

**PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON
*VIBURNUM GRANDIFLORUM***



**Ph.D Thesis
BY
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**INSTITUTE OF CHEMICAL SCIENCES, UNIVERSITY OF
PESHAWAR, PESHAWAR, PAKISTAN
FEBRUARY, 2014**

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*VIBURNUM GRANDIFLORUM***



**A dissertation submitted for the partial
fulfillment of the degree of
Doctor of Philosophy in Chemistry**

BY

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FEBRUARY, 2014



DECLARATIONS

This is to certify that **Mr. Muhammad Alam** has carried out his research and experimental work on the topic entitled as “**Phytochemical and biological studies on *Viburnum grandiflorum***” under my guidance and supervision. His research work is original and his dissertation is worthy of presentation to the University of Peshawar for the award of degree of **Doctor of Philosophy in chemistry**

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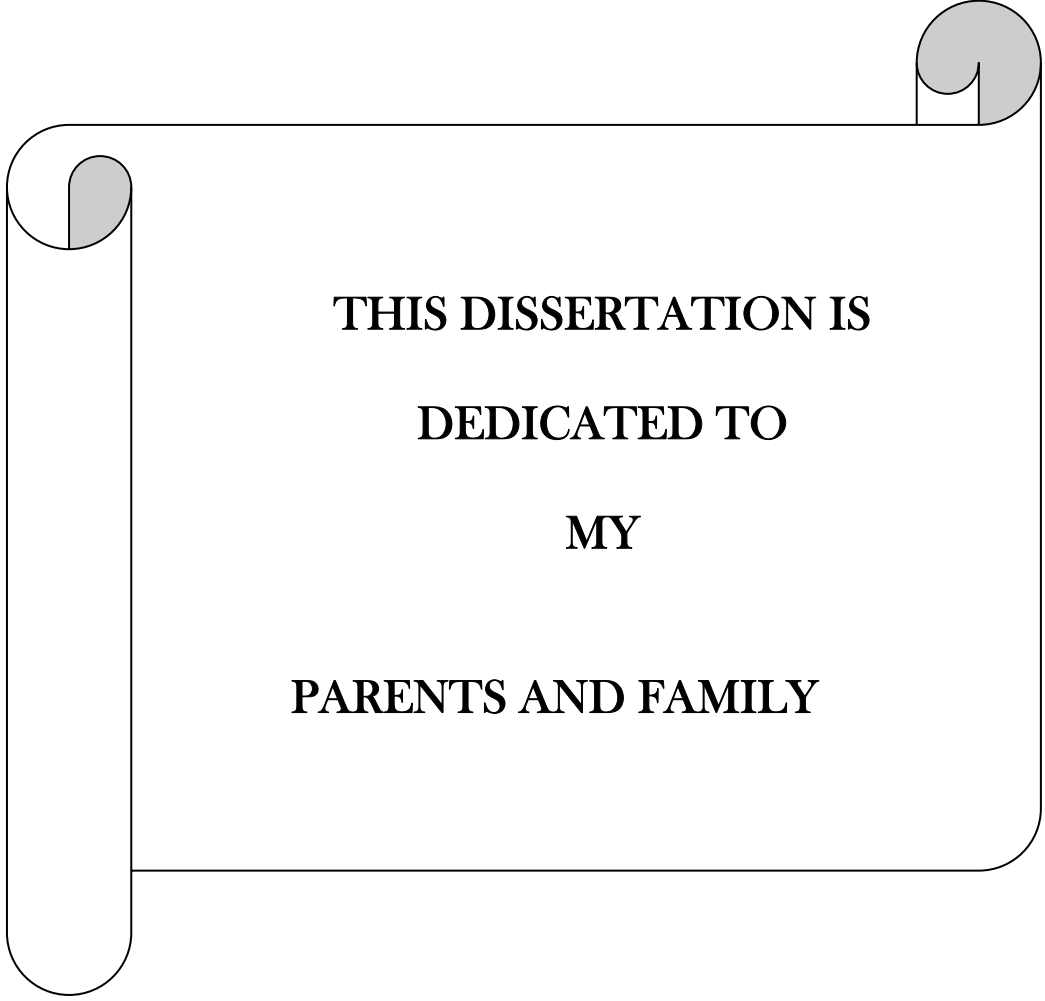
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**THIS DISSERTATION IS
DEDICATED TO
MY
PARENTS AND FAMILY**

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ABSTRACT

This dissertation consists of phytochemical and biological studies of the medicinal plant *Viburnum grandiflorum*, which has been investigated for the first time for their phytochemical constituents. Three new compounds; grandino 5, 8 peroxide (**259**), grandinoid (**263**), grandifloruside (**266**) from the aerial parts and three compounds; viburnoside (**268**), grandiflorinol (**274**) and grandifloronin (**275**) isolated from the roots of *V. grandiflorum*. Ten hitherto unreported compounds; betulinic acid (**257**), ursolic acid (**107**), β - sitosterol (**258**), oleanolic acid (**138**), stigmasterol (**260**), β -sitosterol 3-*O*- β -D-glucopyranoside (**261**), benzofuran derivative (**262**), betulin (**264**), *p*-hydroxy cinnamic acid (**229**), 3, 4, 5-trihydroxy benzoic acid; 3,5 dimethyl ether 4-*O*- β -D-glucopyranoside (**265**) from the aerial parts while six compounds; ethyl gallate (**267**), pentadecyl 3, 4-dihydroxy butanoate (**269**), cedrusin (**270**), koaburaside (**271**), (+) syringaresinol (**272**) and secoisolariciresinol (**273**) were isolated from roots of *V. grandiflorum*.

A cerimide class compound viburnoside (**268**), has reported first time from this genus. Advanced and sophisticated spectroscopic techniques were used for the characterization of compounds i.e. EI-MS, ESI-MS, UV, IR, ^1H NMR, ^{13}C NMR (BB and DEPT), HMBC, HSQC, NOESY, COSY and TOCSY.

The crude ethanolic extracts, fractions and pure compounds were evaluated for their biological potentials. The crude ethanolic extracts and subsequent fractions (aerial parts and roots) showed significant antibacterial activity and exhibited moderate antifungal

activity, while oils from the aerial parts found effective against *A. flavus* and *M. canis*. The fractions and oils (aerial parts) investigated for their phytotoxic activity which indicated significant phytotoxic activity except *n*-hexane fraction of aerial parts and ethyl acetate fraction of roots at low dose, while both samples of oils showed good phytotoxic activity. All fractions of aerial parts and roots exhibited significant insecticidal activity against the *R. dominica*, and *C. analis* except *n*-hexane fraction of roots. Both the tested oil samples showed moderate antileishmanicidal activity. The xanthine oxidase inhibition effect of the tested compound **262** was found moderate against the positive control (Allupurinol).

The crude ethanolic extract (aerial parts) and compound **270** tested for their antinociceptive properties, anti-inflammatory effects, anti-inflammatory effects, sedative effect, muscle relaxant effect and anticonvulsant effects and found active in the antinociceptive activity, moderate anti-inflammatory and antipyretic effects. Both samples showed mild muscle relaxant effects.

It is concluded that roots and aerial parts of *V. grandiflorum* can be safely used as antifungal especially against *M. canis* and *Fusarium* species. Beside the antifungal action the plant is a potential source of insects and weeds control. The roots of *V. grandiflorum* can also be used safely for the treatment of pain and inflammation.

LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BB	Broad band
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
CC	Column Chromatography
CEV	Crude extract of <i>Viburnum</i>
C3S	Cyanidin 3-sambubioside
5-CQA	5-Caffeoyl quince acid
COSY	Correlation Spectroscopy
2D NMR	Two Dimensional Nuclear Magnetic Resonance Spectroscopy
1D NMR	One Dimensional Nuclear Magnetic Resonance Spectroscopy
D	Duplet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra acetate
EI-MS	Electron Impact-Mass Spectrometry
ESI-MS	Electron Spray Ionization-Mass Spectrometry
FAB-MS	Fast Atom Bombardment Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
^1H NMR	Proton Nuclear Magnetic Resonance Spectroscopy

HMBC	Heteronuclear Multiple Bond Correlation
HR-EIMS	High Resolution-Electron Impact Mass Spectrometry
HR-ESIMS	High Resolution-Electro Spray Ionization Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
IC ₅₀	Inhibitory Concentration 50
L	Levo
LC ₅₀	Lethal Concentration 50
LC-MS	Liquid Chromatography-Mass Spectrometry
MS	Mass Spectrometry
MTT	Methyl Thiazol Tetrazolium
M	multiplet
NOESY	Nuclear Overhauser Effect Spectroscopy
Q	quartet
R	Rectus
R _f	Retardation Factor
S	Sinister
S	singlet
TLC	Thin Layer Chromatography
T	triplet
TOCSY	Total correlation spectroscopy
UV	Ultra Violet
VLC	Vacuum Liquid Chromatography

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Muhammad Alam

The author of this dissertation born in May 20, 1977 at Ghano Dheri, a small village of Tehsil Takht bhai, District Mardan (K.P.K). He got primary education at their village from GPS Ghano Dheri while matriculated from Govt. Higher Secondary School Hatian (Mardan) and F.Sc. from Govt. Degree College Takaht Bhai. He did B.Sc. from Govt. Post Graduate College Mardan. He did M.Sc. in Chemistry from Govt. Post Graduate Jehanzeb College Saidu Sharif Swat affiliated with University of Peshawar in 2000. He enrolled under M.Phil leading to Ph.D Programme of HEC in October 2007, at Institute of Chemical Sciences University of Peshawar under the supervision of Prof. Dr. Ghias Uddin. His research in Ph.D focused on Phytochemical and biological studies on *Viburnum grandiflorum*. He is serving against the post of subject specialist (SS in Chemistry) in (E&S) Education Department (K.P.K.) since 2005.

1.1 General introduction

Since time immemorial, human beings are using the products of living organism i.e. plants, animals and microorganisms for folk medicine, which are called “natural products”. The history of natural products use goes far back into antiquity. The use of the plants for medicinal purposes started from *Rig Veda* (4500-1600 B.C) and *Ayurveda* (2500-1600 B.C).¹ Egyptians (1550 B.C.) adopted medicinal plants for the treatment of various human diseases. According to the available history of folk medicine Shen Nung (the emperor of China) and Sekhet Enanach (in Egypt) were the first known and distinct physicians to discovered large number of herbal drugs and poisons (3000 B.C).²⁻⁴ Shen Nung compiled thousands of drugs in his book “Pen Tsao” (Great herbal). Many of them are still in use as folk medicines such as croton aconite, opium and rhubarb which is playing a vital role in modern pharmacopoeias. The book “Nei Ching” has written by Hwang Ti, Chinese emperor (2600 B.C) and considered the basic reference book of Chinese medicine literature. Many modern drugs like ephedrine (*Ephedra sinica*), Ipecac (*Cephaelis acuminata*), quinine (*Cinchona succirubra*), digitalis (foxglove; *Digitalis purpurea*), ajmaline (*Rauwolfia serpentina*) vinblastine (*Catharanthus roseus*), and taxol (*Taxus brevifolia*) were used for the treatment of hypotention, dysentery and reduce poisoning, malaria, heart diseases, Brugada syndrome (genetic disease), leukemia and anticancer respectively.⁵⁻⁸

1.2 Pharmacological development in Indo-Pak sub-continent

The local system of medicine in the Indo-Pak sub-continent is known as Ayurveda and developed primary health system; Charaka and Sushruta cited about 700 medicinal plants.⁹ The ancient Moen Jo Daro civilization of Indus valley played their part in the development of medical field with riverine civilizations of Sumer Egypt and China respectively. Hippocrates (460 B.C.), Theophrastus (370-267 B.C.) and Galen are the great names of Hellenic period and played a vital role in the advancement of herbal medicine.¹⁰⁻¹³

1.3 Developments in medicine in the Muslim period

Muslim scientists provided a great reservoir of medical knowledge during the early and later middle ages in the Muslim period of science and medicine. In this context the names of the great muslim scientist; Abu Bakar Mohammad Bin Zakaria Al-Razi, 932 A.D. (Rhazes) documented medicinal plants in his books; "*Alhavi Kabeer*" (Continents of Rhazes), *Kitab-al-Mansoori* while Bu-Ali Ibn Sina (908-1037 A.D.) an eminent physician, philosopher, astronomer and mathematician of their age reported about 760 herbal drugs in his well-known book "The Cannon of Medicine" and served as a text book of medicine in Europe till the end of 17th century. A famous pharmacist and botanist Zia-ud-din ibn al-Baitar (1248 A.D.), gave a detail description of the medicinal plants and its utilization for the treatment of human ailments in his books; *Kitab al-Jami fial Adwiya al-Mufrada*, *Kitab al-Mughni fial-Adwiya al-Mufrada*, while Al-Idrisi (1099-1166 A.D.) also wrote books; *Kitab al-Jamili-sifat*, *Ashtat-al-Nabatat* on the importance of medicinal plants.¹⁴

F.W. Serturmer (1783-1814) isolated morphine from the dried latex of *Papaver somniferum* L. (opium) and initiated the separation of pure compounds from the crude extract of medicinal plants. Followed the isolation of morphine; Pelleties and Cavantou isolated caffeine, cinchonine, quinine, brucine and strychnine. In 1870, Schiff for first time isolated and identified coniine which was later on synthesized by Ladenburg and considered to be the first isolated compounds from the natural sources in the history of man kind.^{15, 16}

1.4 Developments of medicine in modern period

The innovative studies on the isolation of herbal medicine resulted various classes of secondary metabolites such as reserpine and ajmaline. The promotion of the tradition of Ayurvedic and Unani system of indigenous curing wide range of investigation done by the school of tropical medicine in the 1920 decades at Calcutta (India), on medicinal plants. A research institute was established in 1920 at Tibbi College Delhi, India under the direction of the great physician Hakim Ajmal Khan to carry out multidimensional research on herbal medicines. In 1990, Hakim Muhammad Saeed also established Hamdard University at Karachi, Pakistan for the development of herbal medicines.

Thus a global motivation has developed in the entire world for the research on medicinal plants. Mostly these plants are found in hot and humid regions of the terrestrial world. In this context late eminent scientist Prof. Dr. Salimuzzaman Siddiqui, *F.R.S.*, laid the foundation of Husain Ebrahim Jamal (H.E.J) Research Institute of Chemistry (Karachi, Pakistan), with the provision of the latest instrumental and technical facilities for the

isolation, structure elucidation and bioassay studies of the constituents of medicinal plants.

The improvement in isolation and structural elucidation instruments greatly focuses the interest of the researchers towards medicinal plants. University of Peshawar also took historical discion and established a centre “Phytochemical Nutraceutical Research Laboratory” (PNRL) affiliated with Institute of Chemical Sciences equipped with the latest version of instruments.

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2.0 Classification of *Viburnum grandiflorum*-Guch

Kingdom:	Plantae – Plants
Subkingdom:	Tracheobionta – Vascular plants
Super division:	Spermatophyta – Seed plants
Division:	Magnoliophyta – Flowering plants
Subdivision	Angiosperms
Class:	Magnoliopsida – Dicotyledons
Super order:	Asterides
Order:	Dipsacales
Family:	Adoxaceae
Genus:	<i>Viburnum</i> L.
Species:	<i>Viburnum grandiflorum</i> wall ex. D.C- GÜCH



Viburnum grandiflorum Wall ex. D.C-Güch

Viburnum genus is a native to the temperate or subtropical zones from South America to South East Asia, belongs to the family Adoxaceae previously included in Capripoliaceae consists of more than 230 species. Six species of the genus *Viburnum* (*V. cotinifolium*, *V. cylindricum*, *V. grandiflorum*, *V. mullaha*, *V. opulus*, and *V. tinus*) are present in Pakistan.¹

2.1.1 Physiology of *Viburnum grandiflorum* plant

V. grandiflorum Wall ex. D C. synonyms *V. foetens* Decene and *V. nervosum* D. Don known as Himalayan *Viburnum* deciduous shrubs distributed in Himalaya from Swat to Hazara division between 2700-3600 m. A detailed description of the characteristic parts of the plant is described below:

Stem

Viburnum grandiflorum is a precocious, deciduous, multi-stemmed shrub develops an upright, rounded silhouette shrub which has 3-5 × 2-3 m long stem. The colour of stem and twigs is green to the fairly brown and burgundy.²

Leaves

The leaves of *V. grandiflorum* appear after flowers on stiff stout braches. The leaves of *V. grandiflorum* are 5-12 cm in length and 2.5-6 cm in width, which accumulates as elliptic, oblong, acute, sharply toothed, connate at the base and more are less hairy on the nerves and the leaves emit a bad smell when bruised. Mostly the leaves appear after flowers on stiff stout twigs in April and fallen in November till June.²

Flowers

The flowers of *V. grandiflorum* appear soon after the leaves have fallen which are sweet scented and usually planted in the gardens for ornamental purposes. The flowers of *V. grandiflorum* are hermaphrodite (have both male and female organs), along calyx tube, glabrous; lobes short, ciliate, long tubular corolla, 10-15 cm lobes rounded, spreading white to rose-pink.²

Seeds

V. grandiflorum is a single seeded plant, which is inside the fruits (sweetish and edible) and grooved on one side and deeply grooved on the other with incurved margins. The plant is pollinated by insects.²

Habitat

V. grandiflorum is a deciduous shrub found in the Himalaya at 2700-3600 meters altitude. It is often forming dense scrub but normally in shrubberies or in the form of open forests. The suitable condition for its growing is light (sandy), medium (loamy) and heavy (clay) moist soils. It can grow in semi-shade (light woodland) or no shade.

2.1.2 Cultivation of *Viburnum grandiflorum*

V. grandiflorum is an easily grown plant it succeeds in most soils but is ill-adapted for poor soils and for dry situation; prefers a deep rich loamy soil in sun or semi-shade. A very ornamental plant, the deliciously scented flowers are produced in the winter without damage up to -12 °C and the fruits often produce in this condition.

2.1.3 Medicinal uses

The medicinal uses of *V. grandiflorum* are none known, but others species of *Viburnum* genus have long history in folk medicine. Native used a decoction of black haw (*V.*

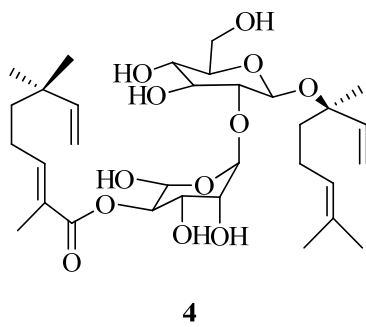
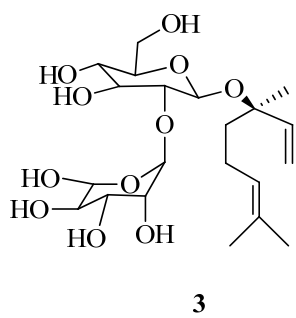
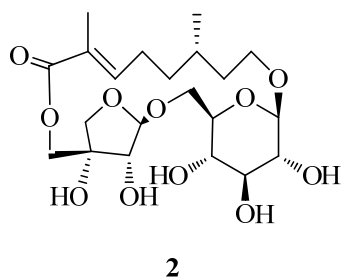
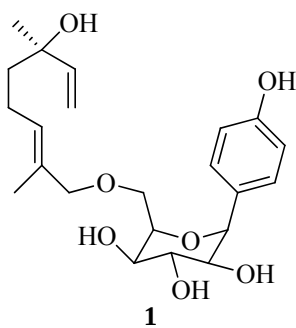
prunifolium) to treat gynecological conditions, including menstrual cramps, aiding recovery after child birth, menopause, morning sickness, prevention of miscarriage. Due to its antispasmodic properties, the plant may also be used in treating cramps of the digestive tract or the bile ducts.³ *Viburnum opulus* bark also known as cramp bark used in herbal medicine with success in cramps and spasms, in convulsions, fits and lock jaw, and also in palpitation, heart disease and rheumatism while the crude extract fluid is used as elixir, for the treatment of nervous tension (nerve sedative) and anti-spasmodic in asthma and hysteria.⁴

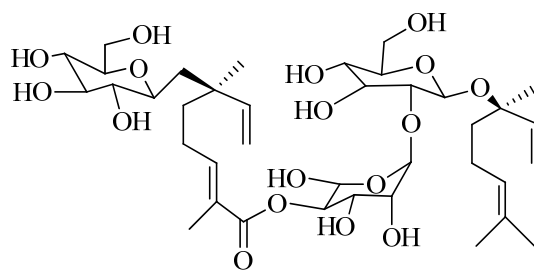
2.2.0 Chemical constituents

V. grandiflorum is previously not investigated for their chemical constituents however different classes of compounds (monoterpenes, diterpenes, sesquiterpenes, triterpenes, iridoid, iridoid glucoside, steroids, saponines, flavonoids, coumarins, alkaloids etc.) have been reported from various species of the genus which are given below:

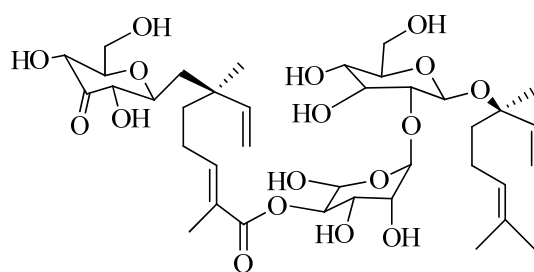
2.2.1 Monoterpene.

Ten monoterpenes were isolated from the genus so far. Hase, et al., in 1982 reported first monoterpene; phlebotricoside (**1**) (*V. phlebotrichum*), subsequently Iwagawa, et al., (1983) isolated urceolid (**2**) from the methanolic extract of *V. urceolatum*.^{5, 6} In 1993 Calis, et al., reported monoterpenes from *V. Orientale* i.e. anatoside (**3**), anatoside A (**4**), anatoside B (**5**), anatoside C (**6**), anatoside D (**7**), anatoside E (**8**), and betulalbuside B (**9**), while betulalbuside A (**10**) by Calis, et al., (1995) isolated from *V. lantana*.⁷⁻⁹

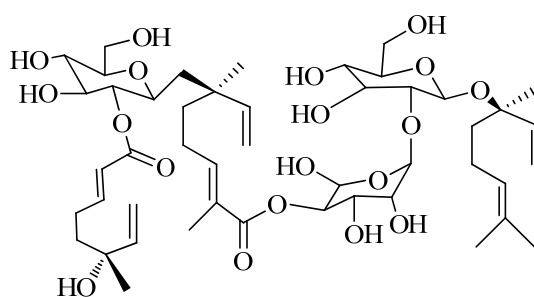




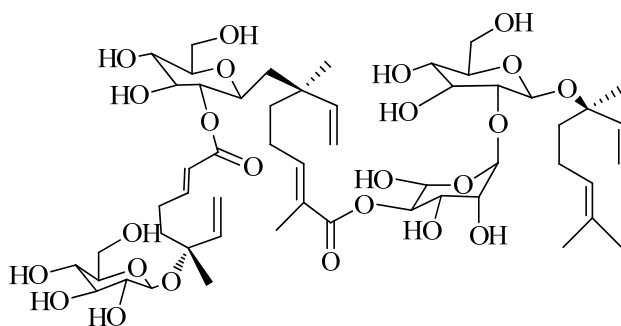
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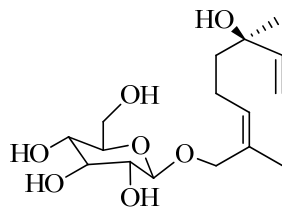
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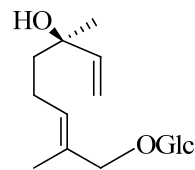
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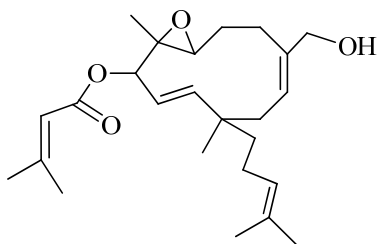


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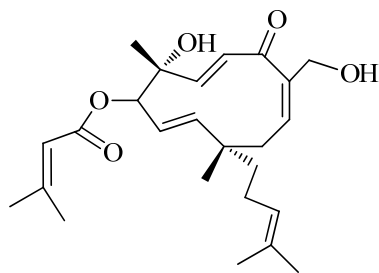
2.2.2 Diterpenes

Vibsanin types of diterpenes are most dominant group of compounds in the genus *Viburnum* reported from *V. odoratissimum*, *V. awabuki*, and *V. suspensum*.²⁷⁻⁴⁵

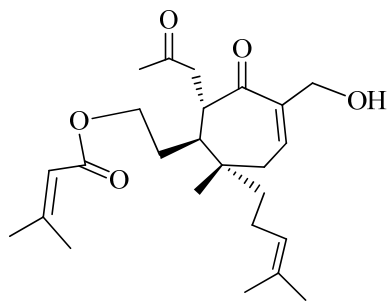
Kawazu, et al., in 1980 reported vibsanin type of diterpenes vibsanin A-F (**11-16**) from *V. odoratissimum*.¹⁰ In 1992, Iwagawa and coworkers isolated labdane-type diglucosides gomojosides A-J (**17-26**) from *V. suspensum*,¹¹ furthermore the same group of researchers in 1993 reported seven gomojosides K-Q (**27-33**) from *V. suspensum*.¹² In 1996 Fukuyama, et al., characterized diterpenes; neovibsanin A (**34**) and neovibsanin B (**35**) from *V. awabuki*.¹³



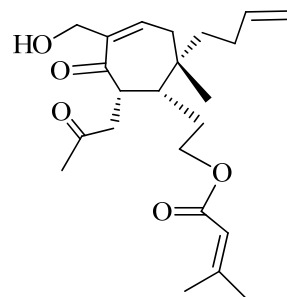
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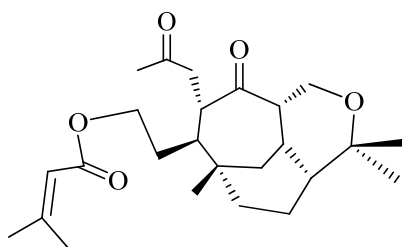
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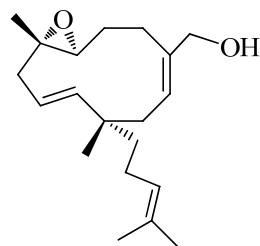
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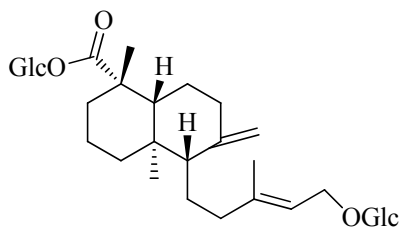
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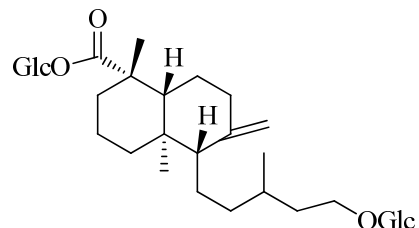
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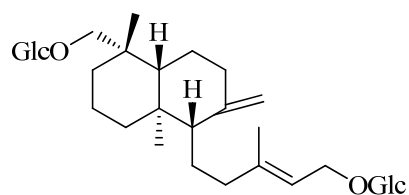
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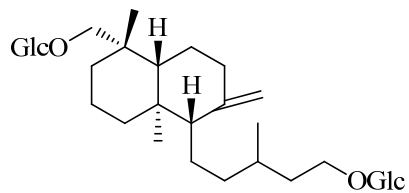
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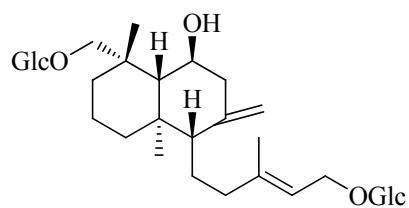
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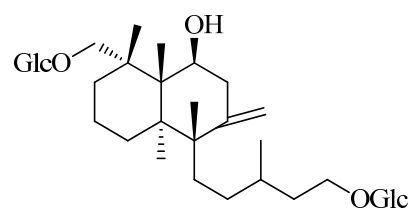
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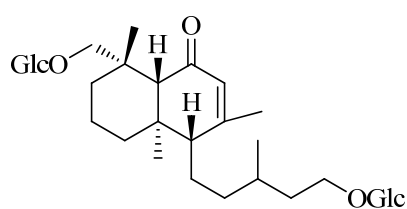
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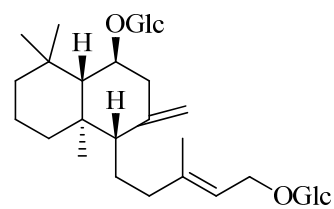
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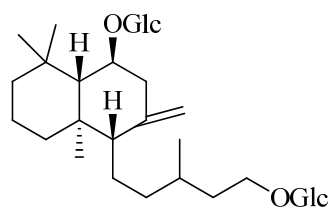
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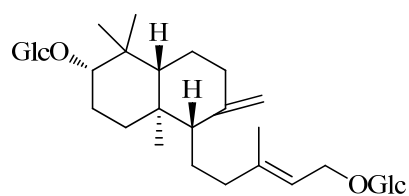
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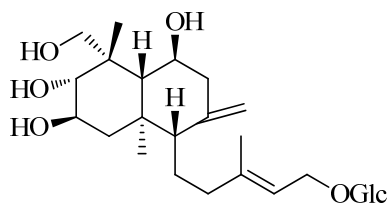
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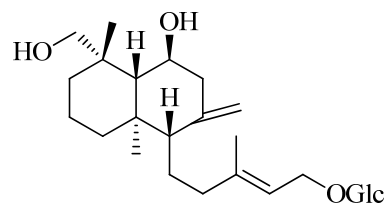
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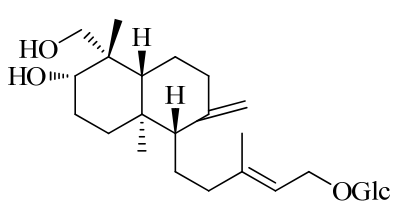
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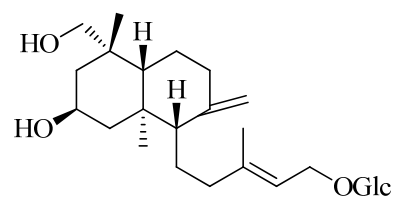
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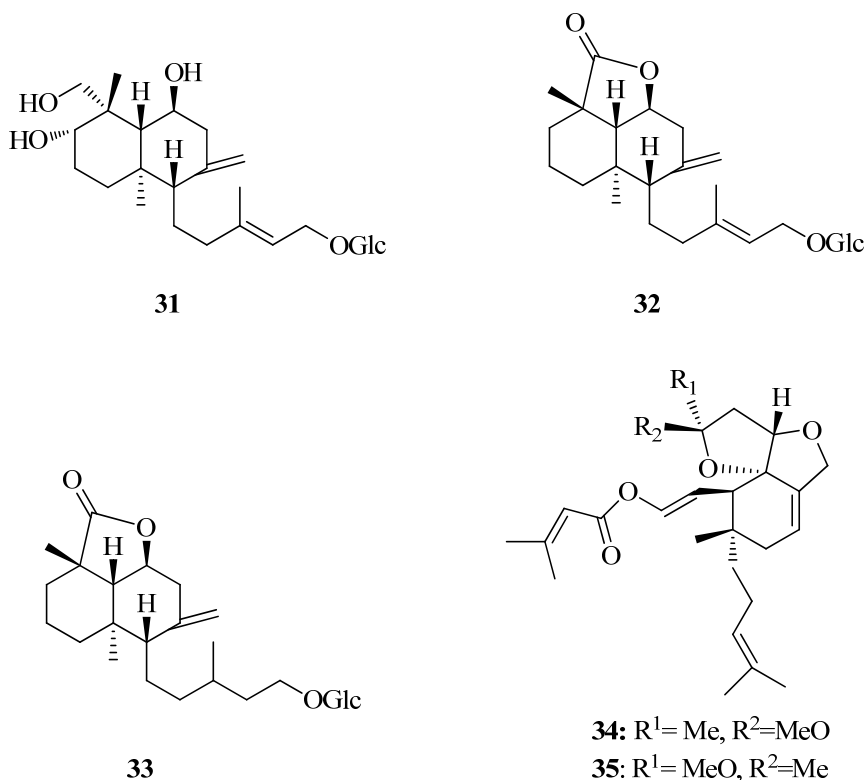
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Subsequently Fukuyama, et al., also reported vibsanin type of diterpenes; neovibsanin H (**36**) and neovibsanin I (**37**), 3-hydroxyvibsanin E (**38**) from *V. awabuki*.^{14, 15.}

Furthermore the same research group isolated furanovibsanines A-G (**39-45**), 3-*O*-methylfuranovibsanin A (**46**), 7-epifuranovibsanin B (**47**), 5-epivibsanin C (**48**), 5-epivibsanin E (**49**), 5-epivibsanin H (**50**), 5-epivibsanin K (**51**), 18-*O*-methyl-5-epivibsanin K (**52**), cyclovibsanin A (**53**), 15-*O*-methylcyclovibsanin A (**54**), 15-*O*-methylcyclovibsanin B (**55**), 3-hydroxy-15-*O*-methylcyclovibsanin A (**56**), neovibsanin D (**57**), neovibsanin G (**58**), 7-epineovibsanin D (**59**), 15-*O*-methylneovibsanin F (**60**), 15-*O*-methyl-14-epineovibsanin F (**61**), 15-*O*-methyl-18-oxoneovibsanin F (**62**), 2-*O*-methylneovibsanin H (**63**), 2-*O*-methylneovibsanin I (**64**), 14-epineovibsanin G (**65**), and neovibsanin F (**66**) from *V. awabuki* and *V. suspensum* respectively.¹⁶⁻²⁰

Minami, et al., in 1998, isolated vibsananin G (**67**) vibsananin H (**68**), vibsananin K (**69**), 15, 18-di-*O*-methylvibsananin H (**70**), 18-*O*-methylvibsananin K (**71**) from *V. awabuki*.²¹

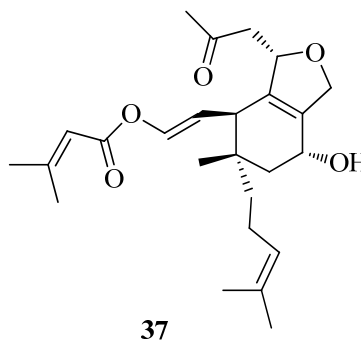
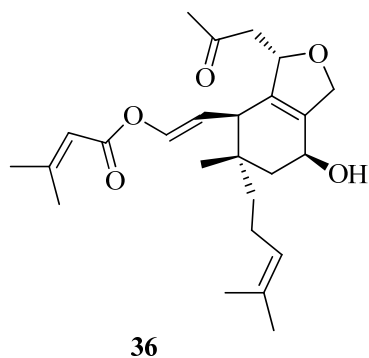
Kubo, et al., isolated diterpenes aldovibsananin A (**72**), aldovibsananin B (**73**), and 7-epialdovibsananin A (**74**) from *V. odoratissimum*.²²

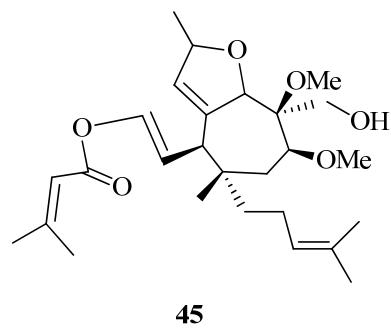
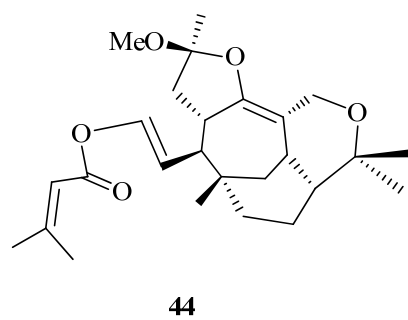
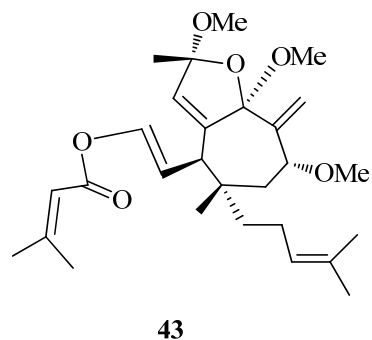
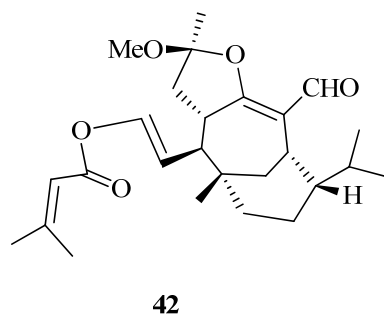
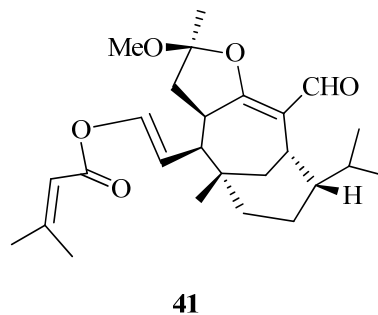
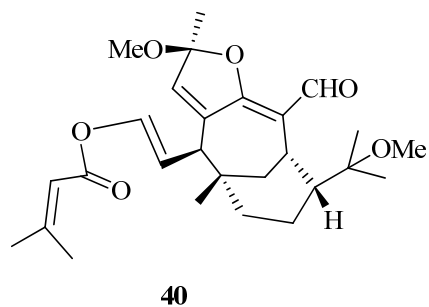
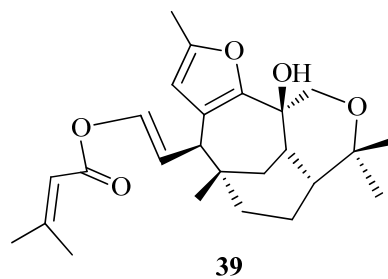
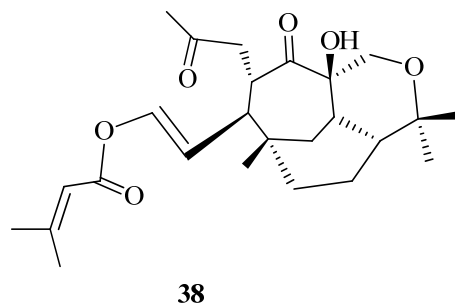
subsequently Kubo research group reported macrocyclized diterpenoid neovibsananin C (**75**), spirovibsananin A (**76**) and vibsananin I (**77**), vibsananin L (**78**), 14-hydroxyvibsananin F (**79**), (14*R**)-14, 15-epoxyvibsananin C (**80**), (14*S**)-14, 15-epoxyvibsananin C (**81**), neovibsananin O (**82**), neovibsananin L (**83**), neovibsananin M (**84**), (8*Z*)- neovibsananin M (**85**), 15-*O*-methylvibsananin H (**86**) and 5-epi-15-*O*-methylvibsananin H (**87**) from *V. awabuki* and *V. odoratissimum* respectively.²³⁻²⁶

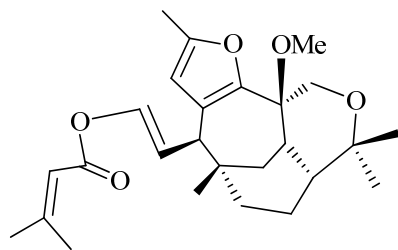
Shen, et al., characterized vibsanol type diterpenes; vibsanol A (**88**), vibsanol B (**89**), vibsananin M (**90**), aldovibsananin C (**91**), 5-epivibsananin G (**92**) and 18-*O*-methylvibsananin G (**93**) from *V. odoratissimum*.^{27, 28}

Duh and El-Gamal research groups isolated vibsananin O (**94**), vibsananines P-W (**95-102**) from *V. awabuki*.^{29, 30}

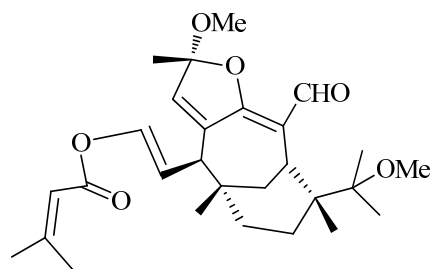
Chen, et al., reported 15-*O*- methyl vibsanol A (**103**) along with two triterpenes from the leaves extract of *V. chingii*.³¹



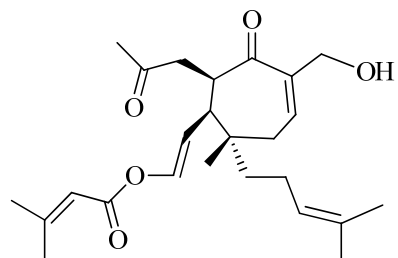




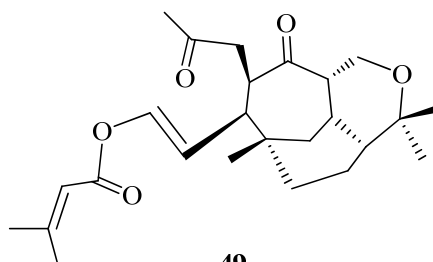
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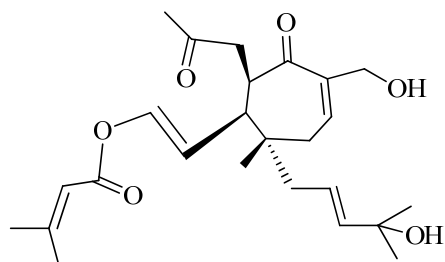
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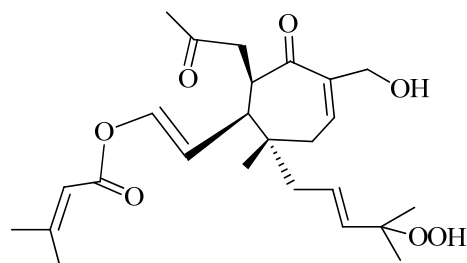
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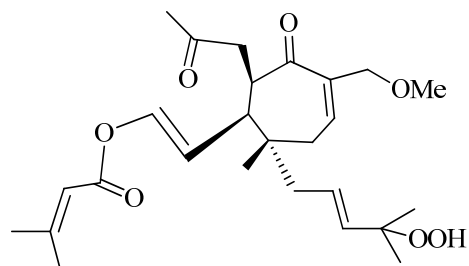
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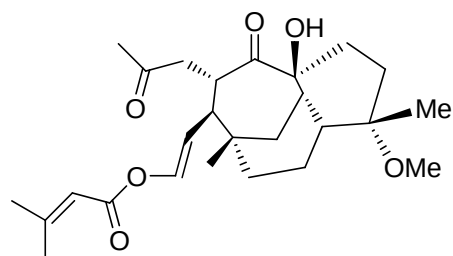
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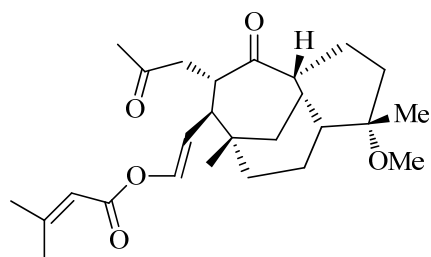
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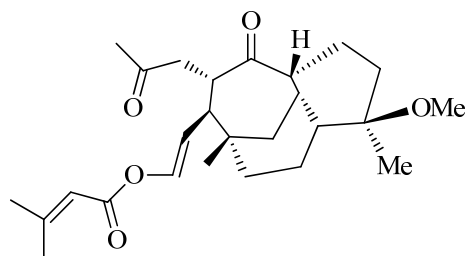
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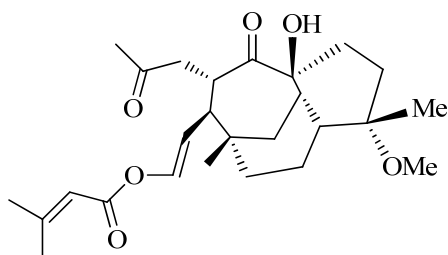
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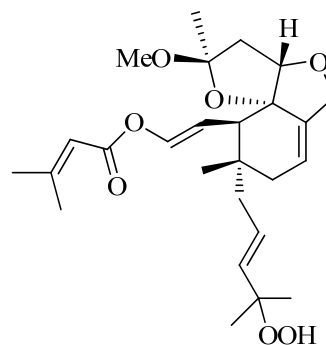
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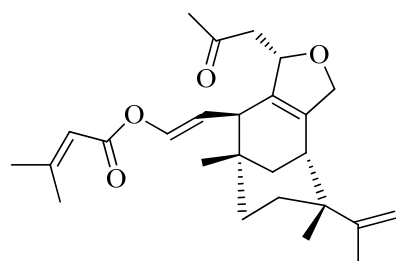
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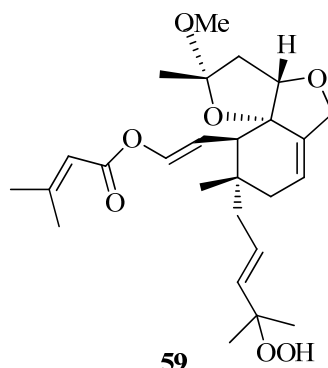
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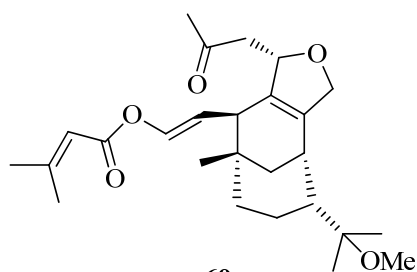
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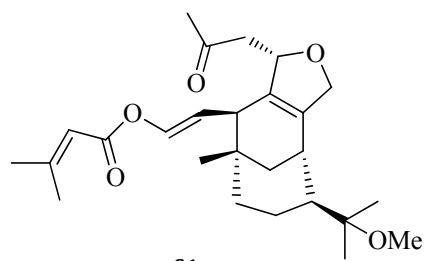
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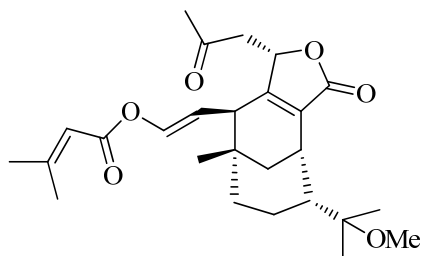
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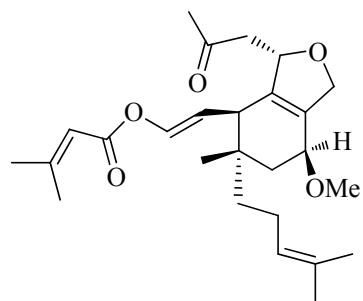
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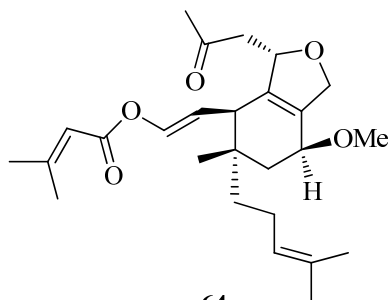
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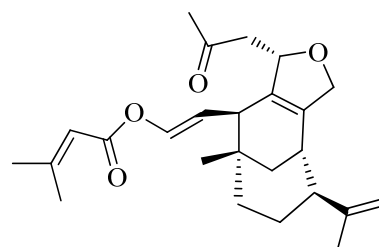
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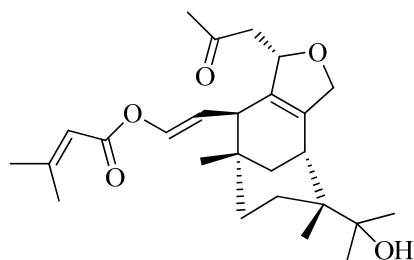
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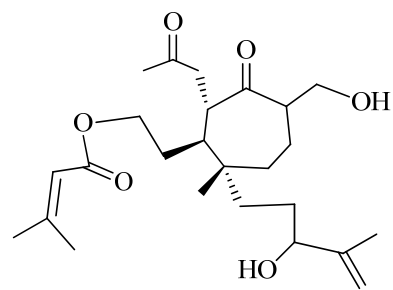
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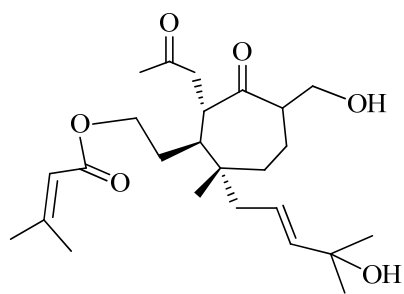
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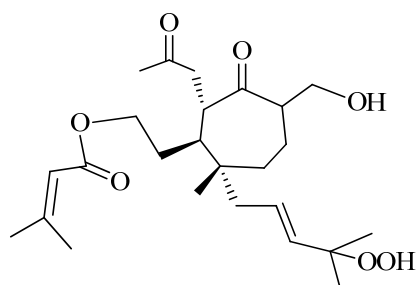
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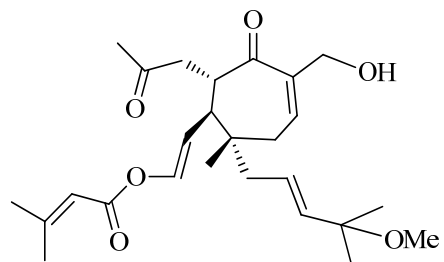
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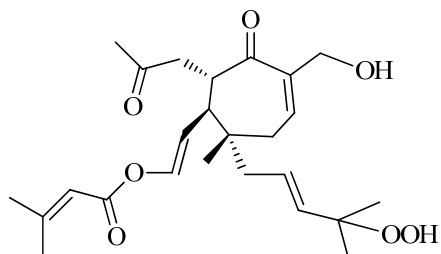
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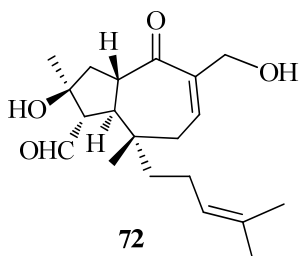
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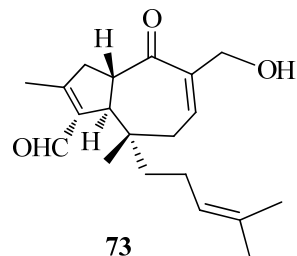
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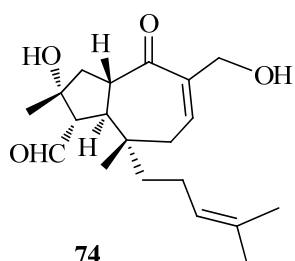
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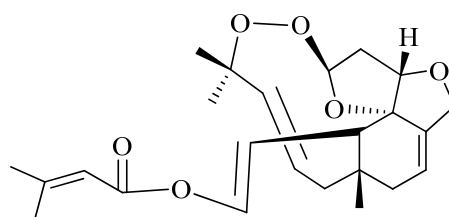
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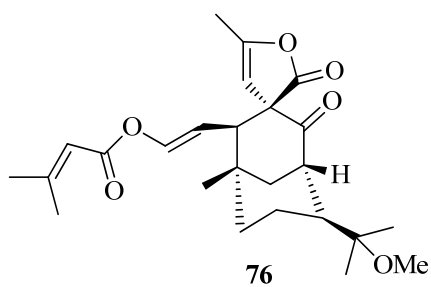
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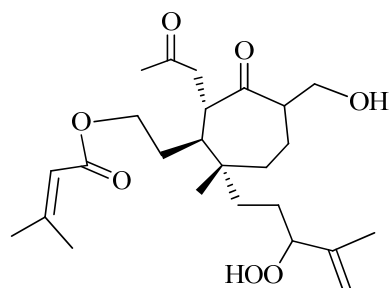
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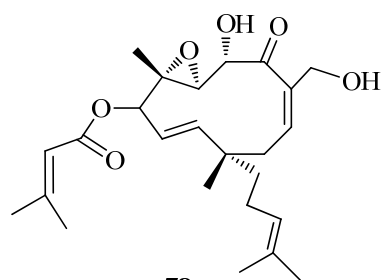
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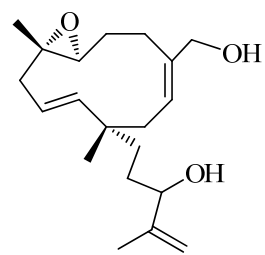
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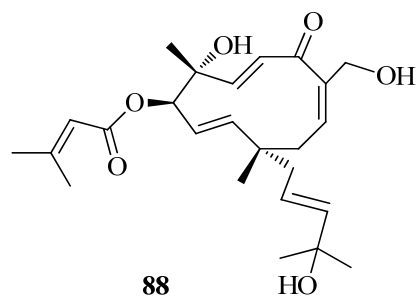
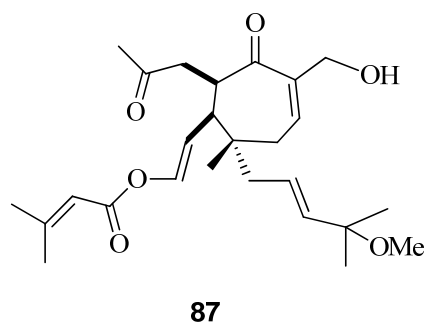
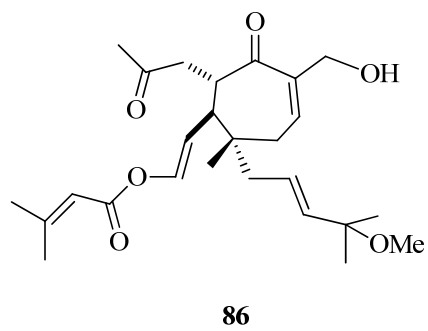
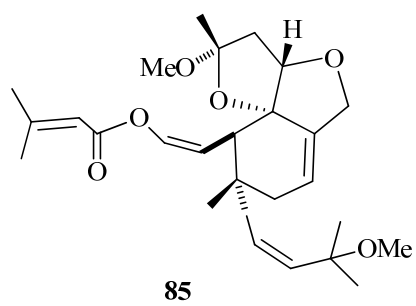
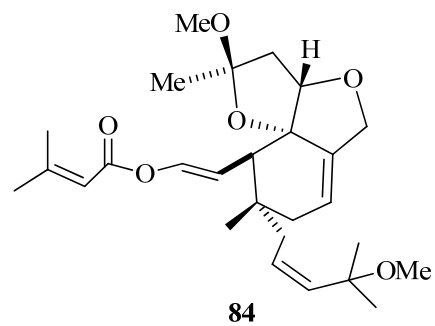
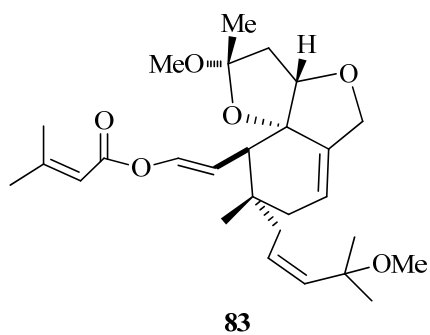
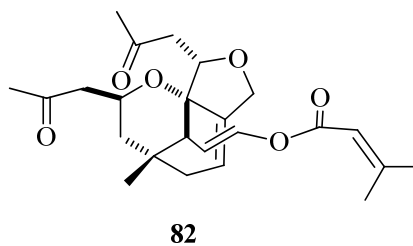
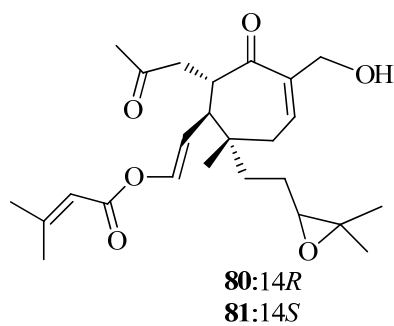
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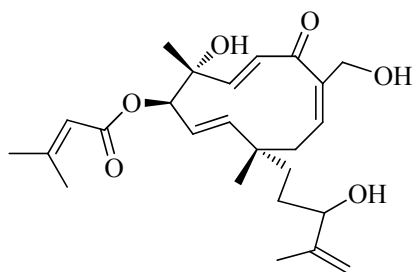


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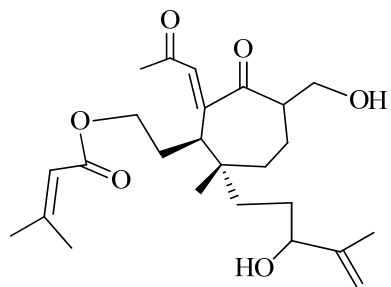


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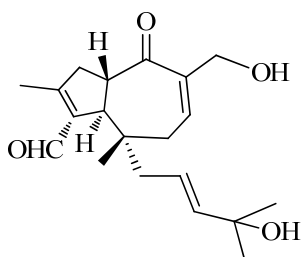




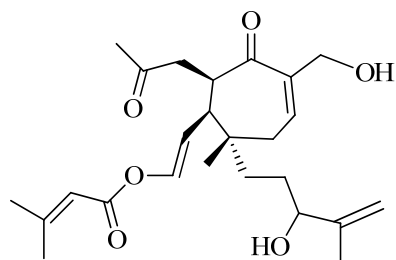
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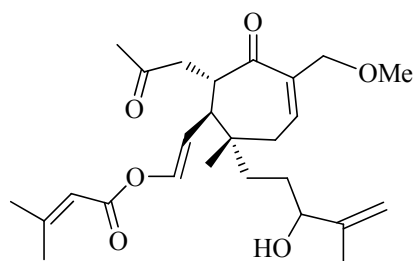
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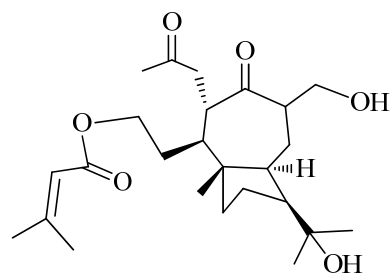
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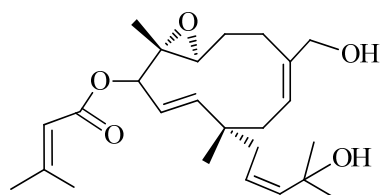
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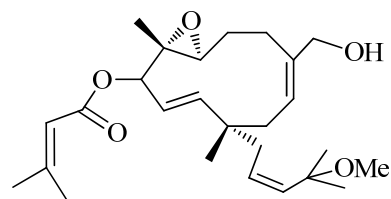
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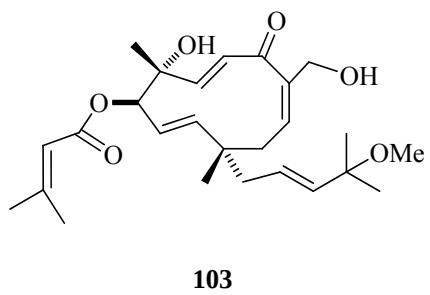
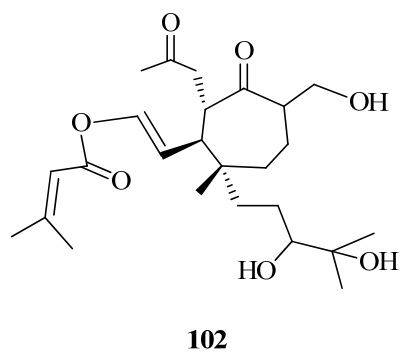
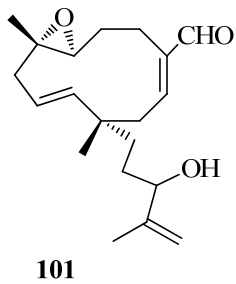
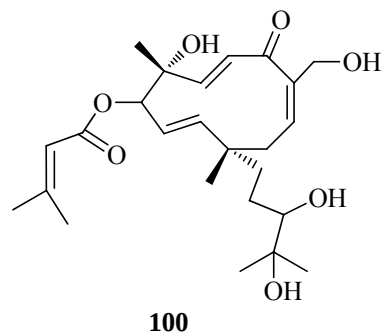
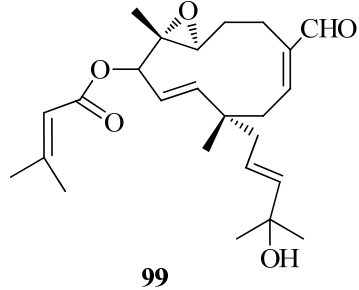
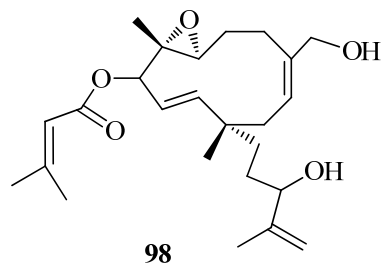
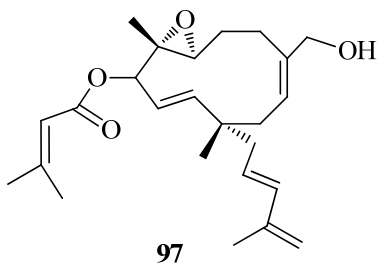
94



95



96



2.2.3 Triterpenes

Iwagawa and coworkers, in 1982 reported triterpenes; α -amyrine palmitate (**104**), lupeol palmitate (**105**) β -amyrine acetate (**106**) and ursolic acid (**107**) from *V. urceolatum* subsequently Iwagawa et al., (1983) isolated triterpene; Y-taraxasterol acetate (**108**) from *V. awabuki*.^{32, 33}

In 1986, Kuroyanagi and his research group reported triterpene; 6α -hydroxy-3-oxolup-20(29)-en-28-oic acid (**109**) from *V. awabuki*.³⁴

Machida and Kikuchi (1997, 1999) isolated viburnols-type triterpenes; viburnols A-K (**110-120**), viburnudienone B1 methyl ester (**121**), viburnudienone B2 methyl ester (**122**), viburnenone B1 methyl ester (**123**), viburnenone B2 methyl ester (**124**), viburnudienone H1 (**125**) and viburnudienone H2 (**126**) from *V. dilatatum*.³⁵⁻³⁷

In 1998, Kagawa and co-workers isolated and characterized oleanine-type triterpenes; 3β , 28-Dihydroxyolean-12-en-11-one (**127**) and 13, 28-epoxyolean-en-3-one (**128**) from *V. awabuki*.³⁸

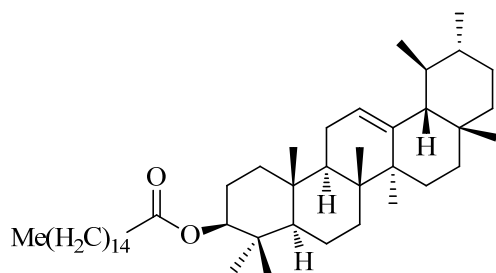
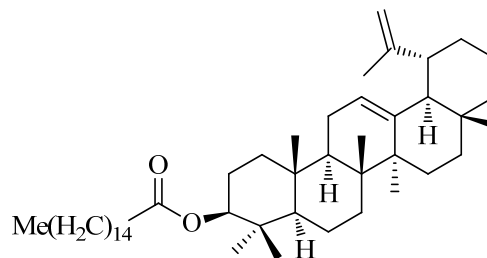
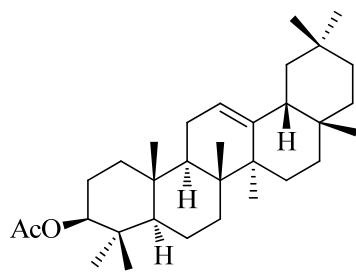
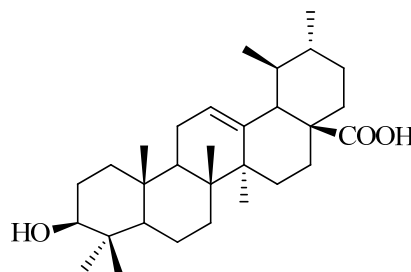
Fukuyama, et al., in 2002 reported three triterpenes; 3-oxooleana-11, 13(18)-dien-28-oic acid (**129**), 24-hydroxy-3-oxooleana-11,13(18)-dien-28-oic acid (**130**) and 6β -hydroxy-3-oxooleana-11,13(18)-dien-28-oic acid (**131**) from *V. suspensum*.³⁹ In the same year, Shen and his research group isolated lupeol-type triterpene; 6β -hydroxy-3-oxolup-20(29)-ene-27,28-dioic acid (**132**) and 6α -hydroxy-3-oxolup-20(29)-ene-27,28-dioic acid (**134**) from *V. odoratissimum*.²³ Ku and his co-researchers in 2003 isolated vibulolide (**135**) from *V. arboricolum*.⁴⁰

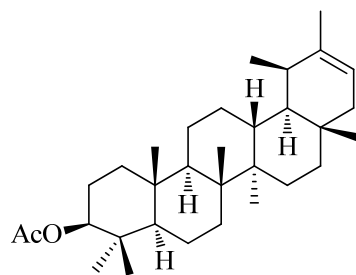
In 2005, Mohamed, et al., reported 3-O- β -D- galactopyranosyl-(1 $\rightarrow$ 2)-O- β -D- glucuronopyranoside oleanolic acid 28-O- β -D-glucopyranosyl ester (**136**), 3-O-(β -D-

glucuronopyranosyl) oleanolic acid (**137**) and oleanolic acid (**138**) from the leaves extract of *V. tinus*.⁴¹

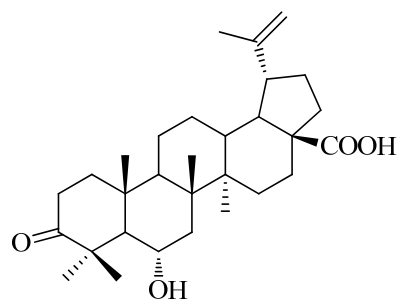
El. Gamal. A. A., in 2008 isolated lupane-type triterpenes; 6β -hydroxy-3, 20-dioxo-30-norlupan-28-oic acid (**139**) and 4, 20-dihydroxy-3, 4-secolupane 3.28-dioic acid methyl ester (**140**) from *V. awabuki*.⁴²

Recently, in 2011 Hu and Chen research groups reported ursal-12-sene-3 β -11 β -diol 3-*O*-palmitate (**141**) ursal-12-sene-1 β -3 β -11 α -triol, 3-*O*-palmitate (**142**), 1 α -3 β -dihydroxy-11 α -methoxy-olean-12-ene (**143**) and 1 α -3 β -dihydroxy-11 α -methoxy-olean-9(11), 12-diene (**144**) from *V. betulifolium* and *V. chingii* respectively.^{43, 31}

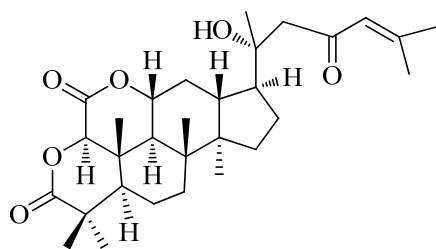
**104****105****106****107**



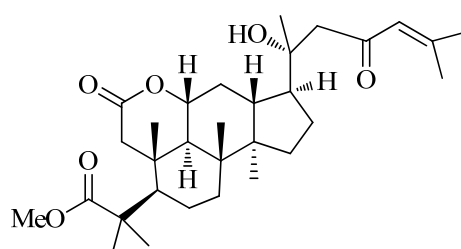
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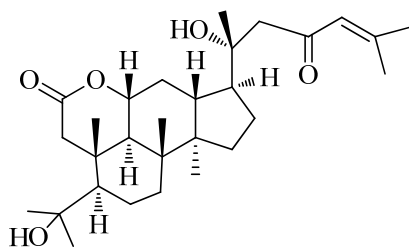
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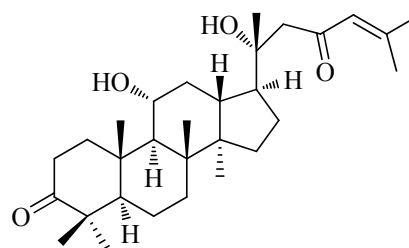
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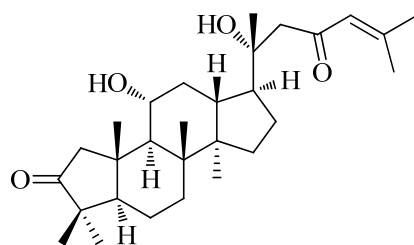
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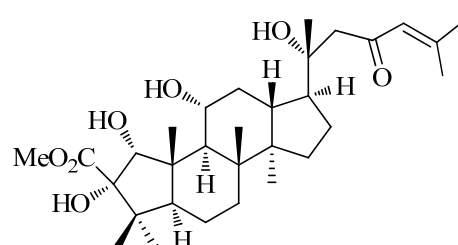
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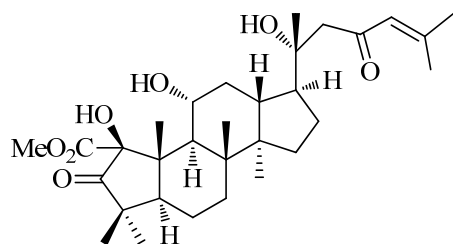
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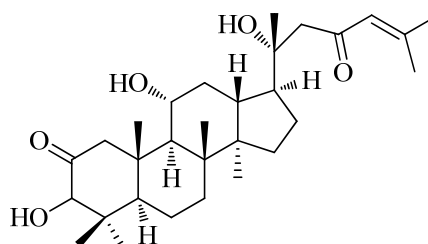
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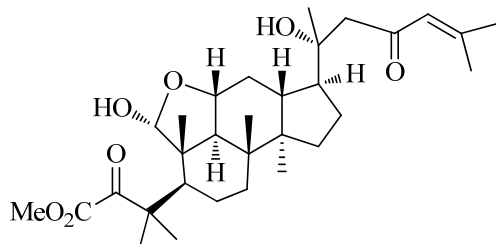
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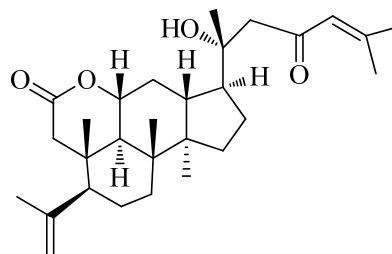
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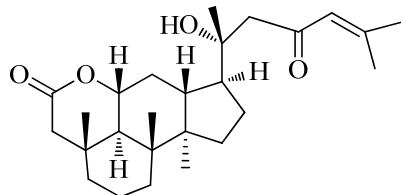
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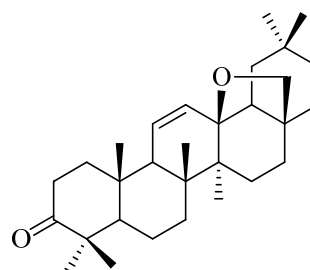
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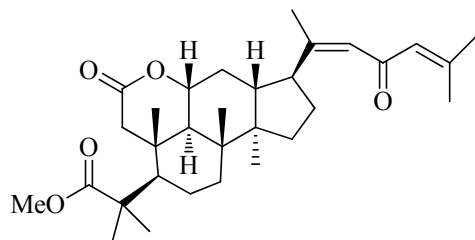
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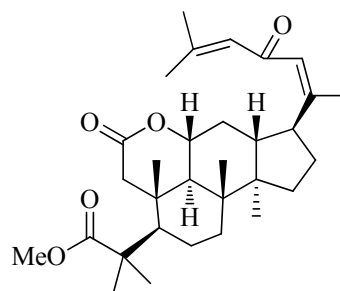
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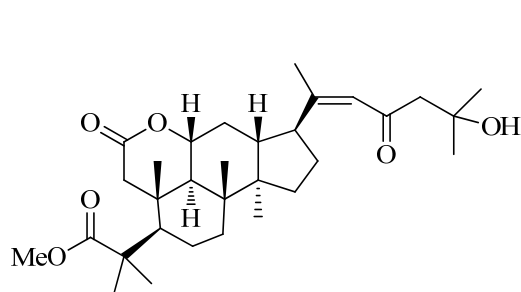
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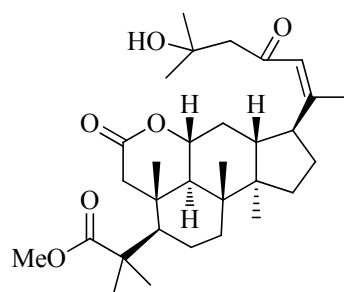
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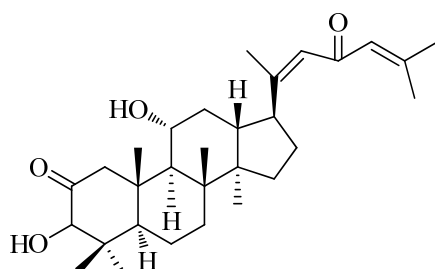
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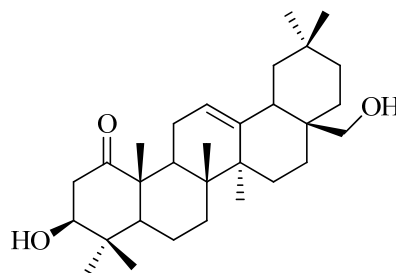
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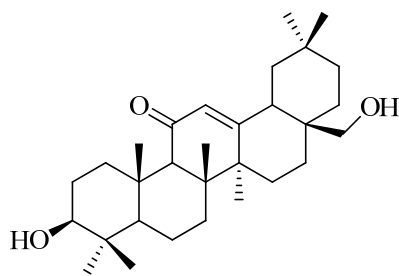
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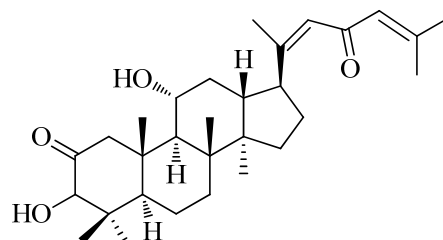
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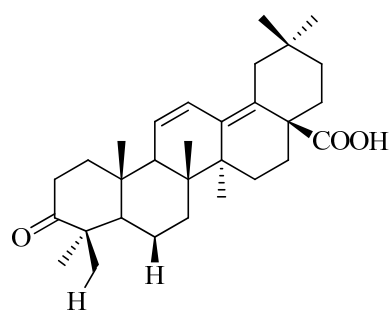
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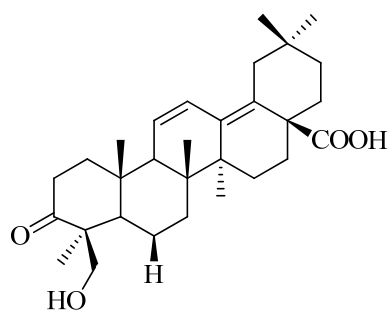
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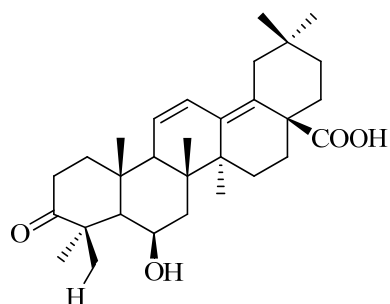
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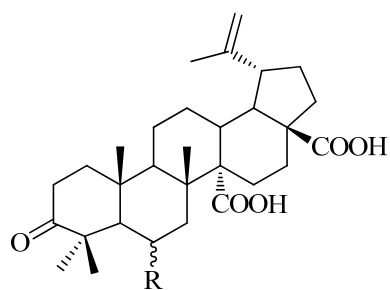
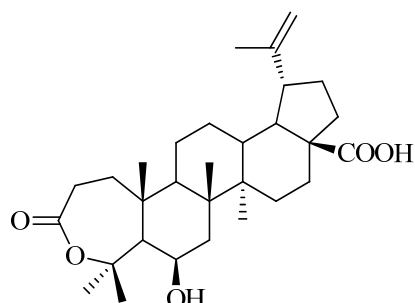
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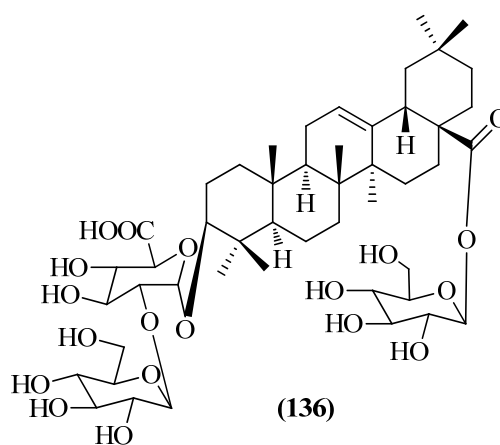
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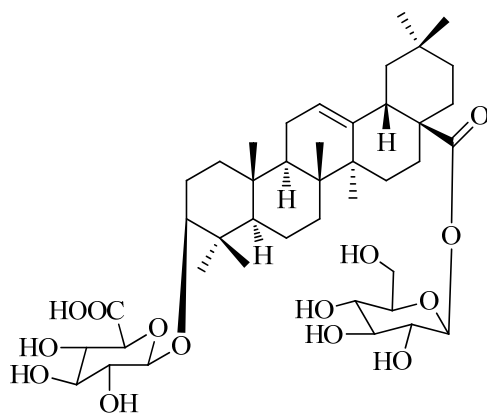
132

133:R = β -OH134:R = α -OH

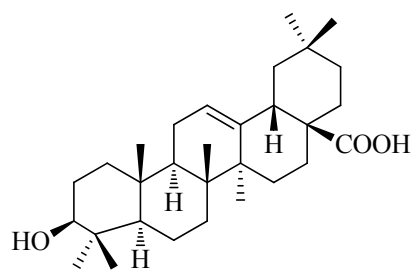
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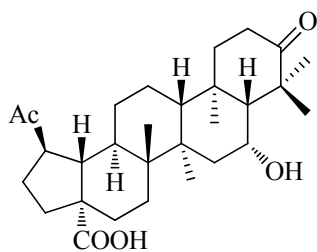
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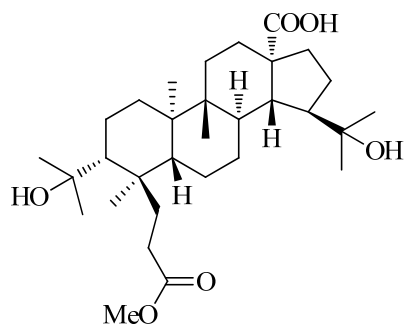
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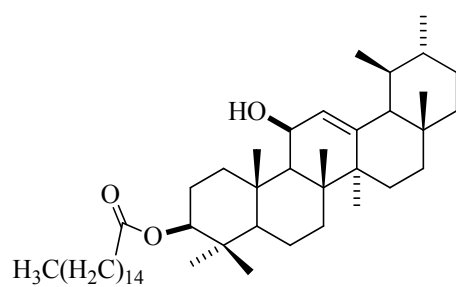
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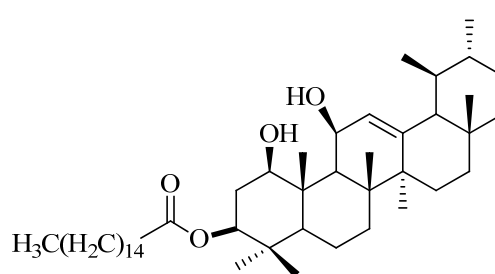
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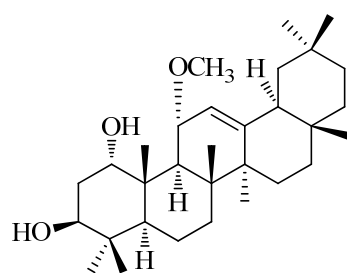
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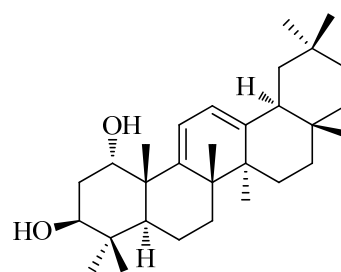
141



142



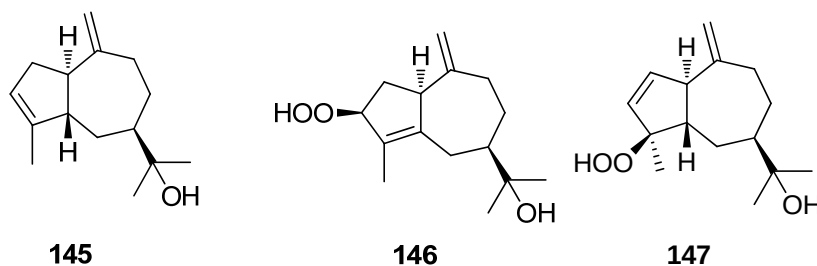
143



144

2.2.4 Sesquiterpines

Fukuyama, et al., in 1996 isolated guaiane-type sesquiterpines; awabukinol [(1 α , 5 β , 7 β)-3, 10 (14)-guaiadien-11-ol] (**145**), 3-hydroperoxyawabukinol [(1 α , 7 β)-3 β -hydroperoxy-4, 10(14)-guaiadien-11-ol] (**146**) and 4-hydroperoxy awabukinol [(1 α , 5 β , 7 β)-4 β -hydroperoxy-2, 10(14)-guaiadien-11-ol] (**147**) from *V. awabuki*.⁴⁴



2.2.5 Flavonoids

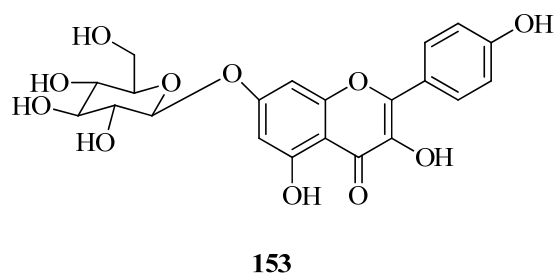
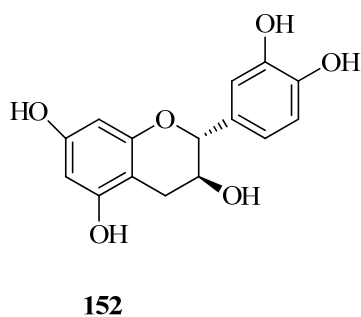
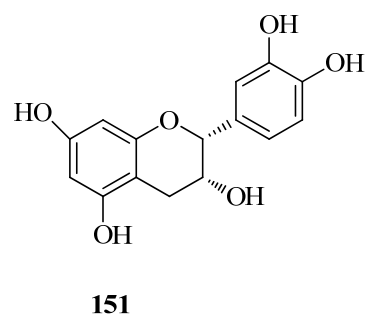
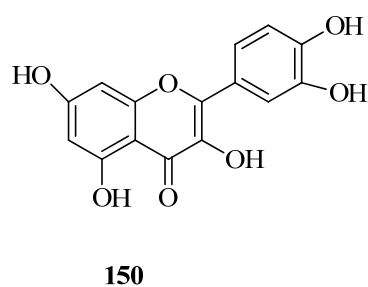
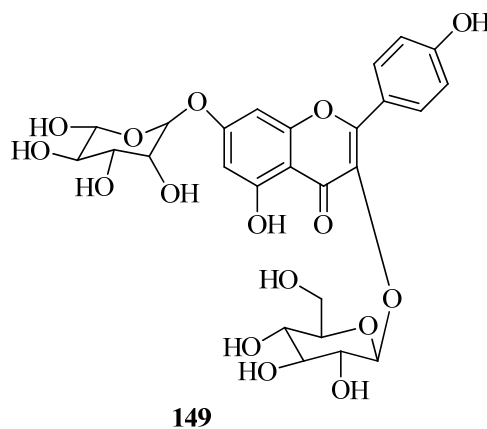
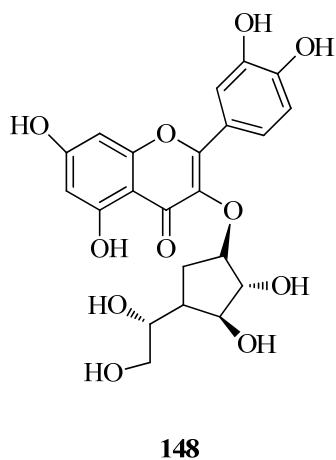
In 1983-1984 Iwagawa, et al., isolated flavonoids; isoquercitroside (**148**), kaempferol 3-*O*- β -D-xylopyranosyl-7-*O*- α -L-rhamnoside (**149**), quercetine (**150**), epicatechin (**151**) and catechin (**152**) and apigenin 7-*O*- β -D-glucoside (**153**) from *V. furcatum*, *V. odoratissimum*, *V. awabuki* and *V. wrightii* respectively.^{33, 45, 46}

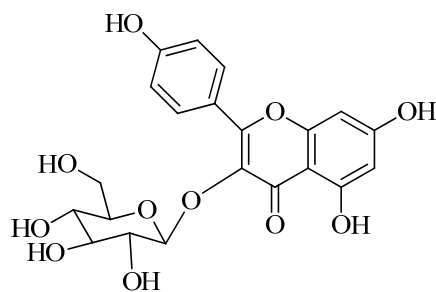
Machida, et al., in 1994 reported flavonoids; astragalin (**154**), kaempferol 3-*O*- β -D-galactopyranoside (**155**) and kaempferol 3-*O*-rutinoside (**156**) from *V. wrightii*.⁴⁷

In 1998 Perveen, et al., isolated flavonoid; luteolin 3-*O*- β -D-xylopyranosyl (1 $\rightarrow$ 2)-*O*- β -D-glucopyranoside (**157**) from *V. grandifolium*.⁴⁸

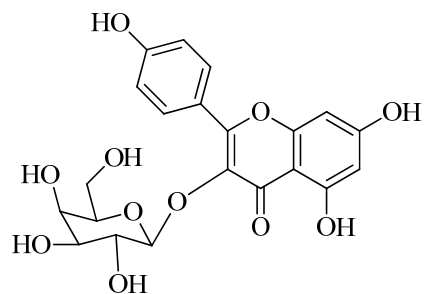
Kim and co-researchers in 2003 characterized flavonoids; cyanidine 3-*O*-sambubioside (**158**) and kuromanin (**159**) from *V. dilatatum*.⁴⁹

In 2005 Mohamed, et al., reported quercetine (**150**), nobiletin (**160**), rutin (**161**) and afzelin (**162**) from *V. tinus*.⁴¹

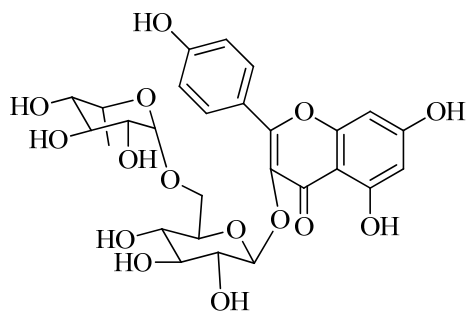




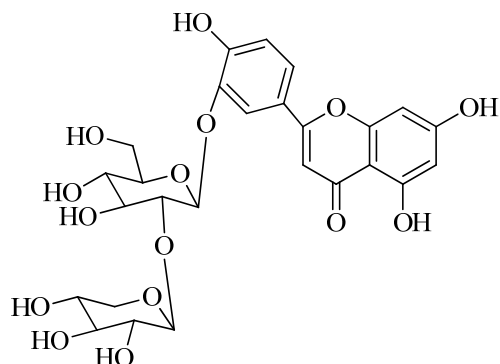
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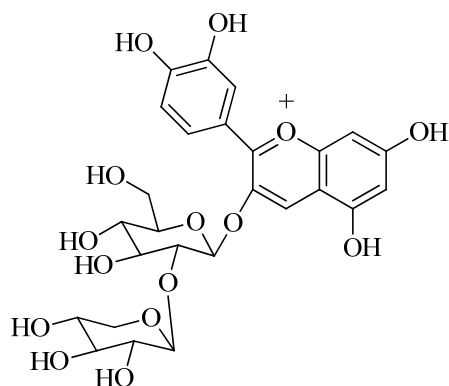
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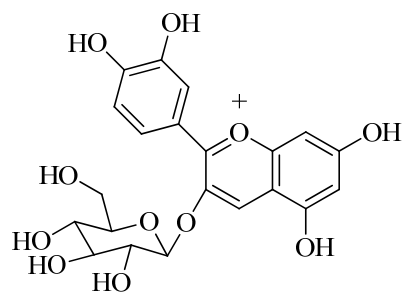
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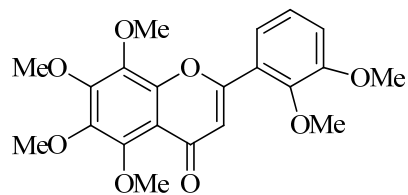
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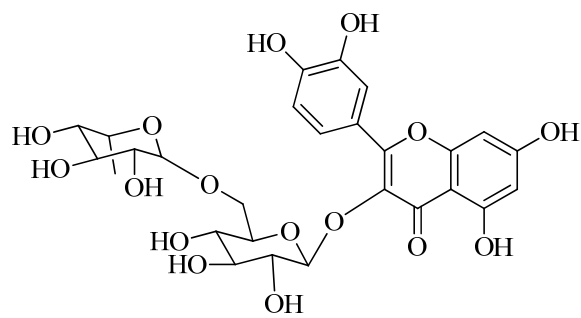
158



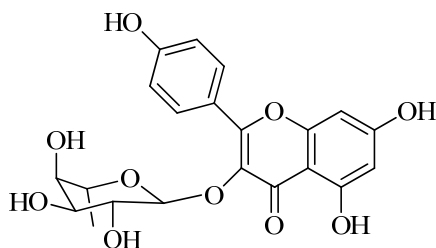
159



160



161



162

2.2.6 Iridoids

Hase, et al., (1983 and 1985) isolated and characterized iridoids glucosides; urceolatoside A (**163**), urceolatoside B (**164**), urceolatoside C (**165**), urceolatoside D (**166**), furcatoside A (**167**), furcatoside B (**168**) and furcatoside C (**169**) from *V. furcatum*.^{50, 51}

In 1985, Jensen research group reported iridoids glucoside; viburnalloside (**170**) and decapetaloside (**171**) from *V. betulifolium*.⁵²

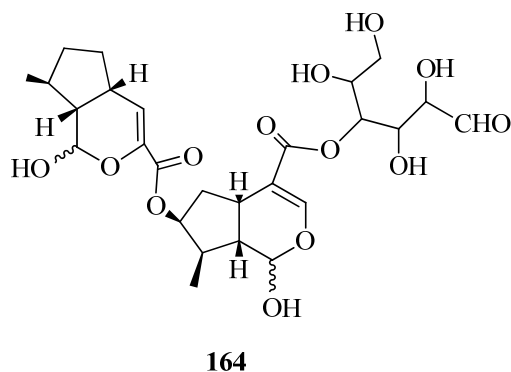
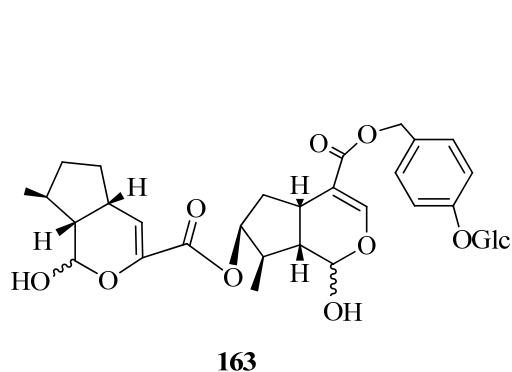
Iwagawa, et al., isolated iridoid glucoside; 2', 3'-*O*-diacetylfurcatoside C (**172**) from *V. japonicum*.⁵³

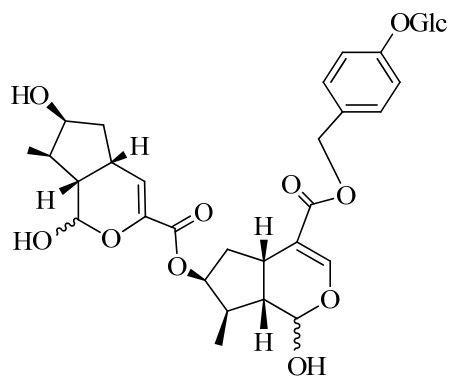
Calis and his co-workers (1995) reported iridoids; lantanoside (**173**) and dihydropenstemide (**174**) along with terpenoids from *V. lantana*.⁹

Tomassini research group (1995, 1997 and 1999) isolated viburtinoside I (**175**), viburtinoside II (**176**), viburtinoside III (**177**), viburtinoside IV (**178**), viburtinoside V (**179**) and suspensolide F (**180**), 2'-acetyldihydropenstemide (**181**), 2'-acetylpatrinoside (**182**), 3'-acetylpatrinoside (**183**), 7,10, 2', 3'-tetraacetylsuspensolide F (**184**), 7, 10, 2', 3'-tetraacetylisosuspensolide F (**185**), 7,10, 2', 6'-tetraacetylisosuspensolide F (**186**), 2', 3'-diacetylvalerosidate (**187**), 2',3'-diacetylisovalerosidate (**188**), isoviburtinoside II (**189**), isoviburtinoside III (**190**), isosuspensolide E (**191**), isosuspensolide F (**192**), 7, 10, 2'-triacetylpatrinoside (**193**), 7-*p*-coumaroylpatrinoside (**194**) and 10-acetylpatrinoside

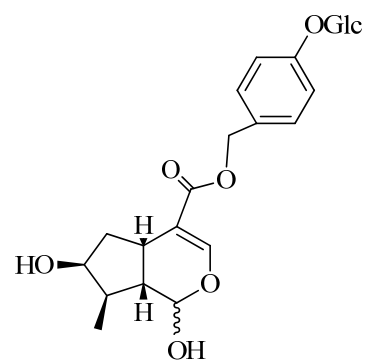
(195), patrinoside (196) and 2'-(*E*)-*p*-coumaroyl dihidropenstemide (197) from *V. tinus*, *V. lantana*, *V. ayavacense*, *V. rhytidophyllum* and *V. prunifolium*.⁵⁴⁻⁵⁸ Eleven iridoids-type compounds, four iridoids glucosides and seven iridoids aglycones were isolated by Fukuyama et al., in 2004 from *V. luzonicum* and characterized as luzonoside A (198), luzonoside B (199), luzonoside C (200), luzonoside D (201), luzonoid A (202), luzonoid B (203), luzonoid C (204), luzonoid D (205), luzonoid E (206), luzonoid F (207) and luzonoid G (208).⁵⁹

In 2005 Mohamed and Fukuyama research groups reported iridoids; viburtinoside A (209), viburtinoside B (210), suspensolide A (211), luzonial A (212), luzonial B (213), luzonidial A (214) and luzonidial B (215) from *V. tinus* and methanolic extract of *V. luzonicum*.^{41, 60}

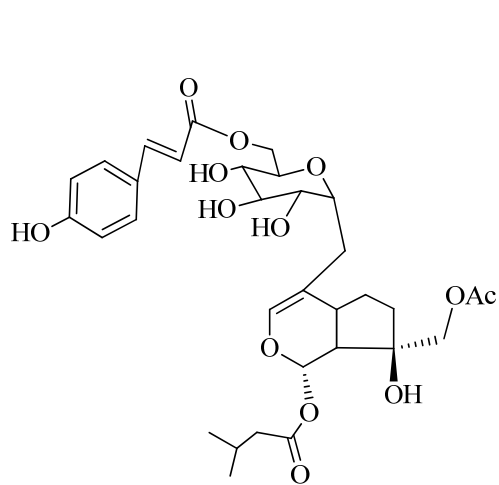




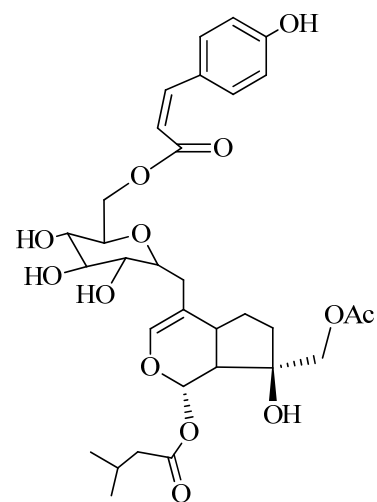
165



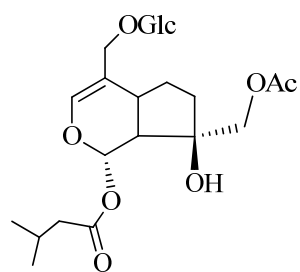
166



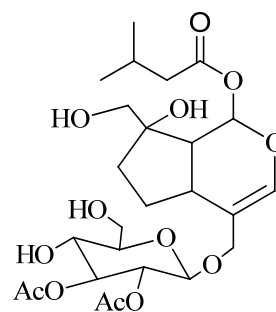
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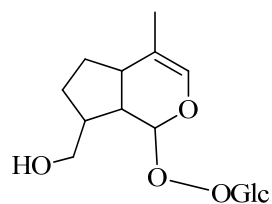
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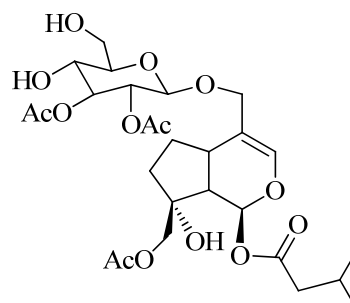
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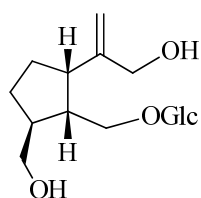
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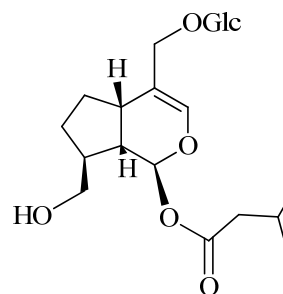
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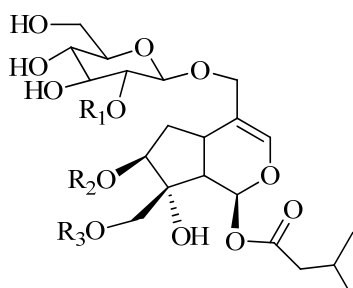
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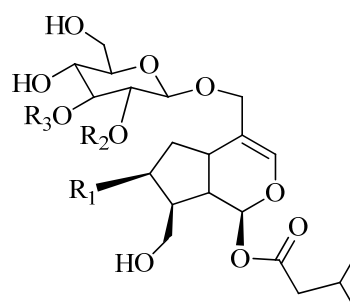
173



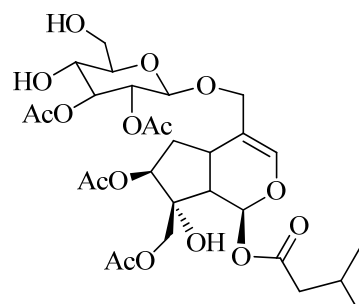
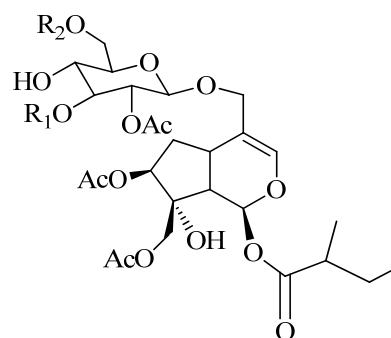
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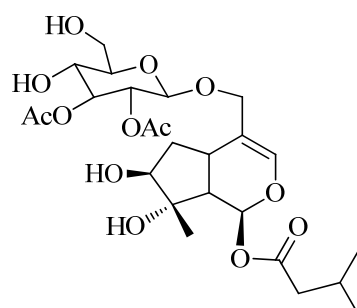
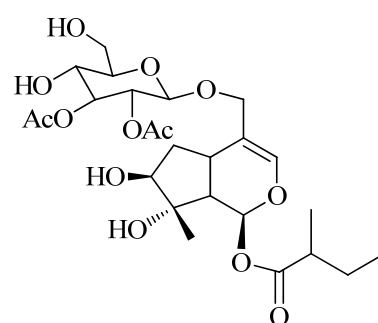
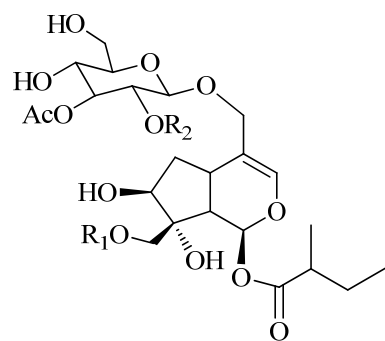
	R ¹	R ²	R ³
175:	Ac	Ac	H
176:	(<i>E</i>)- <i>p</i> -coumaroyl	H	Ac
177:	(<i>Z</i>)- <i>p</i> -coumaroyl	H	Ac
178:	Ac	H	Ac
179:	Ac	Ac	OH
180:	H	H	OH



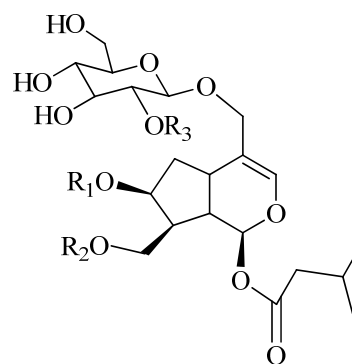
	R ¹	R ²	R ³
181:	H	Ac	H
182:	OH	Ac	H
183:	OH	H	Ac

**184**

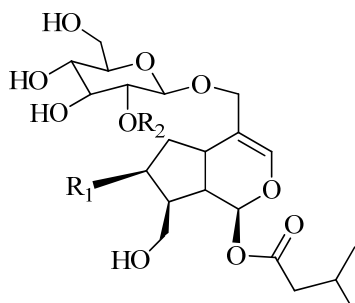
	R ¹	R ²
185:	Ac	H
186:	H	Ac

**187****188**

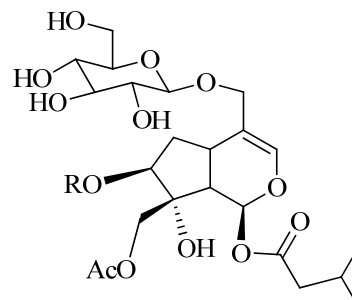
	R ¹	R ²
189:	Ac	(<i>E</i>)- <i>p</i> -coumaroyl
190:	Ac	(<i>Z</i>)- <i>p</i> -coumaroyl
191:	Ac	H
192:	H	H



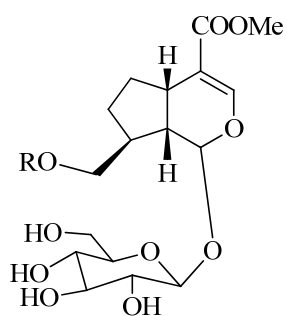
	R ¹	R ²	R ³
193:	Ac	Ac	Ac
194:	<i>p</i> -coumaroyl	H	H
195:	H	Ac	H



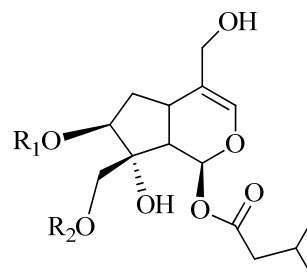
R^1 R^2
196: OH H
197: H *p*-coumaroyl



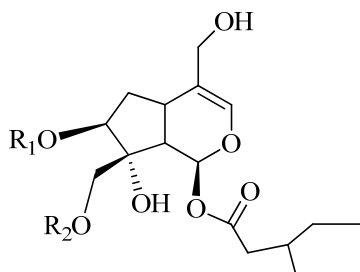
198: $R=(E)$ -*p*-coumaroyl
199: $R=(Z)$ -*p*-coumaroyl



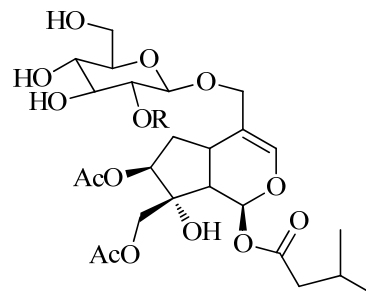
200: (*E*)-*p*-coumaroyl
201: (*Z*)-*p*-coumaroyl



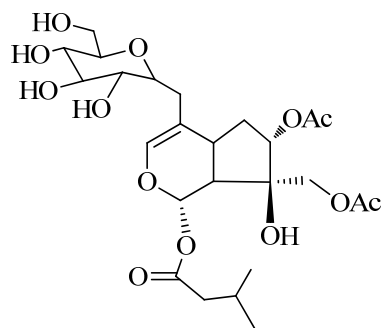
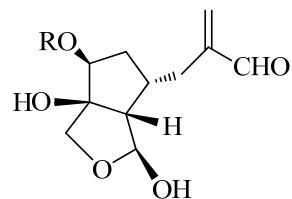
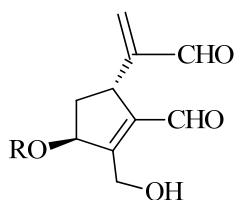
202: $R^1=(E)$ -*p*-coumaroyl, $R^2=H$
203: $R^1=(Z)$ -*p*-coumaroyl, $R^2=H$
204: $R^1=H$, $R^2=(E)$ -*p*-coumaroyl
205: $R^1=H$, $R^2=(Z)$ -*p*-coumaroyl



206: $R^1=(E)$ -*p*-coumaroyl, $R^2=H$
207: $R^1=(Z)$ -*p*-coumaroyl, $R^2=H$
208: $R^1=H$, $R^2=(E)$ -*p*-coumaroyl

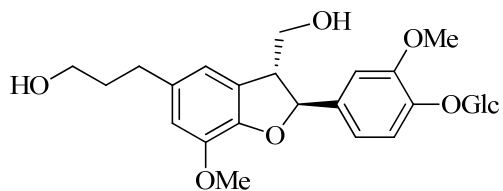
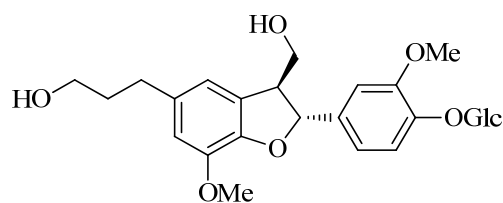


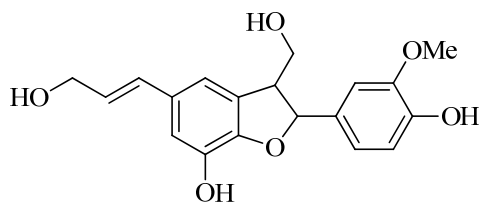
209: $R=(E)$ -*p*-coumaroyl
210: $R=(Z)$ -*p*-coumaroyl

**211****212:**(*E*)-*p*-coumaroyl**213:**(*Z*)-*p*-coumaroyl**214:**R=(*E*)-*p*-coumaroyl**215:**R=(*Z*)-*p*-coumaroyl

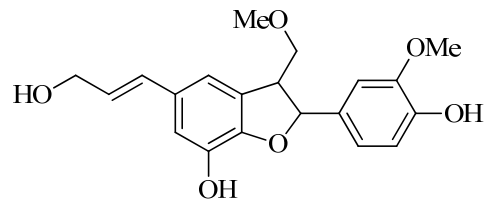
2.2.7 Lignans

Matsuda and Fukuyama research groups in 1996 isolated lignans; 7*R*-dihydro dehydrodiconiferyl alcohol 4-*O*- β -D-glucopyranoside (**216**) and 8*R*-dihydro dehydrodiconiferyl alcohol 4-*O*- β -D-glucopyranoside (**217**), vibsanol (**218**), 9'-*O*-methylvibsanol (**219**) and dihydrodehydrodiconiferyl alcohol (**220**) from *V. awabuki*.^{61, 62}

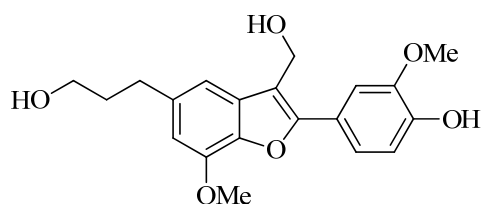
**216****217**



218



219



220

2.2.8 Phenolic compounds

Ohigashi and Koshimizu in 1976, isolated a larva-growth inhibitor compound; chavicol (**221**) from *V. japonicum*.⁶⁴

Hase, et al., in 1982, reported phenolic compounds; *p*-hydroquinone (**222**), arbutin (**223**) and furcatin (**224**) from *V. phlebotrimum* and *V. furcatum*.^{6, 65} while Takido and his co-workers isolated phlebotrigin (**225**) a phenolic compound along with arbutin in 1983 from *V. opulus*.⁶⁵ 5-*O*-Caffeolyquinic acid (**226**) and *p*-hydroxyphenyl β -D-allopyranoside (**227**) isolated from *V. opulus* by Ivanov, et al., in 1983.⁶⁶

In 1984 Iwagawa research group reported phenolic compounds; 6-*O*-acetylarbutin (**228**) and 4-hydroxycinnamic acid (**229**) from *V. wrightii*.⁶⁷

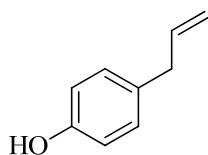
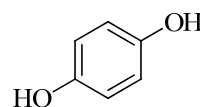
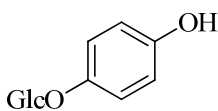
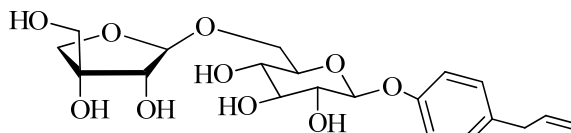
Machida, et al., (1991 and 1992) isolated varieties of compounds; 4-allyl-2-methoxyphenyl 6-*O*- β -D-apiofuranosyl(1 $\rightarrow$ 6)-*O*- β -D-glucopyranoside (**230**), *p*-hydroxyphenyl 6-*O*-(*E*)-caffeoyl- β -D-glucopyranoside (**231**), *p*-hydroxyphenyl 6-*O*-(*E*)-caffeoyl- β -D-allopyranoside (**232**), salidroside (**233**), furcatin (**224**), 2,3,4-trihydroxybutyl 6-*O*-(*E*)-caffeoyl- β -D-glucopyranoside (**234**), 2,3,4,5-tetrahydroxyhexyl

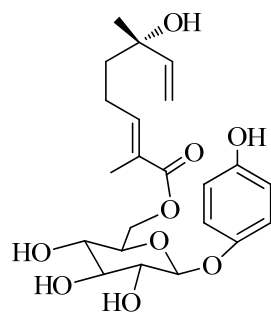
6-*O*-(*E*)-caffeoyl- β -D-glucopyranoside (**235**), 3-*O*-caffeoylquinic acid (**236**) and 4-*O*-caffeoylquinic acid (**237**) from *V. dilatatum*.⁶⁸⁻⁷⁰

Abdallah, et al., in 1995 reported phenolic compounds; henryoside (**238**) and salicin (**239**) from *V. rhytidophyllum*.⁷¹

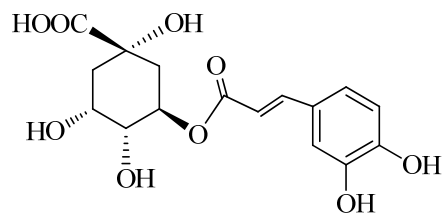
In 2005, Zhu research group reported phenolic constituents; 3-*O*-caffeoylquinic acid methyl ester (**240**), 3-*O*-caffeoylquinic acid methyl ester (**241**) and 5-*O*-caffeoylquinic acid methyl ester (**242**) from the *V. cylindricum*.⁷²

Recently in 2011 Kikuchi and his co-workers isolated compounds; 2-hydroxybenzyl 2-*O*- β -D-glucopyranoside- β -D-glucopyranosyloxy 6-hydroxybenzoate plicatumoside A (**243**) and (+)-neomedioresinol 4, 4'-di- *O*- β -D- glucopyranoside (**244**) from *V. plicatum* while Wu, et al., isolated 1-*O*-[α - L-arabinofuranosyl (1 $\rightarrow$ 6)- β -D- glucopyranoside]-erythro-1,2-bis(4-hydroxy-3-methoxyphenyl) propane-1, 3-diol (**245**) from *V. ichangense*.^{73,74}

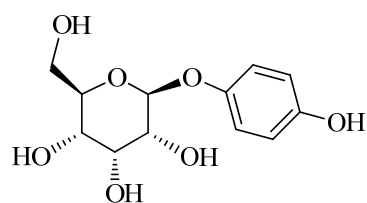
**221****222****223****224**



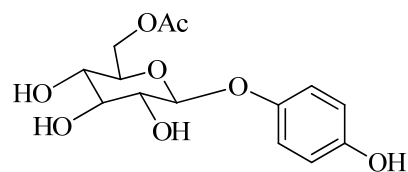
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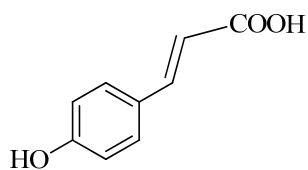
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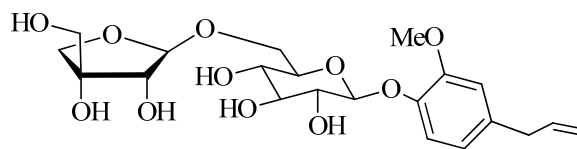
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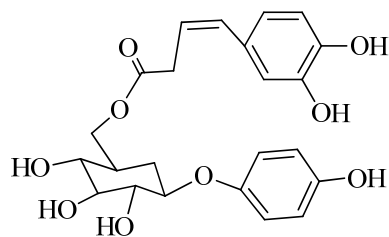
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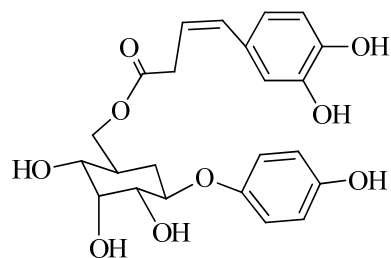
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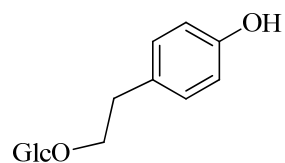
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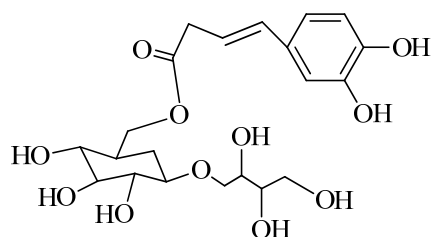
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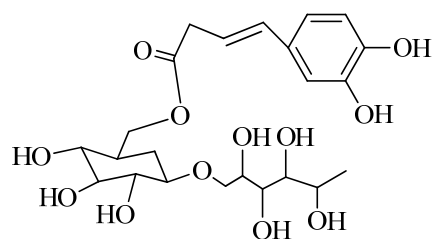
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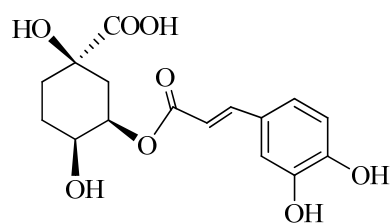
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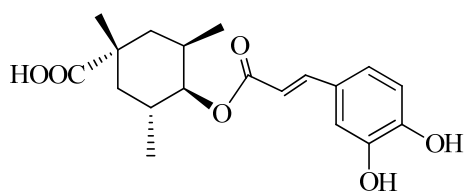
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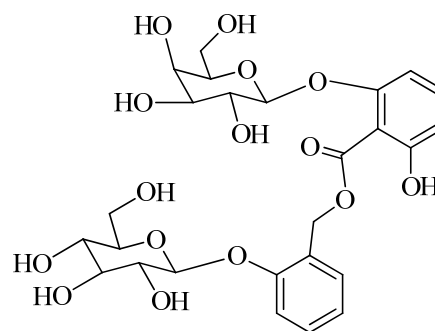
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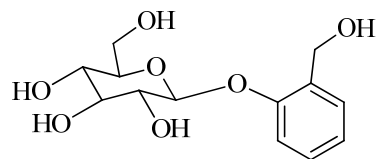
(236)



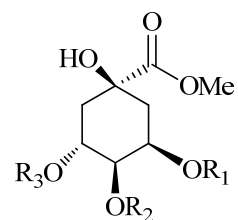
237



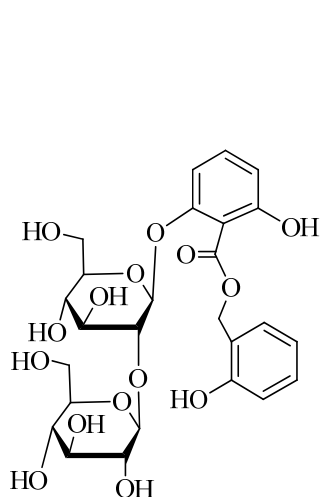
238



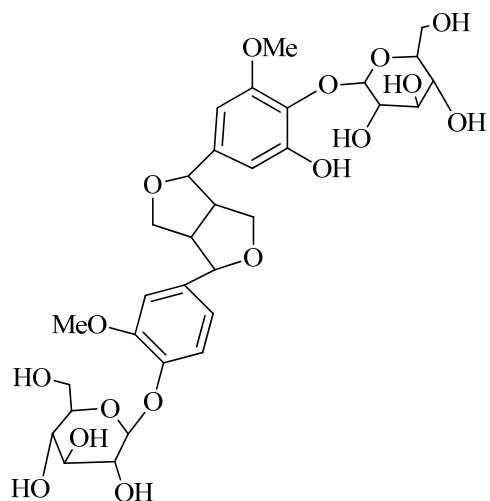
239



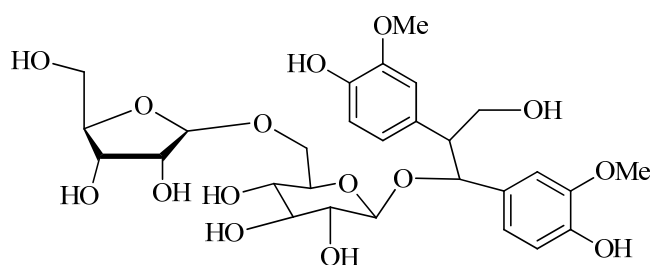
	R ¹	R ²	R ³
240:	H	H	Caffeoyl
241:	H	Caffeoyl	H
242:	Caffeoyl	H	H



243



244



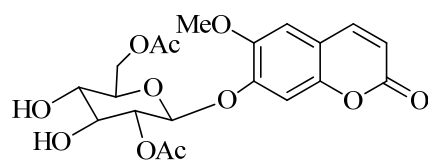
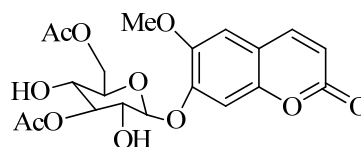
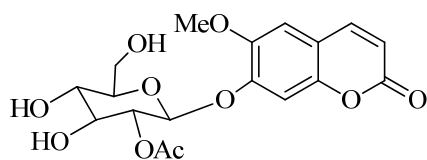
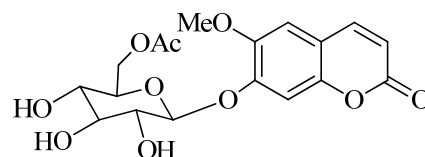
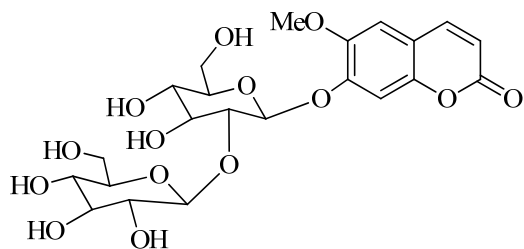
245

2.2.9 Coumarins

Iwagawa, et al., in 1984 isolated acetylated coumarin glucoside from the leaves of *V. suspensum* and characterized their structure as 2', 6'-*O*-diacetylscopolin (**246**).⁷⁵

In 1986, Kuroyanagi, et al., reported coumarins-type compounds; 3' 6'-*O*-diacetylscopolin (**247**), 2'-*O*-acetylscopolin (**248**) and 6'-*O*-acetylscopolin (**249**) from *V. awabuki*.³⁴

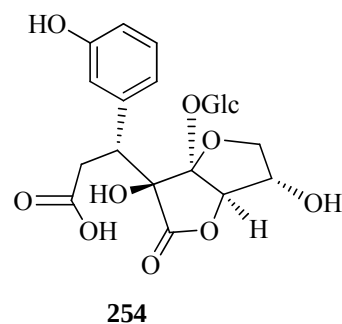
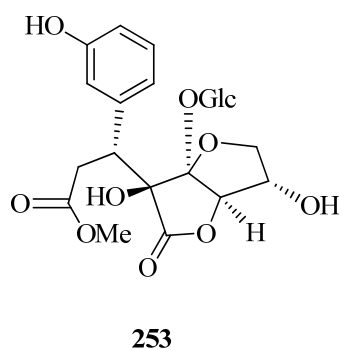
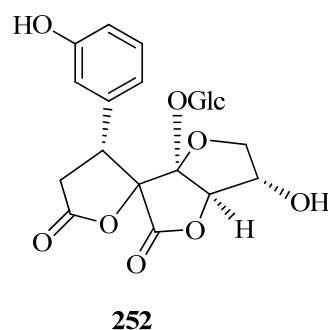
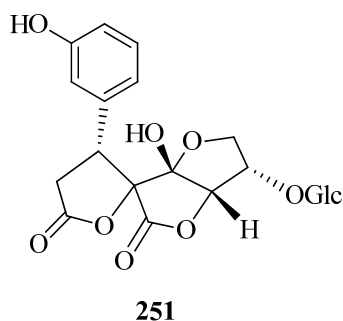
Mohamed, et al., in 2005 reported 7-*O*- β -D-sophoroside (**250**) a coumarin diglucoside scopoletin from *V. tinus* along with iridoids compounds.⁴¹

**246****247****248****249****250**

2.2.10 Lactones

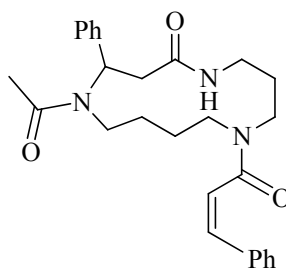
Iwagawa and Hase reported (1984) lactone-type; compound dilaspirolactone (**251**) from *V. dilatatum*.⁷⁵

In 1994, Machida, et al., isolated viburnulides A (**252**), viburnulides B (**253**) and viburnulides C (**254**) from *V. wrightii*.⁷⁶

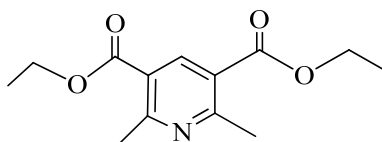


2.2.11 Alkaloids

Two alkaloids have been reported up till now from the *Viburnum* genus. Abdallah, et al., in 1995 isolated viburnine (255) from *V. rhytidophyllum* while 2, 6-Di-C-methyl-nicotinic 3, 5-diethyl ester (256) alkaloid was reported by Mohamed, et al., in 2005 from the leaves of *V. tinus*.^{41, 71}



255



256

Table: 2.1 List of chemical constituents isolated from *Viburnum* genus

S. No.	Name of the Compounds	References
Monoterpenes		
1	Phlebotricoside	5
2	Urceolid	6
3	Anatolioside	7
4	Anatolioside A	7
5	Anatolioside B	7
6	Anatolioside C	7
7	Anatolioside D	7
8	Anatolioside E	8
9	Betulalbuside B	8
10	Betulalbuside A	9
Diterpenes		
11	Vibsanin A	10
12	Vibsanin B	10
13	Vibsanin C	10
14	Vibsanin D	10
15	Vibsanin E	10
16	Vibsanin F	10
17	Gomojosid A	11
18	Gomojosid B	11
19	Gomojosid C	11
20	Gomojosid D	11
21	Gomojosid E	11
22	Gomojosid F	11
23	Gomojosid G	11
24	Gomojosid H	11
25	Gomojosid I	11
26	Gomojosid J	11

27	Gomojosid K	12
28	Gomojosid L	12
29	Gomojosid M	12
30	Gomojosid N	12
31	Gomojosid O	12
32	Gomojosid P	12
33	Gomojosid Q	12
34	Neovibsanin A	13
35	Neovibsanin B	13
36	Neovibsanin H	14
37	Neovibsanin I	14
38	3-Hydroxyvisanin E	15
39	Furanovisanin A	16
40	Furanovisanin B	16
41	Furanovisanin C	16
42	Furanovisanin D	16
43	Furanovisanin E	16
44	Furanovisanin F	16
45	Furanovisanin G	16
46	3- <i>O</i> -Methylfuranovibsanin A	16
47	7-Epifuranovibsanin B	16
48	5-Epivibsanin C	17
49	5-Epivibsanin E	17
50	5-Epivibsanin H	17
51	5-Epivibsanin K	17
52	18- <i>O</i> -Methyl-5-EpivibsaninK	17
53	Cyclovibsanin A	18
54	15- <i>O</i> -Methylcyclovibsanin A	18
55	15- <i>O</i> -Methylcyclovibsanin B	18
56	3-Hydroxy-15- <i>O</i> -methylcyclovibsanin A	18

57	Neovibsanin D	19
58	Neovibsanin G	19
59	7-Epineovibsanin D	19
60	15- <i>O</i> -Methylneovibsanin F	19
61	15- <i>O</i> -Methyl-14-epineovibsanin F	19
62	15- <i>O</i> -Methyl-18-oxoneovibsanin F	19
63	2- <i>O</i> -Methylneovibsanin H	19
64	2- <i>O</i> -Methylneovibsanin I	19
65	14-Epineovibsanin G	19
66	Neovibsanin F	20
67	Vibsanin K	21
68	15, 18-Di- <i>O</i> -methylvibsanin H	21
69	18- <i>O</i> -Methylvibsanin K	21
70	Aldovisanin A	22
71	Aldovisanin B	22
72	7-Epialdovibsanin A	22
73	Neovibsanin C	23
74	Spirovibsanin A	24
75	Vibsanin I	25
76	Vibsanin L	25
77	14-Hydroxyvibsanin F	25
78	(14 <i>R</i> *)-14, 15-Epoxyvibsanin C	25
79	(14 <i>S</i> *)-14, 15-Epoxyvibsanin C	25
80	Neovibsanin O	26
81	Neovibsanin L	26
82	Neovibsanin M	26
83	(8 <i>Z</i>)- Neovibsanin M	26
84	15- <i>O</i> -Methylvibsanin H	26
85	5-Epi-15- <i>O</i> -methylvibsanin H	26
86	Vibsanol A	27

87	Vibsanol B	27
88	Vibsanin M	28
89	Aldovibsanin C	28
90	5-Epivibsanin G	28
91	18-O-Methylvibsanin G	28
92	Vibsanin G	29
93	Vibsanin H	29
94	Vibsanin, O	29
95	Vibsanin, P	30
96	Vibsanin Q	30
97	Vibsanin R	30
98	Vibsanin S	30
99	Vibsanin T	30
100	Vibsanin U	30
101	Vibsanin V	30
102	Vibsanin W	30
103	15-O- Methyl-vibsanol A	31
Triterpenes		
104	α -Amyrine palmitate	32
105	Lupeol palmitate	32
106	β -Amyrine acetate	32
107	Ursolic acid	32
108	Y-Taraxasterol acetate	33
109	6 α -Hydroxy-3-oxolup-20(29)-en-28-oic acid	34
110	Viburnol A	35
111	Viburnol B	35
112	Viburnol C	35
113	Viburnol D	35
114	Viburnol E	35
115	Viburnol F	36

116	Viburnol G	36
117	Viburnol I	36
118	Viburnol J	36
119	Viburnol K	36
120	Viburnol M	36
121	Viburnudienone B1 methyl ester	38
122	Viburnudienone B2 methyl ester	37
123	Viburnenone B1 methyl ester	37
124	Viburnenone B2 methyl ester	37
125	Viburnudienone H1	37
126	Viburnudienone H2	37
127	3 β , 28-Dihydroxyolean-12-en-1-one	38
128	3 β , 28-Dihydroxyolean-12-en-11-one	38
129	13, 28-Epoxyolean-en-3-one	38
130	3-Oxooleana-11,13(18)-dien-28-oic acid	39
131	24-Hydroxy-3-oxooleana-11,13(18)-dien-28-oic acid	39
132	6 β -Hydroxy-3-oxooleana-11,13(18)-dien-28-oic acid	39
133	6 β -Hydroxy-3-oxolup-20(29)-ene-27, 28-dioic acid	23
134	6 α -Hydroxy-3-oxolup-20(29)-ene-27, 28-dioic acid	23
135	Viburolide	40
136	3- <i>O</i> - β -D-Galactopyranosyl-(1 $\rightarrow$ 2)- <i>O</i> - β -D-glucurono pyranosideoleanolic acid 28- <i>O</i> - β -D-glucopyranosyl ester	41
137	3- <i>O</i> -(β -D-glucuronopyranosyl) oleanolic acid	41
138	Oleanolic acid	41
139	6 β -Hydroxy-3, 20-dioxo-30-norlupan-28-oic	42

	acid	
140	4, 20-Dihydroxy-3, 4-secolupane 3.28-dioic acid3 methyl ester	42
141	Ursa-12-sene-3 β -11 β -diol, 3- <i>O</i> -palmitate	43
142	Ursa-12-sene-1 β -3 β -11 α -triol, 3- <i>O</i> -palmitate	43
143	1 α -3 β -Dihydroxy-11 α -methoxy-olean-12-ene	31
144	1 α -3 β -Dihydroxy-11 α -methoxy-olean-9(11), 12-diene	31
Sesquiterpenes		
145	Awabukinol [(1 α , 5 β , 7 β)-3, 10(14)-guaiadien-11-ol]	44
146	3-Hydroperoxyawabukinol [(1 α , 7 β)-3 β -hydroperoxy-4,10(14)-guaiadien-11-ol]	44
147	4-Hydroperoxyawabukinol [(1 α , 5 β , 7 β)-4 β -hydroperoxy-2, 10(14)-guaiadien-11-ol]	44
Flavonoids		
148	Isoquercitroside	45
149	Kaemferol 3- <i>O</i> - β -D-xylopyranosyl-7- <i>O</i> - α -l-amnoside	45
150	Epicatechin	33
151	Catechin	33
152	Apigenin 7- <i>O</i> - β -D-glucoside	46
153	Astragalin	47
154	Kaempferol 3- <i>O</i> - β -D-galactopyranoside	47
155	Kaempferol 3- <i>O</i> -rutinoside	47
156	Luteolin 3- <i>O</i> - β -D-xylopyranosyl (1 $\rightarrow$ 2)- <i>O</i> - β -D-glucopyranoside	48
157	Cyanidine 3- <i>O</i> -sambubioside	49
158	Kuromanin	49
159	Quercetine	41

160	Nobiletin	41
161	Rutin	41
162	Afzelin	41
Iridoids		
163	Urceolatoside A	50
164	Urceolatoside B	50
165	Urceolatoside C	50
166	Urceolatoside D	50
167	Furcatoside A	51
168	Furcatoside B	51
169	Furcatoside C	51
170	Viburnalloside	52
171	Decapetaloside	52
172	2', 3'-O-Diacetylfurcatoside C	53
173	Lantanoside	9
174	Dihydropenstemide	9
175	Viburtinoside I	54
176	Viburtinoside II	54
177	Viburtinoside III	54
178	Viburtinoside IV	54
179	Viburtinoside V	54
180	Suspensolide F	54
181	2'-Acetyldihydropenstemide	55
182	2'-Acetylpatrinoside	55
183	3'-Acetylpatrinoside	55
184	7, 10, 2', 3'-Tetraacetylsuspensolide F	56
185	7, 10, 2', 3'-Tetraacetylisosuspensolide F	56
186	7, 10, 2', 6'-Tetraacetylisosuspensolide F	56
187	2', 3'-Diacetylvalerosidate	56
188	2', 3'-Diacetylisovalerosidate	56

189	Isoviburtinoside II	56
190	Isoviburtinoside III	56
191	Isosuspensolide E	56
192	Isosuspensolide F	56
193	7, 10, 2'-Triacetylpatrinoside	57
194	7- <i>p</i> -Coumaroylpatrinoside	57
195	10- Acetylpatrinoside	57
196	Patrinoside	58
197	2'-(<i>E</i>)- <i>p</i> -Coumaroyldihidropenstemide	58
198	Luzonoside A	59
199	Luzonoside B	59
200	Luzonoside C	59
201	Luzonoside D	59
202	Luzonoid A	59
203	Luzonoid B	59
204	Luzonoid C	59
205	Luzonoid D	59
206	Luzonoid E	59
207	Luzonoid F	59
208	Luzonoid G	59
209	Viburtinoside A	41
210	Viburtinoside B	41
211	Suspensolide A	41
212	Luzonidial A	60
213	Luzonidial B	60
214	Luzonidial A	60
215	Luzonidial B	60
Lignans		
216	7 <i>R</i> -Dihydrodehydrodiconiferyl alcohol 4- <i>O</i> - β -D-	61

	glucopyranoside	
217	8 <i>R</i> -Dihydrodehydrodiconiferyl alcohol 4- <i>O</i> - β -D-glucopyranoside	61
218	Vibsanol	62
219	9'- <i>O</i> -Methylvibsanol	62
220	Dihydrodehydrodiconiferyl alcohol	62
Phenols		
221	Chavicol	63
222	<i>P</i> -Hydroquinone	5
223	Arbutin	5
224	Furcatin	64
225	Phlebotrichin	65
226	5- <i>O</i> -Caffeolyquinic acid	66
227	<i>p</i> - Hydroxyphenyl β -D- allopyranoside	66
228	6- <i>O</i> -Acetylarbutin	67
229	<i>p</i> - Hydroxycinnamic acid	67
230	4-Allyl-2-methoxyphenyl 6- <i>O</i> - β -D-apiofuranosyl (1 $\rightarrow$ 6)- <i>O</i> - β -D-glucopyranoside	68
231	<i>P</i> -Hydroxyphenyl 6- <i>O</i> -(<i>E</i>)-caffeoyl- β -D-glucopyranoside	68
232	<i>P</i> -Hydroxyphenyl 6- <i>O</i> -(<i>E</i>)-caffeoyl- β -D-allopyranoside	68
233	Salidroside	68
234	2, 3, 4-Trihydroxybutyl 6- <i>O</i> -(<i>E</i>)-caffeoyl- β -D-glucopyranoside	69
235	2, 3, 4, 5-Tetrahydroxyhexyl 6- <i>O</i> -(<i>E</i>)-caffeoyl- β -D-glucopyranoside	69
236	3- <i>O</i> -Caffeoylquinic acid	70
237	4- <i>O</i> -Caffeoylquinic acid	70
238	Henryoside	71

239	Salicin	71
240	3- <i>O</i> -Caffeoylquinic acid methyl ester	72
241	3- <i>O</i> -Caffeoylquinic acid methyl ester	72
242	5- <i>O</i> -Caffeoylquinic acid methyl ester	72
243	Plicatumoside A	73
244	(+)-Neomedioresinol 4, 4'-Di- <i>O</i> - β -D-glucopyranoside	73
245	1- <i>O</i> -[α -L-Arabinofuranosyl (1 $\rightarrow$ 6)- β -D-glucopyranoside]-Erythro-1, 2-bis (4-hydroxy-3 methoxyphenyl) propane-1,3-diol	74
Coumarins		
246	2', 6'- <i>O</i> -Diacetylscopolin	75
247	3', 6'- <i>O</i> -Diacetylscopolin	34
248	2'- <i>O</i> -Acetylscopolin	34
249	6'- <i>O</i> -Acetylscopolin	34
250	Scopoletin 7- <i>O</i> - β -D-sophoroside	41
Lactones		
251	Dilaspirolactone	75
252	Viburnulides A	76
253	Viburnulides B	76
254	Viburnulides C	76
Alkaloids		
255	Viburnine	71
256	2, 6-Dimethyl- nicotinic 3, 5-diethyl ester	41

2.3.0 Pharmacological importance of Genus *Viburnum*

2.3.1 Hepatoprotective activity:

In 2005 Mohamed, et al., determined the hepatoprotective activity of the extract of *V. tinus* which showed significant activity at high dose ($LD_{50} = 500$ mg/kg) indicates improvement of serum ALT (alanine aminotransferase) and AST (aspartate aminotransferase).⁴²

2.3.2 Antihyperglycemic activity:

The freeze-dried powder of *V. dilatatum* fruit juice (CEV) was orally administered streptozotocin-induced diabetic rats which showed significantly decrease plasma glucose level in rats. The experimental results of *V. dilatatum* fruits extract also showed α -glucosidase inhibitory activity and the antihyperglycemic action. In 2006, Iwai, et al., reported five chemical constituents from the fruits of *V. dilatatum*. Among these isolated compounds cyanidin 3-sambubioside (C3S) and 5-caffeoyl quince acid (5-CQA) showed significant antihyperglycemic activity. C3S and 5-CQA showed inhibitory activity on sucrase and maltase.⁷⁷

2.3.3 Antimicrobial activity:

Chavicol (**238**) was isolated from the crude methanolic extract of *V. japonicum* by Ohigashi and Koshimizu in 1976, showed activity against *Drosophila larvae* and adults.⁷¹

2.3.4 Antioxidant activity:

Cyanidin 3 sambubioside (**150**) and kuromanin (**151**) isolated from the fruits of *V. dilatatum* in 1984 showed antioxidant activity.⁴⁶ Subsequently Laux, et al., in 2008 tested water extracts of different parts of *V. opulus* and *V. lantana* for their antioxidant properties which exhibited strong antioxidant activity.⁷⁸

In 2008, Wu, et al., evaluated the isolated compounds from *V. fordiae* for their anti-oxidant properties which showed significant antioxidant activity.⁷⁹

2.3.5 Antibacterial activity:

The *Viburnum* species having a potent antibacterial activity. The methanolic extract of *V. foetens* was tested for their antibacterial activity which showed mild antibacterial activity against *Bacillus subtilis*, *Micrococcus leuteus*, *Salmonella setubal*, *Salmonella aureus* and *Pseudomonas picketi* bacterial strains.⁸⁰

Iwagawa, et al.; tested ten labdane-type diglucosides; gomojosides A-J isolated from *V. suspensum* for their antibacterial potential. Among these five gomojosides showed significant antibacterial activity.²⁴

2.3.6 Antinociceptive activity:

Viburnum lantana leaves water extract was investigated for antinociceptive activity in mice and rats, which showed inhibition as similar to aspirin at concentration of 100 mg/kg but the antinociceptive activity of the extract was found stronger than aspirin at 200 mg/kg dose.⁷⁸

2.3.7 Anti-inflammatory activity:

The methanol, ethyl acetate, and chloroform extracts of *V. toronis* were investigated for their analgesic properties against mice by Calle, et al.; in 1999. The results showed that all the tested extracts decreased the writhings induced by 1% of acetic acid. The ethyl acetate extract showed strong analgesic properties than chloroform and methanol extracts.⁸¹

The leaves extract of *V. lantana* evaluated for their anti-inflammatory properties by Yilmaz, et al., showed mild anti-inflammatory activity compared with indomethacin standard drug. The LD₅₀ of *V. lantana* was found to be 2.69 g/kg.⁸²

The water extract of *V. opulus* was tested for their anti-inflammatory properties against mice and rats which showed no significant results.⁷⁸

2.3.8 Uterine relaxant activity:

The crude extracts, fractions and pure compounds isolated from *V. tinus* by Cometa, et al., showed strong antispasmodic activities against mice and subsequently the same group of researcher tested *V. prunifolium* methanolic extract for their spasmolytic properties which also showed positive results.^{82, 83}

The extracts from *V. toronis* were tested for uterine relaxant properties by Calle, et al., which exhibited relaxation of the normal uterine muscles.⁸⁴

2.3.9 Sedative activity:

Cometa, et al., reported the sedative properties of *V. tinus*. The crude extract, fractions and purified constituents of *V. tinus* were investigated for their sedative activity on mice and on isolated rabbits. All the tested samples showed significant sedative properties.⁸²

2.3.10 Cytotoxic activity:

El-Gamal, A. A., reported cytotoxic activity of the chemical constituents isolated from *V. awabuki* which exhibited significant cytotoxic activity.⁴¹

Bibi, et al., evaluated the crude extracts and their subsequent fractions of *V. foetens* for cytotoxic activity which showed highest cytotoxic activity.⁸⁰

Chen, et al., isolated the diterpenes and tri-terpenes from *V. chingii* which showed significant cytotoxic activity.³¹

Triterpenoids (**1-8**) isolated from *V. mongolicum* were evaluated by Wang, et al., for their cytotoxic activity. All the compounds were tested in vitro against seven cell lines. Compounds (**2-6**) were showed significant cytotoxic activity against all tested cell lines.⁸⁵

2.3.11 Purgative activity:

Viburnum genus some species are used for cleansing purposes and also its edible fruits are used as purgative and sedative. These properties of the *Viburnum* species (*V. foetens*) reported by Qureshi, et al.; in 2009. They investigated and collected data about the indigenous medicinal plants of Himalayan region of Pakistan.⁸⁶

2.4.0 References

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3.1.0 Present work

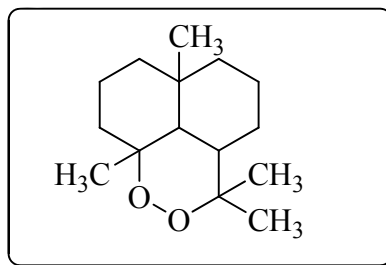
The present studies were focused on phytochemical constituents and their structure elucidation. As a result, six new and sixteen hitherto unreported compounds have been isolated from different parts of *Viburnum grandiflorum* and their structures have been elucidated through advanced spectroscopic techniques i.e. IR, EI-MS, HREI-MS, ESI-MS, ^1H and ^{13}C NMR (BB and DEPT), 2D NMR techniques (HMBC, HMQC, NOESY, COSY and *J*-Resolved). Some of them are biologically active constituents, which may help in primary health care.

The aerial parts and roots of *V. grandiflorum* were separately extracted with ethanol and the crude extract of both parts fractionated into *n*-hexane, chloroform, EtOAc and *n*-butanol fractions. Each fraction was tested for their biological activities on various assays and biologically active fractions were subjected to various column chromatographic techniques (*vide experimental*), resulted purification and characterization of twenty two chemical constituents. Among these chemical constituents six were found new; three from the aerial parts and three from the roots. Sixteen compounds (ten from the aerial part and six from the roots) were identified as hitherto unreported. Thirteen compounds were isolated from aerial parts of *V. grandiflorum* including three new i.e. grandino, 5, 8. peroxide (**259**), grandinoid (**263**) and grandifloruside (**266**) while nine compounds were isolated from roots containing three new i.e. viburnoside (**268**), grandiflorinol (**274**) and grandifloronin (**275**). Structures of these compounds were also determined by advance ID and 2D NMR and comparing their spectral data with related compounds in literature. The literature survey revealed that *V. grandiflorum* has medicinal importance and need to discover biologically active secondary metabolites which may help in primary health care system.

3.1.1 New constituents from *V. grandiflorum* aerial parts

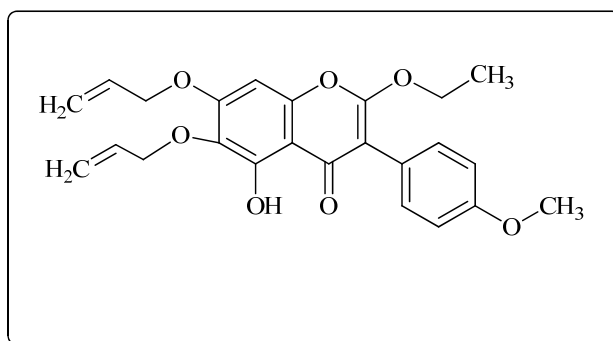
- ❖ Grandino, 5, 8. peroxide (**259**)

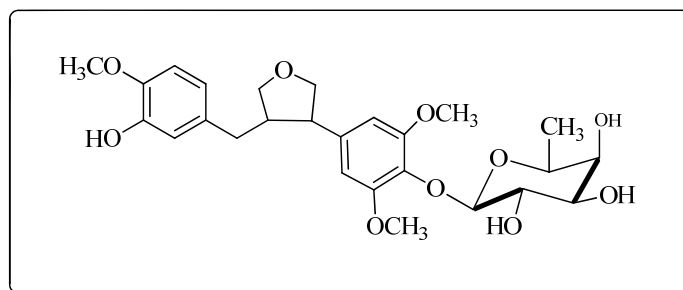
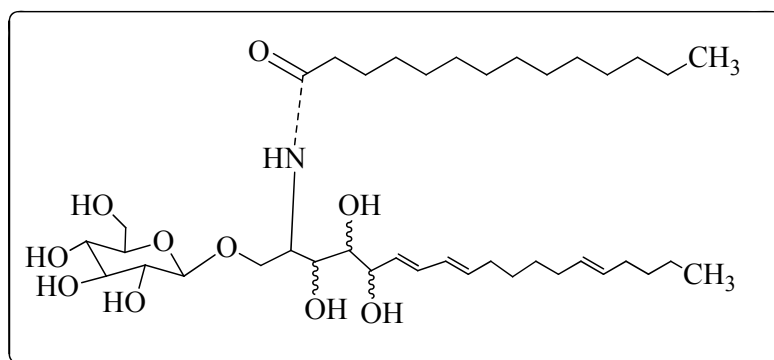
Molecular formula: C₁₅H₂₂O₂

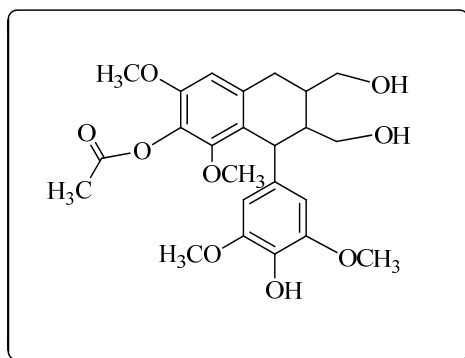
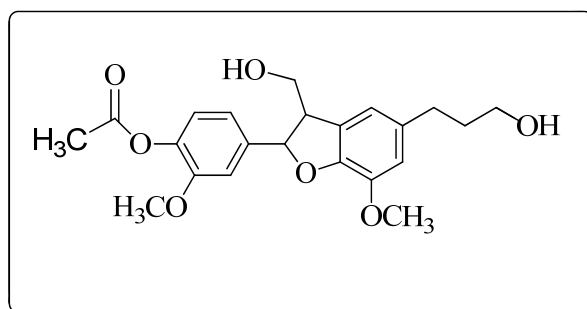


- ❖ Grandinoid (**263**)

Molecular formula: C₁₉H₁₆O₇



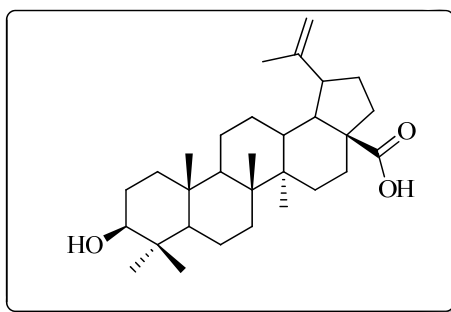
❖ Grandifloruside (**266**)Molecular formula: $C_{26}H_{36}O_{11}$ 3.1.2 New compounds from *V. grandiflorum* roots❖ Viburnoside (**268**)Molecular formula: $C_{39}H_{71}O_{10}N$ 

❖ Grandiflorinol (**274**)Molecular formula: $C_{24}H_{30}O_9$ ❖ Grandifloronin (**275**)Molecular formula: $C_{22}H_{26}O_7$ 

3.1.3 Hitherto unreported constituents from aerial parts of *V. grandiflorum*.❖ Betulinic acid (**257**)

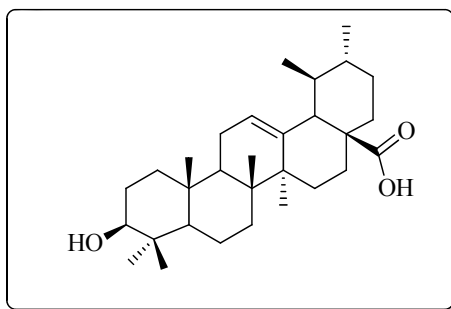
3 β -Hydroxy lup-20(29)-ene-28-oic acid

Molecular formula: C₃₀H₄₈O₃

❖ Ursolic acid (**107**)

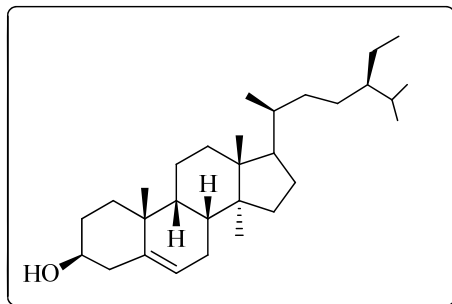
3 β -Hydroxy lup-11(13)-ene-28-oic acid

Molecular formula: C₃₀H₄₈O₃

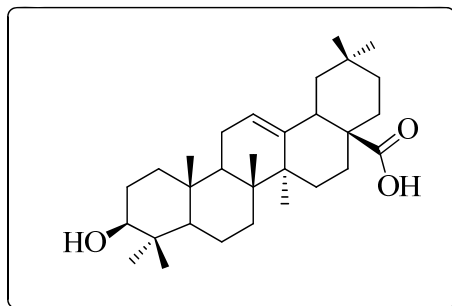


❖ β - Sitosterol (**258**)

Molecular formula: C₂₉ H₅₀O

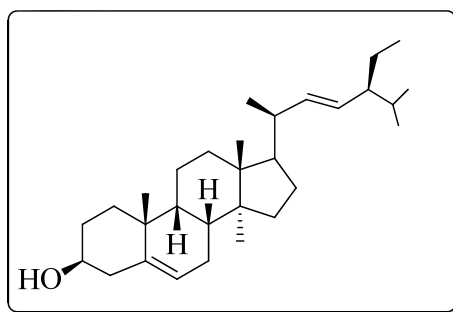
❖ Oleanolic acid (**138**)

Molecular formula: C₃₀H₅₀O₃

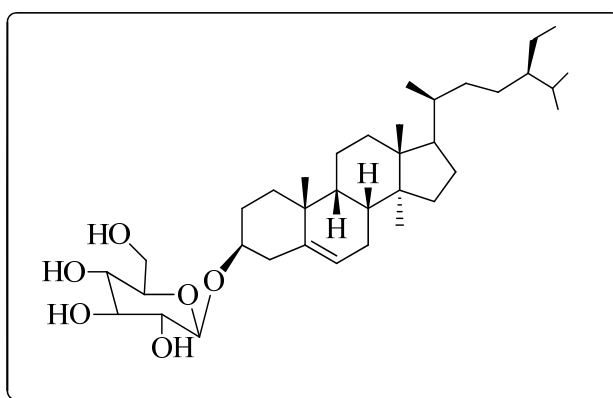


❖ Stigmasterol (260)

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

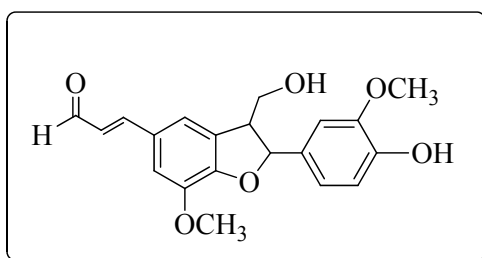
❖ β -Sitosterol 3-O- β -D-glucopyranoside (Glucoside of β -sitosterol) (261)

Molecular formula: $C_{35}H_{60}O_6$



- ❖ 2, 3-Dihydro-2-(4'-hydroxy, 3'-methoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl) 7-hydroxy benzofuran (**262**)

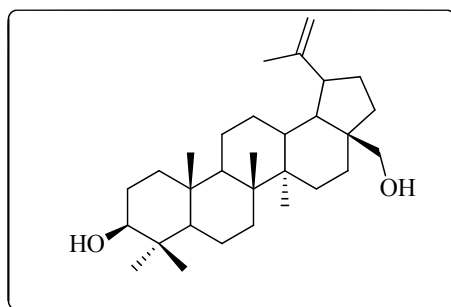
Molecular formula: C₂₀H₂₀O₆

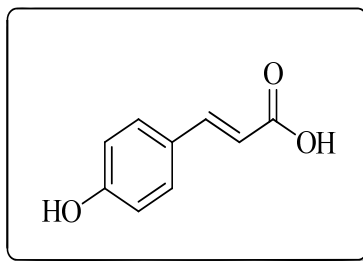
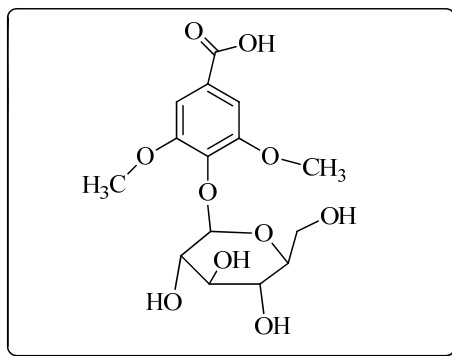


- ❖ Betulin (**264**)

Lup-20(29)-ene-3 β , 28-diol

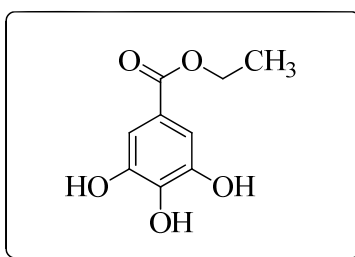
Molecular formula: C₃₀H₅₀O₂



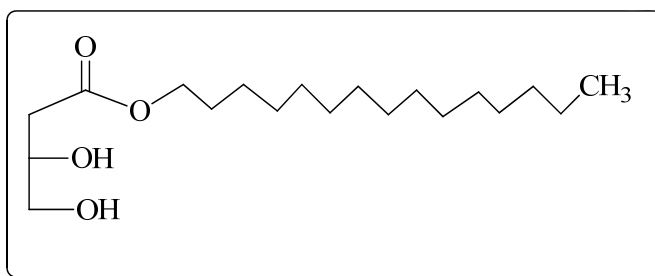
❖ *p*-Hydroxy cinnamic acid (**229**)Molecular formula: C₉H₈O₃❖ 3, 4, 5-Trihydroxy benzoic acid; 3,5 di-Me ether 4-*O*-β-D-glucopyranoside (**265**)Molecular formula: C₁₅H₂₀O₁₀

3.1.4 Hitherto unreported constituents from *V. grandiflorum* roots

- ❖ Ethyl gallate (**267**)
Ethyl 3, 4, 5-trihydroxy benzoate
Molecular formula: C₉H₁₀O₅



- ❖ Pentadecyl 3, 4-dihydroxy butanoate (**269**)
Molecular formula: C₁₉H₃₈O₄

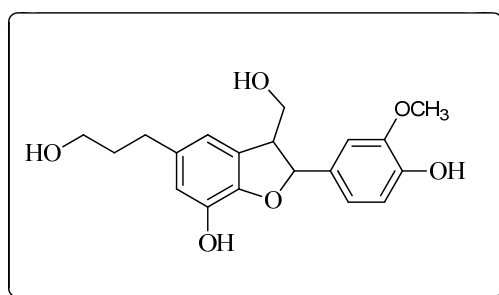


❖ Cedrusin (270)

2-(4'-Hydroxy-3'-methoxyphenyl)-5-(3''-hydroxypropyl)-3-

hydroxymethyl-7-hydroxy 2, 3 dihydro benzofuran

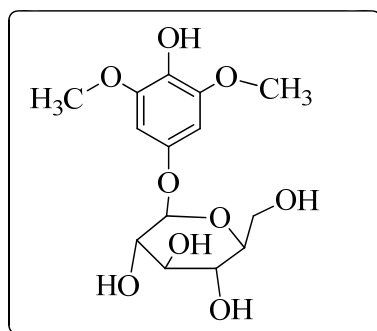
Molecular formula: C₁₉H₂₂O₆

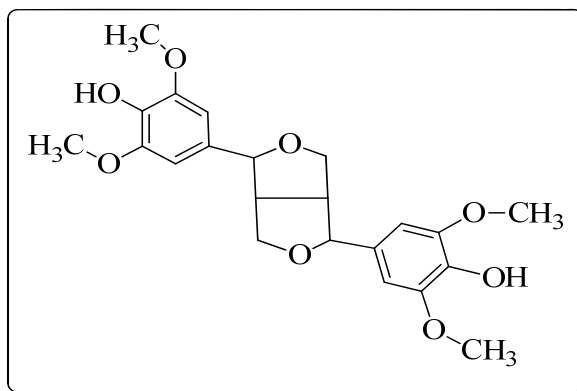
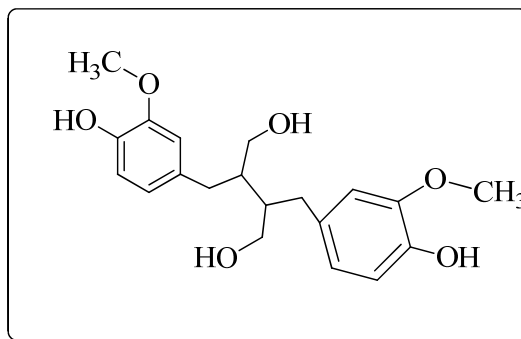


❖ Koaburaside (271)

3, 5-Dimethoxy-4-hydroxy-1-*O*-β-D-glucoside

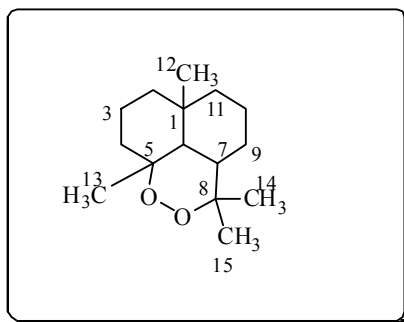
Molecular formula: C₁₄H₂₀O₉



❖ (+) Syringaresinol (**272**)Molecular formula: $C_{22}H_{26}O_8$ ❖ Secoisolariciresinol (**273**)Molecular formula: $C_{20}H_{26}O_6$ 

3.2 Grandino, 5, 8. peroxide (259)

Compound **259** isolated as white amorphous powder. Its molecular mass m/z 234 a.m.u was determined from EI-MS while molecular formula ($C_{15}H_{26}O_2$) was deduced from HREI-MS and ^{13}C NMR (**Table: 4.1**; *vide experimental*). The characteristic IR absorption band at 2915 cm^{-1} indicated the aliphatic C-H stretching. The ^{13}C NMR (BB & DEPT) of **259** (**Table: 4.1**; *vide experimental*) indicated four methyl, six methylene, two methines, and three quaternary carbons signals.



259

The proton chemical shift values for methyl's groups resonated at δ_H 0.84 (s, H-12), 1.09 (s, H-13), 1.19 (s, H-14) and δ_H 1.18 (s, H-15), while carbon chemical shift's values appeared at δ_C 18.6 (C-12), 22.4 (C-13), 27.1 (C-14) and δ_C 27.3 (C-15). The proton signals for methylene group appeared at δ_H 1.36 (t, $J=13$ Hz, H-2), 1.54 (m H-3), 1.79 (m, H-4), 1.91 (m, H-9), 1.27 (m, H-10) and δ_H 1.15 (t, $J=12.5$ Hz, H-11) in the ^1H NMR spectra (**Table: 4.1**; *vide experimental*) while their carbon signals recorded at δ_C 41.0 (C-2), 20.1 (C-3), 43.6 (C-4), 21.5 (C-9), 22.4 (C-10) and δ_C 44.5 (C-11). The two methines signals appeared at δ_H 1.18 (d, $J=11.5$ Hz, H-6) and δ_H 1.34 (m, H-7) while their carbon signals located at δ_C 49.9 (C-6) and δ_C 54.6 (C-7).

respectively. Furthermore quaternary carbons exhibited at δ_c 72.3 (C-5) and δ_c 72.9 (C-8) indicating attachment with peroxide which was supported by the up field value at δ_c 34.5 (C-1). The assignment was further confirmed by comparing 2D NMR with literature showing the peroxide linkage between C-5 and C-8.¹ The HMBC and COSY correlation of **259** (Table: 4.1; *vide experimental*) exhibited the attachment of methyl at C-1, at C-5 and two methyl's showed attachment at C-8. The structure was further supported by mass fragmentations. EI-MS fragmentation pattern showed major peaks at $m/z=204$ with loss of methoxy (Fig: 3.1 (a₁)) furthermore $m/z=85$ fragment showing loss of C₅H₉O (Fig: 3.1(a₂)) and $m/z=99$ peak indicating loss of C₇H₁₅ group (Fig: 3.1(a₃)). These mass fragmentations (Fig: 3.1 (a₁-a₃)) justifying the structure of **259** which was further confirmed by HMBC, NOESY and COSY spectral data (Fig: 3.1 (b, c)). The spectral data of **259** suggesting and comparing with related compound in literature²⁻⁷ characterized the structure of **259** as Grandino 5, 8 peroxide.

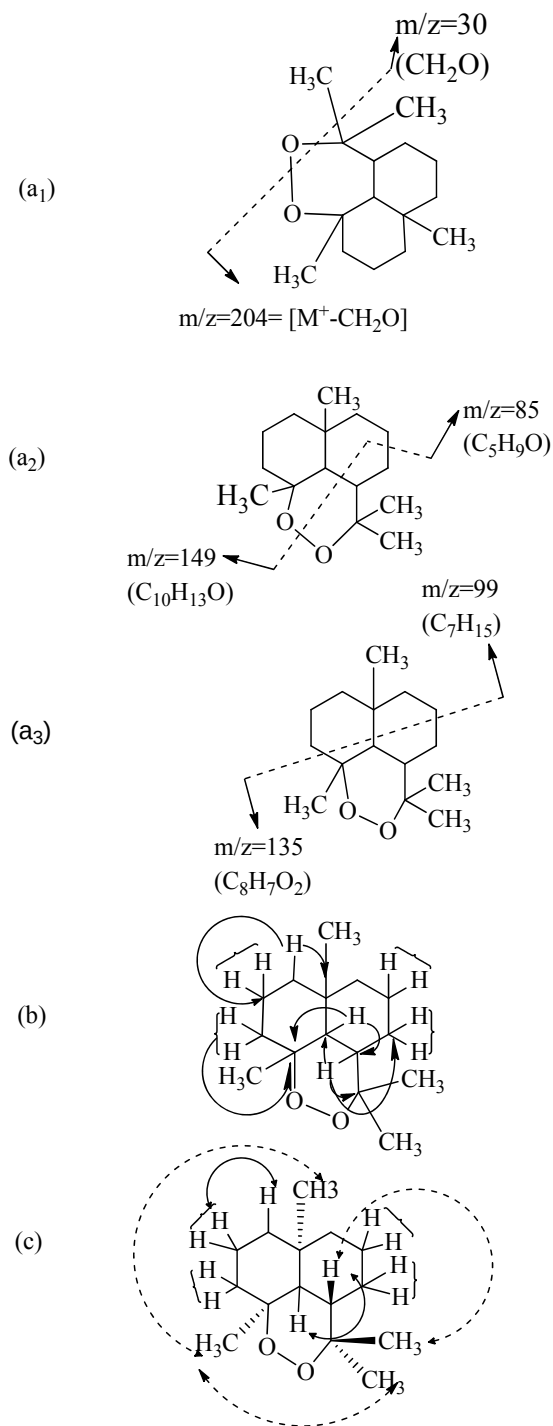
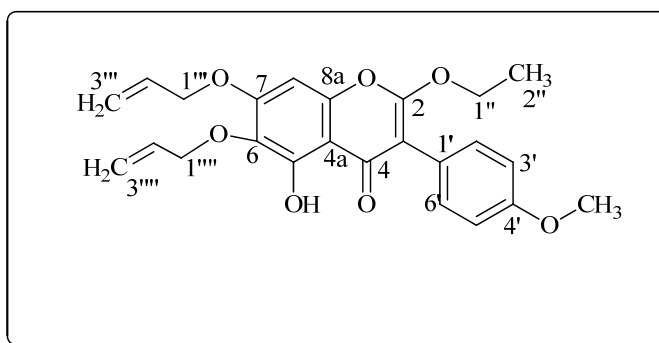


Fig: 3.1 (a) Mass fragmentation pattern, (b) HMBC correlations and (c) Important ^1H - ^1H COSY correlations (bold arrows) and NOESY correlation (dotted arrow) observed for **259**

3.3 Grandinoid (263)

Compound **263** isolated as white amorphous powder from the ethyl acetate fraction of *V. grandiflorum* aerial parts. The EIMS molecular ion peak observed at m/z 424 a.m.u while base peak appeared at m/z 356 a.m.u. The molecular formula of **263** deduced as $C_{24}H_{24}O_7$. The U.V spectrum indicates absorption band at 282 nm and characteristic IR absorption bands appeared at 2915 (C-H stretching aliphatic), 1730 (ester carbonyl), 1610 (aromatic double bonds). The ^{13}C NMR spectra (BB and DEPT) (**Table: 4.2**; *vide experimental*) of **263** exhibited two methyl's, five methylenes, seven methines and eleven quaternary carbons.



263

The protons chemical shift values for methyl's displayed at δ_H 1.01 (t, $J=7.2$ Hz, H-2'') and δ_H 3.81 (s, H-4') while their corresponding carbon values resonated at δ_C 13.5 (C-2'') and δ_C 5.2 (C-4') respectively. The proton NMR values for methylene appeared at δ_H 4.14 (q, $J=7.2$ Hz, H-1''), 4.54 (d, $J=5.6$ Hz, H-1'''), 5.43 (ddd, ($J=17.2, 3.2, 1.6$ Hz), (10.8, 3.2, 1.5 Hz), H-3'''), 4.69 (d, $J=4.8$ Hz, H-1''') and δ_H 5.54 (ddd, $J=17.2, 3.2, 1.2$ Hz, H-3'''). Their carbon NMR absorption peaks displayed at δ_C 62.3 (C-1'), 75.3 (C-1''), 118.4 (C-3'''), 70.2 (C-1''') and δ_C 118.6 (C-3''') respectively. The proton NMR resonated for methines at δ_H 6.67 (s, H-8), 7.18 (dd, $J=7.2, 2.0$ Hz,

H-2'/6'), 6.93 (dd, $J=7.2, 2.0$ Hz, H-3'/5'), 6.23 (ddt, $J=16.4, 10.0, 6.0$ Hz, H-2''') and δ_{H} 6.05 (ddt, $J=15.6, 10.0, 4.8$ Hz, H-2''') while their respective carbon NMR values appeared at δ_{C} 97 (C-8), 131 (C-2'/6'), 113.7 (C-3'/5'), 131.1 (C-2''') and δ_{C} 118.6 (C-2'''). Analysis of the ^1H , ^{13}C NMR, HSQC, and HMBC data (**Table: 4.2**; *vide experimental*) revealed the flavonoid structure; containing substituted aromatic rings, a 5, 6, 7 trisubstituted ring A (δ_{H} 6.67 (s, H-8), δ_{C} 97 (C-8)) and a monosubstituted ring B ((δ_{H} 7.18 (dd, $J=7.2, 2.0$ Hz), δ_{C} 131 (C-2'/6') and δ_{H} (6.93 (dd, $J=7.2, 2.0$ Hz), δ_{C} 113.7 (C-3'/5')). The spectral data and ^{13}C NMR chemical shifts for C-4 (δ_{C} 174.7), C-5 (δ_{C} 113.5), C-6 (δ_{C} 161.4), C-7 (δ_{C} 158.8) showed chrysin nucleus.⁸ The ^1H NMR data further revealed two propenyloxy groups attached, at C-6 ((δ_{H} 5.54 (d, 5.6 Hz), δ_{C} 75.3, C-1''', δ_{H} 6.23 (ddt, 16.4, 10.0, 6.0 Hz), δ_{C} 131.1, CH-2''', δ_{H} 5.43 (ddd, 17.2, 3.2, 1.6 Hz), δ_{C} 118.4, C-3''') and at C-7 (δ_{H} 4.69 (d, $J=4.8$ Hz), δ_{C} 70.2, C-1'''), δ_{H} 6.05 (1H, ddt, $J=15.6, 10.0, 4.8$ Hz), δ_{C} 133.6, CH-2''') and (δ_{H} 5.54, ddd, $J=17.2, 3.2, 1.2$ Hz), δ_{C} 118.6, C-3''') of the ring A. Analysis of the ^1H - ^1H COSY correlation (**Fig: 3.2 (c)**) provided the sequential connectivity of all protons resonances of the propenyloxy chains. The HMBC spectrum exhibited correlations between H-1'''' and C-6 and also between H-1''' and C-7 suggested that one propenyloxy group attached with C-6 and other propenyloxy group attached with C-7. The NOESY correlation between H-1'''' and H-8 further confirmed the position of propenyloxy at C-7. These spectral data and mass fragmentation profile (**Fig: 3.2 (a)**) of **263** and related compound data in literature led to elucidate the structure of **263** as 6,7-bis(propenyloxy)-2-ethoxy-5-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (Grandinoid).

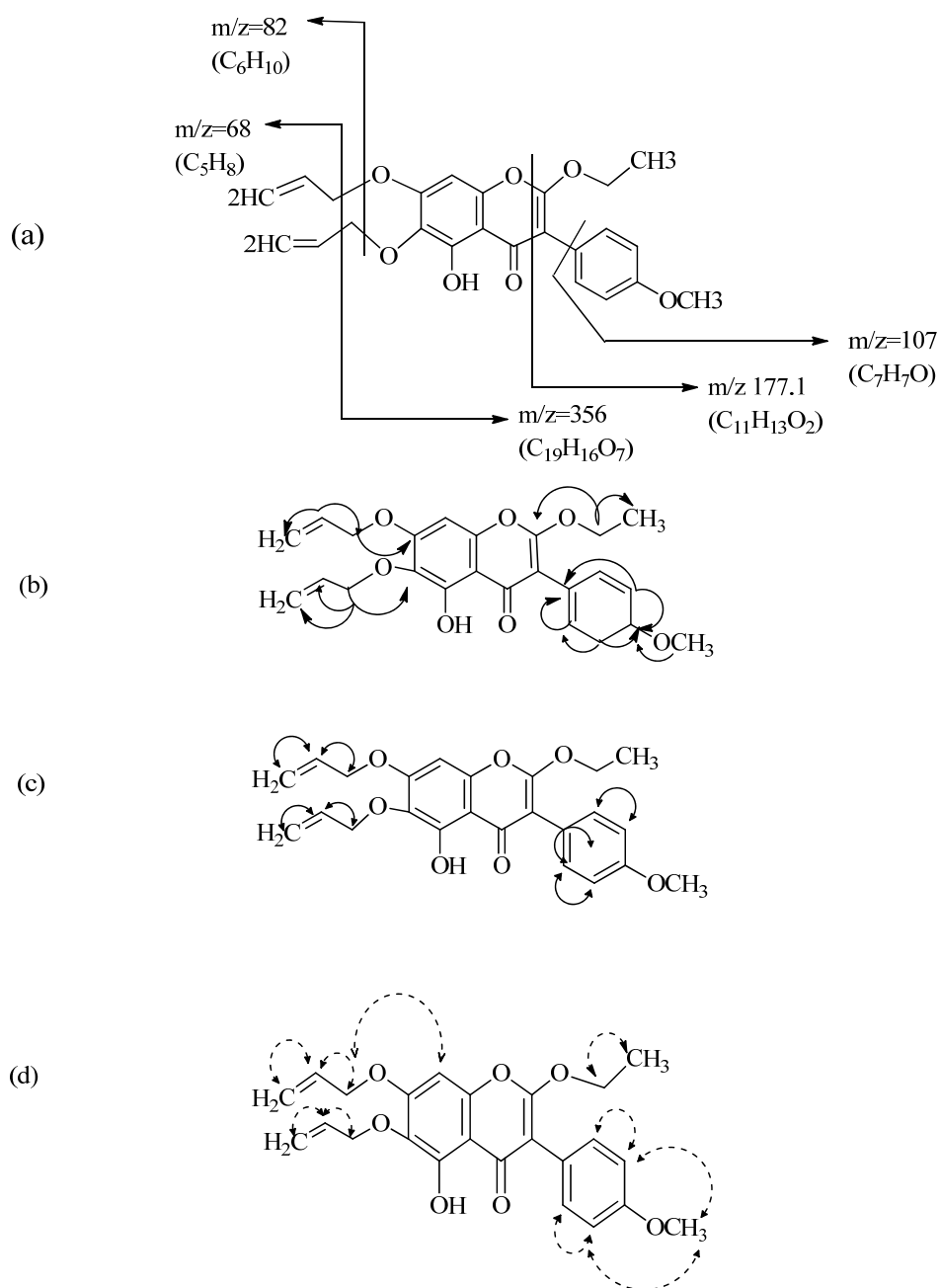
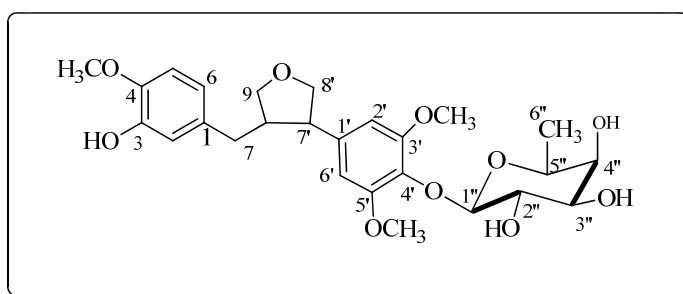


Fig: 3.2 (a) Mass fragmentation pattern, (b) HMBC correlations (c) Important ^1H - ^1H COSY correlations (bold arrows) (d) NOESY correlation (dotted arrow) observed for Grandinoid (263)

3.4 Grandifloruside (266)

Compound **266** isolated from ethyl acetate fraction of *V. grandiflorum* aerial parts as brownish gummy solid. The molecular ion peak at m/z 506 a.m.u and molecular formula $C_{26}H_{34}O_{10}$ was determined from EI-MS profile (*vide experimental*). UV showed absorption bands at 210 nm and 278 nm. The IR absorption bands observed at 2915 (for aliphatic C-H stretching), 3355 (O-H), 1605 (C=C), 1520, 1488 (aromatic region), 1135, 1125, 1044 for (C-O-C). The ^{13}C NMR (BB and DEPT) of **266** showed 4 methyl groups, 3 methylene groups, 12 methines groups and 7 quaternary carbons (**Table: 4.3**; *vide experimental*).



266

The characteristic protons chemical shift values noted for methyl's (δ_H 1.18 (d, $J=6.3$ Hz, H-6''), three methoxy groups (δ_H 3.8, s) attached with carbon C-3, C-3', C-5' respectively while proton of three methylene groups resonated at δ_H 2.83 (d, $J=7.5$ Hz, H-7), 3.65 (m, H-9) and δ_H 3.12 (dd, $J=9.6, 3.6$ Hz, H-8'). The chemical shift values of methines appeared at δ_H 6.67 (s, H-2), 6.75 (d, $J=7.8$ Hz, H-5), 6.59 (dd, $J=8.1, 2.0$ Hz, H-6), 2.02 (m, H-8), 6.63 (s, H-2'), 6.16 (s, H-6'), 1.88 (t, $J=12.8$ Hz, H-7'), 4.5 (m, H-1''), 3.87 (m, H-2''), 3.63 (m, H-3''), 3.30 (t, $J=9.6$ Hz, H-4'') and δ_H 3.50 (m, H-5'') in the ^1H NMR spectrum. The carbon NMR peaks for

methyl's appeared at δ_c 17.9 (C-6'') and δ_c 56.3 (C-3, C-3', C-5'). In the same way carbon values resonated for methylene groups at δ_c 33.8 (C-7), 65.3(C-9) and δ_c 67.9 (C-8'), while methines showed peaks in carbon NMR spectra at δ_c 112.3 (C-2), 116 (C-5), 123.1 (C-6), 40 (C-8), 113.4 (C-2'), 117.1 (C-6'), 45.4 (C-7'), 102.3 (C-1''), 72.3 (C-2''), 72.5 (C-3''), 73.8 (C-4''), and δ_c 70.1 (C-5''). The aromatic methines of **266** at δ_H , 6.67 (s)/ δ_c 112.3 (C-2), 6.75 (d, $J=7.8$ Hz)/ 116 (C-5), 6.59 (dd, $J=8.1, 2.0$ Hz)/ 123.1 (C-6), 6.63 (s)/ 113.4 (C-2') and δ_H 6.16 (s)/ δ_c 117.1 (C-6') (**Table: 4.3**; *vide experimental*) showing two substituted aromatic rings, two non aromatic methines at δ_H 2.02 (m)/ δ_c 40 (C-8) and δ_H 1.88 (t, $J=12.8$ Hz)/ δ_c 45.4 (C-7') and two methylene at δ_H 3.65 (m)/ δ_c 65.3 (C-9), δ_H 3.12 (dd, $J=9.6, 3.6$ Hz)/ δ_c 67.9 (C-8') indicating furan type ring, the methine (C-8) connected with methylene (C-7), which possess δ_H 2.83 (d, $J=7.5$ Hz) and further linked with aromatic ring (C-1) δ_c 134 (**Table: 4.3**; *vide experimental*). The spectral data suggesting lignin skeleton.⁹ The skeleton was further confirmed by ^1H - ^1H NOESY correlation between H-6/H-5 and H-6/H-2 while the H-2'/H-6' also showing strong ^1H - ^1H NOESY correlation (**Fig: 3.3**). In spite of the aromatic ring exhibited substitution at δ_c 147.3 (C-3), 145.2 (C-4), 149.1(C-3'), 146.1 (C-4') and δ_c 149.1(C-5') and substituent's groups were assigned on the bases of HMBC and COSY correlation. Three methoxy groups; δ_c 56.3 were placed at C-3 (δ_c 147.2), C-3' (δ_c 149.1) and C-5' (δ_c 149.1) on the bases of HMBC correlation (**Table: 4.3**; *vide experimental*). One hydroxyl group showed attachment at C-5 (δ_c 145.2), while proton and ^{13}C NMR signals at δ_H 4.5 (m)/ δ_c 102.3 (C-1''), δ_H 3.87 (m)/ δ_c 72.3 (C-2''), δ_H 3.63 (m)/ δ_c 72.5 (C-3''), δ_H 3.30 (t, 9.6 Hz)/ δ_c 73.8 (C-4''), δ_H 3.50 (m)/ δ_c 70.1 (C-5'') and δ_H 1.18 (d, $J=6.3$ Hz)/ δ_c 17.9 (C-6'') indicating the sugar moiety linked at C-4' position with aromatic ring. The anumeric proton at δ_H 4.5 showing correlation

with C-4' and also with C-2'' and C-5'' (**Fig: 3.3(b, c)**). The presence of methyl group at δ_c 17.9 connected to C-5'' which is different from a common glucoside. All these features suggested and mass fragmentation (**Fig: 3.3(a)**) confirmed the structure of **266** as 4-hydroxy-3, 3', 5'-trimethoxy-9, 9'-epoxy, 7- nor lignane-4'-O- β -D-fucoside (grandifloruside).

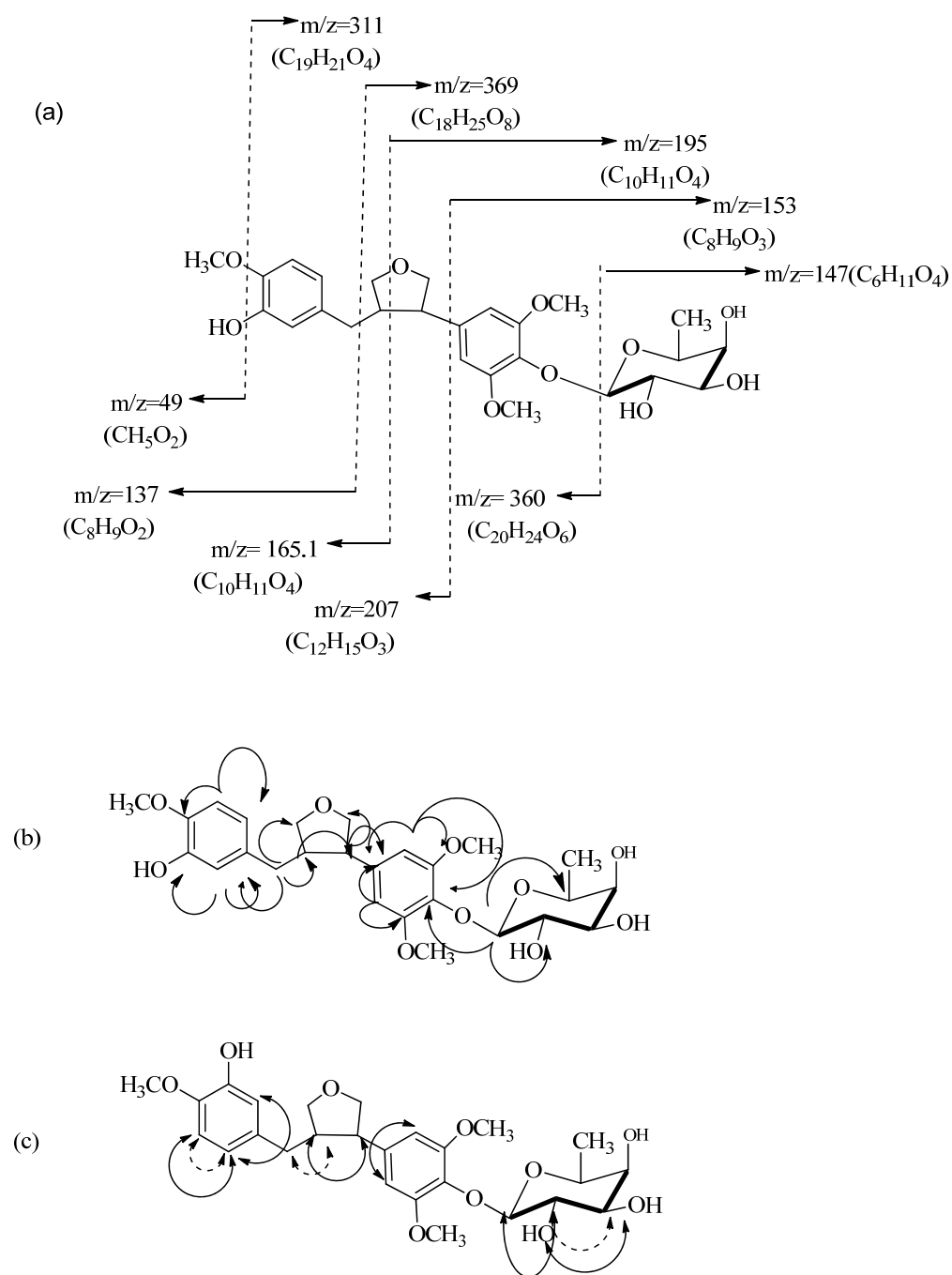
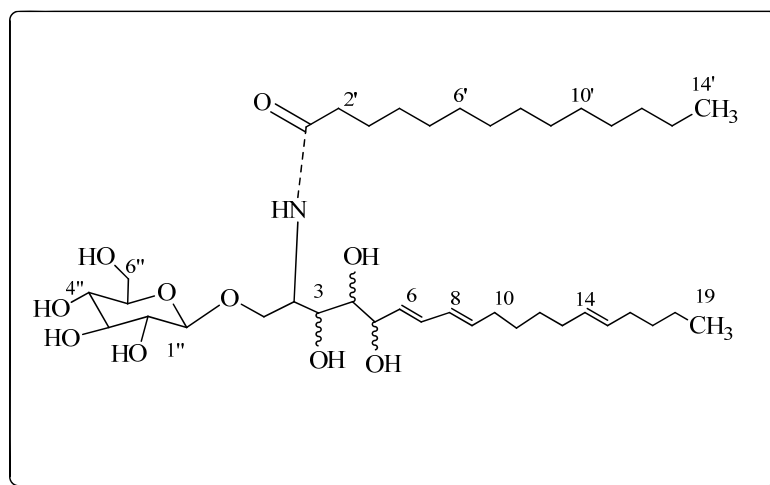


Fig: 3.3 (a) Mass fragmentation pattern, (b) HMBC correlations and (c) Important ^1H - ^1H COSY correlations (dotted arrow) and NOESY correlation (bold arrows) observed for **266**.

3.5 Viburnoside (268)

Compound **268** was purified as white amorphous powder from the chloroform fraction of the crude ethanolic extract of roots (**Scheme 4.5**). Molecular ion peak of **268** obtained in EI-MS profile at m/z 714 a.m.u, while a molecular ion peak appeared at m/z 714.5286 a.m.u in ESI-MS profile and molecular formula was determined as $C_{39}H_{71}O_{10}N$ which containing three degree of unsaturation (*vide experimental*). $[\alpha]_D^{26}$ recorded for **268** was -0.034° and UV absorption bands appeared at 214 nm, 230 nm, 280 nm. The IR absorption peaks recorded for **268** at 2320-3550 for hydroxyl and amide functions, 1735, 1638 were assigned to, C=O, C=C respectively. The ^{13}C NMR (BB and DEPT) and HMQC spectral data (**Table: 4.4**; *vide experimental*) showed two methyls, 21 methylenes, 15 methines and one quaternary carbon.



268

The proton NMR spectrum of **268** possesses a characteristic signal for two methyl groups at δ_H 0.85 (t, $J=7.5$ Hz, H-19, H-14'; 6H) and amide proton resonated at δ_H 8.36 (d, $J=8.1$ Hz, N-H) while the methylene proton signals appeared at δ_H 4.68 (dd, $J=11.0, 6.2$ Hz, H-1a), 4.23 (dd, $J=11.0, 6.2$ Hz, H-1b), 1.23-2.14 (m, H-10 to H-13,

H-16 to H-18 and H-2' to H-13') and δ_{H} 4.36 (m, H-6'') . The ^1H NMR spectrum showed methines proton resonated at δ_{H} 4.79 (m, H-2), 4.56 (m, H-3), 4.77 (dd, $J=10.5, 6.0$ Hz, H-4), 4.77 (dd, $J=10.5, 6.0$ Hz, H-5), 5.48 (t, $J=4.2$ Hz, H-6), 5.95 (m, H-7), 5.93 (m, H-8), 5.93 (m, H-9), 5.95 (m, H-10), 5.48 (t, $J=4.2$ Hz, H-11), 4.96 (d, $J=7.4$ Hz, H-1''), 4.02 (d, $J=7.4$ Hz, H-2''), 3.89 (m, H-3''), 4.2 (m, H-4'') and δ_{H} 4.2 (m, H-5''). The carbon NMR chemical shifts recorded for two methyl's at δ_{C} 14.2 (C-19, C-14'), twenty one methylene δ_{C} 70.1 (C-1), 22.9-32.9 (C-10 to C-13, C-16 to C-18 and C-2' to C-13') and δ_{C} 62.6 (C-6'') while fifteen methines in carbon NMR resonated at δ_{C} 54.6 (C-2), 72.3 (C-3), 72.4 (C-4), 72.5 (C-5), 129.9 (C-6), 132.0 (C-7), 132.0 (C-8), 129.9 (C-9), 105.7 (C-1''), 75.1 (C-2''), 78.4 (C-3''), 71.4 (C-4'') and δ_{C} 78.5 (C-5''). The HMBC (**Table: 4.4**; *vide experimental*) showed amide proton correlation with methine at δ_{H} 4.79 (m)/ δ_{C} 54.6 (C-2), an oxygenated methylene at δ_{H} 4.68 (dd, $J=11, 6.2$ Hz (H-1a)/ δ_{C} 70.1 (C-1) and δ_{H} 4.23 (dd, $J=11.0, 6.2$ Hz (H-1b)/ δ_{C} 70.1 (C-1) and also with carbonyl carbon at δ_{C} 175.1 (C-1') and δ_{H} 4.56 (m, H-3)/ δ_{C} 72.3 C-3). These assignments clearly indicated that compound **268** has sphingosine skeleton.^{10,11} The methines with ^1H and ^{13}C NMR chemical shift values δ_{H} 5.95 (m, H-6)/ δ_{C} 129.9 (C-6), δ_{H} 5.93 (m, H-7)/ δ_{C} 132 (C-7), δ_{H} 5.93 (m, H-8)/ δ_{C} 132 (C-8), δ_{H} 5.95 (m, H-9)/ δ_{C} 129.9 (C-9) and the δ_{H} 5.48 (t, $J=4.2$ Hz, H-14)/ δ_{C} 132.1 (C-14) and δ_{H} 5.48 (t, $J=4.2$ Hz, H-15)/ δ_{C} 131.1 (C-15) (**Table: 4.4**; *vide experimental*) manifested for olefinic (C=C) system. The olefinic protons position and hydroxy methines protons were assigned on the bases of TOCSY experiment. Three adjacent hydroxy methines (CH-OH) at δ_{H} 4.56 (m, H-3)/ δ_{C} 72.3 (C-3), δ_{H} 4.77 (dd, $J=10.5, 6$ Hz, H-4)/ δ_{C} 72.4 (C-4), δ_{H} 5.48 (dd, $J=10.5, 6$ Hz, H-5)/ δ_{C} 72.5 (C-5) (**Table: 4.4**; *vide experimental*) exhibited strong COSY correlation with olefinic proton H-6 and also with H-2 (**Fig: 3.4**). The methine at δ_{H} 4.79 (m, H-2)/ δ_{C} 54.6 (C-

2) showing strong HMBC correlation with C-1, C-3, C-4 and with N-H. Methylene at δ_{H} 4.68 (d, $J=11$ Hz, H-1a), 4.23 (d, $J=6.2$ Hz, H-1b)/ δ_{C} 70.1 (C-1) exhibited strong HMBC correlation with C-2, C-3, and with C-1' (**Fig: 3.4**). The HMBC correlation of the olefinic methines with methylene which appeared as multiplet in the proton NMR spectrum and its chemical shift values ranging from δ_{H} 1.23 - 2.14 (m)/ δ_{C} 22.9 – 32.9 (**Table: 4.4**; *vide experimental*). The structure of **268** was deduced on comprehensive analysis of mass fragmentation, HMBC, NOESY and COSY correlations (**Fig: 3.4**) and named as 2-(N-tetradecanoyl)-6*E*, 8*E*, 14*E*-nonadectrien-3, 4, 5-triol-1-*O*-(β -D-glucopyranoside (viburnoside).

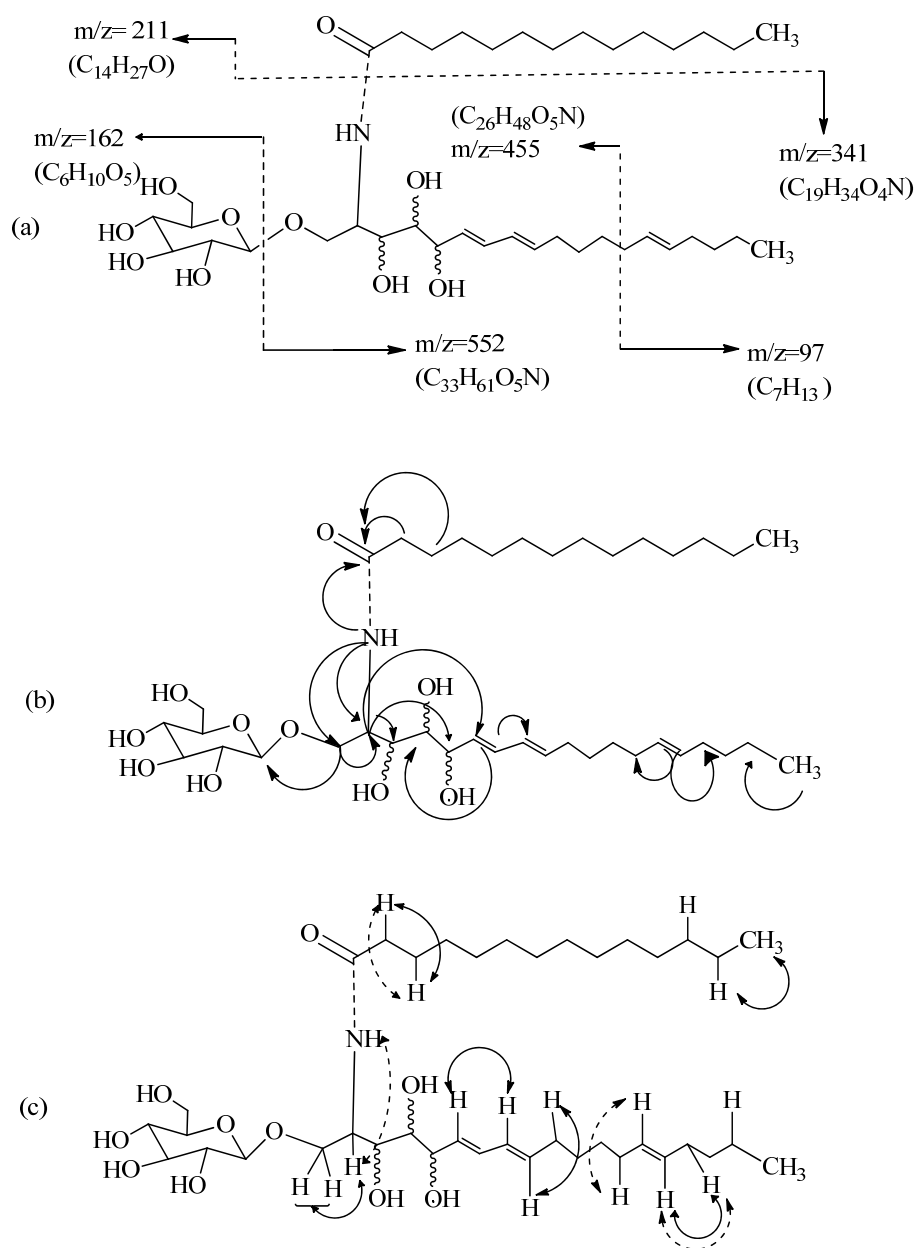
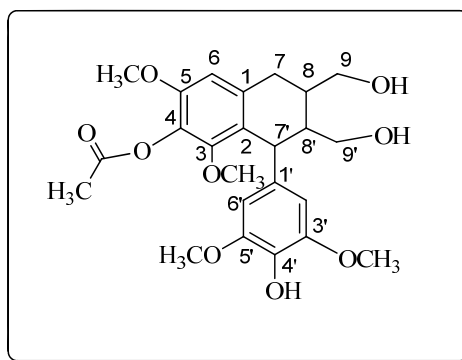


Fig: 3.4 (a) Mass fragmentation pattern, (b) HMBC correlations (c) Important ^1H - ^1H COSY correlations (bold arrow) and NOESY correlation (dotted Arrows) observed for **268**.

3.6 Grandiflorinol (274)

Compound **274** isolated as white amorphous solid from the ethyl acetate fraction of the crude ethanolic extract of *V. grandiflorum*. The purification of **274** through column chromatography and pre-coated thin layer chromatographic plates (**Scheme 4.6**). Molecular ion peak at m/z 462 a.m.u observed in EI-MS, while Molecular formula of **274** determined as $C_{24}H_{30}O_9$. $[\alpha]_D^{26}$ measured for **274** was -12.5° and UV absorption maxima observed at 210 nm and 265 nm. The IR absorption bands recorded at 3420 (O-H), 1580-1620 (aromatic C=C bonds). Analysis of spectra (**Table: 4.5**; *vide experimental*) which indicated five methyles, four methylenes, six methines and nine quaternary carbons



274

The ^1H NMR chemical shifts resonated for methyles at δ_H 1.88 (s), 3.36(s), 3.77 (s) and two methyles at δ_H 3.69 (s) while the methylene protons chemical shifts values appeared at δ_H 3.48 (m, H-9), 2.58 (dd, $J=14.4, 5.4$ Hz, Ha-7') and δ_H 2.70 (dd, $J=15.0, 4.8$ Hz, Hb-7), while methines protons resonated at δ_H 6.37 (s, H-2'/6'), 4.3 (d, $J=5.4$ Hz, H-7'), 1.93-1.98 (m, H-8'), 6.57 (s, H-6) and δ_H 1.61 (m, H-8). The compound consist of two trisubstituted aromatic rings (A and B).The ring B having two aromatic protons which appeared as two protons singlet at δ_H 6.37 (s, H-2'/6'),

while two methoxy groups resonated at δ_{H} 3.72 (s) appeared as six protons singlet in ^1H NMR spectrum. Positions of methoxy groups C-3' and C-5' on ring B determined from HMBC correlations. The ring A possesses one aromatic proton at δ_{H} 6.57 (s, H-6) while the two methoxy groups at δ_{H} 3.84 (s) and 3.36 (s) which indicated HMBC correlations with C-2 and C-5 respectively. The ^1H NMR spectrum showed a methyl at δ_{H} 1.88 (s)/ δ_{C} 24.1 connected with carbonyl carbon in HMBC spectrum. The acetyl group was placed at C-4 on the basis of NOESY spectrum. The non aromatic methines resonated at δ_{H} 4.30 (d, 5.4 Hz, H-7')/ δ_{C} 42.2 (C-7'), δ_{H} 1.93-1.98 (m, H-8')/ δ_{C} 49.8 (C-8') and δ_{H} 1.58-1.65 (m, H-8)/ δ_{C} 40.7 (C-8), while methylenes appeared at δ_{H} 2.58 (dd, $J=14.4$, 5.4 Hz, Ha-7 and δ_{H} 2.70 (dd, $J=14.4$, 4.8 Hz, Hb-7)/ δ_{C} 33.5 (C-7), δ_{H} 3.46-3.50 (m, H-9')/ δ_{C} 63.9 (C-9'), δ_{H} 3.57-3.62 (m, H-9)/ δ_{C} 66.9 (C-9) in the NMR spectrum. (**Table: 4.5**; *vide experimental*) and ^{13}C NMR (BB and DEPT) indicated quaternary carbons at δ_{C} 126.2 (C-1), 130.1 (C-2), 134.3 (C-4'), 138.8 (C-4), 139.3 (C-1'), 147.6 (C-5), 148.6 (C-3) and δ_{C} 148.9 (C-3'/5'), (**Table: 4.5**; *vide experimental*). The ^1H NMR and ^{13}C NMR data of **274** showed similarity with literature data of coniferyl dimer polystachyol.^{12, 13} The **274** spectral data was found similar with polystachyol except acetyl group (methyl δ_{H} 1.88(s)/ δ_{C} 24.1 attached with C=O ^{13}C NMR δ_{C} 180.0), The position of the acetyl group at C-4 indicated from NOESY correlation (**Fig: 3.5 (c)**) and comparatively downfield ^{13}C NMR chemical shift δ_{C} 138.8 of C-4 (**Table: 4.5**; *vide experimental*) also supported the presence of acetyl group at this position. The position of the acetyl group also clear from methyl group which showed HMBC correlation with C=O (**Table: 4.5**; *vide experimental*). HMBC, NOESY and COSY correlations and mass fragmentations (**Fig: 3.5, a, b, c**) characterized the structure of **274** and related compounds data in literature further

confirmed the structure as 9, 4', 9'-trihydroxy-3, 5, 3', 5'-tetramethoxy-2, 7'-cyclo lignan-4-yl acetate (grandiflorinol).

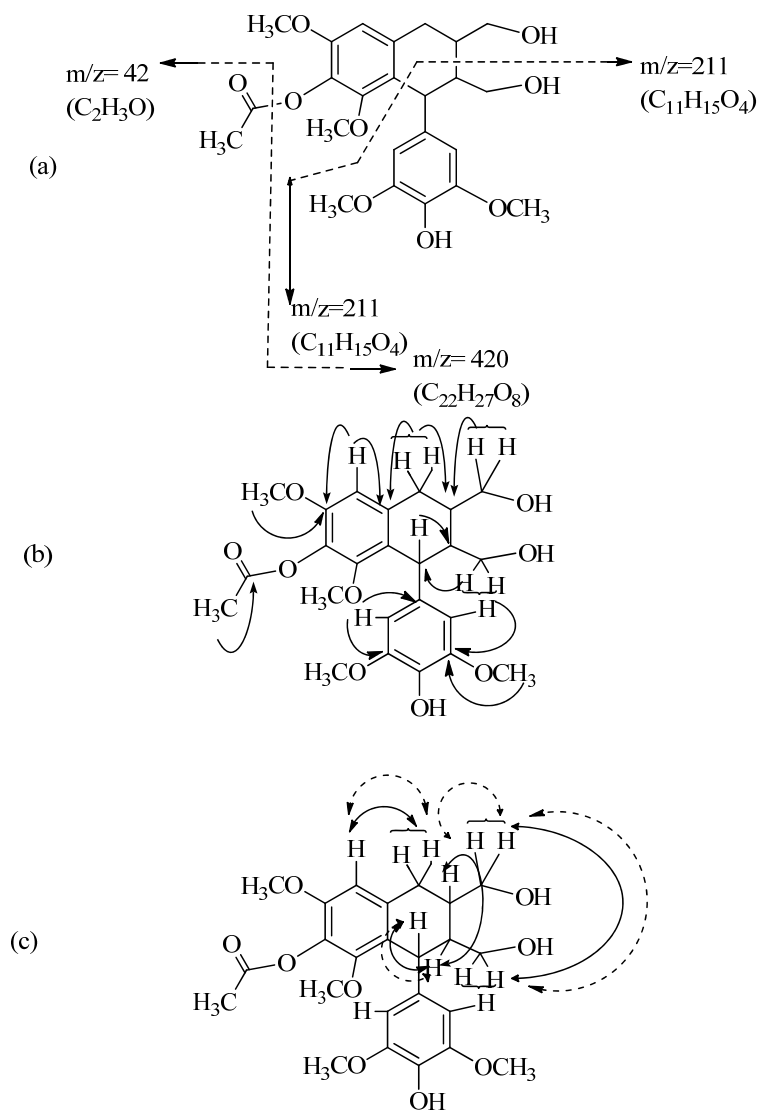
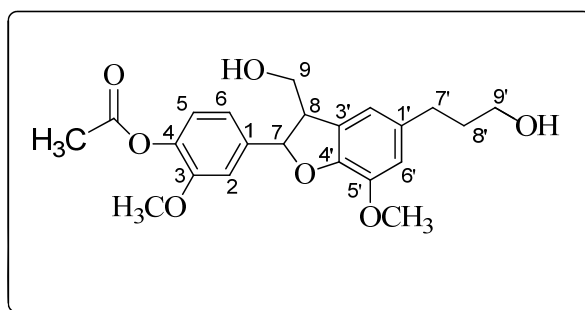


Fig: 3.5 (a) Mass fragmentation pattern, (b) HMBC correlations and (c) Important ^1H - ^1H NOESY (solid arrows) and COSY (dotted arrows) correlations for **274**.

3.7 Grandifloronin (275)

Compound **275** isolated as white amorphous solid from the ethyl acetate fraction of crude ethanolic extract of *V. grandiflorum* roots. Its molecular ion peak m/z at 402 a.m.u and molecular formula $C_{22}H_{26}O_7$ with ten degree of unsaturation was established by HRESI-MS. $[\alpha]_D^{26}$ measured -0.28° for **275** and UV absorption bands showed at 208 and 282 nm. IR exhibited absorption bands at 1728 (C=O) and 1580-1620 (aromatic C=C bonds). The functional groups indicated by UV and IR spectra confirmed by ^1H and ^{13}C NMR data including 2D NMR techniques. The ^{13}C NMR (BB and DEPT) (**Table: 4.6**; *vide experimental*) showed the presence of twenty two carbons in the compound; three methyles, four methylenes, seven methines and eight quaternary carbons.



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HSQC, HMBC, NOESY and COSY experiments were used for the structure elucidation to confirm the structure of **275**. The ^1H NMR chemical shift values (**Table: 4.6**; *vide experimental*) observed for non aromatic methines at δ_H 5.48 (d, $J=6.0$ Hz, H-7) and δ_H 3.43 (m, H-8) while for aromatic methines δ_H 6.55 (s, H-2'), 6.59 (s, H-6'), 6.97 (1H, s, H-2), 6.75 (d, $J=6.6$ Hz, H-5) and δ_H 6.82 (dd, $J=7.6$ Hz, 2.0, H-6). The methylenes protons resonated at δ_H 2.55 (t, $J=7.8$ Hz, H-7'), 1.78 (m, H-8'), 3.54 (t, 6.6, H-9') and δ_H 3.81 (m, H-9). The ^{13}C NMR values for non aromatic

methines at δ_c 88.6 (C-7) and δ_c 55.7 (C-8) while δ_c 116.6 (C-2'), 116.9 (C-6'), 110.3 (C-2), 116.0 (C-5) and δ_c 119.6 (C-6) appeared for aromatic methines in ^{13}C NMR spectra. The ^{13}C NMR spectra for methylenes found at δ_c 32.7 (C-7'), 35.8 (C-8'), 62.2 (C-9') and δ_c 65.1 (C-9). Two methoxy groups resonated at δ_H 3.80 (s)/ δ_c 56.2 while one methyl group appeared as singlet in ^1H NMR spectrum at δ_H 1.88 which appeared at δ_c 24.2. The two methoxy and one methyl groups showed HMBC correlations with C-3, C-5' and C=O respectively. The methylenes at δ_H 2.55 (t, $J=7.8$ Hz, H-7')/ δ_c 32.7 (C-7'), δ_H 1.78 (m, H-8')/ δ_c 35.8 (C-8') and 3.54 (t, $J=6.6$ Hz, H-9')/ δ_c 62.2 (C-9') showed strong HMBC, NOESY and COSY correlation with each other (**Fig: 3.6**) manifested hydroxypropenyl group attached at C-1' with aromatic ring. The spectral data of the **275** exhibited similarity with lignan skeleton.¹⁴⁻¹⁶ The **275** was found similar with 6, 3'-dimethoxy, 2, 3-dihydro dehydrodiconiferyl alcohol but possesses slightly difference in the NMR signal resonated at δ_H 1.88 (s)/ δ_c 24.2 which showed HMBC correlation with carbonyl carbon resonated at δ_c 180.1.¹³ After comprehensive analysis of the NOESY and COSY spectra of **275** acetyl group was placed at C-4 (**Fig: 3.6**). Mass fragmentation profiles (**Fig: 3.6 (a)**) NMR (1D and 2D) experimental data NOESY, COSY spectra (**Table: 4.6; vide experimental**), (**Fig: 3.6 (c)**) and the literature study confirmed the structure of **275**. The structure of **275** characterized as 9, 9'-dihydroxy-3, 5'-dimethoxy-8, 3'-neo-lignan-4-yl acetate (grandifloronin).

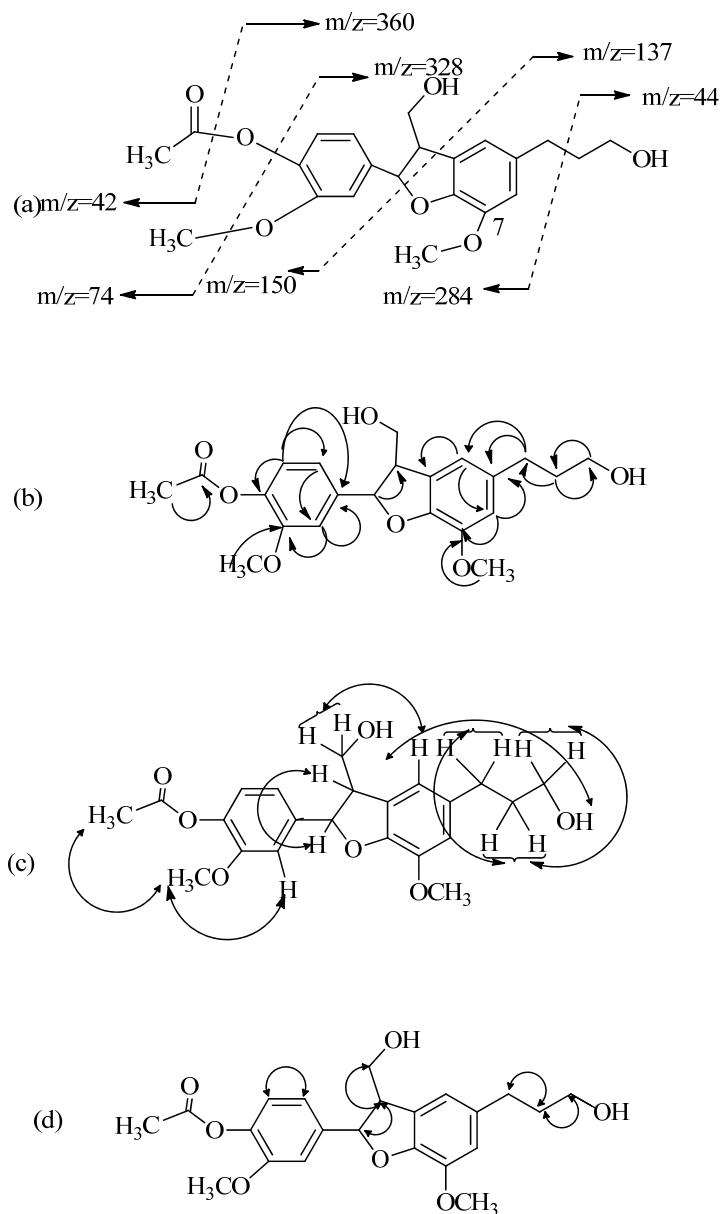
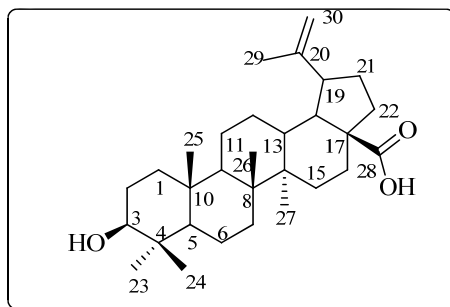
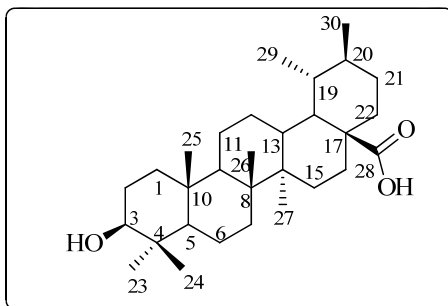


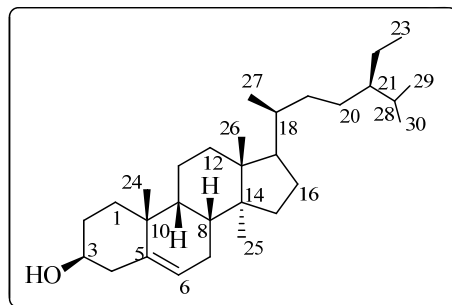
Fig: 3.6 (a) Mass fragmentation pattern, (b) HMBC correlations and (c) Important ^1H - ^1H NOESY (solid arrows) (d) COSY (solid arrows) correlations observed for **275**.

3.8.0 Hitherto unreported constituents from the aerial parts of *V. grandiflorum*.**3.8.1 Betulinic acid (257)** **3β -Hydroxy lup-20(29)-ene-28-oic acid****257**

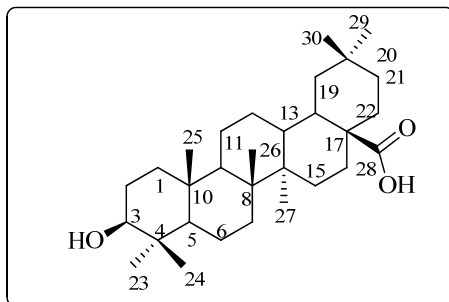
Compound **257** obtained as white amorphous powder from the chloroform fraction of the aerial parts of *V. grandiflorum* crude ethanolic extract. The EI-MS spectrum showed molecular ion peak at m/z 456 a.m.u ($C_{30}H_{48}O_3$). The structure of **257** characterized as betulinic acid (3β -hydroxy lup-20(29)-ene-28-oic acid) by comparison of their spectral data with reported data in literature.^{17, 18}

3.8.2 Ursolic acid (107)**3 β -Hydroxy lup-11(13)-ene-28-oic acid)****107**

Compound **107** isolated as white amorphous powder from chloroform fraction of aerial parts of *V. grandiflorum* crude ethanolic extract. Molecular mass m/z 456 a.m.u ($C_{30}H_{48}O_3$) established through EI-MS. The structure **107** characterized as 3 β -hydroxy lup-11(13)-ene-28-oic acid (ursolic acid) and confirmed through reported data in literature.¹⁹

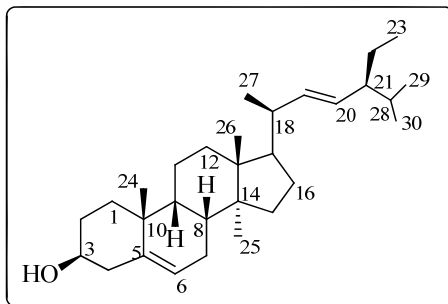
3.8.3 β -Sitosterol (**258**)**258**

Compound **258** afforded as colorless needles chloroform soluble from chloroform fraction of aerial parts of *V. grandiflorum*. The molecular ion peak appeared at m/z 414 a.m.u ($C_{29}H_{50}O$) was established from EI-MS. The structure of **258** was elucidated as β -sitosterol and confirmed by data in literature.²⁰

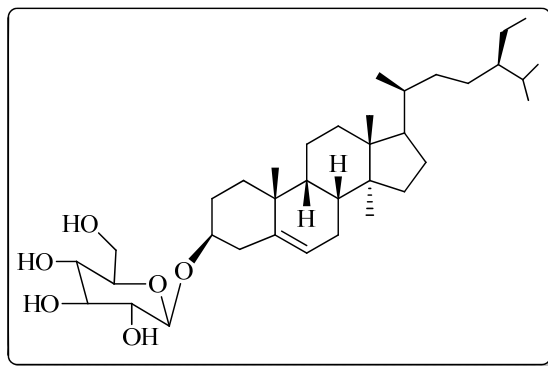
3.8.4 Oleanolic acid (138)**138**

Compound **138** isolated from chloroform fraction as white powder. EI-MS showed molecular ion peak at m/z 456 a.m.u ($C_{30}H_{48}O_3$). The structure of the compound was finalized as oleanolic acid based on the available literature data.²¹

3.8.5 Stigmasterol (260)

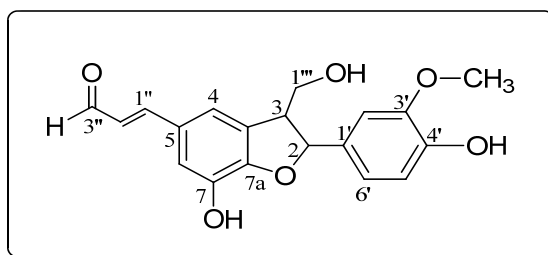
**260**

Compound **260** was afforded as soft crystals from chloroform fraction of *V. grandiflorum* aerial parts. The molecular mass m/z 412 a.m.u ($C_{29}H_{48}O$) of **260** investigated through EIMS experiment. The structure of **260** elucidated as stigmasterol from experimental data and by comparison with related compound in literature.²⁰

3.8.6 Glucoside of β -sitosterol β -Sitosterol 3-*O*- β -D-glucopyranoside) (261)**261**

Compound **261** obtained as colorless needles from chloroform fraction of *V. grandiflorum* aerial parts. The molecular ion peak appeared at m/z 576 a.m.u ($C_{36}H_{61}O_6$) was established from EI-MS and the structure was confirmed by matching with related compound data in literature.²²

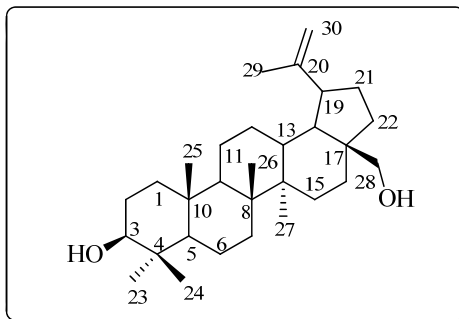
3.8.7 2, 3-Dihydro-2-(4'hydroxy, 3'-methoxy phenyl)-3-hydroxy methyl-5-(2-formylvinyl) 7-hydroxy benzofuran (262)



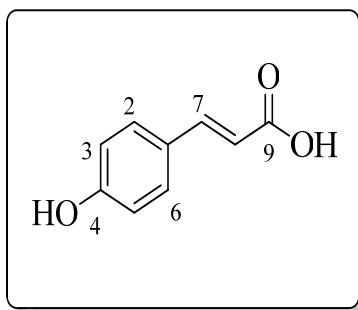
262

Compound **262** isolated as yellow amorphous powder from chloroform fraction of aerial parts of *V. grandiflorum*. The EI-MS and HR-EIMS exhibited molecular mass m/z 342 ($C_{19}H_{18}O_6$). The structure of **262** elucidated as 2, 3-dihydro-2-(4'hydroxy, 3'-methoxy phenyl)-3-hydroxy methyl-5-(2-formylvinyl) 7-hydroxy benzofuran on the bases of NMR data (**Table: 3.7**; *vide experimental*) and comparison with literature.²³⁻²⁵

3.8.8 Betulin (264)

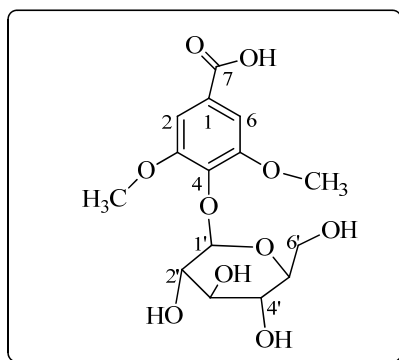
Lup-20(29)-ene-3 β , 28-diol**264**

Compound **264** afforded as white powder from the *V. grandiflorum* chloroform fraction of the crude extract of the aerial parts. The EI-MS exhibited molecular mass m/z 442 a.m.u and HR-EIMS showing exact molecular formula $C_{30}H_{50}O_2$ for 442.3816 (calcd. 442.3811). The structure of the **264** elucidated as lup-20(29)-ene-3 β , 28-diol (betulin) on comparison with reported data in literature.²⁶

3.8.9 *p*-Hydroxy cinnamic acid (**229**)**229**

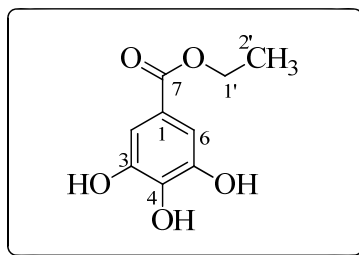
Compound **229** was afforded as white amorphous powder from the chloroform faction of crude ethanolic extract of *V. grandiflorum* aerial parts. The molecular mass m/z 164 a.m.u ($C_9H_8O_3$) was established by HR-EI-MS. The structure of **229** elucidated as *p*-hydroxy cinnamic acid (**229**) on the bases of NMR spectral data (**Table: 3.8**; *vide experimental*) and by comparison with reported data in literature.²⁷

3.8.10 3, 4, 5-Trihydroxy benzoic acid; 3, 5-di-Me ether 4-*O*- β -D-glucopyranoside (265)



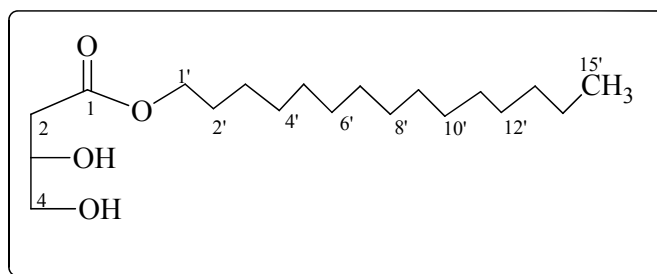
265

Compound **265** isolated as colorless amorphous powder from the ethyl acetate fraction of the crude ethanolic extract of aerial parts of *V. grandiflorum*. EI-MS and elemental analysis measurement HR-EIMS showed the molecular formula as $C_{15}H_{20}O_{10}$. The compound **265** structure characterized as 3, 4, 5-trihydroxy benzoic acid; 3,5 di-Me ether 4-*O*- β -D-glucopyranoside on the bases of NMR spectral data (**Table: 3.9**; *vide experimental*) and related compound data in literature.²⁸

3.9.0 Hitherto unreported constituents from the *V. grandiflorum* roots.**3.9.1 Ethyl gallate (267)****Ethyl 3, 4, 5-trihydroxy benzoate****267**

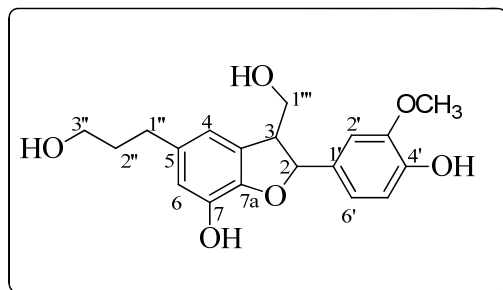
Compound **267** afforded as white amorphous powder from the chloroform fraction of roots of the *V. grandiflorum* crude ethanolic extract. EI-MS and HR-EIMS showing its formula as C₉H₁₀O₅. The structure of **267** was elucidated as ethyl 3, 4, 5-trihydroxy benzoate by comparison of mass profile and NMR spectral data (**Table: 3.10**; *vide experimental*) with reported data in literature.²⁹

3.9.2 Pentadecyl 3, 4-dihydroxy butanoate (269)

**269**

Compound **269** afforded as amorphous gummy solid from the chloroform fraction of roots of *V. grandiflorum*. EI-MS and HR-EIMS exhibited molecular mass m/z 330.0315 a.m.u ($C_{19}H_{38}O_4$) and structure of **269** elucidated as pentadecyl 3, 4-dihydroxy butanoate by matching their spectroscopic data (**Table: 4.11**; *vide experimental*) with reported compound data in literature.³⁰

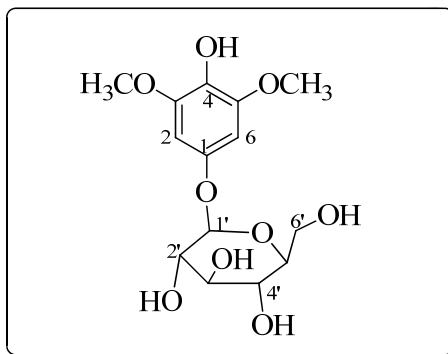
3.9.3 2-(-4'-Hydroxy-3'-methoxyphenyl)-5-(3''-hydroxypropyl)-3-hydroxymethyl-7-hydroxy methyl-7-hydroxy 2,3-dihydro benzofuran (270)



270

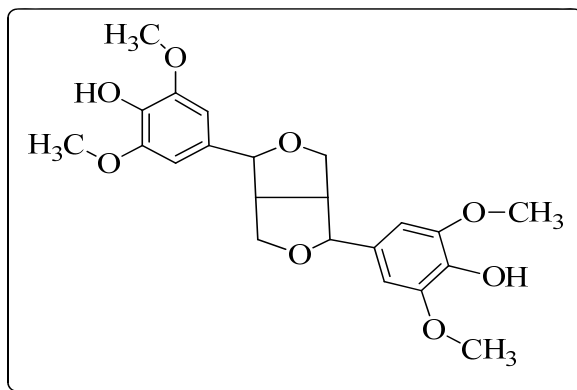
Compound **270** isolated as brownish, amorphous powder from the ethyl acetate fraction of the ethanolic extract of *V. grandiflorum* roots. The molecular ion peak observed at m/z 346 a.m.u (exact molecular mass m/z 346.1464 a.m.u) and molecular formula $C_{19}H_{22}O_6$ by EI-MS and HR-EIMS. The structure of **270** characterized as 2-(-4'-hydroxy-3'-methoxyphenyl)-5-(3''-hydroxypropyl)-3-hydroxymethyl-7-hydroxy 2, 3-dihydro benzofuran by comparing NMR spectral data (**Table: 4.12**; *vide experimental*) and related data in literature.²⁴

3.9.4 Koaburaside (271)

3,5-Dimethoxy-4-hydroxy-1-*O*- β -D- glucoside**271**

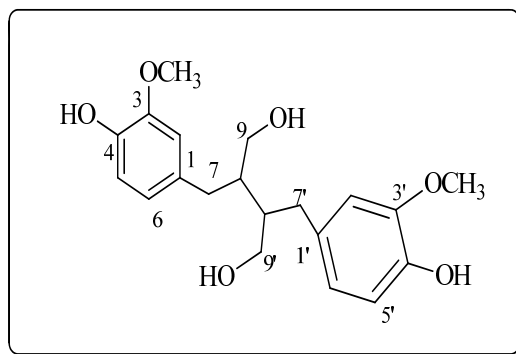
Compound **271** afforded as colorless, amorphous powder from the ethyl acetate fraction of the crude ethanolic extract of *V. grandiflorum* roots. The molecular ion peak observed at m/z 333 a.m.u in the ESI-MS (+TOF MS) and its molecular formula was assigned $C_{14}H_{20}O_9$ by HR-EIMS which gave exact molecular mass as m/z 332.0415 a.m.u. The compound **271** structure was elucidated as 3, 5-dimethoxy-4-hydroxy-1-*O*- β -D-glucoside (**Table: 4.13**; *vide experimental*) and by comparison with related data in literature.³¹

3.9.5 (+) Syringaresinol (272)

**272**

Compound **272** isolated as colorless needless solid from the *V. grandiflorum* ethyl acetate fraction of the roots extract. Molecular mass and molecular formula investigated from HR-EIMS as m/z 418.1623 a.m.u [M^+] ($C_{22}H_{26}O_8$). The compound **272** structure was confirmed as (+) syringaresinol on the bases of NMR spectral data (**Table: 3.14**; *vide experimental*) and comparison with data in literature.^{32, 33}

3.9.6 Secoisolariciresinol (273)

**273**

Compound **273** isolated as white amorphous solid from ethyl acetate fraction of roots crude ethanolic extract of *V. grandiflorum*. Molecular mass m/z 362.0315 a.m.u. and molecular formula ($C_{20}H_{26}O_6$) established from HR-EI-MS. The structure of **273** was elucidated as secoisolariciresinol from NMR experiments data (**Table: 3.15**; *vide experimental*) and comparison with reported data in literature.³⁴

3.10.0 Biological activity

3.10.1 Phytochemical screening of *viburnum grandiflorum*.

The subsequent fractions of the crude ethanolic extract of aerial parts and roots were tested for their phytochemical constituents. Preliminary phytochemical profiling indicated the presence of different classes of secondary metabolites such as alkaloids, steroids, terpenoids, flavonoids etc. Different fractions of aerial parts and roots having different classes of secondary metabolites (**Table: 3.1** and **Table: 3.2**).

Table: 3.1 Phytochemical screening of *V. grandiflorum* stem extracts

S.No	Constituents	<i>n</i> -Hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	-	-	-
2	Terpenoids	-	+	+	+
3	Flavonoids	-	-	-	-
4	Anthraquinones	-	+	+	-
5	Tannins	-	+	+	+
6	Plobotanins	-	-	-	-
7	Saponins	-	+	+	-
8	Glycoside	+	+	+	+
9	Reducing sugars	-	-	-	-
10	steroids	+	+	-	-

Key: - = absent, + = present

Table: 3.2 Phytochemical tests of *V. grandiflorum* root extracts

S.No	Constituents	<i>n</i> -Hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	-	+	-
2	Terpenoids	-	+	+	+
3	Flavonoids	-	-	+	+
4	Anthraquinones	-	+	+	+
5	Tannins	-	+	+	+
6	Plobotanins	-	-	-	-
7	Saponins	-	+	+	-
8	Glycoside	+	+	+	+
9	Reducing sugars	-	-	-	-
10	steroids	+	+	-	-

Key: - = absent, + = present

3.10.2 Antibacterial activity

The plant extracts (stem, roots) were subjected to antibacterial activity for exploring its bioactive chemical constituents. The well-diffusion assay was used for the investigation of the antibacterial activity of *V. grandiflorum* extracts (stem, roots) against selected microorganisms. The plant extracts of *V. grandiflorum* showed significant reduction in bacterial growth in the term of zone of inhibition, indicating that plant extracts exhibited significant antibacterial activity against tested strains of microorganisms. The zone of inhibition was measured, recorded and presented in tables (**Table: 3.3** and **Table: 3.4**). The different extracts of the plant showed significant activity against the selected bacteria such as *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumonia* and *B. subtilis*. All plant extracts (stem) showed significant activity against the five selected bacteria, while root extracts was found less active than stem extracts against these selected microorganisms. The chloroform extracts (stem and roots) exhibited inhibitory effect against all tested microorganisms with zone of inhibition ranging from 8 to 16 mm Ethyl acetate and methanol extracts exhibited significant activity with highest zone of inhibition 20 mm. however the ethyl acetate extracts of roots and stem is non active against *K. pneumonia*, *S. epidermidis*, *B. subtilis* bacteria. Methanol and *n*.hexane extracts found non active against *S. epidermidis*, but effective against microorganisms *S. aureus*, *E. coli*, *K. pneumonia* and *B. subtilis*. The recorded antibacterial bioassay data proves its importance as potential antimicrobial drug (**Table: 3.3** and **Table: 3.4**).

Table: 3.3 Antibacterial activity of *Viburnum grandiflorum* (aerial parts)

Microorganism	Gram	AL1	AL2	AL3	AL4	AL5
<i>S. aureus</i>	+	15	14	14	20	28
<i>S. epidermidis</i>	+	NA	10	12	NA	30
<i>E. coli</i>	-	15	16	14	14	32
<i>K. pneumonia</i>	-	14	8	NA	20	28
<i>B. subtilis</i>	+	12	13	20	14	26

Table: 3.4 Antibacterial activity of *Viburnum grandiflorum* (roots)

Microorganism	Gram	AL1	AL2	AL3	AL4	AL5
<i>S. aureus</i>	+	NA	12	14	10	28
<i>S. epidermidis</i>	+	12	10	NA	12	30
<i>E. coli</i>	-	12	12	16	13	32
<i>K. pneumonia</i>	-	NA	8	NA	8	28
<i>B. subtilis</i>	+	14	12	NA	14	26

Concentration of stock solution 3 mg/mL and 100 μ L was used for assay

Keywords: AL1=*n*-hexane, AL2=Chloroform, AL3=Ethyl acetate, AL4= Methanol, AL5= Streptomycin, NA= Not active

3.10.3 Antifungal activity

The crude ethanolic extract and succeeding solvent fractions of the roots and aerial parts of *V. grandiflorum* were tested against various strains of fungi i.e. *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *Candida glabrata* as shown (Tables: 3.5, 3.6 and 3.7). The crude extract and chloroform fraction of the roots was not active against any of the tested fungi, while *n*-hexane fraction was not active against *M. canis* having 35% zone of inhibition and ethyl acetate fraction were active against *F. solani* with zone of inhibition 10%. The antifungal activity of the aerial parts against the mentioned fungi is presented in table: 3.6. It is interesting that the ethyl acetate fraction has the most significant antifungal effect against *F. solani* with 30 % zone of inhibition while the crude extract was 10% active against *M. cani*. The *n*-hexane and chloroform fractions of the aerial parts were non active against any of the test fungus. The crude

methanolic extract and ethyl acetate fractions of both parts can be used in the management of mycosis caused by *M. canis* and *Fusarium* species

The antifungal effect of both tested oils is presented in **Table: 3.7**. The VSO-01 was only effective against *A. flavus* and VSO-02 was against *M. canis*.

Table: 3.5 Antifungal activity of *V. grandiflorum* roots.

Name of fungus	<i>n</i> -Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction	Miconazole
<i>T. longifusus</i>	-	-	-	-	-
<i>C. albicans</i>	-	-	-	-	110.8
<i>A. flavus</i>	-	-	-	-	-
<i>M. canis</i>	35	-	-	-	98.4
<i>F. solani</i>	-	-	10	-	73.25
<i>C. glabrata</i>	-	-	-	-	110.8

Table: 3.6 Antifungal activity of *V. grandiflorum* aerial parts.

Name of fungus	<i>n</i> -Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction	Miconazole
<i>T. longifusus</i>	-	-	-	-	-
<i>C. albicans</i>	-	-	-	-	110.8
<i>A. flavus</i>	-	-	-	-	-
<i>M. canis</i>	-	-	-	30	98.4
<i>F. solani</i>	-	-	10	-	73.25
<i>C. glabrata</i>	-	-	-	-	110.8

Table: 3.7 Antifungal activity of *V. grandiflorum* oils (aerial parts)

Name of fungus	VSO-01	VSO-02	Control	Std drug Mic µg/mL
	Zone of Inhibition (mm)			
<i>C. albicans</i>	-	-	100	Miconazole= 110.8
<i>A. flavus</i>	75	-	100	Amphotericin B =20.2
<i>M. canis</i>	-	75	100	Miconazole = 98.4
<i>F. solani</i>	-	-	100	Miconazole =73.25
<i>C. glabrata</i>	-	-	100	Miconazole =110.8

Key words: VSO-01 and VSO-02 sample Conc. 400 µg/mL

3.10.4 Phytotoxic activity

The crude methanolic extract along with subsequent solvent fractions of root and aerial part of *V. grandiflorum* were tested for their phytotoxic activity. The *n*-hexane, chloroform and methanolic fraction of the roots were significant phytotoxic having 70, 75 and 80% inhibition respectively as shown in **table: 3.8**. The *n*-hexane and chloroform fractions were the most significant as it shown the phytotoxicity at lower dose. In case of aerial parts the most significant phytotoxic effect was shown by ethyl acetate with 85% inhibition followed by methanolic extract with 80% growth inhibition as shown in **table: 3.9**. The *n*-hexane and chloroform fractions were significant at higher doses. The phytotoxic effect of the roots was weaker than aerial parts which are summarized in **table: 3.8** and **table: 3.9**.

The oils from aerial parts and roots showed significant phytotoxic activity (**table: 3.10** and **table: 3.11**).

Table: 3.8 Phytotoxic activity of *V. grandiflorum* roots.

Extracts/fractions	No of fronds	Percent growth inhibition		
		10 ppm	100 ppm	1000 ppm
Control	20	-	-	-
<i>n</i> -Hexane	20	10	20	80
Chloroform	20	10	25	75
Ethyl acetate	20	-	5	30
Methanolic	20	-	15	70

Table: 3.9 Phytotoxic activity of *V. grandiflorum* aerial parts.

Extracts/fractions	No of fronds	Percent growth inhibition		
		10 ppm	100 ppm	1000 ppm
Control	20	-	-	-
<i>n</i> -Hexane	20	-	-	75
Chloroform	20	-	35	75
Ethyl acetate	20	10	25	85
Methanolic	20	10	15	80

Table: 3.10 Phytotoxic activity of *V. grandiflorum* oil (aerial parts)

Name of plant	Conc. Of compound (µg/ml.)	No. of Fronds		% Growth Regulation	Conc. of std. drug (µg/ml.)
		Sample VSO-01	Control		
<i>Lemna minor</i>	1000	12	20	40	0.015
	100	14	20	30	
	10	17	20	15	

Table: 3.11 Phytotoxic activity of *V. grandiflorum* oil (aerial parts)

Name of plant	Conc. Of compound (µg/ml.)	No. of Fronds		% Growth Regulation	Conc. of std. drug (µg/ml.)
		Sample VSO-02	Control		
<i>Lemna minor</i>	1000	10	20	50	0.015
	100	13	20	35	
	10	15	20	25	

3.10.5 Insecticidal activity

The crude methanolic extract of roots was better insecticidal against all the three tested insects as shown in **table: 3.13**. *Callosbruchus analis* and *Rhyzopertha dominica* were sensitive for all the applied samples while *Tribolium castaneum* was resistant to the tested samples except methanolic extract. The crude methanolic extract of the aerial parts was the most significant insecticidal against all tested extract samples as presented in **table: 3.12**. The crude methanolic extract of the aerial part showed 20, 40 and 40% mortality against *T. castaneum*, *C. analis* and *R. dominica* respectively. The *n*-hexane, chloroform and ethyl acetate fractions showed 20% mortality against *C. analis* and *R. dominica*. All the tested samples were significant insecticidal against *R. dominica*. The *n*-hexane fraction of roots was failed to produce any insecticidal effect, while the chloroform fraction was effective against *C. analis* and *R. dominica* with 20% mortality. The crude methanolic extract and its ethyl acetate fraction showed 40% mortality *C. analis* and *R. dominica*. The use of

methanolic extract as insecticidal for the protection of flour, meals, crackers etc is strongly recommended. As the crude extract is phytotoxic and insecticidal which act both as weedcidal and insecticidal. The crude ethanolic extract is very interesting on agriculture point of view as having double action at the same time.

No insecticidal effect was demonstrated by tested oils as shown in **Table: 3.14**.

Table: 3.12 Insecticidal activity of the *V. grandiflorum* aerial parts.

Extracts/fractions	Percent mortality		
	<i>T. castaneum</i>	<i>R. dominica</i>	<i>C. analis</i>
Control	-	-	-
<i>n</i> -Hexane	-	20	40
Chloroform	-	20	20
Ethyl acetate	-	20	40
Methanolic	20	40	40

Table: 3.13 Insecticidal activity of the *V. grandiflorum* roots.

Extracts/fractions	Percent mortality		
	<i>T. castaneum</i>	<i>R. dominica</i>	<i>C. analis</i>
Control	-	-	-
<i>n</i> -Hexane	-	-	-
Chloroform	-	40	20
Ethyl acetate	20	40	40
Methanolic	20	40	40

Table: 3.14 Insecticidal activity of oil from aerial parts of *V. grandiflorum*

Name of insect	+ve control	-ve control	VSO-01	VSO-02
	% Mortality			
<i>T. castaneum</i>	100	-	-	-
<i>R. dominica</i>	100	-	-	-
<i>C. analis</i>	100	-	-	-

Keywords: Std = standard (permithrin =239.5 µg/cm²), SP= Sample (1019.10 µg/cm²)

3.10.6 Antileishmanial activity of *V. grandiflorum* oils

Both of the tested oils exhibited a moderate leishmanicidal effect. In comparison with positive control the effect was not significant as presented in **table: 3.15**.

Table: 3.15 Antileishmanicidal activity of *V. grandiflorum* oils.

Leishmanicidal activity		VSO-01	VSO-02
		IC ₅₀ (μg/mL)±SD	
Test compound		77.7± 0.1	70.8±0.2
Standard drugs	Amphotericin B	0.29±0.05	0.29±0.05
	Pentamidine	5.09±0.04	5.09±0.04

3.10.7 Xanthine oxidase inhibitory activity

The xanthine oxidase inhibition effect of the tested compound is presented in the **Table: 3.15**. In comparison with positive control (Allupurinol) our tested molecule exhibited a moderate effect with IC₅₀ value 49.52±0.03 μM.

Table: 3.16 Xanthine oxidase inhibitory activity of **262**

Compounds	IC ₅₀ (μM)
Benzofuran	49.52±0.03
Allopurinol	0.59±0.01

3.10.8 Antinociceptive activity

V. grandiflorum crude extract and compound **270** were scrutinized for their antinociceptive potentials using chemical and thermal induced pain paradigm. The antinociceptive results (**Table: 3.15**) revealed ethanolic extract (150 mg/kg) and compound **270** (10 mg/kg) were found to be active in attenuating (70.45 and 82.11 %) the acetic acid induced writhing in mice. In comparison with the analgesic effect (87.23%) of diclofenac sodium (10 mg/kg) the effect of ethanolic extract was somewhat lesser while **270** was dominant antinociceptive. In addition to the pain protected in acetic acid induce experiment both of the tested samples were failed (data

not presented) to increase the latency time of mice in hot plate pain model clearly explain that ethanolic extract has peripheral antinociceptive effect due to the presence of **270**.

Table: 3.17 Antinociceptive activities of ethanolic extract and **270** as determined by the acetic acid induced writhing test in mice.

Samples	Dose (mg/kg)	Percent analgesia
Normal saline	10 mg/kg	-
Diclofenac sodium	10	87.23 $\pm$ 0.44 ***
Ethanolic extract	50	23.26 $\pm$ 1.43*
	100	40.99 $\pm$ 2.98*
	150	72.09 $\pm$ 2.11**
BFD	5	70.45 $\pm$ 2.98**
	10	82.11 $\pm$ 2.88***

3.10.9 Anti-inflammatory activity

The anti-inflammatory effect of the ethanolic extract (50, 100 and 150 mg/kg) and **270** (5 and 10 mg/kg) is presented in **fig: 3.7** and **fig: 3.8** respectively. A dose dependant protection of paw edema was noticed with both tested samples. Ethanolic extract (150 mg/kg) showed anti-inflammatory effect at first (% protection, 60.33, 1st h) and second stage (% protection, 71.34, 4th h) of inflammation, while the anti-inflammatory potential of **270** (10 mg/kg) was weaker than at both stages (% protection, 41.45, 1st h and 54.47 at 4th h). Diclofenac sodium exhibited very strong effect as compared to both tested samples. The crude extract of the roots as well as its isolated compound demonstrated parallel anti-inflammatory results i.e. the protection of induced oedema was weak in the first phase of inflammation as compared to the second phase.

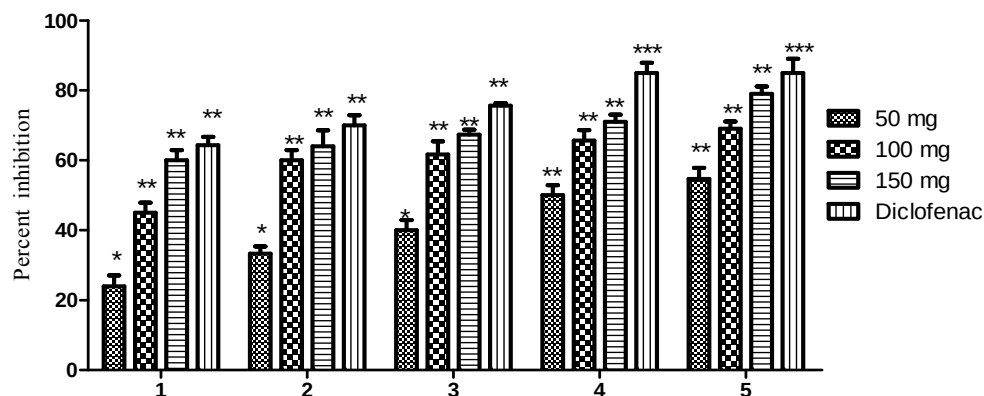


Fig: 3.7 Anti-inflammatory effect of ethanolic extract against carrageenan paws oedema in NMRI mice.

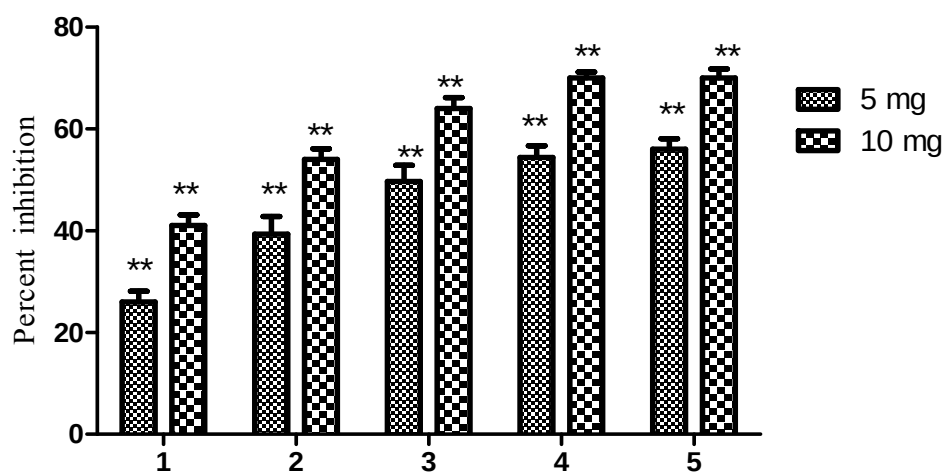


Fig: 3.8 Anti-inflammatory effect of 270 against carrageenan paws oedema in NMRI mice.

3.10.10 Antipyretic activity

The maximum antipyretic effect (71.78%) was exhibited by the ethanolic extract (150 mg/kg) at the 3rd h of post treatment (**Fig: 3.9**), while the pyrexia reducing potential of 270 (10 mg/kg) at the same time was 41.68% (**Fig: 3.10**). Both of tested samples demonstrated attenuation of temperature in fibril mice from the 1st h of post treatment which was maintained throughout the experiment. The fever reducing effect of paracetamol was higher than both of the samples. Regarding the antipyretic effect

of the crude and isolated compound, both significantly attenuated the pyrexia of fibril mice dose dependently.

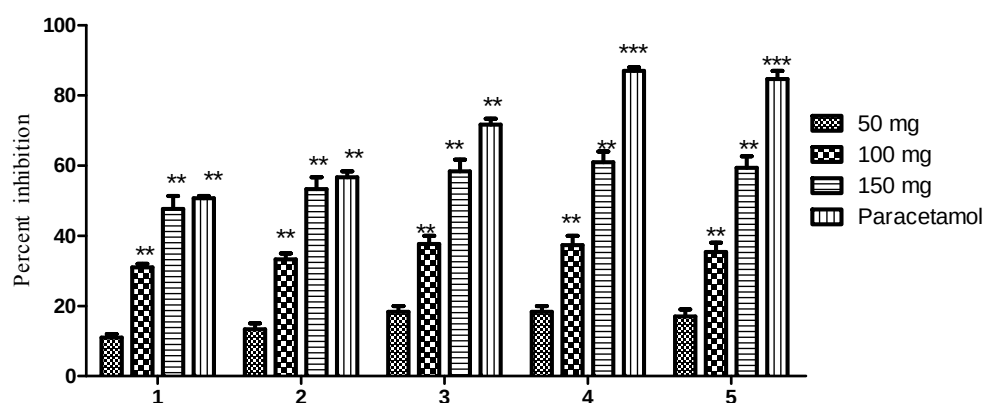


Fig: 3.9 Antipyretic effect of ethanolic extract against brewer's yeast induced pyrexia in NMRI mice.

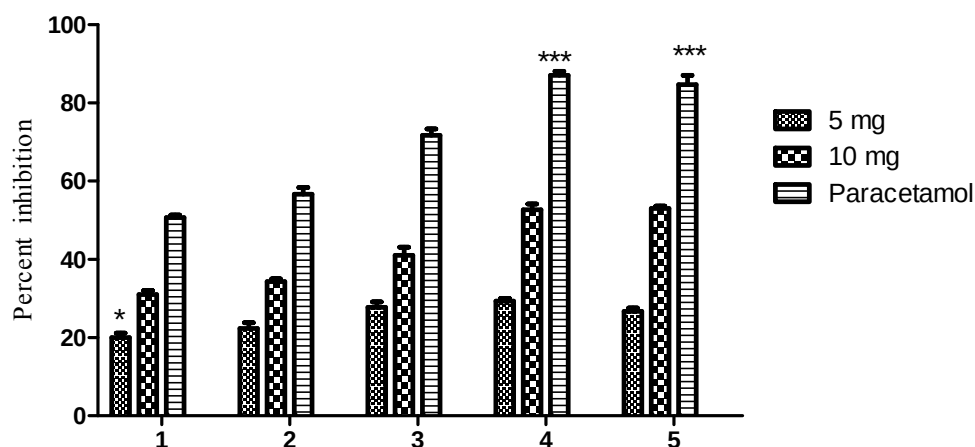


Fig: 3.10 Antipyretic effect of **270** against brewer's yeast induced pyrexia in NMRI mice.

3.10.11 Sedative effect

No significant sedative effect was observed in both of the tested samples ethanolic extract and compound **270**.

3.10.12 Muscle relaxant effect

In rota rod test the compound **270** demonstrated mild muscle relaxant effect as presented in **Fig: 3.11** and the crude extract was devoid of this potential.

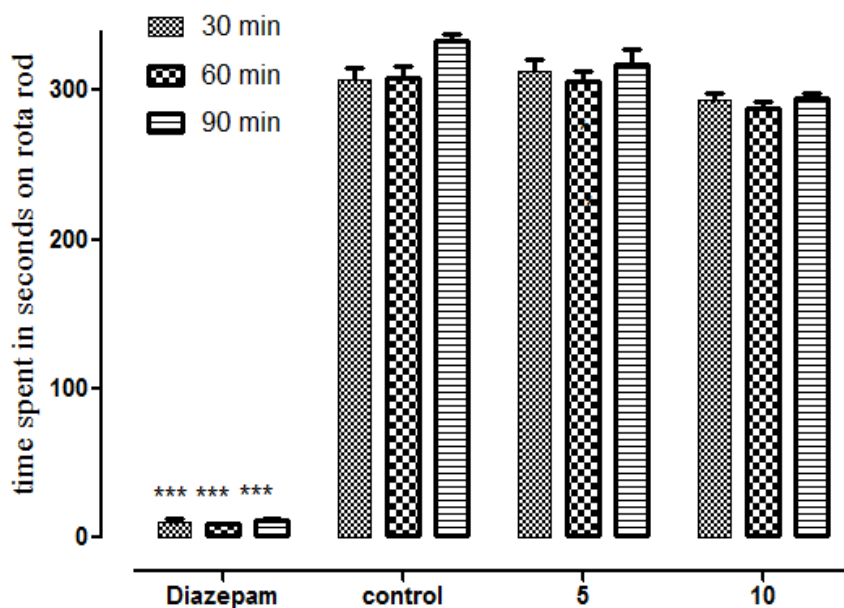


Fig: 3.11 Effects of compound **270** on muscle coordination in rota rod, bars represent the time spent in seconds on rota rod, after 30, 60 and 90 minutes. * $P<0.05$, * $P<0.01$ and *** $P<0.001$.

3.10.13 Anticonvulsant effects

No anticonvulsant effect was observed for both tested samples ethanolic extract and **270**.

3.11.0 References

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4.1 General experimental procedures

Mass spectra were recorded on Finnigan-Mat-311 and Varian MAT 312 mass spectrometer at 250 °C and 70 eV, while Jeol JMS-600H mass spectrometer was used for the measurement of HREIMS (High Resolution Electron Impact mass spectrum), PFK was used as internal standard. For FAB (+ve) mass spectra, JMS HX-110 double focusing mass spectrophotometer used with glycerol (target matrix), methanol (solvent) and Xenon gas as ionization sample. JASCO-A-302 spectrophotometer was used for IR spectra, while Hitachi-U-3200 and SECOMAN spectrophotometers ANTHJRPC Version 4.1h used for the measurement of UV spectra. The measurement of optical rotation of the pure compounds, JASCO-DIP-360 digital polarimeter was used. The ^1H NMR spectra were recorded on Bruker AVANCE 600, 500, 400, 300 spectrometers at 500, 400, 300 MHz were used respectively, while ^{13}C NMR on Bruker AVANCE 600 spectrometer (150 MHz) and Bruker AVANCE 500 spectrometer (125 MHz) using TMS as an internal standard. 2D NMR techniques (HMBC, HSQC, NOESY, COSY, *J*-resolved) of the purified chemical constituents were taken on Bruker AVANCE 600, 500, 400 MHz spectrometer. 1D and 2D TOCSY (total correlation spectroscopy) spectra were recorded on Bruker AVANCE 600.

TLC grade silica gel 60 GF₂₅₄ (Merck) was used for vacuum liquid chromatography (VLC) and various chromatography techniques were used to isolate pure chemical constituents from the crude and semi crude fractions i.e. RP-18 (Merck), Sephadex LH-20, (Sigma-Aldrich), silica gel 60 (70-230 mesh). Silica gel 60 F₂₅₄ on aluminum sheets (Merck, 0.2 mm thickness) and silica gel RP-18 F₂₅₄ coated on glass plate (Merck, 0.25 mm thickness) used for preparative TLC. UV (254 and 365 nm), spraying with 1% Ce

(SO₄)₂ in 10% aqueous H₂SO₄ and I₂ vapours were used for the detection of chemical constituents. HPLC (JAIGEL-ODS-M80) instrument was used for purification of chemical constituents over mobile phase methanol : water (1:1) and flow rate maintained 3.0 mL/min during the recycling preparative (RP) HPLC operation. The peaks observed at 254 nm.

Melting point apparatus Buchii 535 was used for the measurement of melting point of pure chemical constituents. HPLC/analytical grade solvents; CDCl₃, C₃D₆O and CD₃OD and Pyridine solvents were used for recording EIMS, HREIMS, ESIMS, NMR, IR, UV, optical rotation spectral data etc.

4.2 Plant material

V. grandiflorum aerial parts (stem and branches) and roots were collected from Mira Mir Ahmad Khan (Tandyani) Hazara in the month of July 2009 and identified by eminent taxonomist Abdul Majid lecturer in Botany, Department of Botany University of Hazara, and verified by Prof. Dr. Abdur Rashid department of botany, university of Peshawar and a voucher specimen number 02 was deposited in the Hazara University Herbarium.

4.3 Extraction and isolation

The shade dried aerial parts (14.5 kg) of *V. grandiflorum* were pulverized to powder and (×3) repeatedly weekly extracted with ethanol at room temperature. The combined ethanolic crude extract was concentrated under reduce pressure in rotavapor resulted brownish gummy residue 1.5 kg was obtained and partitioned between *n*-hexane and water to remove *n*-hexane soluble fraction (365 g). The *n*-hexane insoluble fraction was further partitioned with chloroform, ethyl acetate and butanol to obtained chloroform

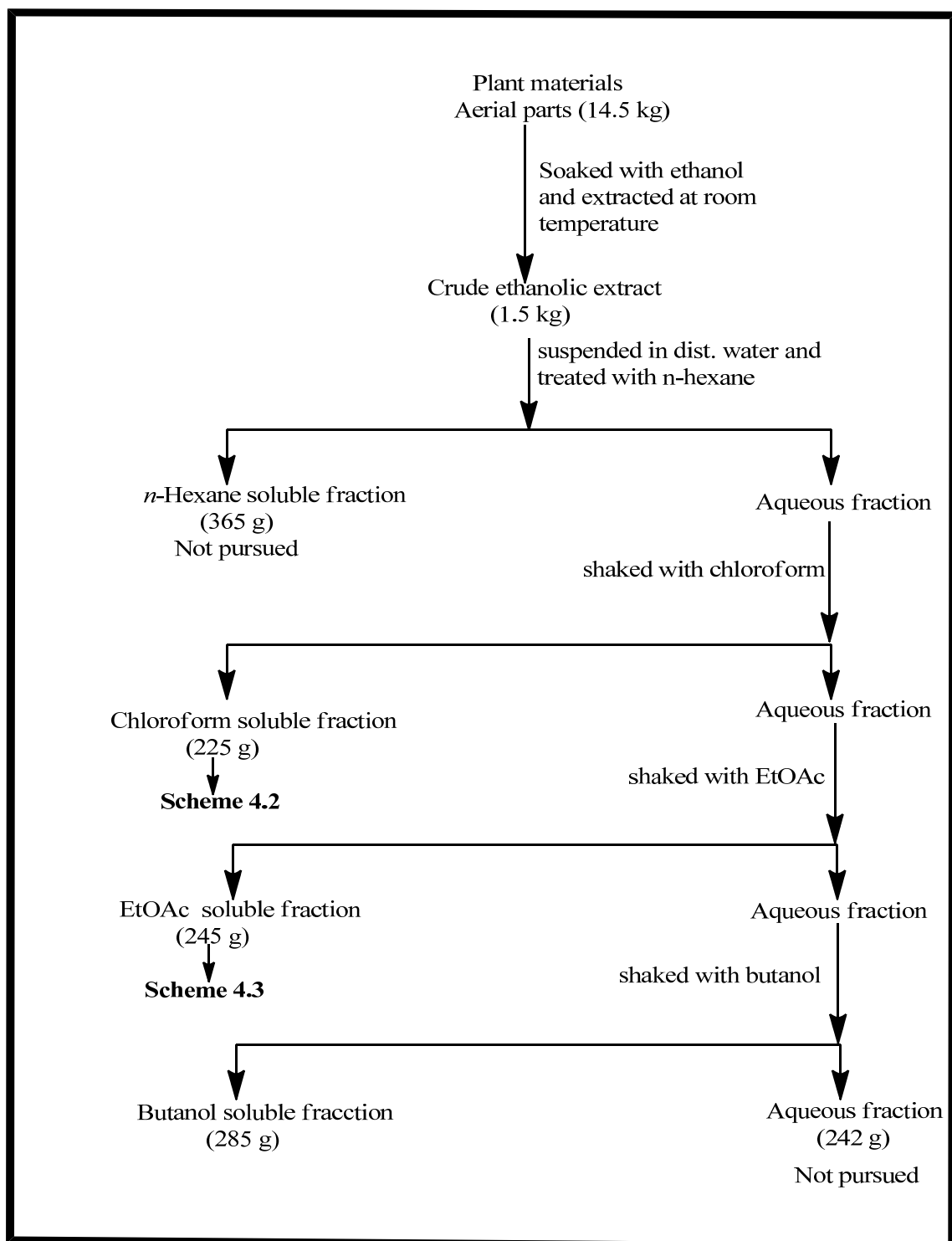
soluble fraction (225 g), ethyl acetate soluble fraction (245 g) and butanolic fraction (285 g) which were concentrated under reduce pressure.

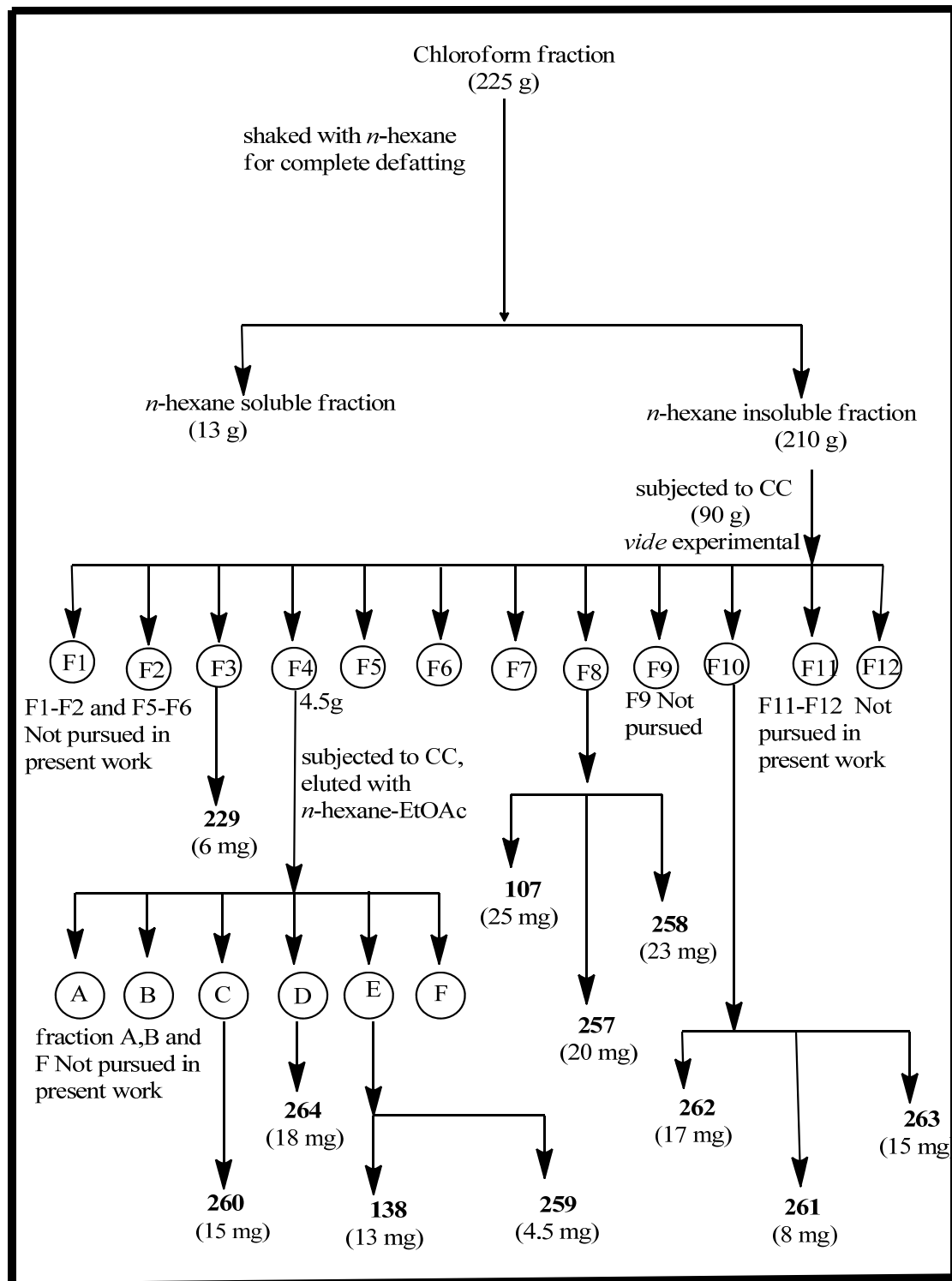
The chloroform fraction (225 g) was again shaken with *n*-hexane to remove as possible *n*-hexane soluble portion. A portion of defatted chloroform fraction (90 g) was subjected to column chromatography (silica gel, 90 mm) and eluted with *n*-hexane-EtOAc with increasing polarity obtained 150 fractions of 20 ml each were collected and compiled to 12 fractions (F₁-F₁₂) based on TLC profile. Fraction F₈ (5 g) has three major spots on TLC which was purified by thin column (30 mm) of silica gel followed by preparative TLC and furnished three major compounds betulinic acid (**257**), ursolic acid (**107**) and β - sitosterol (**258**) afforded (**Scheme 4.2**). The fraction F₄ (4.5 g) was also subjected to CC eluted with ethyl acetate : *n*-hexane with increasing polarity (1:9, 2: 8, 3:7, 4:6) afforded six sub-fractions A-F. Fraction E yielded two pure compounds oleanolic acid (**138**) and sesquiterpine per oxide (**259**) while fraction C and D gave two pure compounds; stigmasterol (**260**) and betulin (**264**) respectively. Glucoside of β - sitosterol (**261**), benzofuran derivative (**262**) and Grandinoid (**263**) were obtained from the fraction F₁₀ followed by column chromatography, subsequently fraction F₃ yielded *p*-hydroxycinnamic acid (**229**).

Ethyl acetate soluble fraction (60 g) of the aerial parts were subjected to CC (*n*-hexane-ethyl acetate in increasing order of polarity) afforded six sub-fractions (E₁-E₆). E₂ (8.5 g) was further divided into six sub-fractions EG₁-EG₆ followed by CC. one chemical constituent (**265**) has been purified from fraction EG₃ followed by preparative TLC while a pure compound grandifloruside (**266**) obtained from sub-fraction EGd of fraction E₅ by means of preparative TLC (**Scheme 4.3**).

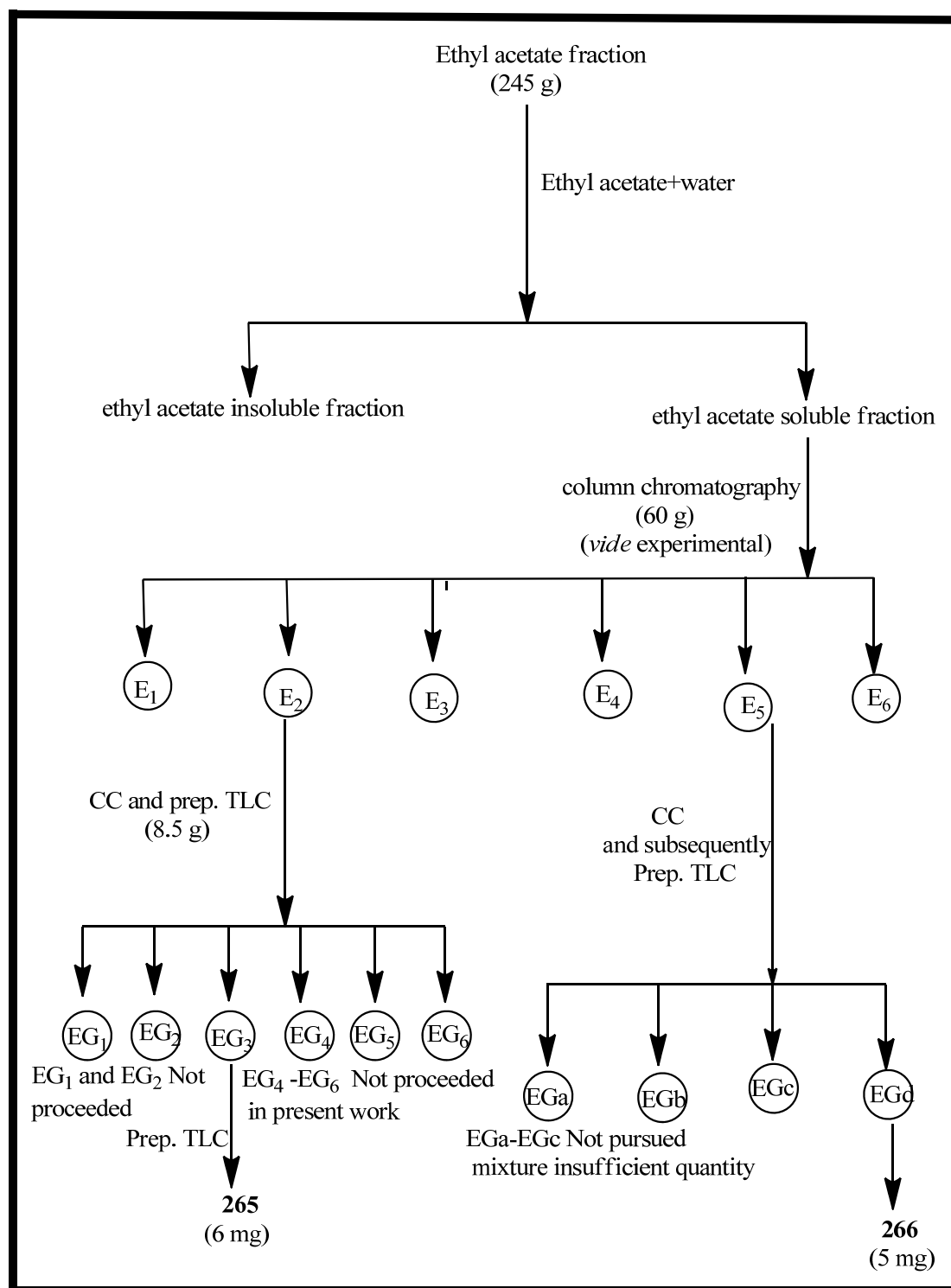
V. grandiflorum roots extract fractionated as given in the (Scheme 4.4). The chloroform fraction (125 g) defatted with *n*-hexane and chloroform soluble portion 50 g was subjected to CC and eluted with *n*-hexane, *n*-hexane-EtOAc solvent system with increasing order of polarity. Ten fractions RC₁ to RC₁₀ obtained from this process. The sub-fraction RC₃ was further processed i.e. CC, prep. TLC and afforded (267) as pure compound while RC₄ furnished a new cerbuside (268). The compound (269) purified from fraction RC₅ by means of CC, preparative TLC followed major spot on TLC while remaining fractions not pursued in present work (Scheme 4.5).

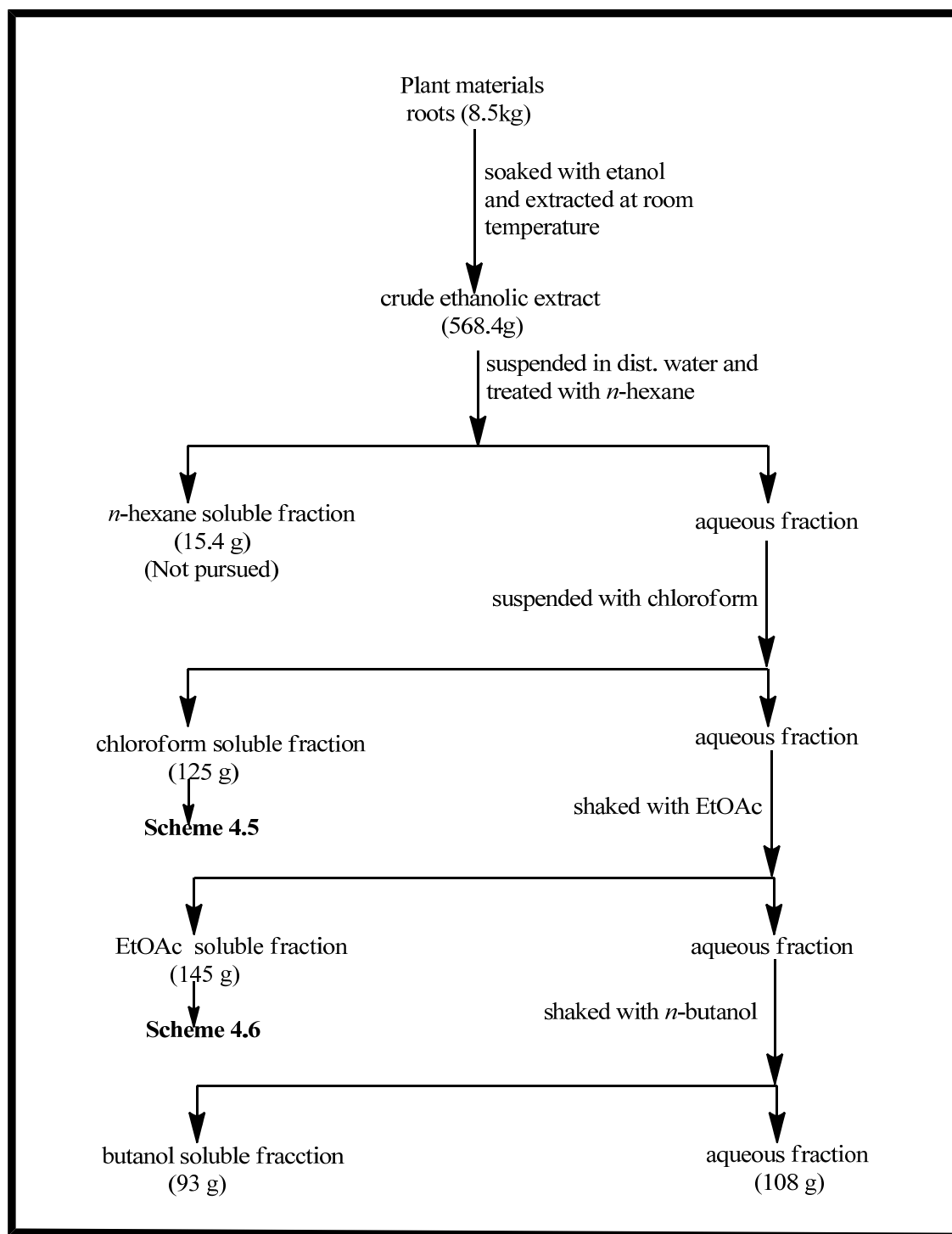
Ethyl acetate fraction (145 g) subsequently divided into two parts, ethyl acetate soluble and insoluble on redissolving in EtOAc. The EtOAc soluble fraction 60 g subjected to CC and seven sub-fractions RE₁ to RE₇ compiled on the basis of TLC profile. The fraction RE₁ was subjected to CC and prep. TLC using 4% MeOH: DCM solvent system furnished three pure compounds; cedrusin (270), koaburaside (271) and (+) syringaresinol (272). Fraction RE₂ was subjected to CC which resulted two pure compounds; secoisolariciresinol (273) and grandiflorinol (274). One pure compound grandifloronin (275) obtained from RE₄ using CC, prep. TLC and RP-18 (Scheme 4.6).

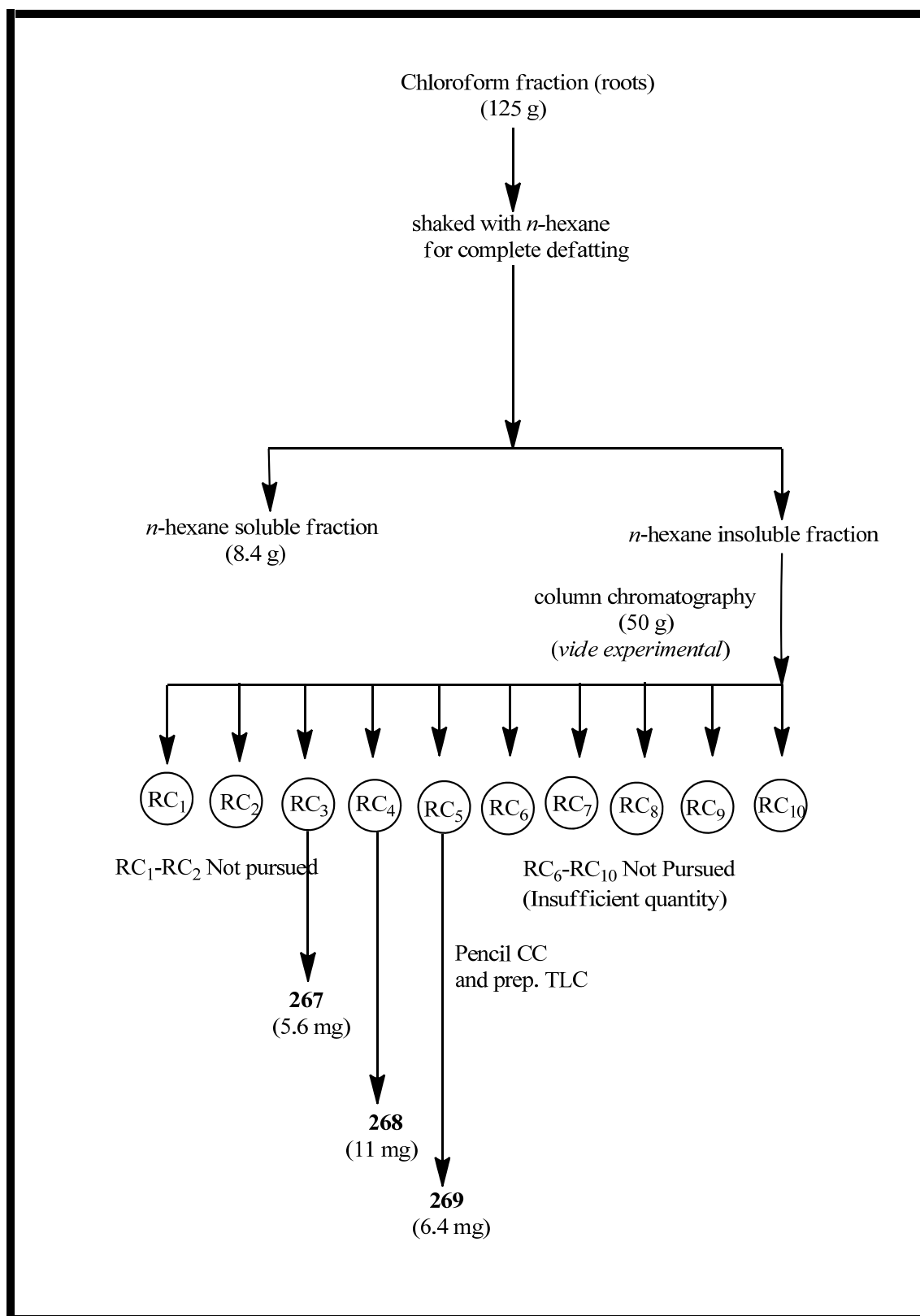
**Scheme 4.1** Extraction and fractionation (aerial parts)

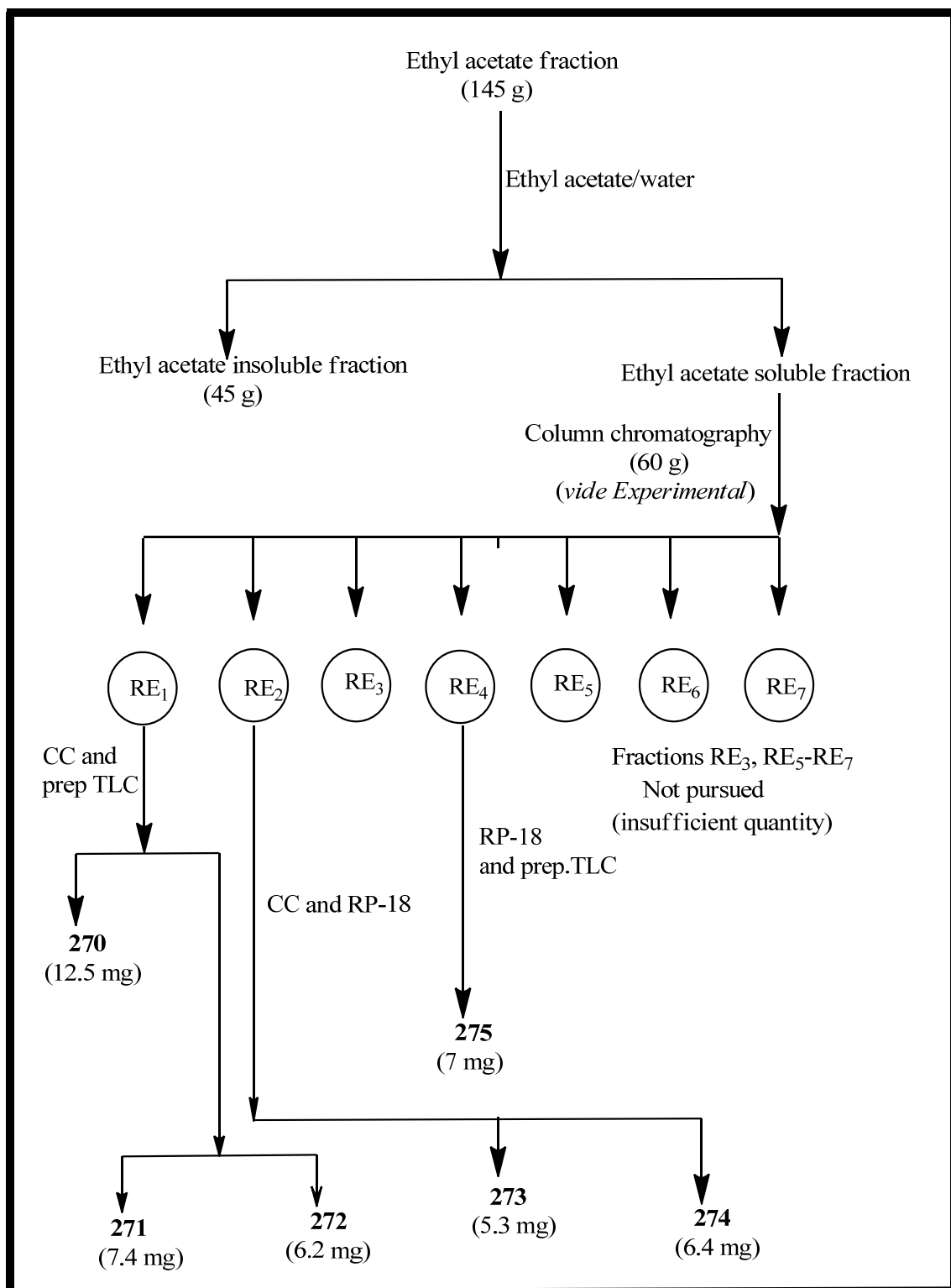


Scheme 4.2 Fractionation, isolation and purification (chloroform fraction)

**Scheme 4.3** Fractionation, isolation and purification (Ethyl acetate fraction)

**Scheme 4.4** Extraction and fractionation (roots)

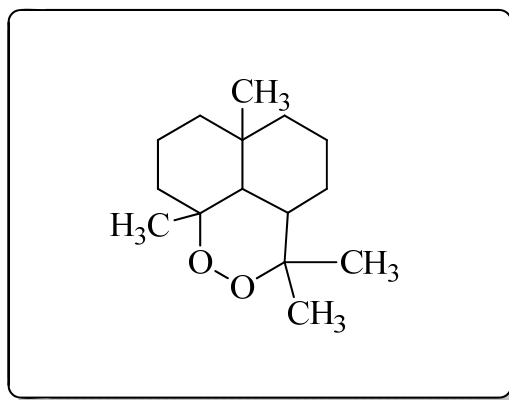
**Scheme 4.5** Fractionation, isolation and purification (Chloroform fraction)

**Scheme 4.6** Fractionation, isolation and purification (Ethyl acetate fraction)

4.4.0 Characterization of constituents from *V. grandiflorum*

4.4.1 New compounds from aerial parts of *V. grandiflorum*

❖ Grandino, 5, 8. peroxide (259)

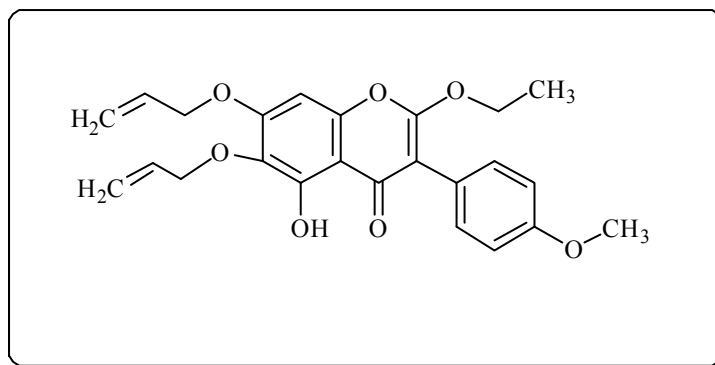


Physical data:

Physical state:	White amorphous powder
Yield:	4.5 mg
EI-MS (m/z):	234 a.m.u.
Molecular formula:	$C_{15}H_{22}O_2$
HR-EIMS:	234.1873 (calcd. 234.1867)
IR $\nu_{\max}$ cm^{-1} ($CHCl_3$):	2915 (C-H aliphatic stretching)
EI-MS m/z (rel int. %):	213(10), 157(23.4), 149(48.7), 123(22), 111(29), 85(69), 71(68), 57(85), 43(100).

Table: 4.1 ^1H and ^{13}C NMR (in CDCl_3) data of compound **259**

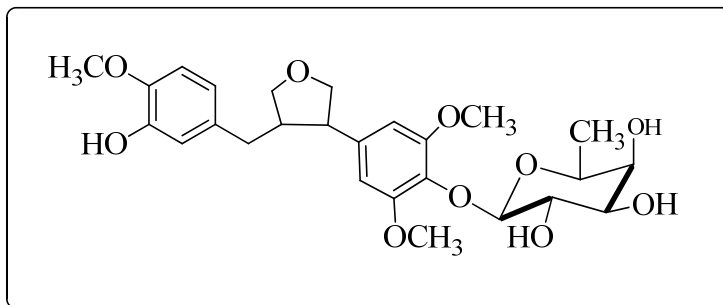
S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	34.5	C	-
2	1.36, t, 13	41	CH_2	C-3, C-11, C-6, C-1
3	1.54, m	20.1	CH_2	C-2, C-4, C-5
4	1.79, m	43.5	CH_2	C-5, C-3, C-6, C-13
5	-	72.3	C	-
6	1.18, d, 11.5	54.8	CH	C-1, C-5, C-7, C-9
7	1.34, d, 5.4	49.9	CH	C-6, C-8
8	-	72.9	C	-
9	1.91, m	21.5	CH_2	C-7, C-10, C-6
10	1.27, m	22.4	CH_2	C-11, C-9
11	1.15, t, 12.5	44.5	CH_2	C-1, C-10, C-7
12	0.84, s	18.6	CH_3	C-1, C-2, C-11, C-6
13	1.09, s	22.6	CH_3	C-4, C-5, C-6
14	1.18, s	27.1	CH_3	C-8, C-7, C-15
15	1.19, s	27.3	CH_3	C-8, C-7, C-14

❖ **Grandinoid (263)****Physical data:**

Physical state:	White amorphous powder
Yield:	15 mg
EI-MS (m/z):	424 a.m.u.
Molecular formula:	$C_{25}H_{24}O_7$
UV $\lambda_{\max}$ (ϵ) in MeOH:	282 (4.3) nm.
IR $\nu_{\max}$ cm^{-1} (CHCl_3):	2915 (C-H aliphatic stretching), 1730 (ester carbonyl), 1610 (aromatic double bonds)
EI-MS m/z (rel.int. %):	356 [$M^+ - C_6H_8 = C_{19}H_{16}O_7$] (100), 327 [$M^+ - C_8H_{13}, C_2H_5; C_{17}H_{11}O_7$] (54), 311 [$M^+ - C_8H_{13}O; C_{17}H_{11}O_6$] (8), 283 [$M^+ - C_9H_{14}O_2; C_{16}H_{10}O_5$] (4), 177 [$M^+ - C_{10}H_{12}O_3; C_9H_5O_4$] (13).

Table: 4.2 ^1H and ^{13}C NMR (in CDCl_3) of compound **263**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
2	-	148.7	C	-
3	-	126.7	C	-
4	-	174.7	C	-
4a	-	84.8	C	-
5	-	161.4	C	-
6	-	113.5	C	-
7	-	158.8	C	-
8	6.67, s	97	CH	C-8a, C-7, C-6, C-4a
8a	-	159.3	C	-
1'	-	123.3	C	-
2'/6'	7.18, dd, 7.2, 2	131	CH	C-1', C-3', C-5', C-4'
3'/5'	6.93, dd, 7.2, 2	113.7	CH	C-1', C-2', C-4', C-6'
4'	-	159.7	C	-
1''	4.14, q, 7.2	62.3	CH_2	C-2''
2''	1.01, t, 7.2	13.5	CH_3	C-1''
1'''	4.54, d, 5.6	75.3	CH_2	C-2''', C-3''', C-7
2'''	6.23, ddt, 16.4, 10, 6	131.1	CH	C-1'''
3'''	5.43, ddd, 17.2, 3.2, 1.6	118.4	CH_2	C-2'''
-	10.8, 3.2, 1.6	-	-	-
1''''	4.69, d, 4.8	70.2	CH_2	C-2'''', C-3''''
2''''	6.05, ddt, 15.6, 10, 4.8	133.6	CH	C-1''''
3''''	5.54, ddd, 17.2, 3.2, 1.2	118.6	CH_2	C-1'''', C-2''''
-	10.8, 3.2, 1.5	-	-	-
4'-OCH ₃	3.81, s	55.2	CH_3	C-4'

❖ **Grandifloruside (266)****Physical data:**

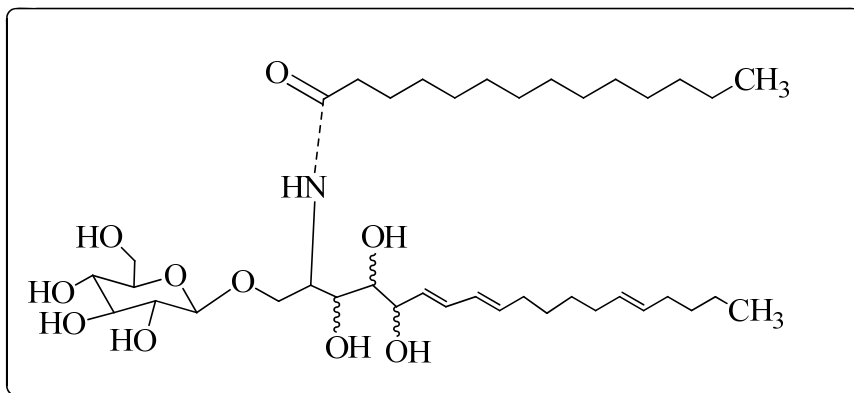
Physical state:	Brownish gummy solid
Yield:	5 mg
EI-MS (m/z):	506 a.m.u.
Molecular formula:	$C_{26}H_{34}O_{10}$
UV $\lambda_{\max}$ (ϵ) in MeOH:	210, 278 (ϵ 4.25×10^6) nm.
IR $\nu_{\max}$ cm^{-1} (CHCl_3):	2915 (C-H stretching aliphatic), 3355(O-H), 1605 (C=C), 1520, 1488 (aromatic region), 1135, 1125, 1044 for (C-O-C)
EI-MS m/z (rel.int%):	506 (2), 360 (65), 342(43), 341(55), 327 (13), 311 (100), 241 (37), 175 (51), 137(69).

Table: 4.3 ^1H and ^{13}C NMR (in MeOD) data of **266**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	134	C	-
2	6.67, s	112.3	CH	C-1, C-4, C-7, C-3
3	-	147.2	C	-
4	-	145.2	C	-
5	6.75, d, 7.8	116	CH	C-3, C-4, C-6
6	6.59, dd, 8.1, 2	123.1	CH	C-1, C-2, C-5
7	2.83, d, 7.5	33.8	CH_2	C-1, C-2, C-8, C-7', C-9
8	2.02, m	40	CH	C-7, C-7', C-9
9	3.65, m	65.3	CH_2	C-7, C-8, C-8'
1'	-	128.8	C	-
2'	6.63, d, 9.6	113.4	CH	C-1', C-4', C-7', C-3'
3'	-	149.1	C	-
4'	-	146.1	C	-
5'	-	149.1	C	-
6'	6.16, s	117.1	CH	C-1', C-4', C-5', C-2'
7'	1.88, t, 12.8	45.4	CH	C-1', C-8', C-8
8'	3.12, dd, 9.6, 3.6	67.9	CH_2	C-9, C-7', C-8
1''	4.5, m	102.3	CH	C-2'', C-5'', C-4''
2''	3.87, m	72.3	CH	C-1'', C-3''
3''	3.63, m	72.5	CH	C-2'', C-4''
4''	3.3, t, 9.6	73.8	CH	C-3'', C-5''
5''	3.50, m	70.1	CH	C-4'', C-6''
6''	1.18, d, 6.3	17.9	CH_3	C-5''
	3.81, s	56.3	OCH_3	-

4.4.2 New compounds from the roots of *V. grandiflorum*

❖ Viburnoside (268)



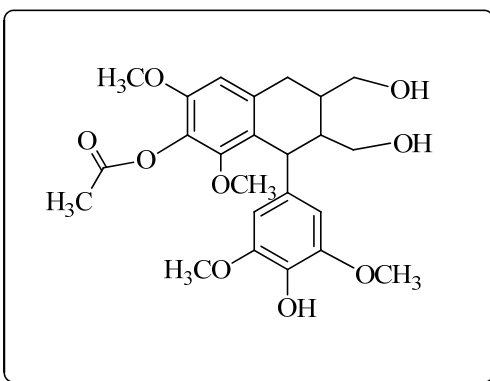
Physical data:

Physical state:	White amorphous powder
Yield:	11 mg
EI-MS (m/z):	713 a.m.u.
Molecular formula:	C ₃₉ H ₇₁ O ₁₀ N
HRESI-MS m/z (rel.int %):	714.5286 ($M^+ + H = C_{39}H_{71}O_{10}N$)
$[\alpha]_D^{26}$:	-0.034°
UV λ_{max} (ϵ) in MeOH:	214 (1.716) nm, 230 (1.586) nm, 280 (0.639) nm.
IR ν_{max} cm ⁻¹ (CHCl ₃):	3385(N-H) 1735 carbonyls respectively), 1638 to 1580 Olefinic C= C bonds
HRESI-MS m/z (rel.int %):	713 (10), 552 (2), 534 (34), 516 (20), 502 (50), 366 (24), 341 (40), 297 (100), 280 (40).

Table: 4.4 ^1H and ^{13}C NMR (in pyridine) data of **268**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
-	8.36, d, 8.1	-	N-H	C-1, C-2, C-1'
1	4.68, dd, 11, 6.2 (Ha)	70.1	CH ₂	C-1'', C-2, C-3
	4.23, dd, 11, 6.2 (Hb)	-	-	-
2	4.79, m	54.6	CH	C-1, C-3, N-H
3	4.56, m	72.3	CH	C-4, C-5, C-2
4	4.77, dd, 10.5, 6	72.4	CH	C-3, C-2, C-5, C-6
5	4.77, dd, 10.5, 6	72.5	CH	C-4, C-6
6	5.93-5.95, m	129.9	CH	C-5, C-4, C-7, C-8
7	5.93-5.95, m	132	CH	C-6, C-8
8	5.93-5.95, m	132	CH	C-7, C-6, C-9
9	5.93-5.95, m	129.9	CH	C-8, C-7, C-10
10-13	1.23- 2.14, m	22.9- 32.9	CH ₂	C-9, C-14
14	5.48, m	132.1	CH	C-13, C-15
15	5.48, m	131.1	CH	C-14, C-16, C-17
16-18	1.22-2.14, m	22.9-32.9	CH ₂	C-17, C-19
19	0.85, t, 7.5	14.2	CH ₃	C-18
1'	-	175.1	C	-
2'- 12'	1.23 - 2.14, m	22.9-32.9	CH ₂	C-1', C-14'
13'	1.22-1.23, m	32.9	CH ₂	C-12', C-14'
14'	0.85, t, 7.5	14.2	CH ₃	C-13'
1''	4.96, d, 7.4	105.7	CH	C-1, C-2'', C-5''
2''	4.02, t, 8.6	75.1	CH	C-1'', C-3''
3''	3.86-3.90, m	78.4	CH	C-2'', C-4''
4''	4.20-4.36, m	71.5	CH	C-3'', C-5''
5''	4.20-4.36, m	78.5	CH	C-4'', C-1'', C-6''
6''	4.20-4.36, m	62.6	CH ₂	C-5''

❖ Grandiflorinol (274)



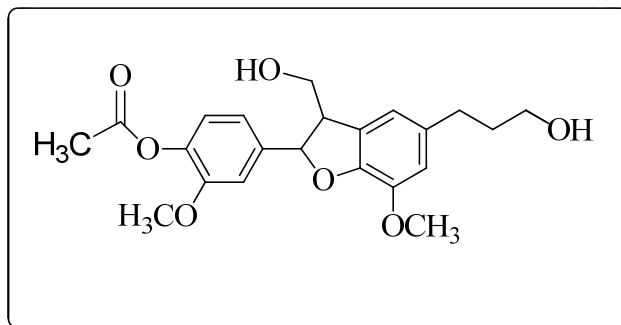
Physical data:

Physical state:	White amorphous solid
Yield:	6.4 mg
EI-MS (m/z):	462 a.m.u.
Molecular formula:	$C_{24}H_{30}O_9$
$[\alpha]_D^{26}$:	-12.5°
UV λ_{max} (ϵ) in MeOH:	265 and 210 nm
IR ν_{max} cm^{-1} ($CHCl_3$):	3420 (O-H), 1580-1620 (for aromatic C= C bonds)
EIMS m/z (rel.int %):	462 [$M^+=C_{24}H_{30}O_9$], 420 [$M^+ - 42$] (100), 371 [$M^+ - 91$] (70), 339 [$M^+ - 123$] (22), 301 [$M^+ - 161$] (21), 217 [$M^+ - 245$] (56), 205 [$M^+ - 257$] (70), 183 [$M^+ - 279$] (77), 167 [$M^+ - 295$] (60).

Table: 4.5 ^1H and ^{13}C NMR (in MeOD) data of **274**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	126.2	C	-
2	-	130.1	C	-
3	-	148.6	C	-
4	-	138.8	C	-
5	-	147.6	C	-
6	6.57, s	107.6	CH	C-3, C-1, C-4, C-7
7	2.58, dd, 15, 5.4, Ha-7'	33.5	CH ₂	C-1, C-8, C-8', C-2, C-6
-	2.70, dd, 15, 4.8, Hb-7'	-	-	-
8	1.58-1.65, m	40.7	CH	C-8', C-7, C-9, C-7', C-9'
9	3.57-3.62, m	66.7	CH ₂	C-8, C-7, C-8'
1'	-	139.3	C	-
2'/6'	6.37, s	106.6	CH	C-1', C-3', C-6', C-4'
3'	-	148.9	C	-
4'	-	134.3	C	-
5'	-	148.9	C	-
7'	4.30, d, 5.4	42.2	CH	C-1', C-8', C-9', C-6'
8'	1.93-1.98, m	49.8	CH	C-7', C-9', C-8
9'	3.46-3.50, m	63.9	CH ₂	C-8', C-7', C-8
CH ₃	1.88, s	24.1	CH ₃	C=O
OCH ₃	3.84, s	56.5	CH ₃	C-3
OCH ₃	3.72, s	56.6	2× CH ₃	C-3', C-5'
OCH ₃	3.36, s	60.1	CH ₃	C-5
C=O	-	180	C	-

❖ Grandifloronin (275)



Physical data:

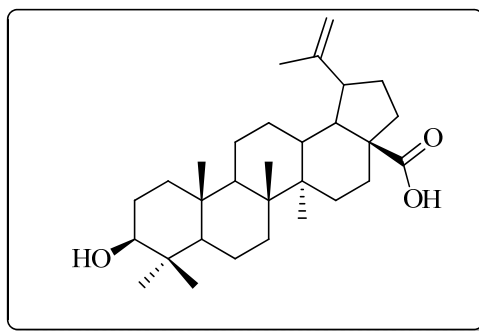
Physical state:	White amorphous solid
Yield:	7 mg
EI-MS (m/z):	402 a.m.u.
Molecular formula:	$C_{22}H_{26}O_7$
$[\alpha]_D^{26}$:	-0.28°
UV λ_{max} (ϵ) in MeOH :	282 and 208 nm.
IR ν_{max} cm^{-1} ($CHCl_3$):	3365 (O-H), 2985 (for aliphatic C-H stretching), 1728 (for ester carbonyl), 1580-1620 (for aromatic C= C bonds).
EI-MS m/z (rel.int. %):	402 [$M^+=C_{22}H_{26}O_7$] (2), 360 [$M^+ - 42$] (7), 328 [$M^+ - 74$] (8), 284 [$M^+ - 118$] (4), 137 [$M^+ - 265$] (100).

Table: 4.6 ^1H and ^{13}C NMR (in MeOD) data of compound **275**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	135	C	-
2	6.97, s	110.3	CH	C-1, C-3, C-6
3	-	149	C	-
4	-	147.3	C	-
5	6.75, d, 6.6	116	CH	C-1, C-4, C-6
6	6.82, dd, 7.6, 2	119.6	CH	C-5, C-2 C-4, C-7
7	5.48, d, 6	88.6	CH	C-8, C-6, C-2, C-1
8	3.43, m	55.7	CH	C-9, C-7, C-1
9	3.81, m	65.1	CH ₂	C-8, C-7, C-3'
1'	-	136.7	C	-
2'	6.55, s	116.6	CH	C-1', C-4', C-7', C-3'
3'	-	129.7	C	-
4'	-	146.4	C	-
5'	-	141.8	C	-
6'	6.59, s	116.9	CH	C-5', C-4', C-7', C-1'
7'	2.55, t, 7.8	32.7	CH ₂	C-5', C-8', C-4', C-6'
8'	1.78, m	35.8	CH ₂	C-7', C-9', C-1'
9'	3.54, t, 6.6	62.2	CH ₂	C-7', C-8'
OCH ₃	3.80, s	56.2	2×CH ₃	C-3, C-5'
4-C=O	-	180.1	C	-
4-CH ₃	1.88, s	24.2	CH ₃	C=O

4.4.3 Hitherto unreported compounds from the aerial parts of *V. grandiflorum*.

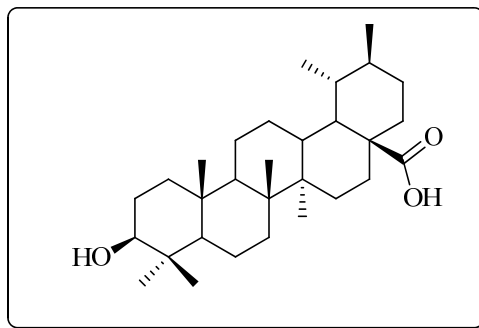
- ❖ **Betulinic acid (257)**
3 β -3-Hydroxy lup-20(29)-ene-28-oic acid



Physical data:

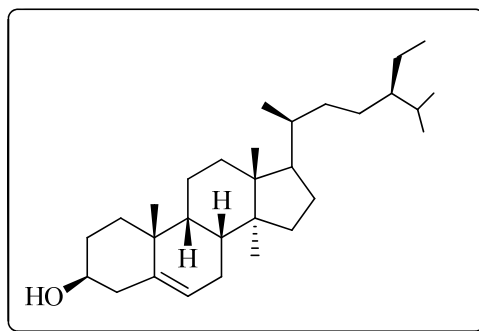
Physical state:	White amorphous powder
EI-MS (m/z):	456 a.m.u.
Molecular formula:	C ₃₀ H ₄₈ O ₃
Yield:	20 mg
M.P:	316-318 °C
IR $\nu_{\max}$ cm ⁻¹ (CHCl ₃):	3425 (O-H), 1712 (carboxylic carbonyl carbon) 1620 (olefinic C= C bonds).
EIMS m/z (rel.int. %):	456 [M^+ =C ₃₀ H ₄₈ O ₃] (15), [M^+ -18] (21), [M^+ - 249] (46), [M^+ -267] (100).

❖ **Ursolic acid**
3 β -3-Hydroxy lup-11(13)-ene-28-oic acid (107)



Physical data:

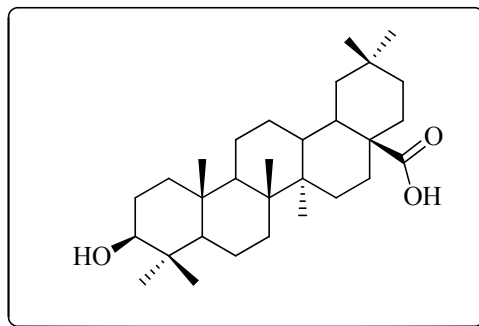
Physical state:	White amorphous powder
EI-MS (m/z):	456 a.m.u.
Molecular formula:	$C_{30}H_{48}O_3$
Yield:	25 mg
M.P:	316-318°
IR $\nu_{\max}$ cm^{-1} (CHCl_3):	3442 (O-H), 1722 (carbonyl carbon carboxylic acid), 1636 (olefinic C=C bonds).
EI-MS m/z (rel.int %):	456 [$M^+ = C_{30}H_{48}O_3$] (17), [$M^+ - 17$] (25), [$M^+ - 46$] (30), [$M^+ - 61$] (21), [$M^+ - 156$] (28) [$M^+ - 199$] (25), [$M^+ - 208$] (100).

❖ β -Sitosterol (258)

Physical data:

Physical state:	Colorless needles
Yield:	23 mg
EI-MS (m/z):	414 a.m.u.
Molecular formula:	$C_{29}H_{50}O$
M.P:	136-140 °C
IR $\nu_{\max}$ cm^{-1} (CHCl_3):	3380 (O-H), 2978 (aliphatic C-H Stretching) 1640 (olefinic C= C bonds).
EI-MS m/z (rel.int %):	414.4 [$M^+=C_{29}H_{50}O$] (29), [M^+-18] (100), [M^+-159] (44), [M^+-269] (48).

❖ Oleanolic acid (138)



Physical data:

Physical state:

White amorphous powder

Yield:

13 mg

EI-MS (m/z):

456 a.m.u.

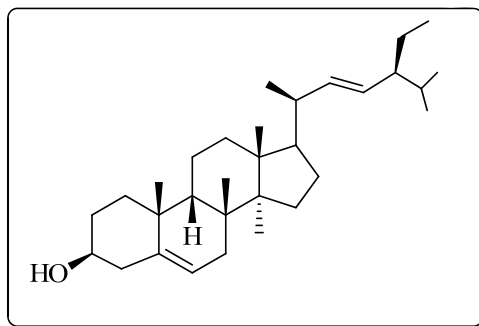
Molecular formula:

$C_{30}H_{48}O_3$

EI-MS m/z (rel.int %):

456 [$M^+=C_{30}H_{48}O_3$] (17), [M^+-17] (25),
[M^+-46] (30), [M^+-61] (21), [M^+-156]
(28), [M^+-199] (25), [M^+-208] (100).

❖ Stigmasterol (260)



Physical data:

Physical state: White soft crystals

Yield: 15 mg

EI-MS (m/z): 412 a.m.u.

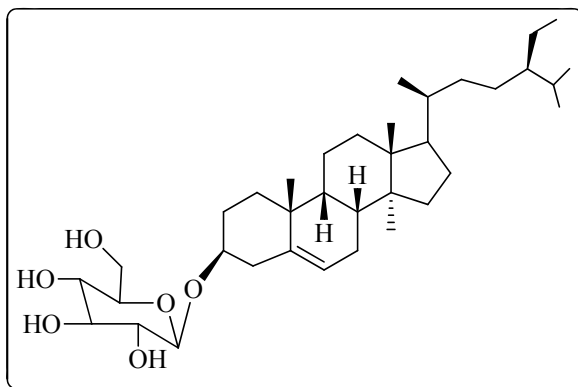
Molecular formula: C₂₉H₄₈O

UV $\lambda_{\max}$ (ϵ) in MeOH: 236 nm.

IR $\nu_{\max}$ cm⁻¹ (CHCl₃): 3425 (hydroxyl), 2970, 2840 (C-H stretching),
1620 (C=C).

EI-MS m/z (rel.int. %): 412 [M^+ =C₂₉H₄₈O](8), 273(60), 181 (45).

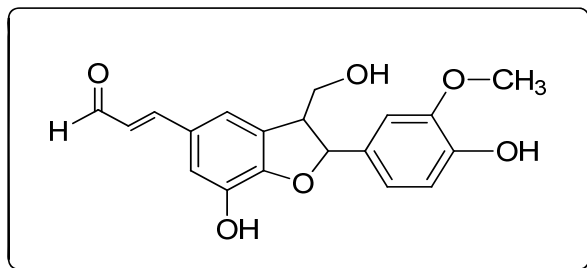
❖ **Glucoside of β -Sitosterol (261)**
 β -Sitosterol 3-O- β -D-glucopyranoside



Physical data:

Physical data:	Colorless needles
Yield:	8 mg
EI-MS (m/z):	576 a.m.u.
Molecular formula:	$C_{36}H_{61}O_6$
IR $\nu_{\max}$ cm^{-1} (CHCl_3):	3420 (O-H), 3045 (aliphatic C-H stretching) 1650 (olefinic C= C bonds).
EI-MS m/z (rel.int %):	576 [$M^+=C_{36}H_{61}O_6$] (29), [M^+-180] (100).

- ❖ 2, 3-Dihydro-2-(4'-hydroxy, 3'-methoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl) 7-hydroxybenzofuran (262).



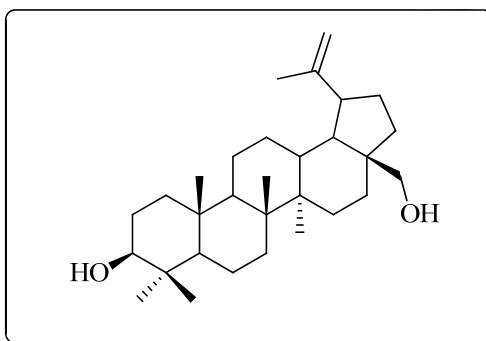
Physical data:

Physical state:	Yellow amorphous powder
Yield:	17 mg
EI-MS (m/z):	342 a.m.u.
Molecular mass:	$C_{19}H_{18}O_6$
$[\alpha]_D^{26}$:	-14
IR $\nu_{\max}$ cm^{-1} (CHCl_3):	3405 (O-H), 3045 (for aromatic C-H stretching) 1728 (for aldehydic carbonyl) 1575-1625 (for aromatic C= C bonds)
EIMS m/z (rel.int %):	342.2 [$M^+=C_{19}H_{18}O_6$] (35), [$M^+ - 18$] (100), [$M^+ - 30$] (98), [$M^+ - 50$] (22).

Table: 4.7 ^1H and ^{13}C NMR (in MeOD) data of **262**

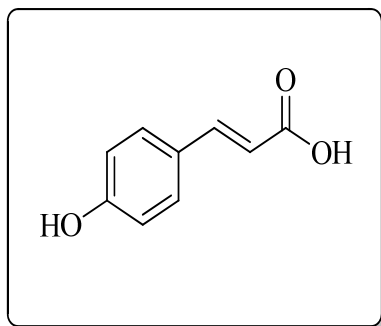
S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
2	5.60, d, 6.5	89.8	CH	C-3, C-3a, C-1', C-2',
-	-	-	-	C-6',C-11, C-7a
3	3.55, q, 6.5	54.9	CH	C-11, C-2, C-3a, C-1'
3a	-	131.2	C	-
4	7.06, s	117.4	CH	C-3a, C-7a, C-5, C-6,
5	-	129.5	C	-
6	7.16, br s	118.5	CH	C-7, C-4, C-5
7	-	142.9	C	-
7a	-	152	C	-
8	7.59, d, 15.6	156.3	CH	C-9, C-10, C-5, C-4
9	6.59, dd, 15.6, 7.8	126.8	CH	C-10, C-8, C-5
10	9.55, d, 7.8	196.1	CH	C-9, C-8
11	3.85, dd, 11.5, 6.5 (H-11a)	64.7	CH ₂	C-3a, C-3, C-2
	3.55, dd, 11.5, 6.5 (H-11b)	-	-	-
1'	-	134.2	C	-
2'	6.96, d, 2	110.5	CH	C-3', C-4', C-2, C-1'
3'	-	149	C	-
4'	-	148	C	-
5'	6.78, d, 8.1	116.2	CH	C-1', C-6', C-4', C-3',
6'	6.85, dd, 8.1, 2	119.7	CH	C-2, C-2', C-4

❖ **Betulin (264)**
Lup-20 (29)-ene-3 β , 28-diol



Physical data:

Physical state:	White powder
EI-MS (m/z):	442 a.m.u.
Molecular formula:	C ₃₀ H ₅₀ O ₂
HR-EIMS:	442.3816 (calculated 442.3811)
M.P:	256-257°C
IR $\nu_{\max}$ cm ⁻¹ (CHCl ₃):	3418 (O-H), 2885 (aliphatic C-H stretching), 1620 (olefinic C= C bonds).
EIMS m/z (rel.int %):	442 [M ⁺ =C ₃₀ H ₅₀ O ₂] (65), [M ⁺ - 18] (55), [M ⁺ - 239] (94), [M ⁺ -51] (55), [M ⁺ -253] (100), [M ⁺ -267] (60)
¹ H NMR:	δ_H 0.74 (H-23), 0.80 (H-24), 0.95 (H-25), 0.96 (H-26), 1.00 (H-27) 1.66 (H-29), 3.16 (H-3), δ_H 3.32, 3.76 (H-28), δ_H 4.66 and 4.56 (H-30).

❖ *p*-Hydroxy cinnamic acid (**229**)

Physical data:

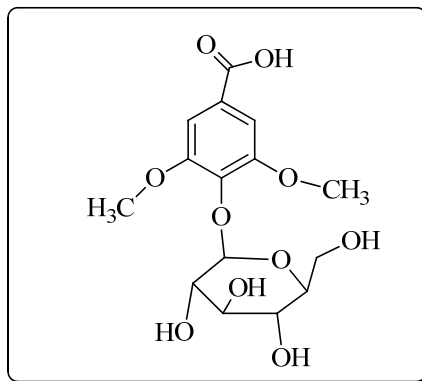
Physical state: White amorphous powder

Yield: 6 mg

EI-MS (*m/z*): 164 a.m.u.Molecular formula: C₉H₈O₃**Table: 4.8** ¹H and ¹³C NMR (in MeOD) data of **229**

S.No.	δ _H , Multiplicity, <i>J</i> (Hz)	δ _C	TYPE	HMBC
1	-	128.6	C	-
2	7.36, d, 8.4	130	CH	C-1, C-7, C-3, C-4
3	6.75, d, 8.4	116	CH	C-2, C-4, C-5, C-1
4	-	159.7	C	-
5	6.75, d, 8.4	116	CH	C-3, C-4, C-6, C-1
6	7.36, d, 8.4	130	CH	C-1, C-5, C-2, C-4
7	6.33, d, 15.4	123	CH	C-1, C-8, C-9, C-2
8	7.32, d, 15.4	141.1	CH	C-9, C-7, C-1
9	-	176.2	C	-

- ❖ 3, 4, 5 - Trihydroxy benzoic acid; 3, 5-di-Me ether 4-*O*- β -D-glucopyranoside (265)



Physical data:

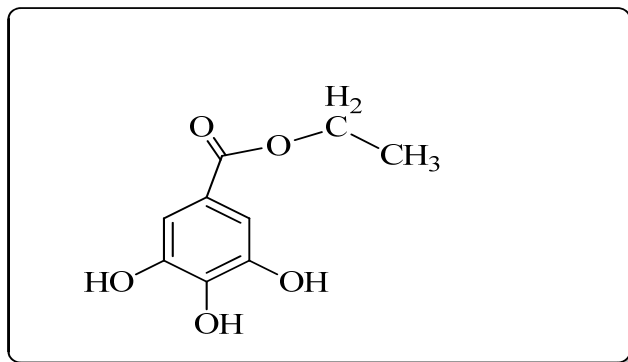
Physical state:	Colorless amorphous powder
Yield:	6 mg
EI-MS (<i>m/z</i>):	360 a.m.u.
Molecular formula:	C ₁₅ H ₂₀ O ₁₀
EIMS <i>m/z</i> (rel.int %)	360 [M^+ =C ₁₅ H ₂₀ O ₆] (6), 198 [M^+ -162] (100), 183 [M^+ -177] (85).

Table: 4.9 ^1H and ^{13}C NMR (in MeOD) data of compound **265**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1		153.9	C	
2	7.34, s	108.4	CH	C-1, C-3, C-4, C-7
3		129	C	
4		139.6	C	
5		129	C	
6	7.34, s	108.4	CH	C-1, C-5, C-7, C-2
7		170.2	C	
8	5.05, d, 7.8	104.5	CH	C-2', C-3', C-5'
9	3.49, t, 7.8	75.6	CH ₂	C-1', C-3'
10	3.40, m	71.2	CH ₂	C-2', C-4'
11	3.41, m	77.8	CH ₂	C-3', C-5'
12	3.21, m	78.4	CH ₃	C-1', C-6', C-4'
13	3.65, dd, 5.4, 4.8	62.4	CH ₃	C-4', C-5'

4.4.4 Hitherto unreported constituents from the roots of *V. grandiflorum*

- ❖ **Ethyl gallate**
Ethyl 3, 4, 5-trihydroxy benzoate (267)



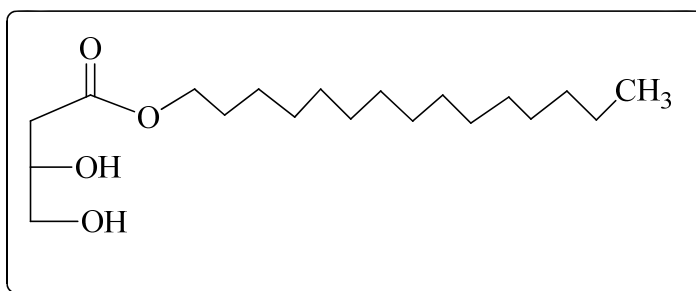
Physical data:

Physical state:	White amorphous powder
Yield:	5.6 mg
EI-MS (m/z):	198 a.m.u.
Molecular formula:	$C_9H_{10}O_5$
M. P:	149-153 °C
EIMS m/z (rel.int %):	198 [$M^+=C_9H_{10}O_5$](80), [M^+-46](100), [M^+-72] (33).

Table: 4.10 ^1H and ^{13}C NMR (in MeOD) data of compound **267**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	146.4	C	-
2	6.95, s	109.9	CH	C-1, C-3, C-4, C-7
3	-	121.7	C	-
4	-	139.6	C	-
5	-	121.7	C	-
6	6.95, s	109.9	CH	C-1, C-2, C-5, C-7
7	-	168.5	C	-
1'	4.15, q, 7.2	61.6	CH_2	C-2'
2'	1.23, t, 7.2	14.6	CH_3	C-1'

❖ Pentadecyl 3, 4-dihydroxybutanoate (269)



Physical data:

Physical state: Gummy solid

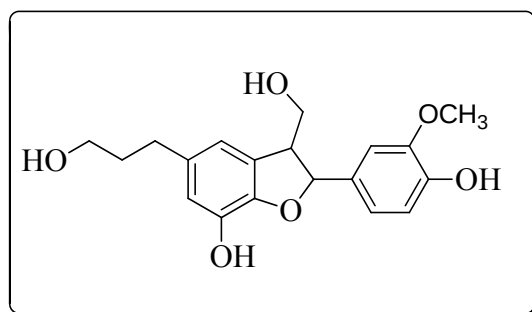
Yield: 6.4 mg

Molecular mass: 330 a.m.u.

Molecular formula: C₁₉H₃₈O₄**Table: 4.11** ¹H and ¹³C NMR (in MeOD) data of compound **269**

S.No.	δ _H , Multiplicity, <i>J</i> (Hz)	δ _c	TYPE	HMBC
1	-	175.5	C	-
2	4.13, dd, 11, 4.2 (Ha-2)	66.4	CH ₂	C-1, C-3, C-4
-	4.05, dd, 11, 6 (Hb-2)	-	-	-
3	3.54, dd, 5.4, 3	42.2	CH	C-2, C-4, C-1
4	4.30, d, 5.4	49.8	CH ₂	C-2, C-3
1'	2.34, t, 7.2	34.9	CH ₂	C-1, C-2', C-3'
2'-14'	1.27- 1.59	23.7-33.1	12×CH ₂	C-13', C-14'
15'	0.89, t, 7.2	14.4	CH ₃	-

❖ **2-(-4'-Hydroxy-3'-methoxy phenyl)-5-(3''-hydroxypropyl)-3-hydroxymethyl-7-hydroxy 2,3 dihydro benzofuran (270)**



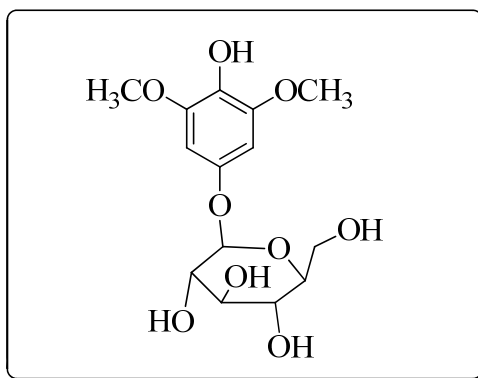
Physical data:

Physical state:	Brownish, amorphous powder
Yield:	12.5 mg
EI-MS (m/z):	346 a.m.u.
Molecular formula:	C ₁₉ H ₂₂ O ₆
HR-EIMS	346.1464
IR $\nu_{\max}$ cm ⁻¹ (CHCl ₃):	3340 (O-H), 1610-1415 cm ⁻¹ for aromatic (C=C).
HR-EI-MS m/z (rel.int %):	346 [M ⁺ =C ₁₉ H ₂₂ O ₆] (1), [M ⁺ - 18] (33), [M ⁺ -30] (10), [M ⁺ -62] (32), [M ⁺ -253] (100), [M ⁺ -74](5), [M ⁺ -152] (11), [M ⁺ -209] (16).

Table: 4.12 ^1H and ^{13}C NMR (in MeOD) data of **270**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
2	5.48, d, 6	88.6	CH	C-3, C-1''', C-2', C-3a, C-1'
3	3.43, t, 6.6	55.7	CH	C-1''', C-3a, C-1', C-2
3a	-	129.7	C	-
4	6.59, s	116.6	CH	C-1'', C-3, C-6, C-7a
5	-	136.7	C	-
6	6.55, s	116.9	CH	C-1'', C-4, C-7, C-7a
7	-	141.8	C	-
7a	-	146.4	C	-
1'	-	135	C	-
2'	-	110.3	CH	C-1', C-3', C-4', C-6'
3'	-	149	C	-
4'	-	147.3	C	-
5'	6.96, s	116	CH	C-2, C-3', C-4', C-6'
6'	6.76, d, 7.8	119.6	CH	C-1', C-4', C-6'
1''	6.83, d, 7.2	32.7	CH ₂	C-2, C-2', C-4'
2''	2.55, t, 7.2	35.5	CH ₂	C-2'', C-3'', C-5, C-4
3''	1.78, t, 7.2	62.2	CH ₂	C-1'', C-3'', C-5
1'''	3.54, t, 6.6	65	CH ₂	C-1'', C-2'', C-2, C-3, C-3a
	3.73, t, 7.8	-	-	-
OCH ₃	3.80, s	56.2	CH ₃	C-3'

❖ **Koaburaside (271)**
3, 5-Dimethoxy-4-hydroxy-1-*O*- β -D- glucoside



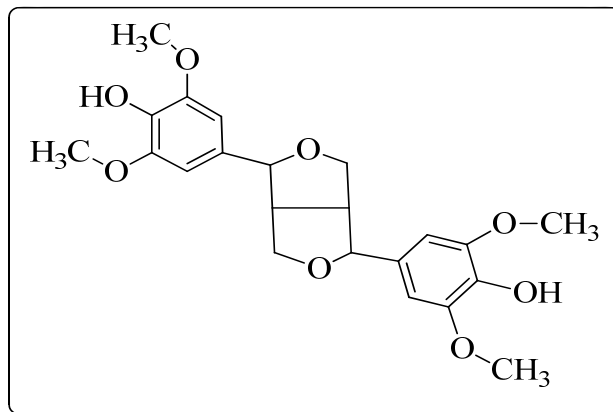
Physical data:

Physical state:	Colorless amorphous powder
Yield:	7.4 mg
EI-MS (m/z):	333 a.m.u.
Molecular formula:	C ₁₄ H ₂₀ O ₉
HR-EIMS:	333.0417
IR $\nu_{\max}$ cm ⁻¹ (CHCl ₃):	3320 (O-H), 1610-1415 cm ⁻¹ (C=C)
EI-MS m/z (rel.int %):	332 [M ⁺ =C ₁₄ H ₂₀ O ₉](3), [M ⁺ -162] (100), [M ⁺ -15] (48), [M ⁺ -30] (66), [M ⁺ -45] (16).

Table: 4.13 ^1H and ^{13}C NMR (in MeOD) data of **271**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	152.4	C	-
2	6.5, s	107.9	CH	C-6, C-1, C-3
3	-	149.3	C	-
4	-	152.4	C	-
5	-	149.3	C	-
6	6.5, s	107.9	CH	C-4, C-5, C-1, C-2
1'	4.73, d, 7.2	103.8	CH	C-1', C-3'
2'	3.40, m	74.9	CH	C-2', C-4'
3'	3.26, t, 2.4	78	CH	C-3', C-5'
4'	3.25, m	78.3	CH	C-4', C-6'
5'	3.29, m	71.7	CH	C-5'
6'	3.92, d, 1.8	62.7	CH ₂	C-5
OCH ₃	3.80, s	56.6	CH ₃ ×2	-

❖ (+) Syringaresinol (272)



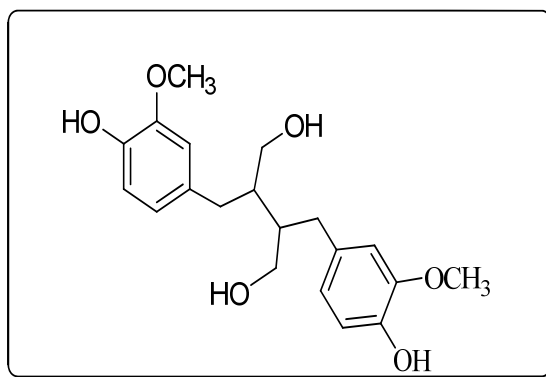
Physical data:

Physical state:	Colorless needles
Yield:	6.2 mg
EI-MS (m/z):	418 a.m.u.
Molecular formula:	C ₂₂ H ₂₆ O ₈
HR-EIMS:	418.1624
$[\alpha]_D^{26}$:	+24.4°
M.P:	184-186°C
IR $\nu_{\max}$ cm ⁻¹ (CHCl ₃):	3425 (O-H), 1630 (C=C)
EI-MS m/z (rel.int %):	418 [M^+ =C ₂₂ H ₂₆ O ₈] (88), [M^+ - 208] (20), [M^+ -210] (12), [M^+ - 225] (30), [M^+ - 237] (100), [M^+ - 251] (82).

Table: 4.14 ^1H and ^{13}C NMR (in MeOD) data of **272**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	133	C	-
2	6.5, s	104.3	CH	C-6, C-1, C-3
3	-	149.3	C	-
4	-	136	C	-
5	-	149.3	C	-
6	6.5, s	104.3	CH	C-4, C-5, C-1, C-2
7	4.71, d, 3	87.6	CH	C-1, C-7, C-8, C-6
8	3.14, , m	55.5	CH	C-7, C-9, C-1
9	3.88, m (H-9a)	72.7	CH ₂	C-8, C-7, C-9'
	4.26, m (H-9b)	-	-	-
1'	-	133	C	-
2'	6.5, s	104.3	CH	C-2', C-4'
3'	-	149.3	C	-
4'	-	136	C	-
5'	-	149.3	C	-
6'	6.5, s	104.3	CH	C-4', C-6'
7'	4.71, d, 3	87.6	CH	C-1', C-8'
8'	3.14, , m	55.5	CH	C-7', C-9'
9'	3.88, m (H-9a)	72.7	CH ₂	C-8', C-9, C-7'
	4.26, m (H-9b)	-	-	-
OCH ₃	3.80, s	56.7	CH ₃ ×2	-

❖ Secoisolariciresinol (273)



Physical data:

Physical data: White amorphous powder

Yield: 5.3 mg

EI-MS (m/z): 362 a.m.u.

Molecular formula: $C_{20}H_{26}O_6$

IR $\nu_{\max}$ cm^{-1} (CHCl_3): 3328 (O-H) and 1610-1520 cm^{-1} (C=C).

EI-MS (m/z rel.int %): 362 [$M^+ = C_{20}H_{26}O_6$] (86), [$M^+ - 18$] (23),
[$M^+ - 168$] (15), [$M^+ - 173$] (28), [$M^+ - 225$] (100).

Table: 4.15 ^1H and ^{13}C NMR (in MeOD) data of **273**

S.No.	δ_{H} , Multiplicity, J (Hz)	δ_{C}	TYPE	HMBC
1	-	133.8	C	-
2	6.57, s	113.2	CH	C-6, C-1, C-3, C-4
3	-	148.7	C	-
4	-	145.4	C	-
5	6.54,d, 7.8	122.6	CH	C-5, C-1, C-2, C-4
6	6.65, d, 7.8	115.7	CH	C-4, C-5,C-1, C-7
7	2.55, dd, 13.2,72 H-7a	35.9	CH ₂	C-8, C-1,C-2, C-6
-	2.66, dd,13.8,7.2 H-7b	-	-	-
8	1.90, m	44	CH	C-7, C-9, C-8'
9	3.58,dd, 12, 4.8	62	CH ₂	C-8, C-8', C-7
1'	-	133.8	C	-
2'	6.57, s	113.2	CH	C-6', C-1', C-3', C-4'
3'	-	148.7	C	-
4'	-	145.4	C	-
5'	6.54,d, 7.8	122.6	CH	C-6', C-1', C-2', C-4'
6'	6.65, d, 7.8	115.7	CH	C-5',C-1', C-2', C-7'
7'	2.55, dd, 13.2,7.2 Ha-7	35.9	CH ₂	C-8', C-9', C-1', C-2', C-
-	2.66, dd,13.2, 7.8 Hb-7	-	-	6'
8'	1.90, m	44	CH	C-7', C-9'
9'	3.58,dd, 12, 4.8	62	CH ₂	C-8, C-8', C-7
OCH ₃	3.80, s	56.1	CH ₃	-

4.5.0 Experimental of biological activities

4.5.1 Phytochemical screening

Standard chemical tests (Sofowora, Trease & Evans and Harborne) were carried out for the qualitative analysis of the constituents isolated from the aerial parts of *V. grandiflorum*.¹⁻⁵

Alkaloids: About 0.2 gm of the each extract was wormed with 2% H₂SO₄ for 2 minutes. Filtered and a few drops of dragendroffs reagent were added. Orange red precipitate indicates presence of alkaloids.

Steroids: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H₂SO₄. The colour changed from violet to blue or green indicates the presence of steroids.

Terpenoids Test: 0.2 g of the each extract was mixed with 2 ml of chloroform and concentrated H₂SO₄ (3 ml) was carefully added form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

Anthraquinones: About 0.5 g of each extract was boiled with 10 % HCl for few minutes in water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Appearance of the rose pink colour indicates the presence of anthraquinone.

Tannins: A small quantity of each extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added the filtrate. A dark green solution indicates the presence of tannins.

Saponins: About 0.2 g of the extract was shaken with 5 ml water and then heated to boil. Frothing temperature (appearance of creamy mass of small bubbles) shows the presence of saponins.

Reducing Sugars: The extracts were shaken with distilled water and filtered. The filtrate was boiled with few drops of Fehling solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Glycosides: The extracts were hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycosides.

Phlobatanins: The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

Flavonoids: Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of Flavonoids.

4.5.2 Antibacterial activity

Antibacterial activity was carried out on the modified agar well diffusion method to study antibacterial activity of the stem extract of *V. grandiflorum*. The muller-hinton agar medium was used.

The cultures used in this study were prepared in triplicates and was incubated at a temperature of 37 °C for 24 to 72 hours. The broth culture (0.6 ml) of the test organism was taken in a sterile petri-dish to which 20 ml of sterile molten MHA was added. Wells were bored in the medium and 0.2 ml of the extracts (*n*-hexane, chloroform, ethyl acetate, methanol) were added.

Streptomycin (2 µgm) used as antibacterial standard. Inoculation was done for 1hr to make possible the diffusion of the antibacterial agent into the medium. The inoculation plates were incubated at 37°C for 24 hours. The diameter of the zone of inhibition of bacterial growth was measured in the plates in millimeter.³

4.5.3 Antifungal activity

Stock solution of each extract was prepared by dissolving at concentration of 24 mg/ml in DMSO and stored in a refrigerator till further used. Sabouraud dextrose agar (SDA) media for fungal growth was prepared by mixing sabouraud 40% glucose agar and agar in distilled water with acidic pH (pH 5.5 to 5.6) and then autoclaved at 121°C for 15 min (Atta-ur-Rahman 1991). Media was then cooled to 45 to 50°C and 20 ml of molten SDA medium was aseptically transferred into each sterilized Petri dish (4 cm diameter). All dishes were then inoculated with 4 mm diameter piece of inoculums detached from a seven days old culture of fungus. Once the agar was hardened, 8 mm wells were bored using a sterile cork borer, then from each stock solution, extracts were transferred to a separate well having a final concentration of 400 µg/ml and the plates were incubated for 24 h at 29°C. Two wells in each petri dish were supplemented with DMSO and reference antifungal drug miconazole (0.2 mg/ml) dissolved in DMSO (sigma) serve as negative and positive control.^{6, 7}

4.5.4 Phytotoxic activity

The phytotoxic activity of the crude and all subsequent solvent fractions of *V. grandiflorum* stem and roots were evaluated using *Lamina minor* plant. 15 mg of respective extract was dissolved in 1.5 ml of respective solvent and from the stock solution transfer 5, 50 and 500 µl to the flask equivalent to 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight under sterilized condition in

laminar flow. 20 ml of E. medium was added to each flask. Other flasks (3 for each) were supplemented with E. medium and standard drug (paraquat, 0.015 µg/ml) served as negative and positive control. To each flask ten plants with 2-3 fronds were transferred and kept all the flasks under about 12 hours day light conditions. Plants were observed daily and on the seventh day the numbers of fronds were counted. The % growth inhibition was recorded with reference to the negative control using the following formula.^{6,7}

$$\text{Inhibition \%} = 100 - \frac{\text{Number of fronds in test sample}}{\text{Number of fronds in negative control}} \times 100$$

4.5.5 Insecticidal activity

In vitro insecticidal assay was carried out for the crude extract and its various solvent fractions against *Tribolium castaneum*, *Rhyzopertha dominica* and *Callosobruchus analis* followed method available in literature.^{6,7} The test sample was prepared (200 mg of crude extract was dissolved in 3 ml of methanol and served as stock solution). The sample (1019.10 µg/cm²) was loaded over the filter paper of appropriate size (9 cm or 90 mm) on Petri plate using micropipette. The plate was left overnight (24 h) to evaporate the solvent. Next morning, 10 healthy and active insects of each species of same size and age were added to each plate including control (methanol) and standard drug (permethrin, 239.50 µg/cm²). Thereafter the plates were incubated in growth chamber at 27°C for 24 h with 50% relative humidity. The percent mortality was calculated using the following formula.

$$\% \text{ Inhibition} = 100 - \frac{\text{Number of insect in test sample}}{\text{Number of insects in control}} \times 100$$

4.5.6 Xanthine oxidase inhibitory assay

The xanthine oxidase activity with xanthine as the substrate was spectrophotometrically measured using the Owens and John's procedure with some modifications.⁸ The allopurinol solution used as positive control and prepared by dissolving 5 mg of allopurinol in 5 ml of 0.15 M phosphate buffer (pH 7.5). Xanthine oxidase from bovine milk was purchased from Sigma ($\times 4500$). The enzyme solution was prepared by diluting 30 μ l of a 5.0 U/0.2 ml xanthine oxidase solution to a final volume of 3 ml. The substrate solution was prepared by addition of 5 drops of 1 M NaOH to 22.7 mg of xanthine to aid its dissolution with deionized water to a final volume of 250 ml. The plant extracts were dissolved in 1% dimethyl sulfoxide (DMSO) to a final concentration of 1 mg/ml. All solutions were prepared immediately before use.

Total volume of the assay mixture is 3.4 ml and consists of the plant extract under study (apportioned concentrations of 200, 100, and 75 μ g/ml), 0.15 M phosphate buffer (pH 7.5) and 100 μ l of 0.03 U/ml xanthine oxidase enzyme solution. After preincubation of the test solution at 25°C for 10 min, the reaction was initiated by addition of 1 ml of 0.6 mM substrate solution of xanthine, mixed thoroughly, and monitored through absorption increments read every 30 sec for 10 min at 295 nm indicating the formation of uric acid using a Shimadzu UV-1700 series spectrophotometer. Allopurinol was used at a final concentration of 30 μ g/ml in the assay mixture. The percent xanthine oxidase inhibitory activity of the assayed samples was determined through the slope of the plot of absorbance against time (seconds). IC₅₀ values were obtained through linear regression analysis the plot of concentration (200, 100, 25 μ g/mL) against percent inhibition.

4.5.7 Analgesic activity

Acetic acid induced writhing test

All the animals (18 – 22 g) of either sex were withdrawn from food 2 h before the start of experiment and divided in various groups. Group I was injected with normal saline (10 ml/kg) as control, group II received standard drug diclofenac sodium (10 mg/ kg) while the remaining groups were administer the ethanolic extract (50, 100 and 150 mg/kg) and compound **270** (5 and 10 mg/kg). After 30 min of above administration, the animals were treated i.p. with 1% acetic acid, for induction of writhing. The number of abdominal constrictions (writhes) were counted after 5 min of acetic acid injection for the period of 10 minutes.⁹

Hot plat test

Mice of either sex (n=6) weighing 18-22 g were acclimatized to laboratory conditions one hour before the start of experiment with food and water *ad libitum*. Animals were then subjected to pre-testing on hot plate (Havard apparatus) maintained at $55 \pm 0.1^{\circ}\text{C}$. Animals having latency time greater than 15 sec on hot plate during pre-testing were rejected (latency time). All the animals were divided in various groups each of six mice. Group I was treated with saline (10 ml/kg), group II was treated with tramadol (30 mg/kg i.p) and rest of the groups were treated with ethanolic extract (50, 100 and 150 mg/kg) and compound **270** (5 and 10 mg/kg, i.p). After 30 min, the animals were placed on hot plate and the latency time (time for which mouse remains on the hot plate ($55 \pm 0.1^{\circ}\text{C}$) without licking or flicking of hind limb or jumping) was measured in seconds. In order to prevent the tissue damage a cut-off time of 30 seconds were imposed for all animals. To find out the opiodergic mechanism in the analgesic activity of ethanolic extract and **270**,

a number of groups were treated with naloxone (0.5 mg/kg s.c.), ethanolic extract (100 and 150 mg/kg, i.p) and **270** (5 and 10 mg). After 10 min, these groups were treated with tramadol (30 mg/kg i.p.) and naloxone injection. The latency time for all groups was recorded at 0, 30, 60, 90 and 120 min. Percent analgesia was calculated using the following formula.⁹

$$\% \text{ Analgesia} = (\text{Test latency} - \text{control latency}) / (\text{Cut-off time} - \text{control latency}) \times 100$$

4.5.8 Anti-inflammatory activity

The anti-inflammatory activity was performed on Wister rats of either sex (150–290 g). The animals were randomly divided in various groups each of six animals Group I was treated with normal saline (10 ml/kg) while group II with diclofenac sodium (10 mg/kg) and rest of the groups were treated with ethanolic extract (50, 100 and 150 mg / kg, i.p) and **270** (5 and 10 mg/kg). After thirty minutes of the above intraperitoneal B administration, carrageenan (1%, 0.05 ml) was injected subcutaneously in the sub-plantar tissue of the right hind paw of each rat. The inflammation was measured using plethysmometer (LE 7500 plan lab S.L) immediately after injection of carrageenan and then 1,2,3,4 and 5 h. The average foot swelling in drug treated animal as well as standard was compared with control and the percent inhibition (anti-inflammatory activity) of edema was determined using the formula.⁹

$$\text{Percent inhibition} = A-B/A \times 100$$

Where A represent edema volume of control and B as paw edema of tested group.

4.5.9 Antipyretic test

The antipyretic activity was evaluated for the ethanolic extract (50, 100 and 150 mg/kg) and **270** (5 and 10 mg/kg) using mice (25–30 g) of either sex. The selected animals were healthy and were acclimatized to laboratory conditions before the start of experiment. The animals were divided into various groups each of six rats. The normal body temperature of each rat was recorded using digital thermometer and then pyrexia was induced by injecting 20% aqueous suspension of brewer's yeast (10 ml/kg s.c.). All groups were fasted overnight but allowed free accesses to drinking water and after 24 h rectal temperature of each mouse was recorded. The induction of pyrexia was confirmed by rise in temperature more than 0.5 °C, while animals showed rise in temperature less than 0.5 °C were excluded from experiment. Group I received saline (10 ml/kg) as a negative control and group II received paracetamol (150 mg/kg) as a standard drug while the remaining groups were treated with ethanolic extract (50, 100 and 150 mg/kg) and **270** (5 and 10 mg/kg). After drugs administration, rectal temperature was again recorded periodically at 1, 2, 3, 4 and following formula.⁹

$$\text{Percent reduction} = (T_a - T_b)/T_b \times 100$$

Where, T_a represents normal body temperature; T_b temperature after yeast injection.

4.5.10 Sedative activity

The apparatus used for this activity was consisted of an area of white wood (150-cm diameter) enclosed by stainless steel walls and divided in 19 squares by black lines. The open field was placed inside a light and sound-attenuated room. Balb-C mice of either sex (n=6) weighing 22 ± 2 g were used. Animals were acclimatized under red light (40 Watt red bulb) one hour before the start of experiment in laboratory with food and water

available *Ad libitum*. Animals were administered with distilled water and the ethanolic extract (50, 100 and 150 mg / kg, i.p) and compound **270** (5 and 10 mg/kg). After 30 min each animal was placed in the center of the box and the numbers of lines crossed were counted for each mouse.⁸

4.5.11 Muscle relaxant activity

The Rota rod used in this test is a metallic rod (3 cm diameter) coated with rubber and connected to a motor. The rod was rotate at a constant speed i.e. 9 rpm and was about 60 cm above the tabletop in order to prevent the mice from jumping off the roller. Mice were exposed to Rota rod as a pretest before the experiment and only those mice included in the study that remain on the rod for 5 min at a speed of 9 rpm. All the groups (n=6) were treated (i.p.) with diazepam (1 mg/kg), distilled water (10 ml/kg) and ethanolic extract (50, 100 and 150 mg / kg, i.p) and compound **270** (5 and 10 mg/kg). Before 30, 60 and 90 min of the exposure to Rota rod. Each mouse was allowed for 5 minutes on the revolving rod and time spent on the rod was recorded.⁸

4.5.12 Pentylenetetrazole (PTZ) induced seizure

NMRI mice (18 – 25 g) of either sex were used for screening of VBE (0.3, 0.4 and 0.5 mg/kg), VBHF (0.3, 0.4 and 0.5 g/kg) and 4HC (25, 50 mg/kg). The animals were divided in various groups (n = 6). Normal saline was injected to group I (negative control) and diazepam (7.5 mg/kg) was administered to group II (positive control). Similarly, ethanolic extract (50, 100 and 150 mg / kg, i.p) was injected to group some of the groups and compound **270** at the dose of 5 and 10 mg/kg was given to rest of group. After 30 min. of the above administration, animals of all groups were treated with PTZ (90 mg/kg, s.c), for the induction of convulsions. Each animal was observed for the onset

of various stages of convulsion for 30 min. The mortality rate of animals were also recorded after 24 h and the resulting data were analyzed statistically and compared with negative control.^{10, 11}

4.6.0 References

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