*,7(11)-trien-12,6*α*-olide	*V. holstii*[379]
182	16*E*-Brachycalyxolide	*V. zanzibarensis*[379]
183	16*E*-23-Desoxybrachycalyxolide	*V. zanzibarensis*[379]
184	16*E*-Isobrachycalyxolide	*V. zanzibarensis*[379]
185	Vernozanzibarolide	*V. zanzibarensis*[379]
186	Isovernozanzibarolide	*V. zanzibarensis*[379]
187	19-Hydroxyvernodalin	*V. colorata*[379]
188	19-Hydroxyglaucolide A	*V. colorata*[379]
189	(4*R**,8*S**,10*R**)-1-Oxo-4-hydroxy-8-tiglyloxy-13-acetoxygermacra-5*E*,7(13)-dien-6,12-olide	*V. nudiflora*[380]
190	(4*R**,8*S**,10*R**)-1,4-Epoxy-8-tiglyloxy-10-hydroxy-13-acetoxygermacra-1,5*E*,7(13)-trien-6,12-olide	*V. nudiflora*[380]
191	(1*R**,4*R**,5*R**,8*S**,10*R**)-1,4-Epoxy-1-ethoxy-5-hydroxy-8-methacryloxy-13-acetoxygermacra-5*E*,7(11)-dien-6,12-olide	*V. nudiflora*[380]
192	(1*S**,4*R**,5*S**,6*S**,8*S**,10*R**)-1,4-Dihydroxy-5,10,13-triacetoxy-8-tiglyloxy-cadin-7(11)-en-6,12-olide	*V. nudiflora*[380]
193	8-Tiglyloxycadin-7(11)-en-6,12-olide	*V. nudiflora*[380]
194	(4*R**,8*S**,10*R**)-1,4-Epoxy-8,10-diacetoxy-1-formyl-oxy-13-hydroxygermacra-5*E*,7(13)-dien-6,12-olide	*V. nudiflora*[380]
195	Prevernocistifolide-8-*O*-senecioate	*V. galamensis*[381]
196	14-*O*-Acetylprevernocistifolide-8-*O*-senecioate	*V. galamensis*[381]
197	2*α*,3*α*-Epoxyprevernocistifolide-8-*O*-senecioate	*V. galamensis*[381]
198	Prevernocistifolide-8-*O*-isobutyrate	*V. galamensis*[381]
199	14-*O*-Acetylprevernocistifolide-8-*O*-isobutyrate	*V. galamensis*[381]
200	Benzylsenecioate	*V. galamensis*[381]
201	Vernonioside A_4	*V. amygdalina*[382]
202	Vernonioside B_2	*V. amygdalina*[382]
203	Vernonioside B_3	*V. amygdalina*[382]
204	3*β*-Acetoxyurs-19-ene	*V. cinerea*[383]
205	Luteolin	*V. amygdalina*[384]
206	Luteolin7-*O*-*β*-glucuronoside	*V. amygdalina*[384]
207	luteolin 7-*O*-*β*-glucoside	*V. amygdalina*[384]
208	Zaluzanin A isobutyrate	*V. leopoldi*[385]
209	(2R,3R,4R,5S,6S,8S)-13,14-Diacetoxy-3,4,5,6-diepoxy-8-(2-methyl-2,3-dihydroxypropanoyloxy)-melampo-1(10),11(13)-dien-6,12-olide	*V. syringifolia*[385]
210	(2R,3R,4R,5S,6S,8S)-13,14-Diacetoxy-3,4,5,6-diepoxy-8-(2-methyl-2,3-epoxypropanoyloxy)-melampo-1(10),11(13)-dien-6,12-olide	*V. syringifolia*[385]
211	3*β*-Friedelinol	*V. zeylanica*[386]

212	Pseudotaraxasterol acetate	*V. zeylanica*[386]
213	Vernonioside D	*V. amygdalina*[387]
214	Vernonioside E	*V. amygdalina*[387]
215	Lupeol palmitate	*V. cinerea*[388]
216	*α*-Amyrin palmitate	*V. cinerea*[388]
217	Octacosanoic acid	*V. cinerea*[388]
218	Stigmasterol-*β*-D-glucopyranoside	*V. cinerea*[388]
219	Isorhamnetin 3-*O*-*β*-D-apio-D-furanosyl(1→2)-*β*-D-galactopyranoside	*V. galamensis*[389]
220	Quercetin-3-galactoside	*V. galamensis*[389]
221	Quercetin-3-apiosyl(1→2)galactoside	*V. galamensis*[389]
222	Quercetin-3-rhamnosyl(1→6)galactoside	*V. galamensis*[389]
223	Isorhamnetin	*V. fruticulosa*[390]
224	Acacetin	*V. fruticulosa*[390]
225	Tamarixetin	*V. fruticulosa*[390]
226	(1*S**,4*R**,5*S**,6*S**,8*S**,10*R**)-1,4,5,8,10-Penta-hydroxy13-*O*-methylcadin-7(11)-en-6,12-olide	*V. fruticulosa*[390]
227	5-Methylcoumarins,2′-epicycloisobrachycoumarinone epoxide	*V. brachycalyx*[391]
228	Cycloisobrachycoumarinone epoxide	*V. brachycalyx*[391]
229	Luteolin-4′-*O*-glucoside	*V. cinerea*[392]
230	Chlorogenic acid	*V. cinerea*[392]
231	3,5-Dicaffeoylquinic acid	*V. cinerea*[392]
232	3,4-Dicaffeoylquinic acid	*V. cinerea*[392]
233	4,5-Dicaffeoylquinic acid	*V. cinerea*[392]
234	Methyl caffeate	*V. cinerea*[392]
235	11*β*,13-Dihydrovernolide	*V. colorata*[393]
236	Vernoguinosterol	*V. guineensis*[394]
237	Vernoguinoside	*V. guineensis*[394]
238	Ethyl-3,4-dicaffeoylquinate	*V. patula*[395]
239	Protocatechuic acid	*V. patula*[395]
240	Caffeic acid	*V. patula*[395]
241	16*β*,22*R*;21,23*S*-Diepoxy-3*β*-*O*-*β*-D-glucopyranosyl-oxy-21*S*,24-dihydroxy-5*α*-stigmasta-8,14-dien-28-one	*V. guineensis*[396]
242	16*β*,22*R*;21,23*S*-Diepoxy-21*S*,24-dihydroxy-5*α*-stigmasta-8,14-diene-3,28-dione	*V. guineensis*[396]
243	1′,3,3′,4′,6′-Pentakis-*O*-(3-methylbutanoyl)-*β*-D-fructofuranosyl-*α*-D-glucopyranoside	*V. guineensis*[396]
244	1′,2,3′,6,6′-Pentakis-*O*-(3-methylbutanoyl)-*β*-D-fructofuranosyl-*α*-D-glucopyranoside	*V. guineensis*[396]
245	Zaluzanin D	*V. arborea*[397]
246	Bauerenyl acetate	*V. patula*[398]
247	Epifriedelanol	*V. patula*[398]
248	20(30)-Taraxastene-3*β*-21*α*-diol	*V. patula*[398]
249	3-*O*-{*β*-D-Galactopyranosyl-(1-2)-[*β*-D-gluco-pyranosyl-(1→6)]-*β*-D-glucopyranoside}-5*α*,14*α*-androst-8-ene	*V. colorata*[399]
250	3-*O*-[*β*-D-Glucopyranosyl-(1→6)-*β*-D-gluco-pyranoside]-5*α*,14*α*-androst-8-ene	*V. colorata*[399]

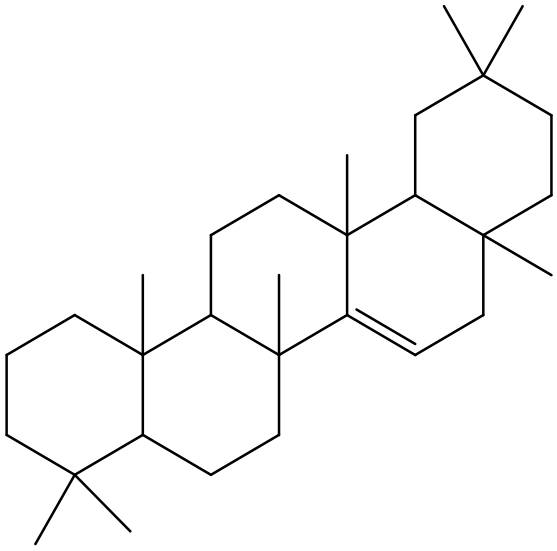
251	3*β*,21,24-Trihydroxy-21,23;22,28;26,28-triepoxy-5α-stigmasta-8(9),14(15)-dien-3-*O*-*β*-D-galacto-pyranosyl-(1→2)-*β*-D-glucopyranoside	*V. colorata*[399]
252	3*β*,21,24-Trihydroxy-21,23;22,28;26,28-triepoxy-5α-stigmasta-8(9),14(15)-dien-3-*O*-*β*-D-galacto-pyranosyl-(1→2)-*β*-D-(6-acetyl)glucopyranoside	*V. colorata*[399]
253	3*β*,25,29-Trihydroxy-5α-stigmasta-8(9),14(15),24*Z*(28)-triene	*V. colorata*[399]
254	3*β*,23,25-Trihydroxy-24,28-epoxy-5*α*-stigmasta-8(9),14(15)-diene	*V. colorata*[399]
255	2′,3,4,4′-Tetrahydroxychalcone	*V. anthelmintica*[400]
256	5,6,7,4′-Tetrahydroxyflavone	*V. anthelmintica*[400]
257	Butin	*V. anthelmintica*[400]
258	Vernonioside G	*V. cumingiana*[338]
259	24-Methylenelanost-9(11)-en-3*β*-ol acetate	*V. cumingiana*[338]
260	Ursolic acid	*V. cumingiana*[338]
261	Stearic acid	*V. cumingiana*[338]
262	Glaucolides K	*V. pachyclada*[401]
263	Glaucolide L	*V. pachyclada*[401]
264	Glaucolide M	*V. pachyclada*[401]
265	Lup-20(29)-ene-3,30-diol-3-acetate	*V. chunii*[402]
266	Lupenyl acetate	*V. chunii*[402]
267	22*E*,24*R*-Ergosta-5,22-dien-3*β*-ol	*V. chunii*[402]
268	Betulinic acid	*V. chunii*[402]
269	Vernoioside S	*V. cumingiana*[403]
270	Vernoniether S	*V. cumingiana*[403]
271	3,2′-Bis(3,4-dihydroxyphenyl)-5,3′-dihydroxy-6,7-combine dihydropyran-3,4-dihydro-benzopyrone	*V. chunii*[404]
272	1,3-Di-*O*-caffeoylquinic acid	*V. chunii*[404]
273	1,5-Di-*O*-caffeoylquinic acid	*V. chunii*[404]
274	1-(3,4-Dimethoxyphenyl)-2,3-dimethanol-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene	*V. chunii*[404]
275	Ferulic acid	*V. chunii*[404]
276	(+)-Lirioresinol B	*V. cinerea*[405]
277	Stigmasterol-3-*O*-*β*-D-glucoside	*V. cinerea*[405]
278	4-Sulfo-benzocyclobutene	*V. cinerea*[405]
279	4*α*,10*α*-Epoxyaromadendrane	*V. chalybaea*[406]
280	Taraxasteryl acetate	*V. chalybaea*[406]
281	Pseudotaraxasteryl acetate	*V. chalybaea*[406]
282	2,3-dimethyl-1,2,3-butanetriol	*V. chalybaea*[406]
283	Angophorol	*V. chalybaea*[406]
284	Angophorol-7-*O*-glucoside	*V. chalybaea*[406]
285	Angophorol-7-*O*-rutinoside	*V. chalybaea*[406]
286	3,7-Dimethoxy-5,3′,4′-trihydroxyflavone	*V. chalybaea*[406]
287	Lupenyl propionate	*V. chunii*[407]
288	Vernocuminoside A	*V. cumingiana*[408]
289	Vernocuminoside B	*V. cumingiana*[408]
290	Vernocuminoside C	*V. cumingiana*[408]
291	Vernocuminoside D	*V. cumingiana*[408]

292	Vernocuminoside E	*V. cumingiana*[408]
293	Vernocuminoside F	*V. cumingiana*[408]
294	Vernocuminoside G	*V. cumingiana*[408]
295	Quercetin-3-*O*-*β*-D-xylopyranosyl-(1-4)-*β*-D-gluco-pyranoside	*V. ferruginea*[409]
296	Quercetin-3-*O*-*β*-D-apiofuranosyl-(1-4)-*β*-D-gluco-pyranoside	*V. ferruginea*[409]
297	Kaempferol 3-*O*-*β*-D-apiofuranosyl-(1-4)-*β*-D-gluco-pyranoside	*V. ferruginea*[409]
298	Kaempferol-4′-methyl ether-3-*O*-*β*-D-xylopyranoside	*V. ferruginea*[409]
299	(-)-Clovane-2,9-diol	*V. cinerea*[410]
300	Caryolane-1,9*β*-diol	*V. cinerea*[410]
301	Apigenin	*V. cinerea*[410]
302	Chrysoeriol	*V. cinerea*[410]
303	Thermopsoside	*V. cinerea*[410]
304	Luteolin-7-*O*-*β*-D-glucoside	*V. cinerea*[410]
305	Apigenin-4'-*O*-*β*-D-glucoside	*V. cinerea*[410]
306	Hyperin	*V. cinerea*[410]
307	Incaspitolide D	*V. patula*[411]
308	(*S*)-*N*-benzoylphenylalanine-(*S*)-2-benzamido-3-phenyl-propyl ester	*V. patula*[411]
309	Indole-3-carboxylic acid	*V. patula*[411]
310	Diosmetin	*V. patula*[411]
311	*cis*-(12*S*,13*R*)-(3-Methylpentyl) vernolate	*V. galamensis*[412]
312	*cis*-(12*S*,13*R*)-(2,3-Propanediol) vernolate	*V. galamensis*[412]
313	Vernocuminoside H	*V. cumingiana*[413]
314	Vernocuminoside I	*V. cumingiana*[413]
315	Vernocuminoside J	*V. cumingiana*[413]
316	Vernocuminoside K	*V. cumingiana*[413]
317	Vernocuminoside L	*V. cumingiana*[413]
318	Vernocuminoside M	*V. cumingiana*[413]
319	Vernocuminoside N	*V. cumingiana*[413]
320	Vernodalidimer A	*V. anthelmintica*[414]
321	Vernodalidimer B	*V. anthelmintica*[414]
322	5,7,4'-Trihydroxy-6,3'-dimethoxyflavone	*V. spirei*[415]
323	5,3'-Dihydroxy-6,7,4'-trimethoxyflavone	*V. spirei*[415]
324	Methyl-3,5-dicaffeoylquinate	*V. cumingiana*[416]
325	Methyl-3,4-dicaffeoylquinate	*V. cumingiana*[416]
326	Ethyl-3,4-dicaffeoylquinate	*V. cumingiana*[416]
327	Methyl-3,4,5-tricaffeoylquinate	*V. cumingiana*[416]
328	24-Methylene-lanosta-9(11)-en-3*β*-acetate	*V. cumingiana*[416]
329	Ethyl gallate	*V. cumingiana*[416]
330	Di-butyl phthalate	*V. cumingiana*[416]
331	Myricetin	*V. anthelmintica*[417]
332	Astragalin	*V. anthelmintica*[417]

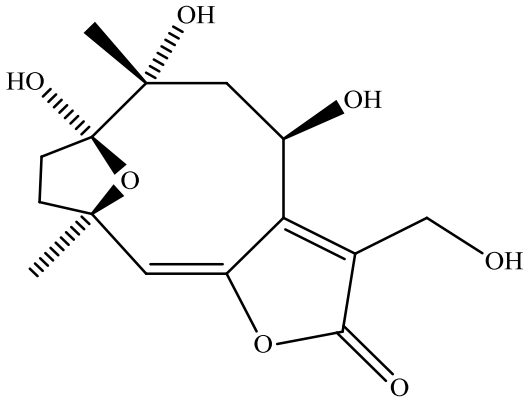
9.3 Structures of some compounds reported from genus *Vernonia*



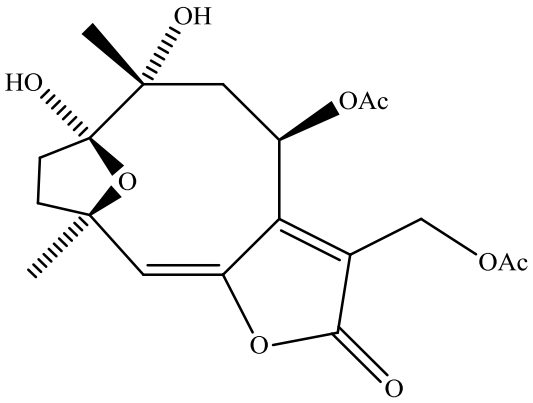
24-Hydroxytaraxer-14-ene[360]



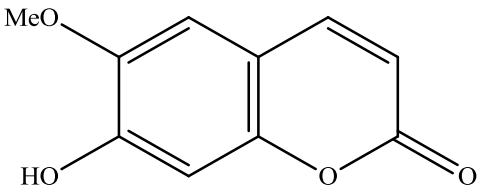
Taraxer-14-ene[360]



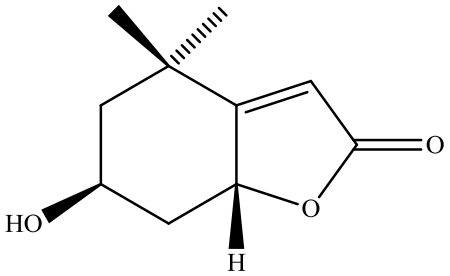
Piptacarphol[365]



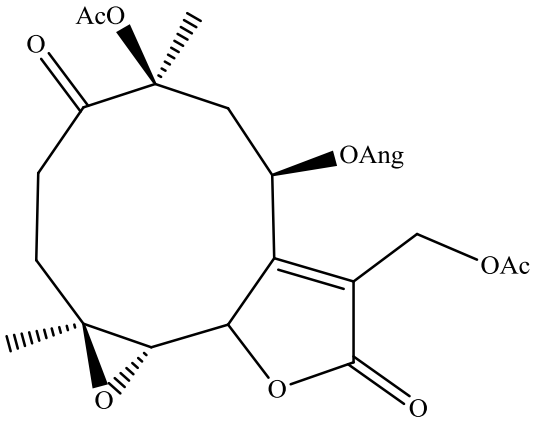
Diacetylpiptacarphol[365]



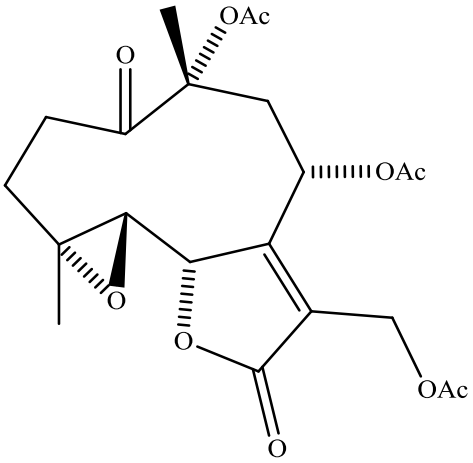
Scopoletin[365]



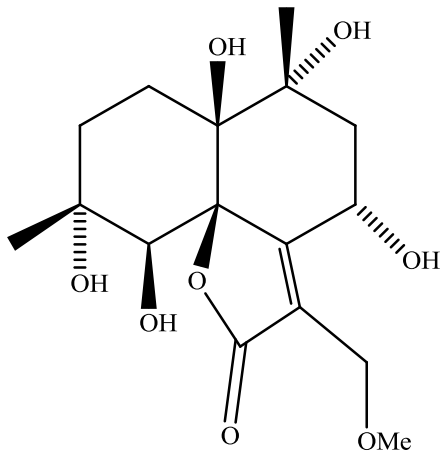
Loliolide[365]



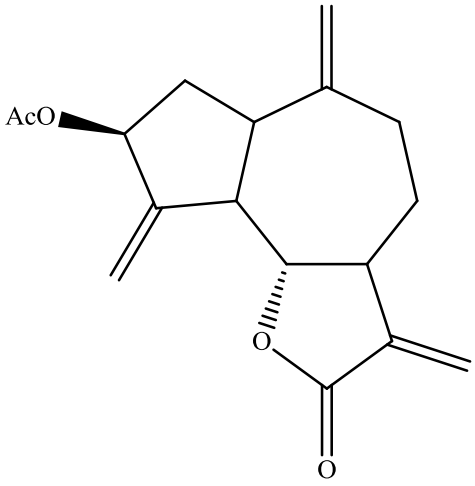
Glaucolide G[365]



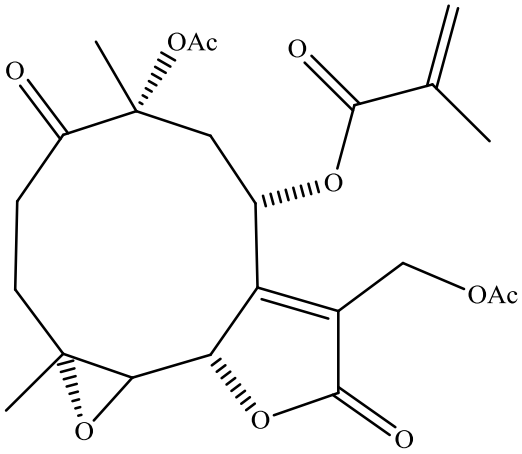
Glaucolide B[371]



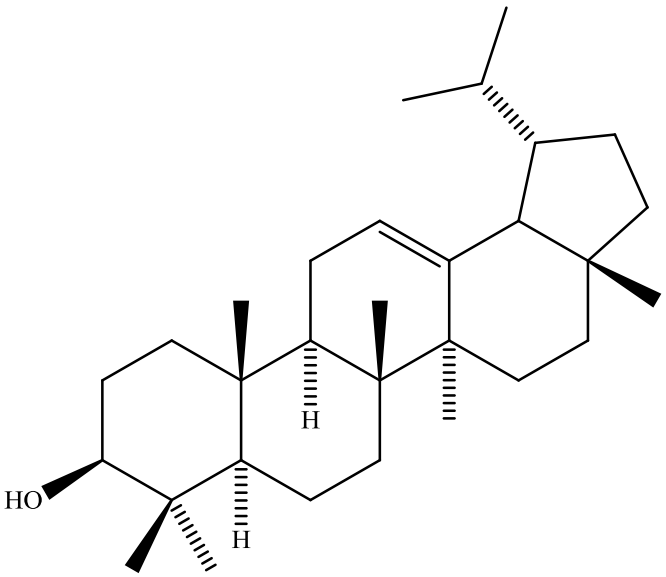
(1*S**, 4*R**, 5*S**, 6*S**, 8*S**, 10*R**)-1,4,5,8,10-Pentahydroxy-13-*O*-methylcadin- 7(11)-en-6,12-olide[390]



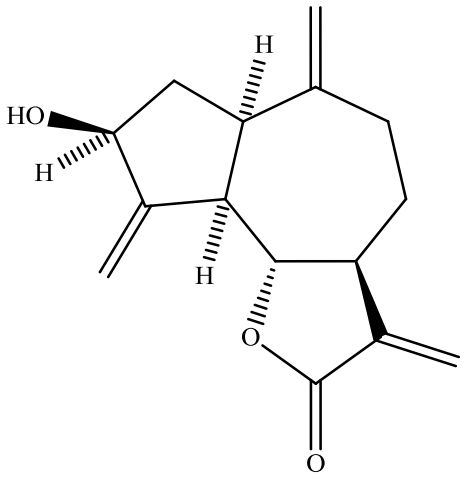
Zaluzanin D[397]



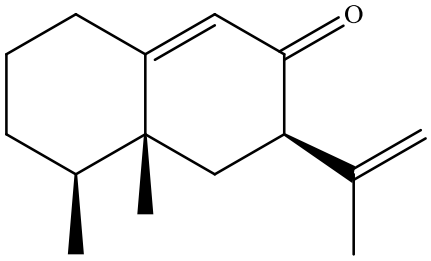
Glaucolide A[418]



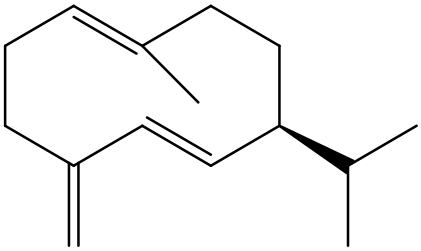
Aplotaxene[351]



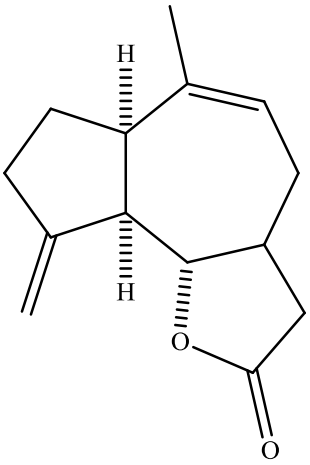
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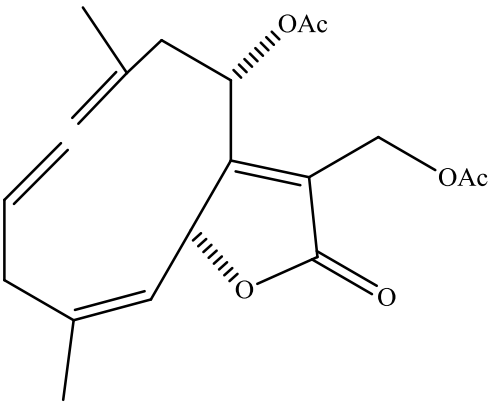
Eremophilone[351]



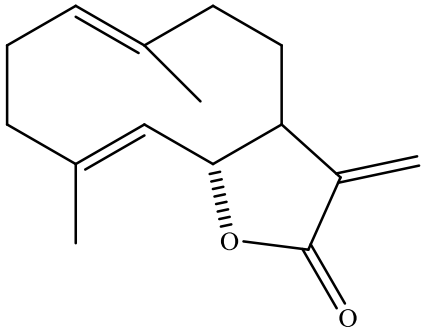
Germacrene D[351]



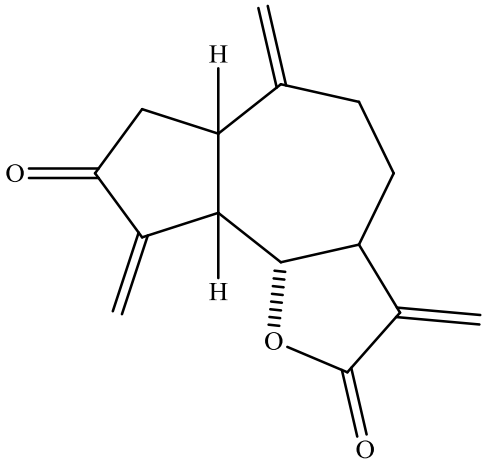
Guaianolide[351]



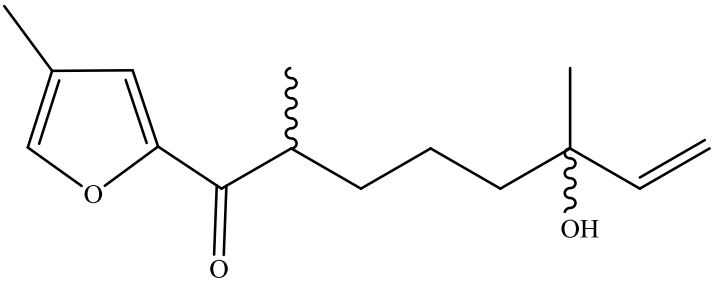
Vernonallenolide[352]



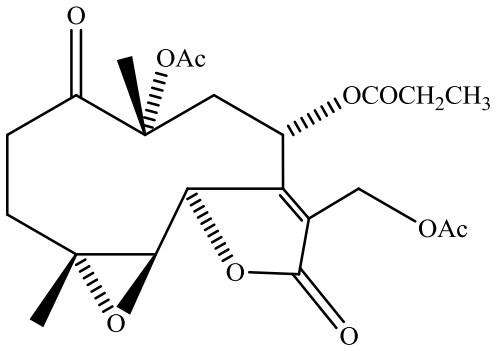
Costunolide[352]



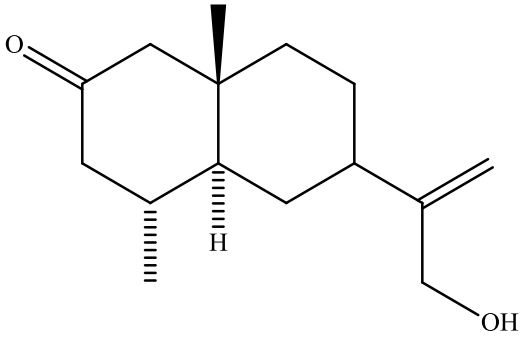
Dehydro-zaluzanin C[352]



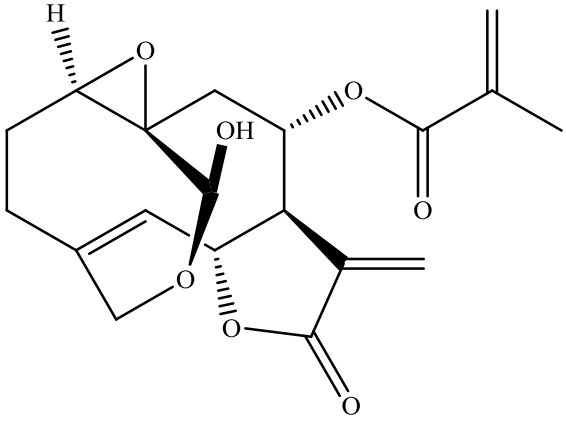
Vernopolyanthofuran[352]



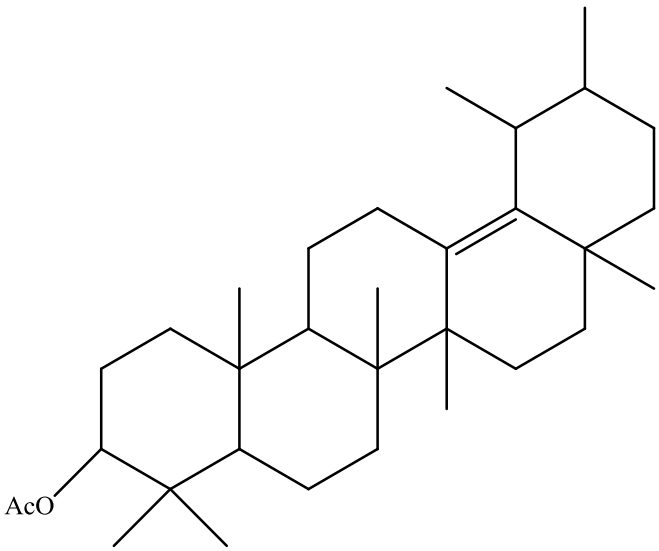
8-*O*-Desacetyl-8-*O*-glaucolide B propionate[352]



13-Hydroxy-4*β*,15*H*-*α*-selinen-2-one[352]



Vernolide[330]



3*β*-Acetoxyurs-13(18)-ene[339]

9.4 Medicinal and pharmacological properties of genus *Vernonia*

Several *Vernonia* species are used as traditional medicines in different parts of the world.[419] *V. patula* has been used in folk remedies for curing of acute gastroenteritis, headache, rheum and malaria.[420] *V. amygdalina* exhibit anti-bacterial, antifungal, nematicidal, anti-plasmodial, insecticidal, antibiotic, antioxidant, anticancer, antiviral, antimalarial, anti-helminthic and anti-inflammatory activities. It has also been used for the treatment of schistomiasis, amoebic dysentery, gastrointestinal problems, venereal diseases, wounds, hepatitis, diabetes, against menstrual pain, fever, hiccups, kidney problems, stomach pain, skin infections, as anthelmintic against leishmaiasis, as a purgative, as laxatives, as fertility inducers in barren women, as well as in the treatment of eczema.[330-337] *V. cumingiana* has been used to cure malaria, sore throat, cold, tooth aches, rheumatism aches, strain of the lumbar muscles, injuries and eye keratitis.[338] *V. cinerea* used for the treatment of tridosa, consumption, asthma, bronchitis, stomachic and astringent.[339] *V. kotschyana* has been used to treat gastritis, gastro duodenal ulcers, as an aid to ameliorate digestion and in wound healing.[421] The leaves of *V. colorata* have been used for tonsillitis, earache and febrifuge.[399] *V. cumingiana* has been used for the treatment of lumbocrural pain, rheumatic arthritis, fracture and malaria.[422] Some *Vernonia* species have been used to treat menstrual disorders, hair regeneration, dysentery, as a coagulant and to prevent stomach and liver disturbances.[423] *V. anthelmintica* has been used as vermicide, for the treatment of vitiligo and breast cancer.[400] *V.guineensis* used as an aphrodisiac, an anthelmintic, an antidote to poison and to treat malaria and jaundice in Cameroon.[394]

10.1 *Vernonia anthelmintica*

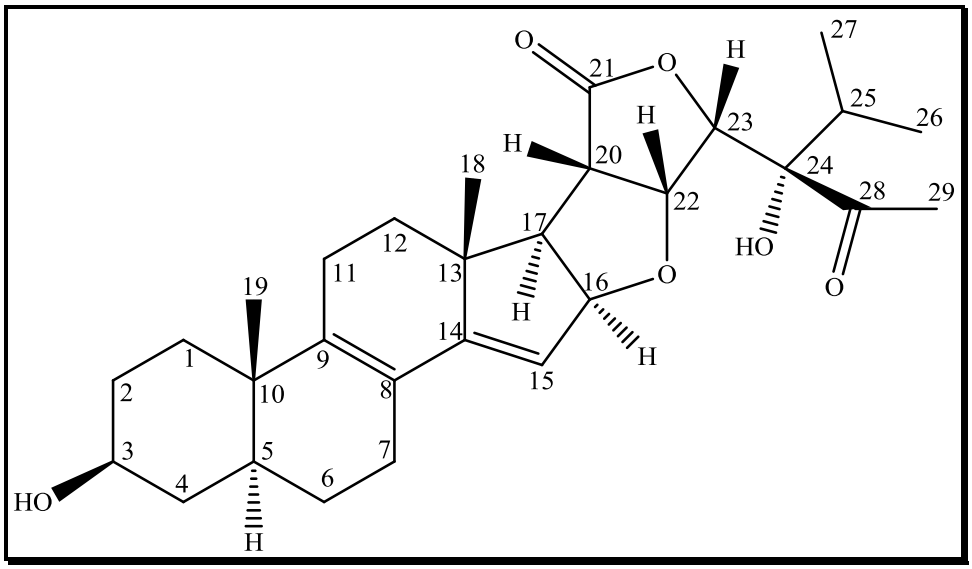
This part of the thesis describes phytochemistry and bioactivities of the aerial parts of *V. anthelmintica*. As a result eleven new compounds (**62**-**72**) have been isolated[424]. Their structures were elucidated through advanced spectroscopic techniques.



Vernonia anthelmintica

10.1.1 Structure elucidation of new compounds isolated from *V. anthelmintica*

10.1.1.1 Vernoanthelcin A (62)



Compound **62** was obtained as colorless crystals with a melting point of 274-276 ^{o}C, showed a molechular formula $C_{29}H_{40}O_6$ (ten degrees of unsaturation) having a molecular mass of 484 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 485.2904, calcd. for $[M + H]^+$ 485.2898). The IR spectrum revealed the absorption bands of hydroxyl group (3476 cm^{-1}), carbonyl groups (1766 and 1738 cm^{-1}) and a conjugated C=C band (1697 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-10.1**) of **62** was typical of a sterol structure, displaying two methyl singlets at δ_H 0.94 (H-18) and 1.01 (H-19), a characteristic multiplet for H-3 at δ_H 3.63 (m) and two methyl groups of an isopropyl group at δ_H 1.00 (d, J = 7.2 Hz, H-26) and 1.03 (d, J = 7.2 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.1**) indicated 29 carbon signals, including an ester carbonyl group at δ_C 177.4 (C-21), a ketonic carbonyl group at 214.8 (C-28) and four sp^2 and five oxygenated carbon atoms. Combined analysis of DEPT and HSQC spectra

revealed a conjugated diene, which consisted of a trisubstituted carbon at δ_C 114.8 (C-15) and three tetrasubstituted carbons at 156.6 (C-14), 144.3 (C-9) and 123.0 (C-8). The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 indicated a sterol nucleus. Moreover, the UV spectrum showed an absorption maximum at 246 nm, a typical UV characteristic of $\Delta^{8,14}$ steroidal nucleus compounds and confirmed the position of the conjugated diene.[396,425-429] The ^1H–^1H COSY spectrum of **62** demonstrated the connectivities: H-15/H-16/H-17/H-20/H-22/H-23. The HMBC correlation between H-16/C-22 indicated that a 16,22-oxygen bridge was present in **62**. The ether bond between C-16 and C-22 was confirmed by the carbon chemical shifts at δ_C 89.7 and 83.0. The ester carbonyl at δ_C 177.4 (C-21), showing HMBC correlations with δ_H 2.95 (H-17), 3.25 (H-20), 4.82 (H-22) and 4.85 (H-23) was unambiguously connected to C-20 at δ_C 48.8 and C-23 at 80.3. Furthermore, the HMBC correlation of δ_C 84.1 (C-24) with δ_H 4.85 (H-23), 1.00 (H_3-26), 1.03 (H_3-27) and 2.35 (H_3-29) indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 at δ_C 35.2 and C-28 at δ_C 214.8, respectively. Consequently, the planar structure of **62** was deduced to be as shown in **Figure-10.1**.

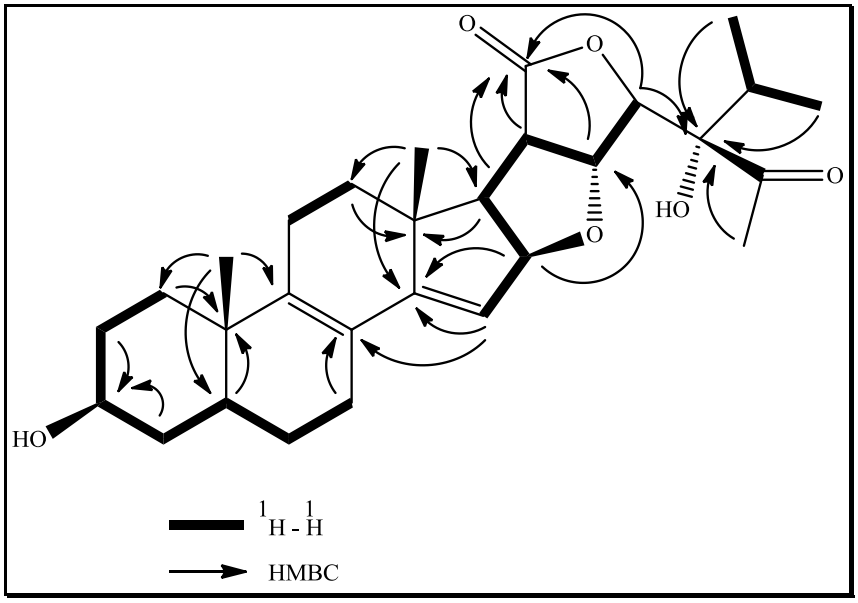


Figure-10.1: Selected COSY (^1H ─ ^1H) and HMBC (H→C) correlations of **62**

The relative configurations for C-17, C-20 and C-23 were determined by NOE difference experiments, which displayed NOEs for H-17 (4.2%) by irradiating H-16, for H-23 (3.4%) and H_3-18 (5.2%) when irradiating H-20, as well as for H-20 (3.9%) and H_3-27 (5.8%) by irradiating H-23, indicating the *α*, *β*, *β*-orientation of H-17, H-20, and H-23, respectively. Meanwhile, the NOESY correlation of H-3 with H-5 showed the *β*-orientation of HO-3. The structure of **62** and its absolute configuration was confirmed by X-ray diffraction analysis (**Figure-10.2**).[430,431]

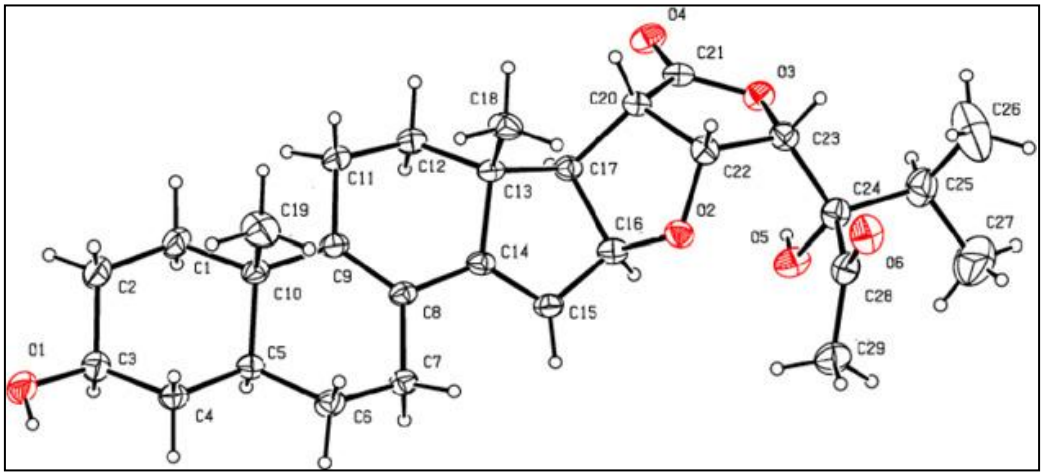


Figure-10.2: The X-ray structure of **62**

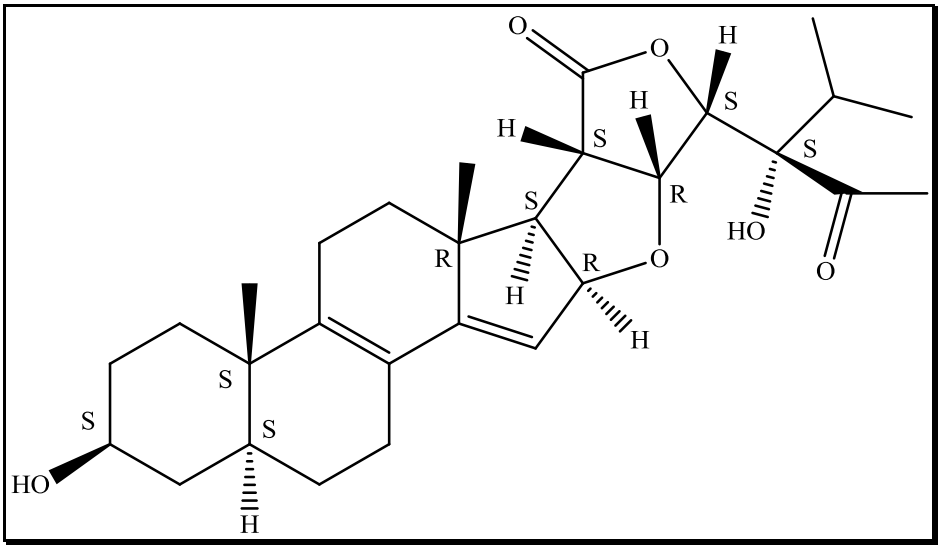


Figure-10.3: The absolute configuration of **62**

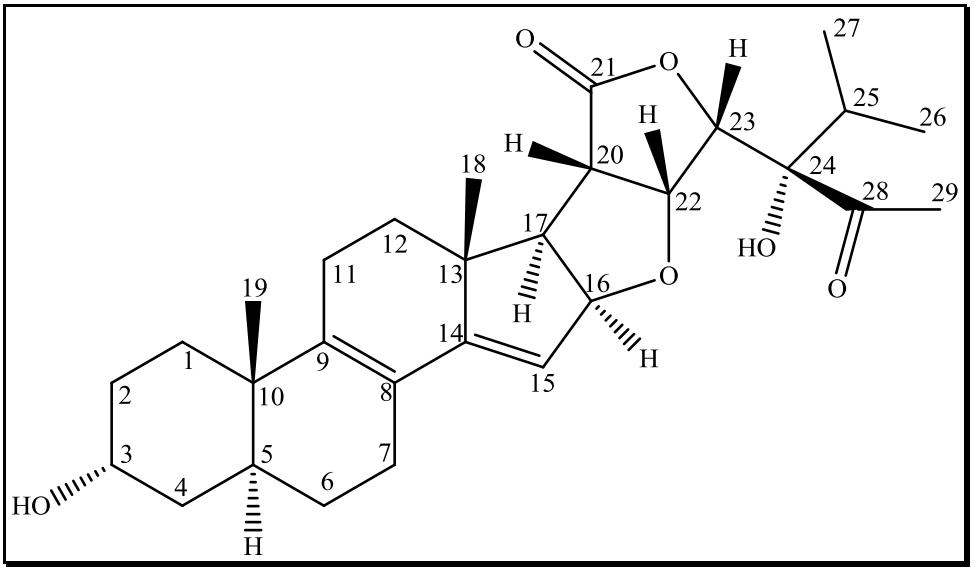
The absolute configuration of **62** was also demonstrated by applying the CD exciton chirality method.[432] Vernoanthelcin A **(62)** showed a CD split in the region of

210–250 nm corresponding to a negative chirality, which arose from the exciton coupling of the chromophores of the *trans*-conjugated double bond and the *γ*-lactone. The absolute configurations of the ten chiral centers in **62** were thus determined as 3*S*, 5*S*, 10*S*, 13*R*, 16*R*, 17*S*, 20*S*, 22*R*, 23*S* and 24*S*. The positive cotton effect at 290 nm due to the $n \rightarrow \pi^*$ transition of the carbonyl group of **62**. Thus, vernoanthelcin A **(62)** was identified as (3*S*,5*S*,10*S*,13*R*,16*R*,17*S*,20*S*,22*R*,23*S*,24*S*)-16,22-epoxy-21,23-lactone-3,24-dihydroxy-stigmasta-8,14-dien-28-one.

Table-10.1:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of vernoanthelcin A **(62)** in $CDCl_3$		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	34.9	CH_2	1.24, m
			1.82, m
2	31.5	CH_2	1.46, m
			1.90, m
3	70.7	CH	3.63, m
4	38.1	CH_2	1.35, m
			1.70, m
5	40.6	CH	1.42, m
6	24.9	CH_2	1.46, m
			1.63, m
7	26.8	CH_2	2.09, m
			2.40, m
8	123.0	C	--
9	144.3	C	--
10	36.8	C	--
11	22.2	CH_2	2.21, m
			2.31, m
12	36.2	CH_2	1.47, m
			1.94, m
13	44.9	C	--
14	156.6	C	--
15	114.8	CH	5.34, (d, 2.4)
16	89.7	CH	5.10, (dd, 2.4, 6.8)
17	56.3	CH	2.95, (d, 6.8)
18	20.5	CH_3	0.94, s
19	18.4	CH_3	1.01, s
20	48.8	CH	3.25, (d, 5.2)
21	177.4	C	--
22	83.0	CH	4.82, (dd, 3.6, 5.2)
23	80.3	CH	4.85, (d, 3.6)
24	84.1	C	--
25	35.2	CH	2.18, m

26	17.0	CH_3	1.00, (d, 7.2)
27	17.1	CH_3	1.03, (d, 7.2)
28	214.8	C	--
29	28.0	CH_3	2.35, s
24-OH	--	--	4.70, s

10.1.1.2 Vernoanthelcin B (63)



Compound **63** as white, amorphous solid, showed a molechular formula $C_{29}H_{40}O_6$ (ten degrees of unsaturation) having a molecular mass of 484 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 485.2909, calcd. for $[M + H]^+$ 485.2898). The IR spectrum revealed the absorption bands of hydroxyl group (3400 cm^{-1}), carbonyl groups (1772 and 1703 cm^{-1}) and a conjugated C=C band (1657 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-10.2**) of **63** displayed two methyl singlets at δ_H 0.94 (H-18) and 0.97 (H-19), a characteristic multiplet 4.08 (H-3), and two methyl groups of an isopropyl group 1.02 (d, *J* = 7.2 Hz, H-26) and 1.04 (d, *J* = 7.2 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.2**) indicated 29 carbon signals, including an ester carbonyl group at δ_C 177.4 (C-21), a ketonic carbonyl group 214.9 (C-28), and four sp^2 and five oxygenated carbon atoms. HSQC spectra revealed a

conjugated diene, which consisted of a trisubstituted carbon at δ_C 114.6 (C-15) and three tetrasubstituted carbons at 56.8 (C-14), 144.6 (C-9) and 123.0 (C-8). The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 indicated the presence of diene in the sterol nucleus. Moreover, the UV spectrum showed typical absorption maximum at 246 nm, characteristic of $\Delta^{8,14}$ steroidal nucleus and confirmed the position of the conjugated diene.[396,425-429] The ^1H–^1H COSY spectrum of **63** demonstrated the following connectivities: H-15/H-16/H-17/H-20/H-22/H-23. The HMBC correlation between H-16/C-22 indicated that a 16,22-oxygen bridge was present in **63**. The ether bond was confirmed by the carbon chemical shifts at δ_C 89.8 (C-16) and δ_C 83.0 (C-22). The ester carbonyl at δ_C 177.4 (C-21), showing HMBC correlations with δ_H 2.95 (H-17), 3.23 (H-20), 4.80 (H-22), and 4.84 (H-23) was unambiguously connected to C-20 at δ_C 48.9 and C-23 at δ_C 80.3. Furthermore, the HMBC correlation of C-24 at δ_C 84.1 with H-23, H_3-26, H_3-27, and H_3-29 indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 at δ_C 35.0 and C-28 at δ_C 214.9, respectively. Consequently, the planar structure of **63** was deduced to be as shown in **Figure-10.4**.

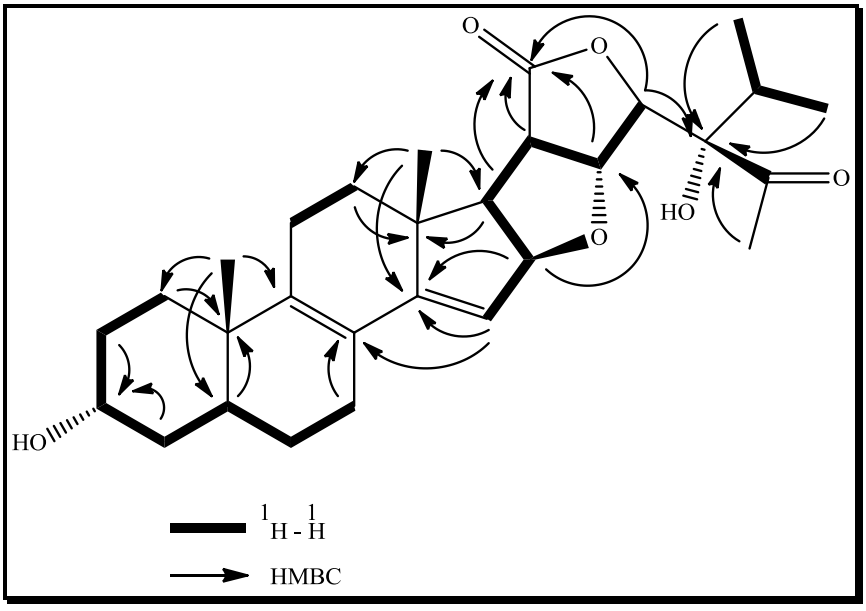


Figure-10.4: Selected COSY (^1H ─ ^1H) and HMBC (H→C) correlations of **63**.

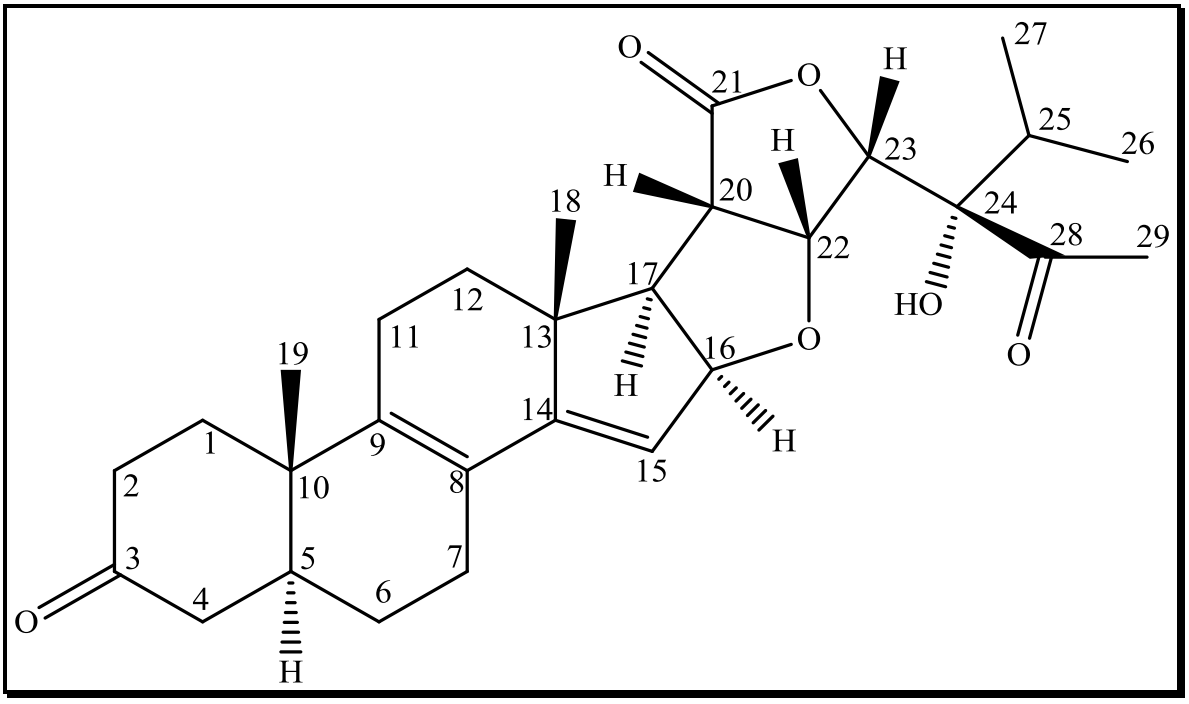
The relative configurations for C-17, C-20 and C-23 were assigned through NOE difference experiments, which displayed NOEs for H-17 (4.2%) by irradiating H-16, for H-23 (3.4%) and H_3-18 (5.2%) when irradiating H-20, as well as for H-20 (3.9%) and H_3-27 (5.8%) by irradiating H-23, indicating the *α*, *β*, *β*-orientation of H-17, H-20, and H-23, respectively.

The absolute configuration of **63** was also demonstrated by applying the CD exciton chirality method.[432] Vernoanthelcin B **(63)** showed a CD split in the region of 210–250 nm corresponding to a negative chirality, which arose from the exciton coupling of the chromophores of the *trans*-conjugated double bond and the *γ*-lactone. The absolute configurations of the ten chiral centers in **63** were thus determined as 3*R*, 5*S*, 10*S*, 13*R*, 16*R*, 17*S*, 20*S*, 22*R*, 23*S* and 24*S*. The positive cotton effect at 290 nm due to the $n\rightarrow\pi^*$ transition of the carbonyl group of **63**. Thus, vernoanthelcin B **(63)** was identified as (3*R*,5*S*,10*S*,13*R*,16*R*,17*S*,20*S*,22*R*,23*S*,24*S*)-16,22-epoxy-21,23-lactone-3,24-dihydroxy-stigmasta-8,14-dien-28-one.

Table-10.2:	^{1}H- (400 MHz) and ^{13}C- (150 MHz) NMR data of vernoanthelcin B **(63)** in $CDCl_3$		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	30.4	CH_2	1.50, m
			1.54, m
2	29.2	CH_2	1.67, m
			1.70, m
3	66.1	CH	4.08, br s
4	36.0	CH_2	1.49, m
			1.56, m
5	35.2	CH	1.85, m
6	24.8	CH_2	1.38, m
			1.48, m
7	26.7	CH_2	2.10, m
			2.40, m
8	123.0	C	--
9	144.6	C	--

10	37.3	C	--
11	21.9	CH_2	2.15, m
			2.32, m
12	36.2	CH_2	1.42, m
			1.89, m
13	45.0	C	--
14	156.8	C	--
15	114.6	CH	5.33, (d, 2.4)
16	89.8	CH	5.10, (dd, 2.4, 7.2)
17	56.4	CH	2.95, (d, 7.2)
18	20.5	CH_3	0.94, s
19	19.3	CH_3	0.97, s
20	48.9	CH	3.23, (d, 5.4)
21	177.4	C	--
22	83.0	CH	4.80, (dd, 3.6, 5.4)
23	80.3	CH	4.84, (d, 3.6)
24	84.1	C	--
25	35.0	CH	2.17, m
26	17.0	CH_3	1.02, (d, 7.2)
27	17.1	CH_3	1.04, (d, 7.2)
28	214.9	C	--
29	28.1	CH_3	2.35, s
24-OH	--	--	4.73, s

10.1.1.3 Vernoanthelcin C (64)



Compound **64** was isolated from *n*-hexane fraction as a light yellow amorphous powder, which showed a molechular formula $C_{29}H_{38}O_6$ possessing a molecular mass of 482 a.m.u. as confirmed by its positive-mode HRESIMS (*m/z* 483.2746, calcd. for $[M + H]^+$ 483.2741). The IR spectrum revealed the absorption bands of hydroxyl group (3396 cm^{-1}), carbonyl groups (1775 and 1709 cm^{-1}) and a conjugated C=C band (1650 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-10.3**) of **64** showed two methyl singlets at δ_H 0.97 (H-18) and 1.20 (H-19) and two methyl groups of an isopropyl group at 0.99 (d, *J* = 6.8 Hz, H-26) and 1.03 (d, *J* = 7.2 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.3**) indicated 29 carbon signals, including an ester carbonyl group at δ_C 177.6 (C-21), two ketonic carbonyl group at 211.0 (C-3) and 215.0 (C-28), and four sp^2 and four oxygenated carbon atoms. DEPT and HSQC spectra revealed a conjugated diene, which consisted of a trisubstituted carbon at δ_C 115.8 (C-15) and three tetrasubstituted carbons at 156.3 (C-14), 142.6 (C-9) and 124.0 (C-8). The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 implied that the diene is in the sterol nucleus, which was confirmed by UV absorption maximum at 245 nm, a typical UV characteristic of $\Delta^{8,14}$ steroidal nucleus compounds, and confirmed the position of the conjugated diene.[396,425-429] The ^{1}H–^{1}H COSY spectrum of **64** demonstrated the following connectivities: H-15/H-16/H-17/H-20/H-22/H-23. The carbon chemical shifts at δ_C 89.9 (C-16) and 83.2 (C-22) indicated the presence of ether bond in **64**, which was further confirmed by the HMBC correlations between H-16/C-22. The ester carbonyl at δ_C 177.6 (C-21), showing HMBC correlations with δ_H 2.96 (H-17), 3.25 (H-20), 4.81 (H-22) and 4.85 (H-23) was unambiguously connected to C-20 at δ_C 49.1 and C-23 at δ_C 80.5. The HMBC correlations between H-2 at δ_H 2.45 and C-3 at δ_C 211.0, H-4 at 2.24, 2.38 and C-3 revealed that the ketonic

group was located at C-3 in **64**. Furthermore, the HMBC correlation of C-24 at δ_C 84.3 with H-23, H_3-26, H_3-27 and H_3-29 indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 at δ_C 35.4 and C-28 at δ_C 215.0, respectively. Consequently, the planar structure of **64** was deduced to be as shown in **Figure-10.5**.

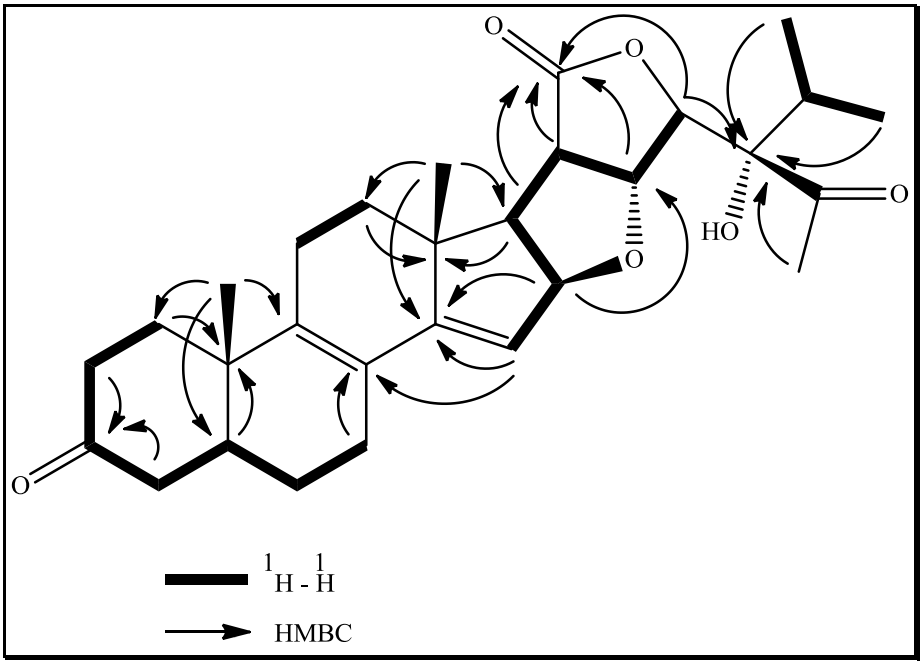


Figure-10.5: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **64**.

NOE difference experiment was used to identify the relative configurations for C-17, C-20 and C-23. Thus NOEs were observed for H-17 (4.2%) by irradiating H-16, for H-23 (3.4%) and H_3-18 (5.2%) when irradiating H-20, as well as for H-20 (3.9%) and H_3-27 (5.8%) by irradiating H-23, indicating the *α*, *β*, *β*-orientation of H-17, H-20, and H-23, respectively.

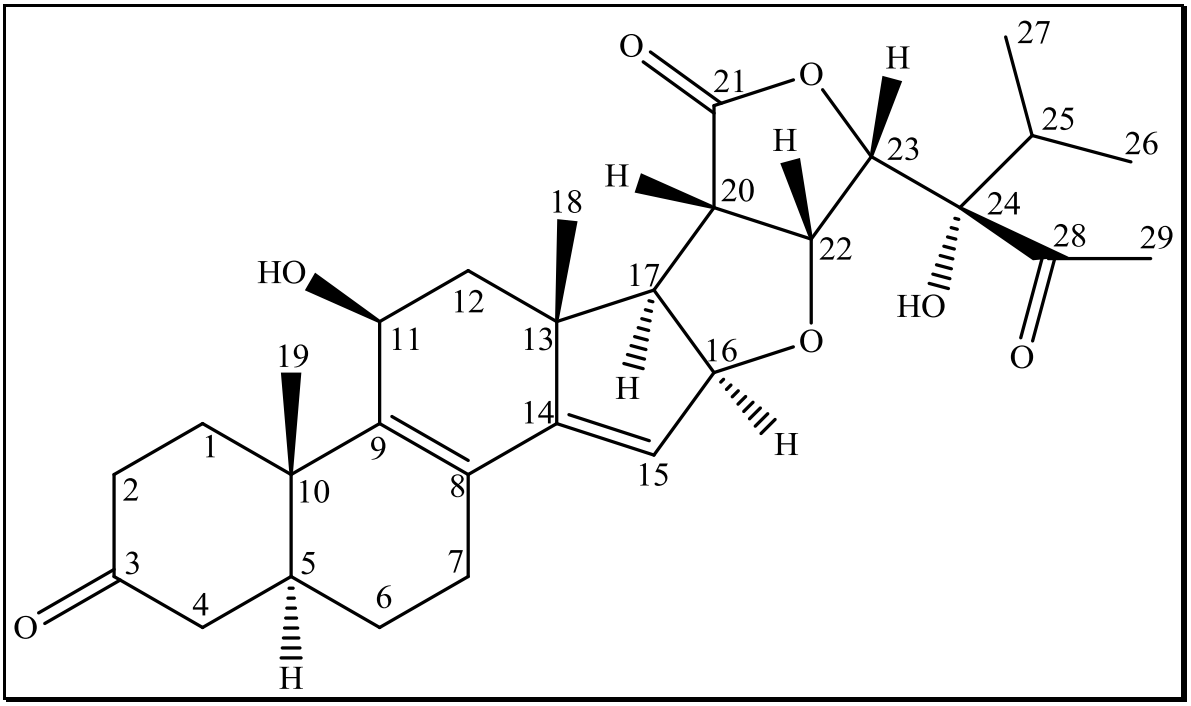
The absolute configuration of **64** was also demonstrated by applying the CD exciton chirality method[432] which showed a CD split in the region of 210–250 nm corresponding to a negative chirality, that arose from the exciton coupling of the chromophores of the *trans*-conjugated double bond and the *γ*-lactone. The absolute

configurations of the nine chiral centers in **64** were thus determined as 5*S*, 10*S*, 13*R*, 16*R*, 17*S*, 20*S*, 22*R*, 23*S* and 24*S*. The positive cotton effect at 290 nm due to the $n\rightarrow\pi^*$ transition of the carbonyl group of **64**. These spectral data led to its characterization as (5*S*,10*S*,13*R*,16*R*,17*S*,20*S*,22*R*,23*S*,24*S*)-16,22-epoxy-21,23-lactone-24-dihydroxy-stigmasta-8,14-dien-3,28-dione.

Table-10.3:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of vernoanthelcin C **(64)** in $CDCl_3$		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	36.5	CH_2	1.52, m
			2.16, m
2	38.3	CH_2	2.13, m
			2.45, m
3	211.0	C	--
4	44.7	CH_2	2.24, m
			2.38, m
5	42.7	CH	1.86, m
6	25.2	CH_2	1.46, m
			1.65, m
7	26.9	CH_2	2.15, m
			2.42, m
8	124.0	C	--
9	142.6	C	--
10	37.0	C	--
11	22.6	CH_2	2.32, m
			2.38, m
12	36.4	CH_2	1.62, m
			1.93, m
13	45.2	C	--
14	156.3	C	--
15	115.8	CH	5.38, (d, 2.4)
16	89.9	CH	5.11, (dd, 2.4, 6.8)
17	56.5	CH	2.96, (d, 6.8)
18	20.7	CH_3	0.97, s
19	17.7	CH_3	1.20, s
20	49.1	CH	3.25, (d, 5.2)
21	177.6	C	--
22	83.2	CH	4.81, (dd, 3.6, 5.2)
23	80.5	CH	4.85, (d, 3.6)
24	84.3	C	--
25	35.4	CH	2.17, m
26	17.2	CH_3	0.99, (d, 6.8)
27	17.3	CH_3	1.03, (d, 7.2)

28	215.0	C	--
29	28.2	CH_3	2.35, s
24-OH	--	--	4.68, s

10.1.1.4 Vernoanthelcin D (65)



A white amorphous powder, showed a molechular formula $C_{29}H_{38}O_7$ (eleven degrees of unsaturation) having a molecular mass of 498 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 521.2514, calcd. for $[M + Na]^+$ 521.2519). The IR spectrum revealed the absorption bands of hydroxyl group (3471 cm^{-1}), and carbonyl groups (1763 and 1699 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-10.4**) of **65** displayed methyl singlets at δ_H 1.24 (H-18), 1.40 (H-19), 0.99 (d, J = 6.8 Hz, H-26) and 1.03 (d, J = 7.2 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.4**) indicated 29 carbon signals, including an ester carbonyl group at δ_C 177.2 (C-21), two ketonic carbonyl groups at

211.0 (C-3) and 214.7 (C-28), and four sp^2 and five oxygenated carbon atoms. The spectra of DEPT and HSQC revealed a conjugated diene, which consisted of a trisubstituted carbon at δ_C 117.4 (C-15) and three tetrasubstituted carbons at 155.6 (C-14), 142.4 (C-9) and 126.6 (C-8). The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 implied that the diene is in the sterol nucleus as confirmed by UV spectrum which showed an absorption maximum at 245 nm, a typical UV characteristic of $\Delta^{8,14}$ steroidal nucleus.[396,425-429] The ^1H–^1H COSY spectrum of **65** demonstrated the connectivities: H-15/H-16/H-17/H-20/H-22/H-23. The HMBC correlation between H-16/C-22 indicated that a C-16 (δ_C 89.6) and C-22 (δ_C 83.4)-oxygen bridge was present in **65**. The ester carbonyl at δ_C 177.2 (C-21), showing HMBC correlations with δ_H 2.99 (H-17), 3.28 (H-20), 4.81 (H-22), and 4.86 (H-23), was unambiguously connected to C-20 at δ_C 48.9 and C-23 at δ_C 80.4. The HMBC correlations between H-2 at δ_H 2.50 and C-3 at δ_C 211.0, H-4 at δ_H 2.19, 2.30 and C-3 revealed that the ketonic group was located at C-3 in **65**. The ^{13}C-NMR spectrum showed another oxymethine signal at δ_C 65.8 (C-11) which was supported by the downfield value of ^1H-NMR signals at δ_H 1.24 (H_3-18) and 1.40 (H_3-19). This revealed the presence of one more hydroxyl group in **65**. HMBC correlations between H-12 at δ_H 2.12 and C-11 at δ_C 65.8, HO-11 and C-11, combined with ^1H-^1H COSY correlations of HO-11/H-11/H-12, suggested that the hydroxyl group was at C-11 **(Figure-10.6)** Furthermore, the HMBC correlation of C-24 with H-23, H_3-26, H_3-27, and H_3-29 indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 and C-28. Consequently, the structure of **65** was deduced to be as shown in **Figure-10.6**.

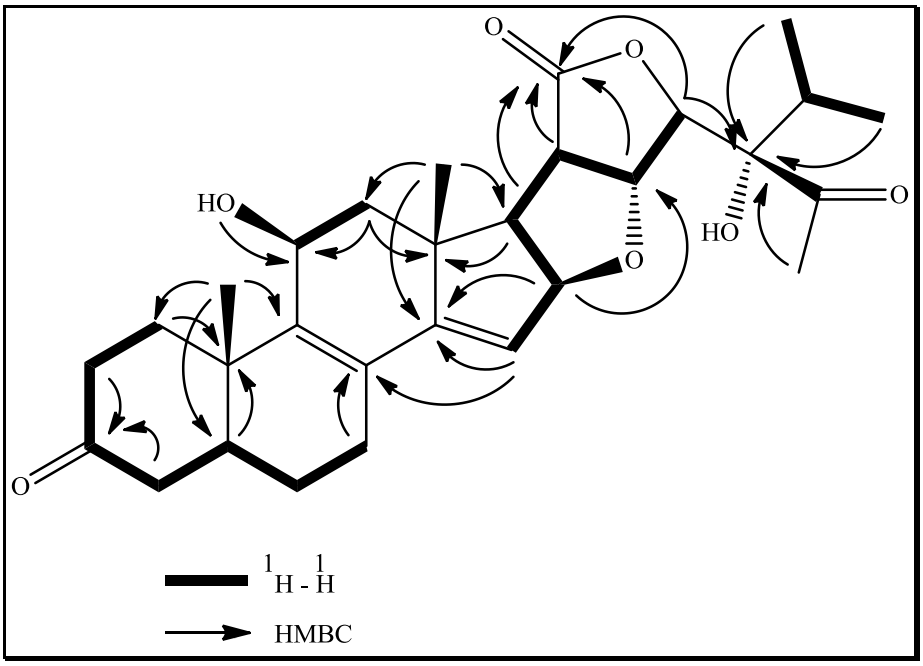


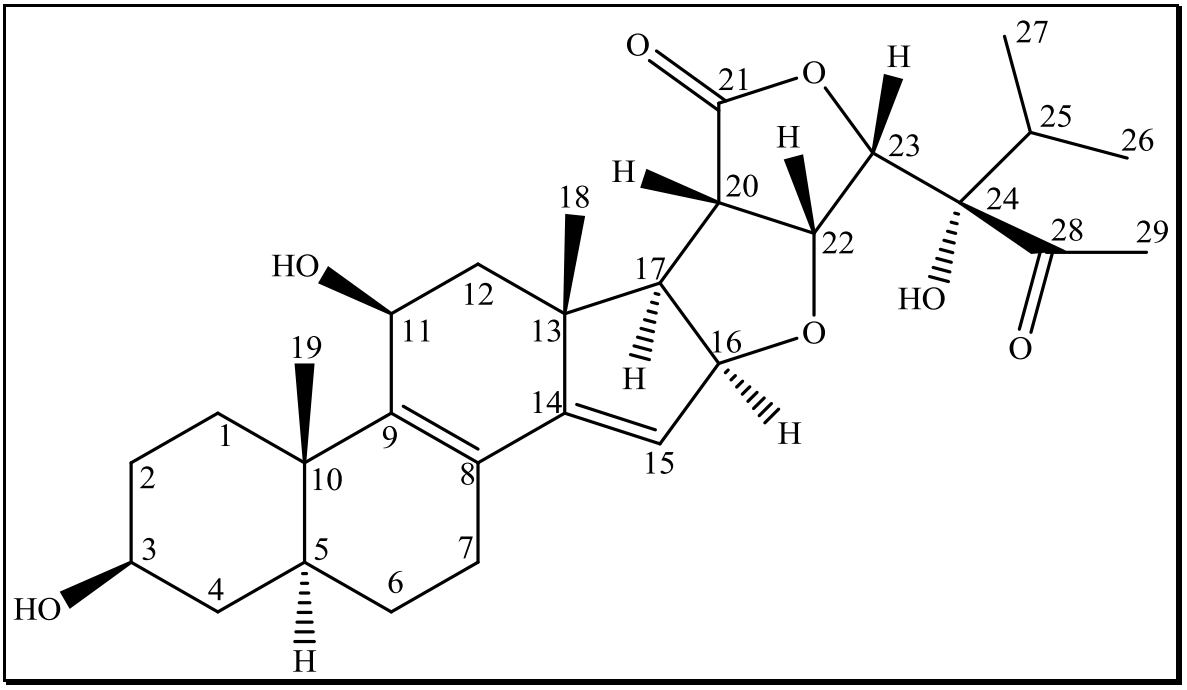
Figure-10.6: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **65**.

The relative configuration of **65** was established by NOESY spectrum. The NOESY correlations of H-11 with H-5 and H-16, and of H-17 with H-5 indicated that they were co-facial and *a*-configurations. Consequently, the NOESY cross-peaks of H-20 with H-23 and H_3-18, and of H-22 with H_3-18 revealed that bore *β*-orientations.

The absolute configuration of **65** was also demonstrated by applying the CD exciton chirality method.[432] Vernoanthelcin D **(65)** showed a CD split in the region of 210–250 nm corresponding to a negative chirality, which arose from the exciton coupling of the chromophores of the trans-conjugated double bond and the *γ*-lactone. The absolute configurations of the ten chiral centers in **65** were thus determined as 5*S*, 10*S*, 11*S*, 13*R*, 16*R*, 17*S*, 20*S*, 22*R*, 23*S* and 24*S*. The positive cotton effect at 290 nm due to the n→π^* transition of the carbonyl group of **65**. All the spectral data of **65** led to its characterization as (5*S*,10*S*,11*S*,13*R*,16*R*,17*S*,20*S*,22*R*,23*S*,24*S*)-16,22-epoxy-21,23-lactone-11,24-dihydroxy-stigmasta-8,14-dien-28-one.

Table-10.4:	^{1}H- (400 MHz) and ^{13}C- (150 MHz) NMR data of vernoanthelcin D **(65)** in $CDCl_3$		
C. No.	δ_C	**Multiplicity**	δ_H **(multiplicity, *J* in Hz)**
1	35.3	CH_2	1.63, m
			2.40, m
2	37.9	CH_2	2.39, m
			2.50, m
3	211.0	C	--
4	44.5	CH_2	2.19, m
			2.30, m
5	43.0	CH	1.85, m
6	24.8	CH_2	1.52, m
			1.56, m
7	26.2	CH_2	2.28, m
			2.33, m
8	126.6	C	--
9	142.4	C	--
10	36.3	C	--
11	65.8	CH	4.56, br s
12	46.8	CH_2	1.80, m
			2.12, m
13	44.2	C	--
14	155.6	C	--
15	117.4	CH	5.47, (d, 2.8)
16	89.6	CH	5.13, (dd, 2.8, 6.8)
17	56.6	CH	2.99, (d, 6.8)
18	23.4	CH_3	1.24, s
19	19.3	CH_3	1.40, s
20	48.9	CH	3.28, (d, 5.2)
21	177.2	C	--
22	83.4	CH	4.81, (dd, 3.2, 5.2)
23	80.4	CH	4.86, (d, 3.2)
24	84.0	C	--
25	35.3	CH	2.15, m
26	17.0	CH_3	0.99, (d, 6.8)
27	17.1	CH_3	1.03, (d, 7.2)
28	214.7	C	--
29	28.0	CH_3	2.34, s
24-OH	--	--	4.51, s

10.1.1.5 Vernoanthelcin E (66)



Compound **66** was isolated from the ethyl acetate fration as a white amporphous solid. The molechular formula $C_{29}H_{40}O_7$ (ten degrees of unsaturation) having a molecular mass of 500 a.m.u. was established through positive-mode HRESIMS which showed *m/z* 501.2854, calcd. for $[M + H]^+$ 501.2847. The IR spectrum revealed the absorption bands for hydroxyl group (3469 cm^{-1}) and carbonyl groups (1760 and 1692 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-10.5**) of **66** showed methyl signals at δ_H 1.22 (s, H-18), 1.20 (s, H-19), 1.00 (d, *J* = 7.2 Hz, H-26) and 1.04 (d, *J* = 7.2 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.5**) displayed 29 carbon signals, including an ester carbonyl group at δ_C 178.1 (C-21), a ketonic carbonyl group at δ_C 214.3 (C-28), and four sp^2 and six oxygenated carbon atoms. Combined analysis of DEPT and HSQC spectra revealed a conjugated diene, which consisted of a

trisubstituted carbon at δ_C 115.6 (C-15) and three tetrasubstituted carbons at δ_C 156.0 (C-14), 144.0 (C-9) and 125.0 (C-8). The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 attributed to the steroidal nucleus, which was confirmed by UV spectrum (246 nm), a typical UV characteristic of $\Delta^{8,14}$ steroidal nucleus.[396,425-429] The ^{1}H–^{1}H COSY spectrum of **66** demonstrated the connectivities: H-15/H-16/H-17/H-20/H-22/H-23. The HMBC correlation between H-16/C-22 indicated that a 16,22-oxygen bridge was present in **66**. The ether bond was confirmed by the carbon chemical shifts of C-16 at δ_C 88.8 and C-22 at δ_C 83.2. The ester carbonyl at δ_C 178.1 (C-21), showing HMBC correlations with H-17 at δ_H 2.87, H-20 at δ_H 3.43, H-22 at δ_H 4.83, and H-23 at δ_H 4.94, was connected to C-20 at δ_C 47.8 and C-23 at δ_C 81.5. The ^{13}C-NMR spectrum showed another oxymethine signal at C-11 (δ_C 64.2) which was supported by the downfield value of ^{1}H-NMR signals at H_3-18 (δ_H 1.22) and H_3-19 (δ_H 1.20). This revealed the presence of one more hydroxyl group in **66**. HMBC correlations between H-12 (δ_H 1.90) and C-11 (δ_C 64.2), HO-11 and C-11, combined with ^{1}H-^{1}H COSY correlations of HO-11/H-11/H-12, suggested that the hydroxyl group was at C-11. Furthermore, the HMBC correlation of C-24 at δ_C 82.6 with H-23, H_3-26 at δ_H 1.00, H_3-27 at δ_H 1.04, and H_3-29 at δ_H 2.34 indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 at δ_C 35.0 and C-28 at δ_C 214.3, respectively. The structure of **66** was deduced to be as shown in **Figure-10.7**.

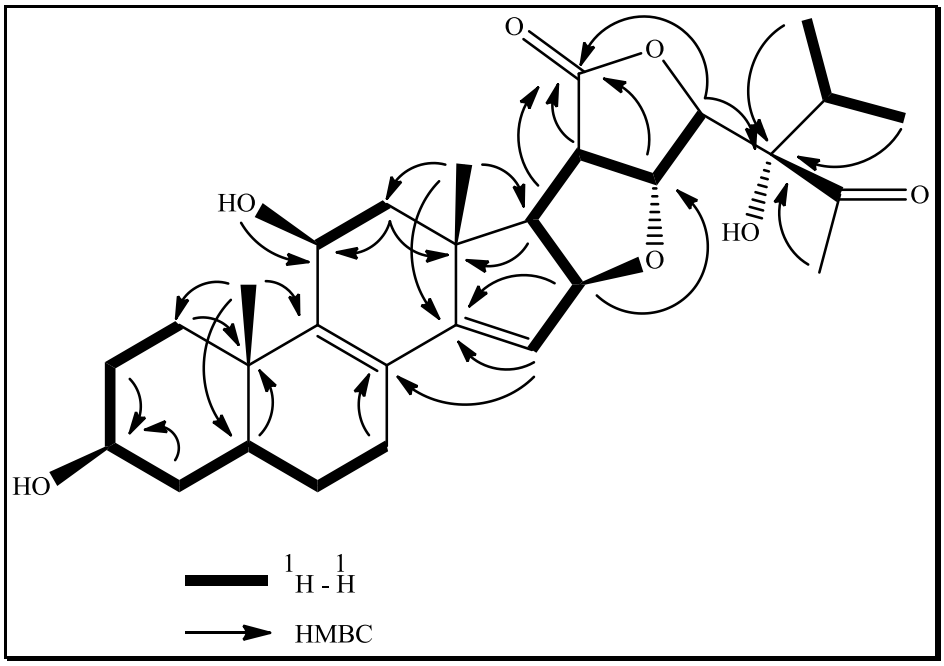


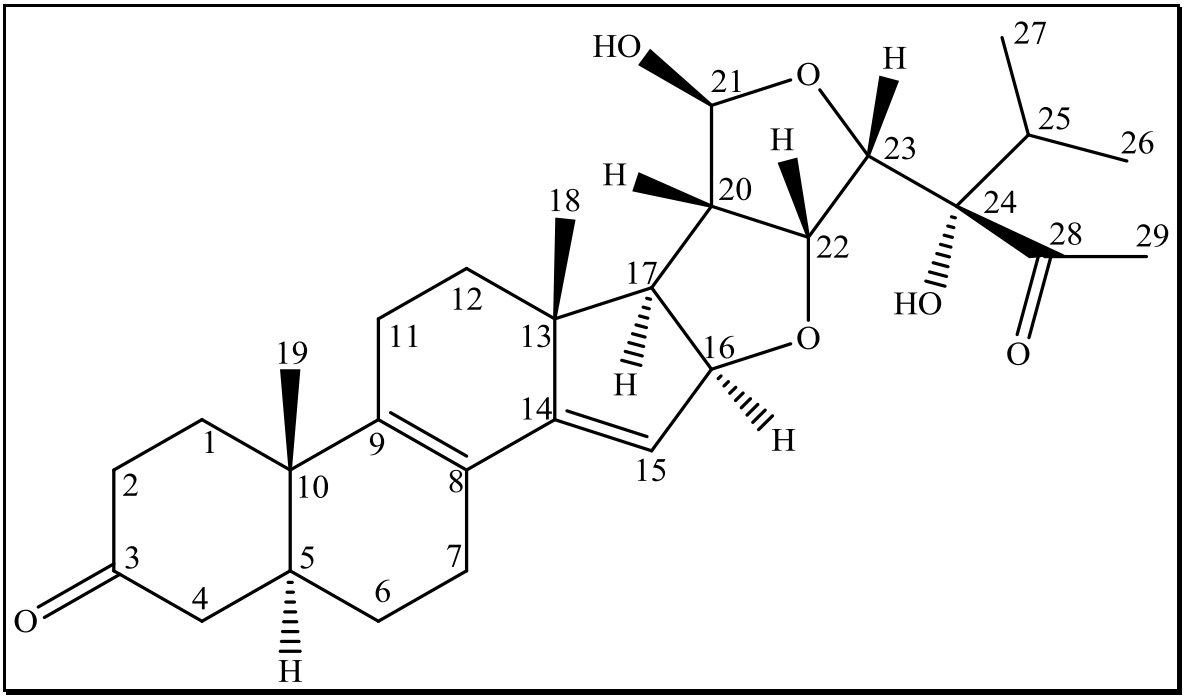
Figure-10.7: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **66**.

The relative configurations for C-17, C-20 and C-23 were determined by NOE difference experiments, which displayed NOEs for H-17 (4.2%) by irradiating H-16, for H-23 (3.4%) and H_3-18 (5.2%) when irradiating H-20, as well as for H-20 (3.9%) and H_3-27 (5.8%) by irradiating H-23, indicating the *α*, *β*, *β*-orientation of H-17, H-20, and H-23, respectively.

The absolute configuration of **66** was also demonstrated by applying the CD exciton chirality method.[432] Compound **(66)** showed a CD split in the region of 210–250 nm corresponding to a negative chirality, which arose from the exciton coupling of the chromophores of the trans-conjugated double bond and the *γ*-lactone. The absolute configurations of the eleven chiral centers in **66** were thus determined as 3*S*, 5*S*, 10*S*, 11*S*, 13*R*, 16*R*, 17*S*, 20*S*, 22*R*, 23*S* and 24*S*. The positive cotton effect at 290 nm due to the n→π^* transition of the carbonyl group of **66**. Based on these spectral data the structure of **66** was characterized as (3*S*,5*S*,10*S*,11*S*,13*R*,16*R*,17*S*,20*S*,22*R*,-23*S*,24*S*)-16,22-epoxy-21,23-lactone-3,11,24-trihydroxy-stigmasta-8,14-dien-28-one.

Table-10.5:	^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR data of vernoanthelcin E **(66)** in $CDCl_3$:CD_3OD (1:1)		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
1	33.2	CH_2	1.32, m
			2.19, m
2	30.1	CH_2	1.53, m
			1.85, m
3	69.6	CH	3.56, m
4	37.0	CH_2	1.35, m
			1.65, m
5	40.5	CH	1.43, m
6	24.1	CH_2	1.52, m
			1.68, m
7	25.6	CH_2	2.20, m
			2.45, m
8	125.0	C	--
9	144.0	C	--
10	35.7	C	--
11	64.2	CH	4.52, br s
12	45.6	CH_2	1.70, m
			2.12, m
13	43.6	C	--
14	156.0	C	--
15	115.6	CH	5.47, (d, 2.4)
16	88.8	CH	5.14, (dd, 2.4, 7.2)
17	57.3	CH	2.87, (d, 7.2)
18	22.5	CH_3	1.22, s
19	19.1	CH_3	1.20, s
20	47.8	CH	3.43, (d, 6.0)
21	178.1	C	--
22	83.2	CH	4.83, (dd, 3.6, 6.0)
23	81.5	CH	4.94, (d, 3.6)
24	82.6	C	--
25	35.0	CH	2.13, m
26	16.1	CH_3	1.00, (d, 7.2)
27	16.1	CH_3	1.04, (d, 7.2)
28	214.3	C	--
29	27.0	CH_3	2.34, s
24-OH	--	--	4.67, s

10.1.1.6 Vernoanthelcin F (67)



Compound **67** obtained as white amorphous powder, assigned a molechular formula $C_{29}H_{40}O_6$ (HRESIMS *m/z* 507.2711, calcd. for $[M + Na]^+$ 507.2717). The IR spectrum showed the absorption bands at 3392 cm^{-1} (OH) and 1741 cm^{-1} (carbonyl groups).

In ^{1}H-NMR spectrum (**Table-10.6**) of **67** three methyl singlets at δ_H 0.94 (H-18), 1.18 (H-19) and 2.34 (H-29) alongwith geminal methyl doublets at 0.96 (*J* = 6.8 Hz, H-26) and 1.02 (*J* = 7.2 Hz, H-27) were assumed. The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.6**) showed 29 carbon signals, including a hemiacetal carbon at δ_C 104.1 (C-21), two ketonic carbonyl group at δ_C 210.9 (C-3) and δ_C 215.6 (C-28), and four sp^2 and five oxygenated carbon atoms. The DEPT and HSQC spectra revealed a conjugated diene, consisted of a trisubstituted carbon at δ_C 116.0 (C-15) and three tetrasubstituted carbons at δ_C 156.4 (C-14), 142.5 (C-9) and 123.8 (C-8). The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 indicated that the

diene is in the sterol nucleus. Moreover, the UV spectrum showed an absorption maximum at 246 nm, which is a typical UV characteristic of $\Delta^{8,14}$ steroidal nucleus compounds and confirmed the position of the conjugated diene.[396,425-429] The $^{1}H–^{1}H$ COSY spectrum of **67** showed the following connectivities: H-15/H-16/H-17/H-20/H-21/H-22/H-23. The HMBC correlation between H-16/C-22 indicated that a 16,22-oxygen bridge was present in **67**. The ether linkage in the nucleus was established between C-16 and C-22 chemical shifts and multiplicities of H-16 at δ_H 4.96 (dd, *J* = 2.4, 7.2 Hz) and H-22 at 4.78 (dd, *J* = 3.0, 6.0 Hz) which was further confirmed by the HMBC correlation. The HMBC correlations of H-21 (δ_H 5.44) with C-17 (δ_C 59.8), C-20 (δ_C 53.3), C-22 (δ_C 88.2) and C-23 (δ_C 80.6) suggested that the hemiacetal carbon C-21 is connected to C-20 and C-23 through an oxygen atom, respectively. The HMBC correlations between H-2 (δ_H 2.44) and C-3 (δ_C 210.9), H-4 (δ_H 2.20, 2.32) and C-3 revealed that the ketonic group was located at C-3 in **6**. Furthermore, the HMBC correlation of C-24 at δ_C 84.5 with H-23, H_3-26 at δ_H 0.96, H_3-27 at δ_H 1.02, and H_3-29 at δ_H 2.34 indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 at δ_C 35.5 and C-28 at δ_C 215.6, respectively. Consequently, the planar structure of **67** was deduced to be as shown in **Figure-10.8**.

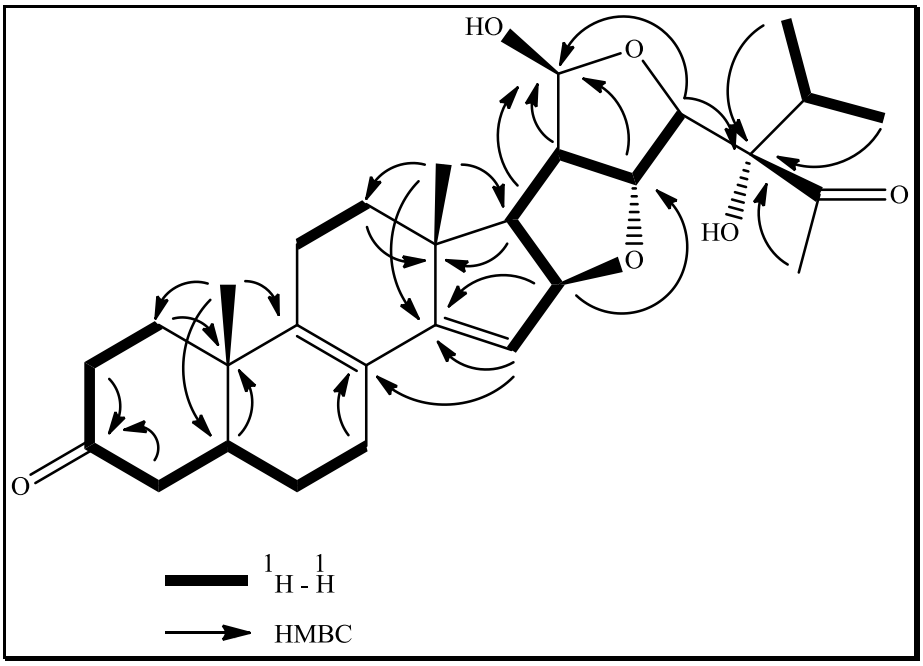


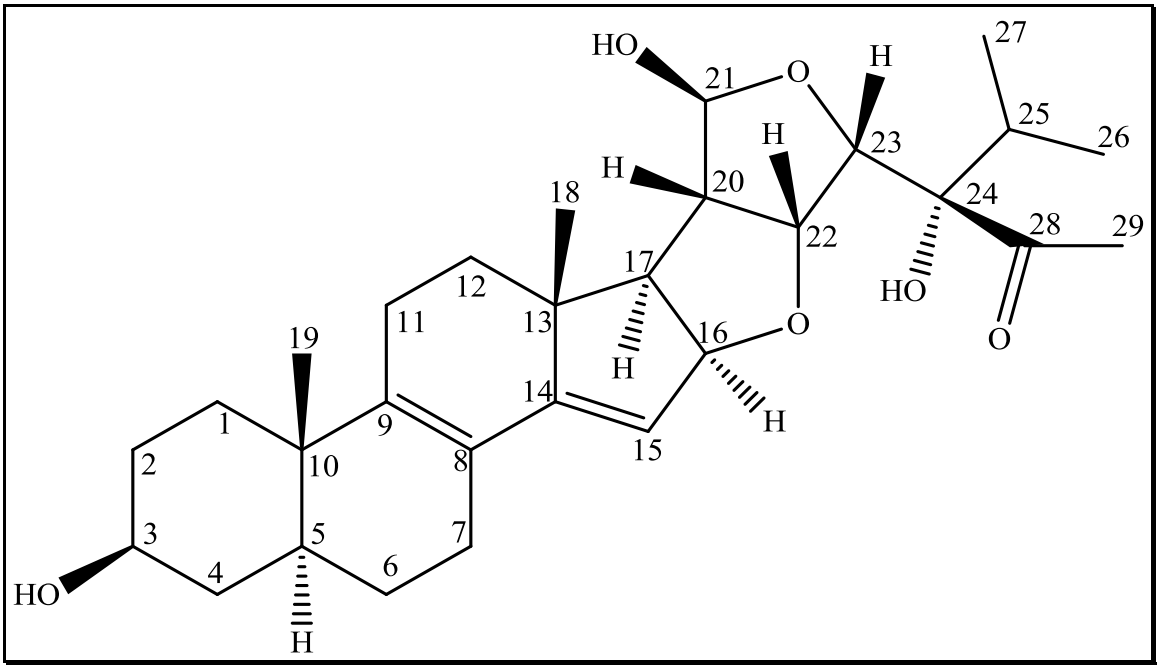
Figure-10.8 Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **67**.

The NOESY correlations of H_3-18 with H-20, H-22 and H-23 implied the H-20, H-22 and H-23 have *β*-orientations. Meanwhile, the NOESY correlations of H-21 with H-16 and H-17 showed the *β*-orientation of the 21-OH.

The absolute configuration of **67** was also demonstrated by applying the CD exciton chirality method,[432] and determined the chiral centers as 5*S*, 10*S*, 13*R*, 16*R*, 17*S*, 20*S*, 21*S*, 22*R*, 23*S* and 24*S*. The positive cotton effect at 290 nm due to the n→π* transition of the carbonyl group of **67**. Thus, vernoanthelcin F **(67)** was identified as (5*S*,10*S*,13*R*,16*R*,17*S*,20*S*,21*S*,22*R*,23*S*,24*S*)-16,22:21,23-diepoxy-21,24-dihydroxy-stigmasta-8,14-dien-3,28-dione.

Table-10.6:	^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR data of vernoanthelcin F **(67)** in $CDCl_3$		
C. No	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
1	36.4	CH_2	1.38, m
			2.15, m
2	38.1	CH_2	2.13, m
			2.44, m
3	210.9	C	--
4	44.5	CH_2	2.20, m
			2.32, m
5	42.6	CH	1.88, m
6	25.1	CH_2	1.52, m
			1.64, m
7	26.6	CH_2	2.18, m
			2.40, m
8	123.8	C	--
9	142.5	C	--
10	36.9	C	--
11	22.2	CH_2	2.24, m
			2.30, m
12	36.1	CH_2	1.61, m
			1.90, m
13	45.1	C	--
14	156.4	C	--
15	116.0	CH	5.44, br s
16	89.3	CH	4.96, (dd, 2.4, 7.2)
17	59.8	CH	2.49, (d, 7.2)
18	20.6	CH_3	0.94, s
19	17.5	CH_3	1.18, s
20	53.3	CH	2.82, (d, 6.0)
21	104.1	CH	5.44 br s
22	88.2	CH	4.78, (dd, 3.0, 6.0)
23	80.6	CH	4.50, (d, 3.0)
24	84.5	C	--
25	35.5	CH	2.12, m
26	17.0	CH_3	0.96, (d, 6.8)
27	17.1	CH_3	1.02, (d, 7.2)
28	215.6	C	--
29	28.7	CH_3	2.34, s
24-OH	--	--	5.01, s

10.1.1.7 Vernoanthelcin G (68)



Compound **68** was obtained from ethyl acetate fraction as a white amorphous powder, showed a molechular formula $C_{29}H_{42}O_6$ (nine degrees of unsaturation) having a molecular mass of 486 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 509.2880, calcd. for $[M + Na]^+$ 509.2874). The IR spectrum revealed the absorption bands of hydroxyl groups (3468 and 3274 cm^{-1}) and carbonyl group (1696 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-10.7**) of **68** displayed methyl singlets at δ_H 0.94 (H-18), 1.00 (H-19) and 2.34 (H-29) while geminal methyl doublets appeared at 0.98 (*J* = 7.2 Hz, H-26) and 1.02 (*J* = 6.6 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.7**) showed 29 carbon signals, including a hemiacetal carbon at δ_C 103.4 (C-21), a ketonic carbonyl group at δ_C 215.9 (C-28), and four sp^2 and six oxygenated carbon atoms. The ^{13}C-NMR and HSQC spectra revealed a conjugated diene, consisted of a trisubstituted carbon at δ_C 114.6 (C-15) and three tetrasubstituted

carbons at δ_C 156.6 (C-14), 144.1 (C-9) and 122.6 (C-8). The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 suggested that the diene is in the sterol nucleus. A typical UV characteristics of $\Delta^{8,14}$ steroidal nucleus was confirmed by UV spectrum which showed an absorption maximum at 248 nm.[396,425-429] The ^{1}H–^{1}H COSY spectrum of **68** demonstrated the connectivities: H-15/H-16/H-17/H-20/H-21/H-22/H-23 (**Figure-10.9**). The HMBC correlation between H-16/C-22 suggested that a 16,22-oxygen bridge was present in **68** which was further confirmed by the carbon chemical shifts (C-16 at δ_C 89.1 and C-22 at δ_C 87.8) and multiplicities of H-16 at δ_H.4.95 (dd, *J* = 2.4, 6.0 Hz) and H-22 at 4.73 (dd, *J* = 3.0, 6.0 Hz). Furthermore HMBC correlations of H-21 (δ_H 5.36) with C-17 (δ_C 59.6), C-20 (δ_C 53.0), C-22 (δ_C 87.8) and C-23 (δ_C 79.8) suggested that the hemiacetal carbon C-21 is connected to C-20 and C-23 through an oxygen atom, respectively. C-24 (δ_C 84.2) showed correlation with H-23 (δ_H 4.44), H_3-26 (0.98), H_3-27 (1.02) and H_3-29 (2.34) clearly indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 at δ_C 35.5 and C-28 at δ_C 215.9, respectively in the HMBC spectrum.

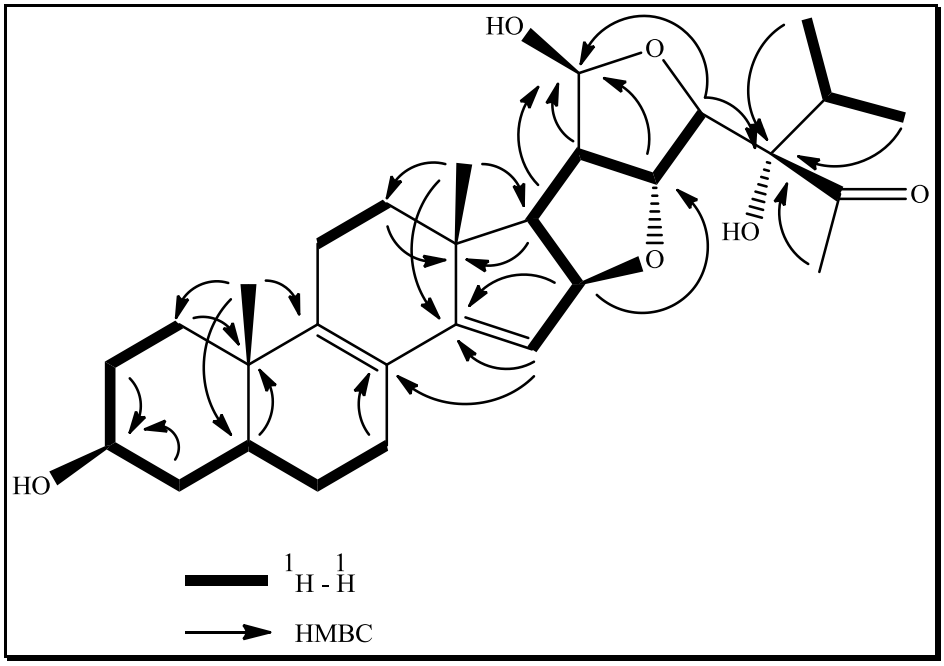


Figure-10.9: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **68**.

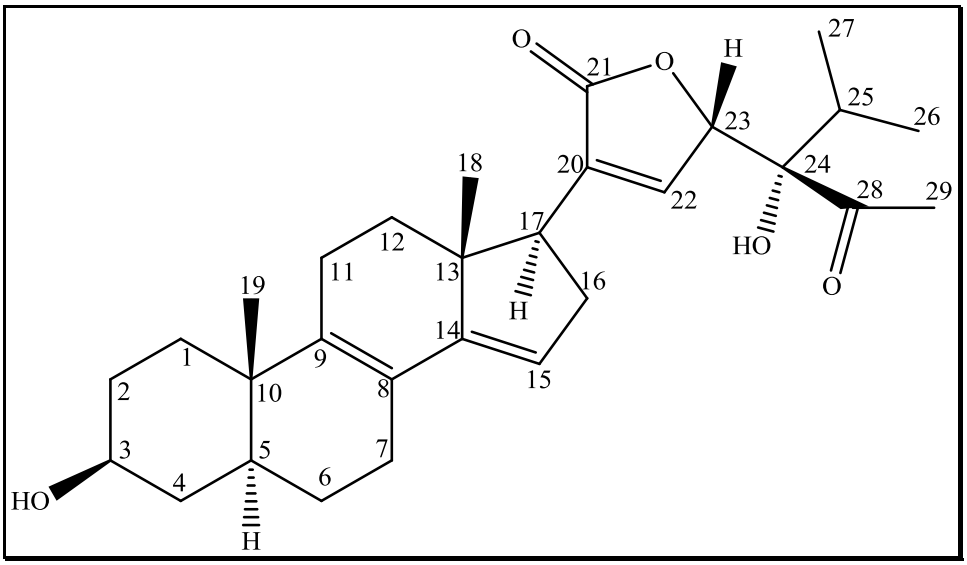
The NOESY connectivities of H_3-18 with H-20, H-22 and H-23 implied the H-20, H-22 and H-23 have *β*-orientations. Meanwhile, the NOESY correlations of H-21 with H-16 and H-17 showed the *β*-orientation of the 21-OH. Similarly the NOESY correlation of H-3 with H-5 showed the *β*-orientation of HO-3.

In the light of these spectral data, the structure of **68** has been deduced as (3*S*,5*S*,10*S*,13*R*,16*R*,17*S*,20*S*,21*S*,22*R*,23*S*,24*S*)-16,22:21,23-diepoxy-3,21,24-tri-hydroxy-stigmasta-8,14-dien-28-one which was also confirmed by applying CD exciton chirality method.[432]

Table-10.7:	^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR data of vernoanthelcin G **(68)** in $CDCl_3$:CD_3OD (1:1)		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
1	35.7	CH_2	1.38, m
			1.69, m
2	30.6	CH_2	1.51, m
			1.85, m
3	69.8	CH	3.58, m
4	37.2	CH_2	1.38, m
			1.68, m
5	40.3	CH	1.49, m
6	34.6	CH_2	1.47, m
			1.59, m
7	26.3	CH_2	2.12, m
			2.40, m
8	122.6	C	--
9	144.1	C	--
10	36.4	C	--
11	21.6	CH_2	2.22, m
			2.32, m
12	34.7	CH_2	1.27, m
			1.87, m
13	44.6	C	--
14	156.6	C	--
15	114.6	CH	5.43, (d, 2.4)
16	89.1	CH	4.95, (dd, 2.4, 6.0)
17	59.6	CH	2.49, (d, 6.0)
18	20.0	CH_3	0.94, s
19	17.8	CH_3	1.00, s
20	53.0	CH	2.79, (d, 6.0)
21	103.4	CH	5.36 br s

22	87.8	CH	4.73, (dd, 3.0, 6.0)
23	79.8	CH	4.44, (d, 3.0)
24	84.2	C	--
25	35.2	CH	2.15, m
26	16.3	CH_3	0.98, (d, 7.2)
27	16.4	CH_3	1.02, (d, 6.6)
28	215.1	C	--
29	28.1	CH_3	2.34, s

10.1.1.8 Vernoanthelcin H (69)



A white, amorphous powder, showed a molechular formula $C_{29}H_{40}O_5$ (ten degrees of unsaturation) having a molecular mass of 468 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 469.2854, calcd. for $[M + H]^+$ 469.2847). The IR spectrum exhibited the absorption bands at 3398 (OH), 1756 and 1708 (carbonyl groups) and 1654 (conjugated C=C) cm^{-1}.

Three methyl singlets appeared in the ^1H NMR spectrum (**Table-10.8**) at δ_H 0.61 (H-18),0.98 (H-19) and 2.14 (H-29) alongwith geminal methyls at 0.78 (d, *J* = 6.8 Hz, H-26) and 1.16 (d, *J* = 6.8 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.8**) indicated 29 carbon signals, including a ketonic carbonyl group at δ_C 209.1, (C-28), an ester carbonyl group (δ_C 173.1, C-21), and a conjugated diene (δ_C

116.2, C-15; δ_C 149.9, C-14; δ_C 123.0, C-8; δ_C 141.9, C-9). These data suggested that compound **69** was a vernoanthelcin-type steroid. Careful analysis of 1D and 2D NMR found the presence of an isopropyl group (C-25, C-26 and C-27), an acetyl group (C-28 and C-29) and an *α*,*β*-unsaturated-*γ*-lactone (C-20, C-21, C-22 and C-23) moiety. In the HMBC spectrum, correlations were observed between H-23 (δ_H 5.12) and C-24 (δ_C 83.4), between H_3-26/H_3-27 (0.78, 1.16) and C-24, between H_3-29 (2.14) and C-24, and between H-17 (2.85) and C-20 (δ_C 136.0) (**Figure-10.10**).[396,425-429] The relative stereochemistry of **69** was determined by using NOESY spectrum, in which the H-17 correlated with H-5, indicating that they possessed *α*-orientation. Based on a biogenetic analogy and comparison of molecular models, the seven chiral centers in **69** were determined as 3*S*, 5*S*, 10*S*, 13*R*, 17*S*, 23*S*, and 24*S*. Thus, vernoanthelcin H **(69)** was characterized as (3*S*,5*S*,10*S*,13*R*,17*S*,23*S*,24*S*)-21,23-lactone-3,24-dihydroxy-stigmasta-8,14,20-trien-28-one.

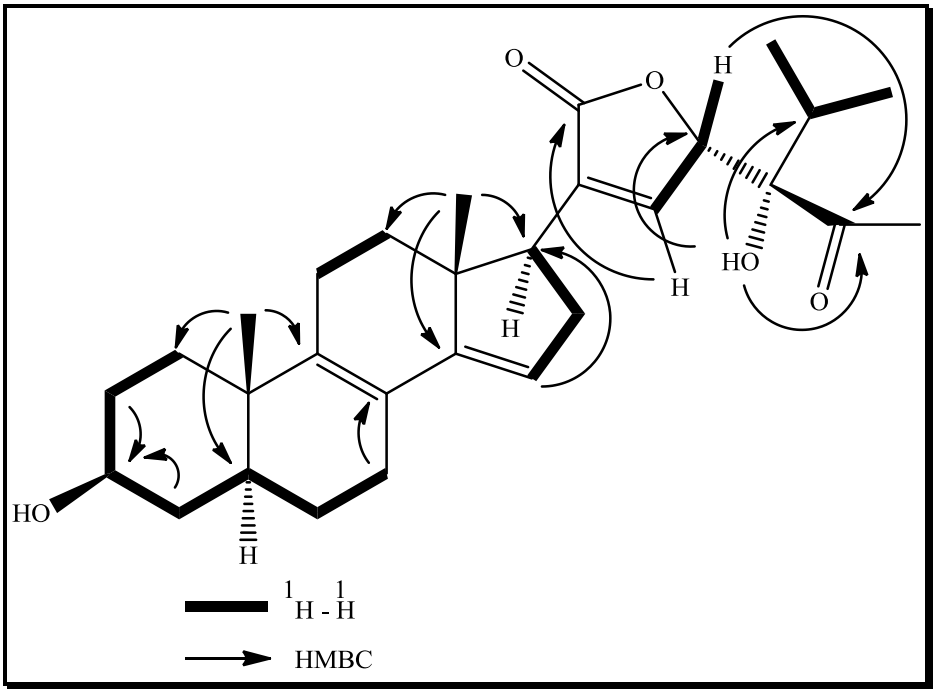
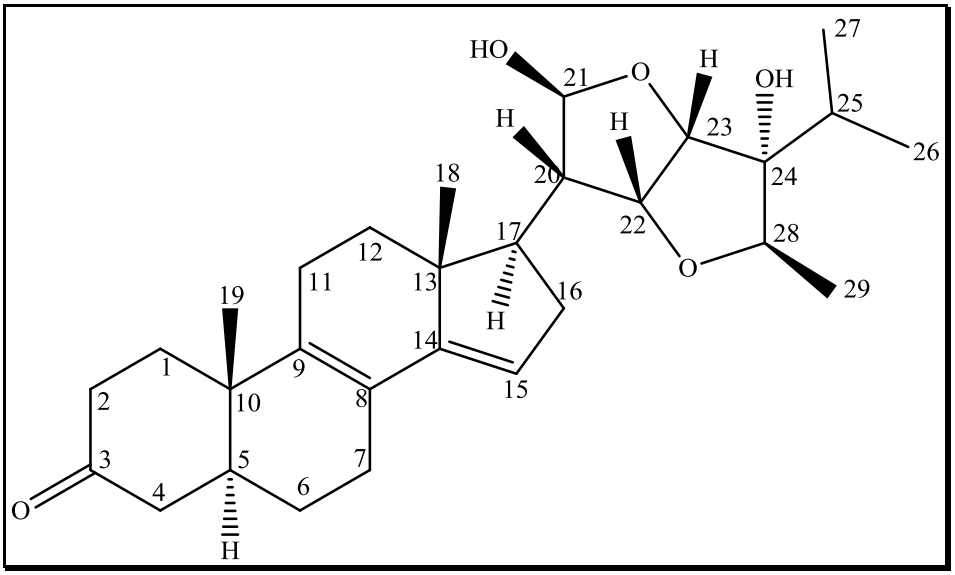


Figure-10.10: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **69**.

Table-10.8:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of vernoanthelcin H **(69)** in $CDCl_3$		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
1	35.2	CH_2	1.25, m
			1.79, m
2	31.7	CH_2	1.45, m
			1.85, m
3	70.9	CH	3.63, m
4	38.3	CH_2	1.38, m
			1.85, m
5	40.8	CH	1.46, m
6	25.2	CH_2	1.46, m
			1.64, m
7	26.6	CH_2	2.32, m
			2.48, m
8	123.0	C	--
9	141.9	C	--
10	36.6	C	--
11	21.9	CH_2	2.09, m
			2.23, m
12	35.8	CH_2	1.53, m
			1.98, m
13	46.0	C	--
14	149.9	C	--
15	116.2	CH	5.37, br s
16	35.0	CH_2	2.38, m
			2.59, m
17	47.7	CH	2.85, (dd, 8.8, 9.2)
18	17.6	CH_3	0.61, s
19	18.4	CH_3	0.98, s
20	136.0	C	--
21	173.1	C	--
22	147.1	CH	7.15, br s
23	81.5	CH	5.12, br s
24	83.4	C	--
25	31.4	CH	2.50, m
26	16.4	CH_3	0.78, (d, 6.8)
27	16.4	CH_3	1.16, (d, 6.8)
28	209.1	C	--
29	26.1	CH_3	2.14, s
24-OH	--	--	4.08, s

10.1.1.9 Vernoanthelcin I (70)



Compound **70** was isolated as white amorphous powder which showed a molecular formula $C_{29}H_{42}O_5$ (nine degrees of unsaturation) having a molecular mass of 470 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 493.2939, calcd. for $[M + Na]^+$ 493.2924). The IR spectrum showed the absorption bands of hydroxyl group (3389 cm^{-1}) and carbonyl group (1712 cm^{-1}). Analysis of the IR and UV, together with the 1D and 2D NMR data of **70** (**Table-10.9**) and comparison with previously isolated compound suggested that **70** was a vernoanthelcin-type steroid. The ^{1}H-NMR spectrum (**Table-10.9**) displayed three methyl doublets at δ_H 0.97 (*J* = 6.4 Hz, H-26), 0.98 (*J* = 6.8 Hz, H-27)0.87 (H-18) and 1.29 (*J* = 6.8 Hz, H-29) alongwith two methyl singlets at 0.87 (H-18) and 1.17 (H-19. The connections shown in bold lines in **Figure-10.10** were deduced from a detailed analysis of the ^{1}H-^{1}H COSY spectrum.

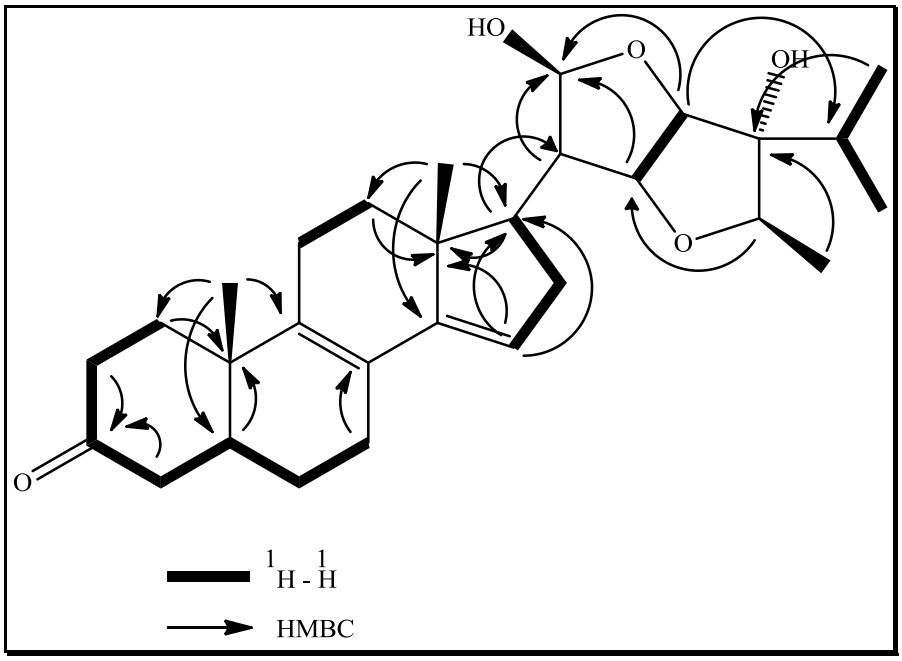
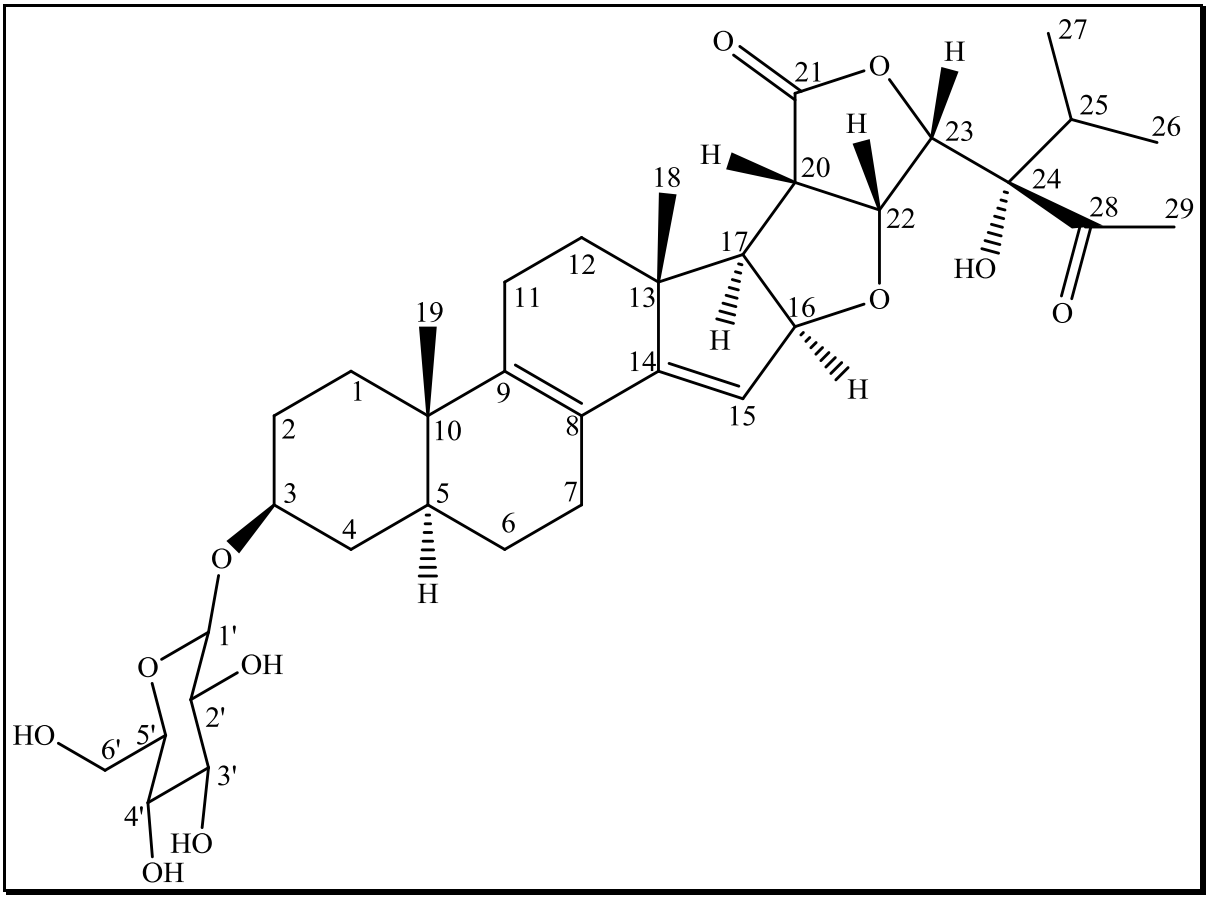


Figure-10.11: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **70**.

The HMBC correlations of C-24 (δ_C 83.0) with H-23 (δ_H 4.34), H_3-29 (1.18), H_3-26 (0.97) and H_3-27 (0.98) indicated that the oxygenated quaternary carbon (C-24) connected with side chain of steroid. Moreover, the HMBC correlations from H-28 (δ_H 4.20) to C-22 (δ_C 82.1), and from H-23 (4.34) to C-21 (δ_C 100.8) revealed that C-28 and C-22 and C-23 and C-21 were linked via an oxygen atom, respectively. The side chain was connected to C-17, which was established by HMBC correlations of H-17 (δ_H 2.20) with C-20 (δ_C 48.8) **(Figure-10.11)**. In the NOESY spectrum, H-22 showed the correlation with H-20, H-23 and H_3-29, meaning that these hydrogens were co-facial and *β*-oriented. The isopropyl group was assigned with *β*-orientation by the correlation of H-23/H-25 in the NOESY spectrum. The absolute configuration of **70** was also demonstrated by applying the CD exciton chirality method,[432] and determined the chiral centers as 5*S*, 10*S*, 13*R*, 17*R*, 20*S*, 21*R*, 22*R*, 23*S*, 24*R*. Thus, the structure of **70** was assigned as (5*S*,10*S*,13*R*,17*R*,20*S*,21*R*,22*R*,23*S*,24*R*)-21,23;22,28-diepoxy-21,24-dihydroxy-stigmasta-8,14-dien-3-one.

Table-10.9:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of vernoanthelcin I **(70)** in $CDCl_3$		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
1	36.7	CH_2	1.60, m
			2.14, m
2	38.2	CH_2	2.39, m
			2.48, m
3	211.4	C	--
4	44.5	CH_2	2.20, m
			2.32, m
5	42.7	CH	1.95, m
6	25.3	CH_2	1.49, m
			1.58, m
7	26.4	CH_2	2.14, m
			2.34, m
8	123.7	C	--
9	139.5	C	--
10	36.6	C	--
11	21.9	CH_2	2.23, m
			2.30, m
12	36.1	CH_2	1.46, m
			1.92, m
13	44.6	C	--
14	150.2	C	--
15	117.9	CH	5.41,br s
16	35.4	CH_2	2.24, m
			2.53, m
17	45.7	CH	2.20, m
18	17.1	CH_3	0.87, s
19	17.4	CH_3	1.17, s
20	48.8	CH	2.20, m
21	100.8	CH	5.39, (d, 4.0)
22	82.1	CH	4.54, (dd, 4.8, 4.8)
23	87.4	CH	4.34, (d, 4.8)
24	83.0	C	--
25	31.8	CH	1.74, m
26	17.1	CH_3	0.97, (d, 6.4)
27	17.3	CH_3	0.98, (d, 6.8)
28	85.2	CH	4.20, (q, 6.8)
29	14.2	CH_3	1.29, (d, 6.8)

10.1.1.10 Vernoantheloside A (71)



Compound **71** was obtained as white amorphous powder from aqueous methanolic fraction. It showed a molechular formula $C_{35}H_{50}O_{11}$ (eleven degrees of unsaturation) having a molecular mass of 646 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 669.3252, calcd. for $[M + Na]^+$ 669.32458). The IR spectrum revealed the absorption bands of hydroxyl group (3295 cm^{-1}), carbonyl group (1773 cm^{-1}) and a conjugated C=C band (1630 cm^{-1}).

The ^{1}H-NMR spectrum (**Table-10.10**) of **71** exhibited two methyl singlets at δ_H 0.97 (H-18) and 1.03 (H-19), a characteristic multiplet for H-3 at δ_H 3.72 (m), and geminal methyl groups of an isopropyl group at δ_H 1.00 (d, *J* = 7.2 Hz, H-26) and

1.04 (d, *J* = 7.2 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.10**) indicated 35 carbon signals, including an ester carbonyl group at δ_C 178.1 (C-21), a ketonic carbonyl group at δ_C 214.3 (C-28), and four sp^2 and eleven oxygenated carbon atoms. Combined analysis of DEPT and HSQC spectra revealed a conjugated diene, which consisted of a trisubstituted carbon at δ_C 114.2 (C-15) and three tetrasubstituted carbons at δ_C 156.0 (C-14), 143.5 (C-9) and 122.5 (C-8). In the ^{I}H- and ^{13}C-NMR spectra of **71**, the signals at δ_H 3.21–3.87, 4.42 (H-1′, d, *J* = 7.8) and δ_C 100.4, 73.1, 76.1, 69.7, 75.6, 61.0 were ascribable to a hexose unit. The D-glucose assignment was confirmed by GC analysis of its derivative after acid hydrolysis.[433] The NMR data of **71** clearly indicated an anomeric carbon signal at δ_C 100.4, and an anomeric proton signal at δ_H 4.42 (1H, d, *J* = 7.8 Hz). The *β*-configuration of the anomeric proton of the glucose residue was assigned based on its coupling constant (*J* = 7.8 Hz). The HMBC correlations between H-1′ at δ_H 4.42 of the glucose and C-3 at δ_C 77.2 of the aglycone indicated that the D-glucose was attached to the C-3 position of the aglycone. The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 implied that the diene is in the sterol nucleus. Moreover, the UV spectrum showed an absorption maximum at 246 nm, a typical UV characteristic of $\Delta^{8,14}$ steroidal nucleus compounds, and confirmed the position of the conjugated diene.[396,425-429] The ^{1}H–^{1}H COSY spectrum of **71** showed the connectivities: H-15/H-16/H-17/H-20/H-22/H-23. The HMBC correlation between H-16/C-22 indicated that a 16,22-oxygen bridge was present in **71**, which was confirmed by the carbon chemical shifts of C-16 at δ_C 88.7 and C-22 at δ_C 82.2 and multiplicities of H-16 at δ_H 5.11 (dd, *J* = 2.4, 6.6 Hz) and H-22 at 4.84 (dd, *J* = 4.2, 6.0 Hz). The ester carbonyl at δ_C 178.1 (C-21), showing HMBC correlations with H-17 at δ_H 2.85, H-20 at δ_H 3.0, H-22 at δ_H 4.84, and H-23 at δ_H 4.94, was unambiguously connected to C-20 (δ_C 47.6) and C-23

(81.5). Furthermore, the HMBC correlation of C-24 (δ_C 83.1) with H-23 (4.94), H_3-26 (1.00), H_3-27 (1.04) and H_3-29 (2.34) indicated that the oxygenated quaternary carbon C-24 was connected to C-23 (81.5), C-25 (35.1) and C-28 (214.3), respectively. Consequently, the planar structure of **71** was deduced to be as shown in **Figure-10.12**.

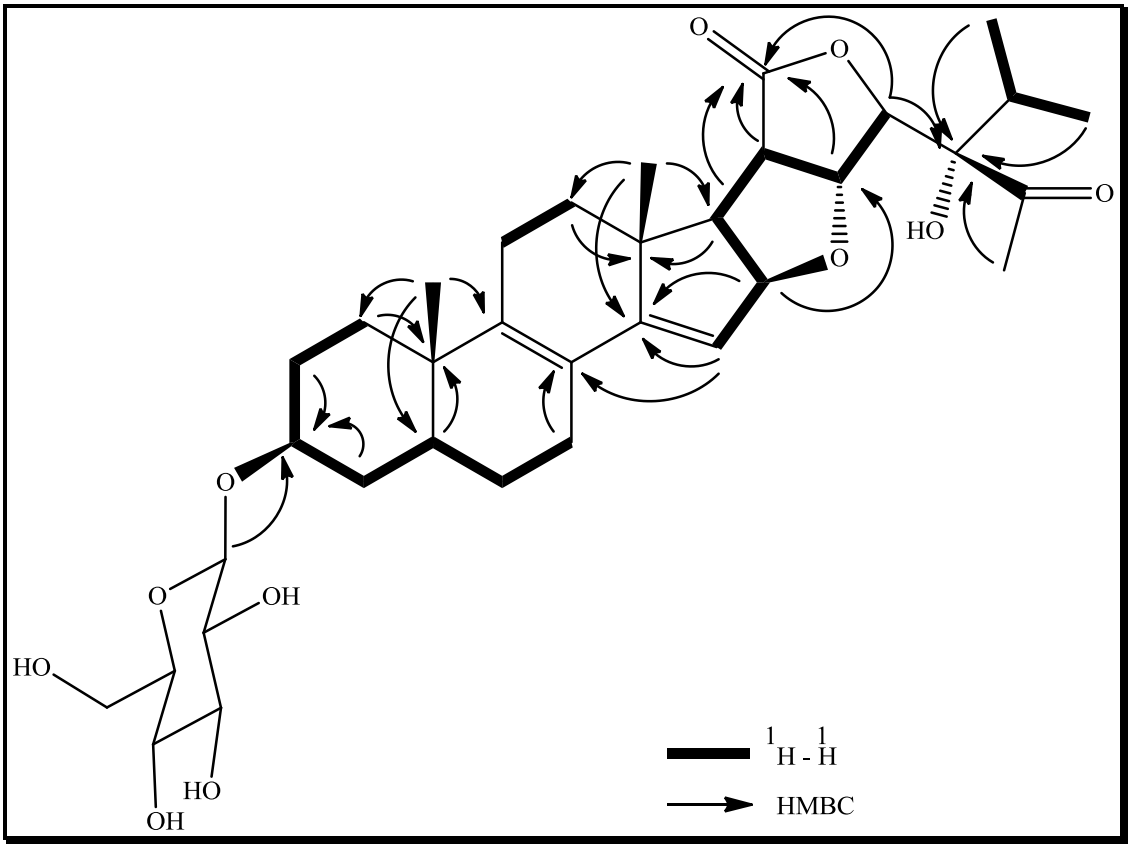


Figure-10.12: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **71**.

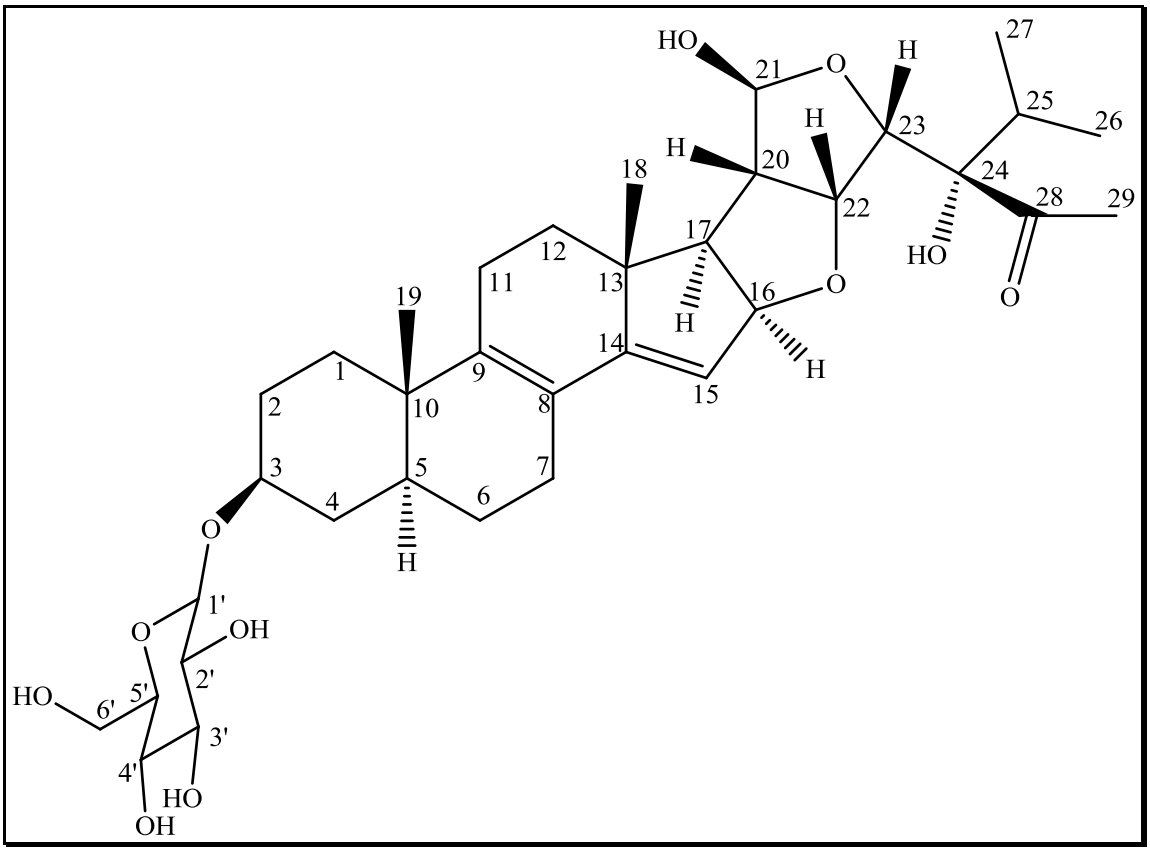
The relative configurations for C-17, C-20 and C-23 were determined by NOE difference experiments, which displayed NOEs for H-17 (4.2%) by irradiating H-16, for H-23 (3.4%) and H_3-18 (5.2%) when irradiating H-20, as well as for H-20 (3.9%) and H_3-27 (5.8%) by irradiating H-23, indicating the *α*, *β*, *β*-orientation of H-17, H-20, and H-23, respectively. Meanwhile the NOESY correlation of H-3 with H-5 showed the *α*-orientation of H-3.

The absolute configuration of **71** was also demonstrated by applying the CD exciton chirality method.[432] Vernoantheloside A **(71)** showed a CD split in the region of 210–250 nm corresponding to a negative chirality, which arose from the exciton coupling of the chromophores of the trans-conjugated double bond and the γ-lactone. The absolute configurations of the ten chiral centers in **71** were thus determined as 3*S*, 5*S*, 10*S*, 13*R*, 16*R*, 17*S*, 20*S*, 22*R*, 23*S* and 24*S*. The positive cotton effect at 290 nm due to the $n\rightarrow\pi^*$ transition of the carbonyl group of **71**. Thus, vernoantheloside A **(71)** was identified as (3*S*,5*S*,10*S*,13*R*,16*R*,17*S*,20*S*,22*R*,23*S*,24*S*)-16,22-epoxy-21,23-lactone-24-hydroxy-3-*O*-β-D-glucostigmasta-8,14-dien-28-one.

Table-10.10:	^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of vernoantheloside A **(71)** in $CDCl_3$:CD_3OD (1:1)		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
1	33.7	CH_2	1.42 , m
			1.84, m
2	28.8	CH_2	1.62, m
			1.98, m
3	77.2	CH	3.72, m
4	35.4	CH_2	1.46, m
			1.96, m
5	40.0	CH	1.47, m
6	24.5	CH_2	1.50, m
			1.64, m
7	26.1	CH_2	2.12, m
			2.41, m
8	122.5	C	--
9	143.5	C	--
10	36.3	C	--
11	21.4	CH_2	2.26, m
			2.35, m
12	34.5	CH_2	1.28, m
			1.88, m
13	44.3	C	--
14	156.0	C	--
15	114.2	CH	5.39, (d, 2.4)
16	88.7	CH	5.11, (dd, 2.4, 6.6)
17	56.9	CH	2.85, (d, 6.6)
18	19.8	CH_3	0.97, s
19	17.5	CH_3	1.03, s

20	47.6	CH	3.0, ov
21	178.1	C	--
22	82.2	CH	4.84, (dd, 4.2, 6.0)
23	81.5	CH	4.94, (d, 4.2)
24	83.1	C	--
25	35.1	CH	2.12, m
26	16.0	CH_3	1.00, (d, 7.2)
27	16.2	CH_3	1.04, (d, 7.2)
28	214.3	C	--
29	27.8	CH_3	2.34, s
1′	100.4	CH	4.42, (d, 7.8)
2′	73.1	CH	3.21 (dd, 7.8, 7.8)
3′	76.1	CH	3.39, m
4′	69.7	CH	3.38, m
5′	75.6	CH	3.32, m
6′	61.0	CH_2	3.73, (dd, 5.4, 12.0)
			3.87, (dd, 2.4, 11.4)

10.1.1.11 Vernoantheloside B (72)



A white amorphous powder of **72** showed a molechular formula $C_{35}H_{52}O_{11}$ (ten degrees of unsaturation) having a molecular mass of 648 a.m.u. as deduced from its positive-mode HRESIMS (*m/z* 648.3399, calcd. for $[M + Na]^+$ 648.3402). The IR spectrum showed the absorption bands at 3350 (OH), 1760 (carbonyl group) and 1647 (conjugated C=C) cm^{-1}. UV spectrum exhibited an absorption maximum at 246 nm which is a typical UV characteristic of $\Delta^{8,14}$ steroidal nucleus.[396,425-429]

The ^{1}H-NMR spectrum (**Table-10.11**) of **72** revealed methyl singlets at δ_H 0.97 (H-18), 1.03 (H-19) and 2.34 (H-29) and geminal methyl groups of an isopropyl group at δ_H 1.00 (d, *J* = 7.2 Hz, H-26) and 1.04 (d, *J* = 7.2 Hz, H-27). The ^{13}C-NMR (BB and DEPT) spectra (**Table-10.11**) showed 35 carbon signals, including a hemiacetal carbon at δ_C 103.2 (C-21), a ketonic carbonyl group at δ_C 215.8 (C-28), and four sp^2 and eleven oxygenated carbon atoms. Combined analysis of DEPT and HSQC spectra revealed a conjugated diene, consisted of a trisubstituted carbon at δ_C 114.5 (C-15) and three tetrasubstituted carbons at δ_C 156.4 (C-14), 143.7 (C-9) and 122.5 (C-8). In the ^{1}H- and ^{13}C-NMR spectra of **72**, the signals at δ_H 3.21–3.87, 4.42 (H-1′, d, *J* = 7.8) and δ_C 100.4, 73.1, 76.1, 69.7, 75.6, 61.0 were ascribable to a hexose unit. The D-glucose assignment was confirmed by GC analysis of its derivative after acid hydrolysis.[433] The NMR data of **72** clearly indicated an anomeric carbon signal at δ_C 100.4, and an anomeric proton signal at δ_H 4.42 (1H, d, *J* = 7.8 Hz). The *β*-configuration of the anomeric proton of the glucose residue was assigned based on its $J_{1H,2H}$ value (*J* = 7.8 Hz). The HMBC correlations between H-1′ at δ_H 4.42 of the glucose and C-3 at δ_C 77.2 of the aglycone indicated that the D-glucose was attached to the C-3 position of the aglycone. The HMBC correlations from H-15 to C-8, C-9, C-13, C-14, C-16 and C-17 suggested that the diene is in the sterol nucleus. The ^{1}H–^{1}H COSY spectrum of **72** demonstrated the connectivities: H-15/H-16/H-17/H-20/H-

21/H-22/H-23. The HMBC correlation between H-16/C-22 indicated that a 16,22-oxygen bridge was present in **72**, which was confirmed by the carbon chemical shifts of C-16 at δ_C 88.9 and C-22 at δ_C 87.6 and multiplicities of H-16 at δ_H 4.95 (dd, *J* = 2.4, 6.6 Hz) and H-22 at 4.73 (dd, *J* = 3.0, 6.0 Hz) . The HMBC correlations of H-21 (δ_H 5.35) with C-17 (δ_C 59.5), C-20 (δ_C 52.8), C-22 (δ_C 87.6) and C-23 (δ_C 79.8) suggested that the hemiacetal carbon C-21 is connected to C-20 and C-23 through an oxygen atom, respectively. Furthermore, the HMBC correlation of C-24 at δ_C 84.0 with H-23, H_3-26 at δ_H 1.00, H_3-27 at δ_H 1.04, and H_3-29 at δ_H 2.34 indicated that the oxygenated quaternary carbon C-24 was connected to C-23, C-25 at δ_C 35.1 and C-28 at δ_C 215.8, respectively. Consequently, the planar structure of **72** was deduced to be as shown in **Figure-10.13**.

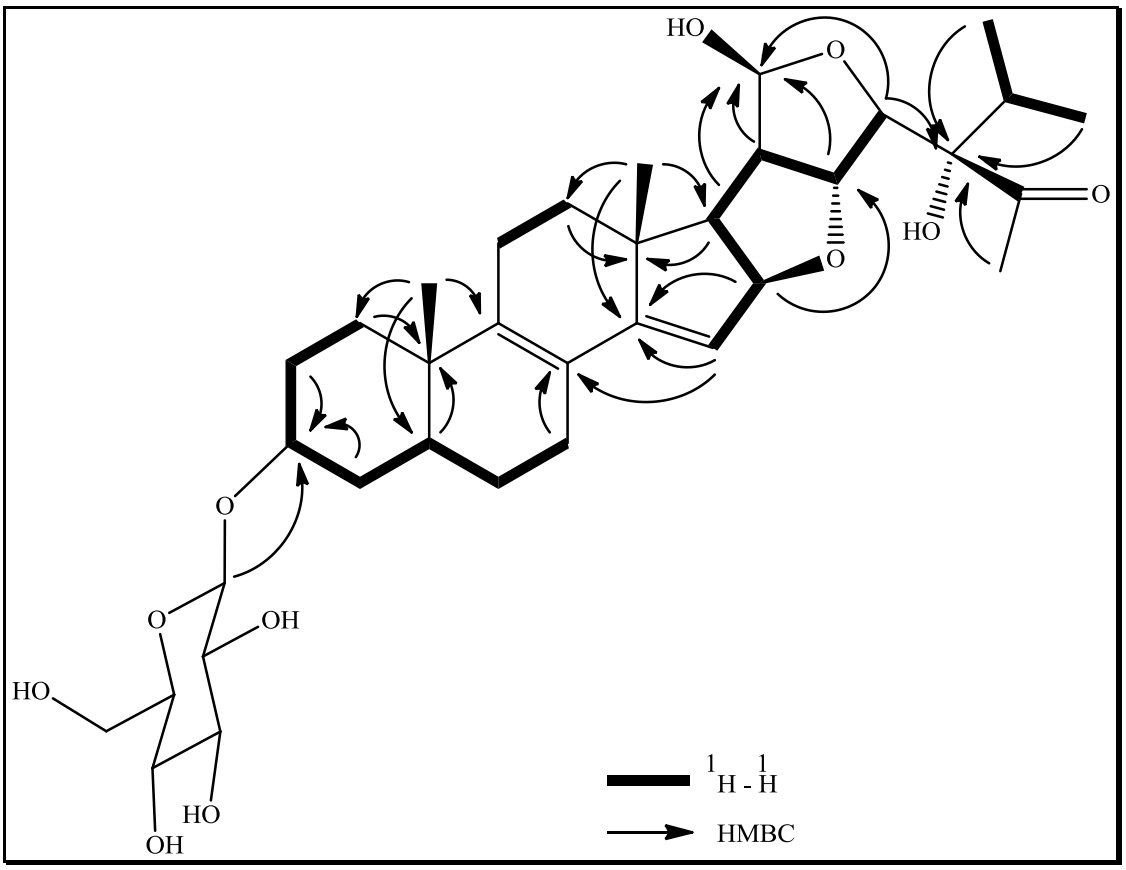


Figure-10.13: Selected COSY (^{1}H ─ ^{1}H) and HMBC (H→C) correlations of **72**.

The NOESY correlations of H_3-18 with H-20, H-22 and H-23 implied the H-20, H-22 and H-23 have *β*-orientations. Meanwhile, the NOESY correlations of H-21 with H-16 and H-17 showed the *β*-orientation of the 21-OH. Similarly the NOESY correlation of H-3 with H-5 showed the *α*-orientation of H-3.

The absolute configuration of **72** was determined as 3*S*, 5*S*, 10*S*, 13*R*, 16*R*, 17*S*, 20*S*, 21*S*, 22*R*, 23*S* and 24*S* by applying the CD exciton chirality method.[432] in the light of these spectral observation the structure of **72** was characterized as (3*S*,5*S*,10*S*,13*R*,16*R*,17*S*,20*S*,21*S*,22*R*,23*S*,24*S*)-16,22:21,23-diepoxy-21,24-dihydroxy-3-*O*-*β*-D-glucostigmasta-8,14-dien-28-one.

Table-10.11:	^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR data of vernoantheloside B **(72)** in $CDCl_3$:CD_3OD (1:1)		
C. No.	**δ_C**	**Multiplicity**	**δ_H (multiplicity, *J* in Hz)**
1	33.7	CH_2	1.40 , m
			1.82, m
2	28.8	CH_2	1.30, m
			1.63, m
3	77.2	C	3.72, m
4	35.5	CH_2	1.30, m
			1.37, m
5	40.0	CH	1.48, m
6	24.5	CH_2	1.51, m
			1.62, m
7	26.1	CH_2	2.12, m
			2.40, m
8	122.5	C	--
9	143.7	C	--
10	36.4	C	--
11	21.4	CH_2	2.22, m
			2.32, m
12	34.5	CH_2	1.38, m
			1.84, m
13	44.5	C	--
14	156.4	C	--
15	114.5	CH	5.43, br s
16	88.9	CH	4.95, (dd, 2.4, 6.6)
17	56.5	CH	2.49, (d, 6.6)
18	19.8	CH_3	0.97, s
19	17.5	CH_3	1.03, s

20	52.8	CH	2.79, (d, 6.0)
21	103.2	CH	5.35 br s
22	87.6	CH	4.73, (dd, 3.0, 6.0)
23	79.8	CH	4.43, (d, 3.0)
24	84.0	C	--
25	35.1	CH	2.13, m
26	16.0	CH_3	1.00, (d, 7.2)
27	16.2	CH_3	1.04, (d, 7.2)
28	215.8	C	--
29	27.8	CH_3	2.34, s
1′	100.4	CH	4.42, (d, 7.8)
2′	73.1	CH	3.21 (dd, 7.8, 7.8)
3′	76.1	CH	3.39, m
4′	69.7	CH	3.38, m
5′	75.6	CH	3.32, m
6′	61.0	CH_2	3.73, (dd, 5.4, 12.0)
			3.87, (dd, 2.4, 11.4)

10.2 Biological studies

10.2.1 Anti-bacterial assay

Various fractions of the seeds and aerial parts of *V. anthelmintica* were subjected for anti-bacterial assay. All of them showed no activity against six selected bacterial stains *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* at the tested concentration (03 μg/mL) (**Table-10.12**).

Table-10.12:	Anti-bacterial assay of various extracts/fractions from the seeds/aerial parts of *V. anthelmintica*					
Aerial parts/seeds						
Fractions	***E. c***	***B. s***	***S. f***	***S. a***	***P. a***	***S. t***
F1	x	x	x	x	x	x
F2	x	x	x	x	x	x
F3	x	x	x	x	x	x
A1	x	x	x	x	x	x
A2	x	x	x	x	x	x
A3	x	x	x	x	x	x
DMSO (–)	x	x	x	x	x	x
Imipenum 10μg/Disc (+)	35	36	36	43	32	40
Keywords:	well size = 6mm, Aerial parts = F, seeds = A, F1=*n*-hexane, F2 = EtOAc, F3 = MeOH, A1 = Acetone, A2 = Methanol, A3 = MeOH:H_2O (1:1), *E. c = Escherichia coli, B. s = Bacillus subtilis, S. f = Shigella flexenari, S. a = Staphylococcus aureus, P. a = Pseudomonas aeruginosa, S. t = Salmonella typhi*					

10.2.2 Anti-fungal assay

Different fractions of the seeds and aerial parts of *V. anthelmintica* showed non-significant anti-fungal activity against five selected fungal strains, *Candida*

albicans, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *candida glabrata* (**Table-10.13)**

Table-10.13:	Anti-fungal assay of various extracts/fractions from the seeds/aerial parts of *V. anthelmintica*		
Fractions/ Extracts	**Name of Fungus**	**% inhibition**	**Std. Drug Mic (*μ*g/mL)**
F1	*C. a*	0	Miconazole (110.8)
	A. f	0	Amphotericin B (20.20)
	M. c	20	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F2	*C. a*	0	Miconazole (110.8)
	A. f	15	Amphotericin B (20.20)
	M. c	15	Miconazole (98.4)
	F. s	20	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
F3	*C. a*	0	Miconazole (110.8)
	A. f	10	Amphotericin B (20.20)
	M. c	15	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
A1	*C. a*	0	Miconazole (110.8)
	A. f	05	Amphotericin B (20.20)
	M. c	25	Miconazole (98.4)
	F. s	20	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
A2	*C. a*	0	Miconazole (110.8)
	A. f	10	Amphotericin B (20.20)
	M. c	30	Miconazole (98.4)
	F. s	20	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
A3	*C. a*	0	Miconazole (110.8)
	A. f	0	Amphotericin B (20.20)
	M. c	15	Miconazole (98.4)
	F. s	15	Miconazole (73.25)
	C. g	0	Miconazole (110.8)
Keywords:	Aerial parts = F, seeds = A, F1 = *n*-hexane, F2 = EtOAc, F3 = MeOH, A1 = Acetone, A2 = Methanol, A3 = MeOH:H_2O (1:1), *C. a = Candida albicans, A. f = Aspergillus flavus, M. c =Microsporum canis, F. s = Fusarium solani, C. g= Candida glabrata*		

10.3.3 Phytotoxicity

Various extracts/fractions of the seeds and aerial parts of *V. anthelmintica* were tested for their *in vitro* phytotoxic bioassay. All the three fractions of the aerial parts of *V. anthelmintica* showed significant activities at highest doses against *Lemna minor* plant. Acetone extract of the seeds of *V. anthelmintica* showed good activity while MeOH and MeOH:H_2O (1:1) extracts showed moderate activities at highest doses (**Table-10.14**).

Table-10.14:	Phytotoxicity of various extracts/fractions from the seeds/aerial parts of *V. anthelmintica*				
Extract/ Fraction	**Compound Conc. (*μ*g/mL)**	**No. of fronds**		**% growth regulation**	**Std. Drug Conc. (*μ*g/mL)**
		Sample	**Control**		
F1	1000	06	20	70	0.015
	100	14		30	
	10	18		10	
F2	1000	06	20	70	0.015
	100	16		20	
	10	18		10	
F3	1000	06	20	70	0.015
	100	14		30	
	10	18		10	
A1	1000	10	20	50	0.015
	100	12		40	
	10	17		15	
A2	1000	11	20	45	0.015
	100	16		20	
	10	18		10	
A3	1000	12	20	40	0.015
	100	16		20	
	10	18		10	
Keywords:	Aerial parts = F, seeds = A, F1 = *n*-hexane, F2 = EtOAc, F3 = MeOH, A1 = Acetone, A2 = Methanol, A3 = MeOH:H_2O (1:1)				

10.3.4 Insecticidal assay

Insecticidal assay of the various fractions of the seeds and aerial parts of *V. anthelmintica* was carried out through contact toxicity method. All of them were proved to be non-significant (**Table-10.15**).

Table-10.15:	Insecticidal assay of various extracts/fractions from the seeds/aerial parts of *V. anthelmintica*	
Fractions	**Name of Insects**	**% Mortality**
F1	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	20
F2	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	0
F3	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	0
A1	*Tribolium castaneum*	0
	Rhyzopertha dominica	20
	Callosbruchus analis	0
A2	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	0
A3	*Tribolium castaneum*	0
	Rhyzopertha dominica	0
	Callosbruchus analis	20
Keywords:	Aerial parts = F, seeds = A, F1 = *n*-hexane, F2 = EtOAc, F3 = MeOH, A1 = Acetone, A2 = Methanol, A3 = MeOH:H_2O (1:1)	

10.3.5 Brine shrimp (*Artemia salina*) lethality bioassay

Different fractions of the seeds and aerial parts of *V. anthelmintica* were subjected for brine shrimp (*Artemia salina*) lethality bioassay. *n*-hexane and EtOAc fractions of the aerial parts of *V. anthelmintica* showed no cytotoxicity while methanolic fraction showed cytotoxicity at highest dose. Similarly acetone fraction

of the seeds of *V. anthelmintica* showed no cytoxicity while MeOH and MeOH:H_2O (1:1) fraction showed cytotoxicity at highest doses only **(Table-10.16).**

Table-10.16:	Brine Shrimp (*Artemia salina*) Lethality bioassay of various extracts/fractions from the seeds/aerial parts of *V. anthelmintica*					
Fractions	**Dose (*μ*g/mL)**	**No. of shrimps**	**No. of survivors**	**LD50 (*μ*g/mL)**	**STD. Drug**	**LD50 (*μ*g/mL)**
F1	1000	30	22	413892.80	Etoposide	7.4625
	100	30	25			
	10	30	26			
F2	1000	30	25	4251653	Etoposide	7.4625
	100	30	27			
	10	30	28			
F3	1000	30	16	1050.50	Etoposide	7.4625
	100	30	21			
	10	30	28			
A1	1000	29	19	1039111.1	Etoposide	7.4625
	100	29	22			
	10	29	23			
A2	1000	29	15	981.641	Etoposide	7.4625
	100	29	22			
	10	29	20			
A3	1000	29	13	607.64	Etoposide	7.4625
	100	29	22			
	10	29	28			
Keywords:	Aerial parts = F, seeds = A, F1 = *n*-hexane, F2 = EtOAc, F3 = MeOH, A1 = Acetone, A2 = Methanol, A3 = MeOH:H_2O (1:1)					

11.1 General experimental

Melting points were determined with an X-4 digital display micro-melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. UV spectra were obtained on a Shimadzu UV-260 spectrophotometer. IR spectra were recorded on a 170SX FT-IR instrument using KBr discs over the range of 400–4000 cm^{-1}. NMR spectra were recorded on a Bruker AM-400 and a Varian Mercury-600BB NMR (600 MHz) spectrometer using TMS as an internal standard in $CDCl_3$ and $CDCl_3$:CD_3OD (1:1). High-resolution electro spray ionization mass spectra (HRESIMS) were recorded on a Bruker Daltonics APEX II 47e spectrometer. EIMS and FAB-MS were taken on an HP5988a GC/MS and a VG-ZAB-HS at 70 eV. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), MCI gel CHP 20P (75-150μ, Mitsubishi Chemical Corp., Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Fractions were monitored by TLC, which were visualized by heating the silica gel plates after being sprayed with 5% H_2SO_4 in EtOH. *n*-Hexane and ethyl acetate fractions were separated by preparative HPLC (Beijing Chuang Xin Tong Heng Science & Technology Co., Ltd.) on a high pressure DAC column (400 x 100 mm, 200-300 mesh) eluting with petroleum ether–acetone at 200 mL/min. Semi-preparative HPLC isolation was performed on a Waters 1525-2998 instrument using either a preparative silica column (150 x 10 mm, 5 μm) eluting with *n*-hexane–ethyl acetate at 4 mL/min or a preparative C-18 column (150 x 10 mm, 10 μm) eluting with H_2O–CH_3OH-MeCN at 3 mL/min.

11.2 *Vernonia anthelmintica*

11.2.1 Plant material

Aerial parts of *V. anthelmintica* plant were collected from Hathi Mera, Mansehra, K.P.K., Pakistan, at 3100 ft above sea level in July 2007. The taxonomic identification of the plant was done by Prof. Dr. Habib Ahmad, Chairman, Department of Genetics, Hazara University, Pakistan. A voucher specimen (No. 123) was deposited in the herbarium of the Botany Department, Hazara University, Pakistan.

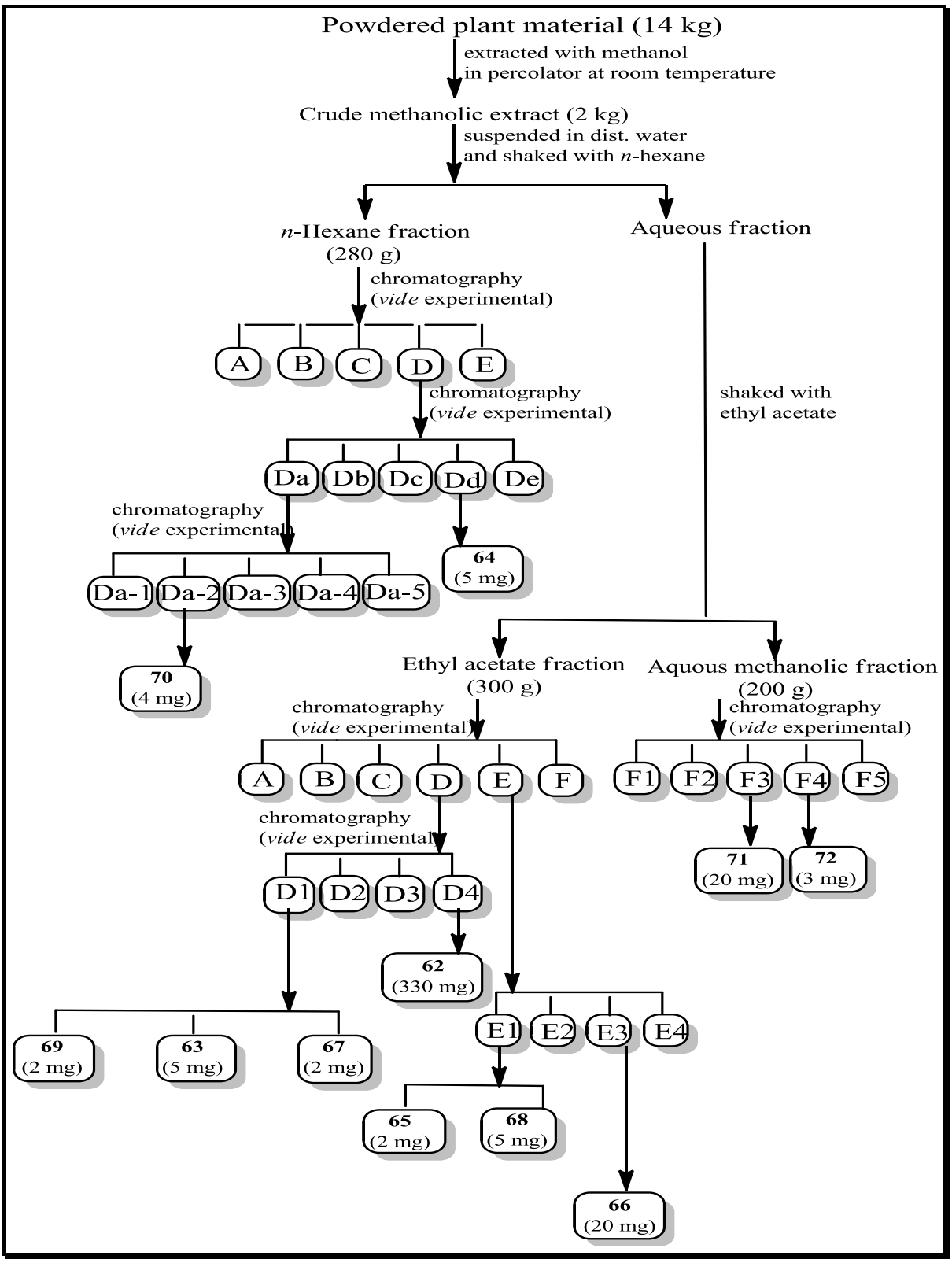
11.2.2 Extraction and isolation

The dried and powder plant material (14 kg) was extracted successively with MeOH at room temperature (x3; 7 days). The combined extract was concentrated under vacuum at temperature below 60 ^{o}C to obtain a methanol extract (2 kg) which was then partitioned between H_2O and *n*-hexane (280 g), H_2O and EtOAc (300 g) and aqueous methanol (200 g). The *n*-hexane fraction (280 g) was separated by preparative HPLC using a gradient of petroleum ether (PE)-acetone (100:0, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1 v/v) at 200 mL/min, and collected several fractions which were combined into five major fractions (A, B, C, D and E) based on TLC profile. Fraction D (obtained from the 5:1 PE:acetone fraction above) was re-chromatographed on a Sephadex LH-20 column, eluting with PE:$CHCl_3$:CH_3OH (5:5:1), which afforded five fractions (Da-De). Fraction Da (2.1 g) was further separated on a silica gel column eluted with PE:EtOAc (5:1, v/v) to give five major sub-fractions (Da-1 to Da-5). Fraction Da-2 (20 mg) was further purified on Sephadex LH-20 eluted with PE:$CHCl_3$:CH_3OH (5:5:1, v/v) to yield **70** (4 mg). Fraction Dd (3

g) was separated on silica gel CC using $CHCl_3$:acetone (15:1) as an eluent to obtain **64** (5 mg).

The ethyl acetate fraction (300 g) was separated by preparative HPLC using a gradient solvent system of PE:acetone (100:0, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1, v/v) followed with MeOH at 200 mL/min to obtaine six major fractions (A–F). Fraction D (obtained from the 5:1 elution fractions, 48.1 g) was further purified on a column of MCI gel (eluted with EtOH:H_2O, 90:10, v/v), which afforded four fractions (D1-D4) based on TLC analysis. The components of fraction D1 (6 g) were separated by chromatography on silica gel eluting with $CHCl_3$:EtOAc (10:1, 5:1, 2:1), followed by chromatography on Sephadex LH-20 (eluted with MeOH:$CHCl_3$ (1:1)), which was furter re-chromatographed by HPLC on a semi-preparative silica column (*n*-hexane:ethyl acetate, 67:33) (total time: 30 min) to afford **69** (tR = 10 min, 2 mg), **63** (tR = 12 min, 5 mg), and **67** (tR = 18 min, 2 mg). Fraction D4 (6.85 g) was also purified by chromatography on Sephadex LH-20 (eluted with MeOH:$CHCl_3$ (1:1)) and re-chromatographed on silica gel (eluted with $CHCl_3$:MeOH (70:1, 50:1, 30:1)) to afford **62** (330 mg). Fraction E (obtained from the PE:acetone (2:1) fraction, 36.8 g) was purified by removing pigment over a column of MCI gel (EtOH:H_2O, 90:10, v/v) as a result four fractions (E1-E4) were obtained by analytical TLC. E1 (4.3 g) showed two major spots alongwith minor spots on the TLC, which resolved by silica gel column chromatography eluting with $CHCl_3$:MeOH (70:1, 50:1, 20:1) to furnish **65** (2 mg) and **68** (5 mg). Fraction E3 (1.4 g) was further purified by silica gel column chromatography using a gradient elution with $CHCl_3$:acetone:MeOH (20:2:0.1-20:1:0.1), which furnished **66** (20 mg). The methanolic fraction (200 g) was filtered and chromatographed on HP-20 polystyrene resin column (using a gradient elution with H_2O, 20% aq EtOH (10 L), 50% aq EtOH (15 L), 80% aq EtOH (15 L), and 95%

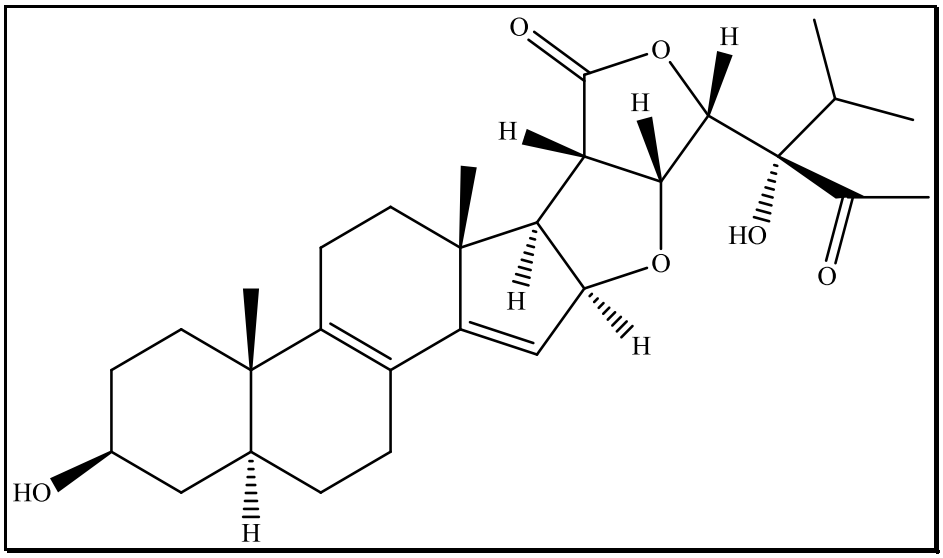
aq EtOH (8 L) to provide five separate fractions (F1-F5) which were concentrated in vacuum under 40 ^{o}C using a rotary evaporator. After evaporation in vacuo, 50% EtOH-eluted residue (20 g) was performed on a silica gel column, using a gradient solvent system from CH_3Cl to CH_3OH. Fraction F3 (0.7 g) was purified by chromatography on Sephadex LH-20 (eluted with MeOH:$CHCl_3$, 1:1, v/v) and then by chromatography on silica gel (gradient elution with $CHCl_3$:acetone:MeOH (5:1:0.1-2:0.1:0.1)) to afford **71** (20 mg). Fraction F4 (39 mg) was purified by HPLC on a semi-preparative C18 column (mobile phase H_2O:CH_3OH:MeCN, 40:48:12) (total time: 40 min) to afford **72** (tR = 16 min; 3 mg) **(Scheme-11.1)**.



Scheme-11.1: Extraction and isolation of pure compounds (**62-72**) from *V. anthelmintica.*

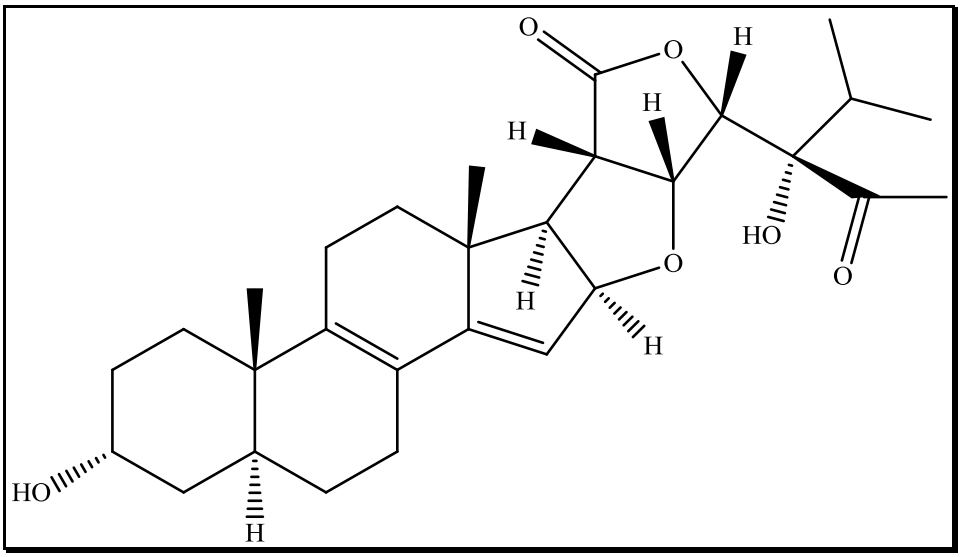
11.2.3 Characterization of the new compounds isolated from *V. anthelmintica*

11.2.3.1 Vernoanthelcin A (62)



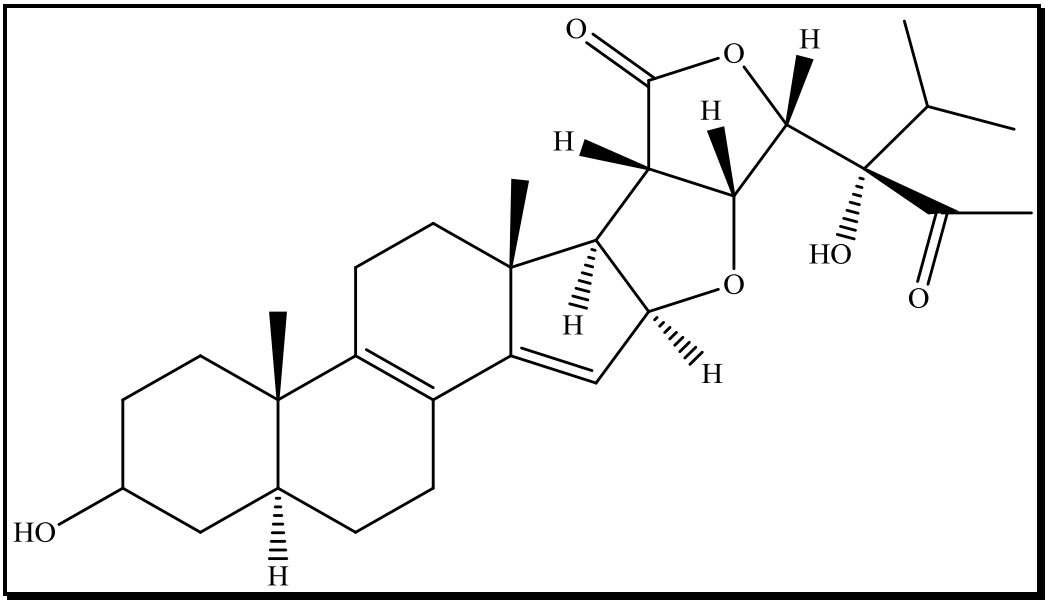
Physical State:	Colorless crystals
M.P:	274-276 ^{o}C
Yield:	330 mg
$[\alpha]_D^{20}$:	$-10.0°$ (c 0.2, MeOH)
UV (MeOH) λ_{max} (log ε):	246 (4.32) nm
IR ν_{max} (MeOH) cm^{-1}:	3476, 2925, 1766, 1738, 1697, 1099, 1056 and 1034
HR-ESI-MS (*m/z*):	485.2904 (calculated for $[M + H]^+$ 485.2898)
^{1}H- and ^{13}C-NMR:	Table-10.1

11.2.3.2 Vernoanthelcin B (63)



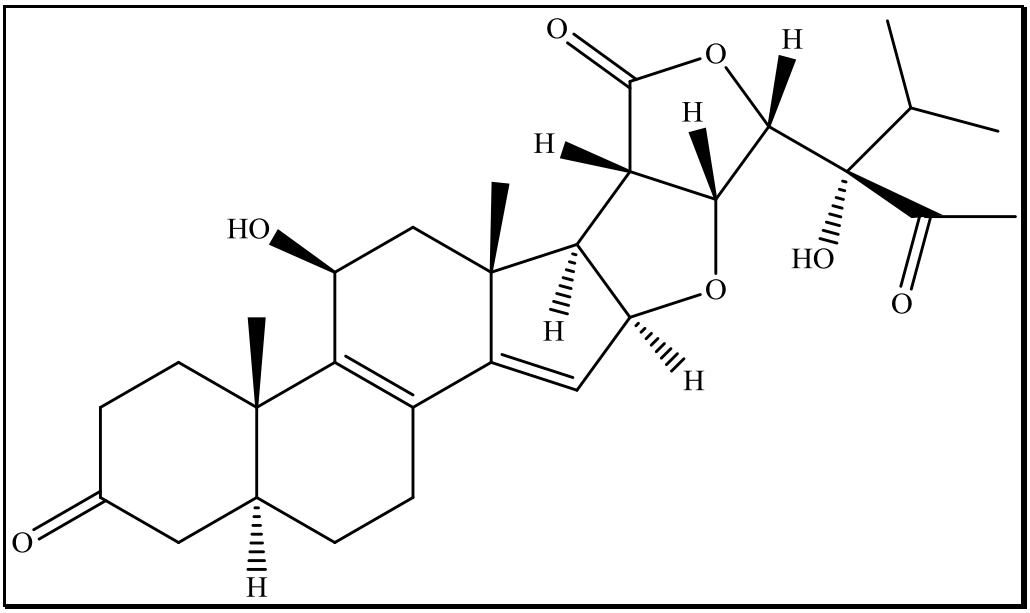
Physical State:	White amorphous solid
Yield:	5 mg
$[\alpha]_D^{20}$:	$+ 10.0°$ (c 0.2, MeOH)
UV (MeOH) λ_{max} (log ε):	246 (4.33) nm
IR ν_{max} (MeOH) cm^{-1}:	3400, 2923, 1772, 1703, 1657, 1458, 1375, 1191, 1089 and 1032
HR-ESI-MS (*m/z*):	485.2909 (calculated for $[M + H]^+$ 485.2898)
^{1}H- and ^{13}C-NMR:	Table-10.2

11.2.3.3 Vernoanthelcin C (64)



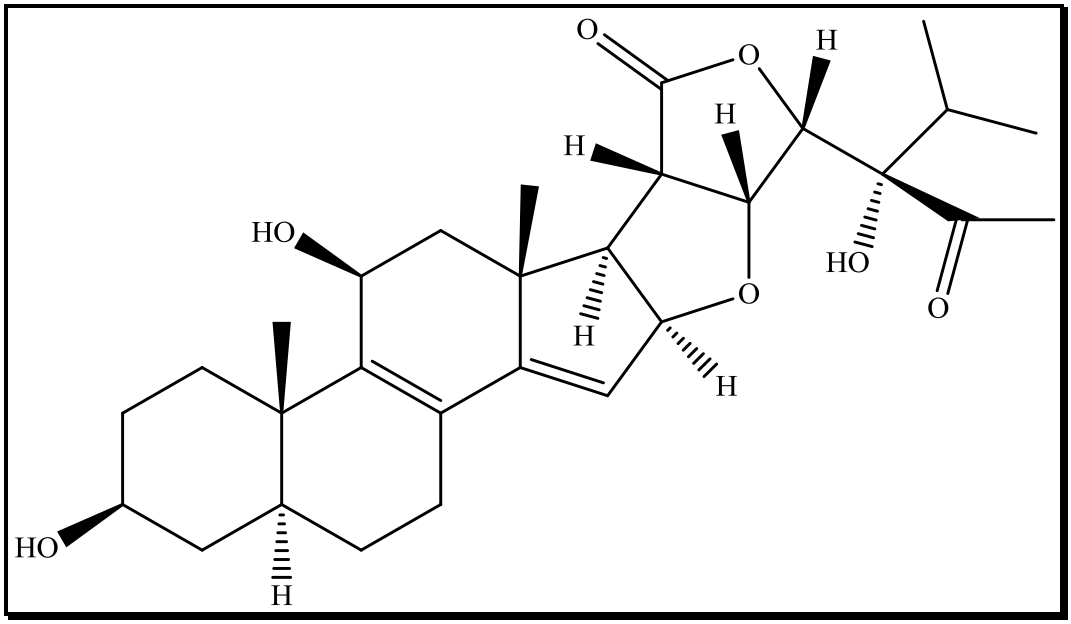
Physical State:	Light yellow amorphous powder
Yield:	5 mg
$[\alpha]_D^{20}$:	$-15.0°$ (c 0.2, MeOH)
UV (MeOH) λ_{max} (log ε):	245 (4.31) nm
IR ν_{max} (MeOH) cm^{-1}:	3396, 2921, 1775, 1709, 1650, 1460, 1093 and 1030
HR-ESI-MS (*m/z*):	483.2746 (calculated for $[M + H]^+$ 483.2741)
^{1}H- and ^{13}C-NMR:	Table-10.3

11.2.3.4 Vernoanthelcin D (65)



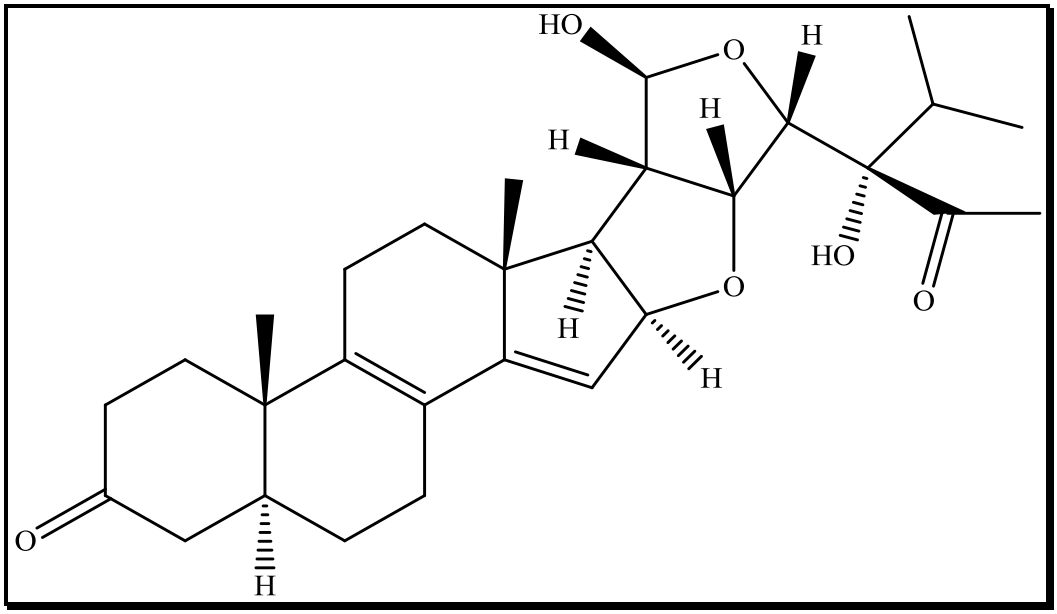
Physical State:	White amorphous powder
Yield:	2 mg
$[\alpha]_D^{20}$:	$-15.0°$ (c 0.2, MeOH)
UV (MeOH) λ_{max} (log ε):	245 (4.31) nm
IR ν_{max} (MeOH) cm^{-1}:	3471, 2923, 1763, 1699, 1092 and 1017
HR-ESI-MS (*m/z*):	521.2514 (calculated for $[M + Na]^+$ 521.2519)
^1H- and ^{13}C-NMR:	Table-10.4

11.2.3.5 Vernoanthelcin E (66)



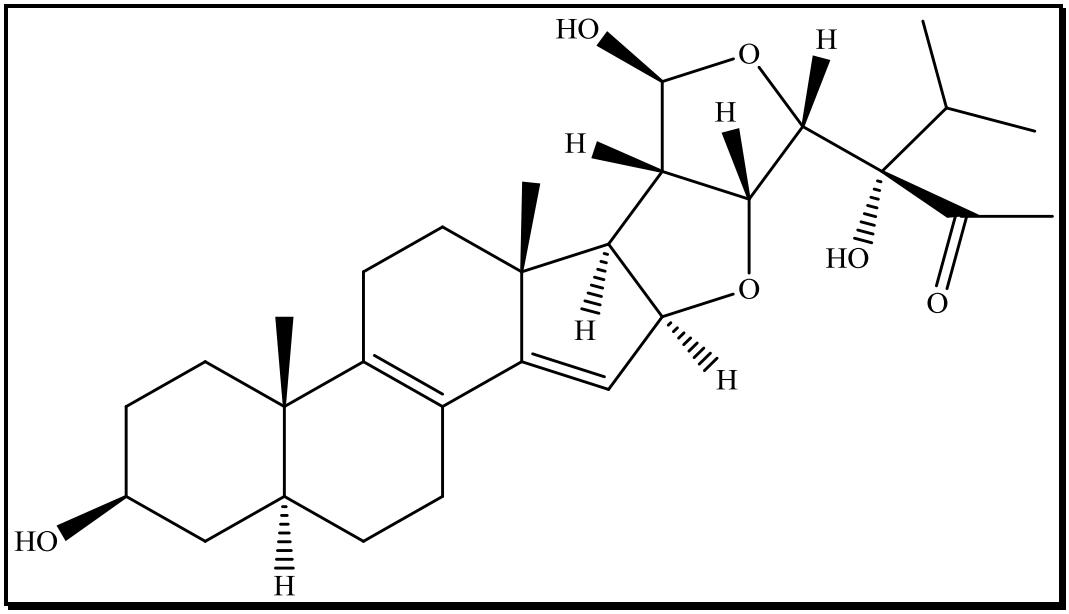
Physical State:	White amorphous solid
Yield:	20 mg
$[\alpha]_D^{20}$:	$-5.0°$ (c 0.2, MeOH)
UV (MeOH) λ_{max} (log ε):	246 (4.34) nm
IR ν_{max} (MeOH) cm^{-1}:	3469, 2928, 1760, 1692, 1097 and 1029
HR-ESI-MS (*m/z*):	501.2854 (calculated for $[M + H]^+$ 501.2847)
^{1}H- and ^{13}C-NMR:	Table-10.5

11.2.3.6 Vernoanthelcin F (67)



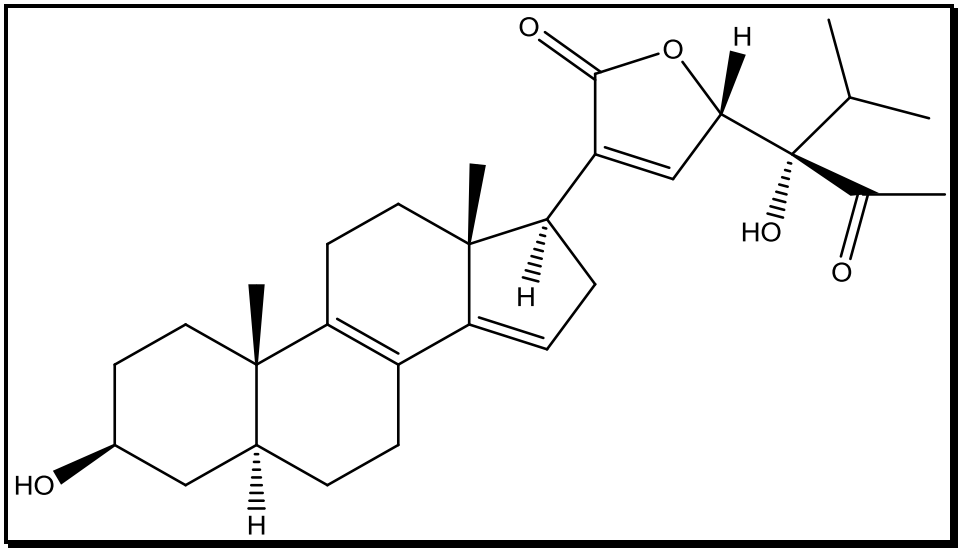
Physical State:	White amorphous powder
Yield:	2 mg
$[\alpha]_D^{20}$:	$+30.0°$ (c 0.1, MeOH)
UV (MeOH) λ_{max} (log ε):	246 (4.26) nm
IR ν_{max} (MeOH) cm^{-1}:	3392, 2922, 2853, 1741, 1649, 1067 and 1025
HR-ESI-MS (*m/z*):	507.2711 (calculated for $[M + Na]^+$ 501.2717)
^1H- and ^{13}C-NMR:	Table-10.6

11.2.3.7 Vernoanthelcin G (68)



Physical State:	White amorphous powder
Yield:	5 mg
$[\alpha]_D^{20}$:	$-2.2°$ (c 0.1, MeOH)
UV (MeOH) λ_{max} (log ε):	248 (4.30) nm
IR ν_{max} (MeOH) cm^{-1}:	3468, 3274, 2925, 1696, 1631 and 1041
HR-ESI-MS (*m/z*):	509.2880 (calculated for $[M + Na]^+$ 509.2874)
^{1}H- and ^{13}C-NMR:	Table-10.7

11.2.3.8 Vernoanthelcin H (69)



Physical State: White amorphous powder

Yield: 2 mg

$[\alpha]_D^{20}$: $+\,10.0°\ (c\ 0.2, \mathrm{MeOH})$

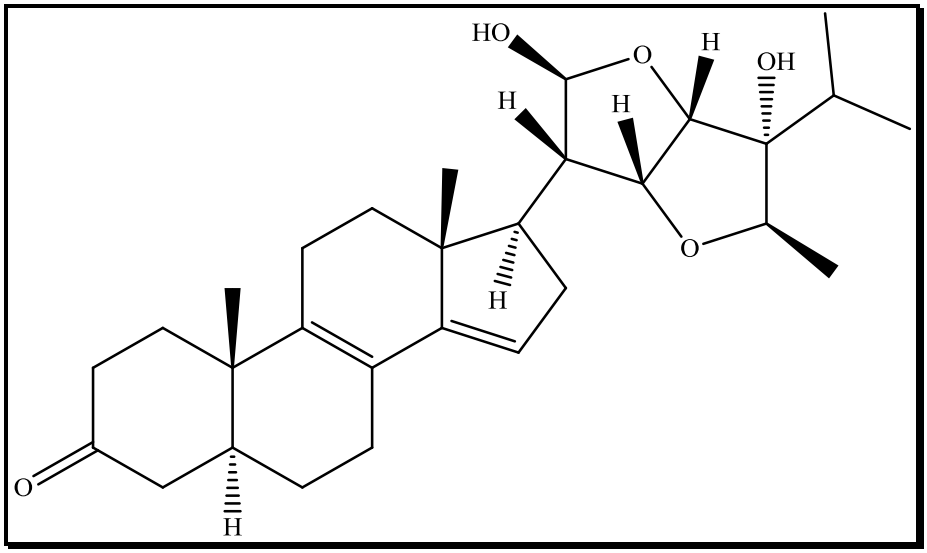
UV (MeOH) λ_{max} (log ε): 246 (4.34) nm

IR ν_{max} (MeOH) cm^{-1}: 3398, 2926, 1756, 1708, 1654, 1071 and 1037

HR-ESI-MS (*m/z*): 469.2854 (calculated for $[M + H]^+$ 469.2847)

^{1}H- and ^{13}C-NMR: Table-10.8

11.2.3.9 Vernoanthelcin I (70)



Physical State: White amorphous powder

Yield: 4 mg

$[\alpha]_D^{20}$: $+\,15.0°\ (c\ 0.2, \mathrm{MeOH})$

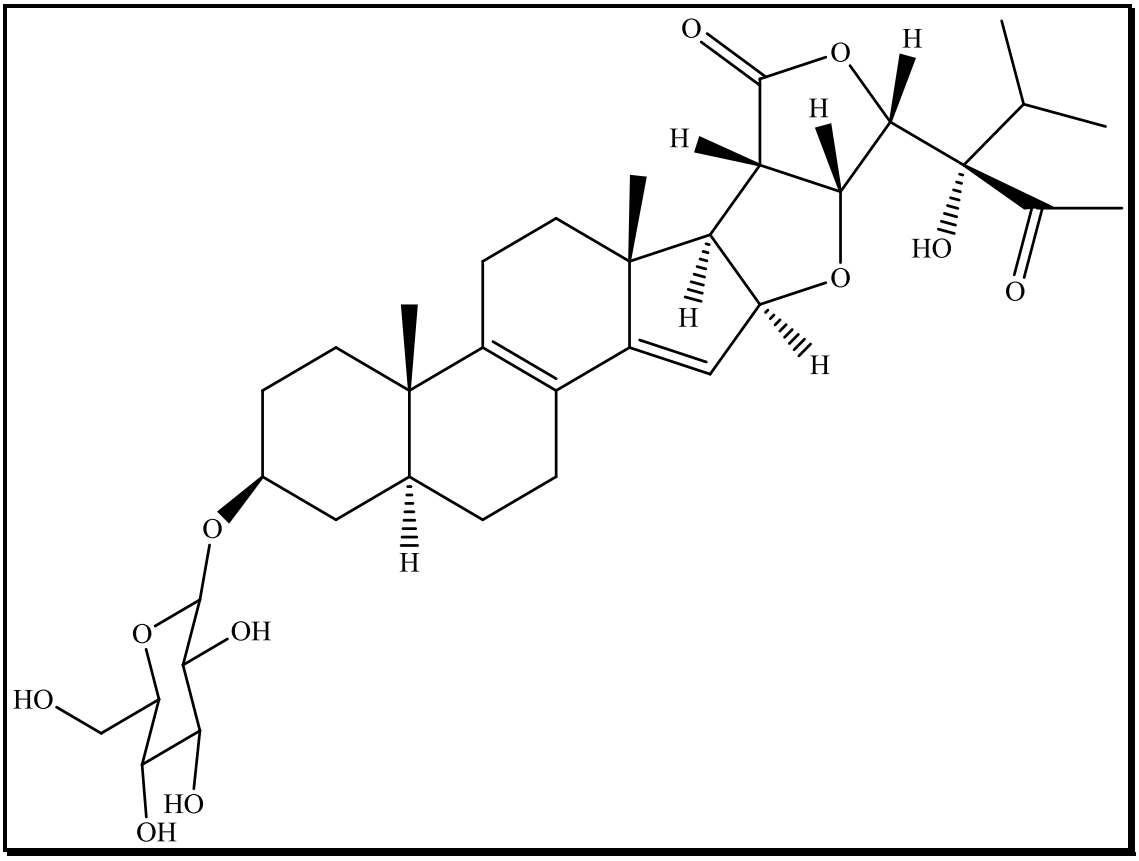
UV (MeOH) λ_{max} (log ε): 250 (4.52) nm

IR ν_{max} (MeOH) cm^{-1}: 3389, 2928, 1712, 1071 and 1038

HR-ESI-MS (*m/z*): 493.2939 (calculated for $[M + Na]^+$ 493.2924)

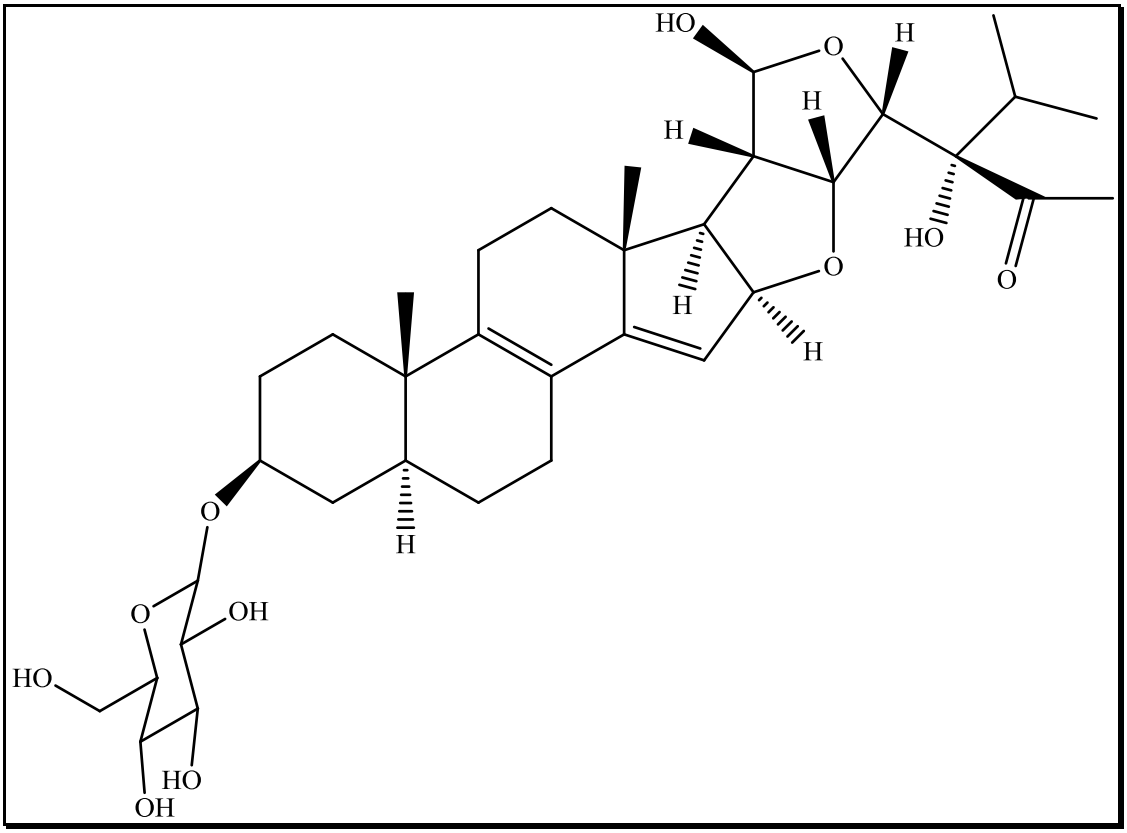
^1H- and ^{13}C-NMR: Table-10.9

11.2.3.10 Vernoantheloside A (71)



Physical State:	White amorphous powder
Yield:	20 mg
$[\alpha]_D^{20}$:	$-31.1°$ (c 0.5, MeOH)
UV (MeOH) λ_{max} (log ε):	246 (4.33) nm
IR ν_{max} (MeOH) cm^{-1}:	3295, 2928, 1773, 1630 and 1029
HR-ESI-MS (*m/z*):	669.3252 (calculated for $[M + Na]^+$ 669.3245)
^1H- and ^{13}C-NMR:	Table-10.10

11.2.3.11 Vernoantheloside B (72)



Physical State:	White amorphous powder
Yield:	3 mg
$[\alpha]_D^{20}$:	$+ 40.0° \ (c \ 0.1, \mathrm{MeOH})$
UV (MeOH) λ_{max} (log ε):	246 (4.34) nm
IR ν_{max} (MeOH) cm^{-1}:	3350, 2944, 2833, 1760, 1647, 1450 and 1027
HR-ESI-MS (*m/z*):	671.3399 (calculated for $[M + Na]^+$ 671.3402)
^{1}H- and ^{13}C-NMR:	Table-10.11

11.3 Biological studies

11.3.1 Anti-bacterial assay

Agar Tube diffusion method[155] was employed for determination of anti-bacterial activity as discussed in **section-5.3.1.**

11.3.2 Anti-fungal assay

Anti-fungal assay was determined through Agar Tube Dilution Method[156,157] as discussed in **section-5.3.2.**

11.3.3 Phytotoxicity

Phytotoxicity of the various fractions was evaluated against the *Lemna minor* L.[159] as discussed in **section-5.3.3.**

11.3.4 Insecticidal assay

Insecticidal assay was determined by Contact Toxicity Method[160] as discussed in **section-5.3.4.**

11.3.5 Brine shrimp (*Artemia salina*) lethality bioassay

Cytotoxicity of the solvent extracted fractions was evaluated against *Artemia salina* (Leach) shrimp larvae as discussed in **section-5.3.5**.

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1. 2-[(1R*,4R*)-1,4-Dihydroxycyclohexyl]-aceticacid
   Mohammad Arfan, **Syed Hamid Hussain**, M. Nawaz Tahir, Jamal Rafique and Farzana Shaheen, *Acta Cryst.* **2011**, *E67***,** o968**.**

2. Highly oxygenated stigmastane-types steroids from the aerial parts of *Vernonia anthelmintica* Willd
   Lei Hua, Wei-Yan Qi, **Syed Hamid Hussain**, Kun Gao, Mohammad Arfan, *Steroids*, **2012**, *77*(7), 811-818

3. Three new phenylpropanoid glucosides from the aerial parts of *Ligularia thomsonii*
   Ghias Uddin, **Syed Hamid Hussain**, Abdul Latif, Mohammad Arfan, Russell J. Cox and Thomas J. Simpson, (submitted)

4. Isolation, structure elucidation and NMR assignments of Senecioside, a new benzoxepin glucoside from the aerial parts of *Senecio desfontainei*
   Ghias Uddin, **Syed Hamid Hussain**, Abdul Latif, Mohammad Arfan, Russell J. Cox and Thomas J. Simpson, (submitted)

5. Isolation, structure elucidation and NMR assignments of Sorbanolic acid, a new triterpenoids from *Sorbus lanata*
   Ghias Uddin, Abdul Latif, **Syed Hamid Hussain**, Mohammad Arfan, Thomas J. Simpson and Russell J. Cox (submitted)

6. Phytochemicals from the stem wood of *Sorbus lanata* (D. Don.) Schauer.
   Ghias Uddin, Abdul Latif, **Syed Hamid Hussain**, Mohammad Arfan, Thomas J. Simpson and Russell J. Cox (submitted)