Phytochemical investigation of Lamiaceae genera *Leucas*, *Lavandula*, *Plectranthus* from Western Ghats

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By

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Research Guide

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CERTIFICATE

It is certified that the work in the Ph.D. thesis entitled **'Phytochemical investigation of Lamiaceae genera *Leucas*, *Lavandula*, *Plectranthus* from Western Ghats'** submitted by **Mr. Roshan R. Kulkarni** was carried out by the candidate under my supervision at National Chemical Laboratory, Pune. The material obtained from other sources and works carried out by other groups have been duly acknowledged in the thesis.

Date: **Dr. Swati P. Joshi**

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DECLARATION

I hereby declare that the thesis entitled **'Phytochemical investigation of Lamiaceae genera *Leucas*, *Lavandula*, *Plectranthus* from Western Ghats'** submitted for Ph.D. degree to the University of Pune has not been submitted by me for a degree at any other university.

Date: Roshan Kulkarni

Pune

Dedicated to my guide

CONTENT

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As the work comes to an end, memories start gathering like clouds ready to burst into rains. It would be impossible to continue without recollecting the countless people who influenced me and my work both directly and indirectly. I have tried to include all that I could gather but if I have missed anyone it is solely due to lapse of my memory.

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Finally, I cannot thank my Spiritual Master, my Guruji, as I slowly realize that It is His work and I cannot do anything but to obey His command.

At His feet,

Roshan

LIST OF ABBREVIATIONS

bd	Broad Doublet
bs	Broad Singlet
bt	Broad Triplet
c	Concentration
°C	Degree Celsius
CC	Column Chromatography
cm^{-1}	Inverse of Centimetre
COSY	COrrelation SpectroscopY
DEPT	Distrotionless Enhancement by Polarization Transfer
d	Doublet
dd	Doublet of Doublet
dt	Doublet of Triplet
2D NMR	2 Dimensional Nuclear Magnetic Resonanance
GC- FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectroscopy
g, mg, µg, kg	gram, milligram, microgram, kilogram
ESIMS	Electrospray Ionization Mass Spectroscopy
HMBC	Heteronuclear Multiple Bond Coherence
HPLC	High-Performance Liquid Chromatography
HREIMS	High Resolution Electron Impact Mass Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	Inhibitory Concentration required for 50% inhibition
IC_{95}	Inhibitory Concentration required for 95% inhibition
IR	Infrared spectroscopy
L, ml, µl	Liter, milliliter, microliter
LRI	Linear Retention Indices
m	multiplicity
NCL	National Chemical Laboratory
NIST	National Institute of Standards and Technology
NMR	Nuclear magnetic Resonance
NOESY	Nuclear Overhauser Effect SpectroscopY
ORTEP	Oak Ridge Thermal-Ellipsoid Plot Program
ppm	Parts Per Millions
s	Singlet
TLC	Thin Layer Chromatography
t	Triplet

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THESIS ABSTRACT

Phytochemical investigation was carried out on five species viz. *Leucas stelligera, Lavandula gibsoni, Anisomeles heyneana, Anisochilus verticillatus* and *Plectranthus mollis* belonging to family Lamiaceae. Analysis of essential oil from *L. gibsoni* and *P. mollis* was carried out. Extracts/essential oil and isolated compounds were evaluated for different biological activities. Salient features of this work are as follows:

1. This is the first report of chemical investigation on *L. stelligera* and *A. verticillatus*.

2. Forty compounds were isolated, eight from *L. stelligera*, thirteen from *L. gibsoni*, six from *A. heyneana*, seven from *A. verticillatus* and six from *P. mollis*.

3. Six new compounds belonging to labdane class were isolated from *L. stelligera.* Cis-8-epi-sclareol belongs to rare class of labdane diterpene with cis decalin ring fusion.

4. Six new compounds were isolated from *L. gibsoni.* Skeleton 2,2-diphenylpropane was shown as naturally occurring. Two new compounds, 2, 19-dimethylcosane-9, 12-diol and its diacetate representing new skeleton were isolated. Two new monoterpenes with rarely occurring ethoxyl substituents were isolated.

5. One new phyllocladane diterpene was isolated from *A. heyneana*. Phyllocladane skeleton is probably first time reported for family Lamiaceae.

6. Seven new compounds belonging to isopimarane class were isolated from *A. verticillatus.*8,9-secoisopimarane is reported as new skeleton.

7. Sitoseryl glucoside palmitate is probably first time isolated from family Lamiaceae so is euscaphic acid D, an ursane class of triterpene.

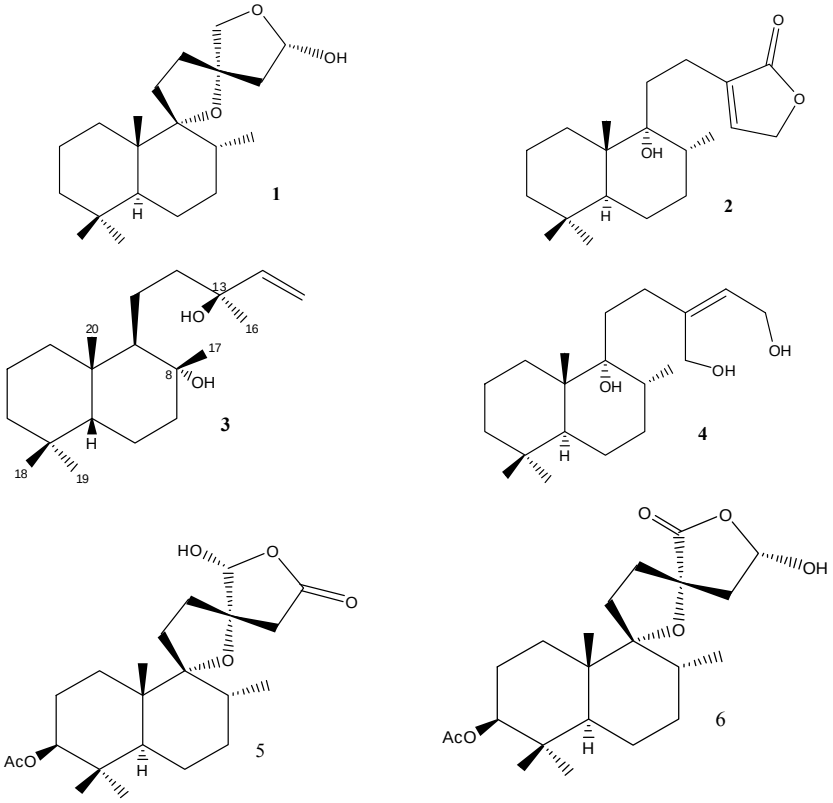
8. 4-Methylresorcinol is probably first time reported as natural product.

9. Other isolated known compounds include flavonoids, velutin, chrysoeriol, salvigenin, apigenin-dimethyl ether, 3'-O-methyleupatorin, eupatorin; phenolic compounds, hydroxychavicol, verbascoside, sesamin, coumarin, 8-hydroxythymol; cembrane diterpene ovatodiolide; phyllocladane diterpene triol; triterpenes ursolic acid, corosolic acid and sterols stigmasterol and sitosterol glucoside.

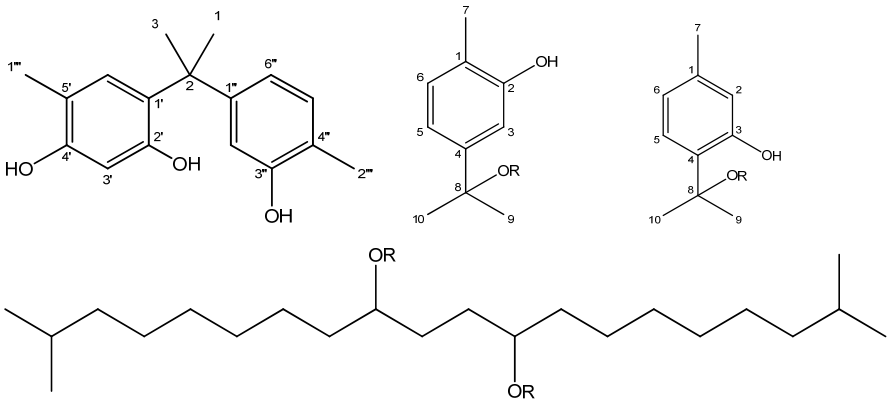
10. Diterpenes **1**, **2**, **3** and **4** isolated from *L. stelligera* and ovatodiolide isolated from *A. heyneana* were potent inhibitors of *Mycobacterial tuberculosis* with compounds **1**, **2** and **4** being selective in inhibiting *M. tuberculosis* and inactive against *M. smegmatis*, *E. coli* and cancer cell lines, MCF-7, THP-1 and HepG2.

11. Essential oil analysis was carried out on two species, *L. gibsoni* and *P. mollis*. This is the first report of essential oil analysis of *L. gibsoni*. It was shown to be dominated by α- terpinolen, 22.22%; thymol, 10.42% and benzenemethanol, 4-(1-methylethyl), 4.52%. This composition is different from that reported for other *Lavandula* species. Strong mosquito repellency of oil against *Aedes aegypti* is first time reported.

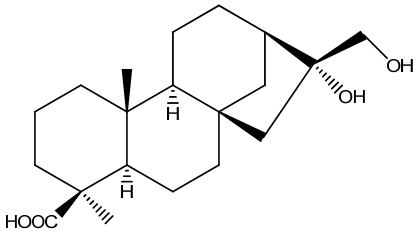
12. Essential oil of *P. mollis* contained piperitone oxide, 23.76%; fenchone, 19.19% and β-caryophyllene, 10.39% as major components. Larvicidal potential of the oil and acetone extract against three vector species viz. *A. aegypti*, *A. stiphensi* and *C. quinquefasciatus* is reported for first time.



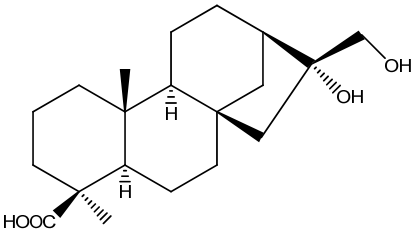
New compounds isolated from *L. stelligera*.



New compounds isolated from *L. gibsoni.*

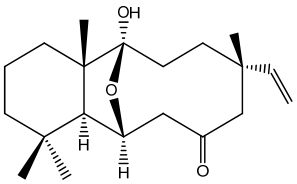


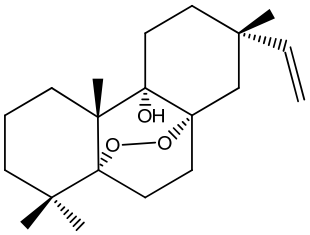
Phyllocladane diterpene



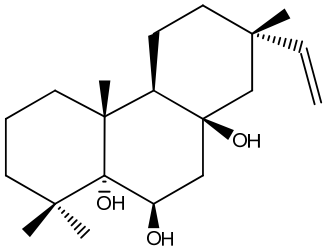
Ovatodiolide- cembrane diterpene

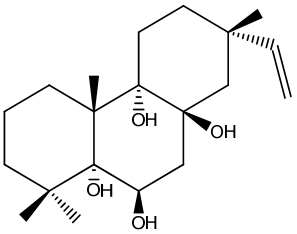
New compounds isolated from *A. heyneana*.





8, 9-Seco isopimarane diterpene





New compounds isolated from *A.verticillatus* belonging to isopimarane and rearranged isopimarane class.

General Experimental Procedure:

a. Insruments: The ^{1}H and ^{13}C NMR spectra were recorded using 400 MHz and 100 MHz Bruker FT-NMR Ultra Shield spectrometers. Chemical shifts are reported in parts per million (δ). Residual solvent peaks in respective deuterated solvents were used as internal reference, 7.28 and 77.00 (central peak) for $CDCl_3$, 3.31 and 49 (entral peak) for CD_3OD and mixture of $CDCl_3$ and CD_3OD, 7.22 and 150.30 for Pyridine-d_5. Optical rotation was recorded with a JASCO P-1020 polarimeter. UV spectra were obtained with CAREY 300. The IR spectra were measured with a Perkin-Elmer FT-IR spectrometer and attenuated total reflectance (ATR) spectra were recorded using Bruker Optics intrument The HREIMS data were obtained using MSI-Autoconcept mass spectrometer. ESIMS was recorded with API-QSTAR-PULSAR spectrometer. X-ray crystallography was carried out using Bruker SMART APEX diffractometer with CCD detector and Mo K_α radiation. Melting point was recorded using Buchi melting point apparatus B-540.

The GC-FID and GC-MS analyses of the essential oils were carried out with Varian CP 3800 apparatus equipped with FID and a GsBP5 capillary column (30m lenght, 0.25 mm i.d., film thickness 0.25 mm).

b. Chemicals: Column chromatography (CC) was carried out on silica gel 100-200# (from Thomas Baker) for first column separarion of extract and 230-400# for all subsequent separations. Precoated plates of silica gel 60 F_{254} from Merck Ltd. were used for analytical and preparative TLC. All the solvents used in CC and preparative TLC were distilled prior to use and HPLC grade solvents from Thomas Baker were used for UV and IR spectroscopy, oprtical rotation measurement and crystallization. H_2 and He used in GC-FID and GC-MS were dry.

c. Collection and processing:

Endemism, abundance and conservation status were considered along with level of scientific exploration before selection of plant members. None of the plants studied in this work are threatened and *Leucas stelligera*, *Lavandula gibsoni*, *Anisochilus verticillatus*, *Anisomeles heyneana* are endemic to Sahyadri (part of Western Ghats in State of Maharashtra).

Healthy individuals in flowering were collected from their natural environment. Collection amount was such as to keep majority of local population at the site intact. Plants were identified in field by Dr. P. Tetali and herbarium speciemens were deposited in Botanical Survey of India, Pune.

They were cleaned off adhereing dust, unwanted plant material, etc. and roots were removed. Aerial parts were dried in shade, cut and pulverized.

c. Extraction, **isolation and structure elucidation of compounds:**

Extract was prepared by keeping the pulverized plant material in contact with 3L of acetone for 14h at room temperature. This procedure was repeated for three days. Respective acetone solubles were filtered and concentrated under reduced pressure and at temperature not exceeding 60^0C to yield acetone extracts. Series of dry Column Chromatographies were used to separate compounds from extract. They were purified by using sinle or multiple preparative TLCs. Details are given in individual chapters.

Combination of NMR spectra and homogeneity on TLC using different mobile phase was considered as criteria for purity of compound. Compounds were nearly complety isolated from all column fractions to determine yields.

d. Essential oil analysis:

For both GC-FID and GC-MS analyses, the oven temperature was programmed rising from 50 to 260^0 at 3^0/min hold at 260^0C for 5min; injector temperature, 250^0C; detector temperature, 300^0C, He (1.0 ml/min); injection volume, 1 μl; split ratio, 6: 4. The Liniear Retention Indices (LRIs) of the constituents were determined relative to the retention times of a series of n-alkanes (C9–C38), and the relative percentages of the individual components of the oils were obtained from the GC-FID peak-area percentages after applying correction factors.

d. Biological assys:

Protocols for all the assys carried out during the present work are given in respective chapters. They were carried out by respective groups in NCL and their efforts are duly recognized in aknowlodgments. Inhibition of *Mycobacterium tuberculosis*, *M. smegmatis*, *Escherishia coli* as well as cytotoxicity againse HeLA, THP-1 and COL cell lines were carried out by Dr. Dhiman Sarkar and his

group. Larvicidal and mosquito repellency was tested by Dr. A. sen and his group though we were actively involved in planning and executing the experiments.

Chapter 1

Chemistry and Pharmacology of family Lamiaceae: A review

Introduction:

Family Lamiaceae is a large family with 180 genera and 3500 species of cosmopolitan distribution. In India the family is represented by 64 genera and 340 species of which 18 % are endemic [1].

A number of species of family Lamiaceae are of medicinal, commercial and of culinary importance. They also find place in the Traditional Systems of Medicines all over the world including Ayurveda. A brief survey of the research carried out on Lamiaceae members reveals importance of this family as a source of potentially bioactive molecules. Hence it is imperative to study the lesser known members including those that havef restricted distribution.

Taxonomically, some authors have combined Lamiaceae and Verbenaceae into one family [2]. Based on this, Lamiaceae is divided into seven sub-families viz. Ajugoideae, Scutellarioideae, Lamioideae, Nepetoideae, Viticoideae, Symphorematoideae and Prostantheroideae [3]. Genera studied in the present work viz. *Anisochilus*, *Lavandula* and *Plectranthus* belong to Nepetoideae while *Anisomeles* and *Leucas* belong to Lamioideae [4]. Except for *Anisomeles* and *Anisochilus*, other genera have been reviewed earlier by other workers and are referred to in respective sections. In short, these previous reviewed work is summarised below:

Genus *Leucas* is represented by many pharmacological activities and with ethnopharmacological background and has been reviewed by Chauhan and Singh [5]. *Plectranthus* is an economically and medicinally important genus. Consequently many reviews [42, 132-134a and b] have appeared dealing with chemical and ethnobotanical aspects of *Plectranthus*. Review by Lukhoba *et. al.*[42] gives account of ethnobotany, Abdel-Mogib *et. al.* reviews chemistry of entire *Plectranthus* up to 1999 [132] and Waldia *et. al.* deal with chemistry of *Plectranthus* reported by Indian workers upto 2010 [133]. Reviews by Alasbahi and co-workers deal with chemistry and ethnobotany of *P. barbatus* [134a, 134b]. Genus *Lavandula* is also well known, especially as a source of essential oil of commercial importance. Book by M. Lis-Balchin reviews commercial angle of *Lavandula* [81].

This is the first attempt to review genera *Anisochilus* and *Anisomeles*. Phytochemical work on genus *Plectranthus* after three reviews mentioned above also has been updated. Literature on *Plectranthus* is compounded by many synonyms. Hence this review is restricted to searches by word "Plectranthus" only. Similarly phytochemical work on genus *Lavandula* not included in the book mentioned above [81] as well as some literature on essential oils with composition different from that normally found in this genus is given here.

The review is divided into three sections representing I) ethnobotany and pharmacology, II) essential oil analysis and III) chemistry and bioactivity of isolated molecules wherever reported. Each section is divided into five parts representing five genera.

Section I. Ethnobotany and pharmacology:

i. *Leucas*:

a. Ethnopharmacology:

Various species of this genus are used in Traditional Systems of Medicine. Decoction of whole plant of *L. aspera* is used in dysentery [6], jaundice and bronchitis [7]. Juice of fresh leaves is used to treat anorexia while young leaves are used in cold, headache and chronic rheumatism [8]. Fried leaf paste is applied on forehead to relieve pain [9]. Leaves are boiled with black pepper and used as gargle to treat throatache [10]. Aerial parts are used in constipation and leaves are used in fever and urinary complaints. Roots are used in mad dog bite and to stop bleeding from nose [10]. *L. aspera* also finds use in ethnoveterinary practice where juice of leaves is added in nose to revert HCN poisoning in cattle whereas leaves along with other herbs are used to treat tympany [11].

L. cephalotes whole herb is a crude drug known as Dronapuspi in the Ayurvedic system of medicine and has been used as diaphoretic, anti-inflammatory, against oedema, and obstinate urinary troubles as well as diabetes and for diseases due to the aggravator of *pitta* [12]. In the Unani system of medicine it is registered as useful drug in the treatment of cough, cold, and gastric complaints [13]. Flowering twigs of *L. cephalotes* is used in a polyherbal combination to treat pus in ears [14]. The herb is also used in scorpion bite [15].

L. lanata leaf infusion is given to cattle along with butter at the time of delivery [16]. Leaves of *L. lavandulaefolia* are used in many situations. Juice is used for wound healing in cattle [17] while young leaves are used to stop bleeding from nose in humans [18]. Juice of whole plant is used in a polyherbal formulation in case of bites by snakes and other venomous insects [19]. Decoction of leaves is used in the treatment of asthma and applied to genitals for treatment of venereal diseases [20]. Whole plant alone is used in conjunctivitis and in combination with turmeric and seeds of mustard for treatment of migraine [21]. Root of *L. lavandulaefolia* is used with turmeric to relieve stomachache [21] while aerial parts of this herb are used as a sedative in nervous disorders [23].

Stems and leaves of *L. zeylanica* are used in headache and roots in treatment of malarial fever [22]. Leaves and paste of roots are used to heal cuts and wounds [22]. Herb *L. aspera* is used as food species by tribal people in certain regions of India [24] so is *L. stelligera* [25]. *L. inflata* is commonly used in local folk medical practices against a variety of ailments in United Arab Emirates [26].

b. Pharmacology:

Various pharmacological studies have been carried out on different species of genus *Leucas*. Anti-inflammatory evaluation of the methanol extract of *L. lavandulaefolia* has been reported by Saha *et al*. The extract was found to possess significant inhibitory activity against carrageenin, histamine, serotonin, and dextran induced hind paw oedema in rats. The effect produced by extract was comparable to that of phenylbutazone [27]. Same group reported wound healing activity of *L. lavandulaefolia*. The results were comparable to standard drug nitrofurazone in terms of wound contracting ability, wound closure time, tensile strength and tissue generation at the site of wound [28]. Mukherjee *et. al.* substantiated folkloric use of *L. lavandulaefolia* as sedative by using various animal models [29]. Same group evaluated methanol extract of this herb for antipyretic potential with yeast induced pyrexia in rats [30]. The effect produced was found to be comparable to that of a standard antipyretic drug paracetamol. But in another study methanolic extract of *L. lavandulaefolia* did not show significant inhibition of NO production [31].

Based on various ethnopharmacologic uses of other members of *Leucas*, Al-Yousuf, *et. al.* studied analgesic activity of the methanol and acetone extracts of *L. inflata*. It was concluded that the crude methanol and acetone extract of *L. inflata* had CNS depressant properties, manifested as anti-nociception and sedation. Both extracts also exhibited anti-inflammatory and antipyretic actions [32]. Muregi *et. al.* studied anti-plasmodium activity of various extracts of *L. calostachys*. The plant did not show significant inhibition in any of the extracts tested [33]. Manjunatha *et. al.* reported wound healing activity of *L. hirta* [34].

ii. *Anisochilus*:

a. Ethnopharmacology:

Genus *Anisochilus* is a small genus represented by sixteen members. Of all the sixteen species, scientific literature is available on only two species viz. *A. carnosus* and *A. harmandii*. *A. carnosus* is used by tribes in north-western Maharashtra for treatment of stomach-ache [35].

b. Pharmacology:

Aqueous extract of leaves of *A. carnosus* is reported to be protecting against rifampicin induced liver damage in rats [36] while ethanolic extract of leaves inhibited *E. coli*. [37]. *A. carnosus* is a part of Chinese patented composition to be used as a feed additive to inhibit avian influenza virus and improve avian immunity [73].

iii. *Anisomeles*:

a. Ethnopharmacology:

A. malabarica is widely used internally for rheumatism and a variety of diseases. An infusion of leaves is used for dyspepsia, while essential oil distilled from the leaves as an embrocation in rheumatic arthritis [38]. *A. indica* is used as anti-inflammatory in Taiwan [39]. *A. malabarica* is used in folk medicine for the treatment of cancer and liver disorders [40]. Other traditional uses documented are as antispasmodic, diaphoretic, antipyretic and antiperiodic properties [41].

b. Pharmacology:

Traditional use of *A. malabarica* in the treatment of cancer has been supported by demonstration of significant protection offered by leaf ethanolic extract against cancer induced in mice [40].

iv. *Plectranthus*:

Ethnopharmacology/pharmacology:

Ethnobotanical uses of *Plectranthus* have been reviewed by Lukhoba *et. al.* revealing varied uses of around 62 species [42]. Traditional applications include treatment for skin conditions, respiratory conditions, digestive disorder, genito-urinary problems, infections and associated fever and musculo-skeletal conditions. Members of this genus are also used in central nervous ailments, psychological problems, insomnia, etc. According to authors, *P. mollis*, a herb found only in India, is used against eight different categories of medicinal problems that include, respiratory stimulant and vasoconstrictor, febrifuge, rheumatism, cardiac depressant, haemorrhage, mental retardation, snakebites and as general tonic. Pharmacologically, *P. mollis* is reported to exhibit relaxant activity on smooth and skeletal muscles as well as cytotoxic and anti-tumour promoting activities. Miscellaneously, *P. mollis* is used as an insect repellent and as instrument to drive evil spirit away. It is an edible plant, leave being cooked and eaten.

Post this review article, a single article has been published by Mgole and co-workers. In it ethnobotanical study has been carried out in six villages in the Bunda district, Mara Region, Tanzania that revealed use of leaf infusion of *P. kilimandschari* in chest pain, cough, dysmenorrhoea and dysentery. The plant is also used in psychiatric problems and ulcers [43].

v. *Lavandula*:

a. Ethnopharmacology:

A glance at recent literature reveals different purposes for which various species of *Lavandula* are used. *L. angustifolia* (= *L. officinalis*) is extensively used for various conditions that include rheumatism, lumbago [44], diabetes, hypertension [45], ailments of urinary system, nervous system and asthma [46]. *L. stoechas* is used for headache, nervous disorder, insomnia, hypertension and inflamed wounds [47], rheumatism [44], diabetes [48], etc. *L. latifolia* is used in general gynaecological disorders [49, 50]. *L. spica* is used in fever, stomachache [51]. *L. multifida* is used in alopecia, as anti-asthmatic, anti-catarrhal, antiseptic, antispasmodic, anti-vertiginous, anxiolytic, bronchidilator, carminative, diaphoretic, diuretic and stomachic [52]. *L. antineae* an endemic herb of central

Sahara is used in chills [53]. *L. dentata* is used in hypertension, diabetes and cardiac disorders [54].

b. Pharmacology:

A number of pharmacological studies have confirmed traditional uses of *L. angustifolia* (= *L. officinalis*) [55-60] as well as have demonstrated new activities and potential uses such as inhibition of acetyl cholinesterase [61], HIV RT inhibition [62] and anti tyrosinase activity as a possible use in skin colour lightening and food preservation [63]. Similarly anticonvulsant and antispasmodic activities of *L. stoechas* have been demonstrated [64]

Section II. Essential oil analysis:

i. *Leucas*:

Analysis of leaf and flower essential oils of *L. martinicensis* revealed 1-hepten-3-ol (7.6-21.5% and 12.8-17.8%) and germacrene D (30.3-39.9% and 29.7-37.0%), as major constituents [65]. GC-MS analysis of petroleum ether fraction of *L. hyssopifolia* revealed fourteen aliphatic long-chain hydrocarbons from heptadecane to hexacosane and nine aliphatic ethyl esters, ethyl tetradecanoate to ethyl heneicosanoate [66]. Essential oil from inflorescence of *L. cephalotes* was found to contain caryophyllene oxide, 26.56%; δ-fenchene, 12.02%; β-ionone, 9.41%; 1-hepten-3-ol, 6.53%; menthol, 6.30%; decahydro naphthalene, 5.15%; α-cadinol, 2.13% and β-caryophyllene 4.05% [67]. Report on volatiles from *L .indica* showed sesquiterpene β-caryophyllene, 24.9%; monoterpene linalool, 24.4%; octane derivatives, 1-octen-3-ol, 4.2%; 3-octanol, 2.3%; trans-2-octen-3-ol, 2.1% and 3-octanone, 2.0% as well as hexane derivatives cis-3-hexen-1-ol, 4.2%; hexanol, 3.1% and trans-2-hexen-l-ol, 2.4% as principle constituents [68]. β-cubebene, 38.15%; α-pinene, 19.71%; β-caryophyllene, 7.41% and α-terpinolene, 5.34% were the major constituents from volatile components obtained from leaves of *L. milanjiana* [69]. Chemical analysis from aerial parts of *L. glabrata* gave *iso*-menthone, 31.8%; pulegone, 11.4%; piperitone, 10.6% and piperitenone, 6.67% as major constituents. The oil also exhibited strong anti-bacterial property [70].

ii. *Anisochilus*:

Essential oil obtained by hydro distillation from aerial parts of *A. carnosus* was active against Gram +ve and Gram –ve bacteria. The oil was essentially terpenic in composition with carvacrol, camphor and alpha cis-begamotene accounting for around 65% of entire composition [71]. Essential oil analysis of oil isolated from *A. carnosus* growing in southern India was also terpenic in composition with sesqueterpene caryophyllene and its oxide accounting for 23% and monoterpenes accounting for 33% of oil composition [72]. Essential oil of *A. carnosus* was found to reduce *in vitro* intestinal manifestations of allergic and anaphylactic disorders.

iii. *Anisomeles*:

Two report on the analysis of essential oil of this genus are available. Analysis of *A. malabarica* essential oil revealed presence of citral, geranic acid and hydrocarbons as main constituents [38]. Essential oil analysis of leaves of *A. indica* led to identification of β-pinene, eugenol, 1,8-cineol as major constituents along with α-pinene, d-limonene, methylchavicol, d-α-thujone, citral, borneol, nerol, α-terpineol, azulene and caryophyllene [130].

iv. *Plectranthus*:

The essential oils of fresh and dried leaves of *P. glandulosus* from Cameroon were analyzed by GC and GC/MS. The oils were characterized by a high percentage of oxygenated monoterpenes (58.6% and 84.6% respectively) represented by cis-piperitone oxide (3.0% and 35.1%), trans-piperitone oxide (0.5% and 12.6%), fenchone (30.8% and 21.6%) and piperitenone oxide (10.9% and 6.0%). The main monoterpene hydrocarbons were terpinolene (25.2% and 7.7%), limonene (3.2% and 1.7%) and myrcene (2.2% and 1.6%). Sesquiterpene derivatives were found in a very low percentage (<2.5%), represented mainly by germacrene D (1.4% and 1.0%) [74]. Analysis of oil of shade dried aerial parts of *P. marrubioides* revealed presence of camphor (49%), 1,8-cineol (9%), p-cymene(3%), α-terpenene (3%), fenchone and isocaryophyllene (both 2%) [75]. The oil was most repellent among the oils tested against 5-7days old females of *Anopheles gambiae* (RD_{50} = 8.9 X 10^{-5} mg/cm^2, 95% CI). Essential oil of same plant was also reported to posseses most potent fumigant toxicity against same

mosquito species by same group [76]. In a similar study, oil of *P. longip*es, was evaluated for repellency on forearms of human volunteers against *A. gambiae*. The oil was found to be quite potent. [77]. Analysis of oil of *P. amboinicus* gave thymol (64.3%), p-cymene (10.3%), γ-terpinene (9.9%) and β-caryophyllene (2.8%) as major components [78]. The leaf oil of this species demonstrated antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris* and *Aeromonas caviae* as well as moderate fungicidal activity against *Aspergillus niger* [78]. In another study, analysis of oil of same species from Tamil Nadu, India showed chemical compostion as carvacrol (28.65%) as major component followed by thymol (21.66%), α-humulene (9.67%), undecanal (8.29%), γ-terpinene (7.76%), ρ-cymene (6.46%), caryophyllene oxide (5.85%), α-terpineol (3.28%) and β-selinene (2.01%) [79]. The oil was tested for its malarial vector control effect against larvae of *Anopheles stephensi* with LC_{50} values were 33.54 (after 12 h) and 28.37 ppm (after 24 h). The LC_{90} values were 70.27 (after 12 h) and 59.38 ppm (after 24 h). Oil of *P. glandulosus* from Cameroon and Germany evoked a maximum percent repellency of 100% for adult *Prostephanus truncatus* and two strains of *Sitophilus zeamais* but the authors concluded that fenchone a [2.2.1]bicycloheptane monoterpene, though a major constituent of *P. glandulosus* oil, might only be a minor component of its bioactivity [80].

v. *Lavandula*:

Compilation of different aspects of essential oil of the genus *Lavandula* has already appeared [81]. In general, *Lavandula* essential oil is characterized by linalol, linalyl acetate, camphor, 1,8-cineol, carvacrol, etc. Here *Lavandula* oils showing compositions different from those normally found are also reviewed below.

Main components of *L. stoechas* ssp. *stoechas* oil were pulegone (40.4%), menthol (18.1%) and menthone (12.6%). The essential oil of the plant was evaluated for anti-bacterial and for panel of cytotoxic activities [82]. It was found to be active against COL-2 (Human Colon Cancer-2) (9.8 μg/ml) and weakly active against LNCaP (hormone-dependent human prostate cancer) (17.6 μg/ml) while the chloroform extract of the same plant was found to be highly active against P-388 (1.4 μg/ml). Both oil and chloroform extract did not show any

activity against the ASK cell line. The essential oil was tested against standard bacterial strains and showed antibacterial activity against most of the tested standard bacterial strains except, *S. epidermidis, E. faecalis, and C. albicans* [82]. Analysis of volatile components of *L. luisieri* collected from different sources, by direct thermal desorption–GCMS revealed camphor and 1,8-cineole (up to 80.9 and 76.7% in leaves; 87.8 and 85.2% in flowers, respectively) as major constituents and 2,3,5,5-tetramethyl-4-methylene-2-cyclopenten-1-one as another major component (up to 60% in flowers and leaves) [83].

By using headspace solvent micro extraction coupled with hydrodistillation, composition of essential oil of *L. angustifolia* was studied which revealed linalool (32.8%), linalyl acetate (17.6%), lavandulyl acetate (15.9%), α-terpineol (6.7%) and geranyl acetate (5.0%) as major constituents [84]. Fifty-five samples of essential oil obtained from individual plants of *L. dentata* var. *dentata* collected from different parts of from Algeria were analysed using GC and ^{13}C-NMR spectroscopy. The results were submitted to chemometric analysis. Two principal clusters of equal importance were distinguished. The samples belonging to cluster I were characterized by a very high content of 1,8-cineole (mean value 48%). Conversely, the mean composition of the samples of cluster II were dominated by 1,8-cineole, β -pinene, trans-pinocarveol and linalool [85]. Analysis of essential oil of *L. angustifolia* from Xinjiang, China, gave linalool (44.54%), geraniol (11.02%), lavandul acetate (10.78%), 3,7-dimethyl-2,6-octadien-1-ol (10.35%), and isoterpineol (6.75%) as the main components [86]. Analysis of essential oil of *L. bipinnata* revealed trans-carveol (18.93%), pulegone (8.45%), camphor (7.09%) and menthol (5.89%) as major components. Other constituents present in fairly good amounts were piperitone (4.65%), caryophyllene oxide (3.68%), linalyl acetate (3.37%) and bicyclogermacrene (3.09%) [87]. The oil was screened for antimicrobial activity against bacteria and fungus. Oil was found to be very active against *B. subtilis*, *S. aureus*, *Micrococcus spp.*, *A. niger*, moderately active against *E. coli*, *S. dysentery*, *E. feacalis*, *VRE*, *C. albicans* and less activity against *P. aureginosa* and *P. notatum*. The minimum inhibiting concentration (MIC) of essential oil ranged from 0.5 to 2.0 µg/L and 2 to 4 µg/L for bacteria and fungi respectively [87].

Section III. Chemistry and bioactive molecules:

i. *Leucas*:

A recent article published by Chauhan *et. al.* gives a review of genus *Leucas* [5]. Work on this genus again again reviewed here.

Labellenic acid (octadeca-5,6-dienoic acid) was shown to be major component of seed oil of *L. cephalotes* [88]. 1-hydroxytetratriacontan-4-one and 32-methyltetratriacontan-8-ol were reported from the shoots of *L .aspera* [89]. Same group reported 28-hydroxypentatriacontan-7-one and 7-hydroxydotriacontan-2-one as well as 5-acetoxytriacontane and β-sitosterol from same plant [90].

Flavonoids, acacetin (**3**) and chrysoeriol (**12**) were isolated from aerial parts of *L. lavandulifolia* [91] while quercitin (**22**) and kaemferol (**23**) were found in leaves of *L. urticaefolia* [92]. Acylated flavone, apigenin 7-O-[6″-O-(p-hydroxy-*t*-cinnamoyl)glucoside] (**8**) was isolated from aerial parts of *L. neufliseana* [93]. From the whole herb of *L. cephalotes*, eight flavones, 5-hydroxy-7,4'-dimethoxyflavone (**4**), pillion (**11**), gonzalitosin I (**13**), tricin (**20**), cosmosin (**5**), apigenin 7-O-β-D-(6"-O-p-coumaroyl)*glucopyranoside* (**9**), anisofolin A (**7**) and luteolin 4-O-β-D-glucuronopyranoside (**14**) were isolated [94].

Four coumarins, siderin (**34**), coumarsabin (**31**), 8-methoxycoumarsabin (**32**) and coumarleucasin (**33**) and one chromone leucasone (**35**), were isolated from roots of *L. inflata* [95]. An isopimarane rhamnoglucoside, linifolioside (**131**) was isolated from whole plant of *L. linifolia* [96]. An acyclic diterpene fatty ester, trans-phytyl palmitate (**92**) was isolated from aerial parts of *L. nutans* [97]. Three labdane type diterpenes, 3-oxo-marrubiin (**108**) and a mixture of two related C-15 epimeric diterpenes 9α,13α,15,16-bisepoxy-15-hydroxy-3-oxo-labdan-6β, 19-olide (**109, 110**) were isolated from aerial parts of *L. neufliseana* [98]. From the whole herb of *L. cephalotes,* new labdane, norlabdane and abietane type diterpenes named leucasdins A (**111**), B (**126**) and C (**173**) respectively, were isolated [94].

An oleane type lactone leucalactone (**225**) was isolated from roots of *L. aspera* [99]. A lupane type triterpene glycoside, leucasin (**230**) was isolated from

whole plant of *L. nutans* [100]. Oleanolic acid (**205**) was isolated from whole herb of *L. cephalotes* [94]. Sterols campesterol (**240**), brassicasterol (**239**), cholesterol (**241**), β-sitosterol (**231**), and stigmasterol (**235**) were isolated from *L. lanata* [101] while 7-oxositosterol (**232**), 7-oxostigmasterol (**236**), 7α-hydroxysitosterol (**233**), 7α–hydroxystigmasterol (**237**) and stigmasterol (**235**) were isolated from whole herb of *L. cephalotes* [94]. Alkaloid nicotine (**69**) was isolated from the aerial parts of *L. aspera* [102].

(*E*)-Phytol (**91**) isolated from *L. volkensi* displayed antimycobacterial activity against *Mycobacterium tuberculosis* [103]. Oleanolic acid 3-acetate (**206**), apigenin (**1**), apigenin-7-O-β-D-(6"-O-p-coumaroyl)glucopyranoside (**9**), cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone) (**15**), mixture of β-sitosterol (**231**) and stigmasterol (**235**), and mixture of β-sitosterol-3-O-β-D-glucoside (**234**) and stigmasterol-3-O-β-D-glucoside (**238**) were isolated from the n-hexane, chloroform and ethyl acetate fractions of the methanolic extract of the whole herbs of *L. mollissima* var. *chinensis* of which apigenin (**1**) exhibited potent anti-inflammatory activity [104]. Bioassay guided separation of *L. aspera* yielded eight lignans (**43-50**) and four flavonoids (**3**, **12**, **7**, **9**) of which lignans **49, 50** and **45** inhibited prostaglandin synthesis while lignans **49, 50 and 43** and all flavonoids exhibited antioxidant activity [105]. Same authors reported isolation of diterpenes leucasperones A (**124**) and B (**125**) and leucasperols A (**112**) and B (**113**), three new isopimarane glycosides, leucasperosides A (**132**), B (**133**), and C (**134**) together with the known compounds asperphenamate (**70**), maslinic acid (**207**), (-)-isololiolide (**82**), and linifolioside (**131**) from same source. Out of these, leucasperone A (**124**), leucasperosides A (**132**) and B (**133**), and linifolioside (**131**) showed inhibition of prostaglandin induced contractions [106]. Flavonoid glucosides leufolins A (**61**) and B (**57**), were isolated from the ethyl acetate soluble fraction of the whole plants of *L. urticifolia*. Both of these compounds exhibited significant inhibitory potential against the enzyme butyrylcholinesterase [107]. Six phenylethanoid glycosides (**51-56**) were isolated from aerial parts of *L. indica*. All these compounds exhibited significant antioxidant activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. These compounds were also found to be moderate inhibitors of enzyme xanthine oxidase [108].

ii. *Anisochilus*:

Few reports on chemical investigation of genus *Anisochilus* are available. Leaves of *A. carnosus* revealed presence of small amount of flavonoid leuteolin (**10**) and apigenin (**1**) [109] while a 3,5,7,4'- tetrahydroxy-8-isoprenyl flavonoid **(25)** was isolated from its aerial parts [110]. A quinone (**243**) toxic to fungi species *Aspergillus*, *Penicillium* and *Fusarium* was characterized from aerial parts of *A. carnosus* [111]. Recently two new diterpenes, 4-epi-triptobenzene L (**145**) and 12-Odeacetyl-6-O-acetyl-19-acetyloxycoleon Q (**174**), as well as eight known diterpenes (**142-144**, **146**, **147**, **175** and **176**) were isolated from the aerial parts of *A. hamandii* [112]. Compounds **143**, **145**, **174** and **176** exhibited antiplasmodial activity against *Plasmodium falciparum*, while **142**, **143**, and **148** showed antimycobacterial activity against *M. tuberculosis*. In addition, **176** showed strong cytotoxicity against NCI-H187 cells.

iii. *Anisomeles*:

Betulinic acid (**226**) was isolated from petroleum ether extract of *A. malabarica* [113]. Ovatodiolide (**93**) and anisomelic acid (**94**) were isolated from hexane extract of whole extract of *A. malabarica* [114] Anisomelolide (**95**), malabaric acid (**96**), 2-acetoxymalabaric acid (**97**), anisomelyl acetate (**98**) and anisomelol (**99**) were isolated from *A. malabarica* [115] so also anisomelin (**17**) [116]. Apigenin 7-O-β-D-(4",6"-di-O-p-coumaroyl)glucoside (**64**) and its 2",6" isomer (**62**) were isolated from stem parts of this plant [117].

Chemical investigations on *A. ovata* led to isolation of ovatodiolide (**93**) [118, 124], anisofolin-A (apigenin 7-O-β-D-(3",6"-di-O-p-coumaroyl)glucoside) (**63**) [119], salvigenin (5-hydroxy-3',4',6,7-tetramethoxylflavone) (**18**), anisomelin (**17**), and apigenin (**1**) [120], flavone glycosides (**62, 64**) [121], 5,6-Dimethoxy-7,3',4'-trihydroxyflavone (**19**) [122], cosmosin hydrate (**6**), cosmosin (**5**), terniflorin (**58**), prunin (**28**), prunin- 6"-p-coumarate (**60**), and prunin-3",6"-di-p-coumarate (**65**) [123] as well as various other terpenoids like glutinone (**213**), friedelin (**214**), glutinol (**212**), betulin (**227**); steroids like β-sitosterol (**231**) and its glucoside (**234**); methyl-p-hydroxycinnamate (**36**) and anisomelic acid (**94**) alongwith n-hentriacontane [124].

Chemical investigation of petroleum ether extract *A. indica* led to isolation of stigmasterol (**235**) and β-sitosterol (**231**) [125], paraffins and fatty acids cerotic, pentacosanoic, lignoceric, tricosanoic, behemic, heneicosanoic, arachic, stearic, and palmitic acids [126]. Benzene extraction of roots of *A. indica* led to isolation of tetracosane, tetracosanol, β-amyrin (**211**), friedelin (**214**), and betulinic acid (**226**) while chloroform fraction of the ethanol extract led to isolation of ovatodiolide (**93**) and anisomelic acid (**94**), along with flavonoid anisomelin (4',5-dihydroxy-3',6,7-trimethoxyflavone) (**17**) [127]. 4,7-Oxycycloanisomelic acid (**100**), 4-methylene-5-hydroxyovatodiolode (**103**) and 4-methylene-5-oxoanisomelic acid (**101**) were isolated from dried whole plant of *A. indica* along with previously known ovatodiolide (**93**), 4,5-epoxyovatodiolide (**102**) and anisomelic acid (**94**) [128]. Another study reported isolation of n-hexacosane, n-hexacosanol, β-amyrin (**211**), ovatodiolide (**93**), anisomelic acid (**94**), β-sitosterol (**231**) along with stearic, palmitic and lignoceric acids from same species [129]. Five new cembrane-type diterpenoids (**103-107**), a new flavonoid glucoside (**59**) and 17 known compdounds such as apigenin (**1**), terniflorin (**58**), 5,8,4′-trihydroxy-7,3′-dimethoxyflavone **(21)**, anisofolin A (**63**), anisofolin B (**62**), and prunin-6″-*p*-coumarate (**60**) were isolated from a methanol extract of *A. indica*. Also were isolated from this plant, maslinic acid (**207**), 3-*O*-*trans*-*p*-coumaroylmaslinic acid (**208**), hederagenin (**209**), and arjunolic acid (**210**); five benzenoids viz. *p*-hydroxybenzoic methyl ester (**67**), *p*-hydroxybenzoic acid (**68**), methyl, *p*-hydroxycinnamate (**36**), methyl, 3,4-dihydroxycinnamate (**37**), and anisovatodside[131].

iv. *Plectranthus*:

So far work on genus *Plectranthus* has been reviewed in three articles viz. Abdel-Mogib *et. al.* review covering chemistry literature up to 1999 [132], another review which covers chemistry of Indian *Plectranthus* covers up to date Indian literature [133]. Also a separate review on *P. barbatus* covers literature up to 2010 [134a and b]has been published. Here literature after 1999 and excluding Indian work is reviewed.

Three new eudesmane sesquiterpenes, plectranthone (**83**), desacetylplectranthone (**84**), isodeacetylplectranthone (**85**) and the three known

flavonols pachypodol (**24**), casticin (**27**), and chrysosplenol D (**26**) were isolated for the first time from the aerial parts of *P. cylindraceus* [135]. Two new antioxidative diterpenoids, plectranthol A (**149**) and plectranthol B (**150**) along with 2 known diterpenoids, parvifloron E (**151**) and F (**152**) were isolated from the leaves of *P. nummularius*. Antioxidative activities of these compounds were measured by the α,α-diphenyl-β-picrylhydrazyl (DPPH) method. All the compounds were more potent radical scavengers than α-tocopherol [136]. Three new diterpenoids, a neoclerodane (**130**) and two labdane derivatives (**114**, **115**) were isolated from an acetone extract of *P. ornatus* [137]. These diterpenes showed moderate anti-candida activity. Six new diterpenoids, three labdane (**116**, **117**, **118**) and three kaurane and one known kaurane (**185**-**188**) derivatives were isolated from an acetone extract of *P. fruticosus* together with β-sitosterol (**231**), stigmasterol (**235**), and β-amyrin (**211**) [138]. Same group later reported isolation of eight new diterpenoids, two labdanes (**119** new and **244** known) and eight kaurane (**189**-**195** new and **196** known) derivatives, a new aromadendrane-type sesquiterpenoid (**88**) along with five flavonoids (**18**, **15**, **16**, **2** and **4**) from the acetone extract of *P. fruticosus* [139].

Antioxidative-activity-guided fractionation of extract of the aerial parts of *P. cyaneus* yielded the two novel abietanoid diterpenoids 11,20-dihydroxysugiol (**155**) and 1,11-epoxy-6,12-dihydroxy-20-norabieta-1(10),5,8,11,13-pentaen-7-one (**156**) in addition to 11-hydroxysugiol (**154**) and the main constituent carnosolon (**153**) [140]. A new trihomo abietane diterpene viz 9R-(2-oxopropyl)abietane derivative (**157**) was isolated from acetone extract of *P. grandidentatus* [141]. Phytochemical investigation of a hexane extract of the aerial parts of *P. ornatus* yielded three new neoclerodane diterpenoids (**127**-**129**), two labdane diterpenes (**122, 123**) for the first time as natural products along with 1α,6β-diacetoxy-8,13*R**-epoxy-14-labden-11-one (**115**) previously reported from the same source, mixture of β-sitosterol (**231**) and stigmasterol (**235**), 3β-acetyl-α-amyrin (**221**) and friedelin (**214**) [142]. Several known compounds ent-16-kauren-19-ol (**197**), ent-16-kauren-19-oic (**198**), xylopic (**199**), and xylopinic (**200**) acids, hinokiol **(242)**, parvifloron D (**168**) and F (**152**), 4β,6β-dihydroxy-1α,5β(H)-guai-9-ene (**86**), 4β,6β-dihydroxy-1α,5β(H)-guai-10(14)-ene (**87**), salvigenin **(18)**,

mixture of hexacosan-1,26-diol and octacosan-1,28-diol ferulate diesters (**39**), mixture of esters from fatty acids and 2-(4-hydroxyphenyl)-ethanol (**66**), esters from ferulic acid and fatty alcohols (**38**), mixture of β-sitosterol (**231**) and stigmasterol (**235**) and ursolic acid **(215)** were identified from *P. strigosus* for the first time [143]. Rana *et. al*. reported analysis of seeds of *P. amboinicus*. It was found to contain about 27% of oil. Linolenic acid (32.5%) was found to be major fatty acid followed by linoleic (26.3%), oleic (22.7%), palmitic (12.8%), and stearic (5.8%) acids [144].

Three new diterpenoids including two pimaranes (**135**, **136**) and a labdane (**121**) were isolated from the whole herb of *P. ernstii*. Structures of these compounds were determined as *rel*-15(ζ),16-epoxy-7α-hydroxypimar-8,14-ene (**135**) and *rel*-15(ζ),16-epoxy-7-oxopimar-8,14-ene (**136**), and compdound **121** elucidated as 1R,11S-dihydroxy-8R,13Repoxylabd-14-ene on the basis of single-crystal x-ray structural analysis. Compdound **135** exhibited moderate anti-staphylococcal activity against a range of multidrug-resistant (MDR) and methicillin-resistant strains of *S. aureus* (MRSA) with MIC 32μg/ml. All three diterpenes exhibited antimycobacterial activity against three strains of rapidly growing mycobacteria with MIC values ranging from 8 to 128μg/ml [145]. New diterpenes **177** and **201** were isolated from *P. saccatus* and *P. porcatus*, respectively [146]. Compound **201** showed a moderate antibacterial activity (MIC, 62.5μg/ml) against *Staphylococcus aureus*. Four abietane (**158-161**), three kaurane (**202-204**) and one labdane (**120**) type diterpenes were isolated from *Plectranthus* spp. [147]. Their anti-mycobacterial activity was reported against MDR bacteria. Bangera *et. al.* analysed carotenoid content of *P. rotundifolius* and found to contain lutein (1.48), zeaxanthin (0.07), α-carotene (0.08), β-carotene (0.01) and neoxanthin (0.04 [148]).

Five known abietane diterpenes of the royleanone and coleon type, namely, fatty acid esters of 7α-acyloxy-6β-hydroxyroyleanone (**162**), grandidone A (**169**), 7α-acetoxy-6β-hydroxyroyleanone (**158**), 6β,7α-dihydroxyroyleanone (**159**) and coleon U (**163**), isolated from *P. grandidentatus*, were evaluated for their effect on the proliferation of human lymphocytes induced by the mitogen PHA [149]. Compounds **158** and **163** yielded the most potent antiproliferative activities

amongst others. Their mechanism of action was suggesting a preferential inhibition of T-lymphocyte proliferation. Ten abietanes (**158-161**, **163-166**, **180**, **181**) active against methicillin- and vancomycin-resistant bacteria were isolated from *P. grandidentatus* (**158-161**, **163-165**) and *P. hereroensis* (**160**, **166**, **180**, and **181**) [150]. Bio-assay guided fractionation of an acetone extract of leaves of *P. saccatus* resulted in the isolation of beyerane diterpenoids (**183, 184**) in 93:7 ratio with insect antifeedant activity [151]. Compound (**183**), characterised as *ent*-3b-(3-methyl-2-butenoyl)oxy-15-beyeren-19-oic acid, showed insect antifeedant activity against *Spodoptera littoralis*.

Known quinonoid abietane diterpenoids were obtained from new sources. These included a mixture of the (4R, 19R) and (4R, 19S) diastereoisomers of coleon A (**170**, **171**) from *P. aff. puberulentus*, coleon A lactone (**172**) from *P. puberulentus* and coleon U (**163**) and coleon U quinone (**167**) from *P. forsteri 'Marginatus'*. These compounds, and the crude acetone extracts from the leaf surfaces of 11 species of *Plectranthus*, were tested for antifeedant activity against *S. littoralis*, antibacterial activity against *Bacillus subtilis* and *Pseudomonas syringae* and antifungal activity against *Cladosporium herbarum*. The coleon A mixture (**170**, **171**) showed potent antifeedant activity against *S. littoralis*, whereas coleon U (**163**) showed the greatest antimicrobial activity [151]. The abietanoid dienedione plectrinone A (**182**) isolated from *P. barbatus* was the active compound responsible for reduced H^+,K^+-ATPase activity [152]. In a different study using zebrafish as a platform for natural product discovery, Crawford *et. al.* isolated Coleon A (**170**) as the active ingredient from *P. barbatus* [153]. The assay searched for ability of extract and compounds to potentiate a sub-effective dose of the anti-angiogenic compound SU5416, an indoline inhibitor of the vascular endothelial growth factor (VEGF) receptor. Coleon U (6,11,12,14-tetrahydroxy-abieta-5,8,11,13-tetraene-7-one, (**163**) a diterpene compound isolated from *P. grandidentatus* was found to possess antiproliferative effect on several human cancer cell lines. The compound selectively induced an apoptotic pathway dependent on nPKC-δ and -ε activation [154].

P. eckloni is traditionally used in South Africa for treating stomach aches, nausea, vomiting and meningitis. Bioassay-guided fractionation of the ethyl

acetate extract of the plant led to the isolation of two known compounds, parvifloron D **(168)** and parvifloron F **(152)**, neither of which were previously reported from this species. These compounds exhibited MIC of 15.6 and 31.2μg/ml, respectively against *Listeria monocytogene*s. Values against a drug-sensitive strain of *M. tuberculosis* were 190 and 95 μg/ml, respectively. Ethyl acetate extract of *P. eckloni* and its isolated compounds were tested for their activity on tyrosinase inhibition. IC_{50} of the extract was found to be 61.7μg/ml. Antibacterial activity of the extract and its isolated compounds correlates with the traditional use of the plant for various ailments such as stomach aches, diarrhoea and skin diseases. The IC_{50} of parvifloron D (**168**) and parvifloron F (**152**) against vero cell lines were found to be 2.9 and 1.6 μg/ml, respectively. This is the first report of the bioactivity of *P. eckloni* extract and its constituents [155]. Rodrigues *et. al*. studied and established the protective role for 3β-hydroxy-3-deoxibarbatusin (**179**) and barbatusin (**178**) affording gastroprotection against gastric damage induced by ethanol for the activity of *P. grandis* [156].

v. *Lavandula*:

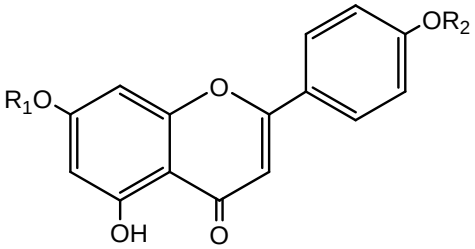
As mentioned earlier, a book by Lis-Balchin gives review of chemistry of genus *Lavandula* [80]. Phytochemical work which has been published after this review has been covered here. Also included is phytochemical work not covered in this book review.

Irregular monoterpenes, necrodanes (**75-81**) were isolated from *L. luisieri* [60, 157-158]. Lavandulol (**74**) was isolated from *L. officinalis* and *L. vera* [159]. Aromatic monoterpenes (**71-73**) were isolated from *L. gibsoni* [160]. Coumarin (**29**) and herniarin (**30**) were demonstrated from *L. officinalis* during biosynthetic studies. [161]. Enol esters of dopaldehyde and caffeic acid (**40**, **41**) along with rosmarinic acid (**42**) were detected in *L. angustifolia* [162]. Longipipene sesqueterpenes (**89**, **90**) were isolated from *L. stoechas* subspp. *stoechas*.

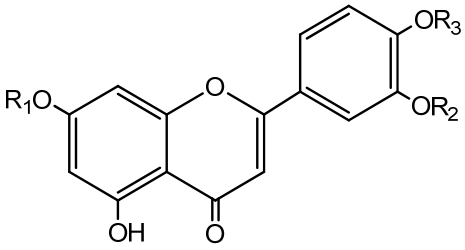
Higher terpenes have only been rarely isolated from genus *Lavandula*. Four new pimarane diterpenes (**137-140**) along with known pimarane glutinosin (**141**) were isolated from *L. multifida* [163]. One new (**223**) and five known triterpenoids (**224**, **228**, **229**, **222** and **217)** were isolated from the aerial parts of *L. spica* [164]. Triterpenic acids, ursolic (**215**), oleanolic (**206**), 2α, 3β-

dihydroxyursolic (**216**), 2α, 3β, 19α, 23-tetrahydroxyursolic (**218**), α- and β-amyrins (**220**, **211**), and the sterol, sitosteryl-3β-D-glucoside (**234**), were isolated and identified form *L. canariensis* [165]. Alkaloid 2-N-phenylaminonaphthalene was isolated from *L. vera* [166]. Other compounds isolated include oxygenated branched fatty acid and its methyl ester [167] and fatty acids [168]. Sosa and co-workers isolated four pimarane diterpenes (**137**-**140**), triterpene acids oleanolic acid (**205**), ursolic acid (**215**), maslinic acid (**207**), 3β,19α, 23-trihydroxy-urs-12-en-28-oic acid (**217**) [169].

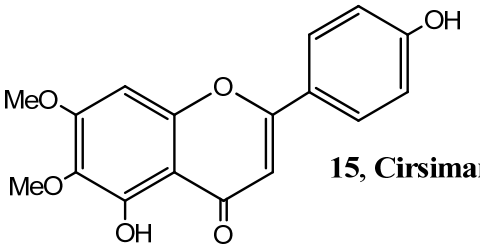
Figure 1: Compounds isolated from genera *Leucas*, *Anisochilus*, *Anisomeles*, *Lavandula* and *Plectranthus*.



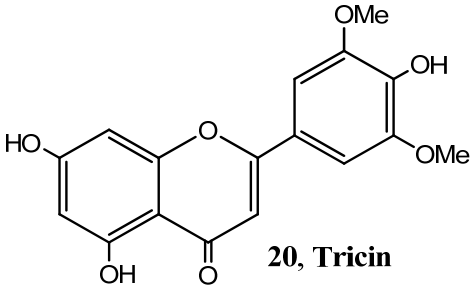
1, R_1=R_2=H, **Apigenin**
2, R_1=Me, R_2=H, **Genwanin**
3, R_1=H, R_2=Me, **Acacetin**
4, R_1=Me, R_2=Me, **Apigenin dimethyl ether**
5, R_1= Glc, R_2=H, **Cosmonsin**
6, R_1= Glc, R_2=H, **Cosmonsin hydrate**
7, R_1= (3,6-di-O-*p*-coumaroyl)glc, R_2=H **Anisofolin A**
8, R_1= 6-O-(*p*-hydroxy-*t*-cinnamoyl)glucoside, R_2=H
9, R_1= 6-O-(*p*-coumaroyl)glucoside, R_2=H



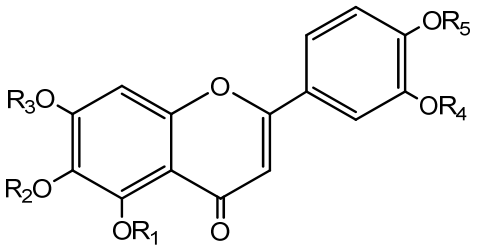
10, R_1=R_2=R_3=H, **Luteolin**
11, R_1=R_3= Me, R_2=H, **Pillion**
12, R_1=R_3= H, R_2=Me, **Chrysoeriol**
13,R_1=R_2=R_3=Me, **Gonzalitosin I**
14, R_1=R_2= H, R_3=GlcUA



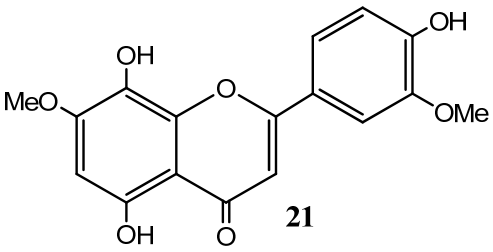
15, Cirsimaritin



20, Tricin



16, R_1=R_4=H, R_2=R_3=R_5=Me, **Eupatorin**
17, R_1=R_5=H, R_2=R_3=R_4=Me, **Anisomelin**
18, R_1=H, R_2=R_3=R_4=R_5=Me, **Salvigenin**
19, R_1=R_2=Me, R_3=R_4=R_5=H



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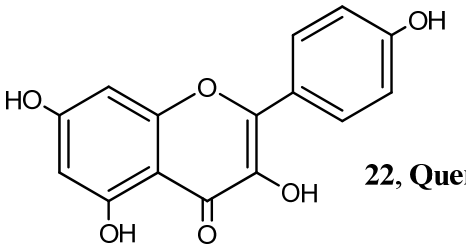
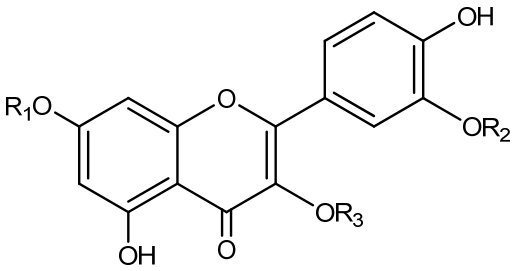
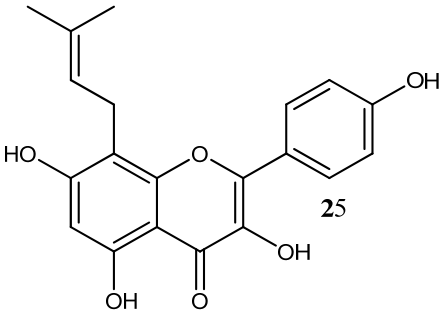


22, Quercitin



23, $R_1=R_2=R_3=H$, **Kaempferol**
24, $R_1=R_2=R_3=Me$, **Pachypodol**



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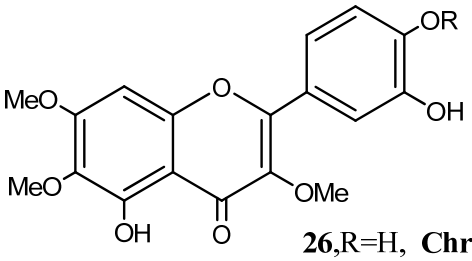
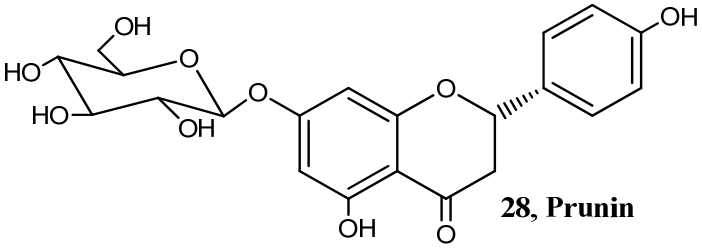
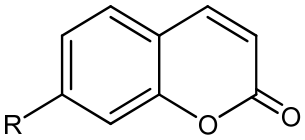


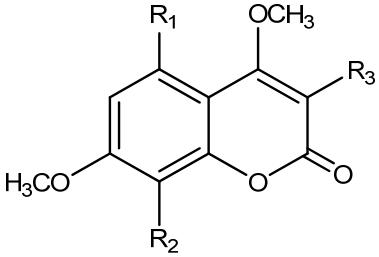
26,R=H, **Chrosophenol D**
27, R=Me, **Casticin**



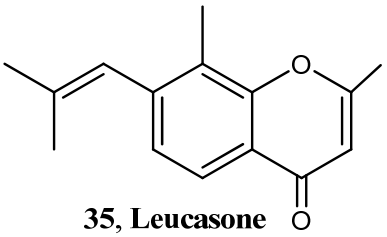
28, **Prunin**



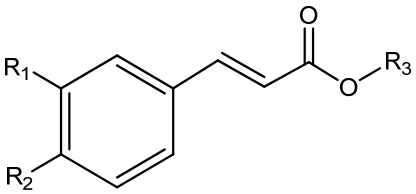
29, R=H, **Coumarin**
30, R=OMe, **Herniarin**



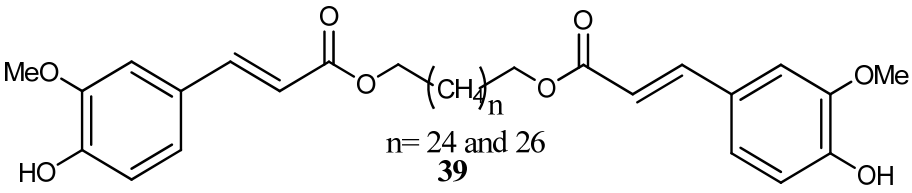
31, $R_1=R_3=CH_3$, $R_2=H$, **Coumarsabin**
32, $R_1=R_3=CH_3$, $R_2=OCH_3$, **8-Methoxycoumarsabin**
33, $R_1=CHO$, $R_2=OCH_3$, $R_3=CH_3$, **Coumarleucasin**
34, $R_1=CH_3$, $R_2=R_3=H$, **Siderin**



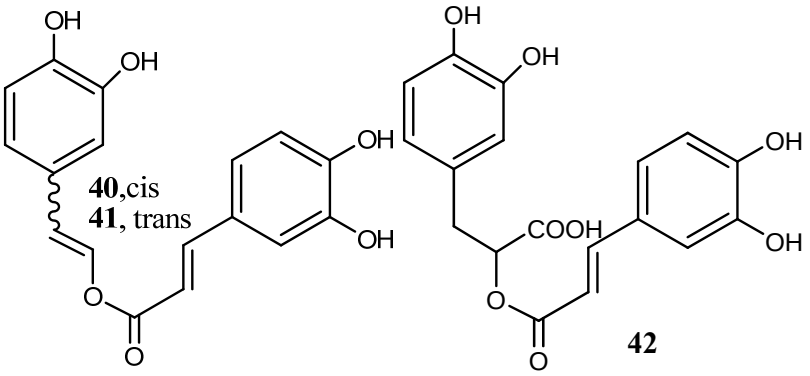
35, **Leucasone**



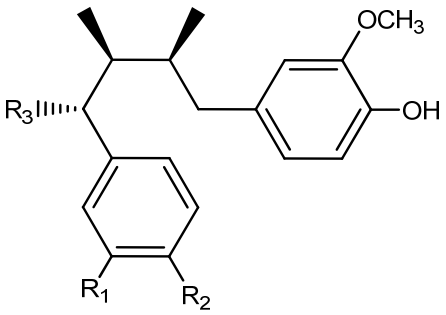
36, R_1= H, R_2= OH, R_3=Me
37, R_1= OH, R_2= OH, R_3=Me
38, R_1= OMe, R_2=OH, R_3= fatty alcohol



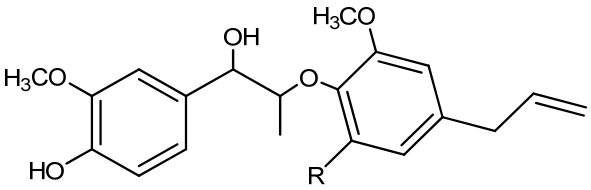
n= 24 and 26
39



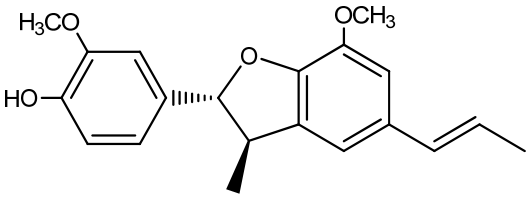
40,cis
41, trans
42



43, R_1= OCH_3, R_2= OH, R_3= H
44, R_1, R_2= -OCH_2O-, R_3= H,
45, R_1= OCH_3, R_2= OH, R_3= OH



46, R=H
47, R=OMe



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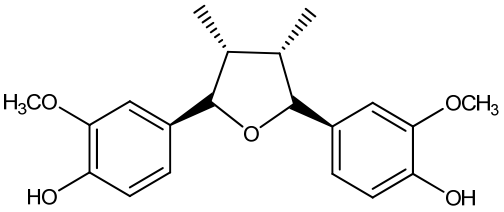

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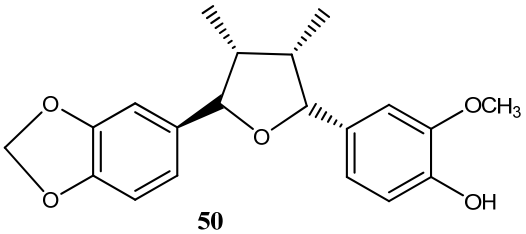

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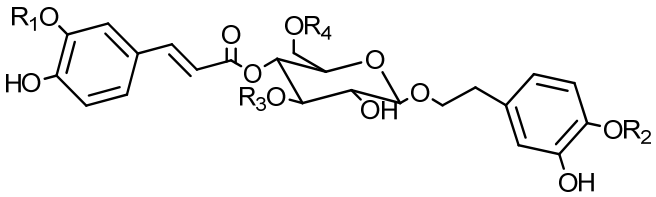
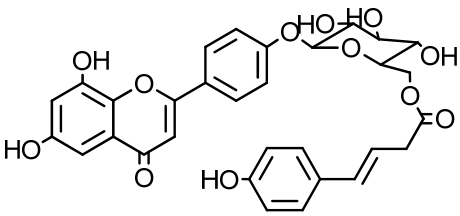
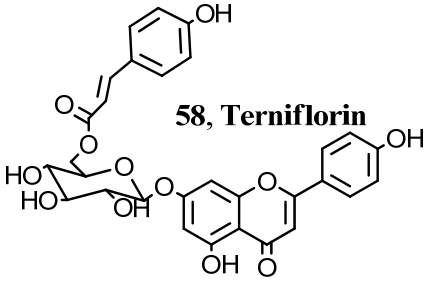


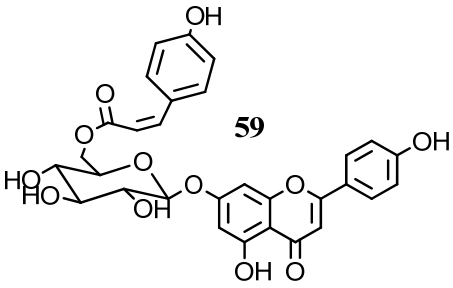
51, R_1=CH_3, R_2= H, R_3= Rhamnose, R_4= Rhamnose
52, R_1=CH_3, R_2=CH_3, R_3= Rhamnose, R_4= Arabinose
53, R_1=CH_3, R_2=CH_3, R_3= Rhamnose, R_4= Rhamnose
54, R_1=CH_3, R_2=CH_3, R_3= (Glc$\xrightarrow{1\,1\,1}$2 Rham), R_4= H
55, R_1=CH_3, R_2=CH_3, R_3= Rhamnose, R_4= H
56, R_1=H, R_2=H, R_3=H, R4=Rhamnose



57, Leofolin B



58, Terniflorin



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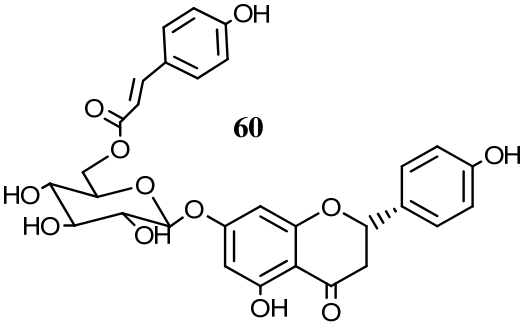

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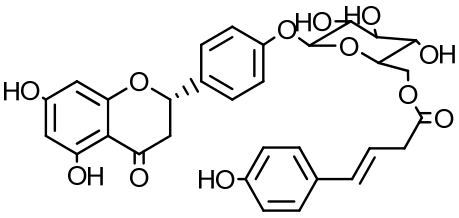
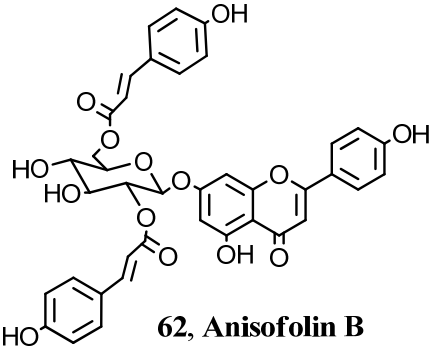
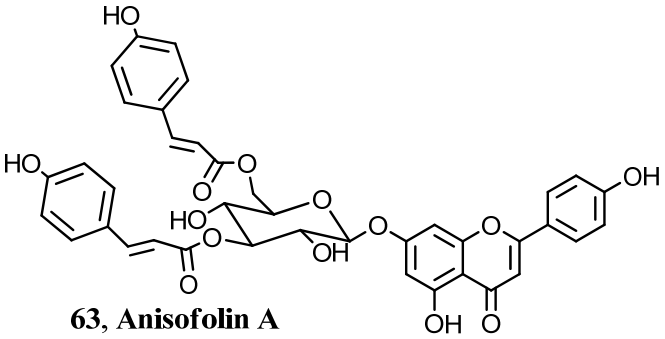


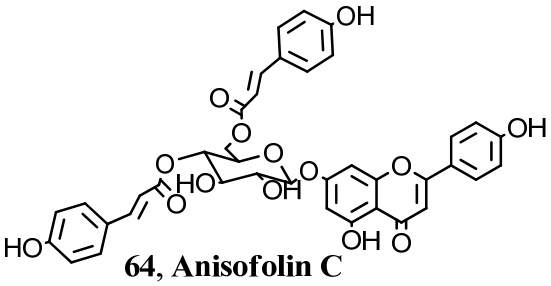
61, Leofolin A



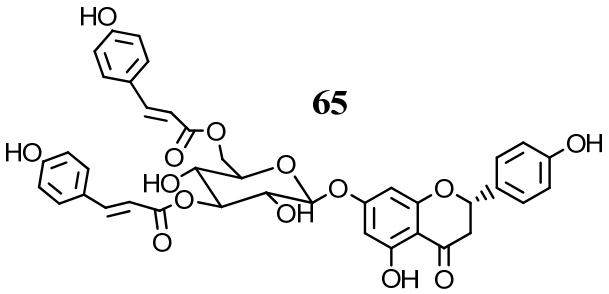
62, Anisofolin B



63, Anisofolin A



64, Anisofolin C



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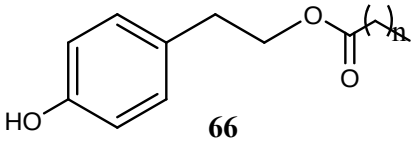
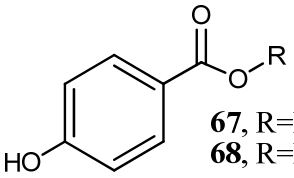
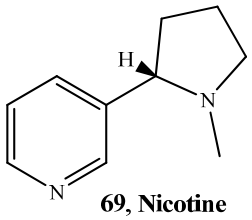


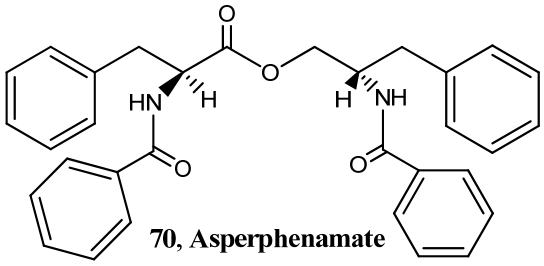
66



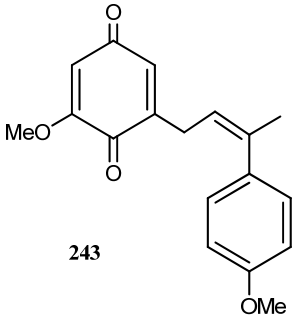
67, R=Me
68, R=H



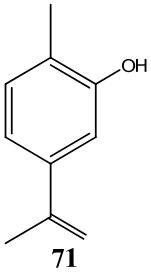
69, Nicotine



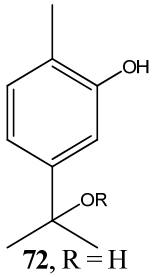
70, Asperphenamate



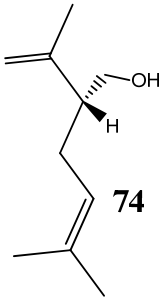
243



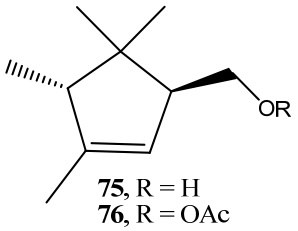
71



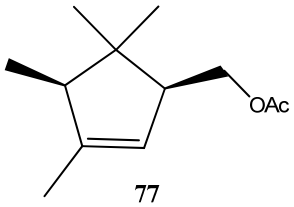
72, R = H
73, R= Me



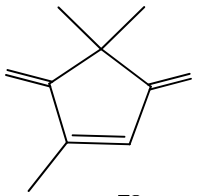
74



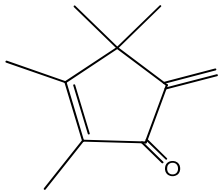
75, R = H
76, R = OAc



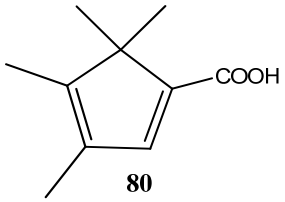
77



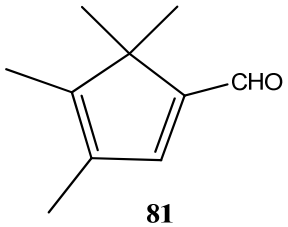
78



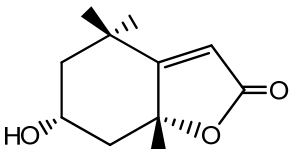
79



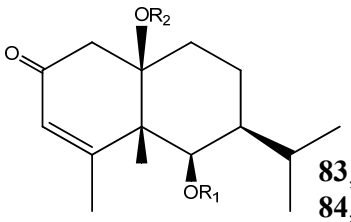
80



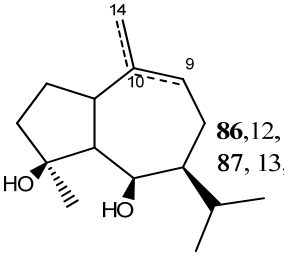
81



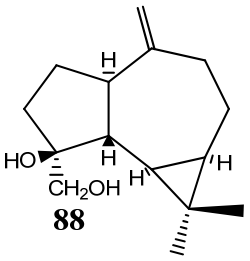
82, (-) Isololiolide



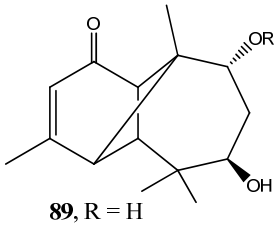
83, R_1= $COCH_3$, R_2= $COCH_3$
84, R_1= $COCH_3$, R_2= H
85, R_1= H, R_2= $COCH_3$



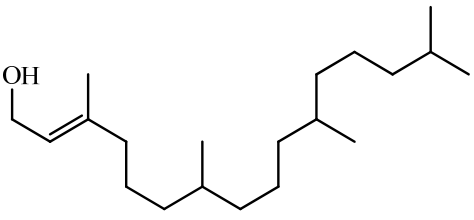
86,12, $\Delta^{9, 10}$
87, 13, $\Delta^{10, (14)}$



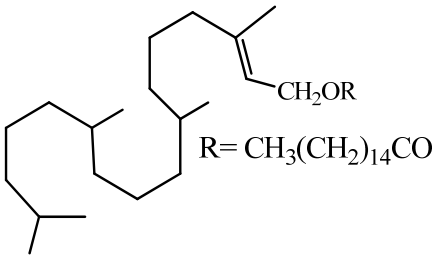
88



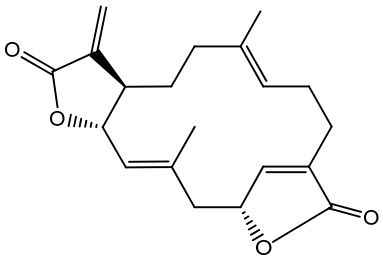
89, R = H
90, R = OAc



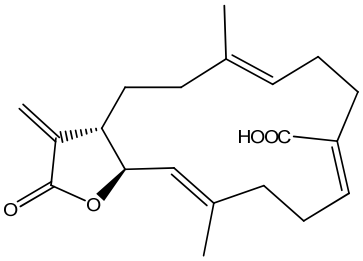
91, ***E*-phytol**



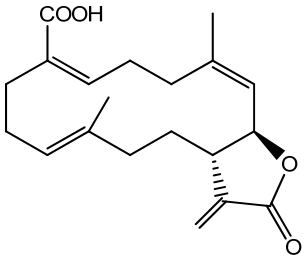
92, *E*-phytyl palmitate



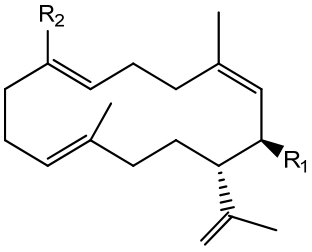
93, **Ovatodiolide**



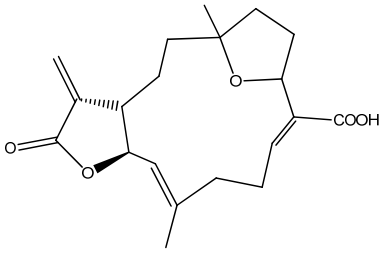
94, **Anisomelic acid**



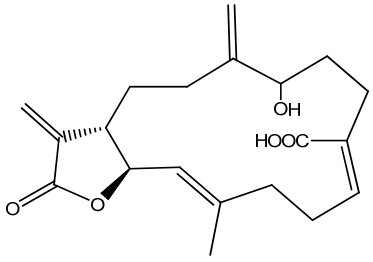
95, **Anismelolide**



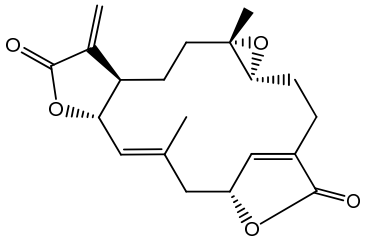
96, 19, R_1= H, R_2= COOH, **Malbaric acid**
97, 20, R_1= OAc, R_2= COOH
98, 21, R_1= H, R_2= CH_2OAc, **Anisomelyl acetate**
99, 22, R_1= H, R_2= CH_2OH, **Anisomelol**



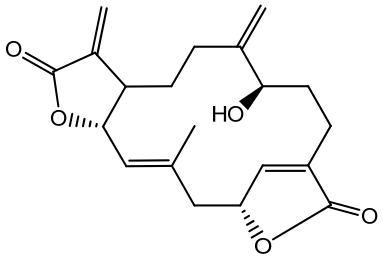
100



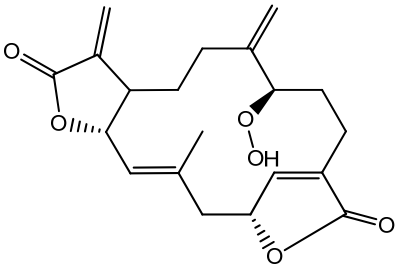
101



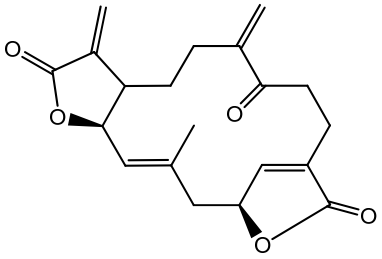
102



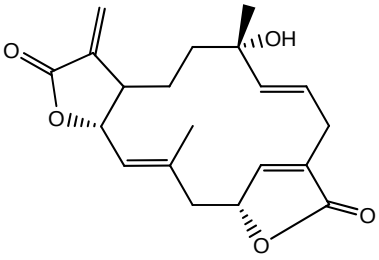
103



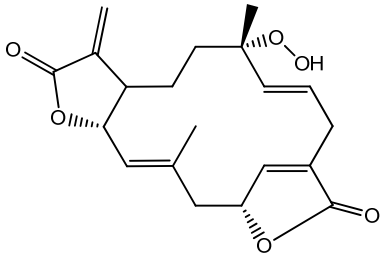
104



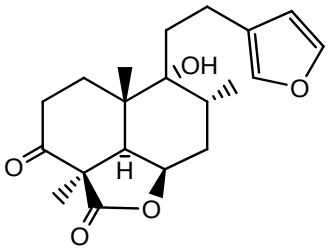
105



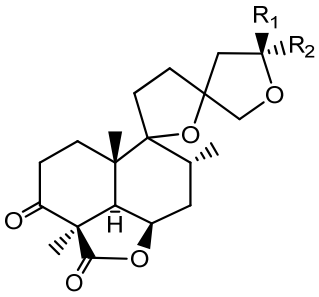
106



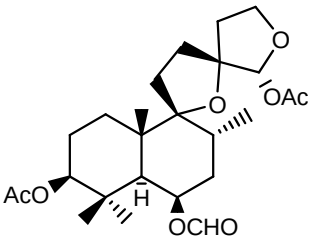
107



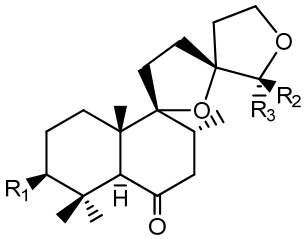
108



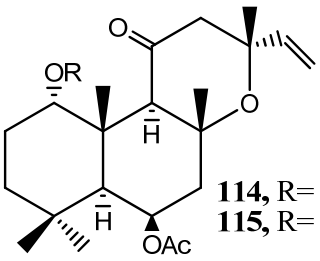
109, R_1=H, R_2=OH; **110**, R_1=OH, R_2=H



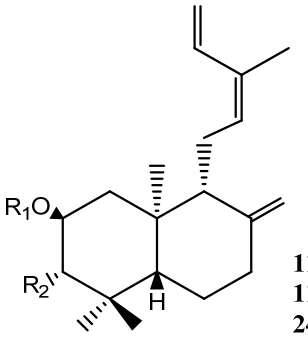
111, **Leucasdin A**



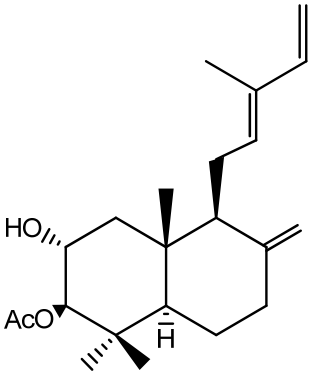
112, R_1= OH, R_2= H, R_3= OH, **Leucasperol A**
113, R_1= OH, R_2=OH, R_3= H, **Leucasperol B**



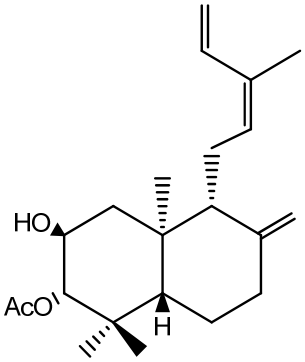
114, R= H
115, R= Ac



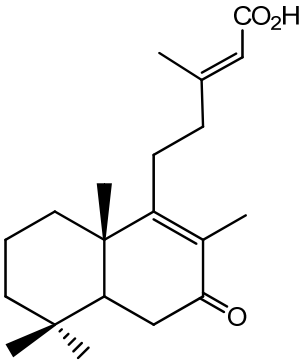
116, R_1, R_2= H
117, R_1= Ac, R_2= OH
244, R_1= H, R_2= OH



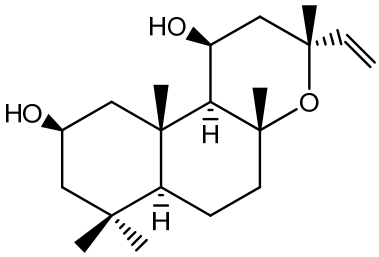
118



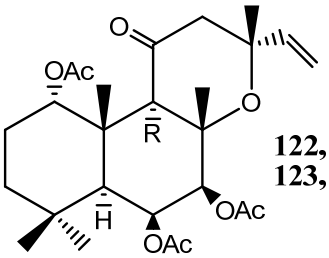
119



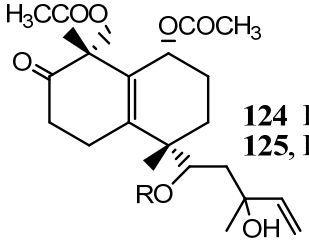
120



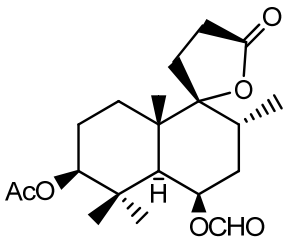
121



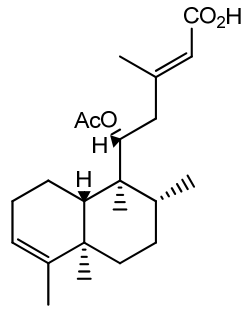
122, R= H
123, R= Ac



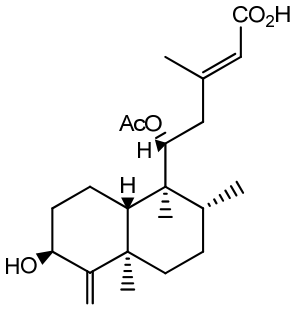
124 R= COCH
125, R=H



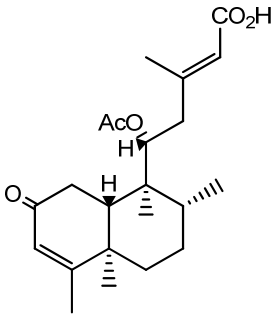
126, **Leucasdin B**



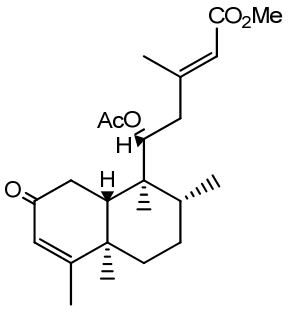
127



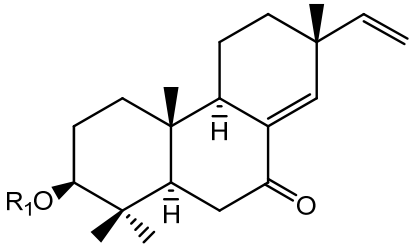
128

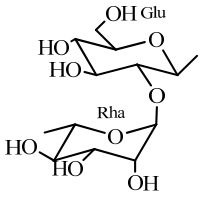


129

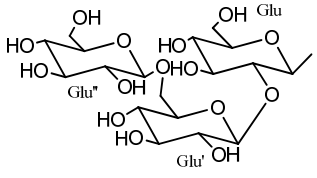


130

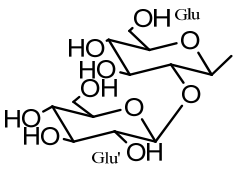




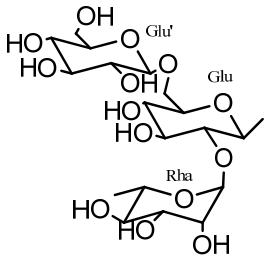
131, R_1, **Linifolioside**



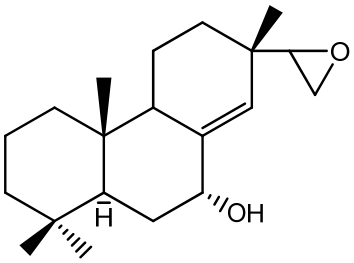
132, R_1, **Leucasperoside A**



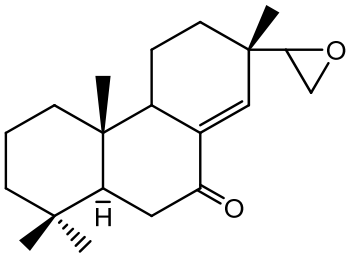
133, R_1, **Leucasperoside B**



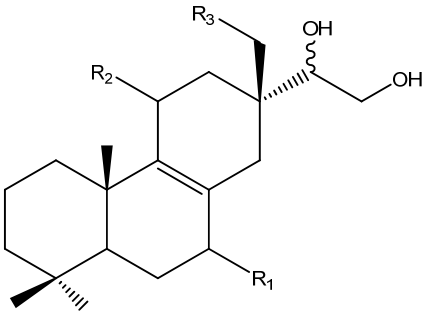
134, R_1, **Leucasperoside C**



135



136

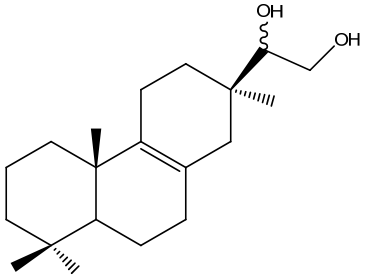


137, R_1 = =O, R_2 = =O, R_3 = H

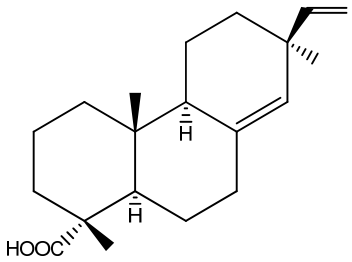
138, R_1 = =O, R_2 = H_2, R_3 = H

139, R_1 = =O, R_2 = H_2, R_3 = OH

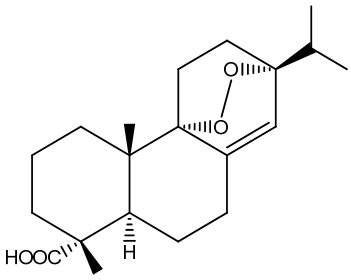
140, R_1 = H_2, R_2 = H_2, R_3 = OH



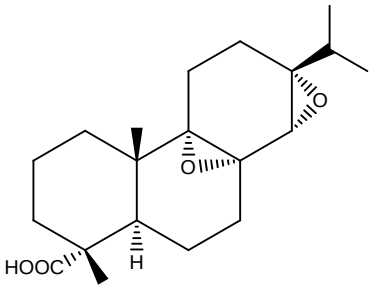
141



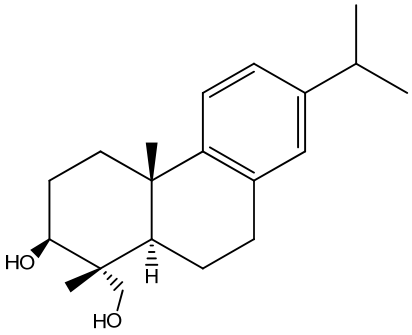
142



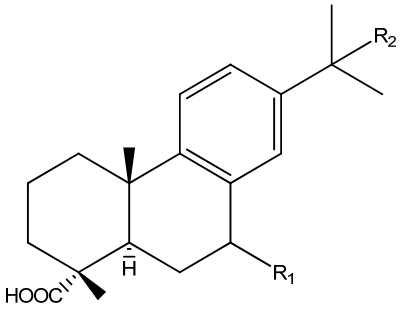
143



144



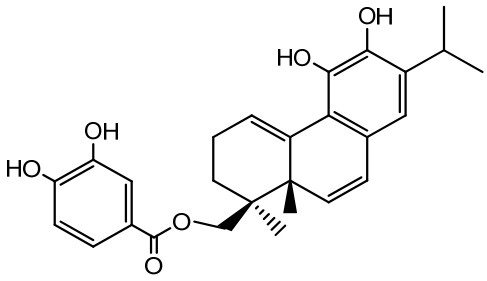
145



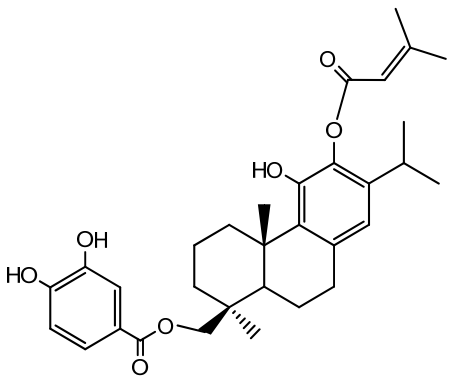
146, R_1= H, R_2= H

147, R_1= O, R_2= H

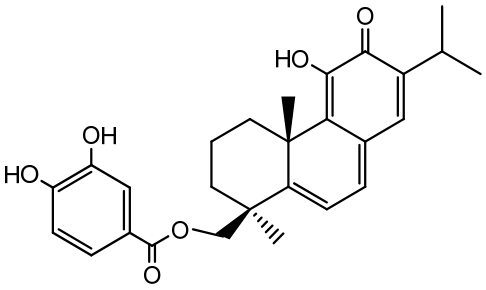
148, R_1= H, R_2= OH



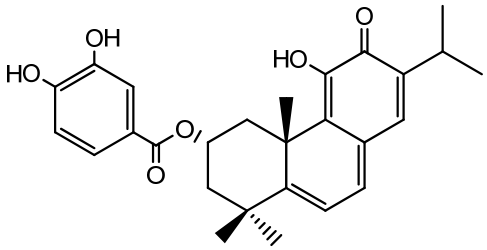
149, **Plectranthol A**



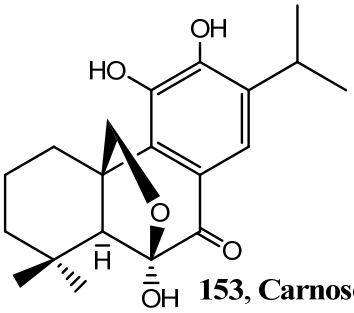
150, **Plectranthol B**



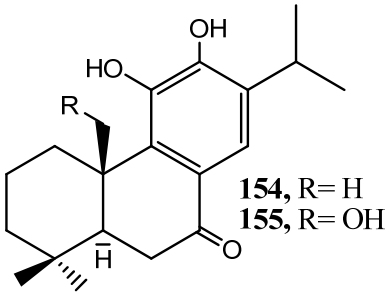
151, **Parvifloron E**



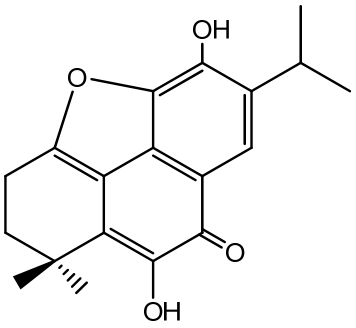
152, **Parvifloron F**



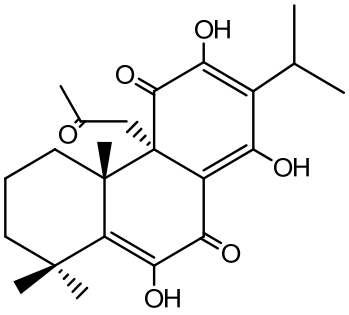
153, **Carnosolon**



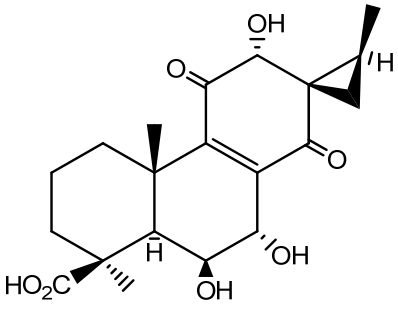
154, R= H
155, R= OH



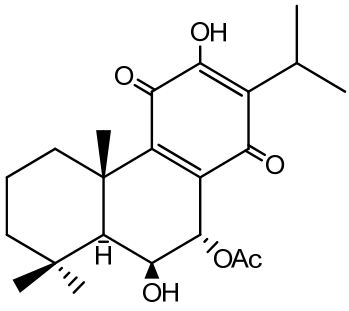
156



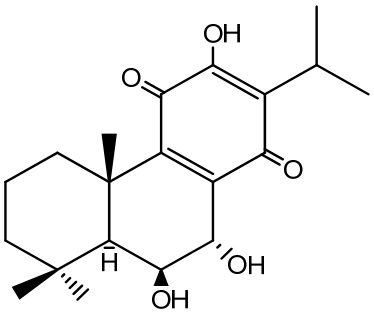
157



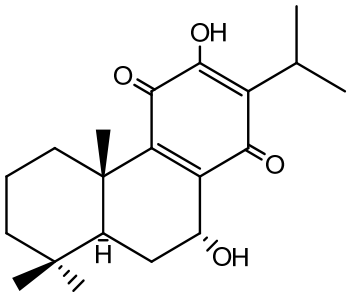
176



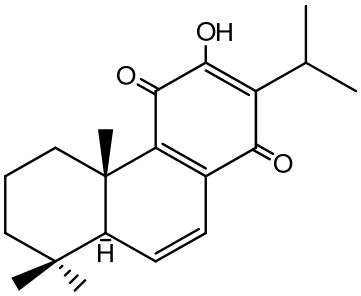
158



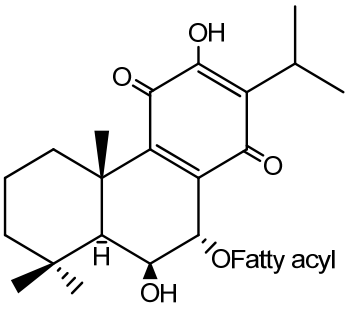
159



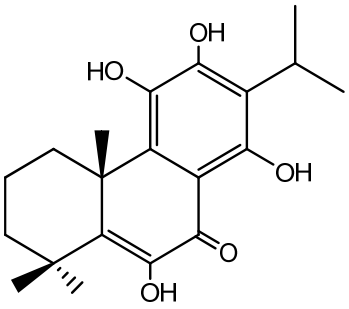
160



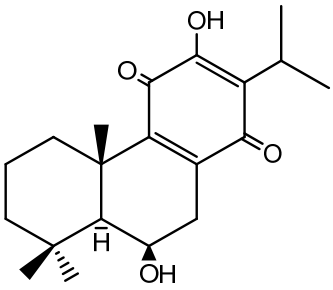
161



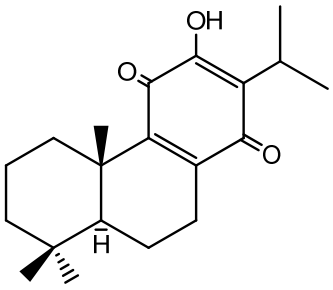
162



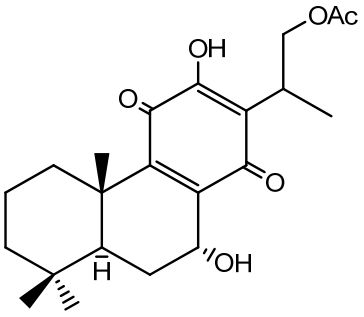
163, Coleon U



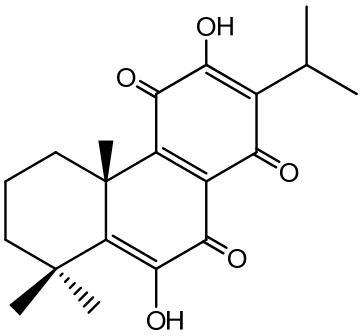
164



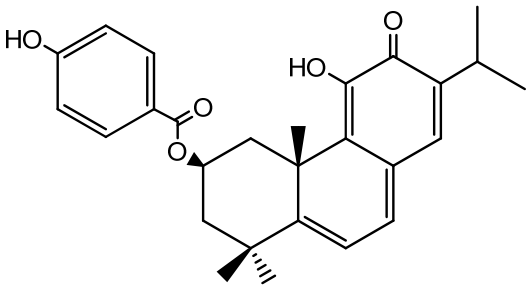
165



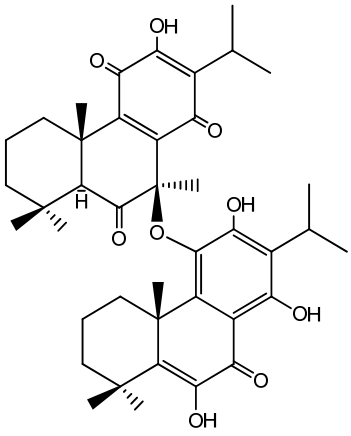
166



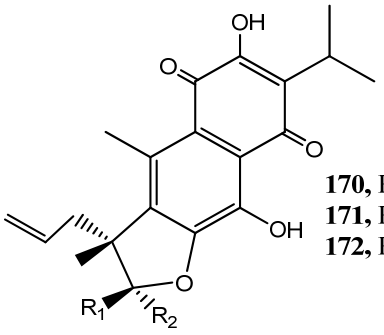
167, **Coeon U quinone**



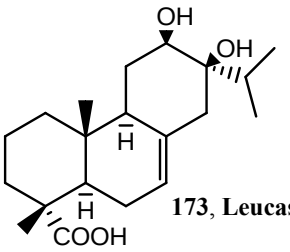
168, **Parvifloron D**



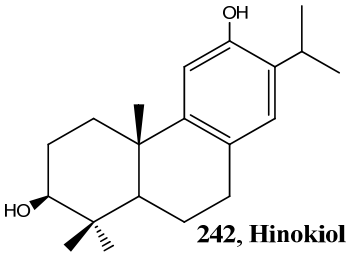
169, **Grandidone**



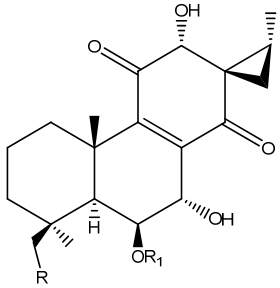
170, R_1=OH, R_2= H
171, R_1=H, R_2= OH
172, R_1, R_2= O



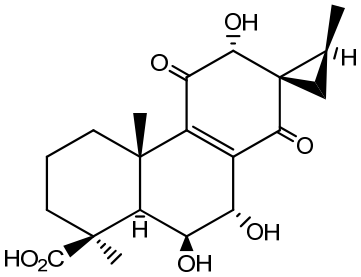
173, **Leucasdin C**



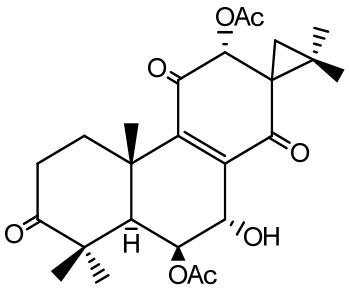
242, **Hinokiol**



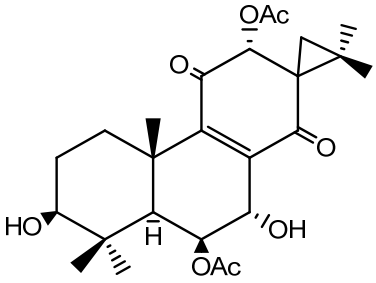
174, R=OAc, R_1=Ac
175, R=H, R_1=H



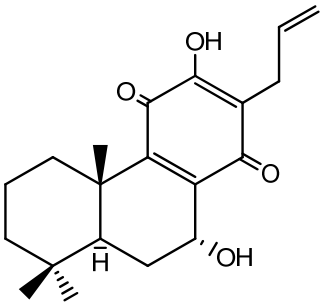
177



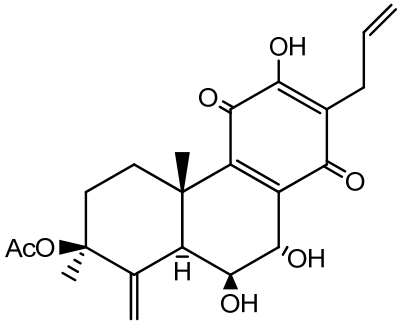
178



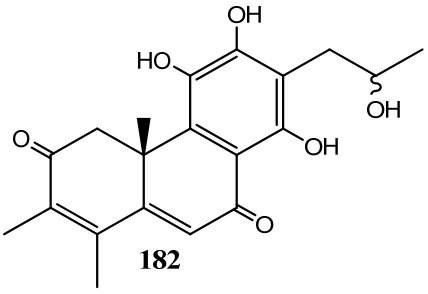
179



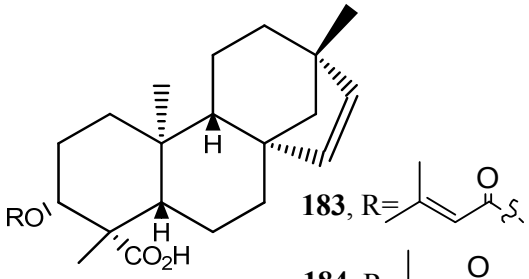
180



181

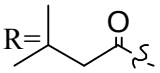


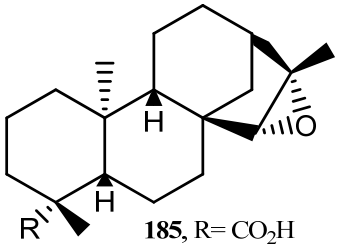
182



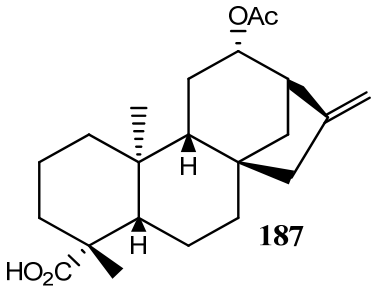
183, R=

184, R=





185, R= CO_2H
186, R= CH_2OH



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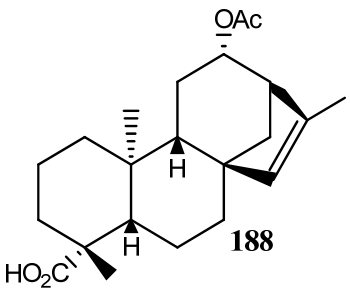

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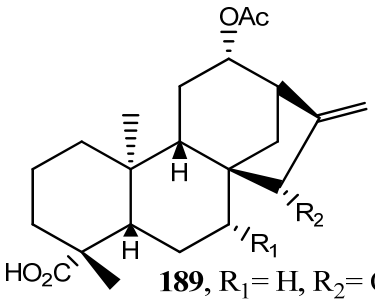
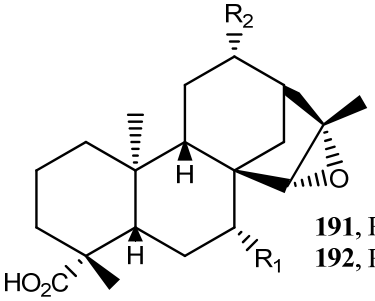
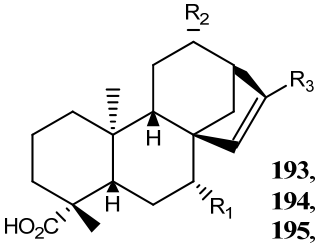


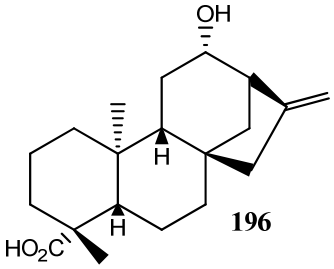
189, R_1= H, R_2= OH
190, R_1= OH, R_2= H



191, R_1= H, R_2= OAc
192, R_1= OH, R_2= H



193, R_1= OH, R_2= H, R_3= Me
194, R_1= OH, R_2= OAc, R_3= Me
195, R_1= H, R_2= OAc, R_3= CHO



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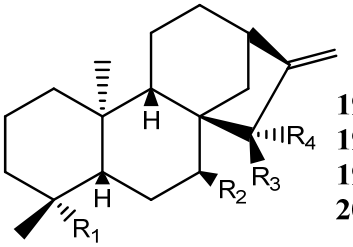
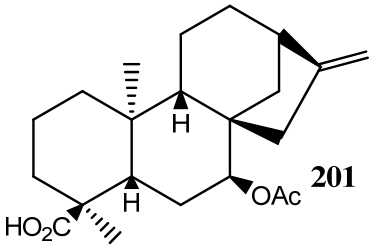


197, R_1= CH2OH, R_2= H, R_3=H, R_4= H
198, R_1= COOH, R_2= H, R_3= H, R_4= H
199, R_1= COOH, R_2= H, R_3= OAc, R_4= H
200, R_1= COOH, R_2= OAc, R_3, R_4= O



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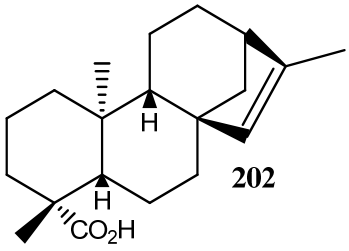

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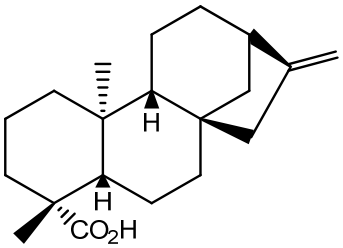

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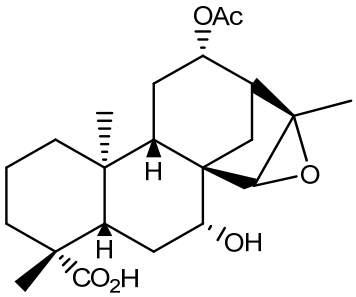

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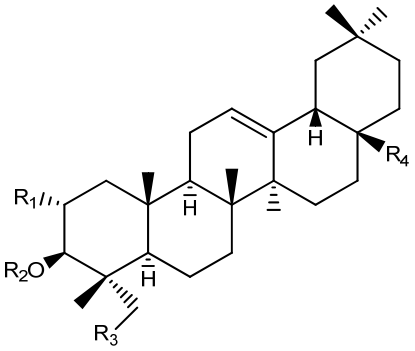
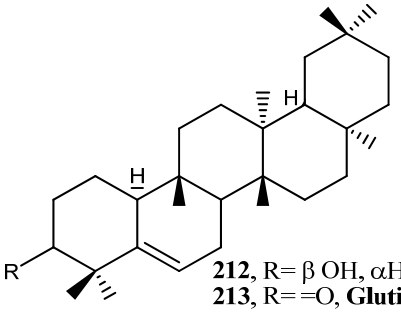
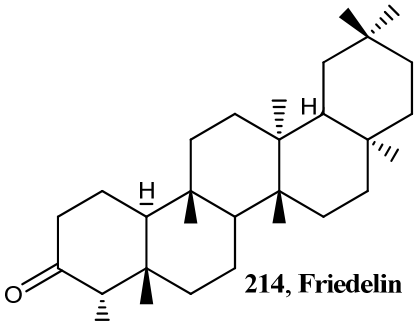


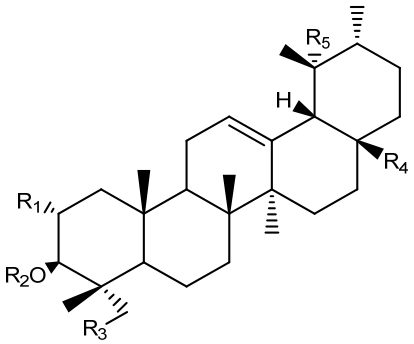
205, R_1=R_2=R_3= H, R_4=COOH, **Oleanolic acid**
206, R_1=R_3= H, R_2=Ac, R_4=COOH, **Oleanolic acid-3-acetate**
207, R_2=R_3= H, R_1=OH, R_4=COOH, **Maslinic acid**
208, R_2= trans-p-coumaroyl, R_3= H, R_1=OH, R_4=COOH
209, R_1=R_2= H, R_3=OH, R_4=COOH, **Hederagenin**
210, R_1=R_3= OH, R_2=H, R_4=COOH, **Arjunolic acid**
211, R_1=R_2=R_3=H, R_4= CH_3, **β amyrin**



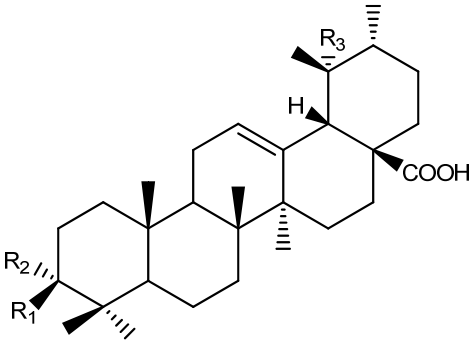
212, R= β OH, αH, **Glutinol**
213, R= =O, **Glutinone**



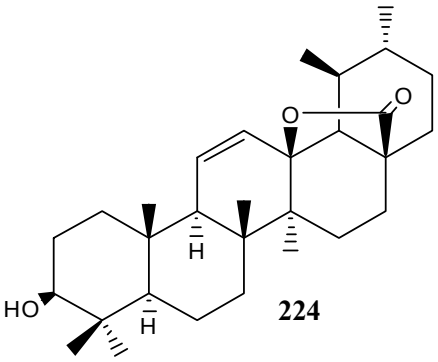
214, **Friedelin**



215, R_1=R_2=R_3= R_5=H, R_4=COOH, **Ursolic acid**
216, R_2=R_3=R_5= H, R_1=OH, R_4=COOH, **Corosolic acid**
217, R_1=R_2=R_3= H, R_5=OH, R_4=COOH
218, R_1=R_2= H, R_3=R_5=OH, R_4=COOH,
219, R_2= H, R_1=R_3=R_5=OH, R_4=COOH,
220, R_1=R_2=R_3= R_5=H, R_4=CH_3, **α amyrin**
221, R_1=R_3= R_5=H, R_2=Ac, R_4=CH_3, **α amyrin-3-acetate**



222, R_1, R_2= =O, R_3=H
223, R_1=OCOH, R_2=R_3=H



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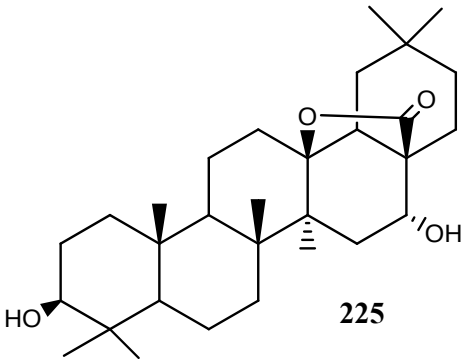
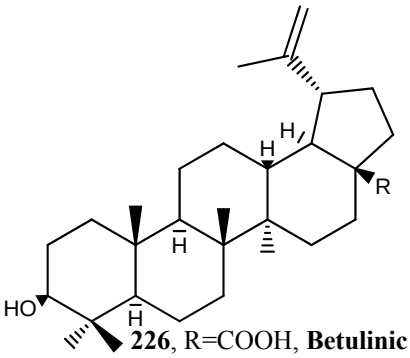
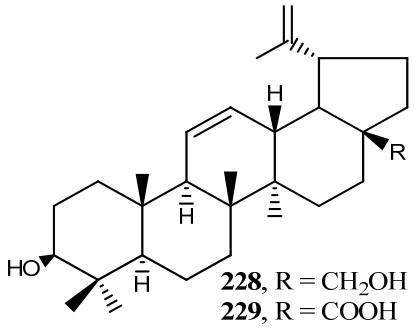


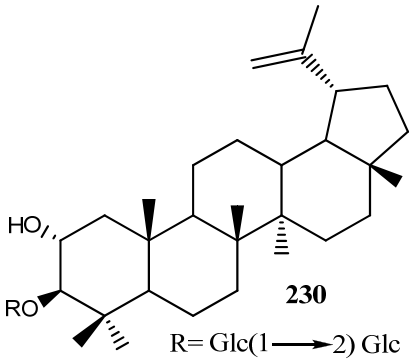
225



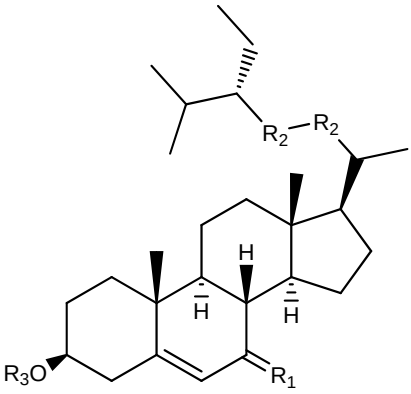
226, R=COOH, **Betulinic acid**
227, R=CH_2OH, **Betulin**



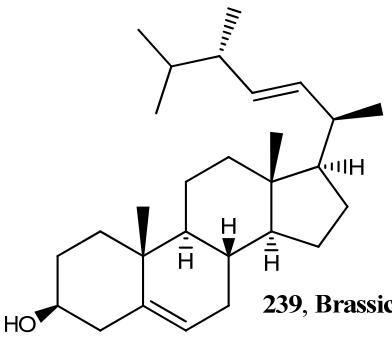
228, R = CH_2OH
229, R = COOH



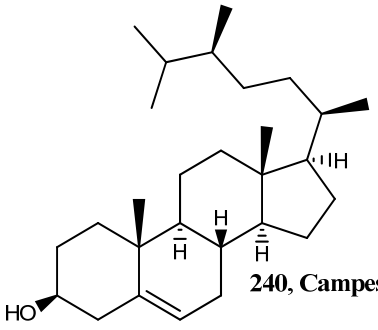
230
R= Glc(1⟶2) Glc



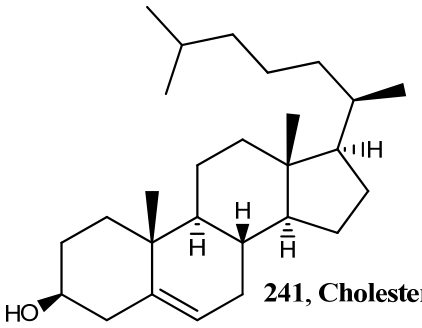
231, R_1= H_2, R_2= CH_2; R_3=H, **Sitosterol**
232, R_1= O, R_2= CH_2; R_3=H,
233, R_1= OH, R_2=CH_2; R_3=H,
234, R_1= H_2, R_2= CH_2; R_3=Glc
235,R_1= H_2, R_2= -CH; R_3=H, **Stigmasterol**
236, R_1= O, R_2= -CH; R_3=H,
237, R_1= OH, R_2= -CH; R_3=H,
238, R_1= H_2, R_2= -CH; R_3=Glc,



239, **Brassicasterol**



240, **Campesterol**



241, Cholesterol

Remarks:

Pharmacology:

1. In genera *Lavandula* and *Leucas*, majority of the pharmacological studies are directed towards confirming the traditional claims with few studies trying to extend traditional use of one species to other.

2. Pharmacological work on genus *Anisochilus* is independent as the genus finds very less space in traditional practices.

3. Work on *Anisomeles* again is of confirmatory nature.

4. Overall there is lack of comprehensive pharmacological work on *Anisochilus*, *Anisomeles* and *Leucas.*

5. Anti-mycobacterial activity is very commonly probed resulting in isolation active molecules across all genera exept *Anisomeles*. Our present wotk has extended it to cembranoids.

6. Majority of the bioassays employed for pure molecules isolated from *Leucas*, probe anti-oxidant potential with very few probing other activities like anti-TB, anti- Alzimer (inhibition of butyrylcholinesterases), etc.

7. Compounds with varied biological activities ranging from anti-oxidant, anti-bacterial, anti-cancer, gastroprotection to insect antifeedant are isolated from genus *Plectranthus*.

Chemistry:

1. 6-substituted flavonones dominate flavonoid composition in *Anisomeles* and *Plectranthus.*

2. Chemistry of all genera, exept *Lavandula* is dominated by diterpenes and phenolic hybrid compounds. *Anisochilus* shows abietanes and pimaranes (from present work) *Anisomeles* is unique in being synthesising monocyclic cembranes,

Leucas has labdanes as major class while *Lavandula* is dominated by volatile components with monoterpenes and necrodane type monoterpenes being unique to entire plant kingdom. Diterpenes are few and restricted to abietane class. *Plectranthus* is prolific in diterpene production and shows labdane, clerodane, kaurane and abietanes as major class of diterpenes.

3. Hybrid phenolic compounds with phenyl propanoids and flavonoids linked to central sugar molecules are commonly found in *Anisomeles* and to lesser extend in *Leucas*.

4. Domination of *Lavandula* chemistry by volatile and other monoterpene components is also supported by present work. This supports the traditional as well as commercial exploitation of *Lavandula* for its essential oil. Genus *Lavandula* produces unusual natural products like necrodane monoterpenes as well as ethoxylated monoterpenes, diphenyl propane and 1,4-dihydroxyl substituted branched alkanes isolated from our work.

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Chapter 2

Phytochemical investigations on Leucas stelligera Wall.

Figure1: *Leucas stelligera* Wall.

2.1. Introduction:

Leucas is a genus of annual or perennial herbs or sub-shrubs with around 100 species growing mostly on dry or disturbed ground and distributed from tropical to southern Africa, Arabian Peninsula, Iran to South China, Taiwan, Japan and South East Asia [1]. Genus *Leucas* in Maharashtra is represented by twenty one species [2]. *Leucas* stelligera called Starry Leucas or Goma in Marathi is an erect branched herb, with hairy, quadrangular stem, found mostly in Western Peninsular India. The hairs are erect and spreading. Leaves are oppositely arranged, elliptic-lanceolate in shape and have serrated margins. Flowers (in November) 1.2 cm long; lips nearly equal occur in dense axillary or terminal whorls [3]. *L. stelligera* is an edible plant with leaves eaten as vegetable. No chemical and pharmacological work is reported on this species.

2.2. Collection and processing:

L. stelligera whole plants, in flowering, were collected from paddy fields from Mulshi area, District Pune on 3rd January, 2008. A herbarium is deposited in Botanical Survey of India, Western Circle, Pune (No. SPJ-5). Roots were separated and aerial parts were cleaned off adhering dust and unwanted plant material, dried in shade, cut and pulverized.

2.3. Extraction and Isolation:

Pulverized aerial parts (1.8 kg) were extracted with acetone (3L ×3 × 14h) at room temperature. The acetone solubles were filtered and concentrated under reduced pressure to yield a greenish extract (57.0 g, 3.0% based on dry plant weight), 55.0g of which was separated by column chromatography (CC) with acetone: petroleum ether gradient to collect 11 fractions LS1-LS11 (Chart 1).

Fractions LS4 (5.5g) and LS5 (2.3g) were subjected separately to CC in 6% acetonitrile in chloroform to collect 10 (LS4a-j) and 13(LS5a-m) fractions respectively. Fractions LS4f, LS4h, LS4i, LS5j and LS5k were combined and subjected further to CC in 15% acetone: pet ether to isolate compound **1**(100mg)

Fraction LS7 (4.1 g) was subjected to CC with acetonitrile 1 to 3% in chloroform to collect 18 fractions (LS7a-r). Fraction LS7i (287.2mg) was subjected to CC in 15% acetone in petroleum ether to isolate compound **2** (100mg) and compound **3** (38mg).

Fraction LS11 (4.9g) was subjected to CC in methanol from 5 to 20% in chloroform to collect 8 fractions (LS11a-h). Fraction LS11b (3g) was subjected to CC in methanol: chloroform from 1 to 3% to collect 6 (LS11bi-bvi) fractions. Fractions LS11biv (670mg) and LS11bv (1.9g) were subjected separately to CC using elution system acetone 5 to 50% in pet ether to collect 25 (LS11biv1-25) and 20 (LS11bv1-20) fractions respectively. Fractions LS11biv22 and LS11bv17 were combined (230mg) and separated by CC using 20% acetone in petroleum ether as elution system. Fractions 7-11 contained compounds **4** and **5**. After repeated attempts to separate them by preparative TLC using different developing systems viz. 1.5% methanol in chloroform, 15% acetone-15% ethyl acetate in petroleum ether and 10% acetonitrile in chloroform, they were obtained as non separable mixture (14mg). Fractions LS11bv18, LS11bv19 and LS11biv23 were combined (372.5mg) and subjected to CC using ethyl acetate in petroleum ether gradient from 30 to 50% as elution system to collect 8 fractions. From fraction 8, compound **7** (15mg) was obtained as pale yellow precipitate. From LS11bvi compound **8** (10mg) precipitated out. It was filtered and filtrate 300mg, was subjected to CC in acetone in petroleum ether (gradient 5-50%) to collect ten fractions, (LS11bvi1-10). Fraction LS11bvi1 was purified by successive preparative TLC using developing systems 25% acetonitrile in chloroform and 35% ethyl acetate in chloroform to isolate **6** (20mg).

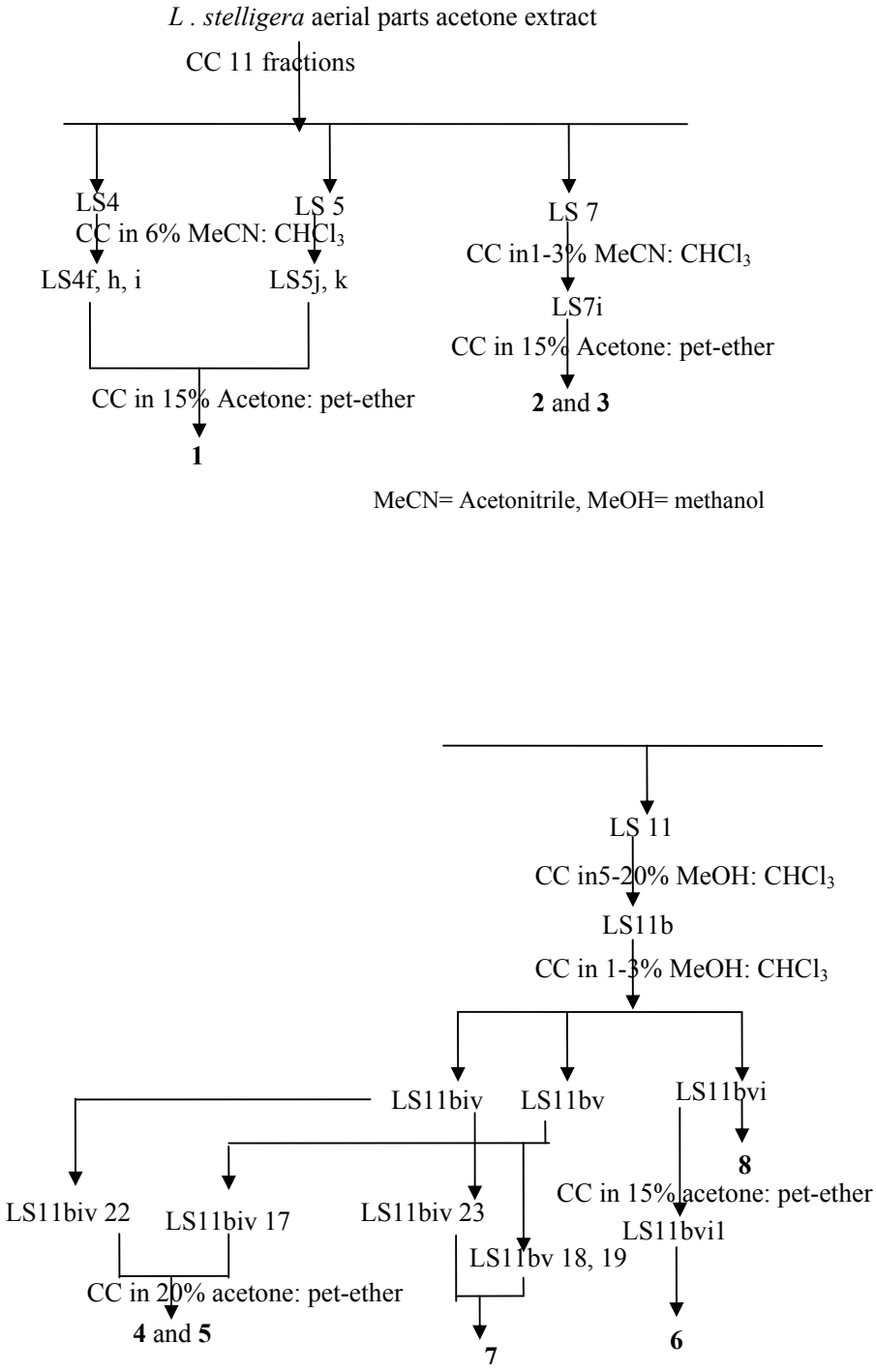


Chart 1: Flow chart for isolation of compounds from *Leucas stelligera.*

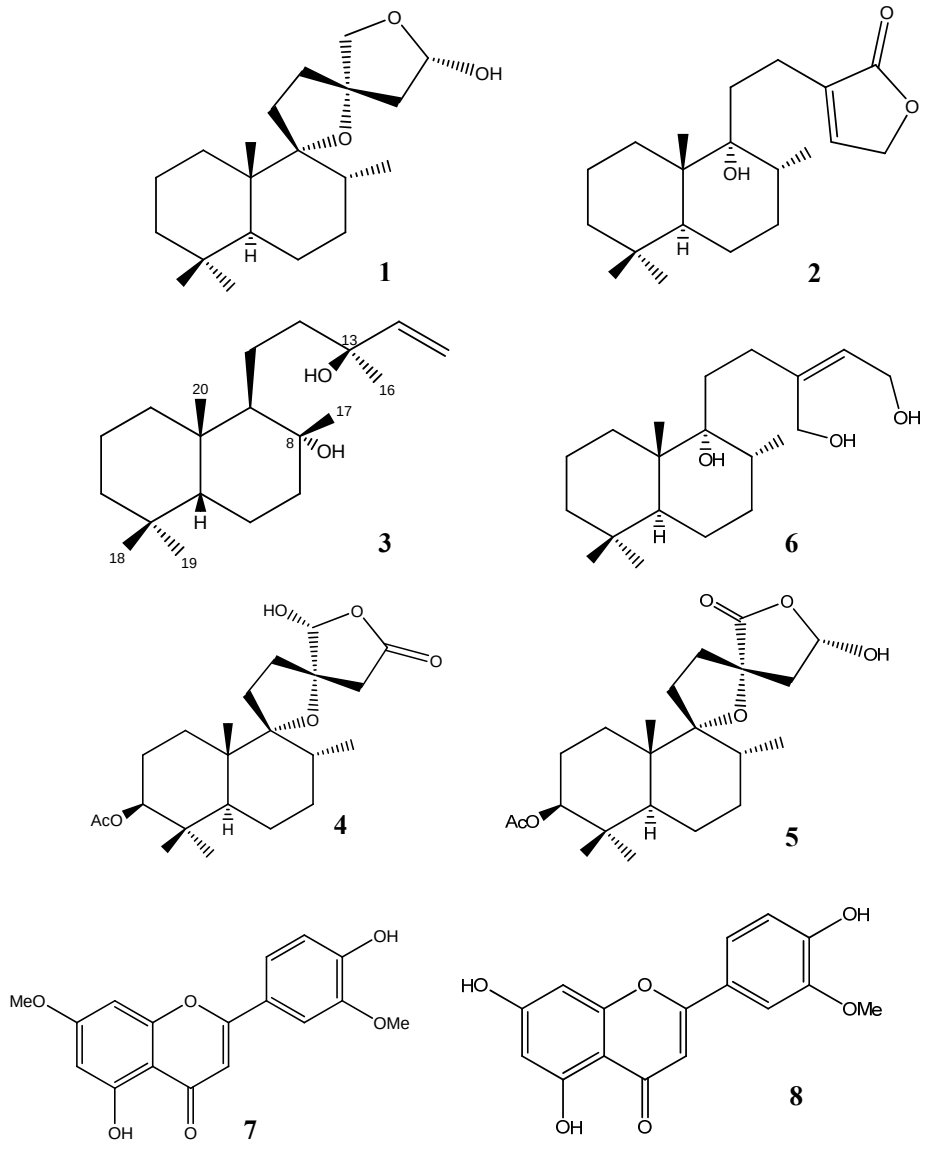


Figure 2: Compounds isolated from *L. stelligera*.

2.4. Structural elucidation:

Compound **1**:

Compound **1** was obtained as a colourless gum. The ESIMS of **1** showed an $[M + 1]^+$ at *m*/*z* 323, $[M + Na]^+$ at *m*/*z* 345 suggesting the molecular formula $C_{20}H_{34}O_3$ with four degrees of unsaturation. The HREIMS of showed $[M]^+$ at *m*/*z* 322.25151, confirming the molecular formula. The IR spectrum showed a stretching frequency of hydroxyl group at 3427cm^{-1}.

The ^{1}H NMR spectrum (Table 1) displayed the presence of three tertiary methyl groups at δ 0.81, 0.86 and 0.91 and one secondary methyl group at δ 0.91 (d, J=6.62Hz). This indicated **1** to be labdane type of diterpene.

The ^{13}C NMR spectrum (Table 1) showed the presence of two quaternary carbons at δ95.02, 89.92 and methine carbon at δ 99.22, indicating towards a prefuran substitution pattern. These spectral characteristics were comparable to that of known molecule **9** isolated from *Leucas nufliseana*, thus confirming the prefuran substitution (Figure 3) [4]**.**

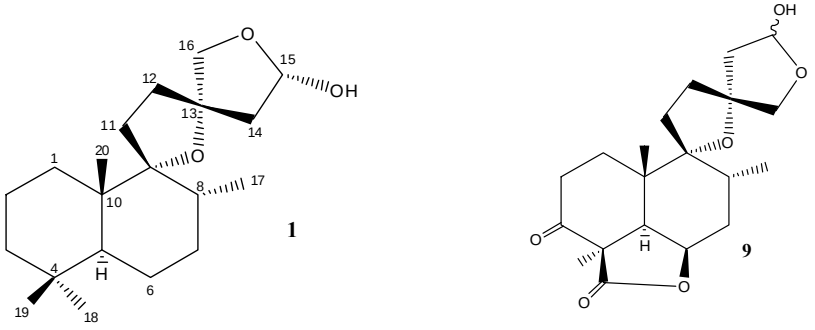
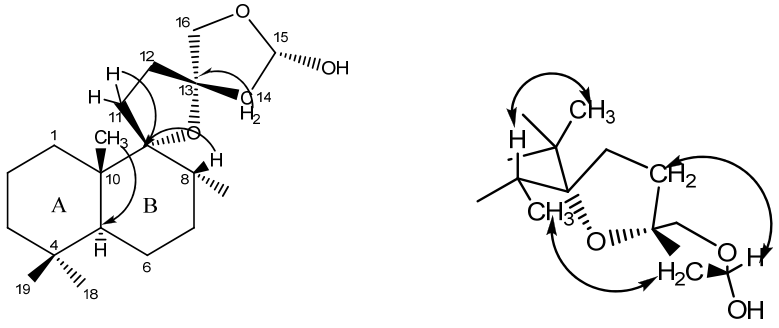


Figure 3: Structure of Compound **1** and reference compound **9**

The observed HMBC correlations (Figure 4a) of H-14 (δ1.98 and 2.32) with C-13 at δ 89.92 and C-15 at δ 99.22 confirmed this substitution. The observed HMBC correlations of H-11 (δ 1.76 and 2.02) and H-8 (δ 1.76) with C-9 at δ 95.02 fixed the prefuran substitution at C-9.



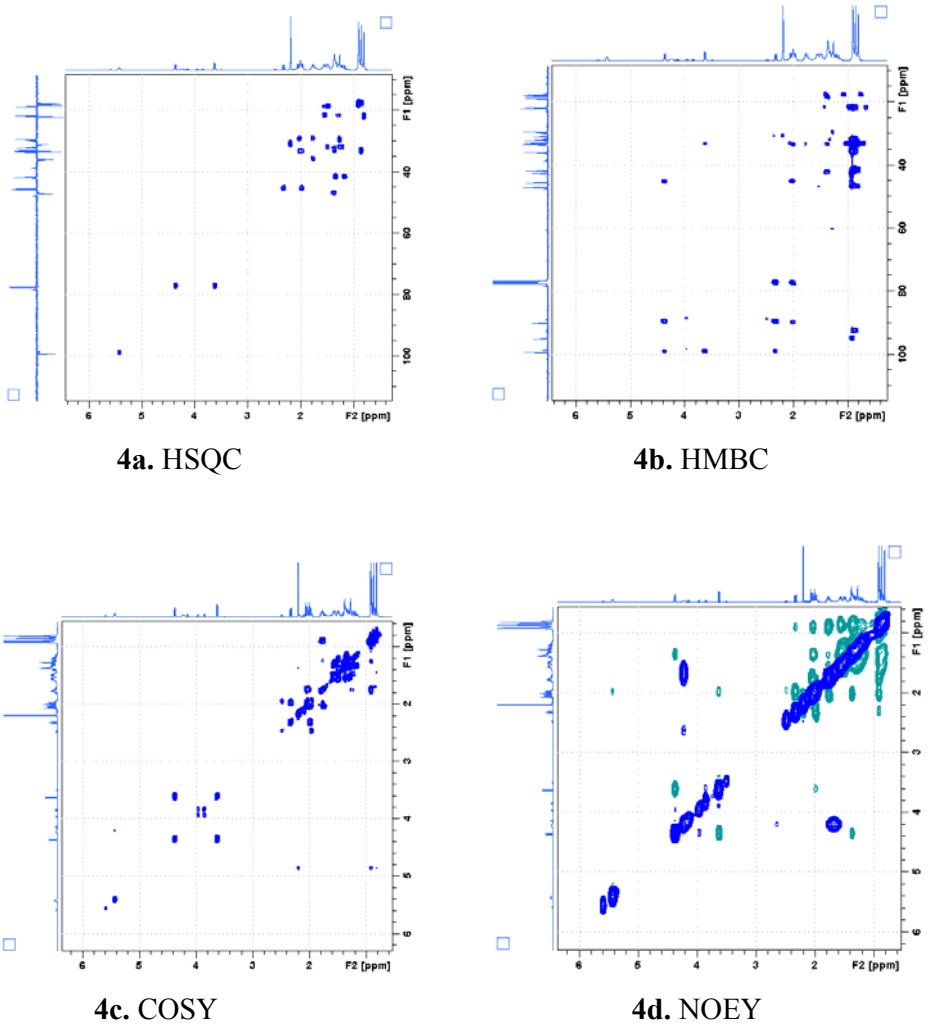
4a. HMBC correlations **4b.** NOESY correlations

Figure 4: 2D correlations for compound **1**

NOE correlation peak between H-20 and H-19 and absence of NOE between H-20 and H-5 confirmed trans-fused A/B ring junction. Presence of NOE correlation between H-20 and H-8 confirmed α position of 17-Me. NOE

correlations between H-20 and H-11, H-17 and H-14, H-12 and H-15 led to the assignment of stereochemistry shown above (Figure 4).

Thus compound **1** was identified as 9, 13, 15, 16-diepoxylabdan-15α-ol.



4a. HSQC **4b.** HMBC

4c. COSY **4d.** NOEY

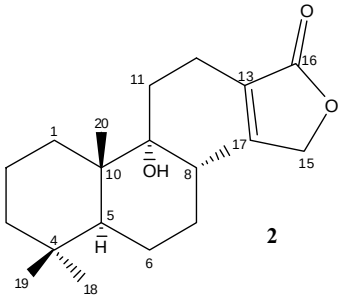
Figure 5: 2D spectra of compound **1**

Compound **2**:

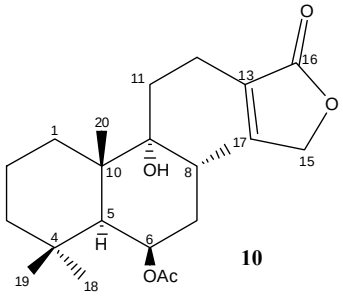
Compound **2** was obtained as white needles. The ESIMS of **2** showed an $[M + 1]^+$ at *m/z* 321 and $[M + Na]^+$ at *m/z* 343 suggesting the molecular formula $C_{20}H_{32}O_3$ with four degrees of unsaturation. The HREIMS of **2** showed an $[M]^+$ at *m/z* 320.23658, confirming the molecular formula. The IR spectrum showed a stretching frequency of hydroxyl (3536cm^{-1}) and α, β- unsaturated lactone (1749cm^{-1})groups.

The ^{1}H NMR spectrum (Table 1) displayed the presence of three tertiary methyl groups at δ 0.84, 0.88 and 0.94, one secondary methyl group at δ 0.92 (d, J=6.7Hz). This indicated **2** to be labdane type of diterpene. DEPT spectrum revealed presence of 4 methyl, 8 methylene, 3 methine and 5 quaternary carbons.

The ^{1}H NMR and ^{13}C NMR data of **2** was nearly superimposable on a known labdane **10** isolated from *Vitex rotundifolia* (family Verbenaceae) [5] (Figure 6) indicating that the structure of **2** was closely related to that of known labdane, except for the absence of acetyloxy group at position 6 in **2**.



2

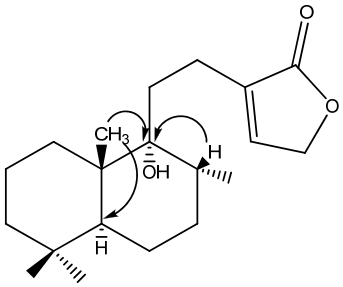


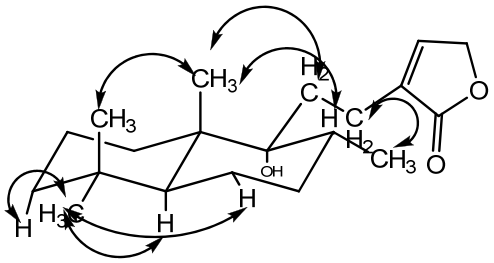
10

Figure 6: Structure of compound **2** and reference compound **10**

H-6 methine and C-6 in **10** appears at δ 5.39 and δ 70.2 respectively. In **2**, H-6 methine is replaced by upfield shifted methylene group at δ 1.29 (1H, m) and 1.54(1H, m) while C-6 appears at δ 21.53 supporting the assigned structure of **2**.

The assignments were confirmed by detailed HSQC and HMBC studies and key correlations are given in Figure 7a.



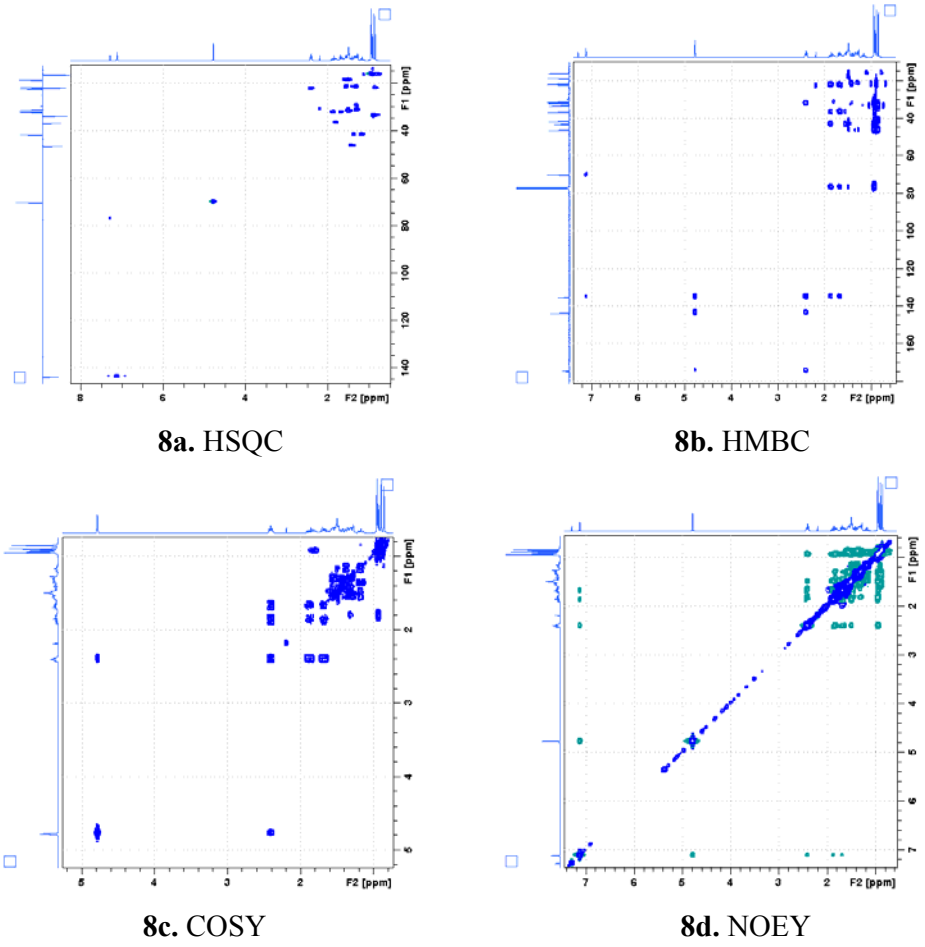


7a. HMBC correlations **7b.** NOESY correlations

Figure 7: 2D correlations for compound **2**

Relative stereochemistry was confirmed by key NOE correlations (Figure 7b). Presence of NOE between H-20 and H-19 and absence of NOE between H-20 and H-5 confirmed trans-fused ring junction. Presence of NOE between H-20/H-8

confirmed α position of 17-CH_3. NOE observed between H-20 and one of the H-11 protons placed the hydroxyl substituent at C-9 in α position.



8a. HSQC **8b.** HMBC

8c. COSY **8d.** NOEY

Figure 8: 2D spectra of compound **2**

Suitable colourless X-ray quality crystals of compound **2** were obtained by crystallization from cyclohexane: acetone (7:3). The X-ray diffraction analysis (Table 2, Figure 9) of **2** confirmed the assigned structure. Thus **2** was identified as labd-9α-ol-13(14)-en-15, 16-olide a new natural product.

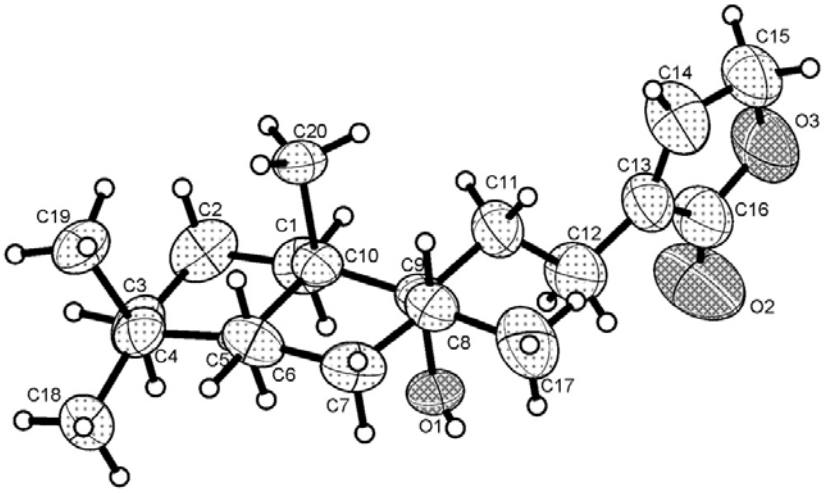


Figure 9: ORTEP diagram of compound **2** (Ellipsoids are drawn at 50% probability)

Compound **3**:

Compound **3** was obtained as colourless crystals. ESIMS gave $[M+1]^+$ at *m/z* 309, $[M+Na]^+$ at *m/z*331, $[M+K]^+$ at *m/z* 347 suggesting the molecular formula $C_{20}H_{36}O_2$ with three degrees of unsaturation. The IR spectrum showed a stretching frequency of hydroxyl (3418cm^{-1}) and olefin (1644cm^{-1}) groups.

The ^{1}H NMR spectrum (Table 3) displayed the presence of five tertiary methyl groups at δ 0.79, 0.87, 1.07, 1.26 and 1.47. This indicated **3** to be diterpene with labdane skeleton..

The ^{13}C NMR spectrum (Table 3) displayed the presence of two quaternary carbons at δ74.8 and 61.18, terminal olefinic group at δ146.15 and 111.11. This indicated compound to be a sclareol **11** or its diastereomers, **12**, **13** (Figure 12)[6].

All the assignments were confirmed by detailed HMBC and COSY correlations. Methyl at δ27.25 showed HMBC correlation with =C*H* at δ 5.95 (H 14) and =CH_2 at δ 5.22 and 5.03 (H 15). Similarly, =C*H* at δ 5.95 exhibited HMBC correlation with *C*H$_2$ at δ 44.96 while CH_2 at δ 1.52-1.63 (H12) exhibited HMBC with Methyl at δ 27.25 and *C*H$_2$ at δ 20.91 (C11). These correlations confirmed side chain connectivity.

Methine at δ 61.19 (C 9) showed correlations with methyl at δ 1.07 and 1.47. Methyl at δ1.07 gave HMBC correlation with methine δ46.25 and

quaternary carbon at δ 38.86 (C10) and methylene at δ 36.48 (C 1). Thus methyl at carbon 10 (C 20, δ 24.78) and carbon 5 (*C*H δ 46.25) were located.

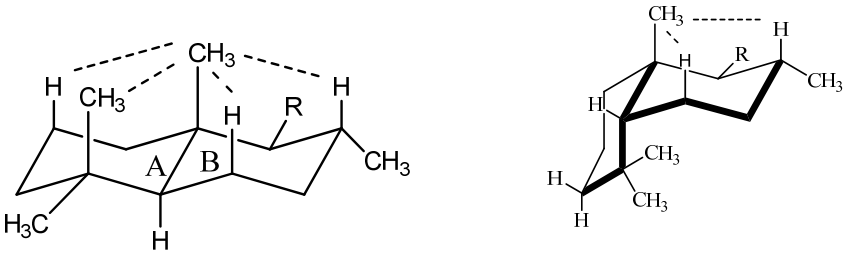
Methyl at δ 0.79 and 0.87 gave HMBC correlations with each other as well as with methine at δ 46.25, methylene at δ 42.22 (C3) and quaternary carbon at δ 32.92 (C4). These correlations established 3-4-5-10-20-1 connectivities. These also identified geminal methyl groups at δ 21.36 and 33.15 and remaining methyl at δ 31.99 was identified as carbon 17. H-17 at δ 1.47 showed HMBC correlation with CH_2 at δ 37.83 and was assigned to C-7. Correlation of proton at δ 1.15 (one of CH_2 at position 3) with CH_2 at δ 18.58 identified later as C2 and remaining CH_2 at δ 20.67 as C 6 confirming sclareol as basic structure of compound **3**.

Examination NOESY revealed methyls at δ 0.79 and 1.07 to be on the same side. Assuming angular methyl at δ 1.07 (C20) to be β on biogenetic grounds, position 19 (β) was ascribed to carbon with δ 21.36 (H at δ 0.79) and position 18 to carbon with δ 33.19 (H at δ 0.87).

Comparative evaluation of ^{13}C values of **3** with reported values of sclareol, 8-episclareol and 13-episclareol (Table 3) [6] revealed deviations in shifts of carbons 1, 2, 5, 7, 11 and 20. Upfield shift of C5 and downfield shift of C20 strongly hinted towards cis-fused A/B ring junction. Chemical shifts of H-5 and H-20 are partially overlapped so their mutual NOE correlation could not be observed. However presence of NOE correlation peak between H-3 at δ1.15 (β) with H-5 at δ1.06 indicated steroid type A/B cis fusion with CH_3-20 axial and H-5 equatorial. Presence of C-17 at δ 31.99, similar to 8-episclareol indicated its α orientation.

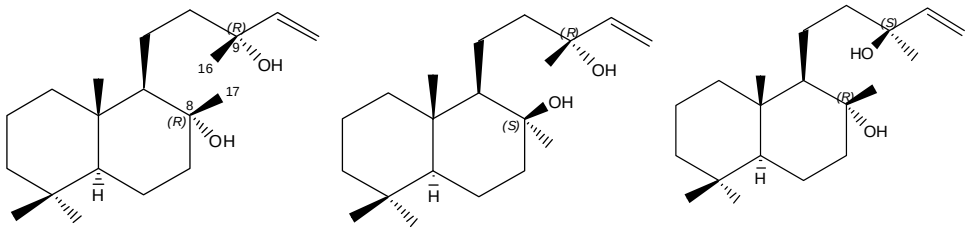
A/B cis fusion in compound **3** can be justified on the basis of shielding 1, 3-diaxial interactions (Figure 10). In 8-episclareol and compound **3**, C-17 methyl is not involved in synaxial (1, 3-diaxial) interaction and hence is not shielded. Comparative evaluation of synaxial interactions in trans and cis diastereomers shows relief obtained in cis configuration, thus explaining downfield shift of C-20 in compound **3**.

Possibility of diastereomer of sclareol with both configurations at 8 and 13 positions reversed was ruled out as the chemical shifts at C-20 and C-5 would be similar to other A/B trans diastereomers with only minimal effects.



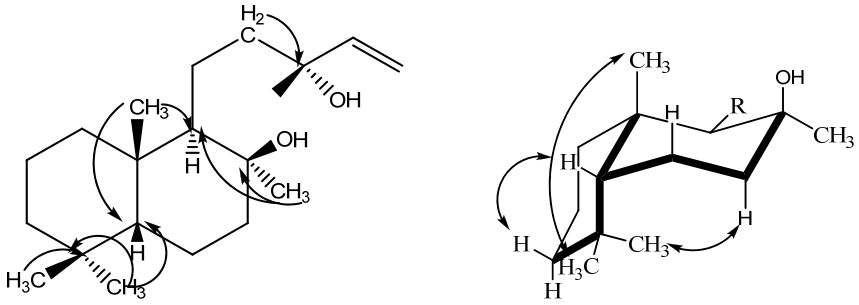
Trans fusion Cis fusion

Figure 10: 1,3-Synaxial shielding interactions in A/B trans-fusion and cis-fusion.



11, Sclareol **12**, 8-epi Sclareol **13**, 13-epi Sclareol

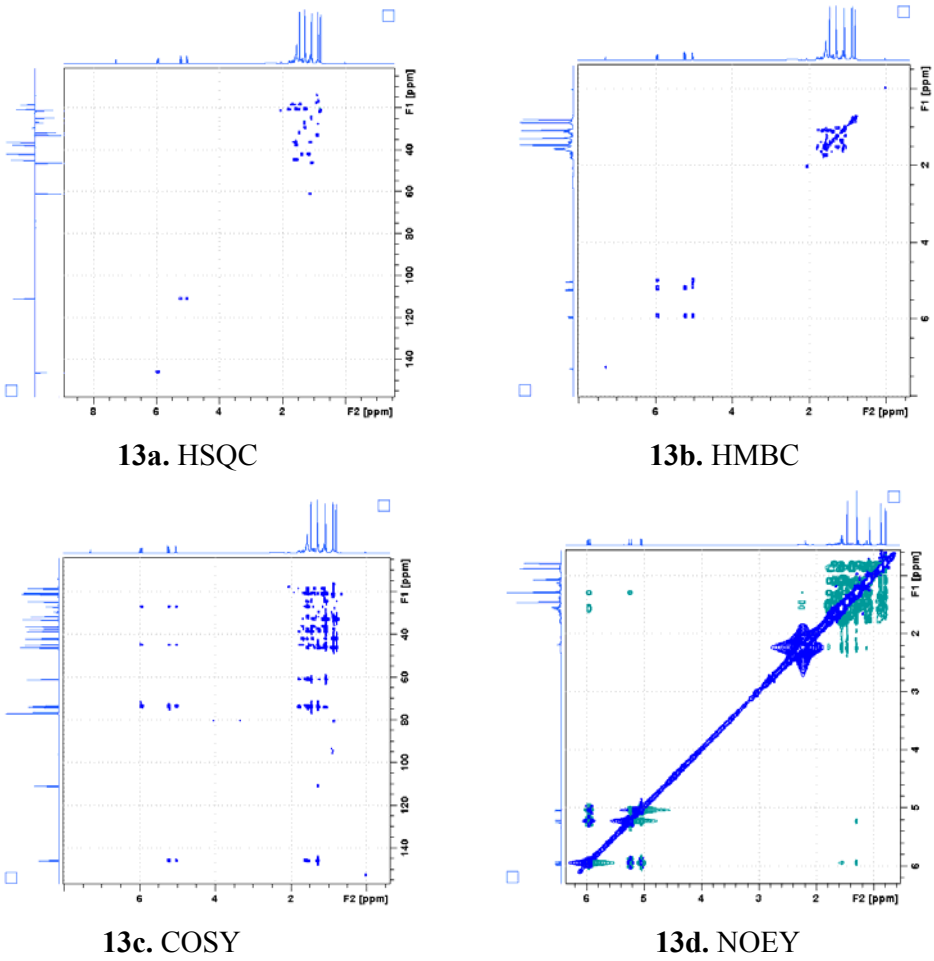
Figure 11: Known A/B trans-fused diastereomers of compound **3**



12a. Key HMBC correlations **12b.** NOESY correlations

Figure 12: Observed HMBC and NOESY correlations for compound **3**.

Key HMBC correlation and NOE correlations are given in Figure 12b. NOE between H-20 and H-19 was observed but the possibility of non-steroid type A/B cis junction -where observation this NOE is more probable- interconverting with steroid type was ruled out as this would lead to two sets of NMR signals which were not observed. Thus by detailed 2D NMR spectroscopy and arguments based on deviations of ^{13}C signals from reference structures, compound **3** was identified as new natural product and named as cis-8-episclareol.



13a. HSQC **13b.** HMBC

13c. COSY **13d.** NOEY

Figure 13: 2D spectra of compound **3**

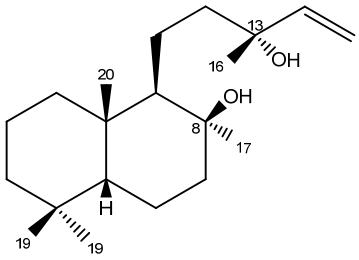


Figure 14: Structure of compound **3**

Compounds **4** and **5**:

Compounds **4** and **5** were isolated as non-separable mixture. CC fraction showing homogeneous spot on TLC using various developing systems was subjected to NMR analysis. ^{13}C NMR spectrum showed 39 resonances at δ 86.13, 88.84, 94.62, 95.38 (quaternary), δ 101.38, 103.03 (methine) and four carbonyl groups at δ 171.20, 171.51, 173.73 and 173.96 with two overlapped methyl signals at δ 21.44 indicated two acetyl groups. This indicated compound under study to be either a dimer with two prefuranic systems with rings D of both monomers joined by two lactone linkages or mixture of two similar prefuranic monomers similar to compound **2**. ESIMS apparently confirmed dimeric structure with well defined $[M+Na]^+$ at *m*/*z* 811 and $[M+K]^+$ at *m*/*z* 827 corresponding to molecular formula $C_{44}H_{68}O_{12}$ with molecular weight of 788. At this point, important peaks at *m*/*z* 417 and 433 were thought to be due to degradation of dimer giving monomer $[M+Na]^+$ and $[M+K]^+$peaks respectively. As dimmerization was thought of at ester linkages, dimer was of mass exactly double that of monomer. At this point 2D NMR data (HSQC and HMBC were scanned and analyzed) and positions of acetyl groups were assigned to carbon 3 in respective monomer following HMBC correlation of protons at δ 4.42 (2H, corresponding to methine at δ 80.74 and 81.11) with quaternary carbons at 37.79 and 37.76 (position 4 of both monomers) (Figure 15)

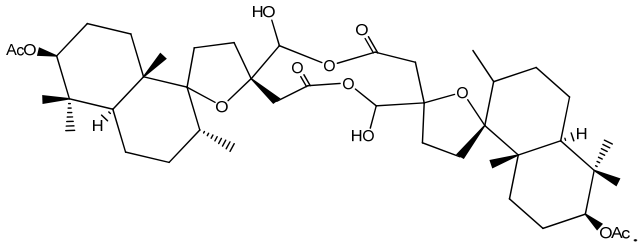


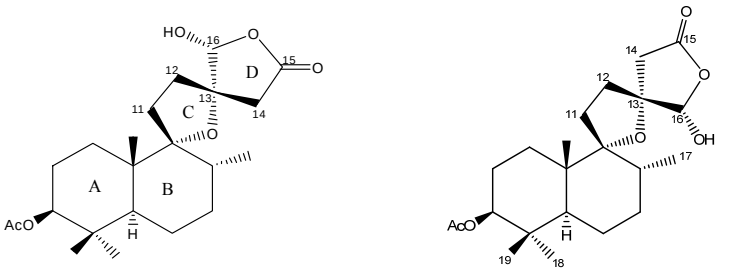
Figure 15: Dimeric structure initially proposed.

We were able to grow X-ray quality crystals in acetone: cyclohexane (30:70). X-ray analysis of randomly taken crystal gave monomeric structure corresponding to compound **4**. This compelling evidence in favour of monomeric structure led us to rethink about our previous dimeric assignment. Analysis of NOESY data revealed strong false peaks (of same phase as diagonal) between protons at δ 5.38 and 5.89 indicating chemical exchange. Also observed was weak

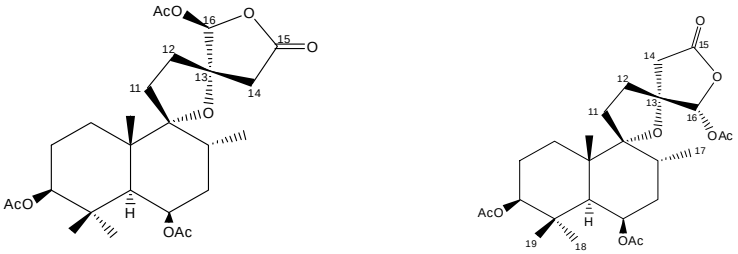
true NOESY peak between these hinting towards a temporary complexing of two monomers with lifetime longer than that required by NOESY experiment. This also explained presence of well defined peaks in ESIMS corresponding to dimer. ^{1}H NMR was scanned in C_6D_6 to avoid possibility of an inter-converting mixture of two compounds with result exactly same as in $CDCl_3$. Thus it was concluded that compound under study was in fact mixture of two compounds forming temporary association in solution while separating on crystallization (it was non-separable on TLC using any combination of mobile phases as mentioned earlier). With this information, analysis of HMBC data was carried out to assign carbon and proton values to individual components as described below:

Use was made of paper by Masateru Ono *et. al.* [5]. Protons on methylene at position 14 resonate as AX system with 17Hz coupling. Depending upon stereochemistry at spiro linkage of rings C and D, their δ separation in ^{1}H NMR varies. Thus in compound **14** (corresponding to **4**, Figure 16), H-14 resonate at δ 2.83 and 2.76 (δ separation of 0.07) while in **15** (corresponging to 5), H-14 resonate at δ 2.89 and 2.57 (δ separation of 0.32). Thus farther placed AX system (δ separation of 1.63) at δ 3.09 (1H, *d*, 17Hz) and 2.46 (1H, *d*, 17Hz)-corresponding to methylene at δ 39.48- was assigned to compound **5** and less separated (δ separation of 0.18) AX system (visually inner placed in NMR spectrum) at δ 2.9 (1H, *d*, 17Hz) and 2.72 (1H, *d*, 17Hz)-corresponding to methylene at δ42.29- was assigned to compound **4**. HMBC correlation of position 14 methylene protons of compound **4** with carbonyl at δ 174.01 identified it to C15 of compound **4** and carbonyl at δ 173.82 to 15 of compound **5**. Compounds **14** and **15** were isolated from *V. rotundifolia*.

Following HMBC correlation of these protons with other carbons, δ 88.9 and 103 were assigned to positions 13 and 16 in compound **4** while carbons at δ 86.4 and 101.4 were assigned to respective positions in compound **5**. Methylene at δ 36.2 and 31.2 were assigned to positions 12 in compounds **5** and **4** respectively. Rest of the resonances being too crowded, could not be separated. Attempts to separate these compounds are underway.



4 **5**



14 **15**

Figure 16: Structures of compounds **4** and **5** with corresponding reference compound **14** and **15**.

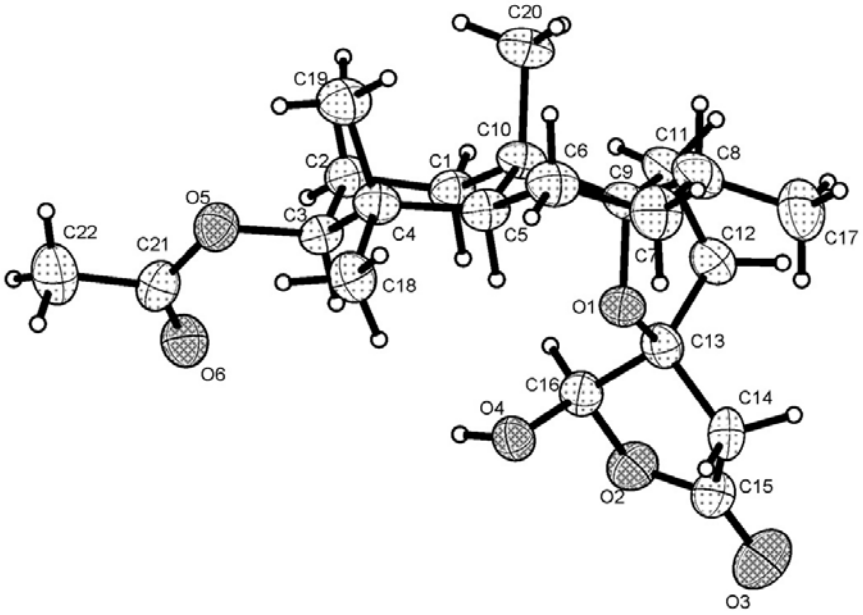
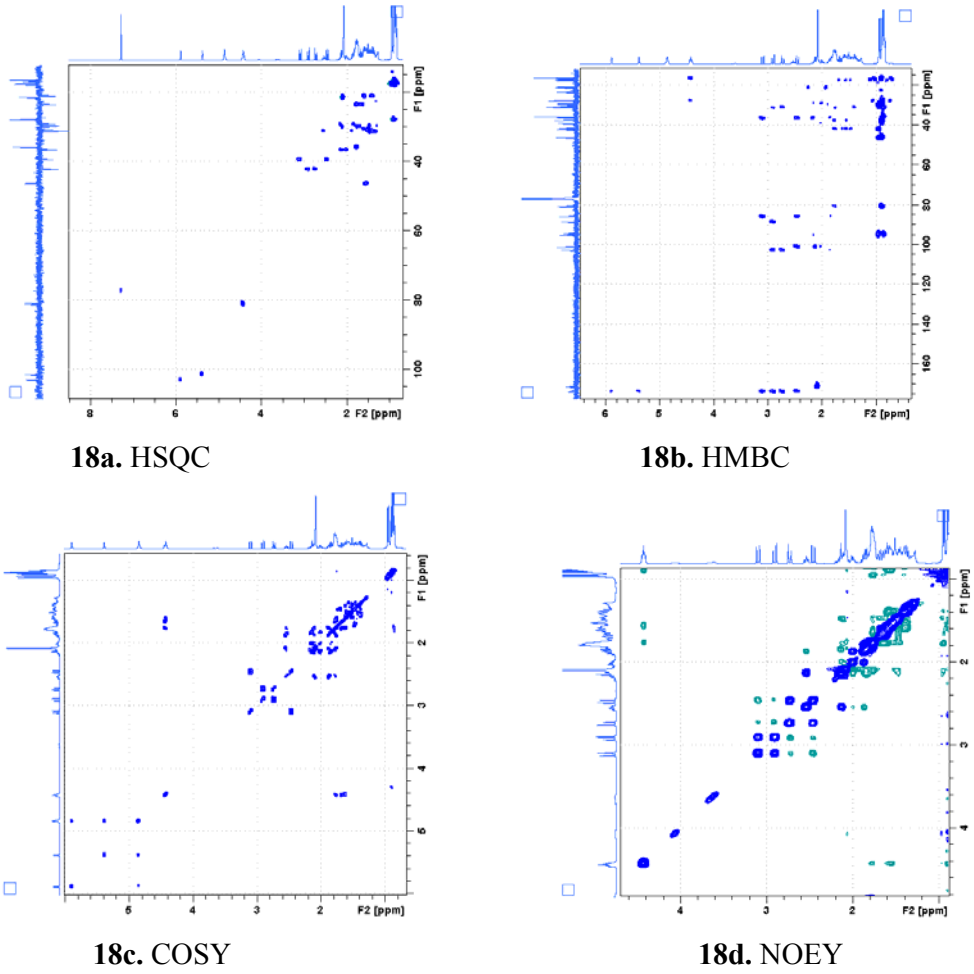


Figure 17: ORTEP diagram of compound **4**. Probabilities are drawn at 50%



18a. HSQC **18b.** HMBC

18c. COSY **18d.** NOEY

Figure 18: 2D spectra of compounds **4** and **5**

Compound **6**:

Compound **6** was obtained as a colourless gum. The ESIMS of **6** showed an $[M + 1]^+$ at *m/z* 325, $[M + Na]^+$ at *m/z* 347 suggesting the molecular formula $C_{20}H_{36}O_3$ with three degrees of unsaturation. The HREIMS of **6** exhibited an $[M]^+$ at *m/z* 324.26895, confirming the molecular formula. The IR spectrum showed a stretching frequency of hydroxyl (3372cm^{-1}) group.

The ^{1}H NMR spectrum (Table 1) displayed the presence of three tertiary methyl groups at δ 0.84, 0.88, 0.94 and one secondary methyl group at δ 0.89 (d, J=6.99Hz, H-17). This indicated **6** to be labdane type of diterpene.

The ^{13}C NMR spectrum (Table 1) displayed the presence of two methylene groups at δ 58.46 and 60.56, a methine carbon at δ 126.03 and a quaternary carbon at δ 144.67. This indicated towards a substituted 2-buten-1, 4–diol system.

This substitution pattern was confirmed by comparison with known molecule bincatriol **16** isolated from *Baccharis incarum* (Family Asteraceae) (Figure 19) [7].

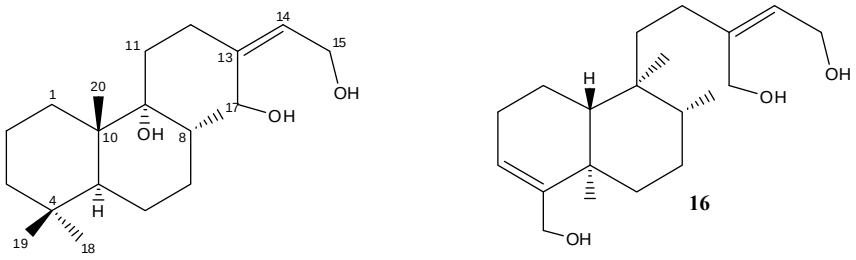
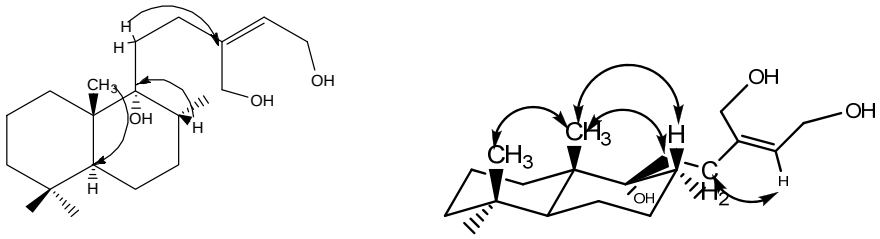


Figure 19: structure of compound **6** and reference compound **10**.

HMBC correlation of H-17 (0.89) with C-9 (δ 77.31) fixed the location of hydroxyl group at C-9. HMBC correlation of H-8 (δ 1.78) with C-9 (δ 77.31), C-10 (δ 43.24) and C-17 (δ 16.52) along with HMBC correlation of H-11 (δ 1.58 and 1.78) with C-9 (δ 77.31), C-8 (δ 36.85) fixed the location of subunit at C-9.

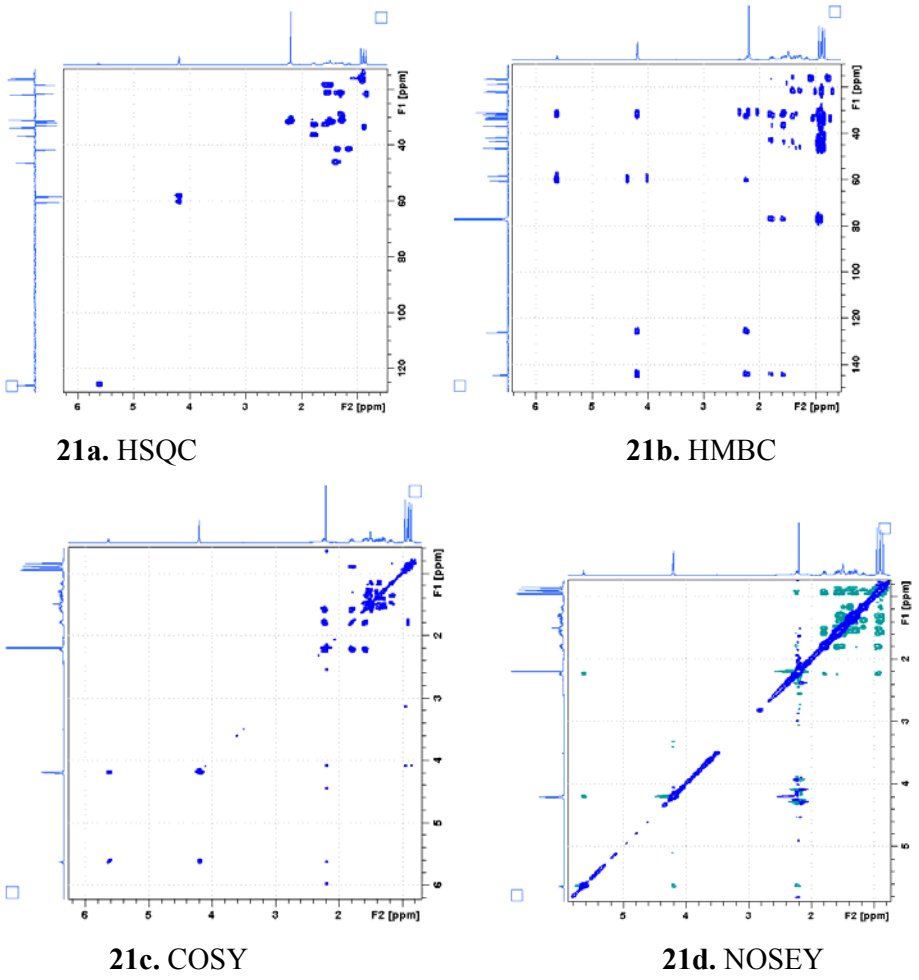


20a. HMBC correlation 21b. NOESY correlation

Figure 20: 2D correlations of compound **6**

Relative stereochemistry was confirmed by key NOE correlations obtained from NOESY data. Presence of NOE between H-20 and H-19 and absence of NOE between H-20 and H-5 confirmed trans-fused ring junction. Presence of NOE between H-20/H-8 α position of 17-CH_3. NOE observed between H-20 and H-11 placed the substituent at C-9 in β position. Z- configuration at double bond was confirmed by NOE between C12 protons and C14 proton.

Thus the compound was identified as a new natural product labd-13(14)-en-9α, 15, 16-triol.



21a. HSQC **21b.** HMBC

21c. COSY **21d.** NOSEY

Figure 21: 2D spectra of compound **6**

Compound **7**:

Compound **7** was isolated as yellow amorphous solid. ESIMS gave $[M+1]^+$ at *m/z* 315, $[M+Na]^+$ at *m/z* 337, $[M+K]^+$ at *m/z* 353 suggesting the molecular formula $C_{17}H_{14}O_6$ with eleven degrees of unsaturation.

The ^1H and ^{13}C NMR spectrum (Table 5) revealed it to be a flavone with 2 methoxyl substituents. Broad one proton singlet at δ12.81 indicated hydroxyl substitution at position 5. Protons at δ 6.97 (d, 8 Hz,), 7.265 (d, 2 Hz,) and 7.425 (dd, 8, 2 Hz,) indicated a 1, 2, 4-trisubtituted pattern. Carbon chemical shifts at δ 92.63 and 98.08 along with above information established oxygen substitution on at 7 on 'A' ring and 3',4'-disbtituted 'B' ring. Bathochromic shift in Band I from 347 to 408nm in its UV spectrum on addition of NaOMe indicated free hydroxyl

at 4'and hence methoxyl at 3'. Thus compound **7** was identified as velutin. This is the first report of velutin from genus *Leucas*.

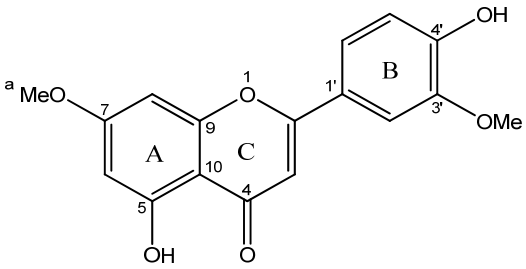


Figure 22: Structure of compound **7**.

Compound **8**:

Compound **8** was isolated as yellow amorphous solid. ESIMS gave $[M + 1]^+$ at *m*/*z* 301 suggesting the molecular formula $C_{16}H_{12}O_6$ with eleven degrees of unsaturation.

The ^1H and ^{13}C NMR spectrum (Table 5) revealed it to be a flavone with one methoxyl substituent. Similarity of ^{13}C NMR data with compound **7** indicated it to be velutin with one methyl less. Downfield shift of carbon 7 indicated loss of methyl at 7 position. Thus compound **8** was identified as chrysoeriol [8]. This is the first report of chrysoeriol from genus *Leucas*.

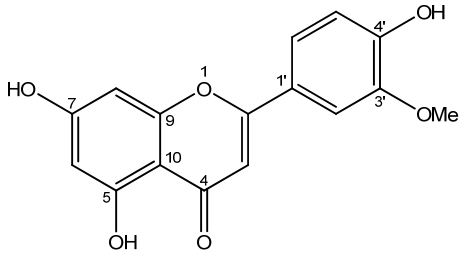


Figure 23: Structure of compound **8**.

2.5: Experimental:

i. Collection and processing:

As described earlier.

ii. Extraction and Isolation:

As described earlier.

Compound **1**:

Colourless gum (100 mg, 0.0055%, based on dry plant weight), HREIMS m/z: 322.25151 $[M]^+$; $[\alpha]_D^{25}$ no rotation (*c* 1.372, acetone); IR (CHCl3) 3427cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1.

Compound **2**:

White crystals (100 mg, 0.0055%), mp 80.5^{0}C; HREIMS m/z: 320.23658 $[M]^+$; $[\alpha]_D^{25}$+14.015 (*c* 1.064, acetone); IR (chloroform) 3536, 1749cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1.

X-ray crystallography:

Single crystals of the compound were grown by slow evaporation of the solution in 30%acetone: cyclohexane. Colourless crystal of approximate size 0.18 x 0.14 x 0.05 mm^3, was used for data collection on *Bruker SMART APEX* CCD diffractometer using MoK_α radiation. Exposure/frame = 10.0sec/frame, Crystals belong to Triclinic, space group P-1, a = 6.5378(15), *b* = 6.5433(14), *c* = 22.339(5) Å, *V* = 922.8(3) $Å^3$, *Z* = 2, D_c = 1.153 g /cc, μ (MoK_α) = 0.71073 Å, *T* = 295 K, 6088 reflections measured, R value 0.0861, wR2 = 0.2080. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL)[23] was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement as per the riding model. X-ray analysis revealed the relative conformation of the molecule at C5, C8, C9, C10 and C13(14) as S, R, R, S, and *Z* configurations. Data collection and refinement parameters are listed in Tables 2a-2d.

Compound **3**:

White crystals (35 mg, 0.002%); mp 69.5.^{0}C; ESIMS m/z: 417$[M + Na]^+$, 433$[M + K]^+$; $[\alpha]_D^{25}$-4 (*c*, 1.0 acetone); IR (chloroform) 3418, 1644cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 2.

Compound **4** and **5**:

White crystals (14mg,0.00077%); ESIMS m/z: 417$[M + Na]^+$, 433$[M + K]^+$; IR (chloroform) 3473, 1783, 1702 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 3.

X-ray crystallography of compound **4**:

Crystals of the compound mixture were grown by slow evaporation of the solution in 30%acetone: cyclohexane. Randomly collected colourless crystal of approximate size 0.49 x 0.08 x 0.06 mm^3, was used for data collection on *Bruker SMART APEX* CCD diffractometer using MoK_α radiation. Exposure / frame = 30.0 sec / frame. Crystals belong to Monoclinic, space group C2, a = 29.084(3), *b* = 7.2886(7), *c* = 14.1287(13) Å, *V* = 2910.4(5)$Å^3$, *Z* = 4, D_c = 1.133 g /cc, μ (MoK_α) = 0.71073 Å, *T* = 296 K, 7410 reflections measured, R value 0.0813, wR2 = 0.0892. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL)[23] was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement as per the riding model. X-ray analysis revealed the relative conformation of the molecule at C3, C5, C8, C9, C10, C13 and C14 as S, S, R, R, S, S and R configurations. Data collection and refinement parameters are listed in Tables 4a-4d.

Compound **6**:

Colourless gum(20mg, 0.0011%), HREIMS m/z: 324.26895 $[M]^+$; $[\alpha]_D^{25}$+12.517 (*c* 0.789, Acetone); IR (chloroform) 3372, cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1.

Compound **7**:

Yellow amorphous powder (8mg, 0.00044%); ESIMS m/z: 327 $[M + Na]^+$; cm^{-1}; UV(in methanol), 268, 308, 347nm; UV(in NaOMe), 266, 394, 408nm; ^1H and ^{13}C NMR spectral data, see Table 5.

Compound **8**:

Yellow amorphous powder (4mg, 0.00022%); ESIMS m/z: 301 $[M + 1]^+$;UV (in methanol) 267, 308, 341 nm; UV (in NaOMe), 269, 307, 328, 340, 347, 404nm; ^1H and ^{13}C NMR spectral data, see Tables 5.

2.6: Anti-mycobacterial activity of compounds isolated from *L. stelligera*.

Tuberculosis is a major and still neglected cause of death and disability with around 2 million deaths and 9 million infections worldwide in 2009. The emergence of drug resistant strains and confluence with HIV epidemic has turned TB into a global public health crisis [9]. Although, available drug regimens can cure most patients [10], emergence MDR, SDR and XDR –TB [11, 12] coupled with insufficient global drug pipeline [13], justifies continued efforts towards development of new drugs with new mode of action and novel structures.

There is currently a re-emerging interest in natural products as being able to provide novel structures for the drug discovery effort and being particularly effective as antibacterial leads [14]. Anti-mycobacterial diterpenes isolated from terrestrial and marine sources and belonging to many different classes, which includes diterpene classes viz. labdane, clerodane, pimarane, kaurane as well as diterpene amide, diterpene alkaloid, phorbol esters, etc., have appeared in many reviews [12, 14-18]. These give accounts of naturally occurring anti-mycobacterial compounds and display us a astonishing variety of structures.

Lamiaceae is a family of great economic and therapeutic importance. Few species [19,20] have demonstrated anti-mycobacterial activity while phytochemical analysis of *Leucas volkensii* [21] and *Anisochillus harmandii* [22] has led to isolation of diterpenes as active principles.

Acetone extract and compounds **1**, **2**, **3**, **6**, **7** and **8** were tested for their effect in *in vitro* models against *M. tuberculosis* H37Ra. Compounds **1**, **2**, **3** and **6** showed significant inhibition against *M. tuberculosis* H37Ra. The IC_{50} and IC_{90} values for the same are shown in Table 6. Specificities of anti-mycobacterial activity of compounds **1**, **2** and **6** were determined by evaluating inhibition of *Escherichia. coli* and *M. smegmatis* at their IC_{90}. The percent inhibition values are given in Table 7. Both extract and compounds **1**, **2** and **6** did not show any significant effect on *E. coli* and *M. smegmatis*; indicating the specificity of these compounds against pathogenic mycobacteria. Compound **1**, **2** and **6** were also tested at their IC_{90} and at 100µg/ml concentrations for their *in vitro* cytotoxicity against MCF-7 (Breast cancer), THP-1(Human acute leukemia cells) and HepG-2(Human hepatocellular liver carcinoma cells) cell lines (Table 18). None of the

compounds showed significant cytotoxic effect against any of these cell lines. Compounds **1** and **2** showed 40.70% and 42.40% inhibition respectively against MCF-7 cell line but only at a high concentration of 100µg/ml. Thus, the cytotoxicity studies indicated no significant toxicity of compounds **1**, **2** and **6** on human cancer cell lines. Evaluation of compound **3** against cancer cell lines, *E. coli* and *M. smegmatis* for probing specific inhibition of *M. tuberculosis* is in progress.

Anti-mycobacterial assay:

Objective: It was intended to study specific inhibition of *M. tuberculosis* by isolated active compounds. Compounds showing inhibition against *M. tuberculosis* were only taken for further assays against *M. smegmatis*, *E. coli* and cancer cell lines. Compounds **1**, **2**, **3**, **6** and **7** were evaluated for these activities while due to paucity of compound **8**, it was not evaluated for these activities. Also **4** and **5** being mixture, were not tested.

A. General experimental procedure:

Mycobacterium smegmatis (ATCC 607) was obtained from Astra Zeneca (Bangalore, India) and *Mycobacterium tuberculosis* H37Ra (ATCC 25177) was obtained from MTCC (Chandigarh, India). *Escherichia coli* strain DH5α was obtained from NCIM (Pune, India).

Cell lines used in this study, MCF-7 (Breast cancer), THP-1 (Human acute leukemia cells) and HepG-2 (Human hepatocellular liver carcinoma cells) were obtained from National Centre for Cellular Sciences (NCCS) - Pune, India.

B. Anti-mycobacterial assay against *M. tuberculosis*- XTT reduction assay:

M. tuberculosis H37Ra (ATCC 25177) cells were grown to logarithmic phase (O.D.595 ~ 1.0) in a defined medium (M. pheli medium) under aerobic conditions in a shaker incubator (Thermo Electron Corporation Model 481) maintained at 150 rpm and 37 °C. After growth, the culture was sonicated for 2 min using a water bath sonicator. Sonicated cells were used for inoculation in micro plate wells. 250 µl of the culture containing ~10^5 cells/ml was added to each well of 96 well plates. 2.5 µl of the test samples dissolved in DMSO was added to the wells to attain a final concentration of 100ug/ml respectively for the preliminary screening. Dose response curve of the active compounds was carried

out by making serial dilutions of the test samples. Then, the plate was incubated in a CO_2 incubator at 37 °C. The plate was taken out on the 8th day of incubation to measure the viable cell counts. The optical density of the culture was measured before addition of XTT at 470 nm which was served as a blank for the MIC calculations. 200 μM XTT was added and incubated for 20 min at 37 °C after shaking for 1 min. After 20 min of incubation, 60μM menadione was added and incubated at 37 °C for 40 min after mixing of 1 min. Finally, the optical density of the suspension was measured at 470 nm by using microplate reader. Results are given in Table 6. Specificity of the active compounds was also checked on *M. smegmatis* using the IC_{90} values found on *M. tuberculosis* (Table 7).

C. Anti-mycobacterial assay against *M. smegmatis*.

This was carried out on compounds **1**, **2** and **6** while **7** being inactive, was not tested further. Bioevaluation of compound **3** is underway. On the 3rd day of incubation, the microplate was taken out to remove the seal and measure the viable cells. Optical density was measured before addition of XTT at 470 nm. 200 μM XTT was added and incubated for 20 min at 37°C after shaking for 1 min. After 20 min incubation, 60 μM menadione was added and mixed for 1 min and then incubated at 37 °C for another 20 min. The optical density was measured at 470nm by using a micro plate reader. For Hypoxia induced XTT reduction microplate assay (HXRMA) on the 7th day of incubation, the plates were taken out and the seal was removed. A similar protocol as mentioned above was repeated for aerobically grown *M. smegmatis* (Table 7).

D. Inhibition assay against *E. coli*.

Effect of the compounds **1**, **2** and **6** was estimated on cultures of *E. coli* using the IC_{90} concentrations obtained against *M. tuberculosis*. The effect on growth was calculated by measuring the absorbance of culture at 620nm after an incubation time of 6h. None of the compounds exhibited any significant effect on growth of organism. Hence, the result confirmed their specific action against *M. tuberculosis* (Table 7). Evaluation of compound **3**, as mentioned above, is underway.

E. Antiproliferative activity-MTT cell proliferation assay

THP-1, HepG2 and MCF-7 cells were 10,000 cells per well in 96- well tissue culture plates. Cells were allowed to adhere for 24h at 37°C and treated with compounds **1**, **2** and **6** diluted in culture medium at their respective IC_{90} concentrations as well as at 100µg/ml, for additional 72h,120h ,and 192h respectively. In the cells in control wells a culture medium consisting of corresponding concentration of DMSO only was added.

After above incubation time of treatment cell proliferation was assessed with 10µl medium containing 5mg/ml MTT and subsequently incubated for additional 1h at 37°C. The formazan crystals were solubilized in 200µl of isopropanol and incubated for another 4 h. The optical density was read on a micro plate reader at 490nm filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the carrier DMSO alone was taken as 100% cell growth. All experiments were performed in triplicate, and the quantitative value was expressed as the average ± standard deviation (Table 8). Evaluation of compound **3** is underway.

2.7. Tables:

Table 1: NMR shifts for compounds **1**, **2** and **6**.

Carbon no	Compound **1**		Compound **2**		Compound **6**	
	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)*	^{13}C (δ)	^{1}H (δ)
1	33.02	1.36 (m)	31.82	1.49 (m)	31.90	1.48 (m)
2	18.72	1.55 (m), 1.48 (m)	18.56	1.48 (m)	18.61	1.54(m), 1.48(m)
3	41.75	1.17 (dt 3.6, 13.0), 1.33 (m)	41.63	1.16 (dt, 3.5 ,12.9), 1.34 (m)	41.66	1.15 (dt 3.5, 13.2), 1.35 (m)
4	33.48	-	33.32	-	33.32	-
5	46.99	1.37 (m)	46.33	1.4 (m)	46.40	1.39 (m)
6	21.66	1.52 (m), 1.26 (m)	21.53	1.54 (m), 1.29 (m)	21.59	1.28 (m),1.53 (m)
7	32.10	1.48 (m), 1.22 (m)	31.24	1.49 (m), 1.29 (m)	31.31	1.27 (m)
8	35.84	1.76 (m)	36.63	1.79 (m)	36.85	1.78 (m)
9	95.02	-	76.76	-	77.31	-
10	42.52	-	43.17	-	43.24	-
11[a]	29.28	1.76(m), 2.03(m)	32.24	1.66(m), 1.85(m)	33.0	1.78 (m), β 1.57 (m)
12 [a]	33.42	2.00 (m)	22.23	2.39(dt 1.34, 8.43)	31.92	2.2 (m)
13	89.92	-	135.14	-	144.67	-
14 14	45.52	2.32(d 13.10), 1.98 (m)	143.74	7.11 (bt 1.6)	126.03	5.63 (t)
15	99.22	5.43 (bs)	70.14	4.774(d 1.89), 4.779 (d 1.89) [a]	60.56	4.19 (bs)
16	77.25	4.37(d 8.5), β3.62(d 8.5)	174.50	-	58.46	4.20 (bs)
17	17.68	0.91(d 6.62)	16.15	0.92(d, 6.65)	16.52	0.89(d 6.99)
18	22.01	0.81 (s)	33.67	0.88 (s)	33.72	0.88 (s)
19	33.30	0.86 (s)	22.02	0.84 (s)	21.96	0.84 (s)
20	18.16	0.91 (s)	16.33	0.94 (s)	16.15	0.94 (s)

* multiplicities in bracket

Table 2a. Crystal data and structure refinement for compound **2**.

Parameter	Value
Temperature	295 K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 6.5378(15)Å α= 87.294(4)°. b = 6.5433(14)Å β= 88.488(4)° c = 22.339(5) Å γ = 75.202(4)°
Volume	922.8(3)Å^3
Z	2
Density (calculated)	1.153 Mg/m^3
Crystal size	0.18 x 0.14 x 0.05 mm^3
Reflections collected	6088
Data / restraints / parameters	3991 / 3 / 431
Final R indices [I>2sigma(I)]	R1 = 0.0861, wR2 = 0.2080

Table 2b. Bond lengths for compound **2**

Carbon (no.)-Carbon (no.)	Bond lengths [Å]	Carbon (no.)-Carbon (no.)	Bond lengths [Å]
C(1)-C(2)	1.499(10)	C(8)-C(17)	1.501(12)
C(1)-C(10)	1.562(8))	C(8)-C(9)	1.540(10)
C(2)-C(3)	1.515(11)	C(9)-C(11)	1.544(10)
C(3)-C(4)	1.535(9)	C(9)-C(10)	1.585(9)
C(4)-C(19)	1.572(8)	C(10)-C(20)	1.549(7)
C(4)-C(18)	1.518(10)	C(11)-C(12)	1.518(9)
C(4)-C(5)	1.550(9)	C(12)-C(13)	1.488(10)
C(5)-C(6)	1.544(8)	C(13)-C(14)	1.345(10)
C(5)-C(10)	1.575(7)	C(13)-C(16)	1.459(10)
C(6)-C(7)	1.515(10)	C(15)-C(16)	1.556(2)
C(7)-C(8)	1.529(10)	C(14)-C(15)	1.452(12)

Table 2c. Bond angles for compound **2**

Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]	Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]
C(2)-C(1)-C(10)	114.6(5)	C(17)-C(8)-C(7)	109.8(7)
C(1)-C(2)-C(3)	111.1(6)	C(11)-C(9)-C(8)	110.4(5)
C(2)-C(3)-C(4)	114.9(6)	C(11)-C(9)-C(10)	111.9(6)
C(19)-C(4)-C(3)	109.0(6)	C(8)-C(9)-C(10)	111.5(5)
C(19)-C(4)-C(18)	106.3(5)	C(1)-C(10)-C(20)	108.9(6)
C(19)-C(4)-C(5)	114.4(5)	C(1)-C(10)-C(5)	106.8(5)
C(3)-C(4)-C(5)	109.1(5)	C(20)-C(10)-C(5)	111.9(4)
C(18)-C(4)-C(3)	107.6(6)	C(1)-C(10)-C(9)	111.1(4)
C(18)-C(4)-C(5)	110.2(5)	C(20)-C(10)-C(9)	109.3(5)
C(6)-C(5)-C(10)	110.0(5)	C(5)-C(10)-C(9)	108.9(5)
C(6)-C(5)-C(4)	113.4(5)	C(12)-C(11)-C(9)	117.5(6)
C(10)-C(5)-C(4)	118.3(5)	C(13)-C(12)-C(11)	113.8(6)
C(7)-C(6)-C(5)	111.5(5)	C(14)-C(13)-C(12)	132.9(7)
C(6)-C(7)-C(8)	115.3(6)	C(14)-C(13)-C(16)	105.7(7)
C(7)-C(8)-C(9)	112.6(5)	C(12)-C(13)-C(16)	121.4(6)
C(17)-C(8)-C(9)	114.1(7)	C(13)-C(14)-C(15)	112.1(7)

Table 2d. Torsion angles for compound **2**

Connectivity	angles [°]	Connectivity	angles [°]
C(10)-C(1)-C(2)-C(3)	-57.8(8)	C(2)-C(1)-C(10)-C(5)	52.2(7)
C(1)-C(2)-C(3)-C(4)	55.8(8)	C(2)-C(1)-C(10)-C(9)	170.8(6)
C(2)-C(3)-C(4)-C(18)	-168.6(6)	C(6)-C(5)-C(10)-C(20)	-61.5(7)
C(2)-C(3)-C(4)-C(5)	-49.1(7)	C(4)-C(5)-C(10)-C(20)	71.0(6)
C(2)-C(3)-C(4)-C(19)	76.6(7)	C(6)-C(5)-C(10)-C(1)	179.4(5)
C(18)-C(4)-C(5)-C(6)	-63.9(6)	C(4)-C(5)-C(10)-C(1)	-48.1(6)
C(3)-C(4)-C(5)-C(6)	178.2(5)	C(6)-C(5)-C(10)-C(9)	59.4(5)
C(19)-C(4)-C(5)-C(6)	55.8(7)	C(4)-C(5)-C(10)-C(9)	-168.1(4)
C(18)-C(4)-C(5)-C(10)	165.1(5)	C(8)-C(9)-C(10)-C(20)	66.0(6)
C(3)-C(4)-C(5)-C(10)	47.2(6)	C(11)-C(9)-C(10)-C(20)	-58.2(6)
C(19)-C(4)-C(5)-C(10)	-75.3(6)	C(8)-C(9)-C(10)-C(1)	-173.8(5)
C(4)-C(5)-C(6)-C(7)	168.0(5)	C(11)-C(9)-C(10)-C(1)	62.0(6)
C(10)-C(5)-C(6)-C(7)	-56.9(6)	C(8)-C(9)-C(10)-C(5)	-56.5(5)
C(5)-C(6)-C(7)-C(8)	51.7(7)	C(11)-C(9)-C(10)-C(5)	179.3(5)
C(6)-C(7)-C(8)-C(17)	-176.9(7)	C(8)-C(9)-C(11)-C(12)	110.5(8)
C(6)-C(7)-C(8)-C(9)	-48.6(8)	C(10)-C(9)-C(11)-C(12)	-124.7(7)
C(17)-C(8)-C(9)-C(11)	-58.4(8)	C(9)-C(11)-C(12)-C(13)	-177.5(7)
C(7)-C(8)-C(9)-C(11)	175.6(6)	C(11)-C(12)-C(13)-C(14)	24.4(15)
C(17)-C(8)-C(9)-C(10)	176.6(6)	C(11)-C(12)-C(13)-C(16)	-153.2(9)
C(7)-C(8)-C(9)-C(10)	50.6(7)	C(16)-C(13)-C(14)-C(15)	-2.3(11)
C(2)-C(1)-C(10)-C(20)	-68.8(7)	C(12)-C(13)-C(14)-C(15)	179.9(9)

Table 3: ^{13}C and ^{1}HNMR data of compound **3** and reference compounds

no	**3**		Sclareol*		8-episclareol*		13-episclareol*	
	^{13}C	^{1}H **	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	**36.48**	1.10, 1.57	39.6	0.95β 1.6 α	39.2	0.86 β 1.65 α	39.7	0.98 β 1.6 α
2	18.58	1.68, 1.43	18.4	1.6 β 1.4 α	18.3	1.6 β 1.4 α	18.4	1.6 β 1.4 α
3	42.22	1.15, 1.37	42.0	1.13 β 1.40 α	42.0	1.14 β 1.40 α	42.0	1.14 β 1.40 α
4	32.92	-	33.2	-	33.2	-	33.2	-
5	**46.21**	**1.06**(dd 12.64, 2.76)	**56.0**	0.91(dd 12.00, 2.55)	**55.9**	0.82	**56.0**	0.94(dd 12.00, 2.55)
6	20.67	1.55,1.25	20.5	1.6 β 1.3 α	18.1	1.35 β 1.35 α	20.5	1.6 β 1.3 α
7	37.83	1.55 (m)	44.1	1.4 β 1.84 α	42.1	1.75 β 1.5 α	44.1	1.4 β 1.82 α
8	74.20	-	74.7	-	73.4	-	74.9	-
9	61.18	1.11	61.7	1.11	59.0	0.76	61.8	1.18
10	38.86	-	39.2	-	39.1	-	39.3	-
11	20.91	1.78, 1.50	19.0	1.5 1.3	19.3	1.3 1.4	19.1	1.45 1.3
12	44.96	1.52-1.63 (m)	44.9	1.65	46.1	1.60	44.8	1.65
13	73.64	-	73.5	-	73.6	-	74.1	-
14	146.15	5.95 (dd, 17.30, 10.38)	146.2	5.93	144.8	5.93	145.2	5.87
15	111.11	5.22 (d,17.30), 5.03 (d,10.38)	111.7	5.21, 5.01	112.0	5.22,5.08	111.8	5.23, 5.06
16	27.25	1.29	26.8	1.27	27.6	1.30	29.1	1.26
17	**31.99**	**1.47**	24.1	1.16	30.5	1.12	24.5	1.15
18	33.11	0.87	33.4	0.86	33.4	0.87	33.4	0.86
19	21.36	0.79	21.5	0.78	21.6	0.82	21.5	0.78
20	**24.78**	**1.07**	**15.4**	0.78	**15.1**	0.96	**15.4**	0.77

* As given in literature [4], ** value in δ

Table 4a. X-ray data for compound **4**:

Parameter	Value
Temperature	296 K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	C2
Unit cell dimensions	a = 29.084(3)Å α= 90°. b = 7.2886(7)Å β= 103.652(2)° c = 14.1287(13) Å γ = 90°
Volume	2910.4(5)Å^3
Z	4
Density (calculated)	1.133 g/cc
Crystal size	0.49 x 0.08 x 0.06 mm^3
Reflections collected	7410
Data / restraints / parameters	4864 / 1 / 376
Final R indices [I>2sigma(I)]	R1 = 0.0813, wR2 = 0.1892

Table 4b. Bond lengths for compound **4**

Carbon (no.)-Carbon (no.)	Bond lengths [Å]	Carbon (no.)-Carbon (no.)	Bond lengths [Å]
C(1)-C(2)	1.500(7)	C(8)-C(17)	1.533(8)
C(1)-C(10)	1.569(7)	C(8)-C(9)	1.542(7)
C(2)-C(3)	1.501(7)	C(9)-C(11)	1.544(7)
C(3)-C(4)	1.529(6)	C(9)-C(10)	1.569(7)
C(4)-C(19)	1.552(7)	C(10)-C(20)	1.551(7)
C(4)-C(18)	1.544(7)	C(11)-C(12)	1.530(7)
C(4)-C(5)	1.545(7)	C(12)-C(13)	1.528(7)
C(5)-C(6)	1.521(7)	C(13)-C(14)	1.526(7)
C(5)-C(10)	1.578(7)	C(13)-C(16)	1.546(6)
C(6)-C(7)	1.505(7)	C(14)-C(15)	1.471(8)
C(7)-C(8)	1.518(9)	C(21)-C(22)	1.503(7)

Table 4c. Bond angles for compound **4**

Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]	Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]
C(2)-C(1)-C(10)	113.5(4)	C(17)-C(8)-C(7)	109.7(6)
C(1)-C(2)-C(3)	110.3(4)	C(11)-C(9)-C(8)	113.2(4)
C(2)-C(3)-C(4)	113.4(4)	C(11)-C(9)-C(10)	112.6(4)
C(19)-C(4)-C(3)	111.1(4)	C(8)-C(9)-C(10)	110.2(4)
C(19)-C(4)-C(18)	108.0(5)	C(1)-C(10)-C(20)	107.5(5)
C(19)-C(4)-C(5)	114.3(4)	C(1)-C(10)-C(5)	108.0(4)
C(3)-C(4)-C(5)	107.5(4)	C(20)-C(10)-C(5)	115.0(4)
C(18)-C(4)-C(3)	107.5(4)	C(1)-C(10)-C(9)	110.1(4)
C(18)-C(4)-C(5)	108.3(4)	C(20)-C(10)-C(9)	108.7(4)
C(6)-C(5)-C(10)	109.6(4)	C(5)-C(10)-C(9)	107.4(4)
C(6)-C(5)-C(4)	115.2(4)	C(12)-C(11)-C(9)	105.1(4)
C(10)-C(5)-C(4)	117.7(4)	C(13)-C(12)-C(11)	104.7(4)
C(7)-C(6)-C(5)	111.6(4)	C(14)-C(13)-C(12)	114.0(4)
C(6)-C(7)-C(8)	114.5(5)	C(14)-C(13)-C(16)	99.7(4)
C(7)-C(8)-C(9)	110.1(4)	C(12)-C(13)-C(16)	112.7(4)
C(17)-C(8)-C(9)	114.2(5)	C(13)-C(14)-C(15)	104.2(5)

Table 4d. Torsion angles for compound **4**

Connectivity	angles [°]	Connectivity	angles [°]
C(10)-C(1)-C(2)-C(3)	-58.3(5)	C(8)-C(9)-C(11)-C(12)	98.3(5)
C(1)-C(2)-C(3)-C(4)	62.4(5)	C(10)-C(9)-C(11)-C(12)	-135.9(4)
C(2)-C(3)-C(4)-C(18)	-172.4(4)	C(9)-C(11)-C(12)-C(13)	23.8(5)
C(2)-C(3)-C(4)-C(5)	-56.0(5)	C(11)-C(12)-C(13)-C(14)	-145.9(5)
C(2)-C(3)-C(4)-C(19)	69.6(5)	C(11)-C(12)-C(13)-C(16)	101.3(5)
C(18)-C(4)-C(5)-C(6)	-62.7(6)	C(16)-C(13)-C(14)-C(15)	30.2(5)
C(3)-C(4)-C(5)-C(6)	-178.6(4)	C(12)-C(13)-C(14)-C(15)	-90.1(5)
C(19)-C(4)-C(5)-C(6)	57.7(6)	C(13)-O(1)-C(9)-C(11)	5.0(5)
C(18)-C(4)-C(5)-C(10)	165.5(4)	C(13)-O(1)-C(9)-C(8)	-115.5(4)
C(3)-C(4)-C(5)-C(10)	49.6(5)	C(13)-O(1)-C(9)-C(10)	125.5(4)
C(19)-C(4)-C(5)-C(10)	-74.1(5)	C(7)-C(8)-C(9)-O(1)	-61.3(5)
C(4)-C(5)-C(6)-C(7)	167.5(5)	C(17)-C(8)-C(9)-O(1)	62.6(6)
C(10)-C(5)-C(6)-C(7)	-57.0(6)	O(1)-C(9)-C(10)-C(20)	-178.9(4)
C(5)-C(6)-C(7)-C(8)	53.8(6)	O(1)-C(9)-C(10)-C(1)	-61.3(5)
C(6)-C(7)-C(8)-C(17)	-179.4(4)	O(1)-C(9)-C(10)-C(5)	56.1(4)
C(6)-C(7)-C(8)-C(9)	-52.9(6)	O(1)-C(9)-C(11)-C(12)	-18.0(5)
C(17)-C(8)-C(9)-C(11)	-52.4(7)	C(9)-O(1)-C(13)-C(14)	135.9(4)
C(7)-C(8)-C(9)-C(11)	-176.4(4)	C(9)-O(1)-C(13)-C(12)	10.3(5)
C(17)-C(8)-C(9)-C(10)	-179.5(5)	C(9)-O(1)-C(13)-C(16)	-113.0(4)
C(7)-C(8)-C(9)-C(10)	56.6(6)	C(11)-C(12)-C(13)-O(1)	-21.2(5)
C(2)-C(1)-C(10)-C(20)	-75.6(5)	O(1)-C(13)-C(14)-C(15)	148.5(4)
C(2)-C(1)-C(10)-C(5)	49.1(5)	C(16)-O(2)-C(15)-O(3)	174.2(5)
C(2)-C(1)-C(10)-C(9)	166.1(4)	C(16)-O(2)-C(15)-C(14)	-6.1(6)
C(6)-C(5)-C(10)-C(20)	-60.6(5)	C(13)-C(14)-C(15)-O(3)	163.1(6)
C(4)-C(5)-C(10)-C(20)	73.7(6)	C(13)-C(14)-C(15)-O(2)	-16.7(6)
C(6)-C(5)-C(10)-C(1)	179.3(4)	C(15)-O(2)-C(16)-O(4)	-92.2(5)
C(4)-C(5)-C(10)-C(1)	-46.4(5)	C(15)-O(2)-C(16)-C(13)	26.0(5)
C(6)-C(5)-C(10)-C(9)	60.6(5)	O(1)-C(13)-C(16)-O(4)	-36.1(5)
C(4)-C(5)-C(10)-C(9)	-165.2(4)	C(14)-C(13)-C(16)-O(4)	83.0(4)
C(8)-C(9)-C(10)-C(20)	64.2(6)	C(12)-C(13)-C(16)-O(4)	-155.7(4)
C(11)-C(9)-C(10)-C(20)	-63.2(5)	O(1)-C(13)-C(16)-O(2)	-153.2(4)
C(8)-C(9)-C(10)-C(1)	-178.3(4)	C(14)-C(13)-C(16)-O(2)	-34.0(5)
C(11)-C(9)-C(10)-C(1)	54.4(5)	C(12)-C(13)-C(16)-O(2)	87.2(5)
C(8)-C(9)-C(10)-C(5)	-60.9(5)	C(3)-O(5)-C(21)-O(6)	-1.4(8)
C(11)-C(9)-C(10)-C(5)	171.8(4)	C(3)-O(5)-C(21)-C(22)	178.5(5)

Table 5: ^{1}H and ^{13}C NMR data for compounds **7** and **8**.

No.	**7**		**8**	
	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
2	164.06	-	164.39	
3	104.47	6.51	102.32	6.48
4	182.41	-	182.08	-
5	162.14	-	161.54	-
6	98.08	6.305 (d, 2 Hz)	99.62	6.06
7	165.45	-	167.53	-
8	92.63	6.425 (d, 2 Hz)	94.33	6.30
9	157.67	-	158.14	-
10	105.51	-	102.97	-
1’	123.34	-	122.06	-
2’	108.28	6.97 (d, 8 Hz)	108.97	7. 37 (d, 2Hz)
3’	146.83	-	148.12	-
4 ‘	149.22		150.95	-
5’	114.97	7.265 (d, 2 Hz)	115.39	6.83 (d, 8 Hz)
6’	120.73	7.425 (dd, 8, 2 Hz)	120.21	7.40 (dd, 8, 2 Hz)
7-Me	55.80	3.82	-	-
4'-Me	56.14	3.94	55.12	3.86

Table 6: *In vitro* antimycobacterial activity of compounds against *M. tuberculosis*

Compound	**IC_{50} (µg/ml)**	**IC_{90}(µg/ml)**
1	5.55	14.88
2	5.02	19.67
3	5.95	10.85
6	9.8	46.52
LS aerial acetone extract	8.94	43.98

Table 7: Percent inhibition of *M. smegmatis* and *E. coli*.

Compound*	***M. smegmatis***	***E. coli***
1	47.83 ± 3.74	23.15 ± 2.45
2	31.56 ± 6.09	0.29 ± 3.56
6	30.57 ± 3.58	11.47 ± 1.65
LS aerial acetone extract	19.47 ± 0.16	3.48 ± 2.561

*at IC_{90}

Three replicates; results are mean ± standard deviation.

Table 8: Cytotoxicity against MCF-7, THP-1 and HepG-2.

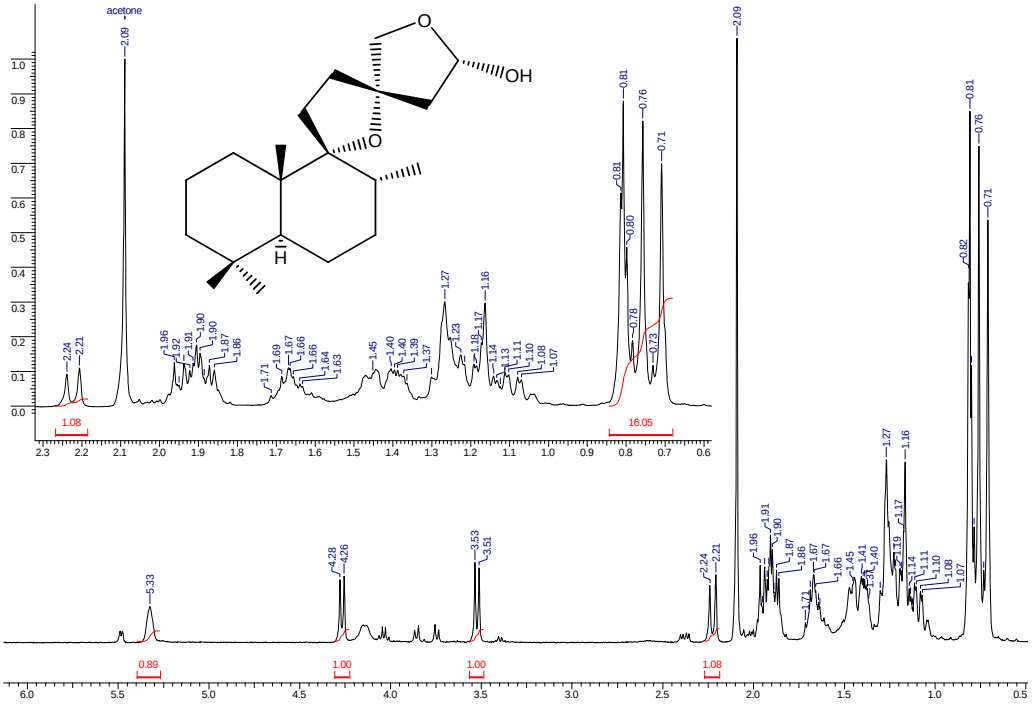
	MCF-7**		**THP-1****		**HepG-2****	
Sr.*	**IC_{90}**	**100**	**IC_{90}**	**100**	**IC_{90}**	**100**
1	28.98 ± 2.23	42.40 ± 1.67	8.18 ± 2.65	21.74 ± 2.78	6.92 ± 1.12	14.43 ± 3.09
2	13.37 ± 2.76	40.70 ± 2.78	10.11 ± 1.67	18.83 ± 2.34	Inactive	16.84 ± 3.25
6	3.54 ± 1.76	3.54 ± 3.45	9.07 ± 3.43	9.07 ± 4.65	21.48 ± 2.13	21.48 ± 2.65
AA[a]	7.01 ± 3.87	Inactive	Inactive	Inactive	4.16 ± 3.21	Inactive

*at IC_{90}, ** values in µg/ml , [a] acetone extract

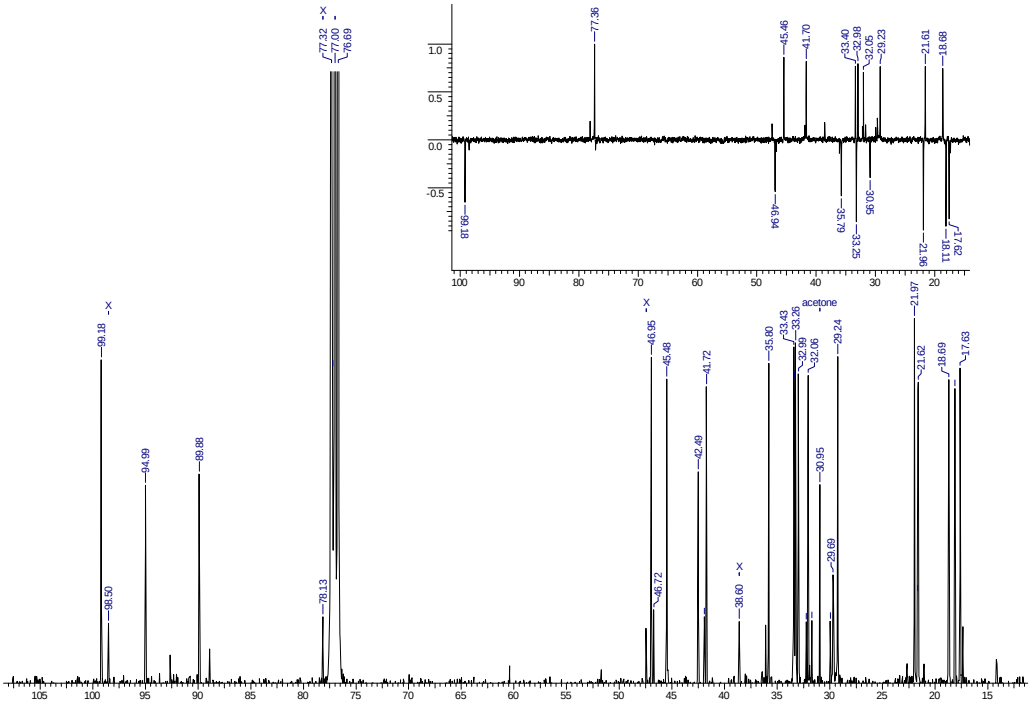
2.8. NMR spectroscopic data:

Compound **1**:

^{1}H NMR:

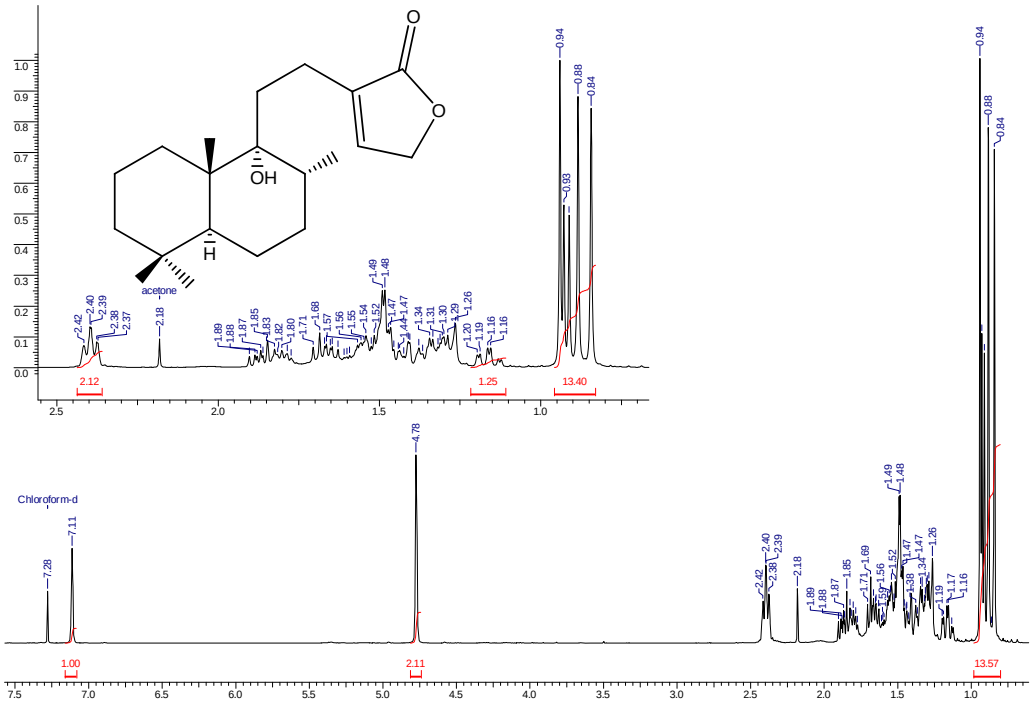


^{13}C NMR and DEPT in inset:

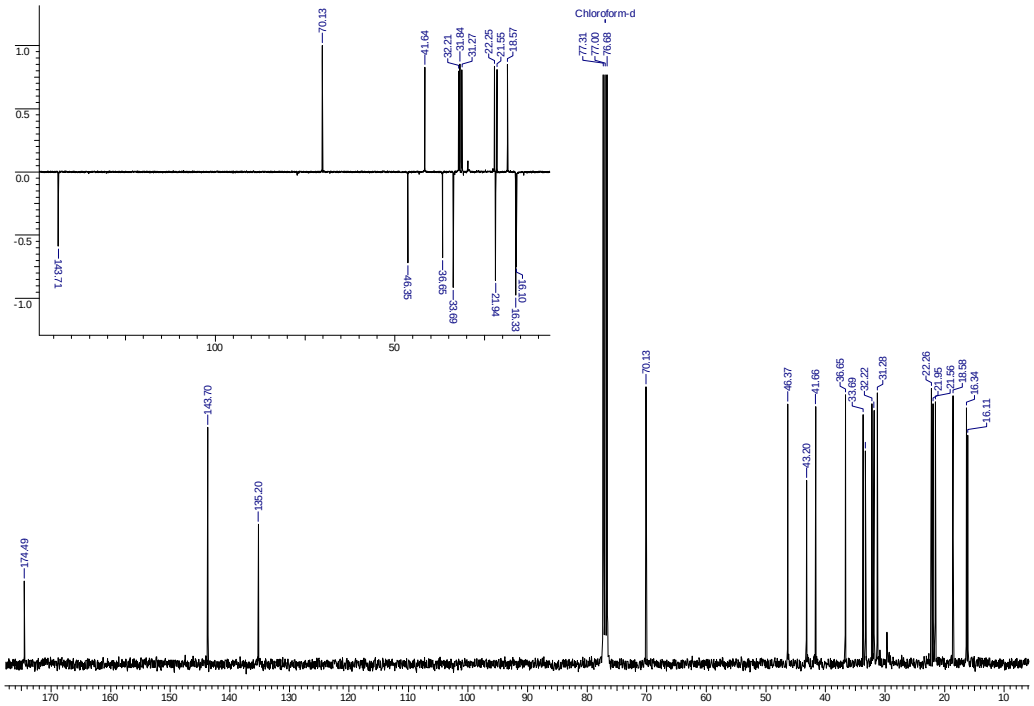


Compound **2**:

^{1}H NMR:

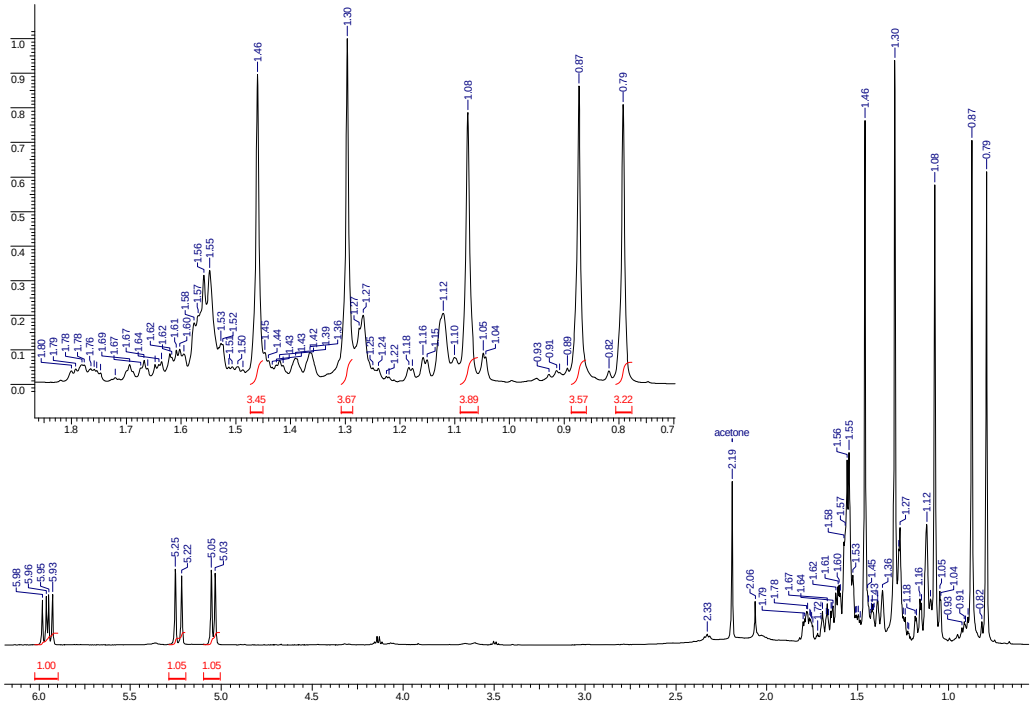


^{13}C NMR and DEPT in inset:

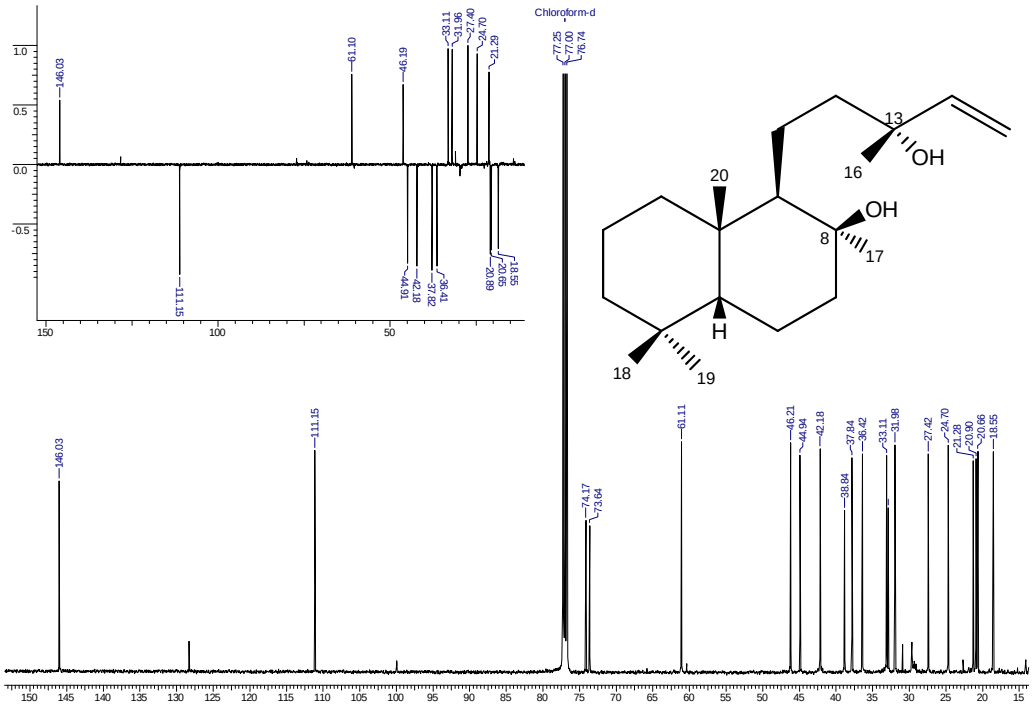


Compound **3**:

^{1}H NMR:

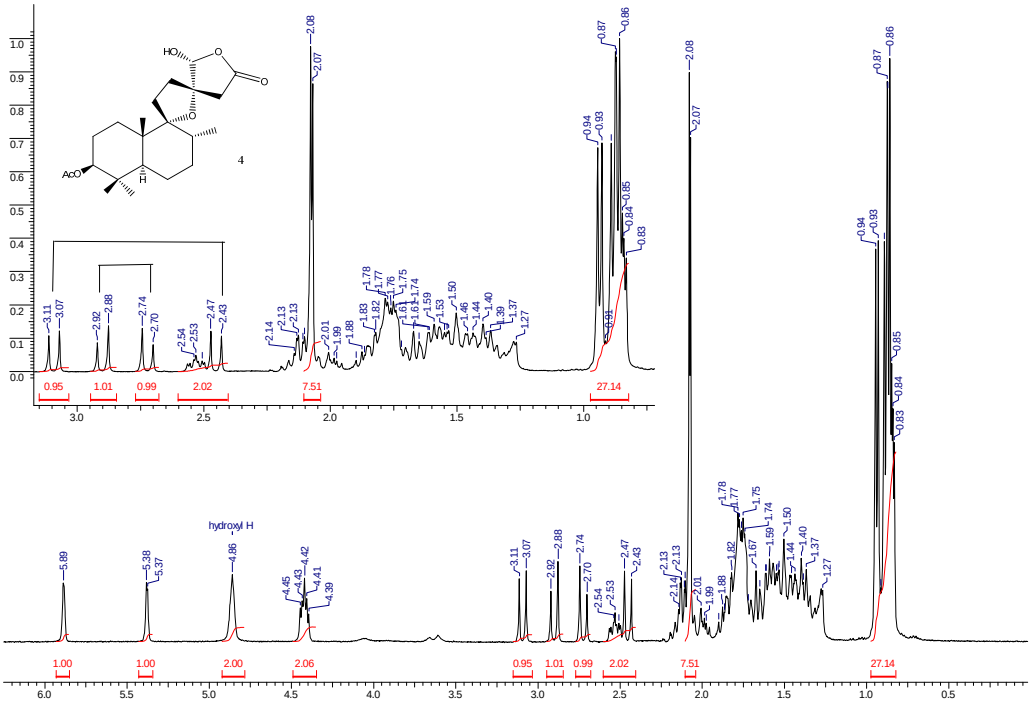


^{13}C NMR and DEPT in inset:

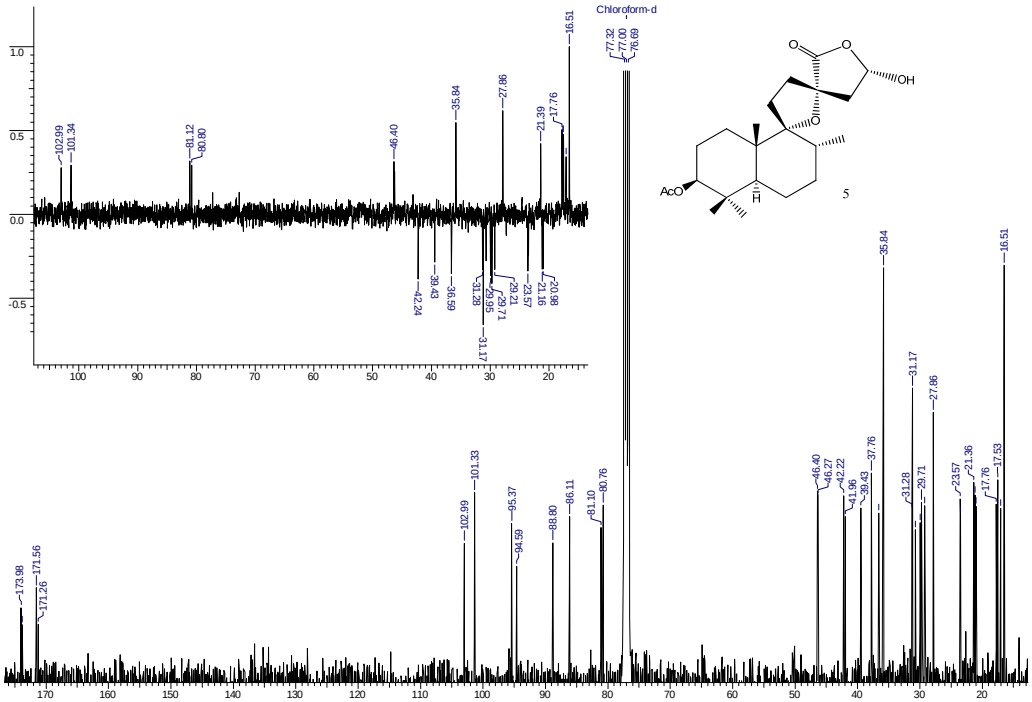


Compounds **4** and **5**:

^{1}H NMR:

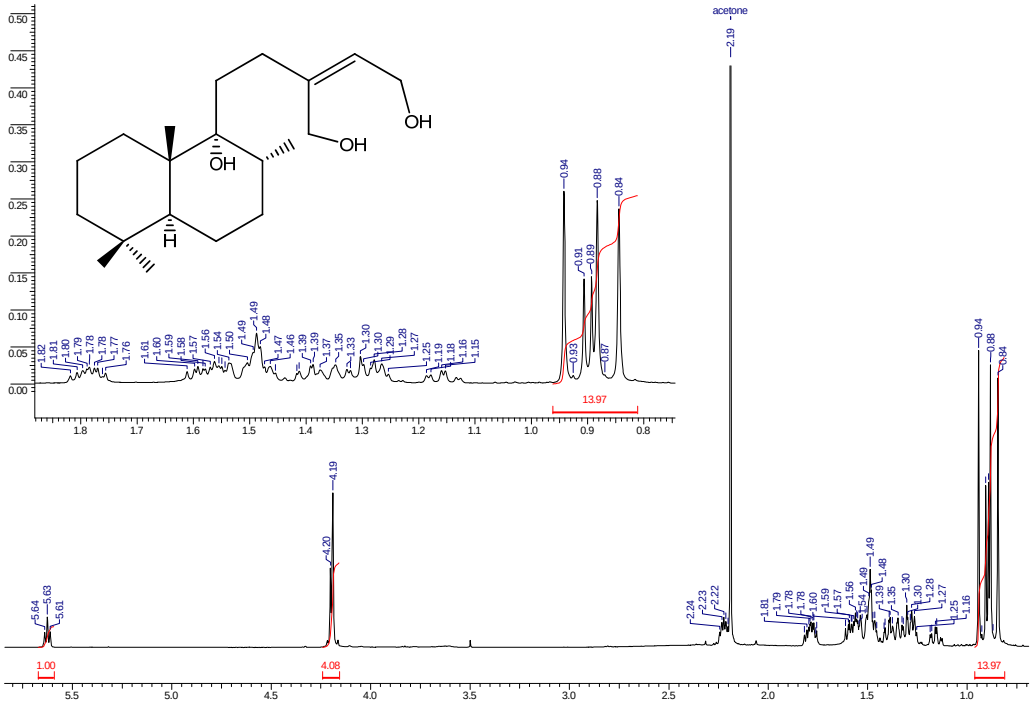


^{13}C NMR and DEPT in inset:

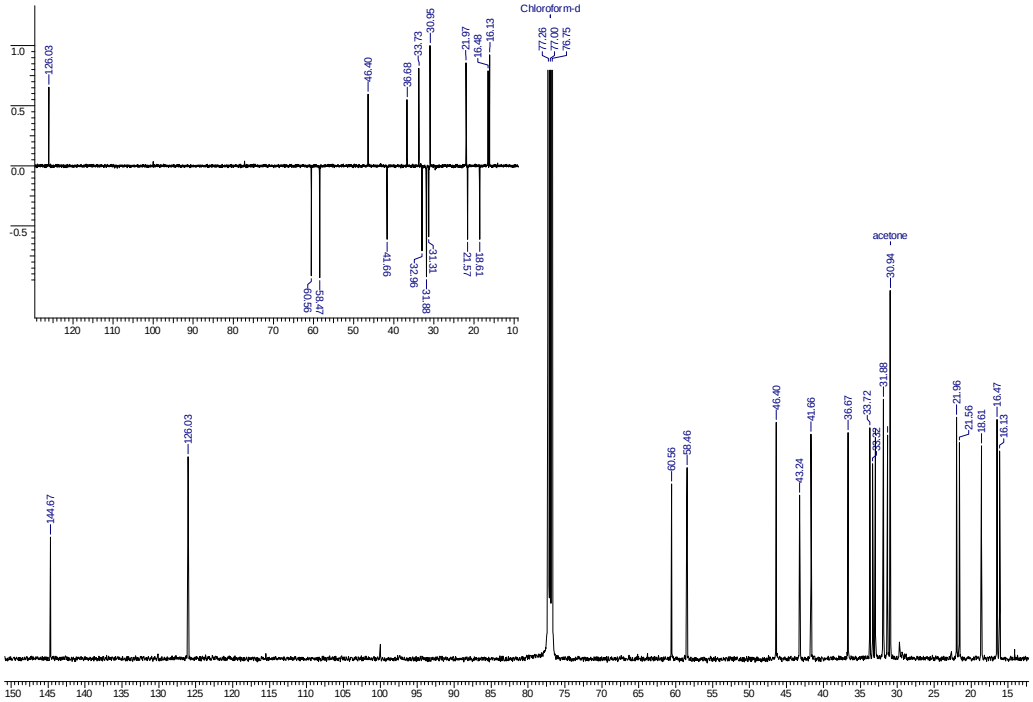


Compound **6**:

^{1}H NMR:

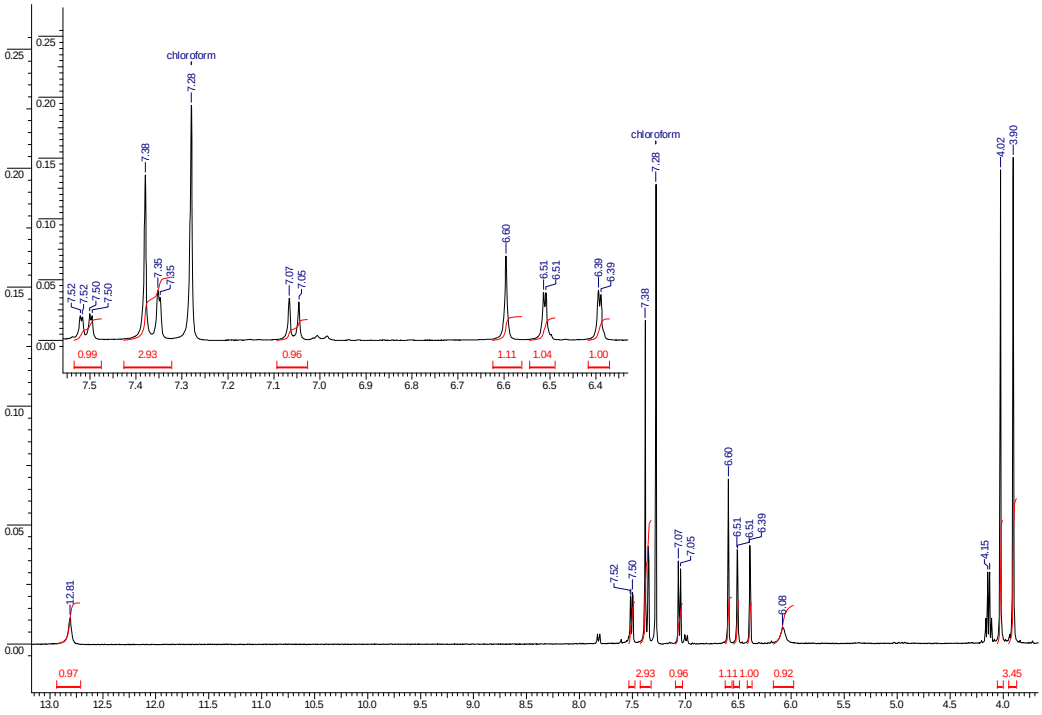


^{13}C NMR and DEPT in inset:

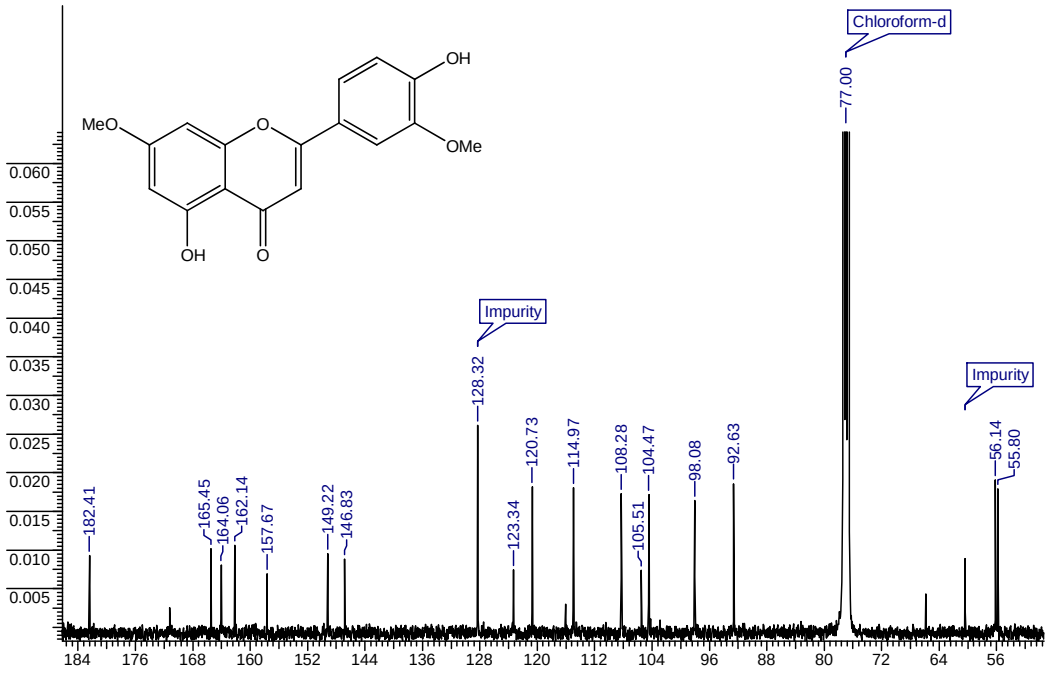


Compound **7**:

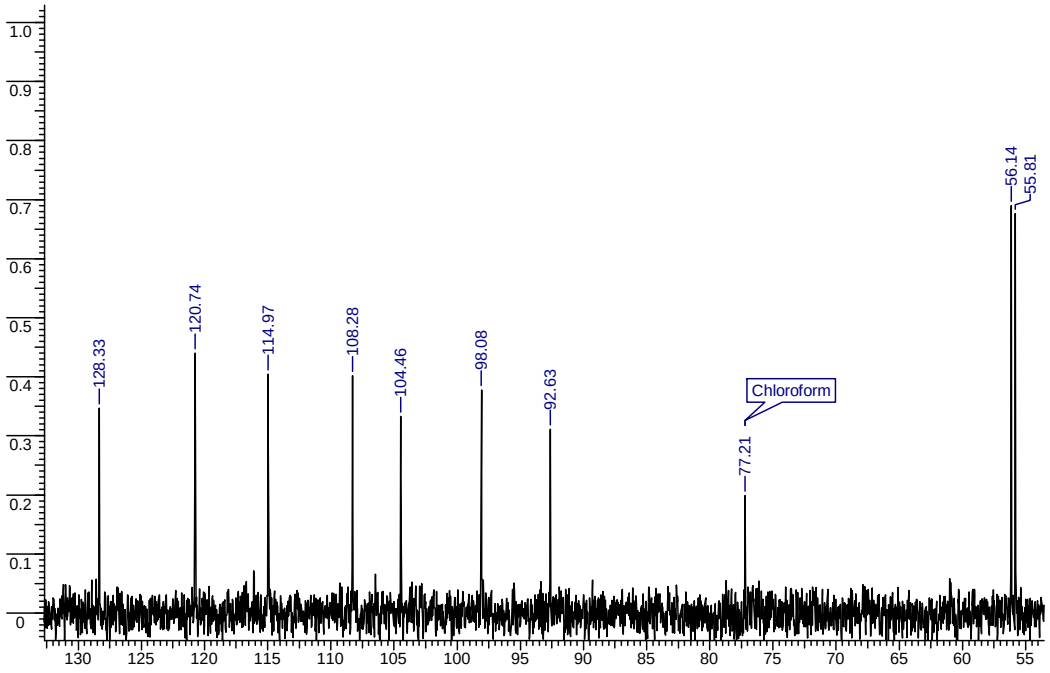
^{1}H NMR:



^{13}C NMR:

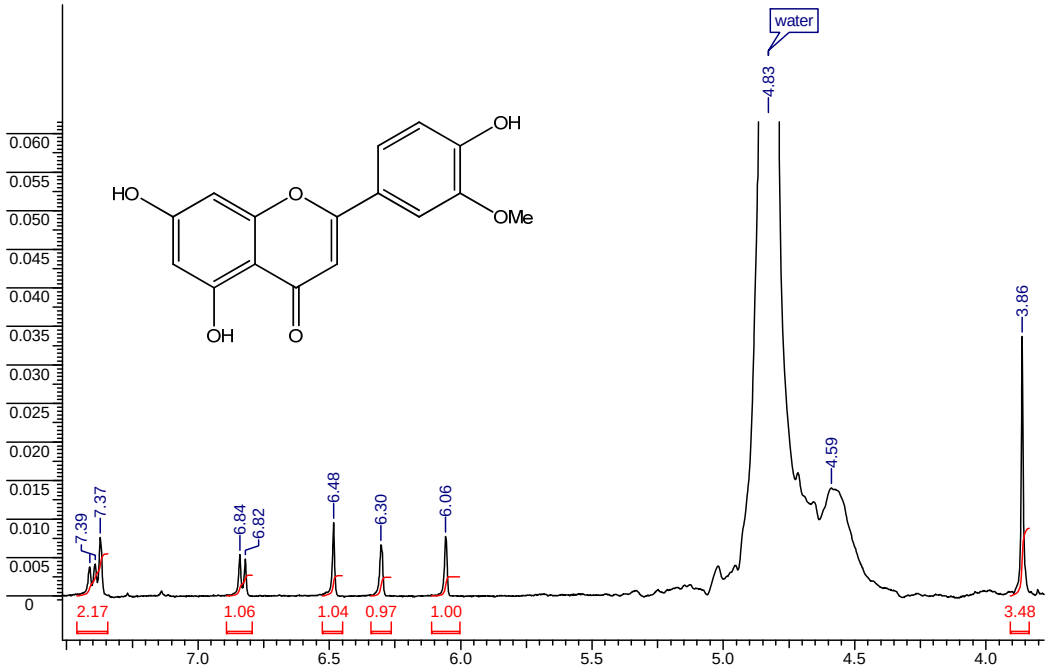


DEPT:

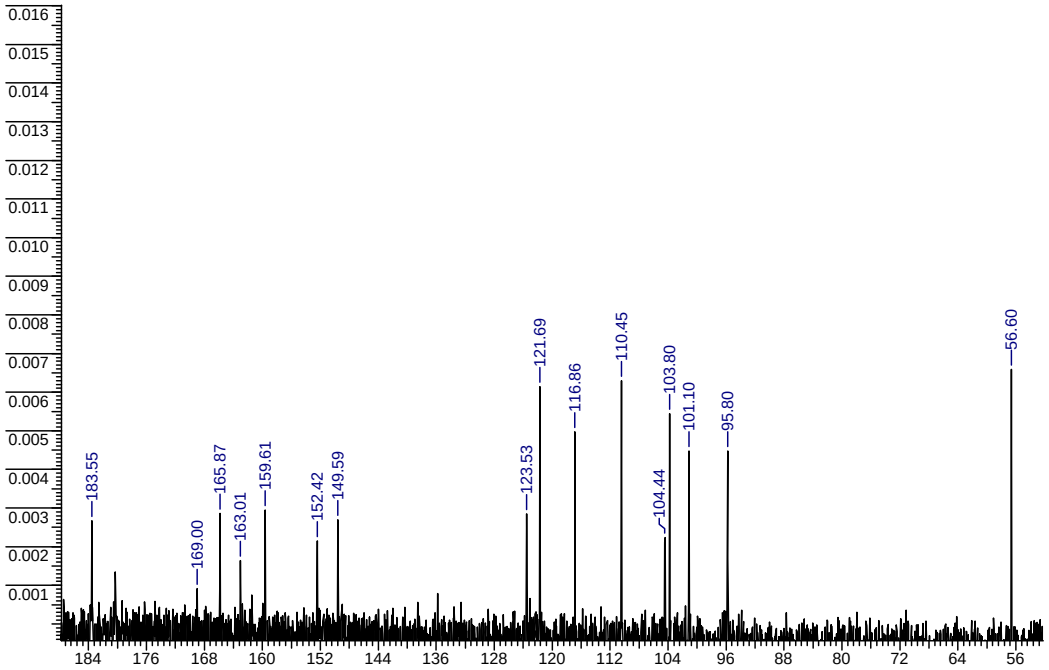


Compound **8**:

^{1}H NMR:



^{13}C NMR: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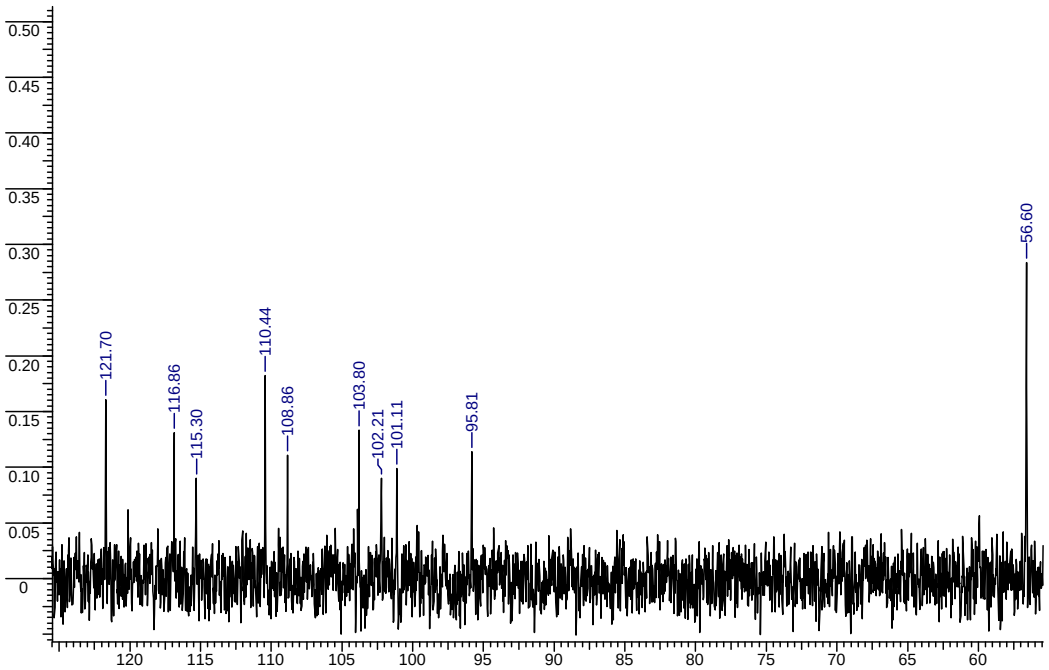
101.10
95.80
56.60

DEPT: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Chapter 3

Phytochemical investigations on *Lavandula gibsoni* Grah.

Figure 1: *L. gibsoni*Grah. Ex Dalz. & Gibs(=*L. lawii* Wight.)

3.1. Introduction:

Genus *Lavandula* is represented by two species in Maharashtra, India viz. *L. pinnata* and *L. gibsoni*. *L. gibsoni* is a medium sized shrub with clusters of tiny violet flowers. It is endemic to Western Ghats and found on the hills of Maharashtra [1].

3.2. Collection and processing:

L. gibsoni, whole plants, in flowering, were collected along roadside at the Purandar Fort region, District Pune in October, 2008. A voucher specimen is deposited in Botanical Survey of India, Western Circle, Pune (No. SPJ-3). Roots were separated and aerial parts cleaned off adhering dust and unwanted plant material. Cleaned aerial parts were divided into two parts. One part was cut into small pieces and processed for obtaining essential oil. Second part was dried in shade, cut and pulverized.

3.3. Extraction and Isolation:

i) Distillation of essential oil:

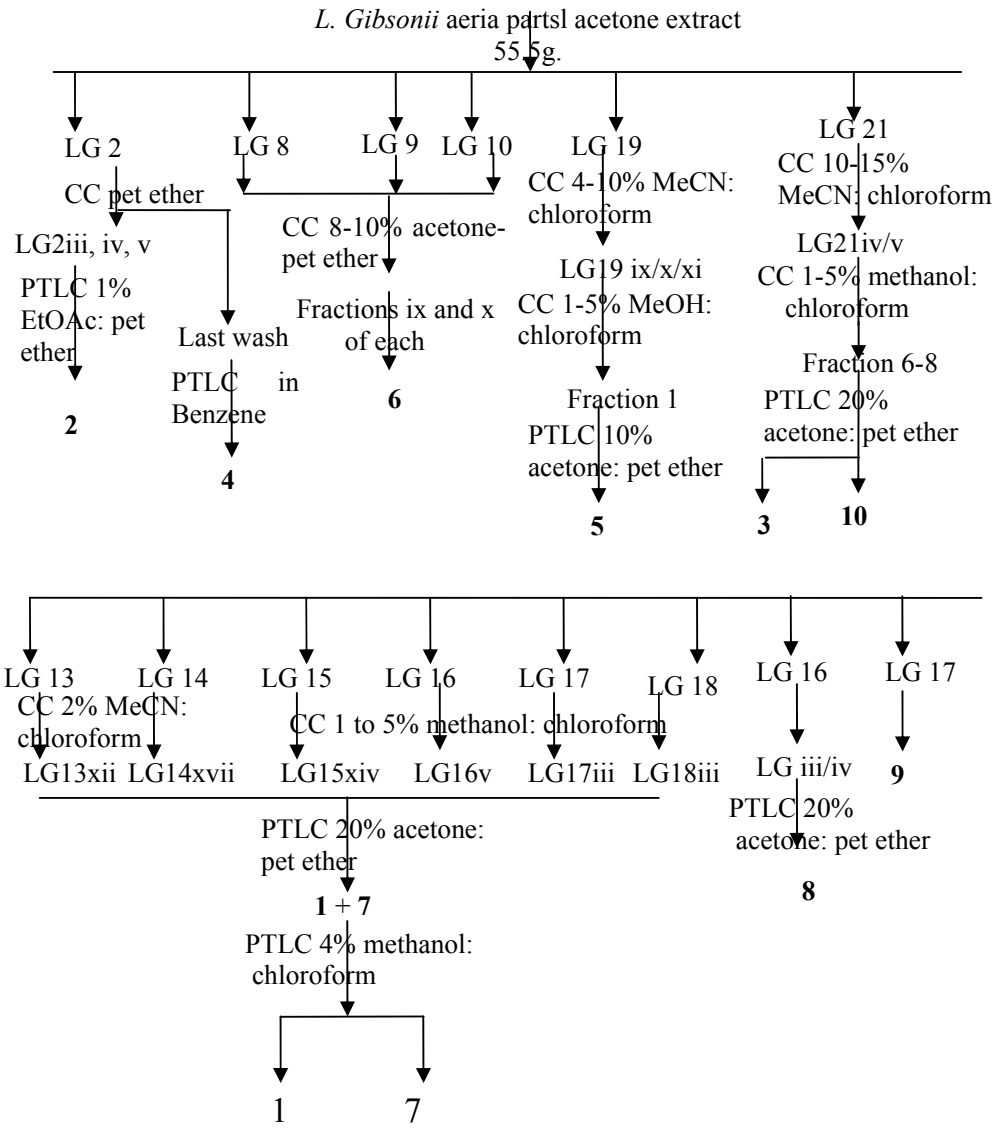
Oil was isolated from fresh aerial parts by hydro-distillation using *Clevenger*-type apparatus with 0.14% yield based on fresh plant material.

ii) Extraction and isolation of compounds from acetone extract:

The pulverized aerial parts of *L. gibsoni* (2.17 kg) were extracted with acetone, (3.0L × 14h × 3), at room temperature. The solvent was evaporated under reduced pressure to yield a dark residue (60.7g, 2.79% on dry plant weight basis) of which 55.5g, was subjected to column chromatography (CC) using acetone gradient in petroleum ether to collect twenty three (LG1-LG23) fractions (Chart 1). Fraction LG2 (1.42 g) was chromatographed on silica gel and eluted with petroleum ether to collect eight fractions (LG2i-viii). Combined fraction LG2iii-v were subjected to preparative TLC using developing system ethyl acetate: petroleum ether (1:9) to isolate compound **2** (7.9 mg). Column washings eluted with acetone gave, on purification by preparative TLC in benzene as developing system gave compound **4** (14mg). Fraction LG8 (538.4mg), fraction LG9 (353.9mg) and fraction LG10 (453.8mg) were subjected separately to CC and eluted with petroleum ether with a gradient of acetone from 8 to 10%. Fractions ix and x from above chromatographic separations were combined and subjected to

preparative TLC in acetone: petroleum ether (15: 85) to obtain compound **6** (5.5mg). Fractions LG13 (844mg), LG14 (548.7mg), LG15 (1.08g), LG16 (456.5mg), LG17(3.15g) and LG18 (1.7g) contained compounds **1** and **7**. LG13 and LG14 were separately subjected to CC using acetonitrile: chloroform (2:8) as eluting system. LG15, LG16, LG17 (after removal of solid by filtration) and LG18 were separately subjected to CC using chloroform with a gradient of methanol from 1 to 5% as eluting system. Fractions LG13xii, LG14xvii, LG15xiv, LG16v, LG17iii and LG18iii from respective columns were combined and subjected to preparative TLC with acetone: petroleum ether (2:8) as developing system to obtain mixture of compounds **1** and **7**. The mixture was separated further by preparative TLC in methanol: chloroform (4: 96) as developing system to isolate pure compounds **1** (30.4mg) and **7**(15mg). Fractions LG16iii and LG16iv contained compound **8** which were combined and subjected to preparative TLC with developing system acetone: petroleum ether (2:8) to obtain pure compound **8** (6.6mg). Solid obtained from LG17 was purified by washing with acetone to obtain compound **9** (110mg) as yellow crystals. Fraction LG19 (1.7g) was subjected to CC using acetonitrile gradient in chloroform from 4 to 10% as mobile phase. Fractions LG19ix, LG19x and LG19xi from this CC were combined (381mg) and subjected to CC and eluted with chloroform with a gradient of methanol from 1 to 5%. Fraction 1 from this chromatography was purified by preparative TLC with developing system acetone: petroleum ether (1:9) to obtain compound **5** (11.9mg) Fraction LG21 (5.0g) was subjected to CC using chloroform with a gradient of acetonitrile from 10 to 15% as mobile phase. Fractions iv and v from this CC were combined (100mg) and again subjected to CC using methanol gradient in chloroform from 1 to 5% as mobile system. Compounds **3**(9 mg) and **10** (47.7 mg) were obtained after purification of combined fractions 6 to 8 of above CC using preparative TLC with developing system acetone: petroleum ether (2:8). Fraction LG23 (4.0g) was separated by CC using methanol gradient 1 to 8% in chloroform as mobile phase. Fractions, LG23vii, viii and ix were combined and compounds **12**(12.0 mg) and **13** (7.0 mg) were separated from it after preparative TLC with developing system 3% methanol in chloroform. Compound **11**(30.0 mg) was isolated from first CC

washings with acetone after successive CCs and preparative TLC in methanol-chloroform.



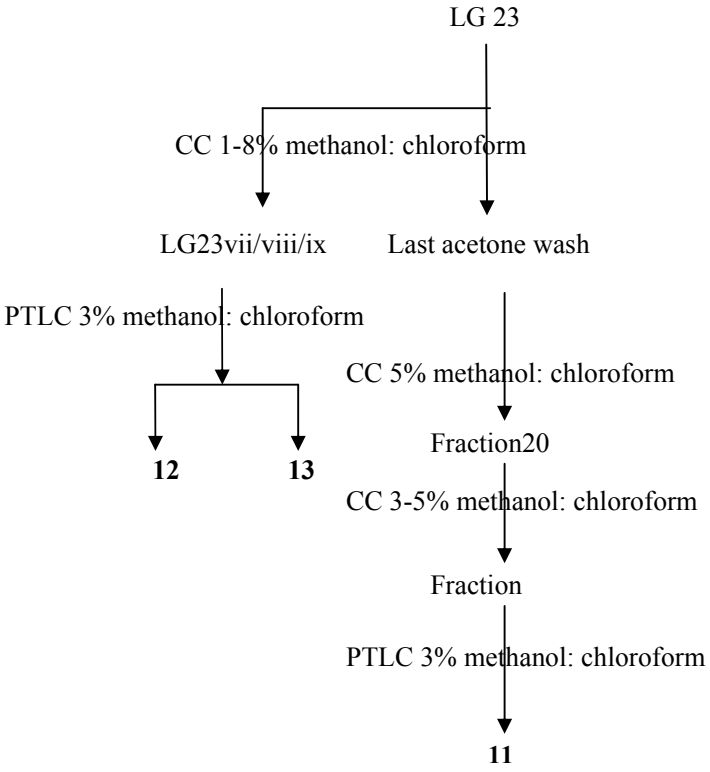
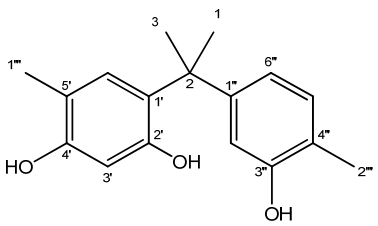
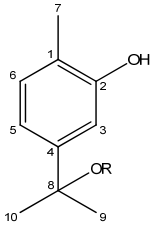


Chart 1: Chromatographic separation of *L. gibsoni*

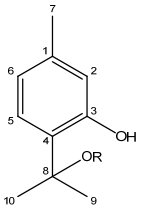


1



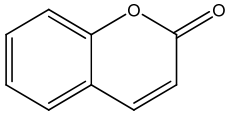
2, **R= ethyl**

3, **R= H**



4, **R= ethyl**

5, **R= H**



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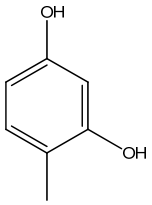

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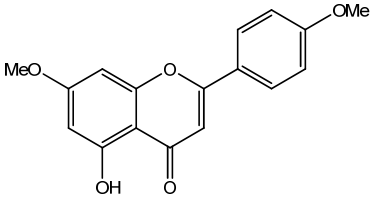

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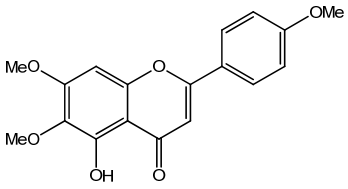

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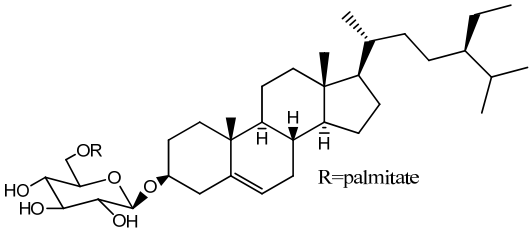

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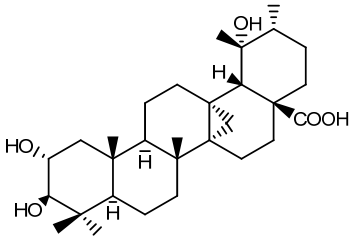

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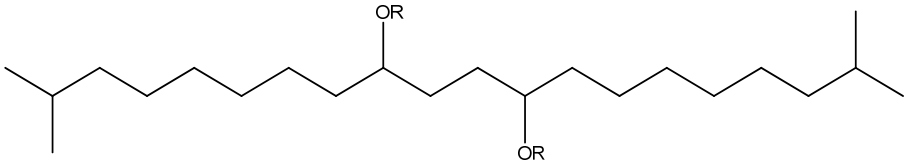


12, **R = H** **13**, **R = OAc**

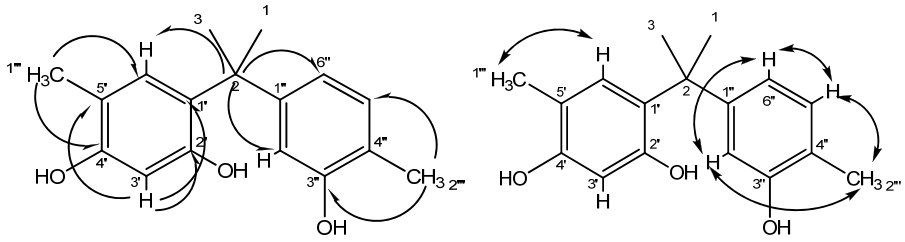
Figure 2: Compounds isolated from *L. gibsoni*

3.4. Structure elucidation:

Compound 1;

Compound **1** was obtained as a light brown gum. The ESIMS of **1** showed an $[M + 1]^+$ at *m/z* 273, $[M + Na]^+$ at *m/z* 295 suggesting the molecular formula $C_{17}H_{20}O_3$ with eight degrees of unsaturation. The HREIMS of **1** showed a molecular ion peak $[M]^+$ at m/z 272.14239 confirming the molecular formula. The IR absorption bands at 3481.20, 1585.14, 1508.05, 1456.70, 1413.21 cm^{-1} indicated presence of hydroxyl group and phenyl ring. The presence of three aromatic protons at δ6.69 (d, J=1.5Hz), 6.87 (dd, J= 7.75, 1.5Hz) and 7.10 (d, J=7.75Hz) in the ^1H NMR spectrum (Table 1) suggested a 1, 2, 4- trisubstituted benzene skeleton. The ^{13}C NMR spectrum (Table 1) showed 17 signals and were identified as four methyls, five methines, and eight quaternary carbons on the basis of the DEPT experiment. Presence of quaternary carbon at δ40.6 and two methyl groups at δ29.79 suggested 2, 2-diphenylpropane skeleton [2]. From the detailed analysis of the COSY, HSQC, and HMBC experiments, structure of compound **1** was assigned as follows:

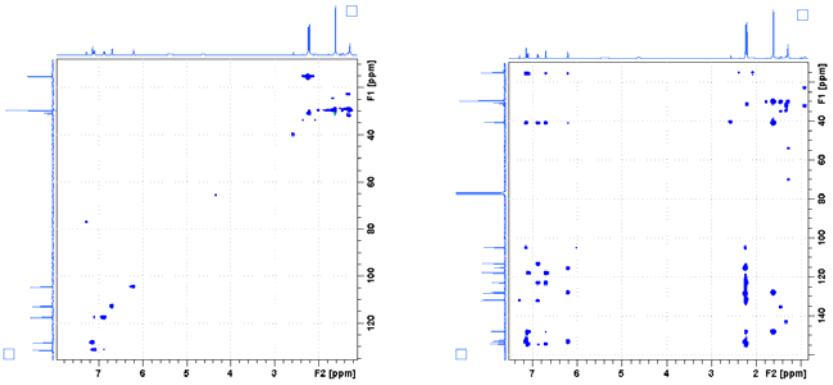
Quaternary carbon at δ40.6 showed three bond correlation with aromatic protons at δ 7.14 (H-6') as well as with δ 6.69 (H-2'') and 6.87 (H- 6''). Methyl at δ 2.23 (C-1''') showed three bond HMBC correlation with carbon at δ 128.34 (C-6') and 153.16 (C-4') (Figure 3). Proton at δ 6.19 (H-3') exhibited three bond correlation with carbon at δ 127.65 (C-1') and 115.21 (C-5') as well as two bond correlation with carbon at δ 152.47 (C-2'). Both carbons at δ152.47 (C-2') and 153.16 (C-4') were correlated with proton at δ 7.14 (H-6') with three bond couplings. This established complete substitution pattern in one ring.



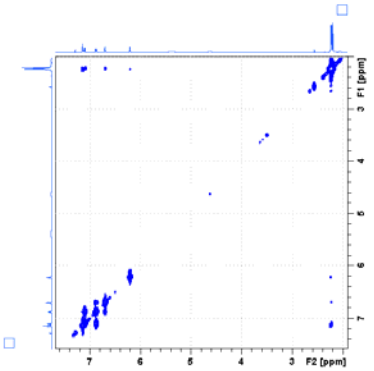
3a. Observed HMBC **3b.** Observed COSY

Figure 3: HMBC and COSY correlations of compound **1**

Substitution pattern on another ring was established as follows. Methyl at δ 2.22 (H-2’’’) showed three bond HMBC correlation with carbons at δ 154.28 (C-3’’) and 131.69(C-5’’) as well two bond correlation with carbon at δ 122.81 (C-4’’). These established substitution pattern in second ring. COSY correlations depicted in Figure2b, additionally confirmed these assignments. Thus compound **1** was isolated as new natural product as [2-(2,4-dihydroxy-5methylphenyl)-2-(3'-hydroxy-4'-methylphenyl)]propane.



4a. HSQC spectrum **4b.** HMBC spectrum



4c. COSY spectrum

Figure 4: 2D spectra of compound **1**.

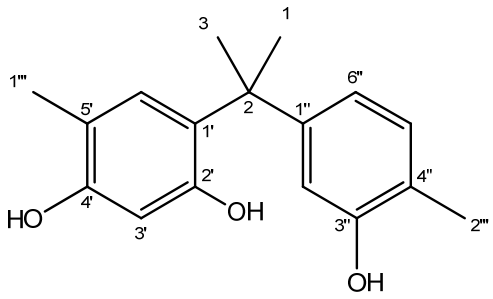
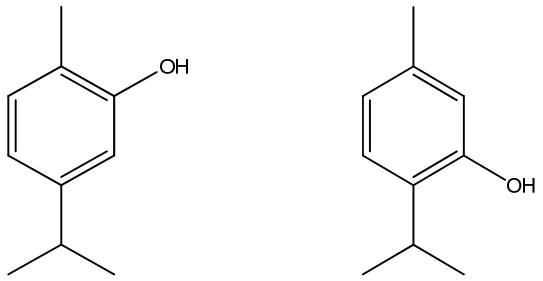


Figure 5: Structure of compound **1**.

Compound **2**:

Compound **2** was isolated as pale yellow gum. The ESIMS of **2** showed an $[M + 1]^+$ at *m/z* 195, $[M + Na]^+$ at *m/z* 217 suggesting the molecular formula $C_{12}H_{18}O_2$ with four degrees of unsaturation probably accounted by one aromatic ring. The HREIMS of **2** showed a molecular ion peak $[M]^+$ at m/z 194.13179 confirming the molecular formula. IR spectrum showed absorption peaks at 3367.17, 1585.14, 1508.05, 1456.70, 1413.21, 1215.74, 756.11, 669.07cm^{-1} indicating presence of hydroxyl group and phenyl ring. The ^1H NMR spectrum (Table 2) exhibited four methyl signals at δ 1.18 (t, J= 7.15Hz, H-2'), 1.55 (s, H-9), 1.55 (s, H-10) and 2.26 (s, H-7). The ^1H NMR spectrum also exhibited three aromatic proton signals δ 7.06 (bs, H-3), 6.85 (dd, J= 7.75, 1.5Hz, H-5) and 7.09 (d, J=7.75Hz, H-6) suggesting a 1, 2, 4- trisubstituted benzene pattern. Presence of one methyl at δ1.18 and methylene at δ3.28 (q, J= 7.15Hz, H-1') suggested an ethoxyl group. The ^{13}C NMR spectrum (Table 2) showed 12 signals and were identified as four methyls, one methylene, three methines, and four quaternary carbons on the basis of the DEPT experiment. Presence of quaternary carbon at δ 76.80 and two methyl groups at δ28.40 suggested 2-alkoxy-2-propyl substitution on phenyl ring. Presence of ethoxyl group on substituent rather than on ring was deduced from presence of one phenolic hydroxyl proton at δ5.79 as well as from HMBC correlation of methylene protons at δ3.28 with quaternary carbon at δ76.80 as shown in Figure 7a. Above data along with biogenetic considerations led to two possible structures corresponding to carvacrol (a) and thymol (b) skeletons respectively (Figure 6).

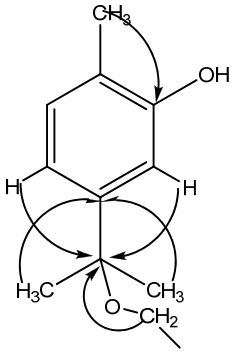


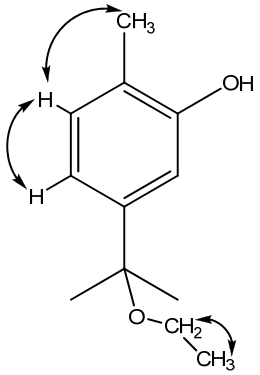
a **b**

Figure 6: Structure of carvacrol (**a**) and thymol (**b**)

Methyl group at δ 2.26 showed three bond HMBC correlation with quaternary carbon at δ154.08 (C-2). Methine protons at δ7.06 (H-3) and δ 6.85

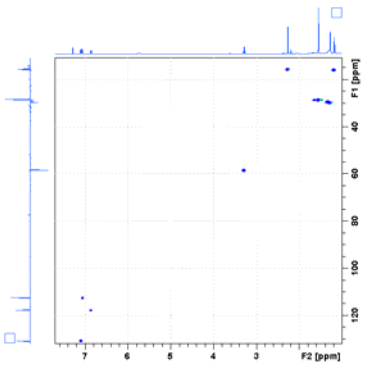
(H-5) showed HMBC correlation with quaternary carbon at δ 76.80 (C-8) (Figure 7a). These two sets of correlations confirmed the carvacrol pattern for compound **2**. COSY correlations shown in Figure 7b confirmed these above assignments. Thus compound **2** was identified as new natural product, 3-(2-ethoxypropan-2-yl)-6-methylphenol or 8-ethoxycarvacrol. No ethanol was used at any stage of processing hence compounds **2** and **4** are in all probability not artifacts and are natural products.

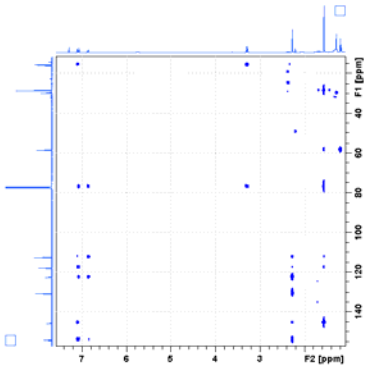




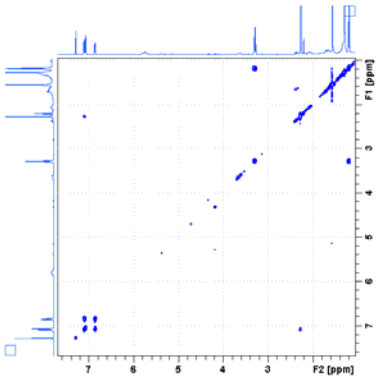
7a. Observed HMBC **7b.** Observed COSY

Figure 7: HMBC correlations for compound **2**





8a. HSQC spectrum **8b.** HMBC spectrum



8c. COSY spectrum

Figure 8: 2D spectra of compound **2**.

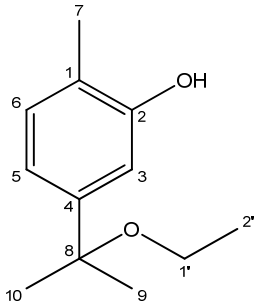


Figure 9: Structure of compound **2**. (Numbering is as per convention and not as found in IUPAC nomenclature.)

Compound 4:

Compound **4** was isolated as brown gum. The ESIMS spectrum of **4** showed a molecular ion peak $[M + 1]^+$ at m/z 195 along with $[M+Na]^+$ peak at 217 and $[M+K]^+$ peak at 233 in agreement with molecular formula $C_{12}H_{18}O_2$ indicating four degrees of unsaturation corresponding to one phenyl ring. ^{1}H NMR spectrum (Table 2) showed signals very similar to compound **2** except for change of positions in aromatic region indicating different substitution pattern. The three aromatic proton signals resonated at δ 7.12 (d, 1.51Hz, H-2), 6.835 (dd, 7.72, 1.5Hz, H-6) and 7.09 (d, 7.72Hz, H-5). ^{13}C NMR spectrum (Table 2) also showed signals very similar to molecule **2** except for interchange of resonance of C- 2 and C-3 as well as C-5 and C-6. This along with the biogenetic considerations confirmed the structure of **4** corresponding to thymol skeleton (Figure 6b) and was identified as new natural product 2-(2-ethoxypropan-2-yl)-5-methylphenol or 8-ethoxythymol.

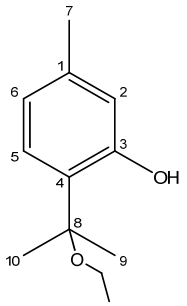


Figure 10: Structure of compound **4**. (Numbering is as per convention and not as found in IUPAC nomenclature.)

Compound **5**:

Compound **5** was isolated as pale yellow gum. The HREIMS of **5** showed a molecular ion peak $[M]^+$ at m/z 166.09957 in agreement with the molecular formula $C_{10}H_{14}O_2$ indicating four degrees of unsaturation corresponding to one phenyl ring. IR spectrum showed absorption peaks at 3609.47, 1585.57, 1522.14, 1457.01, 1414.68 cm^{-1} indicating presence of hydroxyl group and phenyl ring. ^1H NMR and ^{13}C NMR spectra of compound **5** were very similar to those of compound **4** except for absence of ethyl group (Table 2). This indicated similar substitution pattern in aromatic region and ethoxyl group is substituted by hydroxyl group. From this data, compound **5** was identified as 2-(2-hydroxypropan-2-yl)-5-methylphenol or 8-hydroxythymol.

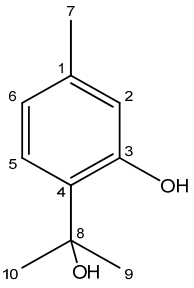


Figure 11: Structure of compound **5**. (Numbering is as per convention and not as found in IUPAC nomenclature.)

Compound 3:

Compound **3** was isolated as brown gum. The ESIMS of **3** showed a molecular ion peak $[M + 1]^+$ at m/z 167 in agreement with the molecular formula $C_{10}H_{14}O_2$ indicating four degrees of unsaturation corresponding to one aromatic

ring. ^{1}H NMR and ^{13}C NMR spectra of compound **3** were very similar to those of compound **2** except for absence of ethyl group (Table 2). This indicated similar substitution pattern in aromatic region and ethoxyl group is replaced by hydroxyl group. Thus compound **3** was identified as 5-(2-ethoxypropan-2-yl)-2-methylphenol or 8-hydroxycarvacrol. It has been isolated earlier from *L. gibsoni* [3].

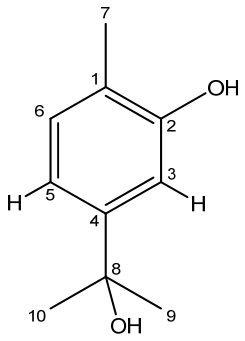


Figure 12: Structure of compound **3**. (Numbering is as per convention and not as found in IUPAC nomenclature.)

Compound **6**:

Compound **6** was isolated as brown gum. ^{13}C NMR spectrum showed 9 signals. Resonances at δ 160.81, 143.43 and 116.70 indicated α, β-unsaturated ester (Table 3). Compound **6** was identifies as coumarin by comparison of its NMR spectra with that of authentic sample available with us as well as with literature [4]. Coumarin has earlier been reported from other *Lavandula* species [5].

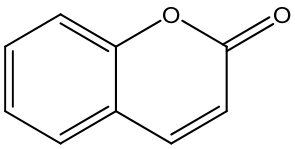


Figure 13: Structure of compound **6**.

Compound **7**:

Compound **7** was isolated as brown amorphous powder. The ESIMS of **7** showed a molecular ion peak $[M + Na]^{+}$ at m/z 147 in agreement with the molecular formula $C_7H_8O_2$ indicating four degrees of unsaturation corresponding

to one aromatic ring. ^{13}C NMR spectrum showed 7 signals which were identified as one methyl, three methines and three quaternary carbons by DEPT experiment. Resonances at δ154.74 and 154.55 indicated resorcinol skeleton for compound **7** as ortho or para hydroxyl substituted carbons resonate upfield due to mutual shield by resonance. Upfield shifted methyl resonance at δ 14.88 indicated its proximity (ortho substitution) with one of the hydroxyl group. Protons at δ 6.97 (d, 8Hz), 6.36 (d, 8 Hz) and 6.35 (bs) indicative of 1, 2, 4-trisubstitution supported resorcinol based structure. Thus compound **7** was identified as 4-methylresorcinol. The assignments were supported by comparison of its spectra with that of 4-methylcatechol [6] and methylhydroquinone [7] as well as with 4-ethylresorcinol [8]. This is probably for the first time that 4-methyresorcinol is reported as natural product.

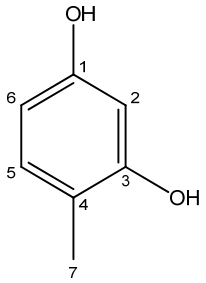


Figure 14: Structure of compound **7**.

Compound **8**:

Compound **8** was isolated as yellow amorphous powder. The ESIMS of **8** showed a molecular ion peak $[M + 1]^+$ at m/z 299 in agreement with the molecular formula $C_{17}H_{14}O_5$ indicating eleven degrees of unsaturation. Examination of its ^{13}C spectrum indicated it to be a flavonoid. Presence of carbonyl at δ182.46 and hydroxyl proton at δ12.79 indicated 5-hydroxyflavone.Three methine carbons at δ 104.34, 98.1 and 92.60 , two methyls at δ 55.53, 55.79 and two doublets in ^1H at δ 6.98 (2H, d, 8.0Hz), 7.81 (2H, d, 8.0 Hz) corresponding to A_2B_2 quartate of ring B, afforded identification of compound **8** as 7, 4'-dimethoxyapigenin [9]. It is probably reported for first time from genus *Lavandula*.

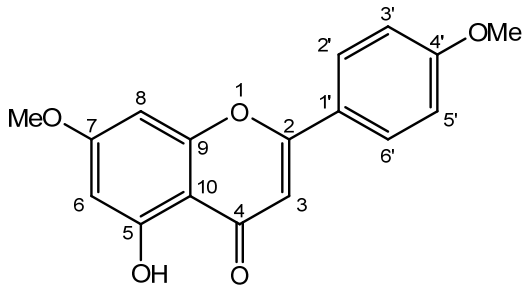


Figure 15: Structure of compound **8**.

Compound **9**:

Compound **9** was isolated as yellow crystals. The ESIMS of **9** showed a molecular ion peak $[M + 1]^+$ at m/z 329 in agreement with the molecular formula $C_{18}H_{16}O_6$ indicating eleven degrees of unsaturation. Examination of its ^{13}C spectrum indicated it to be a flavonoid. Presence of carbonyl at δ182.53 and hydroxyl proton at δ12.78 indicated 5-hydroxyflavone.Two methine carbons at δ103.89 and 90.47 indicated a flavone with substitution at positions either at 3 or 6 or 8, most probably at 6. One of the three methyl resonated at δ60.73, thus confirming methoxyl substitution at position 6 as methyls at 6 methoxyl position in flavones resonate downfield from other methyls at around δ60. Rest two methyls at δ55.44, 56.21 and two doublets in ^1H spectrum at δ 6.99 (d, 9.03 Hz, 2H), 7.80 (d, 9.03 Hz, 2H) afforded identification of compound **9** as 6, 7, 4'-dimethoxyapigenin or salvigenin [10]. It has previously been isolated from *Lavandula* species [11].

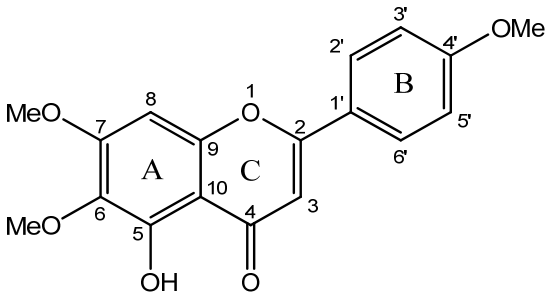


Figure 16: Structure of compound **9**.

Compound **10**:

Compound **10** was isolated as a white amorphous powder. Its ^{13}C NMR spectrum revealed presence of one sugar moiety, signals indicative of a steroid and a fatty acid linkage. Presence of one olefinic proton at δ 5.36 and six methyl

resonances at δ 0.69(3H, s, H- 18), 0.83 (3H, d, *J* = 6.9 Hz, H-26), 0.85 (3H, d, *J* = 6.81 Hz, H-27), 0.89 (3H, t, *J* = 7.4 Hz, H-29), 0.94 (3H, d, *J* = 6.14Hz, H-21) and 1.02 (3H, s, H-19), indicated steroid to be sitosterol. Methylene at δ63.24 in sugar region of ^{13}C NMR spectrum (Table 6) were indicative of glucose with esterification at C-6'. Thus the compound was suspected to be sitosterol glucoside with fatty acid esterified C-6'. ESIMS gave $[M+ 1]^+$ at m/z 815 corresponding to palmitic acid ester. Thus compound **10** was identified as Sitosteryl-6-β-D-glucoside-6'-palmitate after comparison of its NMR spectra with Sitosteryl-6-β-D-glucoside recorded in $CDCl_3$-CD_3OD mixture (isolated from *Anisomeles heyneana*, compound **3**, Chapter 4), reference Sigma-Aldrich spectra for palmitic acid [12] as well as with that of sitosteryl-6-β-D-glucoside-6'-palmitate [13]. It probably is for the first time been isolated from family Lamiaceae.

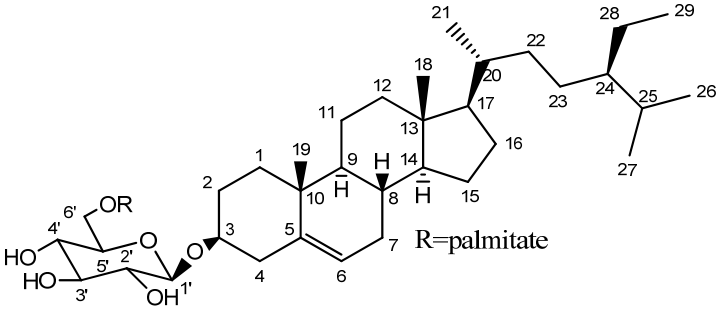


Figure 17: Structure of compound **10**.

Compound **11**:

Compound **11** was isolated as a white amorphous powder. Its ^{13}C NMR spectrum revealed presence of 30 resonances with 6 methyls, 10 methylenes, 6 methines and 8 quaternary carbons. ESIMS gave $[M + Na]^+$ at 509 corresponding to formula $C_{30}H_{48}O_5$ with seven degrees of unsaturation. Carbonyl carbon δ 182.21 and six methyls (Table 7) indicated a pentacyclic triterpene acid with one of the methyl converted to methylene forming a cylopropane ring. Carbons at δ68.6 and 83.8 were indicative of 2α, 3β-diol. Literature survey identified compound **11** as euscaphic acid D [14]. It has been earlier isolated from *Euscaphys japonica* (Family Staphyleaceae) [14] and this is its first report from family Lamiaceae.

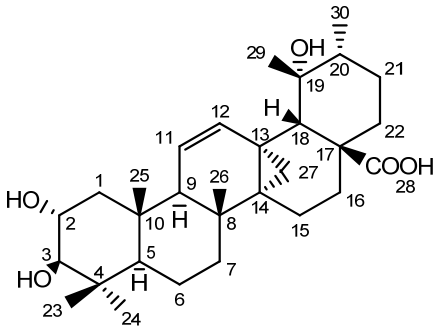
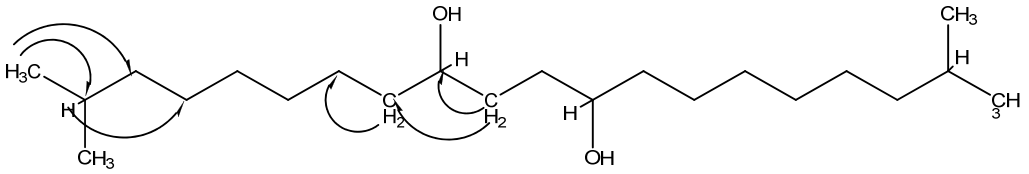


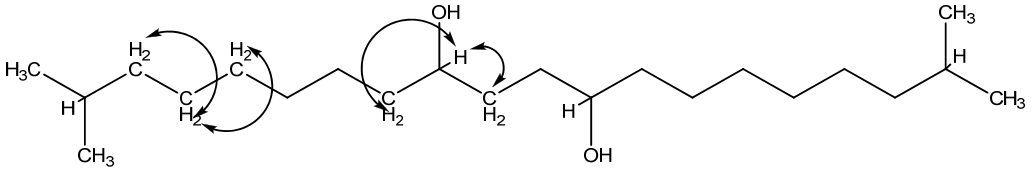
Figure 18: Structure of compound **11**.

Compound **12**:

Compound **12** was isolated as brown amorphous powder, soluble to some extend in both polar and non-polar solvents. ESIMS gave peaks at 343$[M + 1]^+$ and 365 $[M + Na]^+$ corresponding to molecular weight 342 and formula $C_{22}H_{46}O_2$. Upon examination of ^1H NMR spectrum (Table 8) resonances at δ0.87 (d, 6.63Hz, 3H), 0.87 (d, 6.63Hz, 3H) and 1.53 (sep, d 6.63Hz, 1H) revealed presence of isopropyl group Methine at δ 4.05 (bs, 1H) methylene protons at δ 2.58 (d, 16.08Hz, 1H), 2.48 (dd, 16.08, 8.70 Hz, 1H) corresponding to carbon at δ41.04 and rest of the resonances indicated a fatty compound with isopropyl group and methine attached to hydroxyl group. Lack of quaternary carbons and any other methine in ^{13}C spectrum and methylenes at δ 41.04, 39.04 and 36.57 hinted at a symmetrical dimer with 1,4-substituted diols. Literature search [15] gave similar synthetically prepared unbranched straight chain compounds with similar ^{13}C NMR spectra. In COSY spectrum correlation was observed between 4.05 and H8/8' and H10/10' while HMBC confirmed the end connectivities. Thus compound **12** was identified as 2,19-dimethylcosane-9,12-diol. Stereochemistry at both the methines is undetermined as compound with opposite configuration at both carbons have internal plane of symmetry while one with same configuration has S_2 axis of symmetry. In both cases, one set of NMR signals is expected. Thus compound **12** was identified as new natural product. Further confirmation by HRMS is underway.

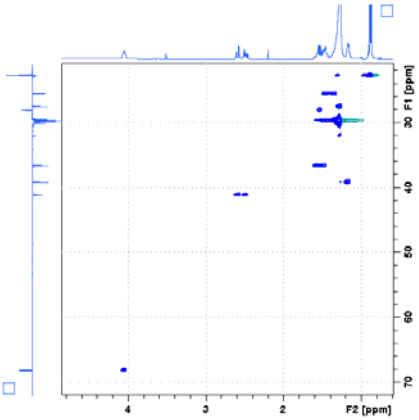


19a. Observed HMBC

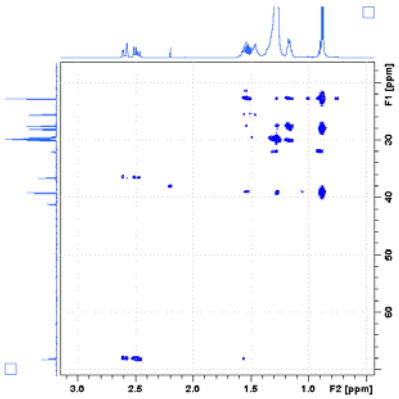


19b. Observed COSY

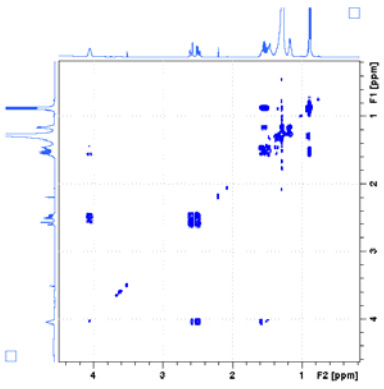
Figure 19: 2D correlations for compound **12**.



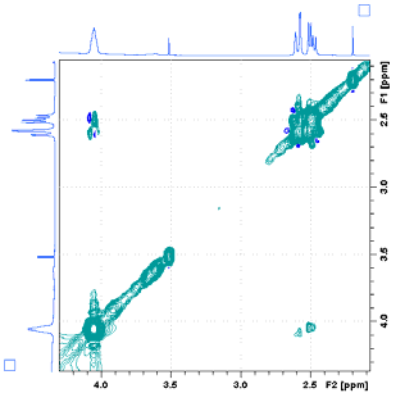
20a. HSQC



20b. HMBC



20c. COSY



20d. NOESY

Figure 20: 2D Spectra of compound **12**.

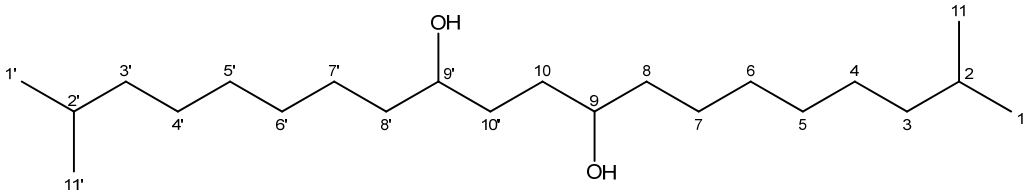


Figure 21: Structure of compound **12**.

Compound **13**:

Compound **13** was isolated as greenish gum insoluble in methanol. Its ^{1}H and ^{13}C NMR spectra (Table 8) were very similar to compound **12** with methine shifted downfield to δ 5.23 and methine at δ1.53 separated from clutter due to downfield shifting of H8/8' protons. There was additional methyl at δ2.24 (s) and carbonyl at δ170.59 indicative of acetylation of compound **12**. Thus compound **13** was identified as new natural product 2, 19-dimethylcosane-9, 12-diacetate. Further confirmation by HRMS and 2D NMR spectroscopy is underway.

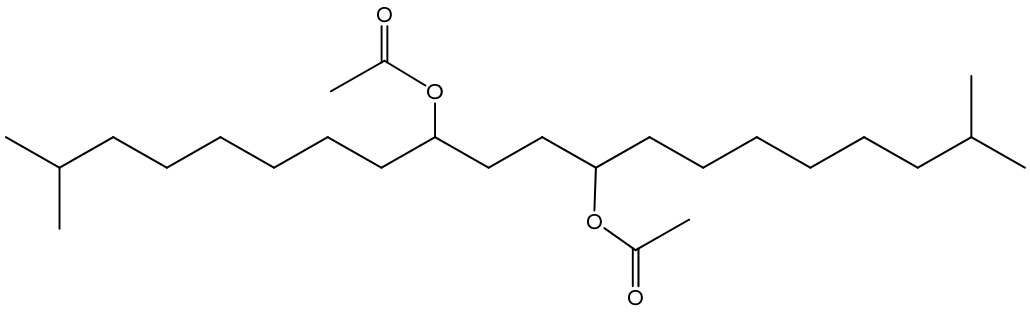


Figure 22: Structure of compound **13**.

3.5. Experimental:

A. Collection and processing:

As described earlier.

B. Extraction and Isolation:

As described earlier.

Compound **1**

Light brown gum (30.4 mg, 0.0014 % based on dry weight basis), IR (CHCl3) 3481, 1584, 1499, 1462, 1415, 1215, cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Table 1; HREIMS m/z: 272.14239 $[M]^{+}$ (calculated for 272.14126).

Compound **2**

Gum (7.9 mg, 0.00036%), IR (chloroform) 3367, 1585, 1508, 1456, 1413, 1215, cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Table 2; HREIMS m/z: 194.13179 [M]+ (calculated for 194.13069).

Compound **3**

Gum (9 mg, 0.00041%), ^{1}H and ^{13}C NMR spectral data, see Table 2.

Compound **4**

Gum (14 mg, 0.00065%), IR (chloroform) 3609.47, 1585.57, 1522.14, 1457.01, 1414.68, 1215.81, 758.43, 669.15cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Table 2; HREIMS m/z: 166.09957 $[M]^{+}$ (calculated for 166.09939).

Compound **5**

Gum (11.9mg, 0.00055%); HREIMS m/z: 166.09957 $[M]^{+}$ (calculated for 166.09939); IR (chloroform) 3610, 1452, 1418, 1210,cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Table 2;

Compound **6**

Gum (5.5mg, 0.00025%); ESIMS 147 $[M + 1]^{+}$, 169 $[M + Na]^{+}$; IR (chloroform), 1734, 1672, 1622, 1565, 1453, 1399cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Table 3;

Compound **7**

Gum (15.0mg, 0.00069%); ESIMS 147 $[M + Na]^{+}$; IR (chloroform); ^{1}H and ^{13}C NMR spectral data, see Table 4;

Compound **8**:

Yellow amorphous powder (6.6mg, 0.00030%); ESIMS, 299 $[M + 1]^+$, 316 $[M + NH_4]^+$, 321 $[M + Na]^+$; ^1H and ^{13}C NMR spectral data, see Table 5;

Compound **9**

Yellow crystals (110 mg, 0.0051%); ESIMS, 329 $[M + 1]^+$, 351 $[M + Na]^+$;^1H and ^{13}C NMR spectral data, see Table 5;

Compound **10**

White amorphous powder(47.7 mg, 0.0022%); ESIMS m/z:, 815 $[M + 1]^+$, 837 $[M + Na]^+$; ^1H NMR, δ, 0.69(3H, s, H- 18), 0.83 (3H, d, *J* = 6.9 Hz, H-26), 0.85 (3H, d, *J* = 6.81 Hz, H-27), 0.89 (3H, t, *J* = 7.4 Hz, H-29), 0.94 (3H, d, *J* = 6.14Hz, H-21), 1.02 (3H, s, H-19), 3.35-3.67 (5H, m), 4.26-4.5 (3H, m), 5.36 (2H, m, H3 and H1'); for ^{13}C NMR spectral data, see Table 6.

Compound **11**

White amorphous powder (30mg, 0.0014%); ESIMS, 509 $[M + Na]^+$; ^1H NMR, 0.87(3H, s, H- 25), 0.89 (3H, d, *J* = 6.78 Hz, H-30), 0.94 (3H, s, H-24), 0.99 (3H, 3, H-26), 1.29 (3H, s, H-23), 1.36 (3H, s, H-29), 3.66 (1H, m, H3), 5.36 (1H, dd, 10.29, 2.26Hz, H11), 5.84 (1H, dd, 10.29, 2.76Hz, H12); for ^{13}C NMR spectral data, see Table 7;

Compound **12**

Green amorphous powder (12 mg, 0.00055%); ESIMS m/z, 343$[M + 1]^+$, 365$[M + Na]^+$; ^1H and ^{13}C NMR spectral data, see Table 9.

Compound **13**

Green gum (7mg, 0.00032%), ^1H and ^{13}C NMR spectral data, see Table 9;

3.6 Analysis of essential oil of *L.gibsoni*:

Mosquitoes are major vectors for the transmission of malaria, filariasis, dengue fever, yellow fever, and several viral diseases [16, 17]. More than two billion people, mostly in tropical countries, are at risk from mosquito-borne diseases, such as malaria, dengue, haemorrhagic fever and filariasis [18]. Personal protective measures, including repellents, are widely used to prevent the transmission of mosquito-borne diseases by minimizing the contact between humans and vectors [19]. Recently, the environmental friendly and biodegradable

natural insecticides of plant origin have been receiving attention as an alternative green measure of control of arthropods of public health importance [20].

Various plant extracts or phytochemicals have been focused as potential sources of commercial mosquito-control agents or as lead compounds [21, 22]. Essential oils obtained from several plant species have been extensively tested to assess their repellent and even insecticidal properties as a valuable natural resource [23].

There is no work reported on the chemistry and biological activity of essential oil of *L. gibsoni*.

Distillation of essential oil:

It is described earlier.

GC analysis of essential oil:

The GC-FID analysis of the essential oil was carried out with an Varian CP 3800 apparatus equipped with FID and a GsBP5 capillary column (30 m length, 0.25 mm i.d., film thickness 0.25 mm). Oven temperature was programmed rising from 50 to 260^0 at 3^0/min hold at 260^0C for 5 min; injector temperature, 250^0C; detector temperature, 300^0C; carrier gas, He (1.0 ml/min); injection volume, 1 µl; split ratio, 6:4 (Figure 23). The Linear Retention Indices (LRIs) of the constituents reported in Table 9 were determined relative to the retention times of a series of n-alkanes (C9–C38), and the relative percentages of the individual components of the oils were obtained from the GC-FID peak-area percentages after applying correction factors.

GC/MS Analysis of essential oil:

The GC/MS analysis of essential oil was performed with an Perkin Elmer Clarus 500 gas chromatograph coupled to a Perkin Elmer Clarus 500 quadruple mass spectrometer equipped with a GsBP5 capillary Column (30 m length, 0.25 mm i.d., film thickness 0.25 mm). Oven temperature was programmed rising from 50 to 280^0C at 5^0C/min with hold at 50^0C for 1min and at 280^0C for 10min.; injector temperature, 280^0C; detector temperature, 300^0C; carrier gas, He (1.0 ml/min); injection volume, 1 µl; mass spectra, positive electron impact mode at 70 eV (Figure 24)

Identification of oil constituents:

The identification of the individual compounds of the oil was based on the comparison of their LRI and mass spectra with those of authentic compounds by means of the NBS and NIST databases and published data on http://www.webbook.nist.gov/.

Mosquito larvicidal assay of essential oil and acetone extract:

Standard WHO method of testing the susceptibility of mosquito larvae to insecticides [33] was followed in all the experiments with slight modification. Larvicidal assay was carried on larvae of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*. Test samples (acetone extract and essential oil) were dissolved in analar grade acetone to prepare stock solutions. Ten early 4^{th} instar mosquito larvae were introduced in 100ml glass beaker containing 50ml water. A known volume of stock solution was added in beaker to get various concentrations of extract and oil. Acetone control was run simultaneously. For each concentration and control, 5 replicates were used and each test was repeated three times. The beakers were kept at 26 $\pm 2^0$C and mortality of larvaewas recorded after 24h. The corrected mortality was analyzed using Abbott's formula wherever required. The mortality were analyzed by log probit method and Lethal Concentration (LC_{50} & LC_{95}) were calculated (Tables 10-12).

Mosquito repellency assay:

Mosquito repellent activity was assessed [34] on the basis of the protection period offered by repellent test sample. For the study, 4-6 days old, blood starved, sucrose fed (0.5M solution) females of *A. aegypti* were taken.

Human hand covered with snugly fitting glove was introduced in the cage containing 100 hungry females. Mosquitoes were allowed to bite on the back of the hand through muslin cloth screen stuck over a small window (2cm X 2cm) cut out in the glove. Test samples (acetone extract, essential oil and standard compounds) were loaded on the muslin cloth screen at concentrations, 0.5, 1.0 and 2.0mg/cm^2. Control muslin cloth screen was treated with solvent alone. After introduction of the hand covered with the glove with treated muslin screen into the mosquito cage, number of bites received in subsequent 5min were counted. In the event of no bites in the initial 5min exposure, the test hand was exposed

repeatedly after every consecutive 30min for 5min test till the time a confirmed bite was received. Protection period was recorded as the time elapsed between repellent application and the time at which a confirmed bite was observed. A control arm was placed in the cage randomly before or after the treated arm to asses mosquitoes bite (Tables 13a-13d).

GC-MS analysis of essential oil of *L. gibsonii*

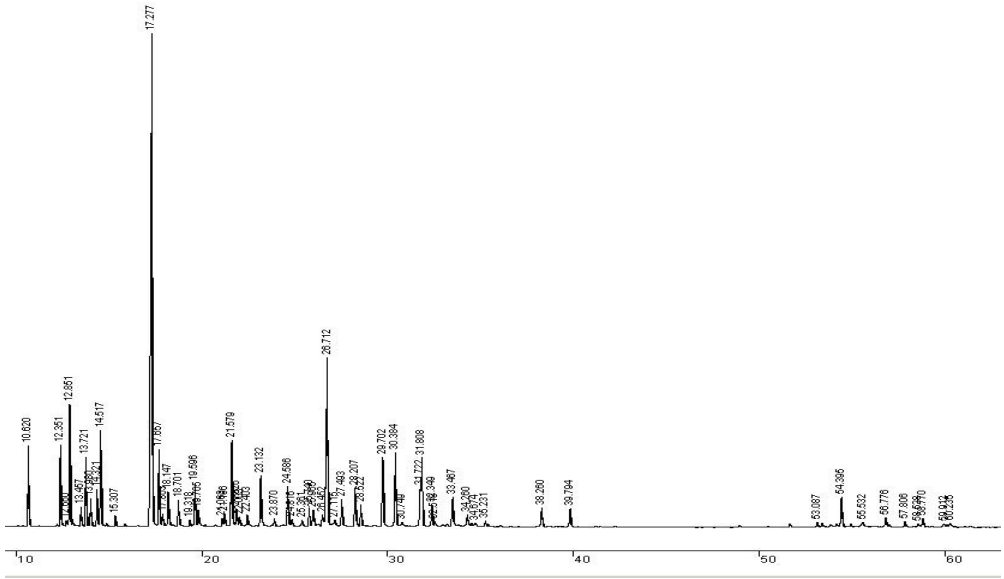


Figure 23: GC-FID of *L. gibsoni* essential oil

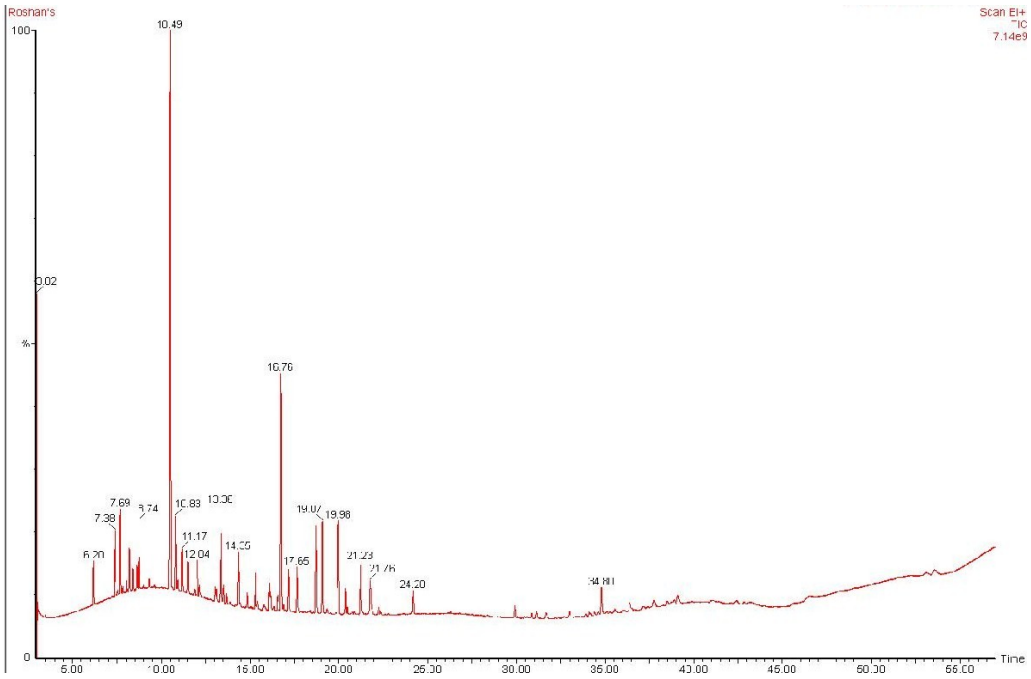


Figure 24: GC-MS of *L. gibsoni* essential oil

Results and Discussion.

Analysis of Essential Oils: The oil was obtained by hydrodistillation and characterized using GC-FID and GC/MS analyses. The identified oil components with their relative contents are reported in Table 9. The essential oil contained α-terpinolen (22.22%), thymol (10.42%) and benzenemethanol, 4-(1-methylethyl) (4.52%) as main components and to a lesser extent β-myrcene, linalool, limonene, 1-octen-3-ol. Reported chemical compositions of essential oil of genus *Lavandula* is found to have linalool, lanalyl acetate, 1,8-cineol, camphor, fenchol, fencone, borneol, terpinen-4-ol, β-pinene, phenylacetaldehyde, α-phellandrene and β-phellandrene as major constituents. For example, oil of *L. angustifolia* was found to contain linalool (50.07%), camphor (12.52% and 1,8-cineol (7.19%) as major constituents [24] while *L. spica* revealed linalool (44.5%), 1,8-cineol (11.0%), borneol (10.9%) and terpinen-4-ol (9.8.5) as main components [25]. 1,8-cineol (48.0%) is the major component of essential oil of *L. dentata* with β-pnene (6.0%) as minor constituent [26]. Oil of *L. stoechas* ssp. *stoechas* had high total content of fenchone and camphor (51-83%) with varying respective ratios based on region [27]. The three oil samples isolated separately from flowering tops, leaves and stems from *L. pinnata* consisted mainly of β-phellandrene (12-32%) and α-phellandrene (6-l6%) as the second most important monoterpene while leaf oil contained phenylacetaldehyde (6-9%) [28].

Mosquito larvicidal activity: The essential was evaluated for larvicidal activity against *A. stephensi, A. aegypti* and *C. quinquefasciatus* larvae at six concentrations ranging from 25 to 150ppm. Essential oil at 150ppm exhibited 100% toxicity for all the three species (Table 10). The acetone extract exhibited 100% toxicity at 250ppm (Table 11). LC_{50} values for essential oil were found to be 48.71, 57.29 and 60.49ppm respectively while LC_{95} values 134.91, 143.58 and 147.49 ppm respectively. However for acetone extract LC_{50} values obtained were 123.16, 135.14 and 144.49 ppm respectively while LC_{95} values 373.19, 320.51 and 350.58 respectively (Table 4). α- terpinolen is reported to show mortality to 4^{th} instar larvae of *A. aegypty* (LC_{50} 28.4µg/ml) and thymol against *C. pipiens* (LC_{50} 37.95µg/ml) [29]. Essential oil of *L. officinalis* exhibited mortality to *A. stephensii* (LC_{50} 83.6ppm) [30] and *L. angustifolia* against *A. albopictus* (LC_{50} >

250ppm). Essential oil composition of the later oil was fenchone 33.9% camphor (13.8%), camphene (13.7%), α-pinene (6.8%), bornyl acetate (5.3%) and limonene (4.4%) [31]. Essential oil of *L. stoechas* exhibited mortality to *C. pipiens molestus* (LC_{50} 89.0ppm) [32].

Mosquito repellent activity: The essential oil was evaluated for repellent activity against *A. aegypti* at 0.5, 1.0 and 2.0mg/cm^2 concentrations (Table 5). Activity at 2.0mg/cm^2 was found to be comparable with standard repellent *N*,*N*-Diethyl-*meta*-toluamide (DEET) (Table 13). Since oil showed α- terpinolen and thymol in around 2:1 ratio, separate study was undertaken to study repellent activity of both these compounds as such as well as 2:1 mixture at concentrations found in oil (Table 13). The results demonstrated that the mixture accounted for 75% activity of the oil.

Conclusion: The results demonstrate promising larvicidal activity of the essential oil and acetone extract against three species of mosquito. The oil was found to be more potent. Essential oil also exhibited potent repellent activity against *A. aegypti* at concentration comparable to standard repellent DEET. Essential oil was found to contain α- terpinolen and thymol as major components which were shown to be accounting for around 75% of repellency, rest being contributed by other minor members. This study provides the first characterization of the essential oils of *L. gibsoni* with chemical composition significantly different than those for essential oils of other *Lavandula* species.

3.7. Tables:

Table 1: ^{1}H and ^{13}C NMR spectral data of compound **1** in $CDCl_3$.

No.	^{13}C (δ)	^{1}H (δ)
1	29.79	1.60 (s)
2	40.6	-
3	29.79	1.60 (s)
1’	127.65	-
2’	152.47	-
3’	104.63	6.19 (bs)
4’	153.16	-
5’	115.21	-
6’	128.34	7.14 (bs)
1’’	147.87	-
2’’	112.98	6.69 (d, *J*= 1.5 Hz)
3’’	154.28	-
4’’	122.81	-
5’’	131.69	7.10 (d, *J*= 7.75 Hz)
6’’	117.70	6.87 (dd, *J*= 7.75, 1.50 Hz)
1’’’	15.34	2.23 (s)
2’’’	15.44	2.22(s)

Table 2: ^{1}H and ^{13}C NMR spectral data of compounds **2**, **3, 4** and **5** in $CDCl_3$.

	2		**3**		**4**		**5**	
No.	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
1	122.54	-	122.29	-	122.54	-	122.61	-
2	154.08	-	153.88	-	112.35	7.125 (d, *J*= 1.51Hz)	111.39	7.08 (bs)
3	112.40	7.06 (bs)	111.33	7.045(d, 1.01Hz)	154.26	-	154.00	-
4	145.70	-	148.40	-	145.38	-	148.11	-
5	117.79	6.85 (dd, *J*= 7.75, 1.5 Hz)	116.36	6.89 (dd, *J*= 7.75, 1.01Hz)	130.62	7.09 (d, *J*= 7.72Hz)	130.69	7.07 (d, *J*= 7.77Hz)
6	130.62	7.09 (d, *J*= 7.75Hz)	130.73	7.09 (d, *J*= 7.75Hz)	117.52	6.835 (dd, *J*= 7.72, 1.51 Hz)	116.18	6.855 (dd, *J*= 7.77 Hz, 1.76 Hz)
7	15.41	2.26	15.36	2.24	15.47	2.27	15.40	2.23
8	76.80	-	72.64	-	77.07	-	72.94	-
9	28.40	1.55	31.57	1.57	28.37	1.56	31.42	1.56
10	28.40	1.55	31.57	1.57	28.37	1.56	31.42	1.56
1’	58.32	3.28 (q, *J*= 7.15Hz)	-	-	58.43	3.30 (q, *J*= 7.21Hz)	-	-
2’	15.72	1.18 (t, *J*= 7.15Hz)	-	-	15.64	1.18 (t, *J*= 7.21Hz)	-	-
OH	-	5.79 (bs)	-	-	-	6.08 (bs)	-	6.33

Table 3: ^{1}H and ^{13}C NMR spectral data of compound **6** in $CDCl_3$.

No.	^{13}C (δ)	^{1}H (δ)
1	160.81	-
2	116.70	6.37 (d, 9.54Hz)
3	143.43	7.65(d, 9.54Hz)
4	118.82	-
5	131.69	7.20-7.50(m)
6	124.42	7.20-7.50(m)
7	127.84	7.20-7.50(m)
8	116.91	7.20-7.50(m)
9	154.04	-

Table 4: ^{1}H and ^{13}C NMR spectral data of compound **7** in $CDCl_3$.

	^{13}C (δ)	^{1}H (δ)
1	154.74	-
2	102.54	6.35 (bs)
3	154.55	-
4	115.72	-
5	131.37	6.97 (d, 8 Hz)
6	107.48	6.36 (d, 8 Hz)
7	14.88	2.18
1-O*H*	-	4.99
3-O*H*	-	4.99

Table 5: ^{1}H and ^{13}C NMR spectral data of compounds **8** and **9** in $CDCl_3$.

	Compound **9**		Compound **8**	
	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
2	163.86	-	164.02	-
3	103.89	6.546	104.34	6.55
4	182.53	-	182.46	-
5	132.47	-	162.56	-
6	152.90	-	98.1	6.35(d, 2.26 Hz)
7	158.61	-	162.14	-
8	90.47	6.52	92.6	6.45 (d, 2,26Hz)
9	153.07	-	157.68	-
10	105.97	-	105.53	-
1’	123.23	-	123.53	-
2’	127.85	7.80 (d, 9.03Hz)	114.48	7.81(d, 8Hz)
3’	114.38	6.99 (d, 9.03Hz)	128.04	6.98(d, 8Hz)
4’	162.52	-	165.4	-
5’	114.38	6.99 (d, 9.03Hz)	114.48	6.98(d, 8Hz)
6’	127.85	7.80 (d, 9.03Hz)	128.04	7.81(d, 8Hz)
6-OMe	60.73	3.96	-	-
7- OMe	56.21	3.89	55.79	3.86
4’- OMe	55.44	3.92	55.53	3.85
5-O*H*		12.78		12.79

Table 6: ^{13}C (δ) NMR spectral data of compound **10** in $CDCl_3$

No.	**10**	No.	**10**	No.	**10**
	Aglycone			Glucose	
1	38.88	16	26.06	1’	101.17
2	28.23	17	56.07	2’	70.10
3	79.60	18	11.84	3’	73.89
4	37.25	19	19.00	4’	73.52
5	140.26	20	36.14	5’	75.94
6	122.16	21	18.76	6’	63.24
7	31.92	22	34.24	Fatty acid	
8	31.84	23	29.58	C=O (C-1’’)	174.66
9	50.14	24	45.80	C-2’’	34.23
10	36.70	25	29.11	C-7’’-12’’	29.73
11	21.05	26	19.81	C-15’’	22.68
12	39.74	27	19.35	Me	14.12
13	42.31	28	23.04		
14	56.74	29	11.84		
15	24.28				

Table 7: $^{13}C(\delta)$ NMR spectral data of compound **11** in CD_3OD

No.	Literature	**11**	No.	Literature	**11**
1	47.9	48.13	16	26.3	26.50
2	68.6	69.55	17	47.3	47.97
3	83.8	84.53	18	47.0	47.71
4	39.8	40.51	19	75.4	76.49
5	55.5	56.55	20	42.4	43.25
6	18.8	19.55	21	26.8	27.18
7	37.3	38.04	22	37.5	38.24
8	33.8	33.59	23	28.9	29.01
9	53.1	53.98	24	17.1	16.95
10	37.8	38.56	25	18.7	18.92
11	119.0	119.47	26	16.7	16.57
12	141.6	141.83	27	16.0	16.39
13	27.8	28.42	28	180.7	182.21
14	33.0	34.48	29	26.8	27.00
15	22.3	22.67	30	15.8	15.65

Table 8: ^{1}H and ^{13}C NMR spectral data of compounds **12** and **13** in $CDCl_3$

No.	**12**		**13**	
	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
1/1’	22.65	0.87 (d,6.63Hz)	22.65	0.87 (d,6.63Hz)
2/2’	28.14	1.53 (sep, 6.63Hz)	28.14	1.53 (sep, 6.63Hz)
3/3’	39.04	1.15 (m)	39.03	1.15 (m)
4/4’	27.51	1.27(m)	27.51	1.27(m)
5/5’	29.93	1.27(m)	29.68	1.27(m)
6/6’	29.68	1.27(m)	29.68	1.27(m)
7/7’	25.44	1.45(m), 1.36 (m)	25.14	1.36(m)
8/8’	36.57	1.58(m), 1.49 (m)	33.98	1.63 (m)
9/9’	68.01	4.05(bs)	70.31	5.23(quint, 6.40Hz)
10/10’	41.04	2.58 (d, 16.08Hz), 2.48 (dd, 16.08, 8.70 Hz)	38.42	2.61(m)
11/11’	22.65	0.87 (d,6.63Hz)	22.65	0.87 (d,6.63Hz)
CH3	-	-	21.09	2.07 (s)
C=O	-	-	170.59	-

Table 9: Chemical composition of essential oil of aerial parts of *L. gibsoni*

Compounds	*LRI*[a])	Composition [%]
α- Pinene	936	1.47
1-Octen-3-ol	980	2.20
β-Myrcene	993	2.78
3-Octanol	997	tr[b])
α-Phellandrene	1007	0.52
+ 3-Carene	1013	1.52
α-Terpinene	1019	0.76
p-Cymeme	1027	0.82
d- Limonene	1031	2.30
cis-Ocemene	1038	tr
Trans ocemene	1049	0.30
α- Terpinolen	1094	22.22
Linalool	1102	2.65
1-Octen-3-ol, acetate	1113	1.49
3-Octanol, acetate	1125	1.23
2, 4-Cycloheptadien-1-one, 2,6,6-trimethyl	1145	1.35
Benzenemethanol, 4-(1-methylethyl)	1189	4.52
α-Terpineol	1194	0.77
1-Octen-3-yl-n-propionate	1207	0.37
3-Isopropyleden-5-methylhex-4-ene-2-one	1241	0.56
Thymol	1305	10.42
Phenethyl-2-methylpropionate	1444	0.23
α-Caryophyllene	1512	tr
Caryophyllene oxide	1590	1.21
Total		59.69

[a]) *LRI*: linear retention index determined on GsBP-5 column ; [b]) tr: trace amounts.

Table 10: Larvicidal activity of essential oil of *L. gibsoni*

Conc.[ppm]	Mortality after 24 hrs		
	A. stephensi ±SE	*A. aegypty* ±SE	*C. quinquefasciatus* ±SE
150	100	100	100
125	94.0±1.31	94.0±1.31	94.0±1.31
100	82.0± 1.06	88.66 ± 0.90	85.33± 1.33
75	58.0± 1.11	75.33± 1.33	61.33± 0.90
50	41.33± 0.90	51.33± 1.65	45.33± 1.92
25	0	15.33± 1.33	11.33±0.90

Table 11: Larvicidal activity of acetone extract of aerial parts of *L. gibsonii*

Conc.[ppm]	Mortality after 24 hrs		
	A. stephensi ±SE	*A. aegypty* ±SE	*C. quinquefasciatus* ±SE
500	100	100	100
250	100	100	100
200	70 ± 1.95	84.61 ± 1.34	77.33 ± 1.18
150	47.33 ± 1.81	54.66 ± 1.33	52.00 ± 0.75
100	31.33 ± 0.90	39.33 ± 0.66	34.00 ± 1.31
75	10.66 ± 0.66	21.33 ± 1.33	15.33 ± 1.33

Table12: LC_{50} and LC_{90} values of *L. gibsoni* essential oil and acetone extract.

	A. stephensi	*A. aegypty*	*C. quinquefasciatus*
Essential oil			
Regression equation ±SE	Y=4.23X -2.55 ± 1.70	Y= 3.71 X -1.27 ± 0.58	Y= 3.95X - 3.42
LC_{50} [ppm] (Fiducial Limits)	60.49 (44.56-82.12)	48.71 (44.58-53.23)	57.29 (41.72-78.68)
LC_{90} [ppm] (Fiducial Limits)	147.83 (81.65-267.65)	134.91 (116.22-156.60)	143.58 (81.18-253.92)
Acetone extract			
Regression equation ±SE	Y= 3.99X -3.62	Y= 3.96X -3.28	Y= 3.95X - 3.42
LC_{50} [ppm] (Fiducial Limits)	144.49 ± 1.52 (84.55 - 246.93)	123.16 ± 1.50 (83.44 - 181.78)	135.14 ± 1.50 (86.12 – 212.08)
LC_{90} [ppm] (Fiducial Limits)	373.19 (1830.6 - 76.0)	320.51 (988.9 - 103.8)	350.68 (1294.9 - 94.9)

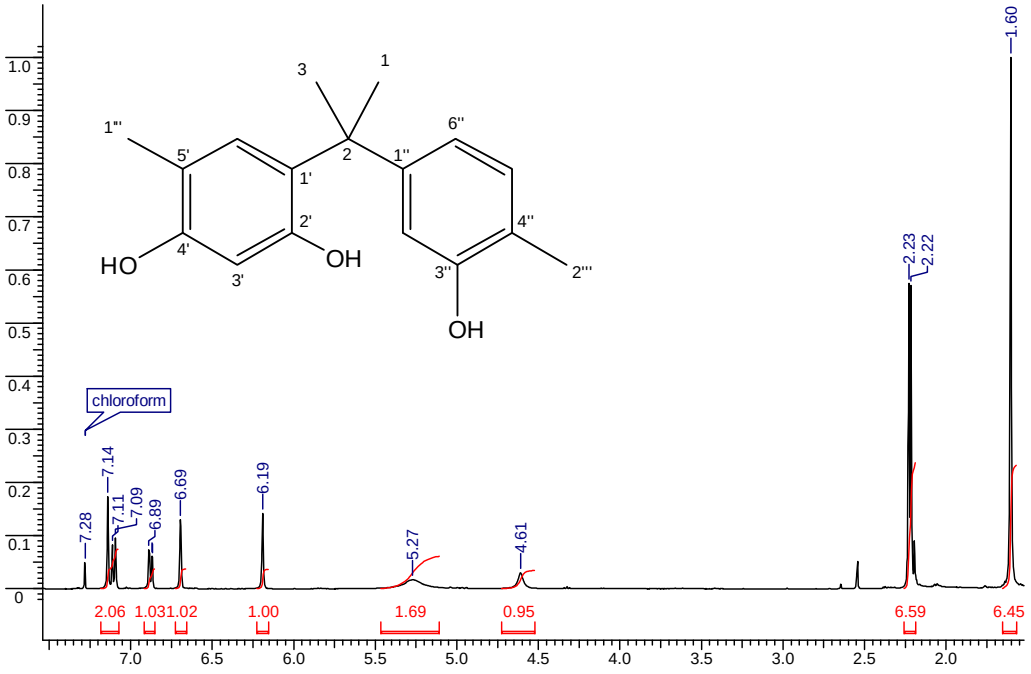
Table 13: Mosquito Repellent activity of essential oil and standard compounds against *A. aegypti females*.

Essential oil	
Concentration (mg/cm^2)	Protection period offered (min)
0.5	140 ± 7.08
1.0	280 ± 12.89
2.0	435 ± 10.27
α- Terpinolen	
0.1	130 ± 3.51
0.2	255 ± 5.25
0.4	310 ± 7.08
Thymol	
0.05	70 ± 3.54
0.1	180 ± 3.27
0.2	250 ± 5.25
2:1 Mixture of α- Terpinolen and Thymol	
0.15	140 ± 3.54
0.3	265 ± 10.39
0.6	370 ± 10.02

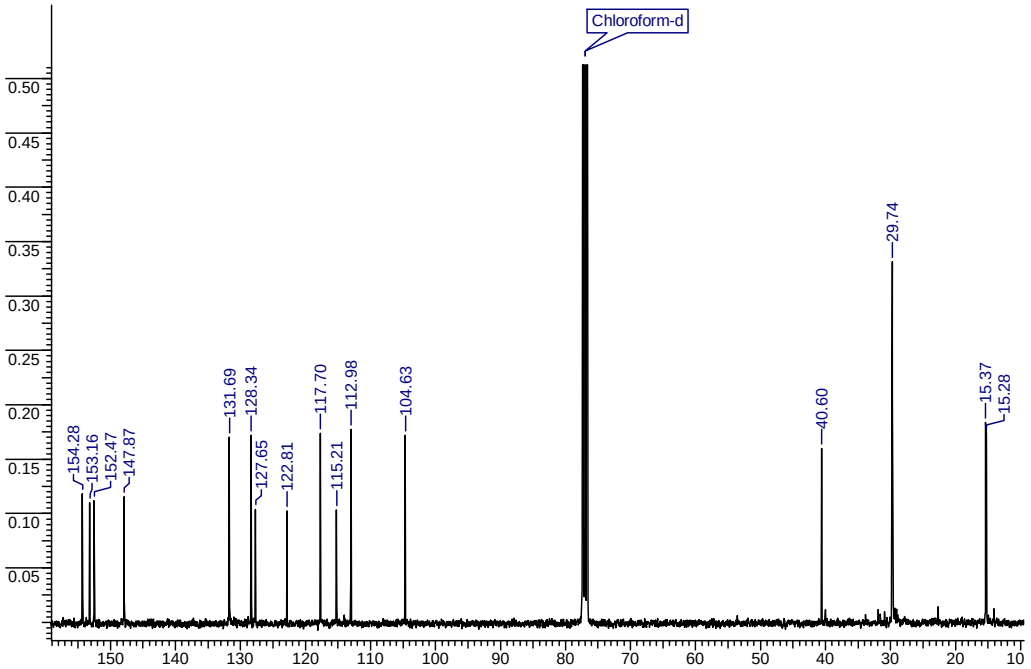
3.8. NMR data:

Compound **1**:

^{1}H NMR:



^{13}C NMR:



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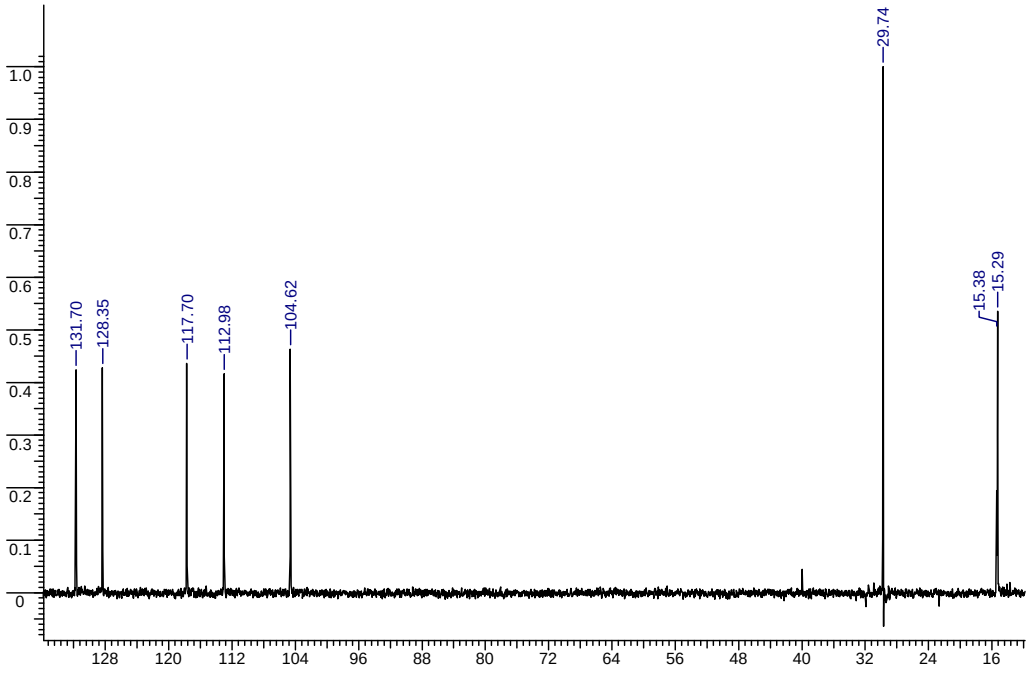
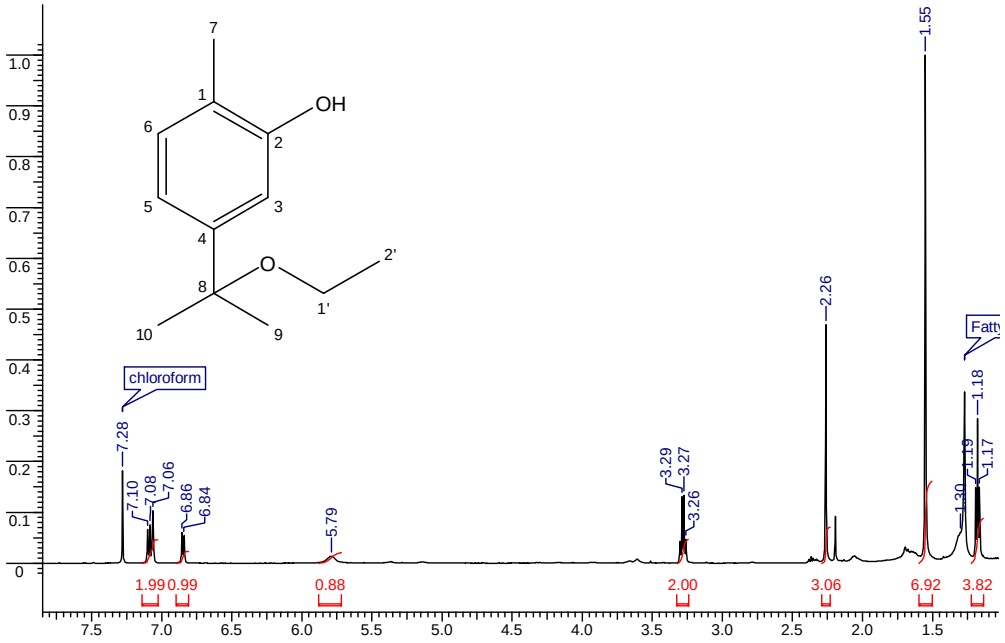
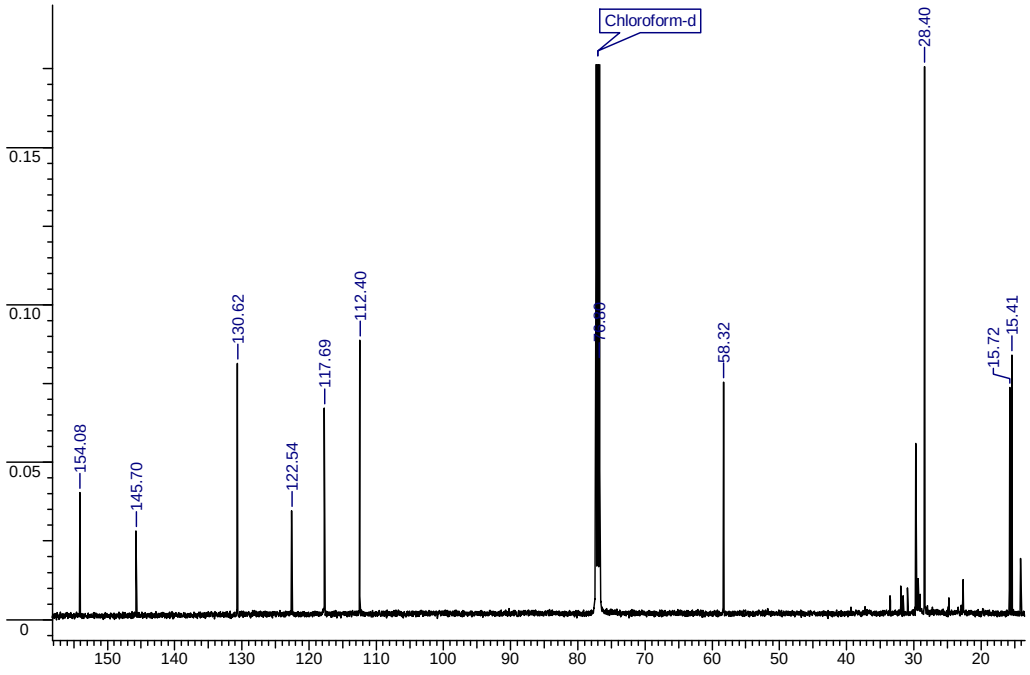


Compound **2**:

^{1}H NMR:



^{13}C NMR: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0.10
0.05
0
130.62
112.40
117.69
154.08
145.70
122.54
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150
140
130
120
110
100
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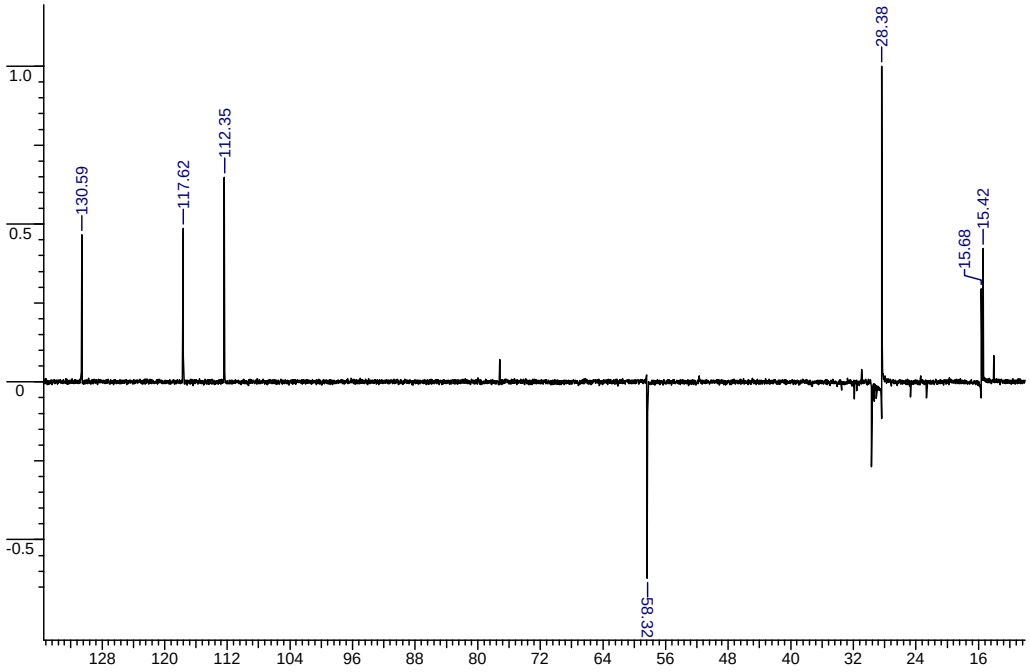
80
70
60
50
40
30
20

DEPT: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0
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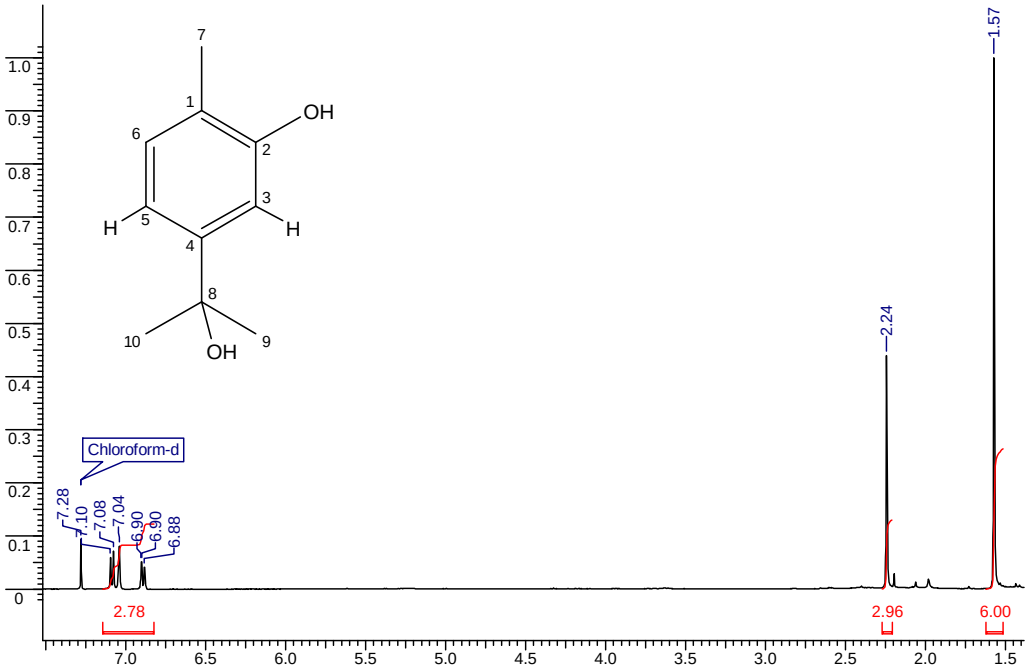
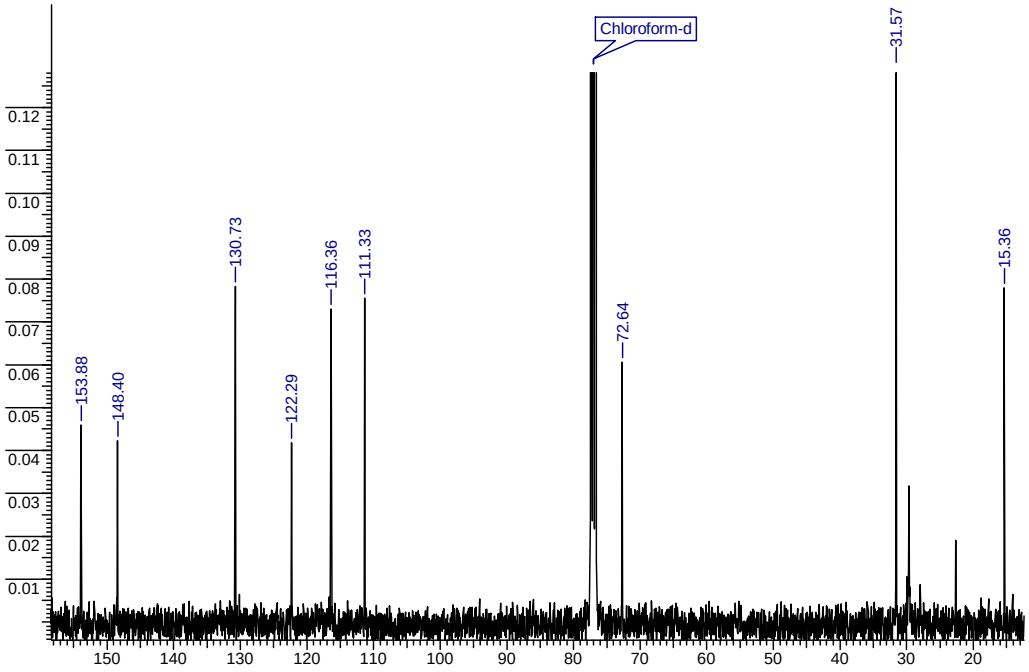
112.35
15.68
15.42
58.32
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16

Compound **3**:

^{1}H NMR:



^{13}C NMR:



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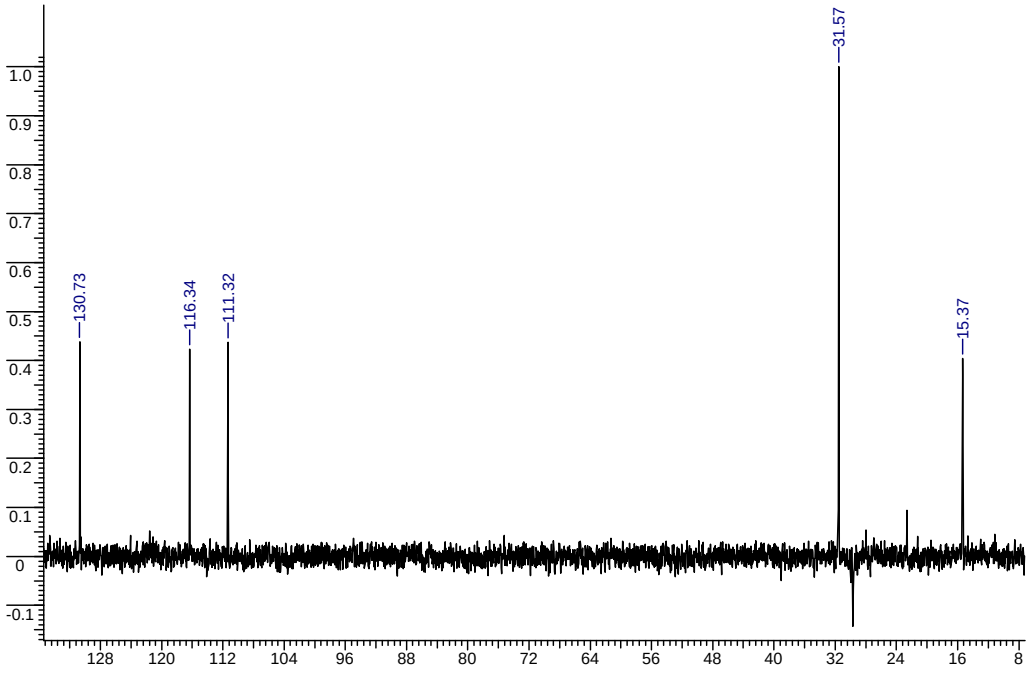
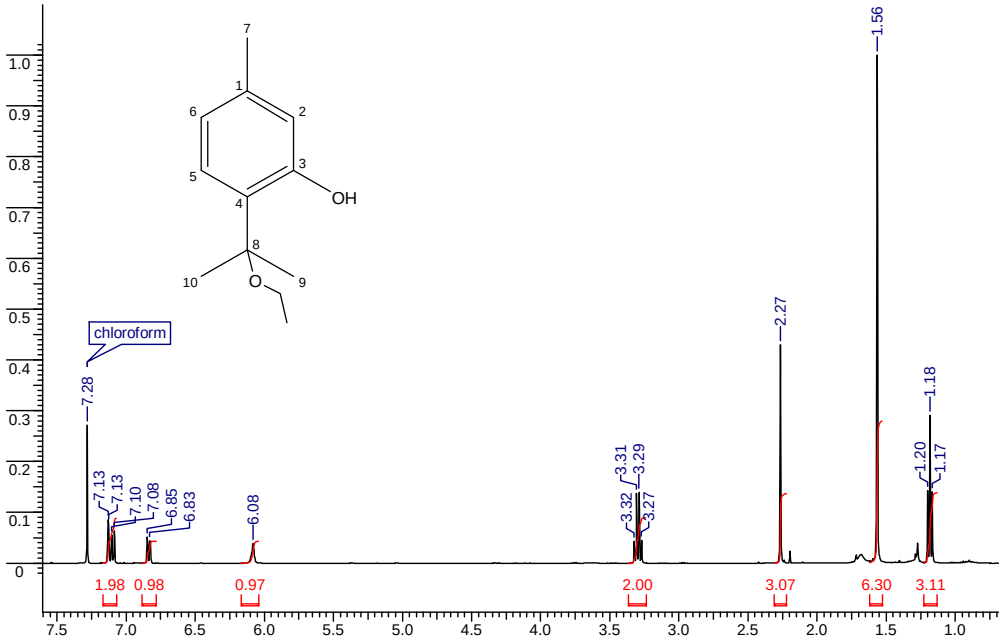
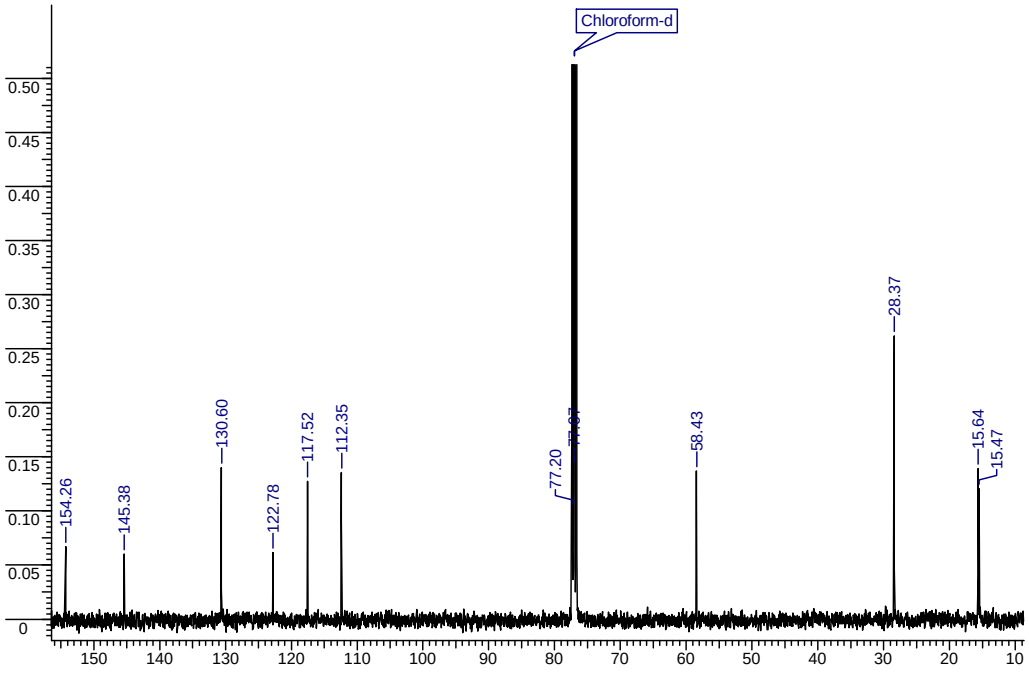


Compound **4**:

^{1}H NMR:



^{13}C NMR:


Chloroform-d
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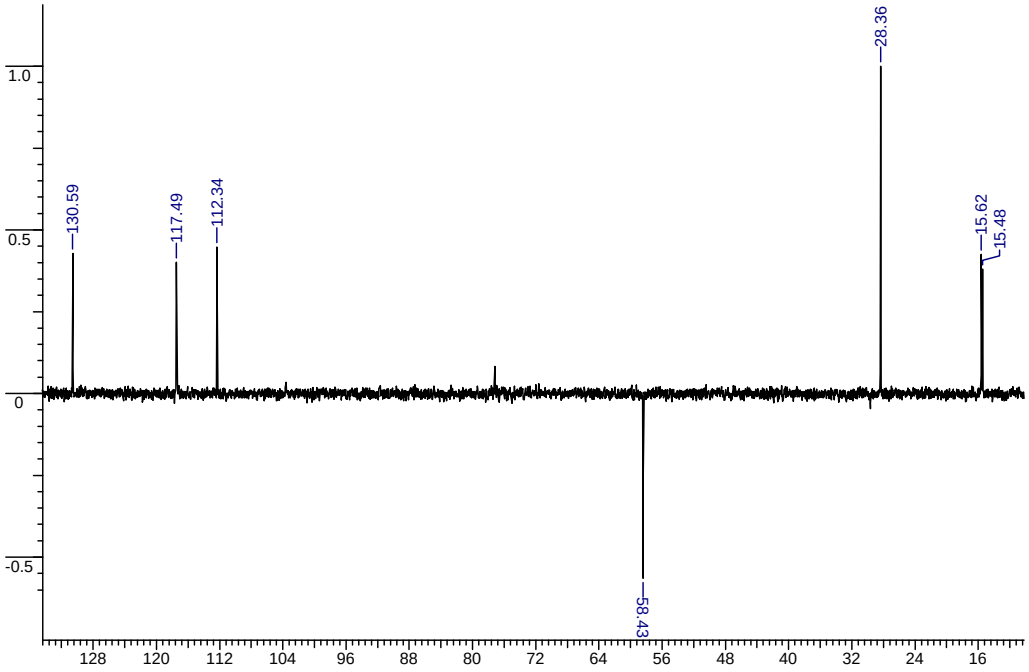
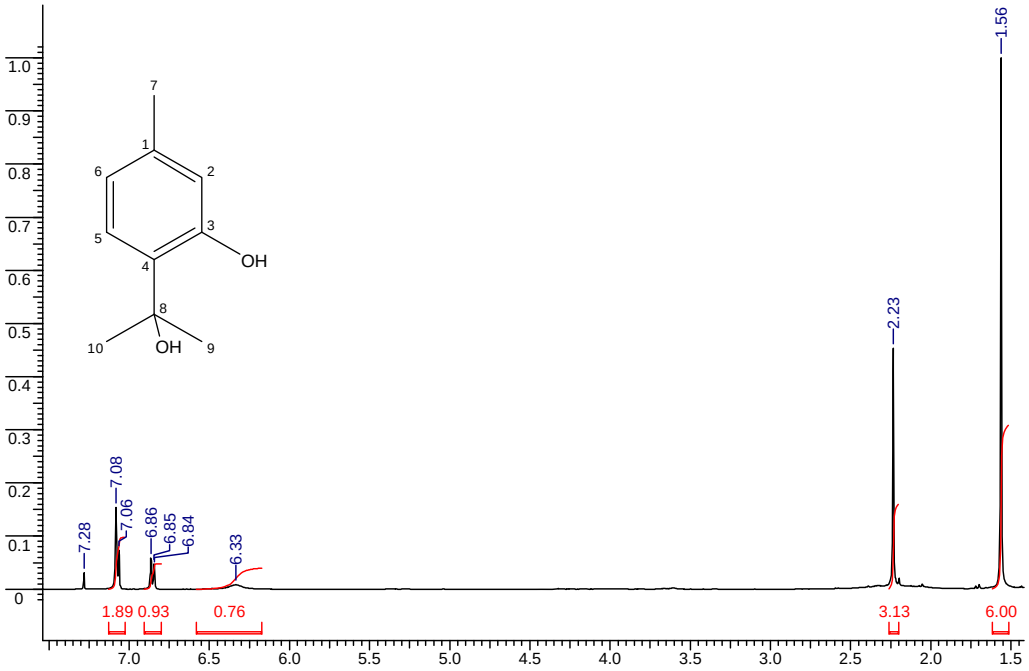
145.38
130.60
122.78
117.52
112.35
77.20
58.43
28.37
15.64
15.47

DEPT:

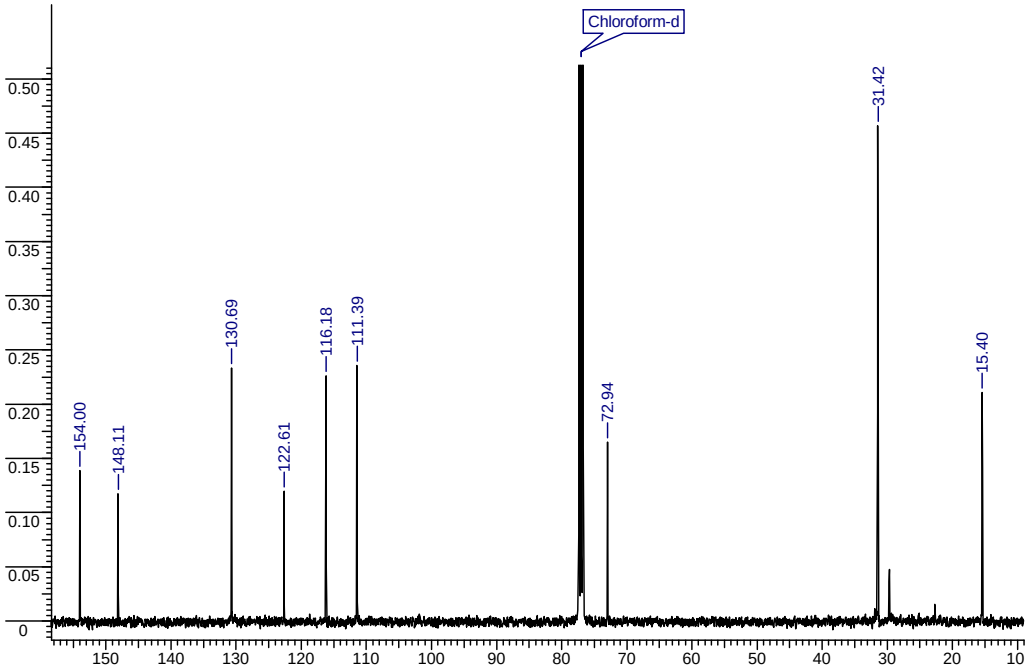

130.59
117.49
112.34
28.36
15.62
15.48
58.43

Compound **5**:

^{1}H NMR:



^{13}C NMR:



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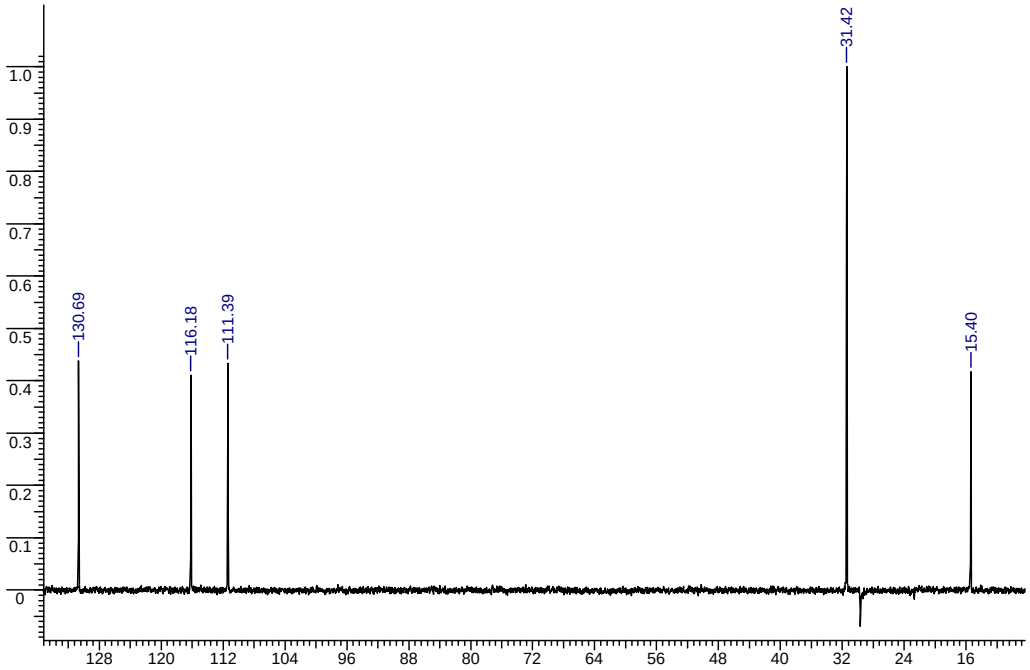
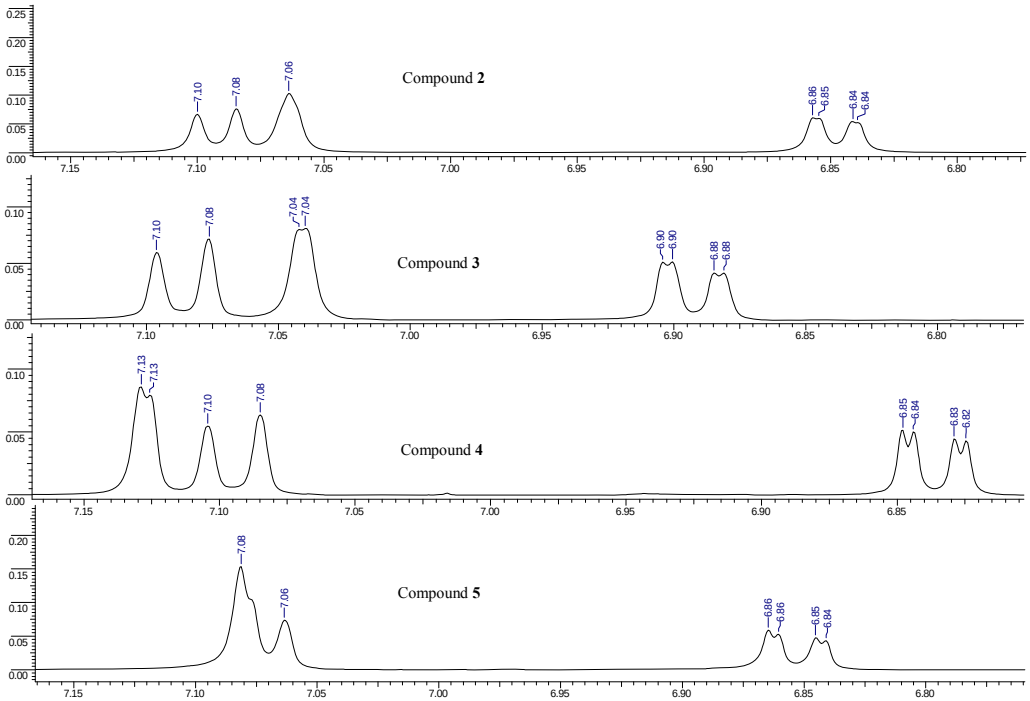


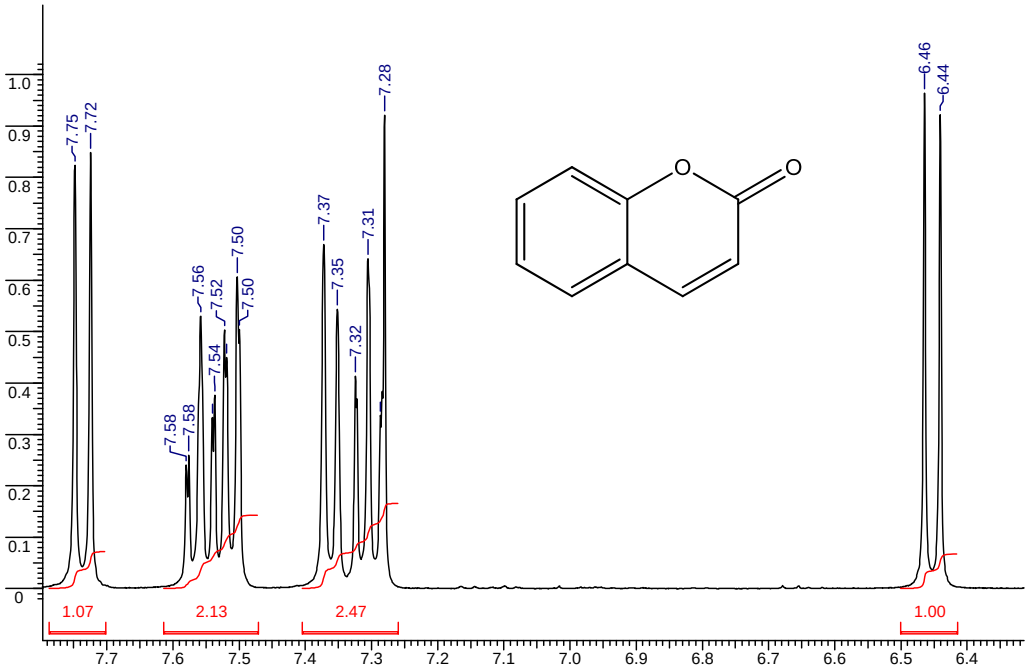
Compounds **2**, **3**, **4** and **5:**

Comparative display of magnified ^{1}H NMR aromatic region.

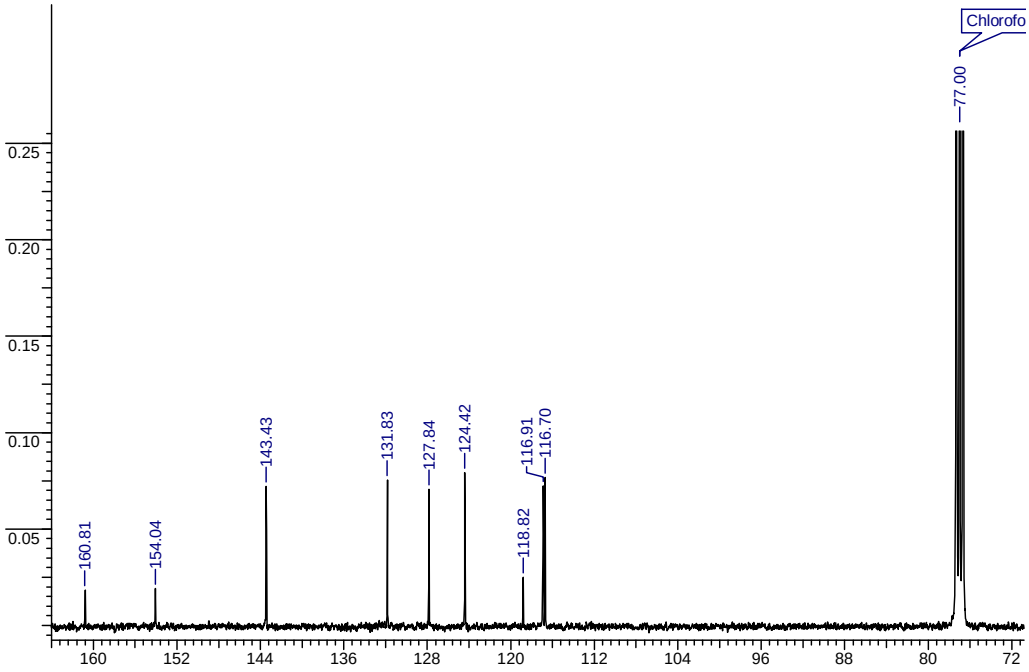


Compound **6**:

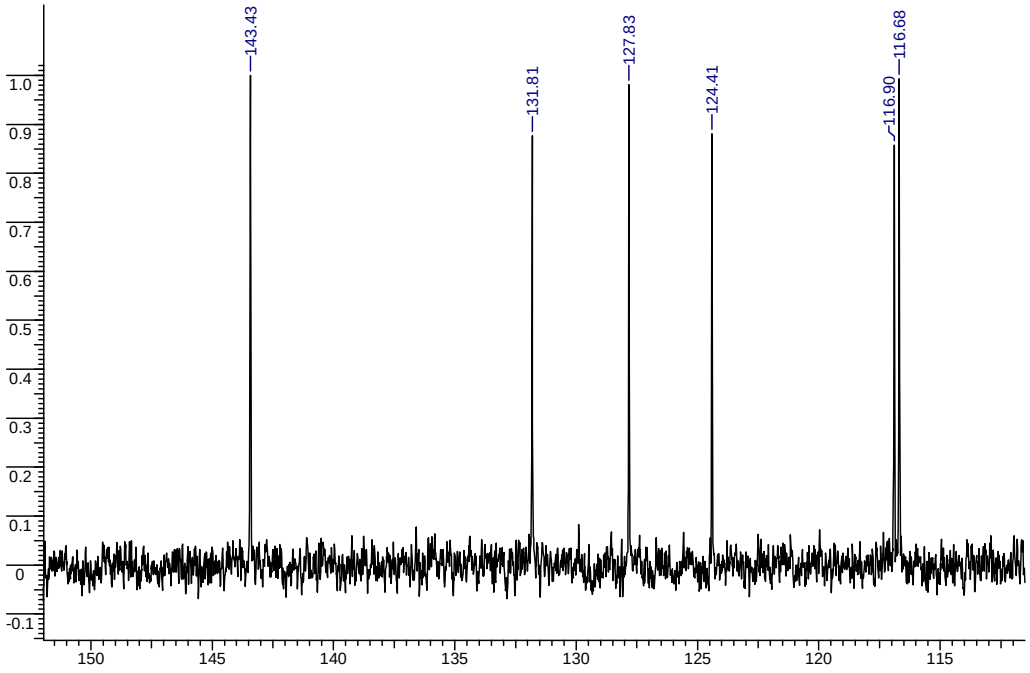
^{1}H NMR:



^{13}C NMR:

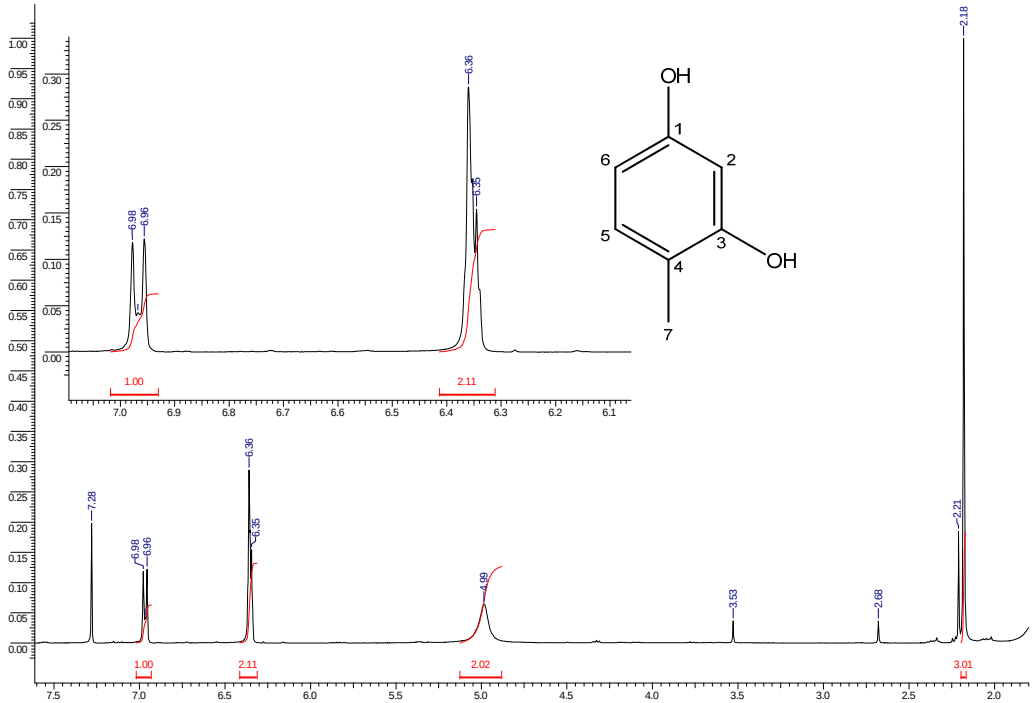


DEPT:

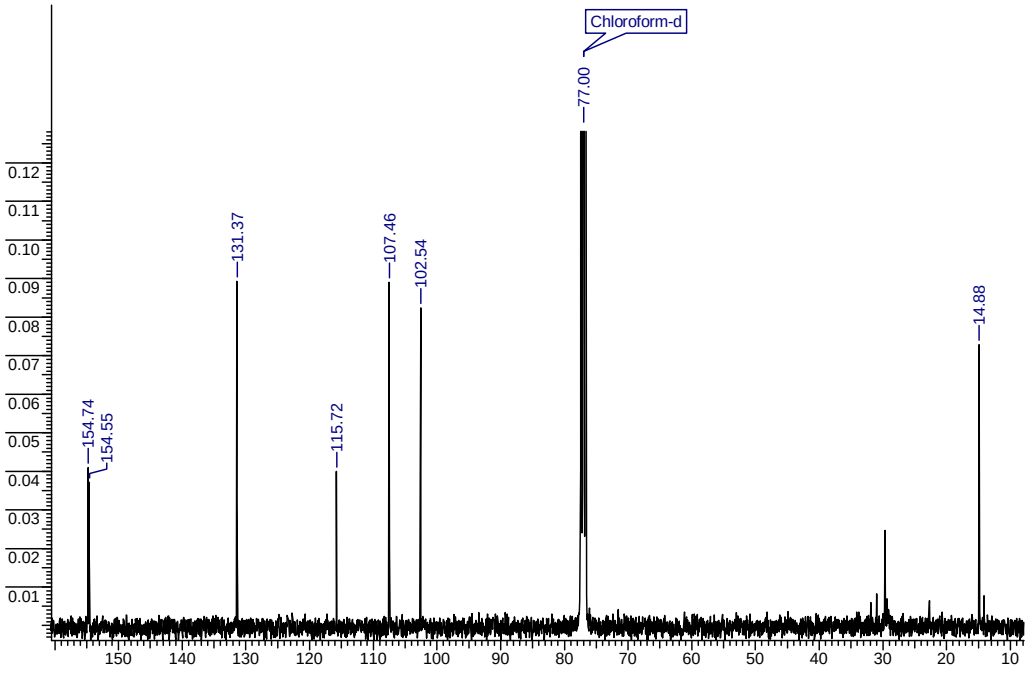


Compound **7**:

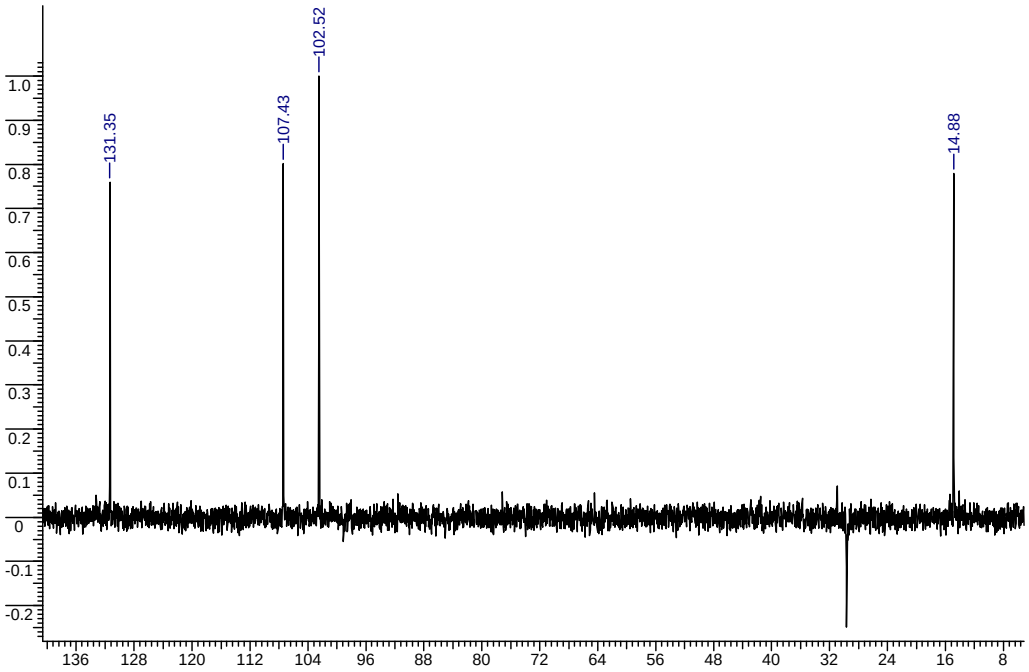
^{1}H NMR:



^{13}C NMR:

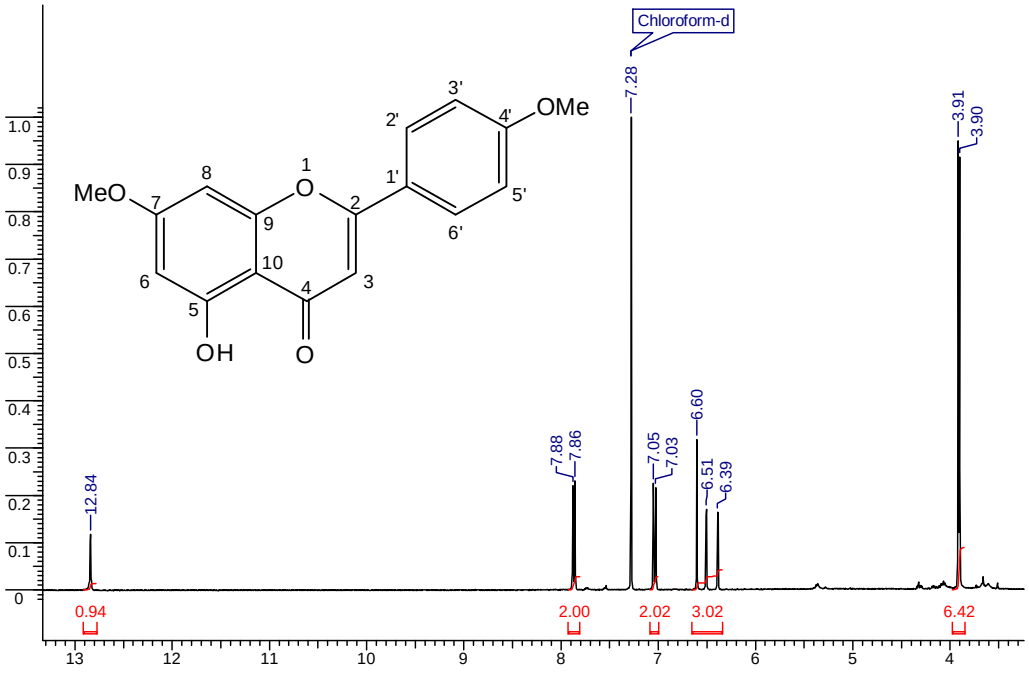

Chloroform-d
77.00
131.37
107.46
102.54
14.88
154.74
154.55
115.72

DEPT:

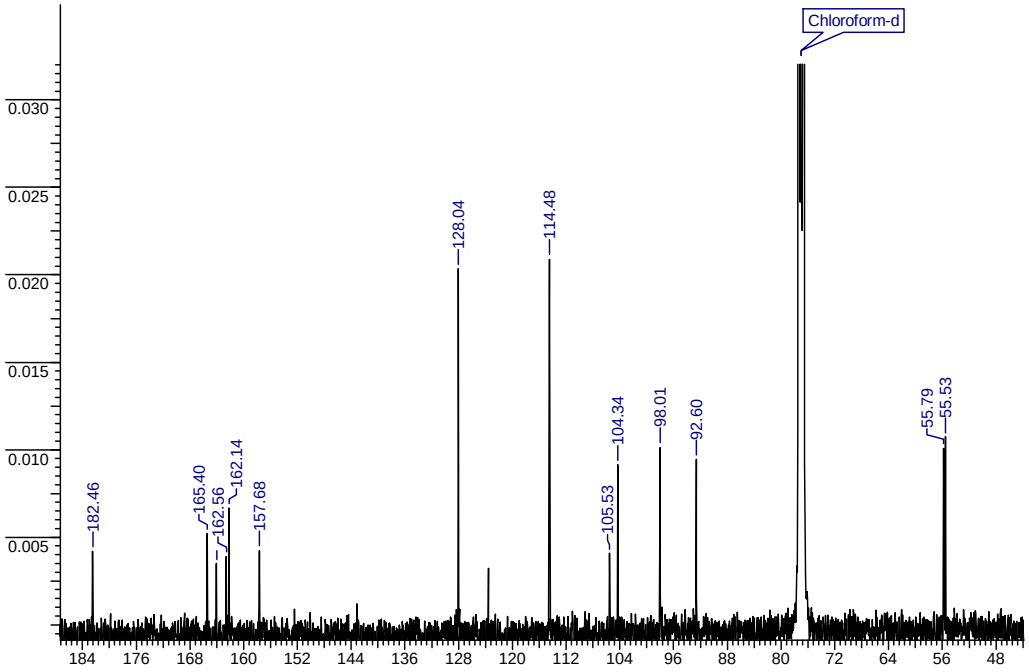

102.52
131.35
107.43
14.88

Compound **8**:

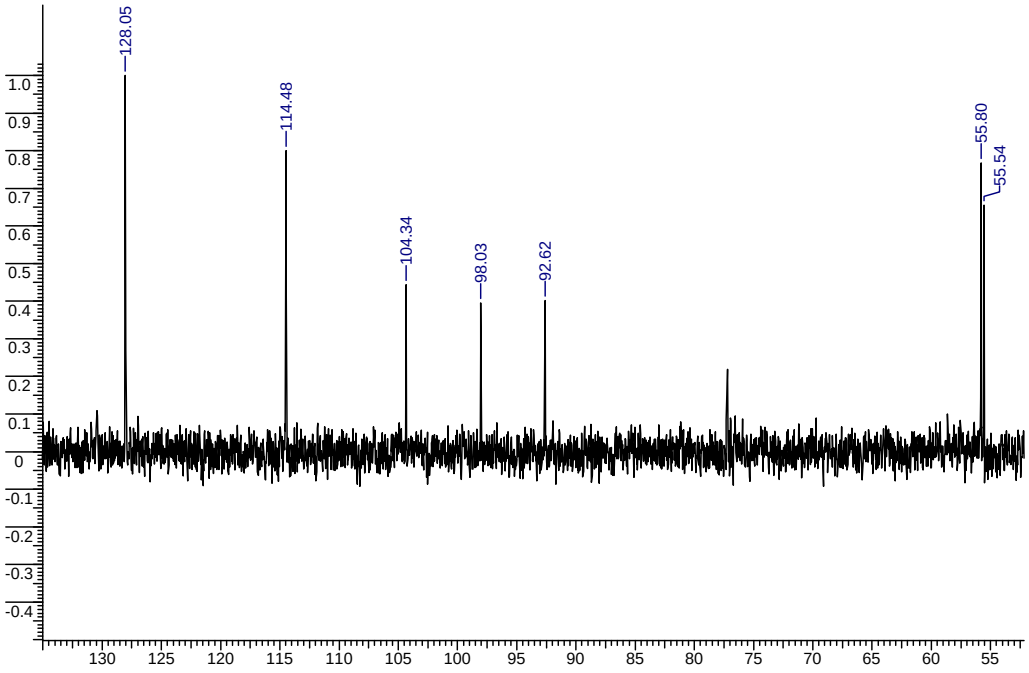
^{1}H NMR:



^{13}C NMR:

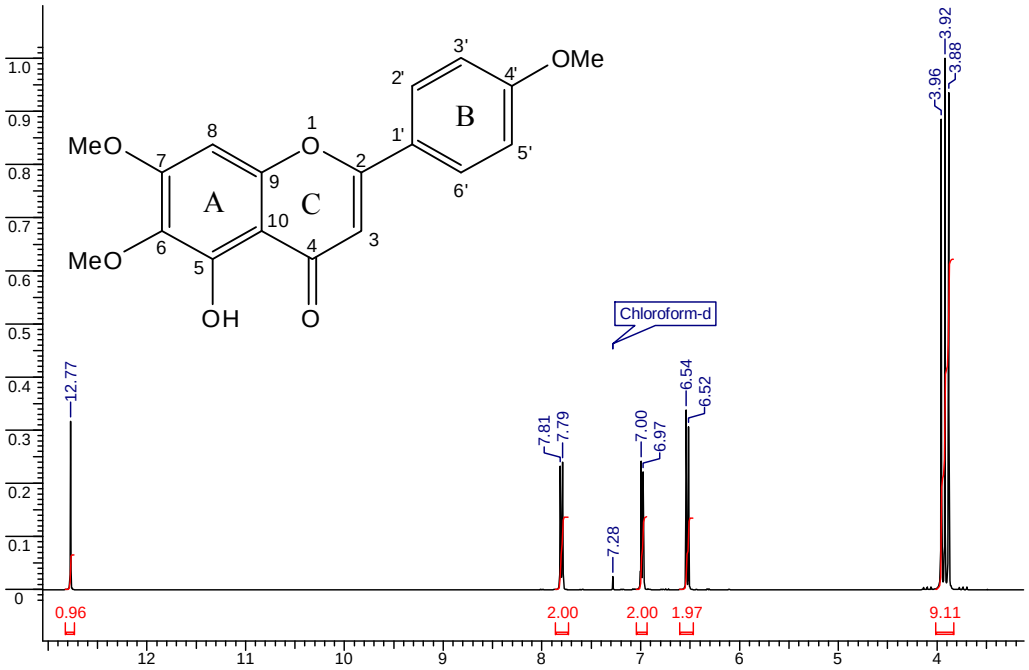


DEPT:

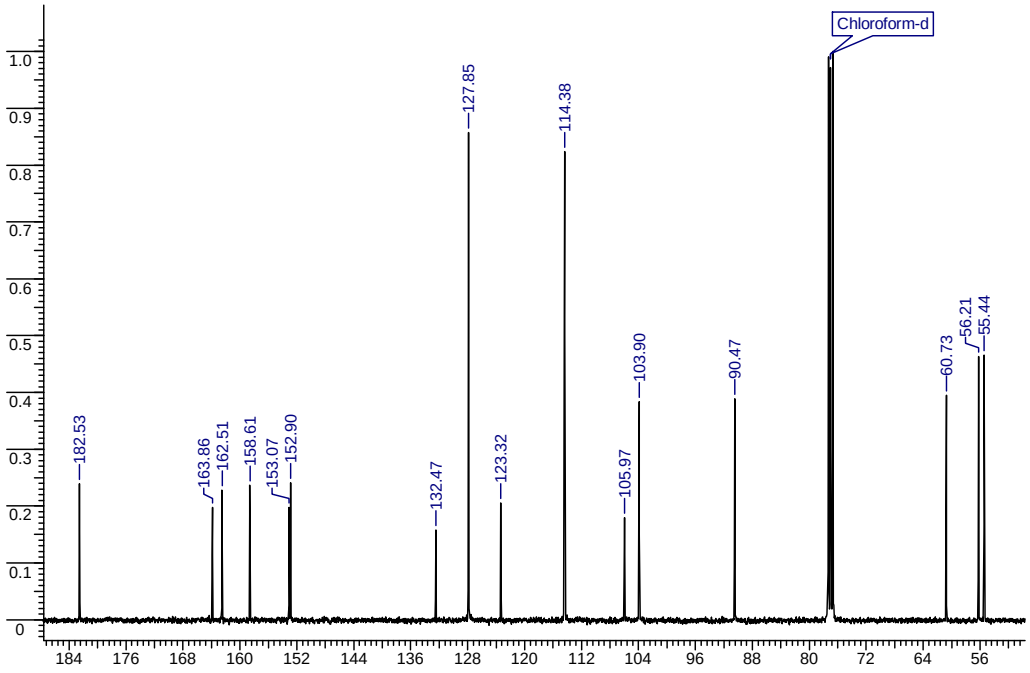


Compound **9**:

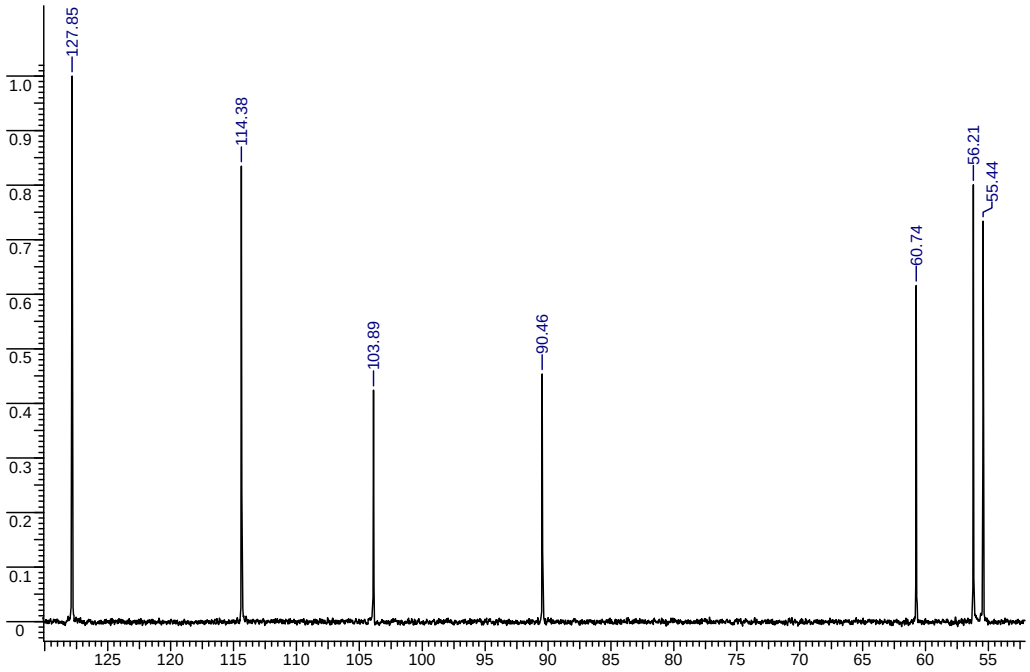
^{1}H NMR:



^{13}C NMR:

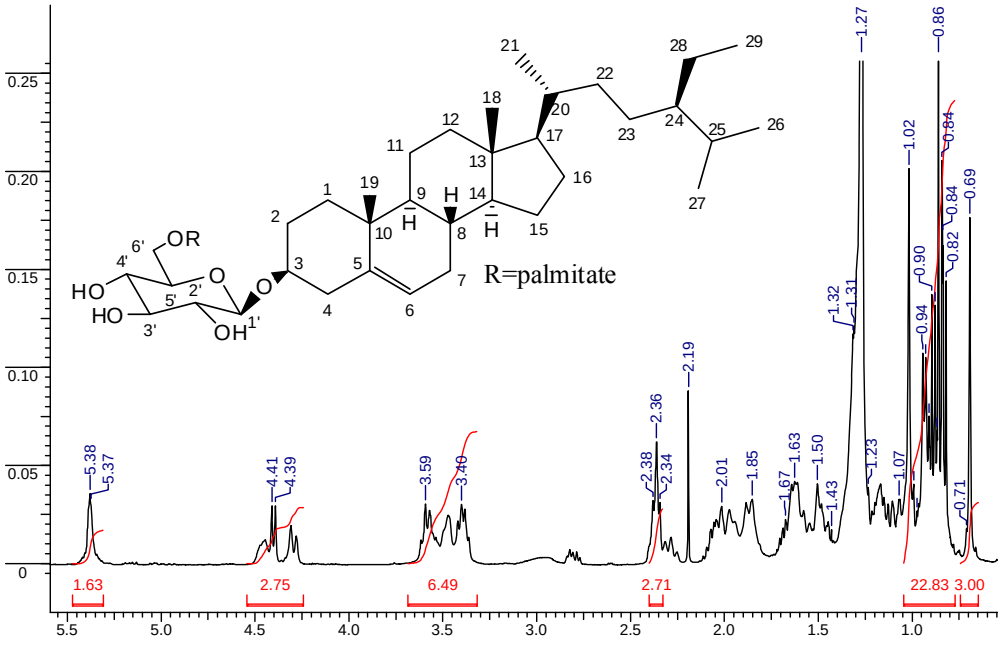

Chloroform-d
182.53
163.86
162.51
158.61
153.07
152.90
132.47
127.85
123.32
114.38
105.97
103.90
90.47
60.73
56.21
55.44

DEPT:

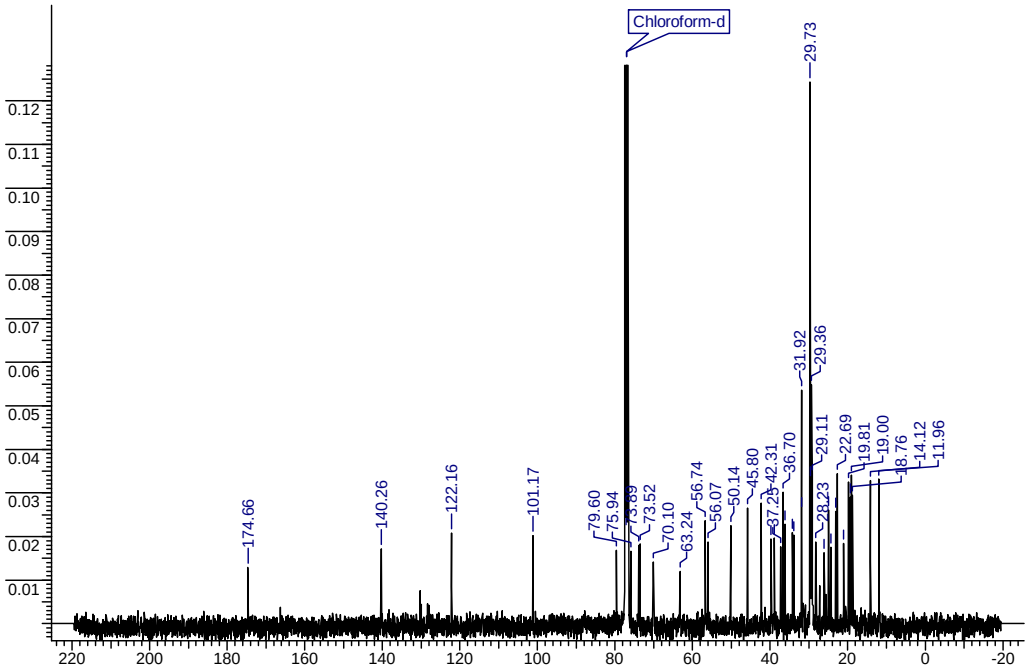

127.85
114.38
103.89
90.46
60.74
56.21
55.44

Compound **10**:

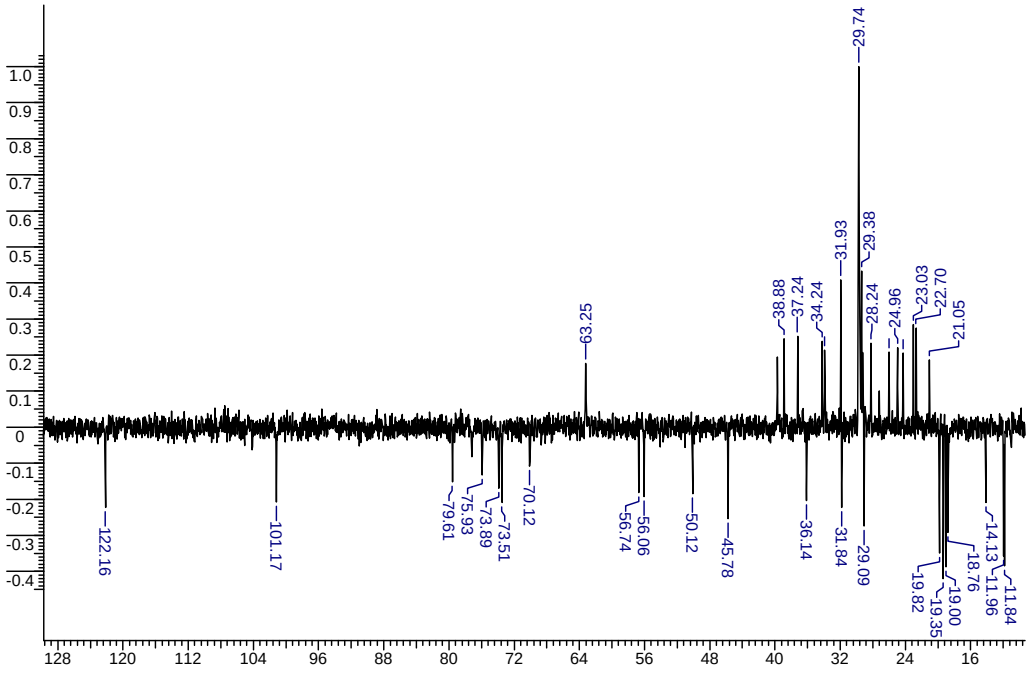
^{1}H NMR:



^{13}C NMR:

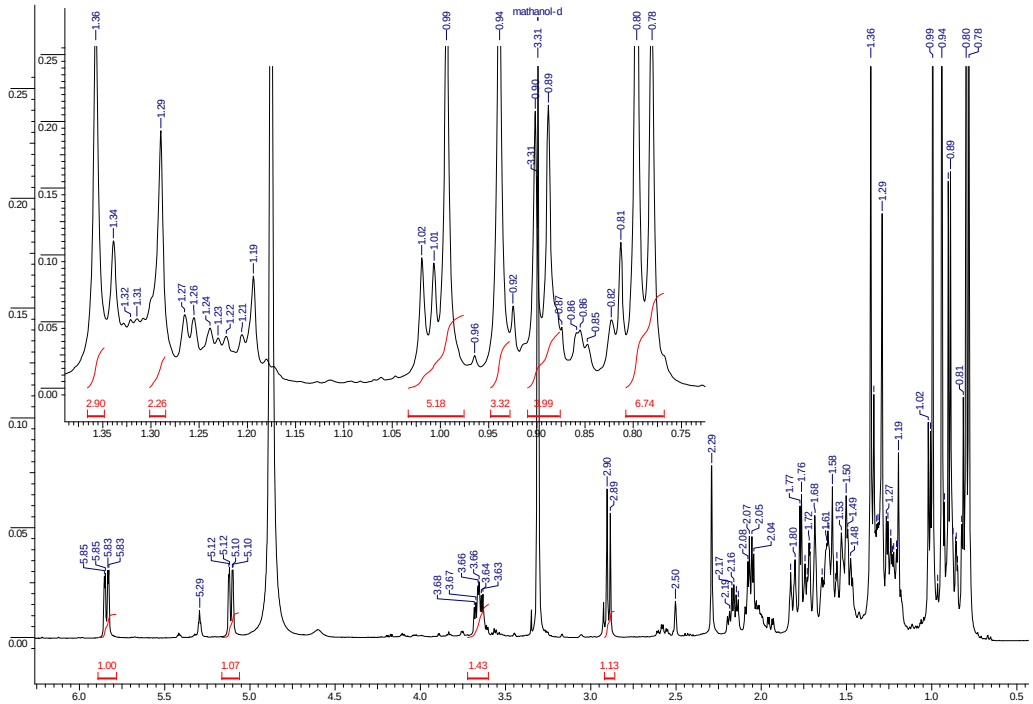


DEPT:

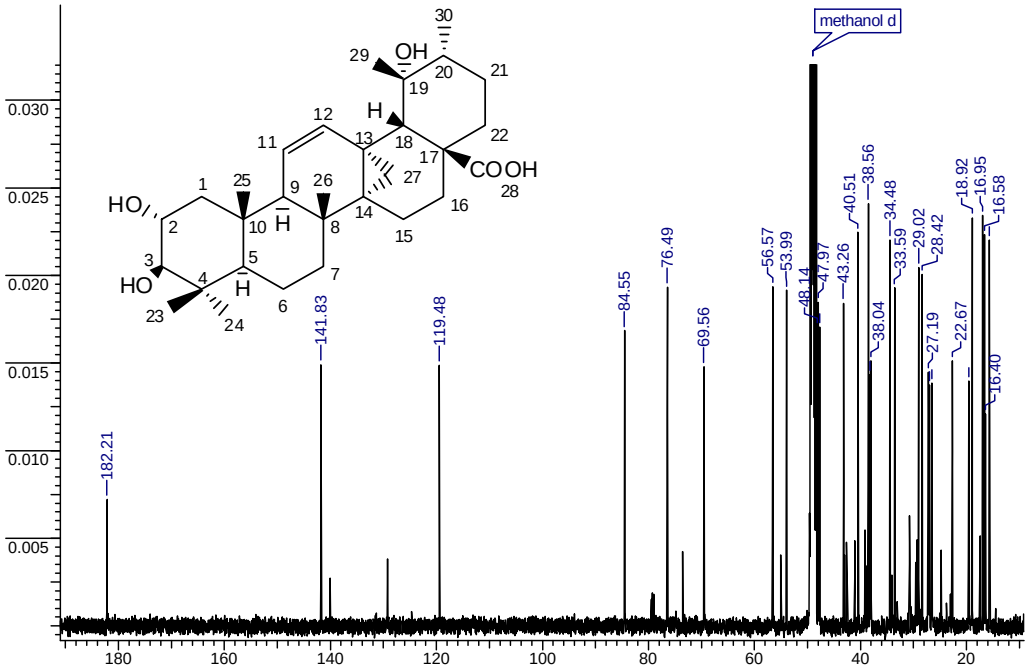


Compound **11**:

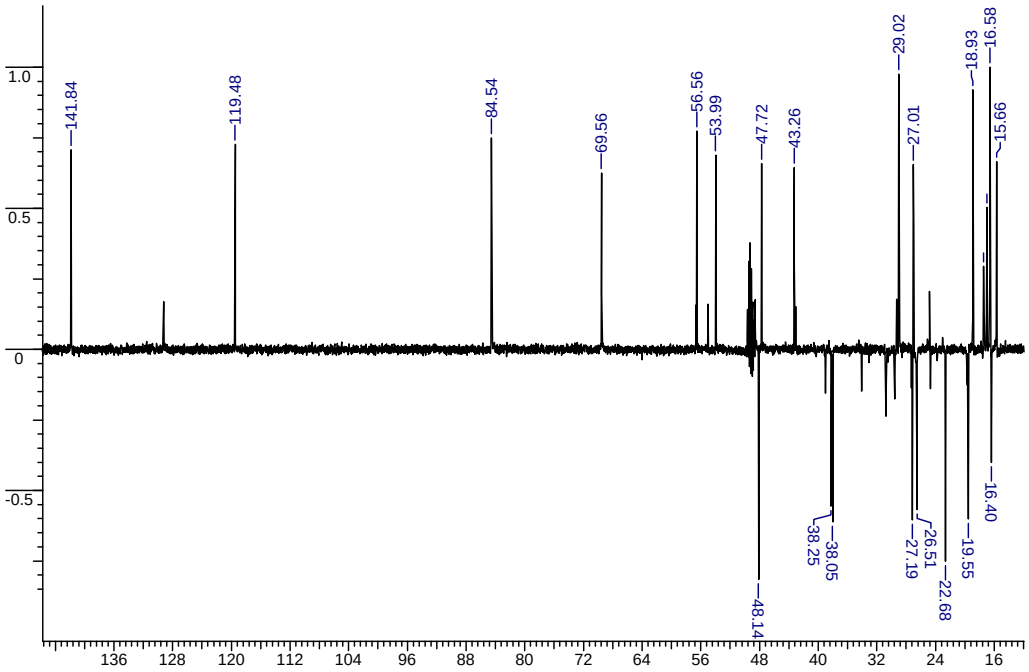
^{1}H NMR:



^{13}C NMR:

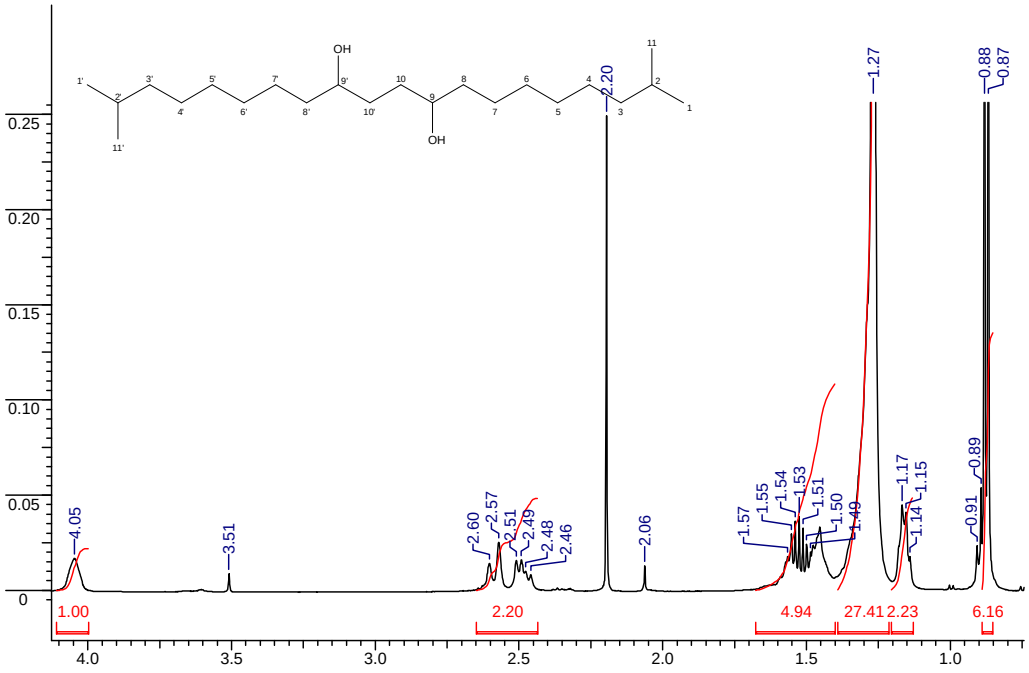

methanol d
182.21
141.83
119.48
84.55
76.49
69.56
56.57
53.99
48.14
47.97
43.26
40.51
38.56
38.04
34.48
33.59
29.02
28.42
27.19
22.67
18.92
16.95
16.58
16.40

DEPT:

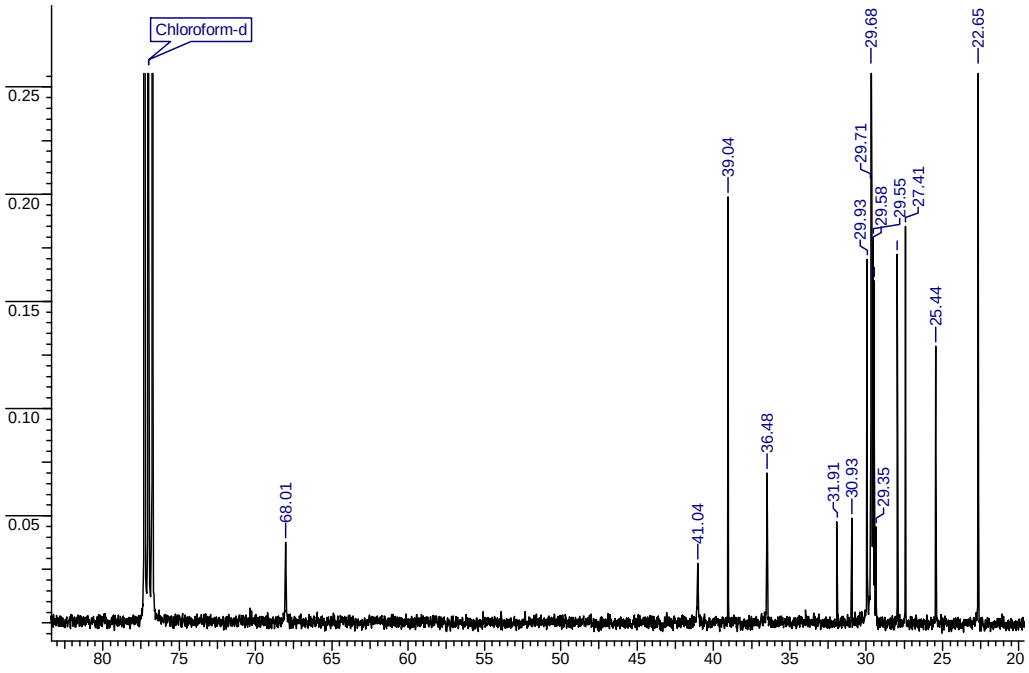

141.84
119.48
84.54
69.56
56.56
53.99
47.72
43.26
29.02
27.01
18.93
16.58
15.66
48.14
38.25
38.05
27.19
26.51
22.68
19.55
16.40

Compound **12**:

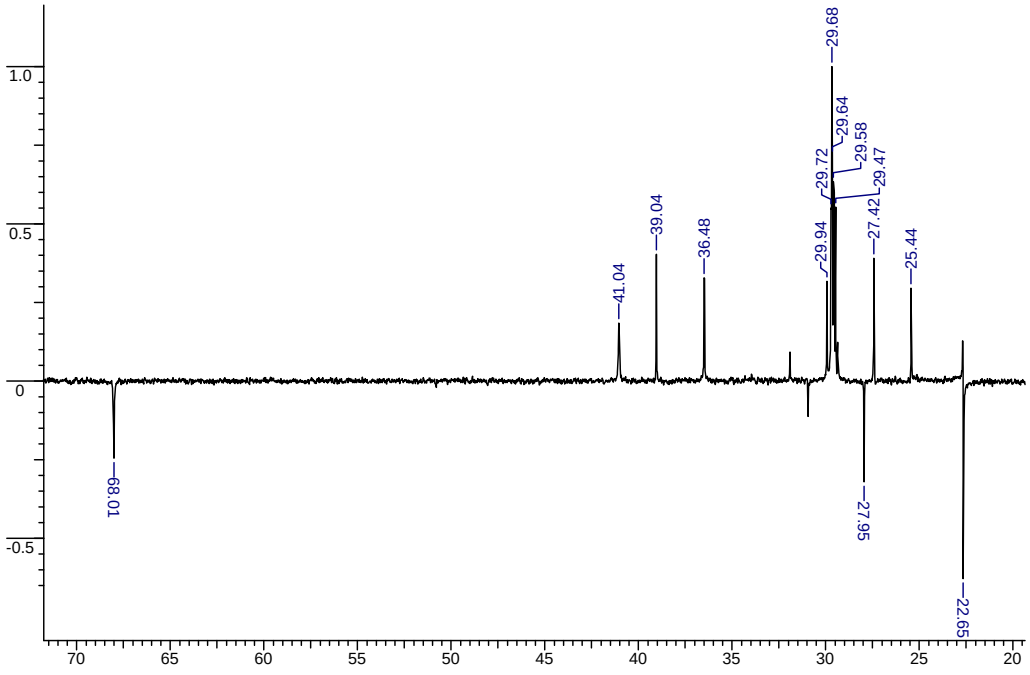
^{1}H NMR:



^{13}C NMR:

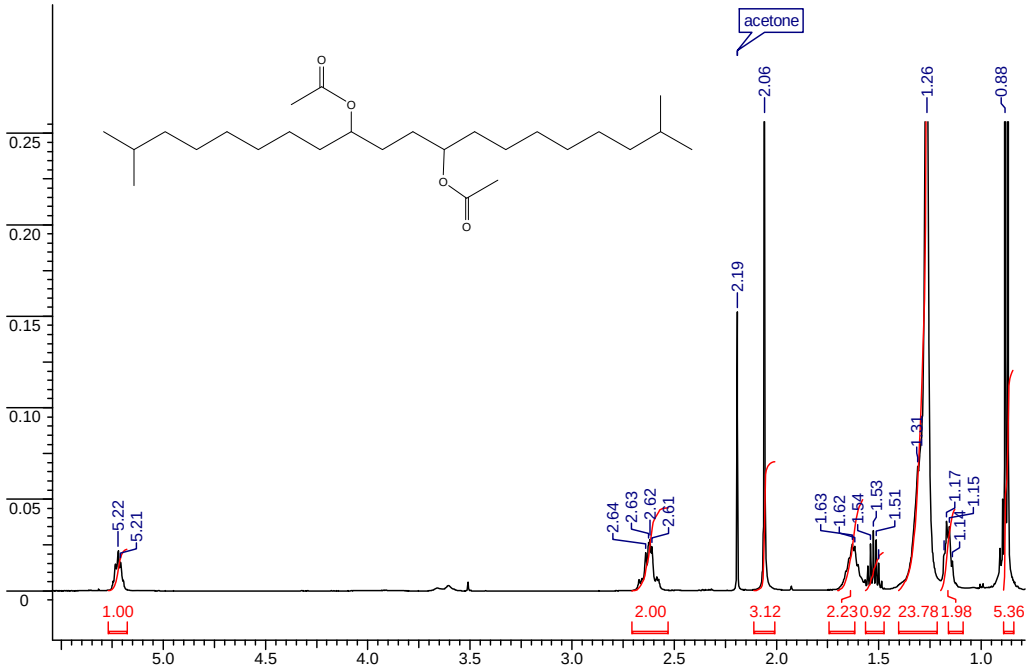


DEPT:

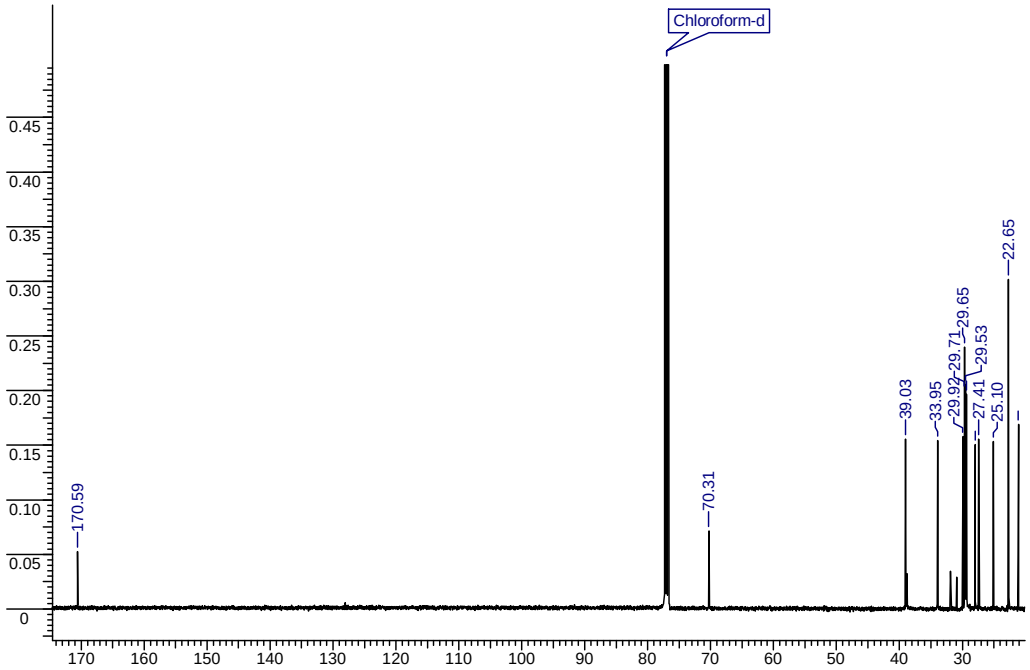


Compound **13**:

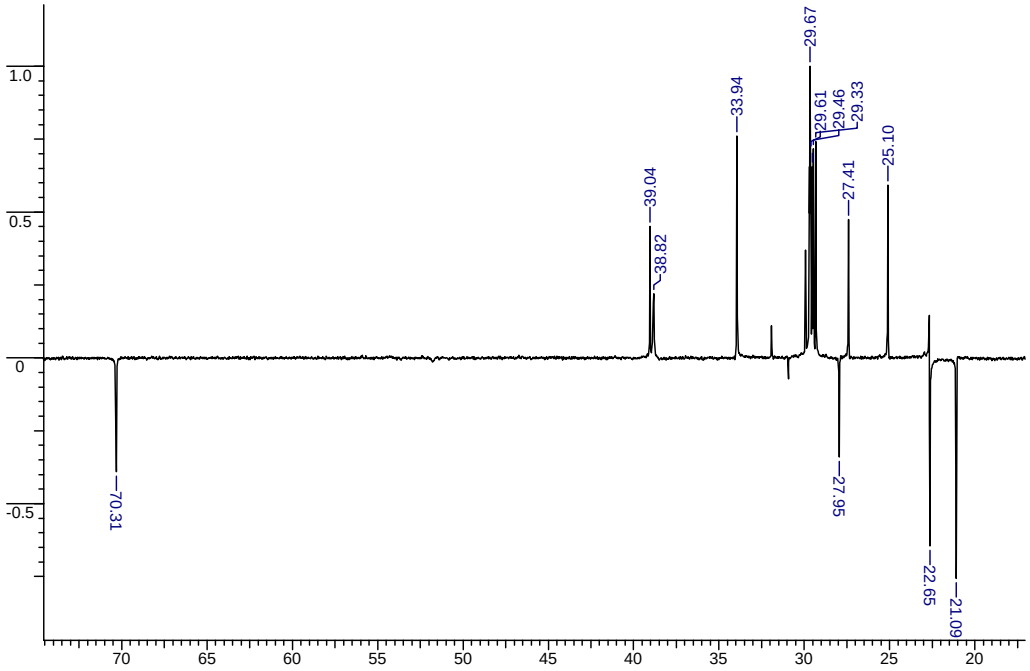
^{1}H NMR:



^{13}C NMR:


Chloroform-d
170.59
70.31
39.03
33.95
29.92
29.71
29.65
29.53
27.41
25.10
22.65

DEPT:


39.04
38.82
33.94
29.67
29.61
29.46
29.33
27.41
25.10
70.31
27.95
22.65
21.09

3.9. Reference:

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Chapter 4

Phytochemical investigations on

Anisomeles heyneana Benth.

Figure 1: *Anisomeles heyneana* Benth.

4.1. Introduction:

Anisomeles is a genus of herbaceous or shrubby plants distributed from Africa to India, South East Asia to North East Australia and east from China to Taiwan, Japan and the Philippines [1]. Genus *Anisomeles* is represented in Maharashtra by three species viz. *A. heyneana*, *A. malabarica* and *A. indica* [2] of which *A. heyneana* is endemic to Maharashtra [3].

A. heyneana called Western Hill Catmint in English or Gopali in Marathi is a tall, erect herb, growing to 1-1.5m high with slender stems and quadrangular braches. Leaves are oppositely arranged, ovate lance-like and 5-12cm long. Flowers (in October-November) are small, 2cm, white tinged with pink and 2-lipped occurring in long cymes. Upper lip is 5 mm and lower lip is 3-lobed. The flowers resemble cow's earlobes, which gives it its Marathi name [4]. Individual plants are found isolated among other plant species and two plants are separated by large distance.

4.2. Collection and processing:

A. heyneana, whole plants, in flowering, were collected from Purandar Fort area, District Pune on 3rd January, 2008. A herbarium is deposited in Botanical Survey of India, Western Circle, Pune (No. SPJ-2). Roots were separated and aerial parts cleaned off adhering dust and unwanted plant material, dried in shade, cut and pulverized.

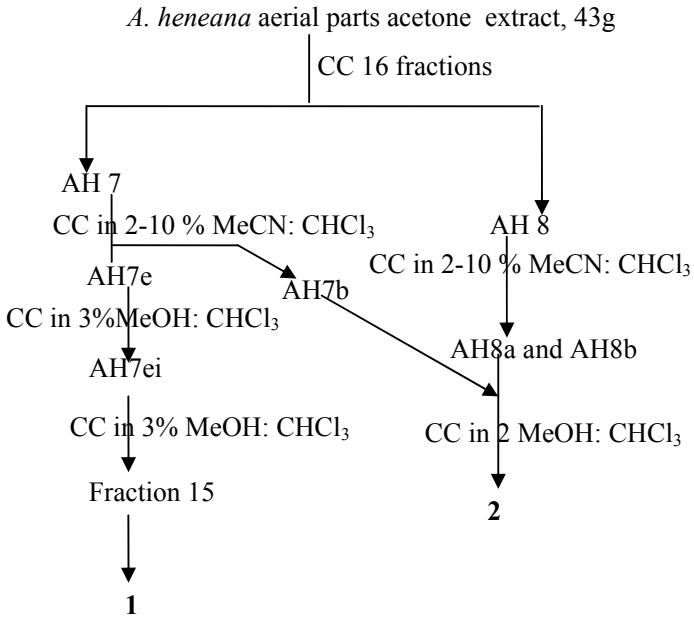
4.3. Extraction and Isolation:

Pulverized aerial parts (1.8 kg) were extracted with acetone (3 L × 3 × 14h) at room temperature. The acetone solubles were filtered and concentrated under reduced pressure to provide a greenish acetone extract (45.0g, 2.5% based on dry plant weight). Acetone extract, 43.0g, was separated by column chromatography (CC) in acetone: pet-ether gradient to collect 16 fractions (AH1-AH16).

Fraction AH7 (1.8g) was subjected to CC in acetonitrile: chloroform gradient from 2 to 10 % to collect six fractions (AH7a-AH7f). From fraction AH7e, compound **1** (8 mg) was obtained by repeated CCs. Fraction AH8 (5.2g) was subjected to CC in acetonitrile: chloroform gradient from 2 to 10 % to collect eight fractions (AH8a-AH8h). Fractions AH8a, AH8b and AH7b were combined and compound **2** (80mg) was obtained after CC in 2% methanol in chloroform.

Fraction AH11 (3.4g) was subjected to CC in 3% methanol in chloroform to collect eleven fractions (AH11a-AH11k). From fraction AH11f, compound **3** (12mg) was obtained as white precipitate which was purified by washing it successively with chloroform and acetone. Fraction AH11g was subjected to CC in 6% methanol in chloroform to collect eight fractions (AH11gi-AH11gviii). From fraction AH11gii, compound **4** was obtained as white crystals (14mg).

Fraction AH14 (15.6g) was subjected to CC in acetonitrile: chloroform from 5 to 50 % to cllect fifteen fractions (AH14a-AH14n). From AH14k, compound **5** was isolated as brown amorphous powder (25mg) after preparative TLC using 25% methanol in chloroform as developing system.



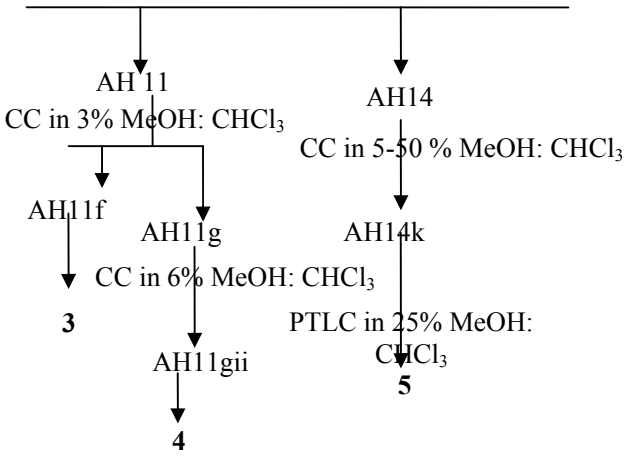
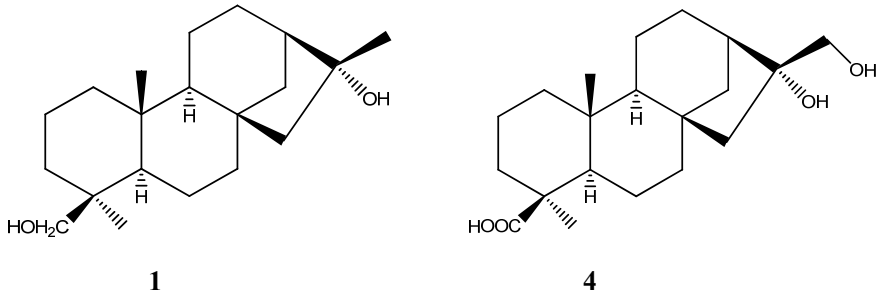
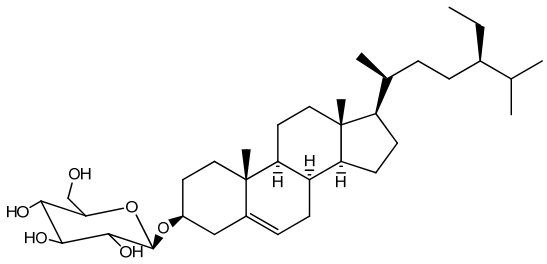


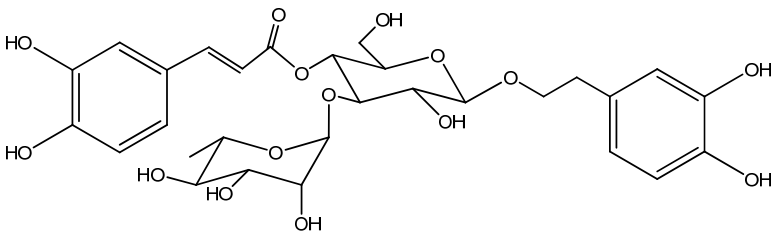
Chart 1: Chromatographic separation of *A. heyneana.*



c



2



3

5

Figure 2: Compounds isolated from *A. heyneana*

4.4. Structure elucidation:

Compound **1**:

Compound **1** was obtained as white needles. The ESIMS of **1** showed an $[M + 1]^+$ at *m*/*z* 307, $[M + Na]^+$ at *m*/*z* 329 suggesting the molecular formula $C_{20}H_{34}O_2$ with four degrees of unsaturation. The IR spectrum showed a stretching frequency of 3419cm^{-1}indicating presence of hydroxyl group.

The ^{1}H NMR and ^{13}C NMR data of **1** (Table 1) was nearly superimposable on a known *ent*-kaurane **6** (Figure 3) isolated as a microbial transformation product [5] indicating that the structure of **1** was closely related to that of **6**, except differing at positions 13, 14, 15, 16 and 20. This indicated compound **1** to be a phyllocladane type diastereomer of **6** with configurations at positions, 4, 5, 9 and 10 all reversed with respect to *ent*-kaurane.

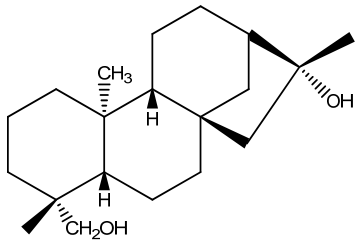
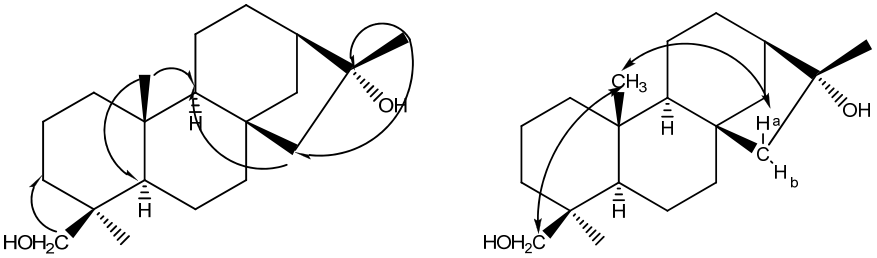


Figure 3: known *ent*-kaurane **6**

The significant upfield shift in C-20, C-15 and downfield shift in C-14 can be rationalized from mutual 1,3-diaxial shielding effect of C-20 and C-15 due to their syn orientation in phyllocladane as well as relief of such shielding in case of C-14. In case of **6**, this 1,3-diaxial shielding interaction between C-20 and C-15 is absent as even though placed 1, 3, they are anti and hence do not shield each other. Observation of NOE correlation peak between H-20 and one of the H-15 protons confirmed the syn configuration (Figure 4b). NOE peak between H-20 and one of H-19 protons confirmed relative orientation of C-20 methyl and C-19 (β) methylene.

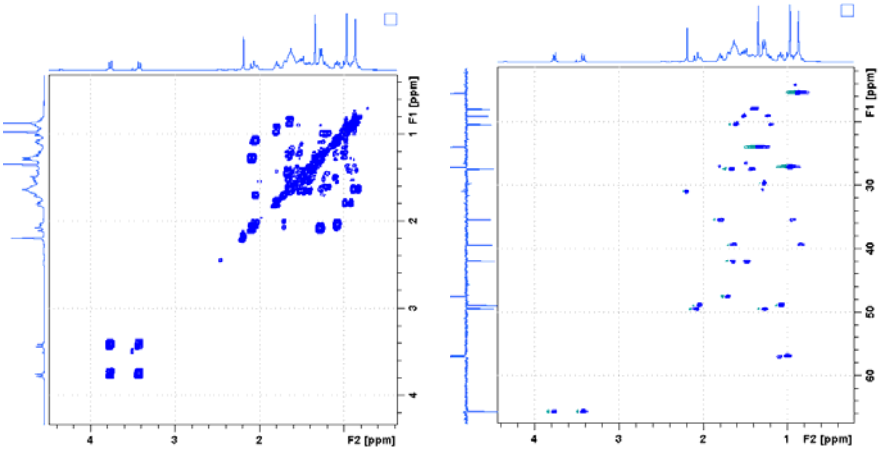
Assigned structure was additionally confirmed by detailed analysis of HMBC spectrum. The observed correlations are shown in Figure 4a.



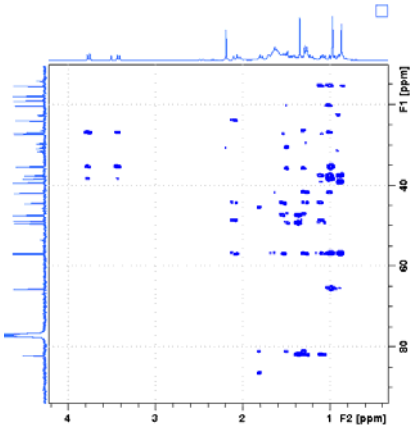
4a. HMBC correlations **4b.** NOESY correlations

Figure 4: 2D correlations for compound **1**

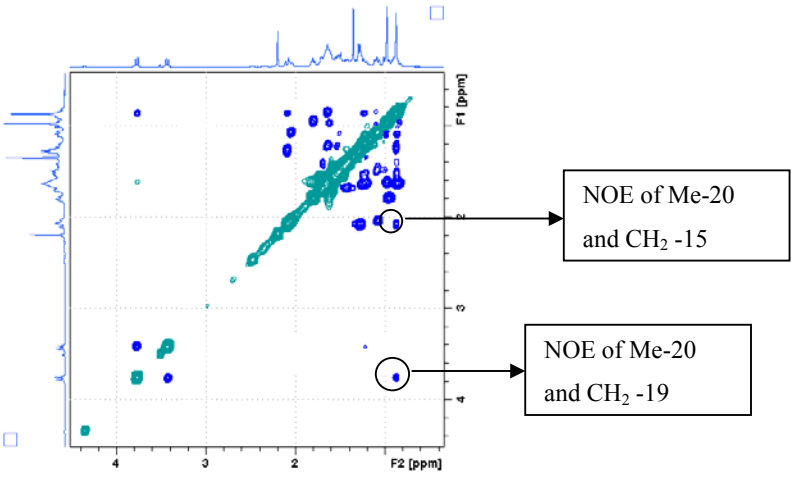
Thus Compound **1**, was identified as phyllocladan-19, 16-diol. This is the first report of this compound from family Lamiaceae. It has been reported from *Bromelia penguin* (Bromeliaceae) [6] and *Ailanthus tryphysa* (Simarubaceae) [7]. Comparison of NMR values with the reported values [7] revealed differences at some places (Table 1) probably due different solvents used to record spectra ($CDCl_3$ for compound **1** and CD_3OD for literature values).



5a. COSY spectrum **5b.** HSQC spectrum



5c. HMBC spectrum



5d. NOESY spectrum with key correlations in text box.

Figure 5: 2D Spectra of compound **1**

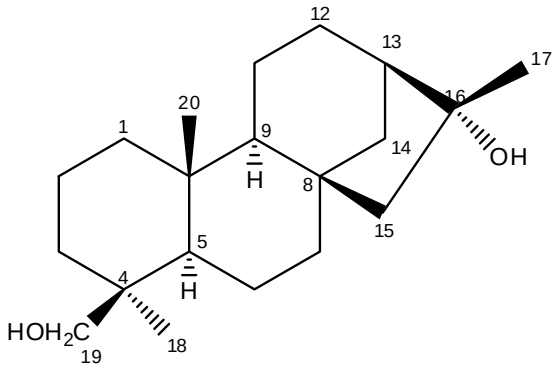


Figure 6: Structure of compound **1**.

Compound **2**:

Compound **2** was obtained as white needles. The ESIMS of **2** showed an $[M + 1]^+$ at *m/z* 329, $[M + Na]^+$ at *m/z* 351 suggesting the molecular formula $C_{20}H_{24}O_4$ with nine degrees of unsaturation. The UV spectrum showed absorbances at 236, 259, 263, 280, 312, 342nm. The IR spectrum showed a stretching frequency of α,β-unsaturated lactone(1755 cm^{-1}) and hydroxyl (3483cm^{-1}) and olefinic (1633, 1437 cm^{-1}) groups.

The ^1H NMR spectroscopic data of compound **2** (Table 2) displayed the presence of two tertiary methyl protons at δ 1.72 and 1.59 while ^{13}C NMR data revealed presence of eight olefinic carbons including an exomethylene group at δ 122.59. These indicated compound **2** to be diterpene with cembrane skeleton. Literature revealed compound **2** to be ovatodiolide [8]. Ovatodiolide is reported from other *Anisomeles* species [8].

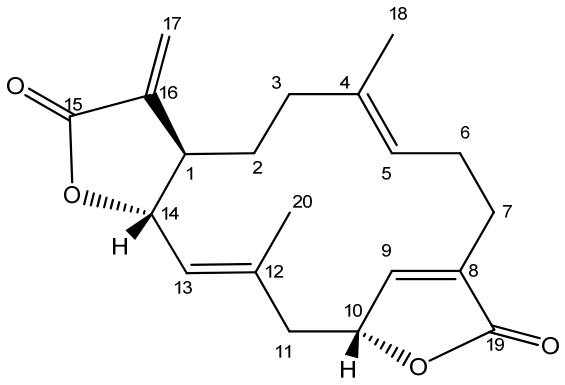


Figure 7: Structure of compound **2**.

Compound **3**:

Compound **3** was obtained as white amorphous powder. The ESIMS of **3** showed an $[M + 1]^+$ at *m/z* 577, $[M + Na]^+$ at *m/z* 599 suggesting the molecular formula $C_{35}H_{60}O_6$ with six degrees of unsaturation. ^{13}C NMR data revealed presence of resonances indicative of glucose moiety. 29 peaks with six methyl, 11 methylene, 9 methine and 2 quaternary carbons including two olefinic carbons at δ122.03 and 141.00 indicated the aglycone to be β-sitosterol. Comparison with literature value (Table 3) [9] identified compound **3** to be β -sitosterol-3-*O*-β-D-glucopyranoside.

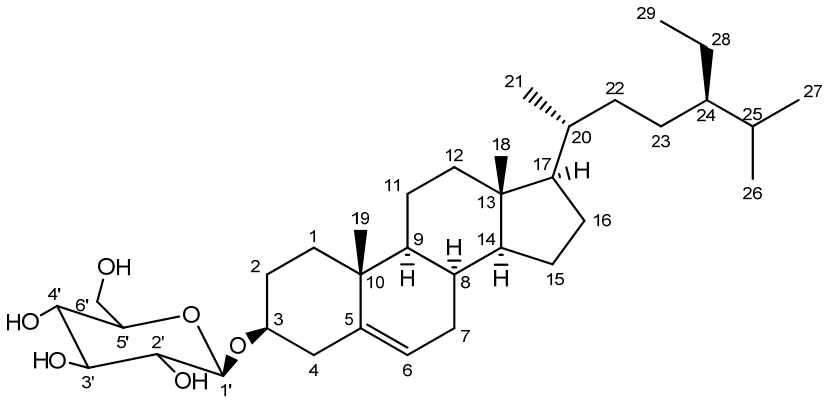


Figure 8: Structure of compound **3**.

Compound **4**

Compound **4** was obtained as white needles. The ESIMS of **4** showed an $[M + 1]^+$ at *m/z* 337, $[M + Na]^+$ at *m/z* 359 suggesting the molecular formula $C_{20}H_{32}O_4$ with five degrees of unsaturation.

The ^1H NMR and ^{13}C NMR data of **4** was nearly superimposable on a known *ent*-kaurane **7** isolated as microbial transformation product [10] (Figure 9)

indicating that the structure of **4** was closely related to that of known *ent*-kaurane, except differing appreciably at positions 14, 15 and 16. By comparing with compound **1**, compound **4** was identified as belonging to phyllocladane skeleton. Presence of quaternary carbon at δ181.8, 85.7 and methylene carbon at δ 66.15 indicated presence of carboxylic acid and two hydroxyl substituted carbons. Again by comparing with compound **1**, they were assigned positions 19, 16 and 17 respectively.

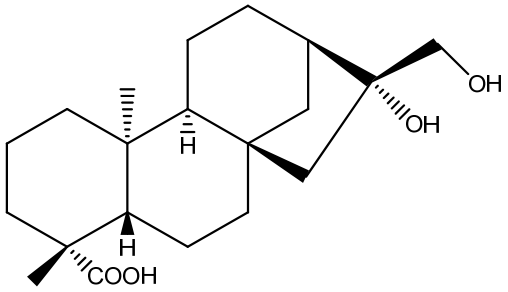


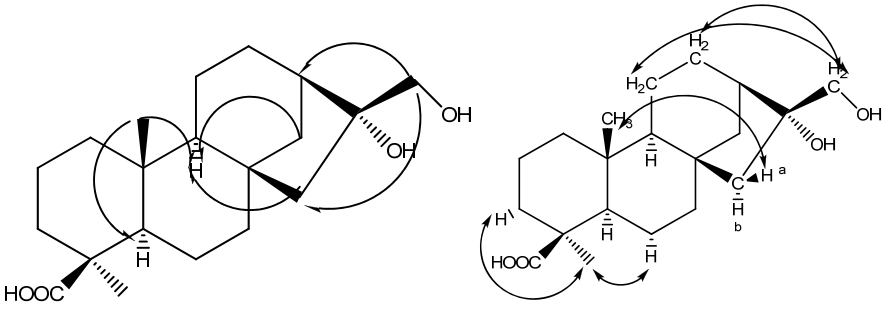
Figure 9: Known *ent*-kaurane **7**

Above assignment was confirmed by detailed analysis of HMBC spectrum as shown in figure 9a.

Syn geometry of H-20 and H-15 was confirmed by observation NOE peak between them. β orientation of C-17 was determined by observation of NOE with C-11 and C-12 methylene protons.

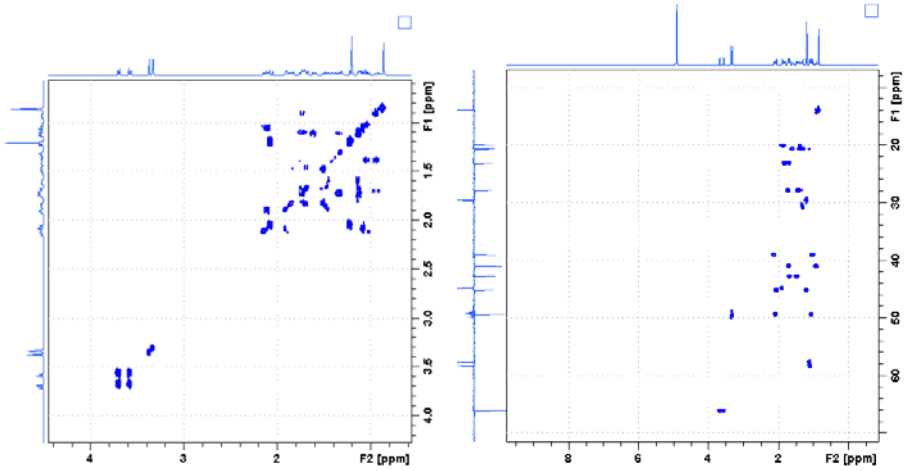
NOE peaks between methyl protons at C-4 with H-3α and H-6α placed methyl α (equatorial) and carboxylic group was assigned β (axial) orientation.

Thus, compound **4** was identified as a new natural product, phyllocladan-16, 17-dihydroxy-19-oic acid. The assigned structure was confirmed by single crystal X-ray crystallography (Figure 12, Table 5).

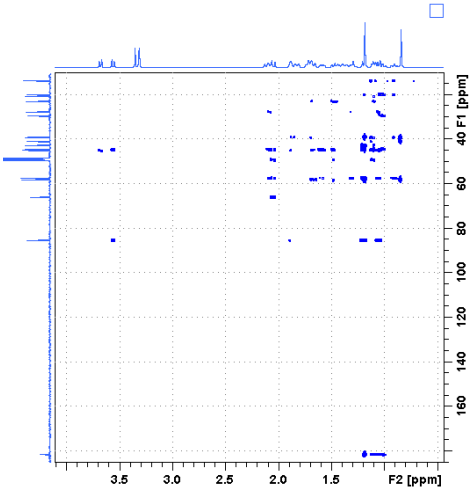


10a. HMBC correlations **10b.** NOESY correlations

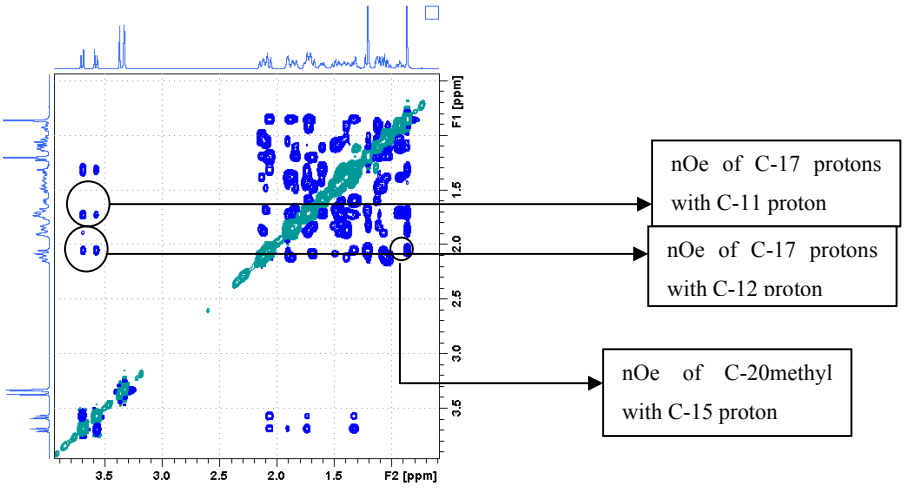
Figure 10: 2D correlations for compound **4**



11a. COSY spectrum **11b.** HSQC spectrum



11c. HMBC spectrum



11d. NOESY spectrum with key correlations in text box.

Figure 11: 2D spectra of compound **4**

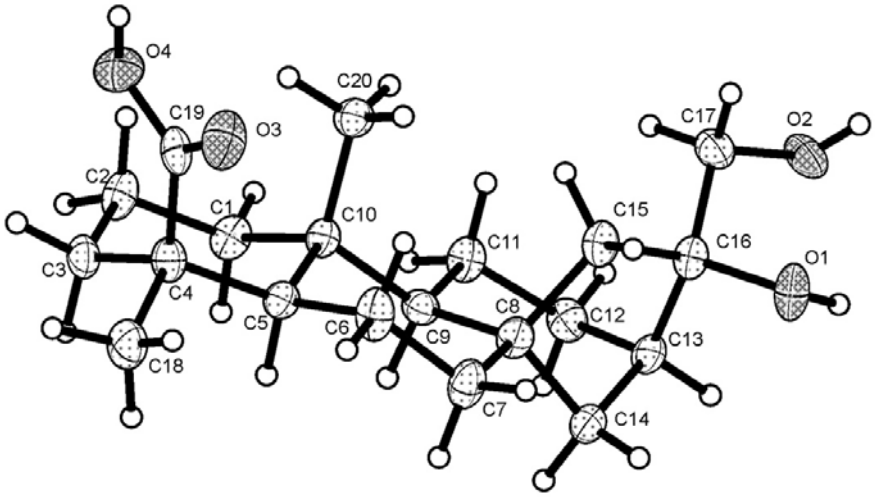


Figure 12: ORTEP diagram of compound **4** (Ellipsoids are at 50% probability).

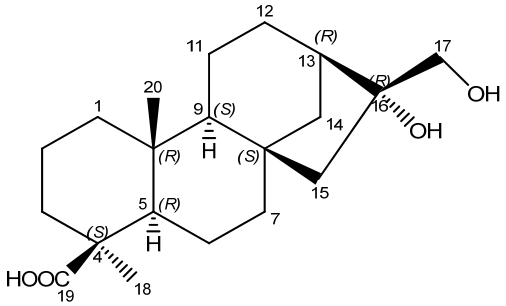


Figure 13: Structure of compound **4**.

Compound **5**:

Compound **5** was obtained as brown amorphous powder. The ESIMS of **5** showed an $[M + Na]^+$ at *m/z* 647 and $[M + K]^+$ at *m/z* 663 suggesting the molecular formula $C_{29}H_{36}O_{15}$ with twelve degrees of unsaturation. The signals in ^{13}C NMR were discernible into two sugar and aromatic regions. Resonances methine carbons at δ114.41, 148.12 and quaternary carbon at δ168.36 indicated one of the aromatic moiety to be cinnamic acid derivative while resonances at δ 72.24 and 36.50 (both methylene) indicated another to be a phenylethylene portion. Coupling of 15.9 Hz of cinnamoyl olefinic protons (at δ6.28 and 7.56) indicated a trans-cinnamoyl moiety. Similarly, methyl resonance at δ18.5 hinted at one of the sugars to be rhamnose and other to be glucose (from methylene at δ 62.3). Literature revealed two compounds, verbascoside (=acteoside) and isoacteoside to be closely matching with compound **5** [11]. Comparison of ^{13}C NMR of compounds isoacteoside and verbascoside is given in Table 6. This

unambiguously established compound **5** to be verbascoside. Verbascoside is of widespread occurrence [11].

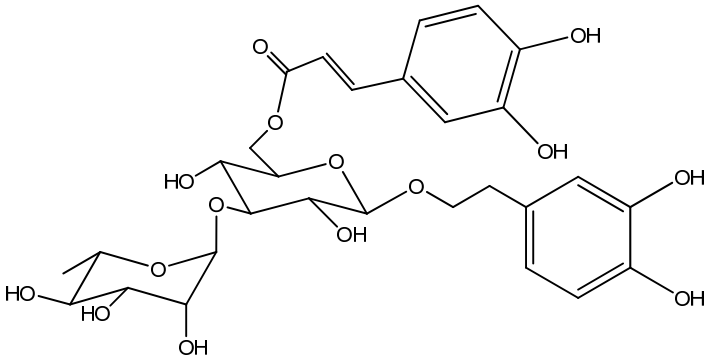


Figure 14: Structure of Isoacteoside.

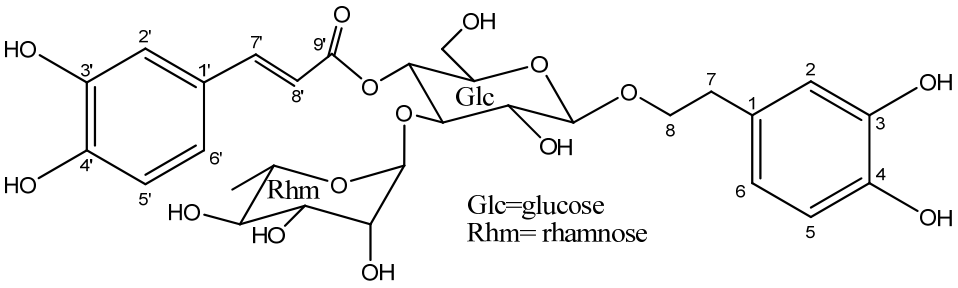


Figure 15: Structure of compound **5**.

4.5: Experimental:

i) Collection and processing:

A. heyneana, whole plant, in flowering, was collected and processed for preparation of acetone extract as described earlier.

ii) Extraction and Isolation:

Extraction and isolation of compounds from acetone extract is described earlier.

Compound **1**:

Colourless crystals (8.0mg, 0.00044% based on dry weight of plant), mp 192^{0}C (reported mp 193-193^{0}C [4]); ESIMS at *m/z* 329 $[M + Na]^+$; $[\alpha]_D^{21..9}$ +6 (0.45% methanol); IR (chloroform) 3419cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data, see Table 1.

Compound **2**:

White crystals (80 mg, 0.0044%), mp139^{0}C (reported mp 150^{0}C [5]); ESIMS at *m/z* 351 $[M + Na]^+$ and at *m/z* 367$[M + K]^+$; UV (acetone), 236, 250, 263, 280, 312, 342nm; $[\alpha]_D^{23.3}$ + 30.09 (*c*1%, acetone), reported $[\alpha]_D^{25}$ + 22.3 09 (*c*1%, chloroform) [5]; IR (chloroform) 3483, 1755, 1633, 1437, 1267, 1121cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data, see Table 2.

Compound **3**:

White amorphous powder (12 mg, 0.00067%); ESIMS at *m/z* 599 $[M + Na]^+$ and at *m/z* 615$[M + K]^+$; ^{1}H NMR, 0.67 (3H, s, H- 8), 0.88 (3H, d, *J* = 7.0 Hz, H-27), 0.89 (3H, t, *J* = 7.4 Hz, H-29), 0.92 (3H, d, *J* = 7.3Hz, H-26), 0.95 (3H, s, H-19), 1.00 (3H, d, *J* = 6.5 Hz, H-21), 3.99 (2H, m, H-3 and H-5'), 4.09 (1H, brt, *J* = 8.1 Hz, H-2'), 4.32 (2H, m, H-3' and H-4'), 4.44 (1H, dd, *J* = 11.7, 5.2 Hz, H-6'), 4.59(1H, dd, *J* = 11.7, 2.4 Hz, H-6'), 5.07 (1H, d, *J* = 7.7 Hz, H-1'), 5.36 (1H, m, H-6); for ^{13}C NMR spectral data, see Table 3.

Compound **4**:

Buff coloured crystals (14 mg, 0.00078%); mp 262^{0}C; ESIMS *m/z* 337 $[M +1]^+$ and *m/z* 359 $[M + Na]^+$; $[\alpha]_D^{24.9}$ 48.53 (0.64% meth) , 32.28 (1.1% methanol); IR (chloroform) 3419, 1640, 1090cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data, see Table 4.

X-ray crystallography:

Single crystals of the compound were grown by slow evaporation of the solution in acetone. Crystal of approximate size 0.13 x 0.05 x 0.04 mm^3 was used for data collection on *Bruker SMART APEX* CCD diffractometer using Mo K_α radiation. Exposure / frame = 15.0 sec / frame. Crystals belong to Monoclinic, space group P21, a =10.9410(6), *b* = 7.1550(4), *c* = 11.8020(6) Å, *V* = 870.33(11) $Å^3$, *Z* = 2, D_c = 1.284 g /cc, μ (Mo K_α) = 0.71073 Å, *T* = 150(2) K, 8336 reflections measured, R value 0.0360, wR2 = 0.0839. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL)[12] was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement as per the riding model. Data collection and refinement parameters as well as compound bond angles, bond lengths and torsion angles are listed in Tables 5a-5d.

X-ray analysis revealed the relative conformation of the molecule at C4, C5, C8, C9, C10, C13, and C16 as S, R, S, S, R, R and R configurations.

Compound **5**:

Brown amorphous powder (25mg, 0.0014%), mp250-265^{0}C; ESIMS at *m*/*z* 647 $[M + Na]^+$ and at *m*/*z* 663$[M + K]^+$; $[\alpha]_D^{23.3}$ -48 to -70 (*c* 0.5, methanol); UV (methanol) 250, 290 and 334nm; ^{1}H NMR and ^{13}C NMR spectral data, see Table 5.

4.6. Evaluation of anti-mycobacterial activity.

General procedure for evaluation of inhibitory activity of compounds against *M. tuberculosis* is given in chapter 2. Results are given in Table 7. Of all the compounds, **2** is found to be very potent (IC_{50} 4.73 µg/ml). Compounds **3**, **4** and **5** were inactive while compound **1** is being evaluated. Further confirmation of activity and evaluation of the compounds against other biological activities is underway.

4.7. Tables:

Table 1: ^{1}H NMR and ^{13}C NMR data for compound **1**

Carbon no	**1** (in $CDCl_3$)		**7** (in $CDCl_3$)*		Literature value of **1** (in CD_3OD)**	
	$\delta_C{}^1$	$\delta_H{}^2$ (multiplicity)	$\delta_C{}^1$	$\delta_H{}^2$ (multiplicity)	$\delta_C{}^1$	$\delta_H{}^2$ (multiplicity)
1	39.21	0.84, 1.63	40.4	1.79, 0.80	40.6	1.65, 0.82
2	17.89	**1.39**	18.3	1.41, 1.58	19.0	1.52
3	35.35	1.79 α, 0.94 β	35.7	1.75, 0.90	36.5	1.81, 0.84
4	38.36	-	39.3	-	38.8	-
5	56.78	0.99 (m)	56.8	0.91	58.4	0.96
6	20.29	1.60 α, 1.20 β	20.7	1.65, 1.31	21.3	1.60, 1.20
7	41.88	1.65(m), 1.49(m)	42.5	1.62, 1.45	43.2	1.62, 1.46
8	44.31	-	45.3	-	45.5	-
9	56.89	1.07	57.0	0.99	58.6	1.08
10	37.58	-	38.6	-	39.6	-
11	18.99	**1.51, 1.23**	18.0	1.48, 1.43	20.3	1.54, 1.27
12	27.3	**1.67**, **1.41**	26.8	1.27, 1.54	28.5	1.65-1.67
13	**47.39**	**1.70**	49.0	1.82	48.1	1.68
14	**48.78**	**2.04, 1.07**	37.5	1.57, 1.88	50	2.05, 1.01
15	**49.38**	**2.09a** (dd 14, 1.70Hz), **1.26b**	57.9	1.55	50	2.02, 1.24
16	**82.04**	-	79.3		82.7	-
17	23.83	1.35 (s)	24.4	1.35	23.9	1.29
18	26.97	0.98 (s)	65.6	3.72, 3.44	27.9	0.88
19	65.64	3.77 (1H, d, J=11Hz), 3. 42 (1H,d, J=11Hz)	27.0	0.95	65.2	3.73, 3.29
20	**15.35**	**0.87** (s)	18.2	1.01	16.1	0.90

* As given in the literature [2], ** as given in the literature [4]

Table 2: ^{1}H NMR and ^{13}C NMR data for compound **2**

No.	**2**		Ovatodiolide*
	^{13}C (δ)	^{1}H (δ)	^{13}C
1	42.58	2.42(m)**	42.6
2	23.60	2.83(m), 2.12(m)	23.7
3	24.76	2.5(m), 2.42(m)	24.7
4	131.25	-	131.3
5	124.87	4.85 (bt, 5.69)	124.8
6	33.14	1.64(m)	33.2
7	36.13	2.19(d, 14.61), 2.04(m)	36.1
8	139.63	-	139.7
9	147.39	7.02(bs)	147.5
10	77.85	4.81 (dd, 10.17, 1.42)	77.8
11	40.11	2.84(dd, 10.34, 3.61), 2.29(dd, 10.41, 3.65)	40.0
12	134.12	-	133.9
13	128.88	5.10(d, 10.43)	128.6
14	78.72	5.08 (bs)	78.7
15	134.30	-	134.0
16	172.96	-	172.8
17	122.59	5.57(d, 1.5), 6.18(d, 1.5)	122.2
18	14.96	1.59(bs)	15.0
19	170.20	-	169.9
20	19.16	1.72 (d, 0.88)	19.2

* As given in the literature [5], **multiplivitie in bracket, coupling constant in Hz

Table 3: ^{13}C NMR data for compound **3**

No.	**3** Pyridine-d_5	**3** ($CDCl_3$ + CD_3OH)	Literature* Pyridine-d_5	No.	**3** Pyridine-d_5	**3** ($CDCl_3$ + CD_3OH)	Literature* Pyridine-d_5
1	37.59	39.06	37.52	19	19.31	19.24	19.26
2	30.35	28.66	30.30	20	36.50	36.58	36.43
3	78.19	79.49	78.16	21	19.12	19.06	19.05
4	39.44	37.71	39.39	22	34.31	34.36	34.26
5	141.00	140.81	140.96	23	26.44	29.98	26.44
6	122.03	122.48	121.96	24	46.14	46.31	46.09
7	32.28	32.32	32.22	25	29.55	29.55	29.51
8	32.15	32.35	32.10	26	19.53	20.03	19.46
9	50.44	50.66	50.39	27	20.09	19.61	20.02
10	37.02	37.14	36.97	28	23.49	23.45	23.44
11	21.39	21.48	21.33	29	12.27	12.17	12.20
12	40.05	40.21	39.99	1’	102.68	101.58	102.62
13	42.58	42.75	42.53	2’	75.45	70.63	75.38
14	56.93	57.22	56.87	3’	78.60	76.47	78.65
15	24.62	24.68	24.55	4’	71.79	74.03	71.75
16	28.66	26.42	28.58	5’	78.72	76.98	78.55
17	56.34	56.50	56.29	6’	62.93	62.13	62.88
18	12.08	12.15	12.02				

* As given in the literature [6]

Table 4: ^{1}H NMR and ^{13}C NMR data for compound **4**

Carbon no	**4** ($CDCl_3$)		**8***	
	δ_C[1]	δ_H[2] (multiplicity)	δ_C[1]	δ_H[2] (multiplicity)
1	41.0	0.89 (dt, 4.1,13.3), 1.70 β	40.5	1.89, 0.86
2	20.06	1.37m, 1.86m β	18.9	1.94, 1.43
3	39.12	2.11,1.00	37.8	2.15, 1.05
4	44.62	-	43.3	-
5	58.30	1.08	56.7	1.10
6	23.16	1.83, 1.70	21.9	1.81-1.90
7	42.84	1.66 (m), 1.46 (m)	41.9	1.65
8	44.84	-	44.4	-
9	57.65	1.1	55.9	1.49
10	39.39	-	39.4	-
11	**20.72**	**1.59α, 1.31β**	18.2	1.04
12	27.89	**1.43 α, 1.71 β**	25.9	1.54-1.67
13	44.81	**1.88**	44.8	1.52
14	**49.40**	**1.04, 2.10**	36.8	2.05, 1.64
15	**45.18**	2.04a((1H,d, J=11Hz), 1.19b(1H, d, J=11Hz),	52.3	1.95, 1.68
16	**85.7**	-	81.5	-
17	66.15	3.76 (1H, d, J=11Hz), 3.58 (1H, d, J=11Hz)	65.4	3.74, 3.63
18	29.56	1.18	180.2	-
19	181.8	-	28.1	1.21
20	13.90	0.84	14.8	1.01

* As given in the literature [7]

Table 5a. Crystal data and structure refinement for compound **4**

Parameter	Value
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 10.9410(6) Å α= 90°. b = 7.1550(4) Å β= 109.60 (10)° c = 11.8020(6) Å γ = 90°
Volume	870.33(8) $Å^3$
Z	2
Density (calculated)	1.284 Mg/m^3
Crystal size	0.13 x 0.05 x 0.04 mm^3
Reflections collected	833
Data / restraints / parameters	3060 / 1 / 222
Final R indices [I>2sigma(I)]	R1 = 0.0360, wR2 = 0.0839

Table 5b. Bond lengths for compound **4**

Carbon (no.)-Carbon (no.)	Bond lengths [Å]	Carbon (no.)-Carbon (no.)	Bond lengths [Å]
C(1)-C(2)	1.529(2)	C(8)-C(9)	1.547(2)
C(1)-C(10)	1.539(2)	C(8)-C(15)	1.557(3)
C(2)-C(3)	1.525(3)	C(9)-C(11)	1.542(2)
C(3)-C(4)	1.538(2)	C(9)-C(10)	1.569(2)
C(4)-C(19)	1.532(3)	C(10)-C(20)	1.542(3)
C(4)-C(18)	1.551(3)	C(11)-C(12)	1.534(2)
C(4)-C(5)	1.566(2)	C(12)-C(13)	1.530(3)
C(5)-C(6)	1.534(3)	C(13)-C(14)	1.528(3)
C(5)-C(10)	1.558(3)	C(13)-C(16)	1.544(3)
C(6)-C(7)	1.525(3)	C(15)-C(16)	1.556(2)
C(7)-C(8)	1.538(3)	C(16)-C(17)	1.521(3)
C(8)-C(14)	1.544(3)		

Table 5c. Bond angles for compound **4**

Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]	Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]
C(2)-C(1)-C(10)	114.50(14)	C(11)-C(9)-C(8)	110.89(14)
C(3)-C(2)-C(1)	111.31(15)	C(11)-C(9)-C(10)	112.82(14)
C(2)-C(3)-C(4)	114.77(15)	C(8)-C(9)-C(10)	117.11(14)
C(19)-C(4)-C(3)	112.79(16)	C(1)-C(10)-C(20)	108.57(15)
C(19)-C(4)-C(18)	104.93(14)	C(1)-C(10)-C(5)	108.62(14)
C(19)-C(4)-C(5)	113.85(15)	C(20)-C(10)-C(5)	111.74(14)
C(3)-C(4)-C(18)	107.02(16)	C(1)-C(10)-C(9)	107.16(14)
C(3)-C(4)-C(5)	109.00(14)	C(20)-C(10)-C(9)	113.88(14)
C(18)-C(4)-C(5)	108.90(15)	C(5)-C(10)-C(9)	106.66(14)
C(6)-C(5)-C(10)	110.64(14)	C(12)-C(11)-C(9)	114.26(15)
C(6)-C(5)-C(4)	116.00(14)	C(13)-C(12)-C(11)	113.22(14)
C(10)-C(5)-C(4)	115.45(15)	C(14)-C(13)-C(12)	108.67(15)
C(7)-C(6)-C(5)	110.22(15)	C(14)-C(13)-C(16)	101.73(15)
C(6)-C(7)-C(8)	114.08(16)	C(12)-C(13)-C(16)	113.99(15)
C(7)-C(8)-C(14)	111.03(15)	C(13)-C(14)-C(8)	102.17(15)
C(7)-C(8)-C(9)	109.62(14)	C(16)-C(15)-C(8)	107.31(14)
C(14)-C(8)-C(9)	107.65(15)	C(17)-C(16)-C(13)	118.14(15)
C(7)-C(8)-C(15)	114.07(15)	C(17)-C(16)-C(15)	110.99(15)
C(14)-C(8)-C(15)	100.18(14)	C(13)-C(16)-C(15)	103.80(14)
C(9)-C(8)-C(15)	113.79(15)		

Table 5d. Torsion angles for compound **4**

Connectivity	angles [°]	Connectivity	angles [°]
C(10)-C(1)-C(2)-C(3)	-53.9(2)	C(6)-C(5)-C(10)-C(20)	-66.78(19)
C(1)-C(2)-C(3)-C(4)	53.1(2)	C(4)-C(5)-C(10)-C(20)	67.49(18)
C(2)-C(3)-C(4)-C(19)	76.42(19)	C(6)-C(5)-C(10)-C(9)	58.26(18)
C(2)-C(3)-C(4)-C(18)	-168.69(15)	C(4)-C(5)-C(10)-C(9)	-167.46(14)
C(2)-C(3)-C(4)-C(5)	-51.1(2)	C(11)-C(9)-C(10)-C(1)	60.28(19)
C(19)-C(4)-C(5)-C(6)	56.5(2)	C(8)-C(9)-C(10)-C(1)	-169.18(16)
C(3)-C(4)-C(5)-C(6)	-176.64(17)	C(11)-C(9)-C(10)-C(20)	-59.81(19)
C(18)-C(4)-C(5)-C(6)	-60.2(2)	C(8)-C(9)-C(10)-C(20)	70.73(19)
C(19)-C(4)-C(5)-C(10)	-75.32(19)	C(11)-C(9)-C(10)-C(5)	176.47(14)
C(3)-C(4)-C(5)-C(10)	51.6(2)	C(8)-C(9)-C(10)-C(5)	-52.99(19)
C(18)-C(4)-C(5)-C(10)	167.99(15)	C(8)-C(9)-C(11)-C(12)	42.0(2)
C(10)-C(5)-C(6)-C(7)	-62.4(2)	C(10)-C(9)-C(11)-C(12)	175.62(15)
C(4)-C(5)-C(6)-C(7)	163.58(17)	C(9)-C(11)-C(12)-C(13)	-40.3(2)
C(5)-C(6)-C(7)-C(8)	57.2(2)	C(11)-C(12)-C(13)-C(14)	56.0(2)
C(6)-C(7)-C(8)-C(14)	-167.24(17)	C(11)-C(12)-C(13)-C(16)	-56.7(2)
C(6)-C(7)-C(8)-C(9)	-48.4(2)	C(12)-C(13)-C(14)-C(8)	-71.26(18)
C(6)-C(7)-C(8)-C(15)	80.5(2)	C(16)-C(13)-C(14)-C(8)	49.30(17)
C(7)-C(8)-C(9)-C(11)	179.40(16)	C(7)-C(8)-C(14)-C(13)	-166.35(15)
C(14)-C(8)-C(9)-C(11)	-59.73(18)	C(9)-C(8)-C(14)-C(13)	73.66(17)
C(15)-C(8)-C(9)-C(11)	50.32(19)	C(15)-C(8)-C(14)-C(13)	-45.49(17)
C(7)-C(8)-C(9)-C(10)	48.0(2)	C(7)-C(8)-C(15)-C(16)	143.69(15)
C(14)-C(8)-C(9)-C(10)	168.84(15)	C(14)-C(8)-C(15)-C(16)	25.04(17)
C(15)-C(8)-C(9)-C(10)	-81.11(19)	C(9)-C(8)-C(15)-C(16)	-89.53(17)
C(2)-C(1)-C(10)-C(20)	-69.1(2)	C(14)-C(13)-C(16)-C(17)	-155.62(16)
C(2)-C(1)-C(10)-C(5)	52.6(2)	C(12)-C(13)-C(16)-C(17)	-38.9(2)
C(2)-C(1)-C(10)-C(9)	167.53(17)	C(14)-C(13)-C(16)-C(15)	-32.27(17)
C(6)-C(5)-C(10)-C(1)	173.47(15)	C(12)-C(13)-C(16)-C(15)	84.49(17)
C(4)-C(5)-C(10)-C(1)	-52.3(2)	C(8)-C(15)-C(16)-C(17)	132.11(16)
		C(8)-C(15)-C(16)-C(13)	4.20(18)

Table 6: ^{1}H NMR and ^{13}C NMR data for compound **5**

	5 (CD_3OD)		Verbascoside* (CD_3OD)		Isoacteoside* (CD_3OD)
Caffeoyl	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	131.42	-	131.5	-	131.4
2	117.10	6.68 m	117.1	6.71 d (2.1)	117.1
3	146.06	-	146.1	-	146.0
4	144.61	-	144.6	-	144.6
5	116.29	6.68 m	116.4	6.7 d (8.4)	116.3
6	121.25	6.56 bd (7.4)	121.3	6.57 dd (2.1, 8.4)	121.3
7	36.50	2.79 bt (9.5)	36.5	2.79 t (9.6)	36.6
8	72.24	4.03 q (8.56), 3.72 q (7.8)	72.33	4.05 m, 3.74 m	72.3
Gucose					
	104.12	4.38 d (7.58)	104.2	4.38 d (7.8)	104.3
	76.14	3.4 bt (8.31)	76.2	3.4 dd (7.8, 9.3)	75.8
	81.67	3.82 t (9.0)	81.7	3.82 t (9.3)	**84.0**
	70.39	4.94 o	70.4	4.94 t (9.6)	70.0
	75.92	3.6-3.5 m	76.0	3.6-3.5 m	75.6
	62.27	3.6-3.5 m	62.3	3.6-3.5 m	**64.6**
Rhamnose					
	103.00	5.19 bs	103.0	5.19 d(1.5)	102.7
	72.29	3.93 bs	72.28	3.93 dd(7.8, 9.3)	72.3
	71.98	3.6-3.5 m	72.0	3.6-3.5 m	72.3
	73.73	3.30 o	73.8	3.30 t(9.6)	74.0
	70.48	3.6-3.5 m	70.6	3.6-3.5 m	70.4
	18.44	1.09 d(6.1)	18.5	1.9 d(6.0)	**17.8**
Cinnamoyl					
1’	127.38		127.7		127.7
2’	115.08	7.06 bs	114.7	7.03 (d 2.0)	115.1
3’	150.18	-	149.8	-	149.5
4’	146.94	-	146.8	-	146.7
5’	116.53	6.79 d(7.09)	116.6	6.79 d(8.1)	116.5
6’	123.30	6.95 bd (7.58)	123.3	6.89 dd(2, 8.1)	123.1
7’	148.12	7.6 d(15.9)	148.1	7.56 d(15.9)	147.2
8’	114.41	6.275 d(15.9)	115.3	6.28 d(15.9)	114.9
9’	168.36		168.4		**169.1**

* As given in the literature [8]

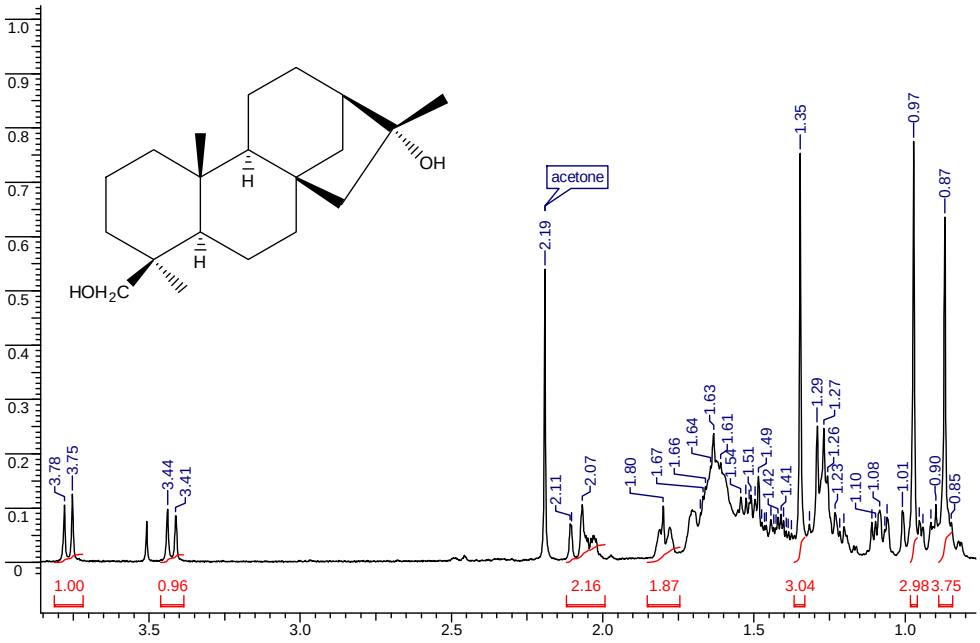
Table 7: Inhibitory activity of isolated compounds against *M .tuberculosis.*

Compounds	IC_{50} (µg/ml)
Compound **1**	Not tested
Compound **2**	4.93
Compound **3**	Not active
Compound **4**	Not active
Compound **5**	Not active

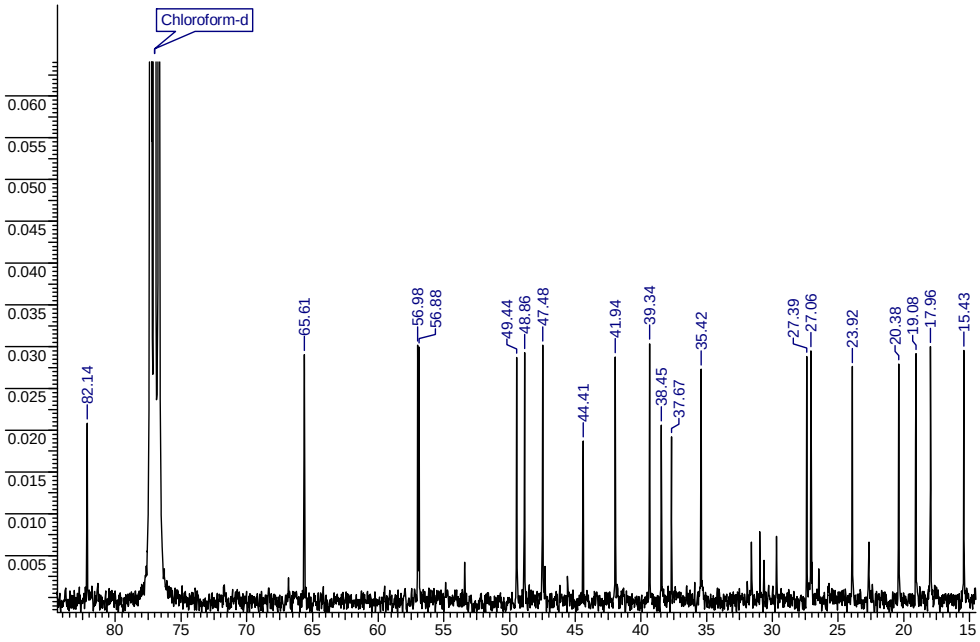
4.8: NMR data:

Compound **1**:

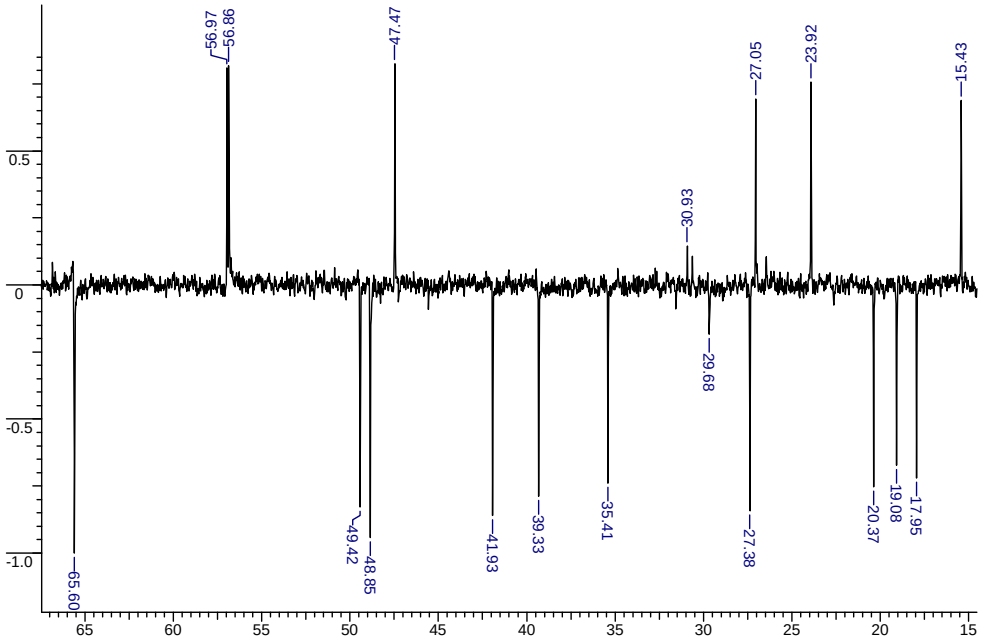
^{1}H NMR



^{13}C NMR:

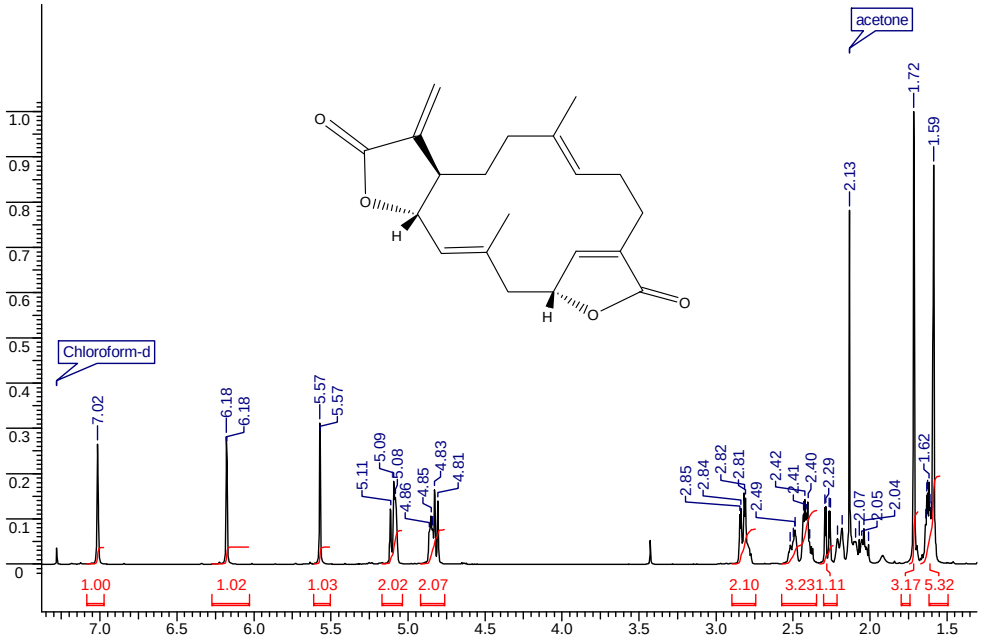


DEPT

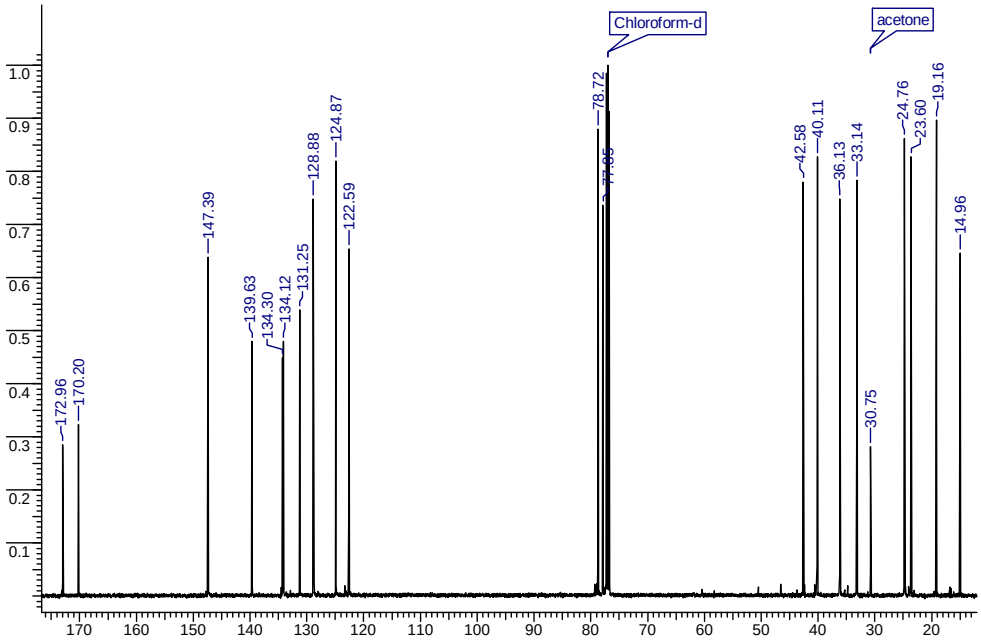


Compound **2**:

^{1}H NMR



^{13}C NMR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acetone
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147.39
139.63
134.30
134.12
131.25
128.88
124.87
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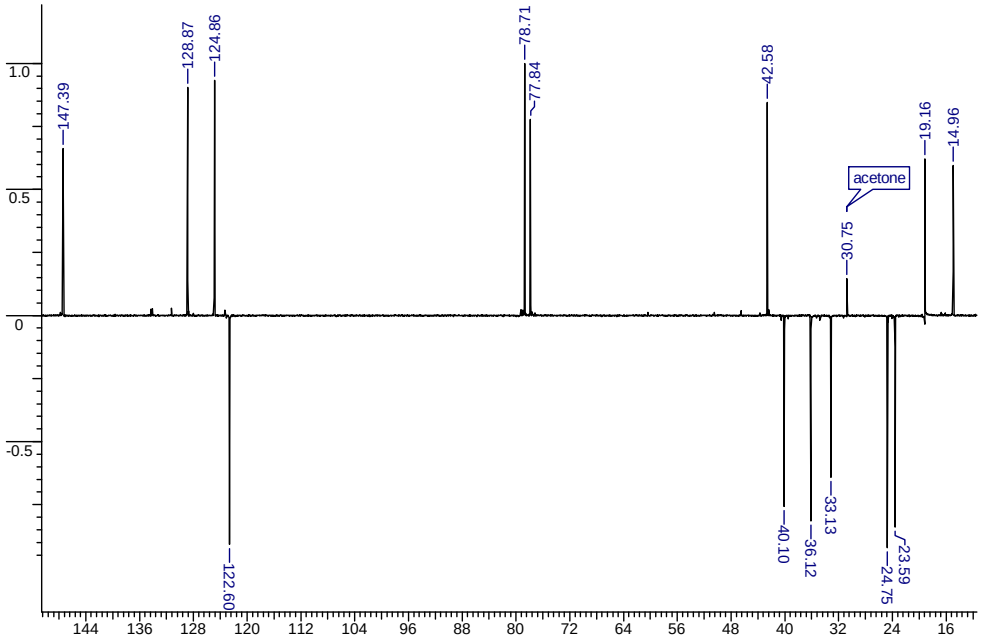
30.75
24.76
23.60
19.16
14.96

DEPT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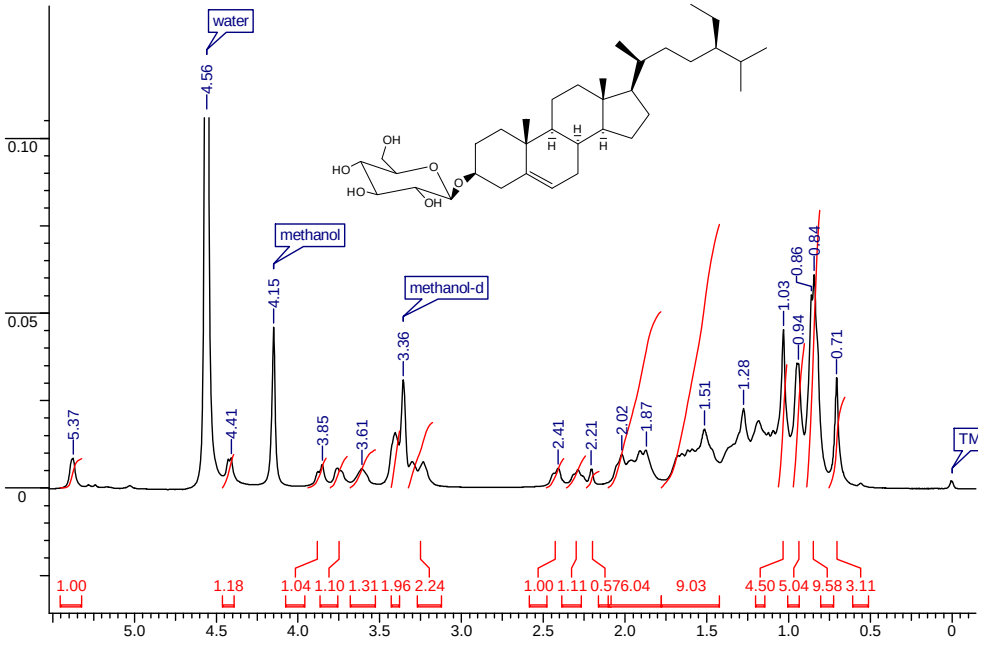
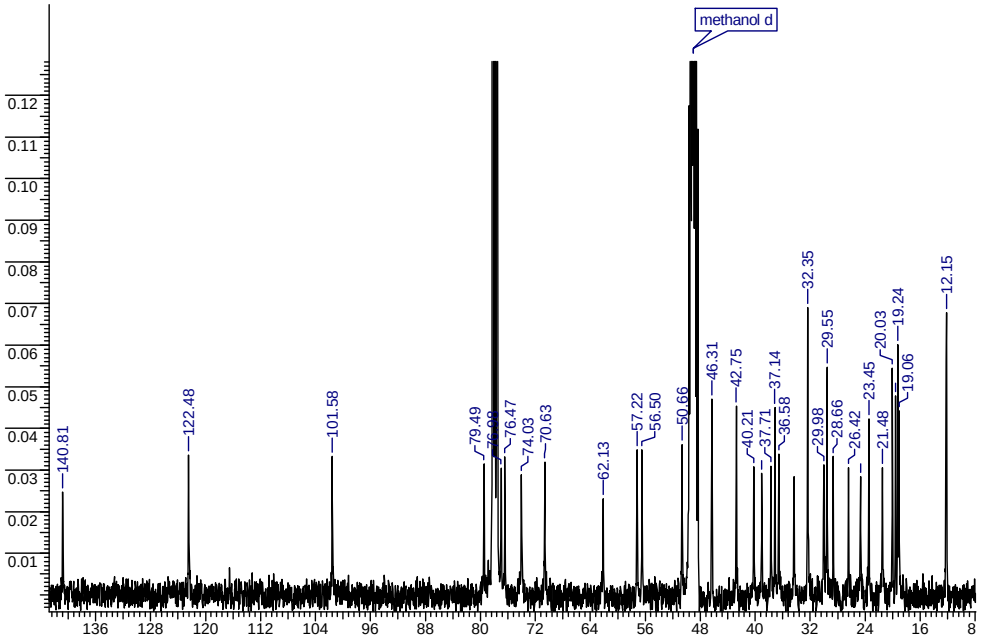
78.71
77.84
42.58
40.10
36.12
33.13
acetone
30.75
24.75
23.59
19.16
14.96

Compound **3**:

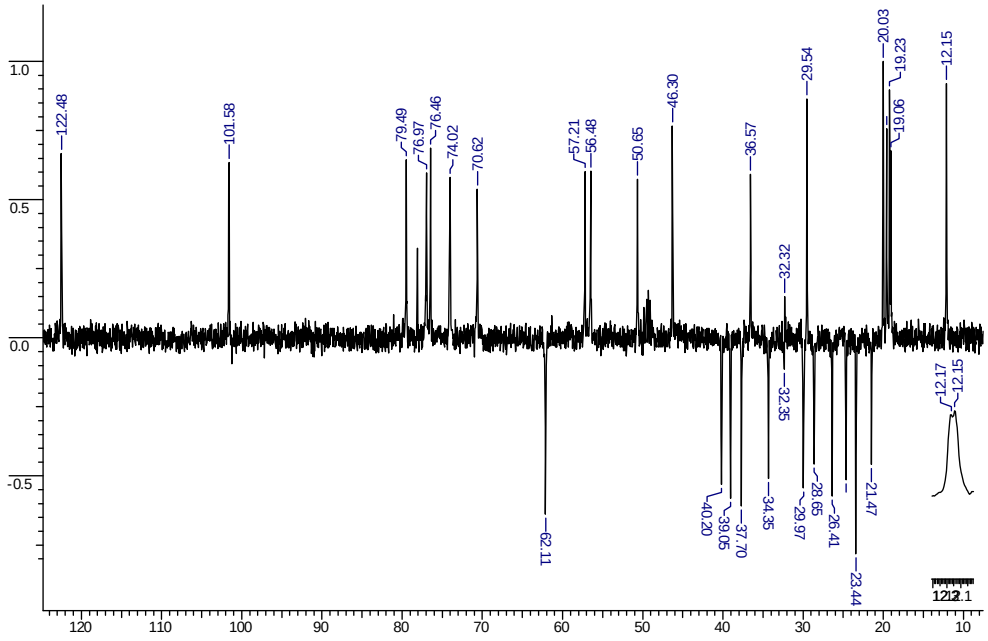
^{1}H NMR in CD_3OD + $CDCl_3$



^{13}C NMR in CD_3OD + $CDCl_3$

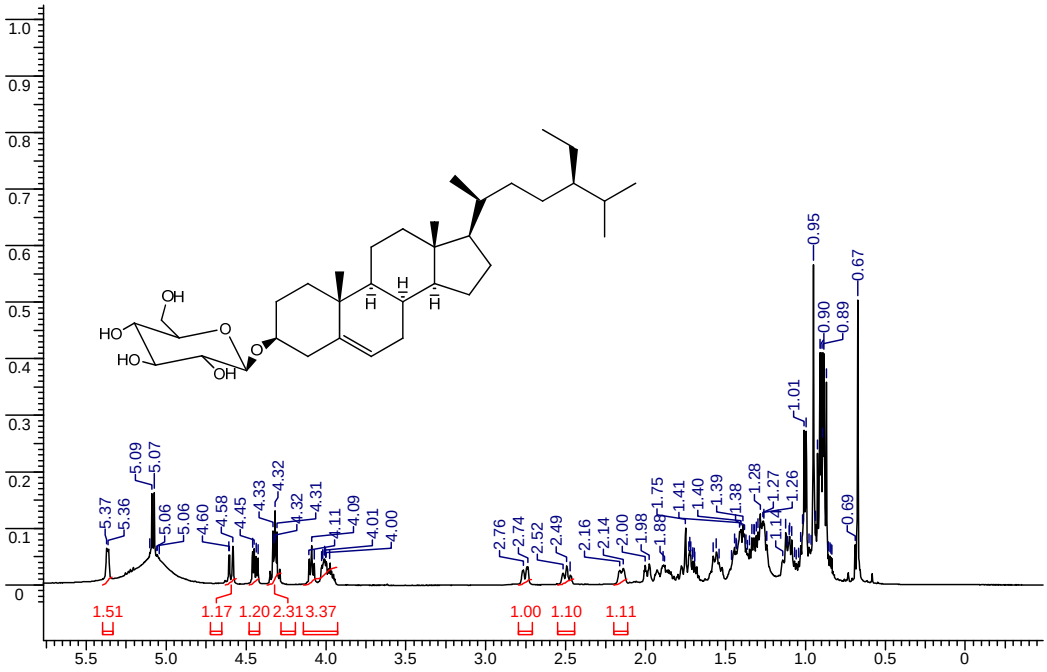


DEPT in $CD_3OD + CDCl_3$

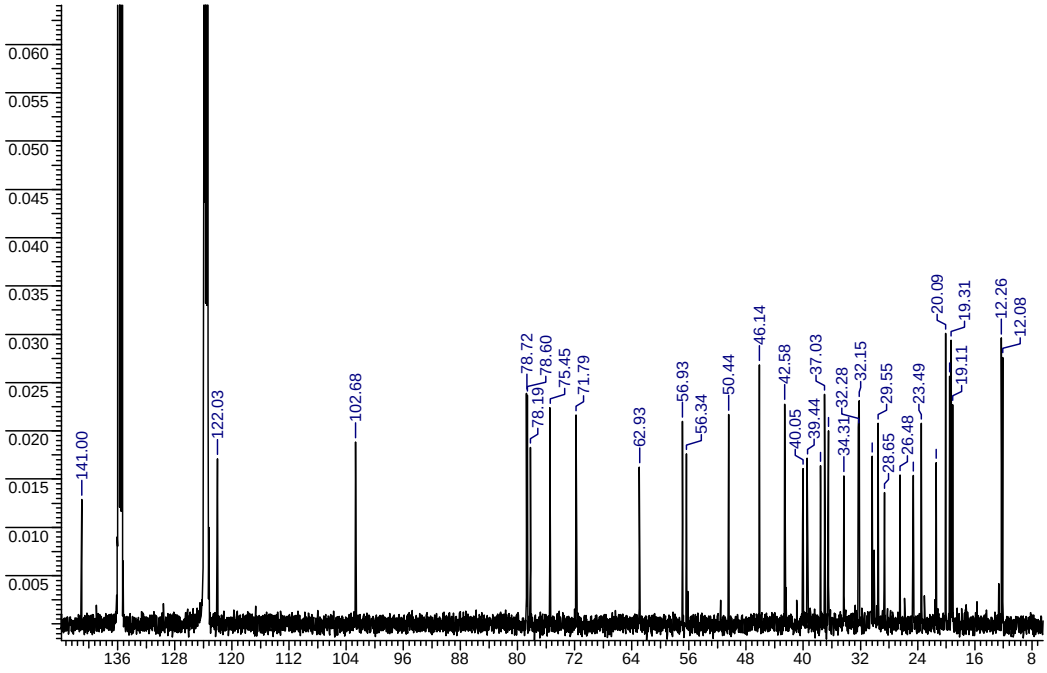


Compound **3**:

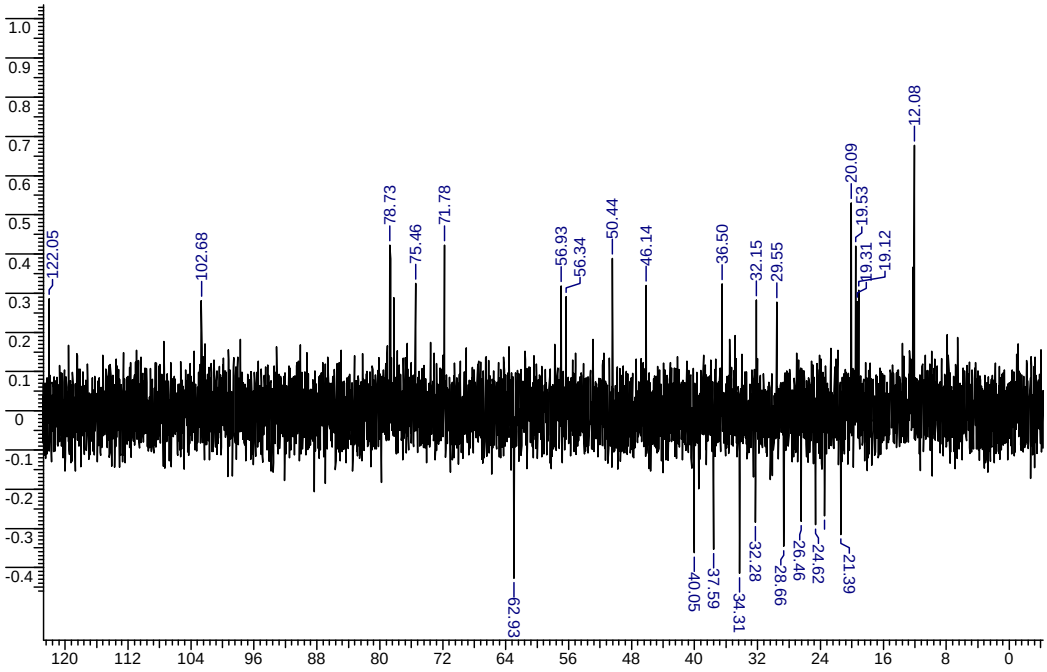
^1H NMR in Pyridine-d_5



^{13}C NMR in Pyridine-d$_5$

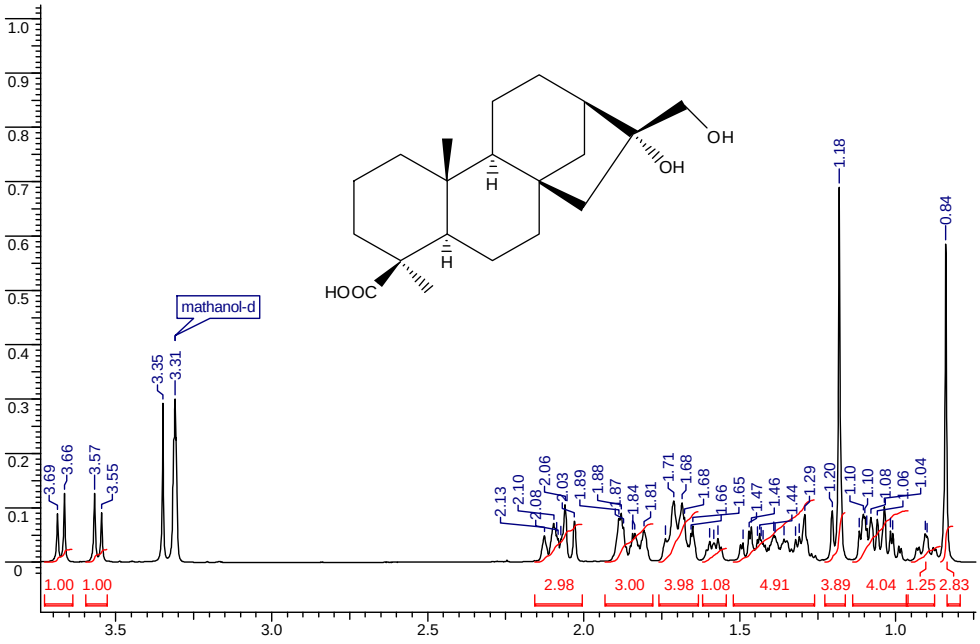


DEPT in Pyridine-d$_5$

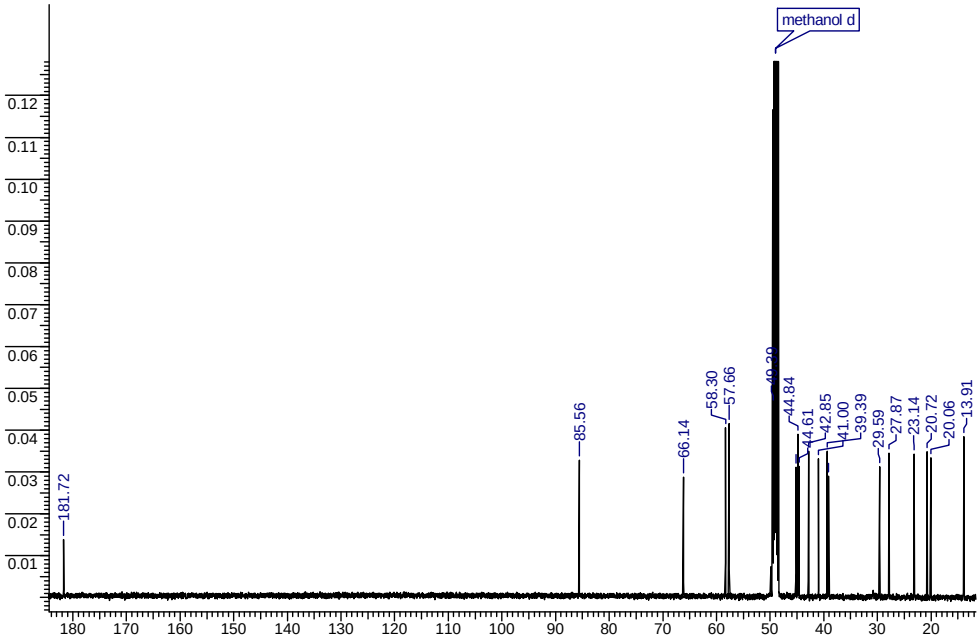


Compound **4**:

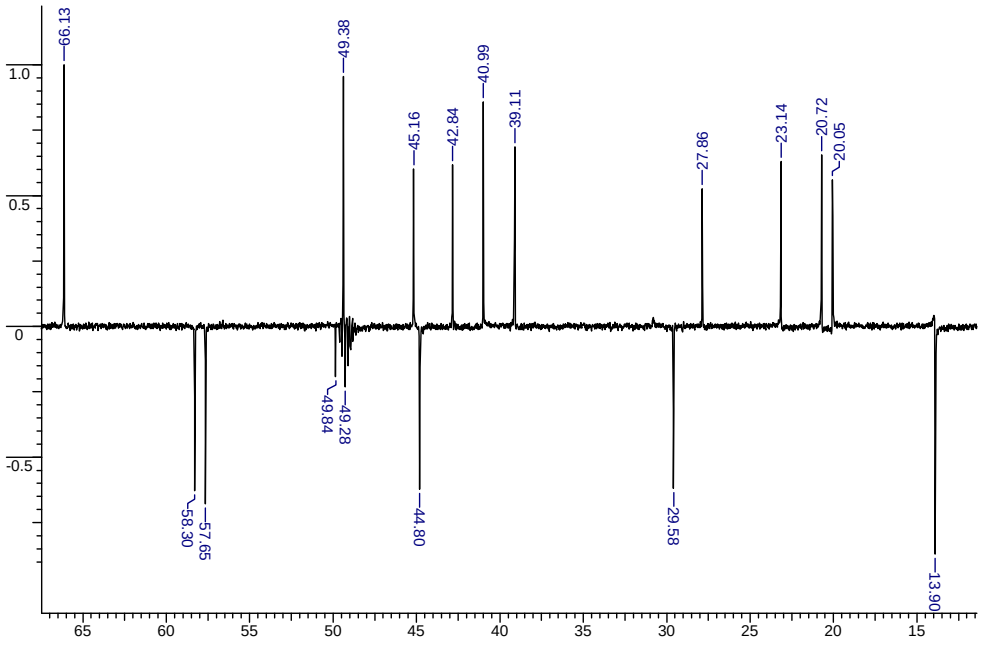
^{1}H NMR



^{13}C NMR

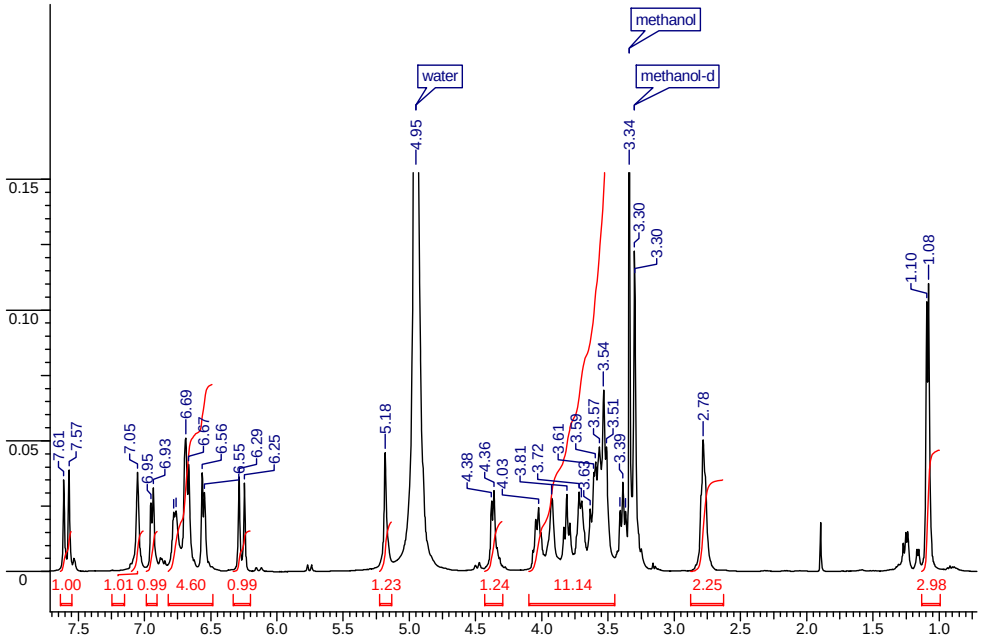


DEPT:

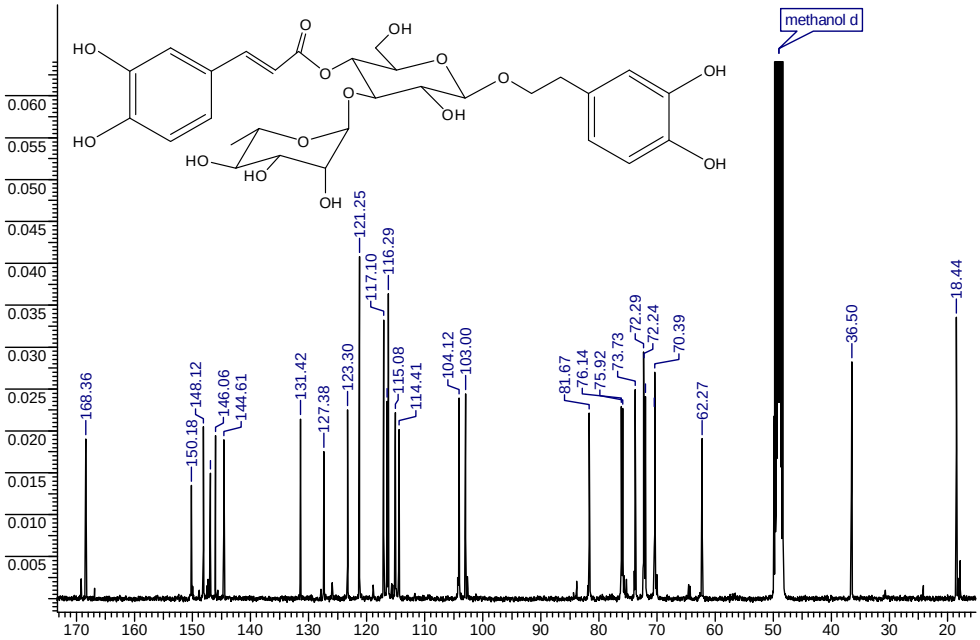


Compound **5**:

^{1}H NMR:



^{13}C NMR: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148.12
146.06
144.61
131.42
127.38
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121.25
117.10
116.29
115.08
114.41
104.12
103.00
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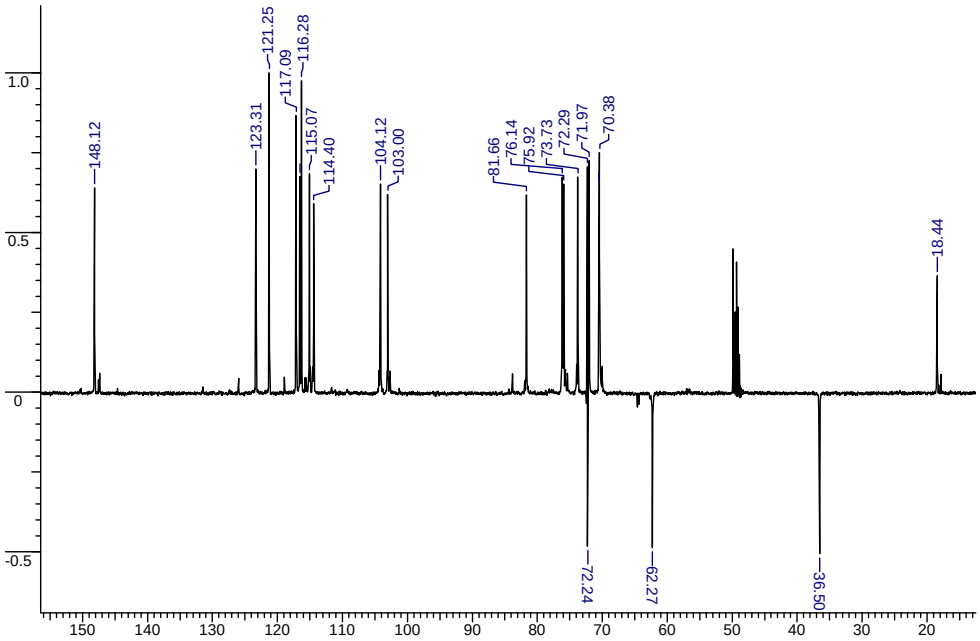
76.14
75.92
73.73
72.29
72.24
70.39
62.27
36.50
18.44

DEPT: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123.31
121.25
117.09
116.28
115.07
114.40
104.12
103.00
81.66
76.14
75.92
73.73
72.29
71.97
70.38
18.44
72.24
62.27
36.50

4.9. Reference:

[1] Bhatti, G. R. and M. J. Ingrouille, *Fontqueria,* **44**, 77 (1996).

[2] *Flora of Maharashtra State – Dicotyledons* (Flora of India series 2), ed. N.P. Singh, P. Lakshminarasimhan, S. Karthikeyan and P. V. Prasanna; published by, The Director, Botanical Survey of India, Culcatta, 2001, Vol **2**, 715.

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Chapter 5

Phytochemical investigations on *Anisochilus verticillatus* Hook. f.

Figure 1: *Anisochilus verticillatus* Hook. f.

5.1. Introduction:

Anisochilus Wall. ex Benth. is an Asian genus of herbs and shrubs. The genus contains 16 species and is chiefly distributed in India, Sri Lanka, Himalaya, Burma, South China, Thailand and Indo-China with 14 species in India. Eight species are endemic to Deccan peninsula with *A. verticillatus* (=*Anisochilus adenanthus* Dalzell) being endemic to Maharashtra [1]. Genus *Anisochilus* is represented in Maharashtra by 3 species viz. *A. carnosus*, *A. verticillatus* and *A. eriocephalus* [2].

A. verticillatus is an erect herb, up to 1.5m tall. Stem is angled, white and tomentose. Leaves, hairy on both sides, are in whorls of 3-6, sessile and oblong-lanceolate. Flowers (in August-October) are pale pink, in dense cylindrical tomentose spikes with spreading corolla lips and persistent calyx [2].

5.2. Collection and processing:

A. verticillatus, whole plants, in flowering, were collected from Purandar Fort area, District Pune, on 21st October, 2008. A herbarium is deposited in Botanical Survey of India, Western Circle, Pune (No. SPJ-1). Roots were separated and aerial parts cleaned off adhering dust and unwanted plant material, dried in shade, cut and pulverized.

5.3. Extraction and Isolation:

Pulverized aerial parts (1.13 kg) were extracted with acetone (3L × 3 × 14h) at room temperature. The acetone solubles were filtered and concentrated under reduced pressure to yield a greenish acetone extract (39.7g, 3.5% based on dry weight of plant), 38.0g of which was separated by column chromatography (CC) eluting with increasing polarity of acetone in petroleum ether to collect 18 fractions (AV1-AV18) (Chart 1).

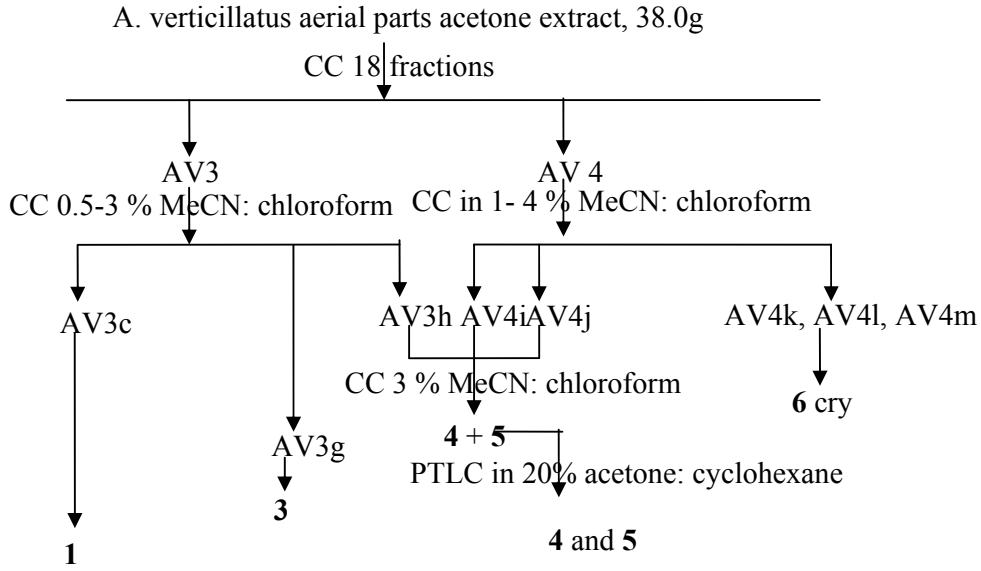
Fraction AV2 (11.8g) was subjected to CC with elution gradient acetonitrile from 0.5% to 3% in chloroform to collect 4 fractions (AV2a-d). Fraction AV2c (287.2mg) was subjected to CC in acetonitrile from 0.5% to 1% in chloroform to isolate compound **2**. This was purified by preparative TLC using benzene as eluting system in one direction and 1% ethyl acetate: benzene by reversing the plate. Fraction AV3 (800mg) was subjected to CC using elution gradient

acetonitrile from 0.5% to 3% in chloroform to collect 8 fractions (AV3a-h). Fraction AV3c contained compound **1** which was purified by preparative TLC using 2% acetonitrile in chloroform and 10% ethyl acetate in cyclohexane as developing systems.

Fraction AV3g contained compound **3** and was purified by preparative TLC using 2% acetonitrile in chloroform and 4% ethyl acetate in benzene as developing systems.

Fraction AV4 (1.8g) was subjected to CC using acetonitrile from 1% to 4% in chloroform as mobile phase to collect 13 fractions (AV4a-m). Fractions AV4i, AV4j and AV3h were combined and subjected to CC in 3% acetonitrile in chloroform to obtain mixture of compounds **4** and **5** which was separated and purified by preparative TLC using 20% acetone in cyclohexane as developing system. From fractions AV4k, AV4l and AV4m, compound **6** was separated by crystallisation.

Fraction AV5 (4.0g) was subjected to CC using elution gradient acetonitrile from 1% to 15% in chloroform to collect 15 fractions (AV5a-o). Fractions AV5n and AV5o contained compound **7**, which was purified by preparative TLC using developing system 10% ethyl acetate in benzene.



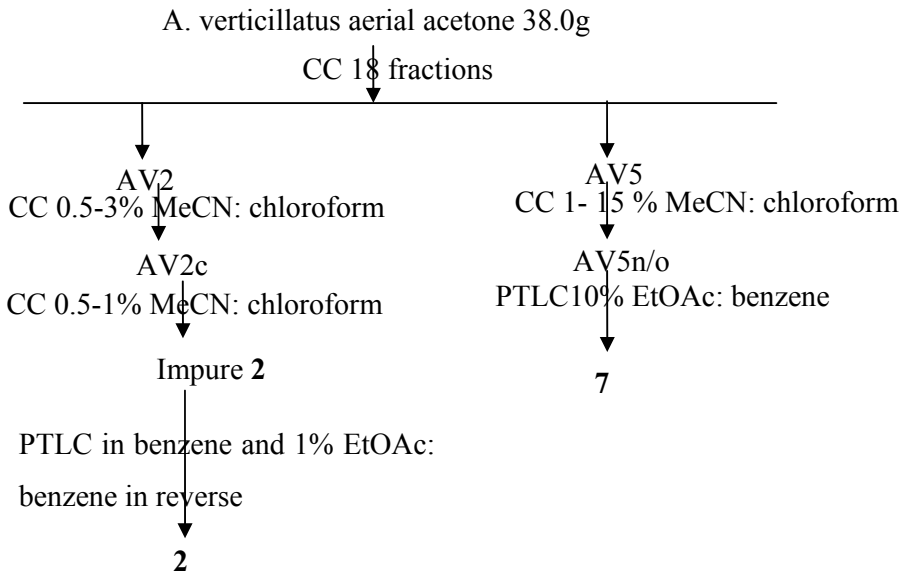


Chart 1: Chromatographic separation of *A. verticillatus*

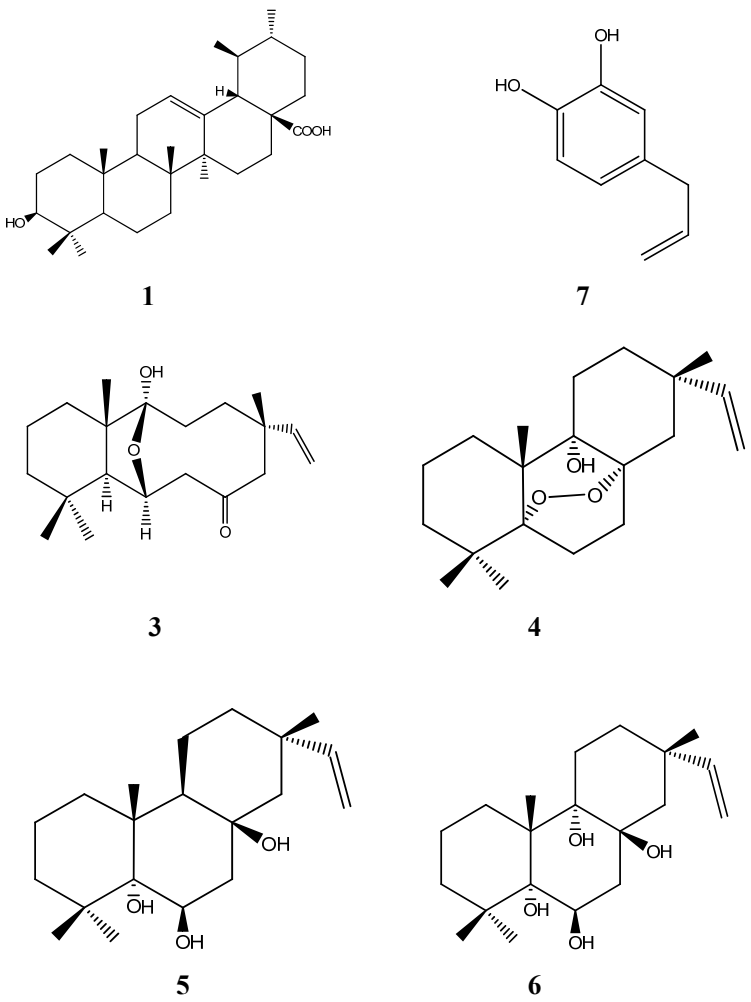


Figure 2: Compounds isolated from *A. verticillatus*.

5.4. Structure elucidation:

Compound 1:

Compound **1** was obtained as white amorphous powder. The ESIMS of **1** showed an $[M + 1]^+$ at *m/z* 457, $[M + Na]^+$ at *m/z* 479 suggesting its molecular formula $C_{30}H_{48}O_3$ with seven degrees of unsaturation. The IR spectrum showed a stretching frequency of carboxylic acid (1688cm^{-1}), hydroxyl (3606cm^{-1}) olefinic (1460, 1148 cm^{-1}) groups.

^{13}C NMR and DEPT spectra revealed presence of 7 methyls, 9 methylene, 7 methine and 7 quaternary carbons. Presence of carbonyl carbon at δ181.6 and one double bond carbons at δ 125.84 (methine) and 137.92 (quaternary) indicated it to be pentacyclic triterpene. Compound **1** was identified as ursolic acid by comparison of its NMR data with that reported in the literature (Table 1) [3].

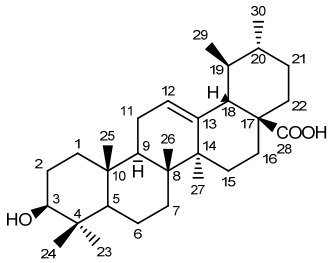


Figure 3: Structure of compound **1**

Compound 2:

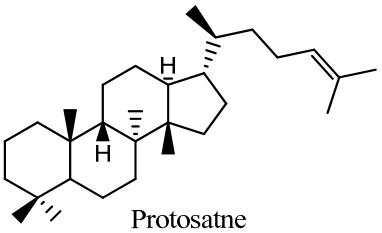
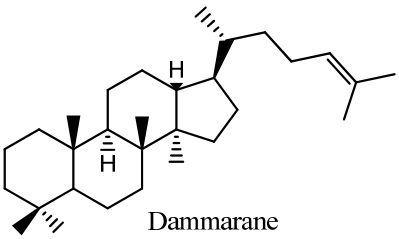
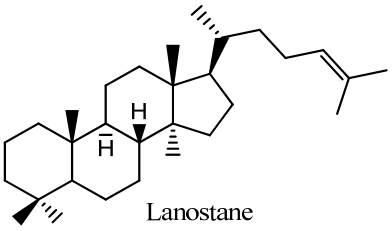
Compound **2** was obtained as white amorphous powder. The ESIMS of **2** showed an $[M + NH_4]^+$ at *m/z* 446, $[M + K]^+$ at *m/z* 467 suggesting its molecular formula $C_{30}H_{52}O$ with five degrees of unsaturation. The IR spectrum showed a stretching frequency of hydroxyl (3409 cm^{-1}), and olefinic (1622, 1457 cm^{-1}) groups.

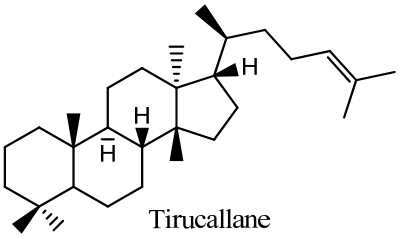
^{13}C NMR and DEPT spectra of **2** revealed presence of 8 methyls, 10 methylene 7 methine and 5 quaternary carbons with two olefinic carbons at δ124.4 (methine) and 139.8 (quaternary). This indicated **2** to be tetracyclic triterpene.

In ^{13}C NMR spectra of tetracyclic triterpenes with pentacyclic D ring, quaternary carbon at C-14 whenever present (protostanes, dammaranes, lanostanes, tirucallanes, euphanes and cucurbitanes), resonates around δ47-50, methines at the ring junctions resonate in the range of δ45 to 55, methine at

position 20 resonates around δ36 while methine of isopropyl resonate at around δ28. Absence of later isopropyl methine and presence of three methine carbons at δ 59.07, 55.18 and 47.72 hinted double bond at C-24. In compound **2**, quaternary carbons resonated at δ 33.74, 36.88, 40.00 and 42.06. First two could be assigned to C-4 or C-10. Quaternary carbon at around δ40.00 is observed for C-8 in dammarane type triterpenes with angular methyl group attached. However quaternary carbon at positions 13 or 14 in 6-6-6-5 type tetracyclic triterpenes resonate in the ranges δ42-45 and δ47-50 respectively. Also methine at δ59.05 is unusual for these type of triterpenes. Also presence of two methines at around δ39 were not explained by any of the known 6-6-6-5 triterpenes (Chart II).

Chart II: 6-6-6-5 tetracyclic triterpenes and characteristic ^{13}C NMR features.

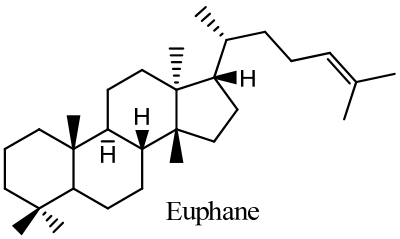
Skeleton	Comment
Compound **2**	*C*H: δ **59.07**, 55.18, 47.72,**39.6, 39.64** C: δ 33.74, 36.88, 40.00, **42.06**
 Protosatne	*C*H: δC5- 45-50, C9/13-40-45, C17- δ 50, C20- δ 36 C: C4/10-δ 37-38; C8- δ 40, **C14- δ 47-50**
 Dammarane	*C*H: δC5- 45-50, C9/13-40-45, C17- δ 50, C20- δ 36 C: C4/10-δ 37-38; C8- δ 40, **C14- δ 47-50**
 Lanostane	*C*H: δC5- 45-50, C8/9-40-45, C17- δ 50, C20- δ 36 C: C4/10-δ 37-38; C13- δ 42-45, **C14- δ 47-50**



Tirucallane

*C*H: δC5- 45-50, C8/9-40-45, C17- δ 50, C20- δ 36

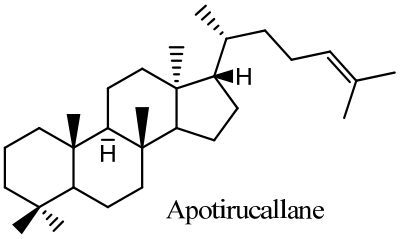
C: C4/10-δ 37-38; C13- δ 42-45, **C14- δ 47-50**



Euphane

*C*H: δC5- 45-50, C8/9-40-45, C17- δ 50, C20- δ 36

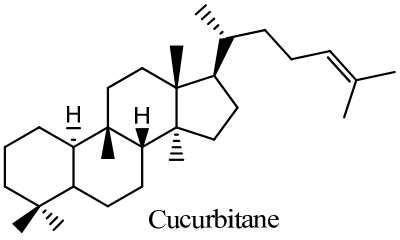
C: C4/10-δ 37-38; C13- δ 42-45, **C14- δ 47-50**



Apotirucallane

*C*H: δC5- 45-50, C9/14-40-45, C17- δ 50, C20- δ 36

C: C4/10-δ 37-38; C8- δ 40, **C13- δ 42-45**



Cucurbitane

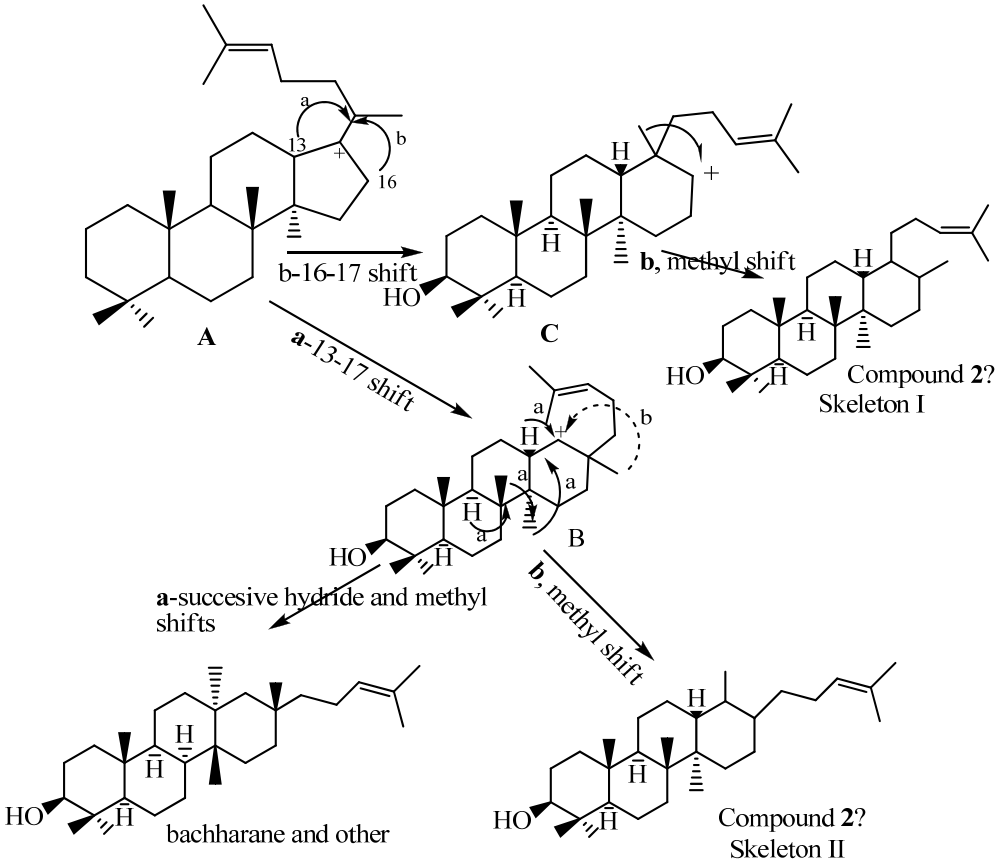
*C*H: δC5- 45-50, C8/10-δ40-45, C17- δ 50, C20- δ 36

C: C4/9-δ 37-38; C13- δ 42-45, **C14- δ 47-50**

These compelling evidences led us to place compound **2** in rare class of tetracyclic triterpenes with 6-6-6-6 rings in which quaternary carbon at C-14 gets upfield shifted to around δ42. Also in the known compounds with this system, quaternary carbon at position 8 resonates at around δ40 as observed in compound **2** [5]. However in known 6-6-6-6 type triterpenes (viz. baccharane and rearranged bachharanes), this leads to increase in quaternary carbon numbers at C-17.

Biosynthesis of 6-6-6-6 type of triterpenes goes plausibly through cationic intermediates **A** and **B** shown in scheme I [4]. Consecutive hydride and methyl shifts lead to currently isolated 6-6-6-6 tetracyclic triterpenes (route a). However methyl shift shown in path b (through intermediate **B** as well as hypothetical intermediate **C**) could lead to new skeletons **I** and **II** with ^{13}C NMR spectral features matching with compound **2**. In fact compounds **8** and **9** conforming to

such skeletons have been generated by an *Alicyclobacillus acidocaldarius* with mutant squalene-hopene cyclase [4].



Scheme I: Biosynthetic approach for compound **2**.

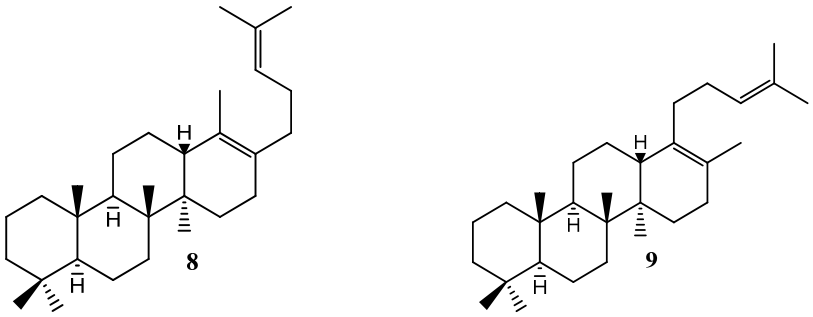
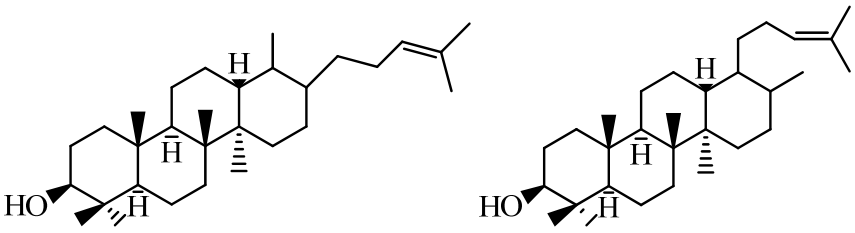


Figure 4: Structure of compounds **8** and **9**.

Thus based on above considerations, compound **2** have been tentatively identified as rearranged bachharane of the type 6-6-6-6 (**2a** or **2b**) (Table 2; Figure 4). Detailed analysis of 2D NMR spectra for confirmation of the srtcture(s) is underway.



2a **2b**

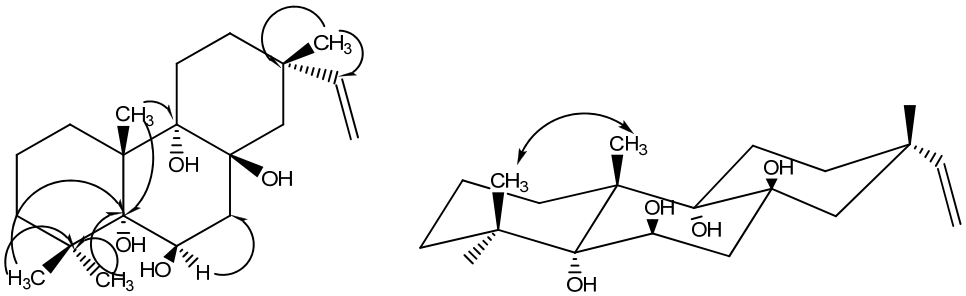
Figure 5: Probable structure of compound **2**

Compound 6:

Compound **6** was obtained as white crystals. The ESIMS of **6** showed an $[M + 1]^+$ at *m/z* 339, $[M + Na]^+$ at *m/z* 361 suggesting the molecular formula $C_{20}H_{34}O_4$ with four degrees of unsaturation. The IR spectrum showed a stretching frequency of hydroxyl (3419 cm^{-1}), olefinic (1634, 1453 cm^{-1}) groups.

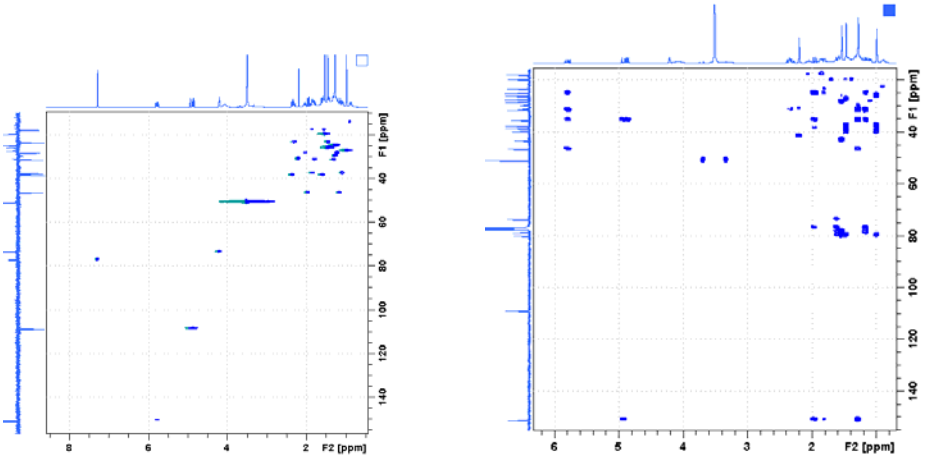
^{13}C NMR and DEPT spectra of **2** revealed presence of 4 methyls, 8 methylene, 2 methine and 6 quaternary carbons. Vinylic carbons at δ 108.9 (=*C*H_2), and 151.04 (=*C*H) revealed this compound to be a pimarane class of diterpene. Presence of 3 quaternary carbons at δ 77.21, 78.71 and 79.79 and methine at δ 73.70 indicated all the three methines at positions 5, 8 and 9 to be substituted by hydroxyl group and one of the methylene converted into *C*HOH (Table 3). Position of this methine was fixed at 6 by observation of correlation of this carbon with one of the C-7 protons at δ1.6 in HMBC spectrum. Figure 6a shows reverse correlation that was also observed between H-6 and C-7 at δ 38.54. Figure6a also shows other correlations that confirmed the assigned structure. Observation of NOE correlation peak between methyl protons at δ1.47 (H-19) and 1.54 (H-20) confirmed their placement on the same face (β orientation) of pimarane skeleton while a peak between H-6 at δ 4.31 and methyl protons at

δ0.99 (H-18) confirmed β orientation of hydroxyl group (Figure 6b). All the 2D spectra are given in Figure 7a-d.

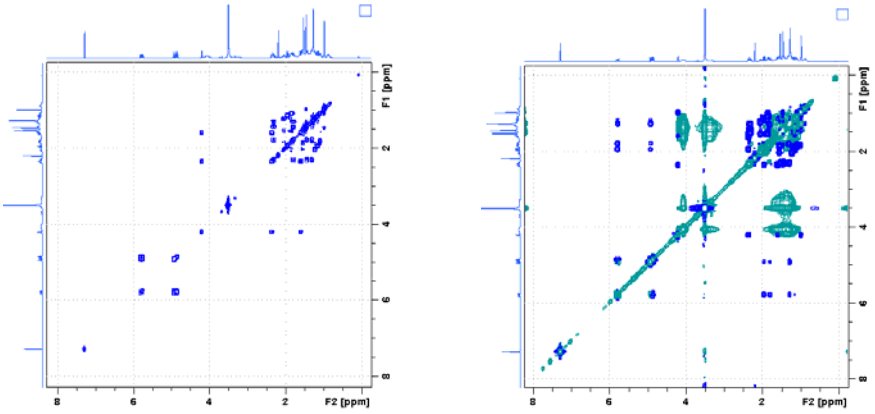


6a. HMBC correlations **6b.** NOESY correlations

Figure 6: 2D correlations for compound **6**.



7a. HSQC spectrum **7b.** HMBC spectrum



7c. COSY spectrum **7d.** NOESY spectrum

Figure 7: 2D Spectra of compound **6**.

Anti configuration at C-8 and C-9 with β orientation of C-8 hydroxyl group was confirmed only after single crystal (in 10%acetone in hexane) X-ray

crystallographic studies. It also revealed anti-orientation of C-5 hydroxyl group and C-20 methyl as well as β orientation of C-17 placing compound 6 in isopimarane skeleton (Figure 8, Tables 4a-4d).

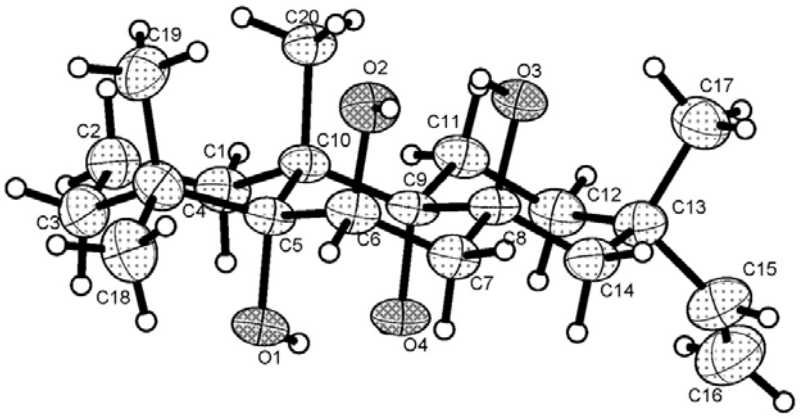


Figure 8: ORTEP diagram of compound **6.** Ellipsoids are at 50% probability.

Thus compound **6** was identified as new natural product with isopimarane skeleton, isopimara-5α,6β,8β,9α-tetraol.

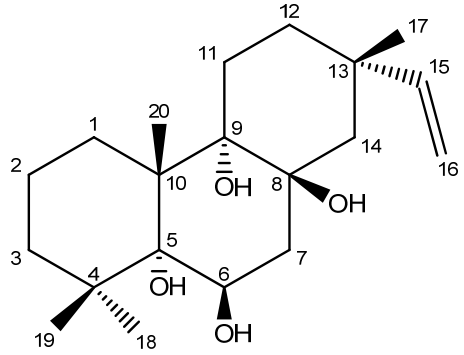


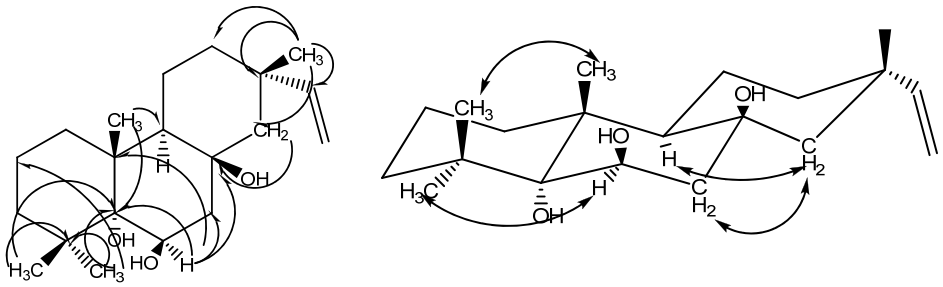
Figure 9: Structure of compound **6**

Compound 5:

Compound **5** was obtained as white crystals. The ESIMS of **5** showed an $[M + 1]^+$ at *m*/*z* 323, $[M + Na]^+$ at *m*/*z* 345 suggesting the molecular formula $C_{20}H_{34}O_3$ with four degrees of unsaturation. The IR spectrum showed a stretching frequency of hydroxyl (3418, cm^{-1}) and olefinic (1634, 1464cm^{-1}) groups.

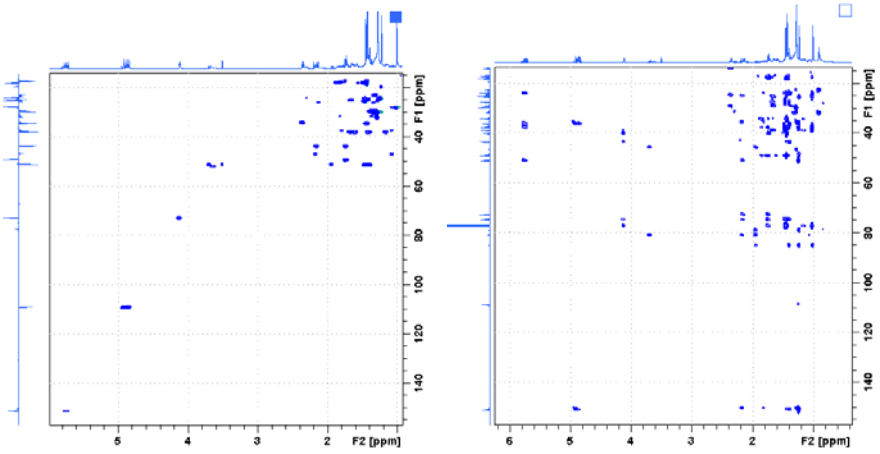
^{13}C NMR and DEPT spectra of **5** revealed presence of 4 methyls, 8 methylene, 3 methine and 5 quaternary carbons. Vinylic carbons at δ108.90 (=CH_2), and 151.12 (=CH) revealed this compound to be a pimarane class of

diterpene (Table 3). Presence of 2 quaternary carbons at δ77.23, 74.65 and methine at δ72.73 and 49.02 indicated compound **5** to be very similar to compound **6** with one hydroxyl group replaced by hydrogen (Table 3). Position of methine at δ 72.73 was fixed at 6 by HMBC correlation of methine proton at δ 4.11 with 7 methylene at δ 43.57 and quaternary carbons at C-5 (δ 77.24), C-8 (δ 74.65) and C-10 (δ 40. 12) as shown in Figure 10a. HMBC correlation of H-20 at δ 1.42 with methine carbon at δ 49.05 confirmed 9 position of later. Figure 10a shows HMBC correlations that confirmed the structure assigned. NOE correlation peak between H-6 methine and methyl at δ1.00 (H-18) confirmed β orientation of hydroxyl group at C-6. Similarly NOE peak between δ 1.73 (either H-9 or H-7 or both) and H-14 proton (δ 1.43) confirmed β orientation of hydroxyl group at C-8. Compound **5** was assigned isopimarane type of skeleton by analogy with compound **6**. From these spectral studies, compound **5** was identified as new natural product, isopimara-5α,6β,8β-triol.

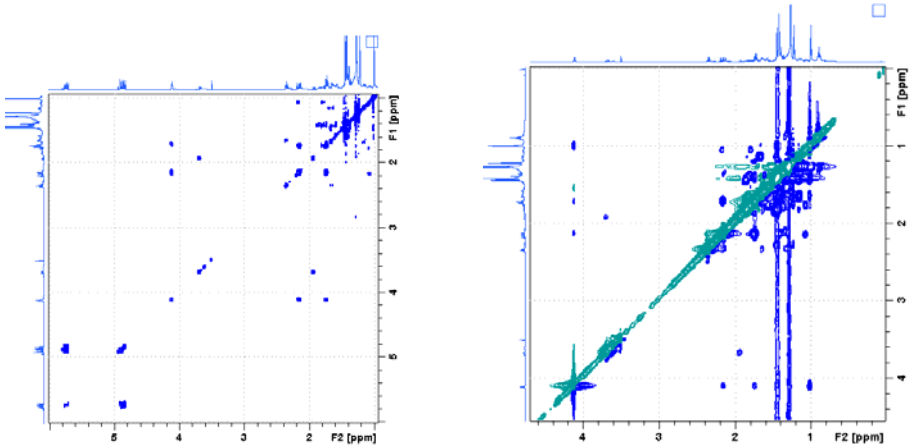


10a. HMBC correlations **10b.** NOESY correlations

Figure 10: 2D correlations for compound **5**



11a. HSQC spectrum **11b.** HMBC spectrum



11c. COSY spectrum **11d.** NOESY spectrum

Figure 11: 2D spectra of compound **5**.

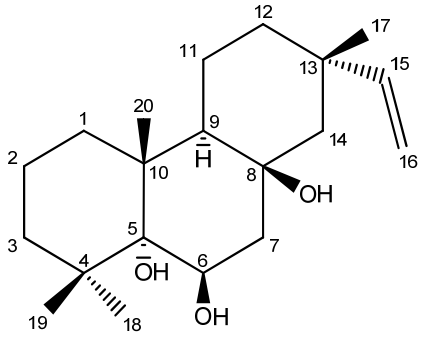


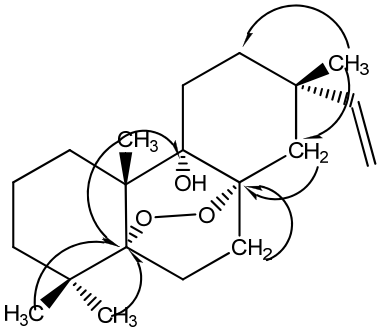
Figure 12: Structure of compound **5**.

Compound 4:

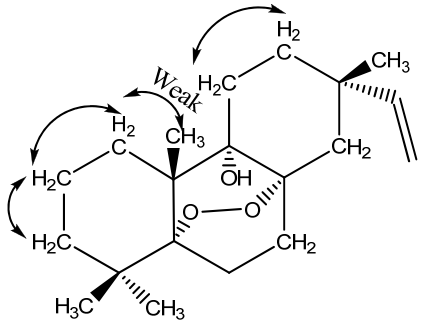
Compound **4** was obtained as a white amorphous powder. The ESIMS of **4** showed an $[M + 1]^+$ at *m/z* 321, $[M + Na]^+$ at *m/z* 343 suggesting the molecular formula $C_{20}H_{32}O_3$ with five degrees of unsaturation. The IR spectrum showed a stretching frequency for hydroxyl group (3422cm^{-1}).

^{13}C NMR and DEPT spectra of **4** revealed presence of 4 methyls, 9 methylene 1 methine and 6 quaternary carbons (Table 3). Vinylic carbons at δ 109.24 (=*C*H$_2$), and 150.49 (=*C*H) revealed this compound to be pimarane class of diterpene (Table 3). Presence of 3 quaternary carbons at δ 78.93, 81.01 and 85.25 indicated compound **4** to be very similar to compound **5** and **6** with one oxygen function less than compound **6**. Presence of same number of methylenes in compound **4** as found in unsubstituted pimaranes indicated no substitution on rings in compound **4**. Downfield shifted carbons at δ81.01 and 85.25 indicated

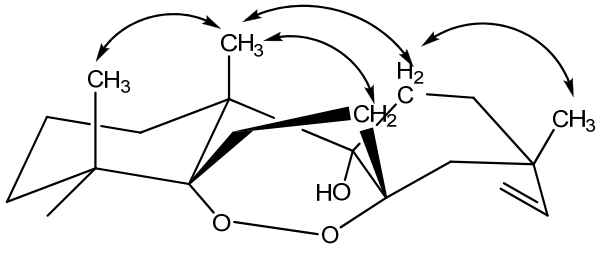
peroxy bridge between C-8 and C-9 or C-5 and C-8 or C-5 and C-9. HMBC correlation was observed between quaternary carbon at δ85.25 with three methyl groups at δ 0.99(H-18), 1.40 (H-19) and 1.21 (H-20) fixed position of this quaternary carbon at 5. Similarly HMBC of H-20 with quaternary carbon at δ 78.93 fixed later at position 9. Comparison of spectral data with those of compound **6** also enabled to place hydroxyl group at C9. This also fixed remaining quaternary carbon at δ81.01 for C-8 and hence position of peroxy bridge between C5-C8. Location of peroxy bridge was also confirmed by strong HMBC of one of the H-7 protons at δ3.69 with δ81.01. This peroxy bridge necessitates both C-5 and C-8 oxygens to be syn. Presence of NOE correlation peak between methyl at δ1.21 (H-20) and proton at δ 1.91 (H-7) as well as NOE correlation peak between 2.29 (H-11) and 1.23 (H-20) confirmed below the plane placement (α orientation) of peroxy bridge. Position of H-17 methyl as β was confirmed by observation of its NOE (weak) with proton at δ 2.29 (H-11). This later correlation revealed compound **4** to be in fact an isopimarane type of diterpene. Thus compound 4 was identified as new diterpene isopimara-5α,8α-peroxy-9α-ol



13a. HMBC correlations

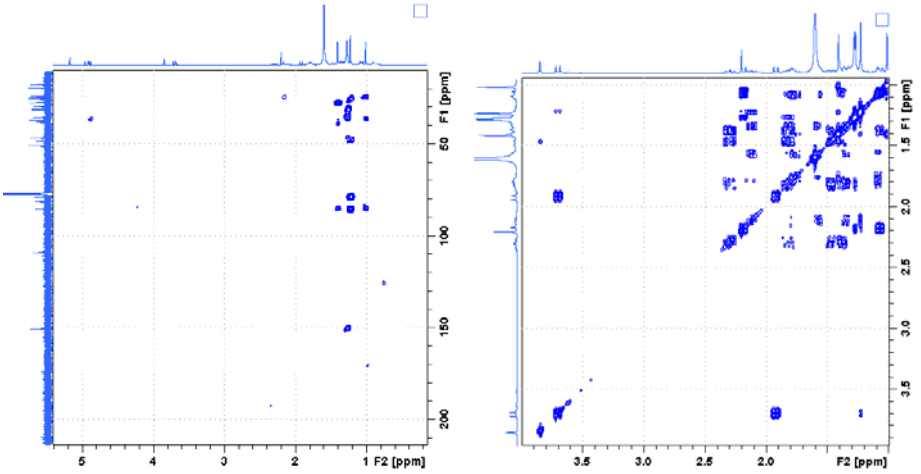


13b. COSY correlations

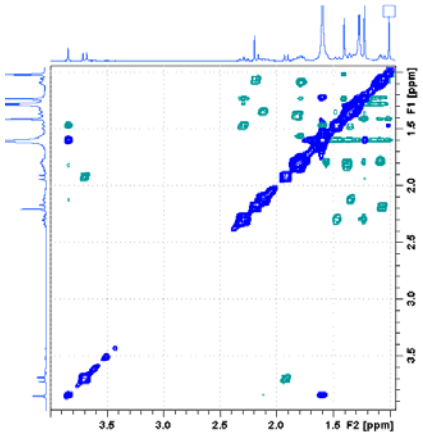


13c. NOESY correlations

Figure 13: 2D correlation of compound **4**



14a. HMBC correlation **14b.** COSY correlation



13c. NOESY correlation

Figure 14: 2D spectra of compound **4**

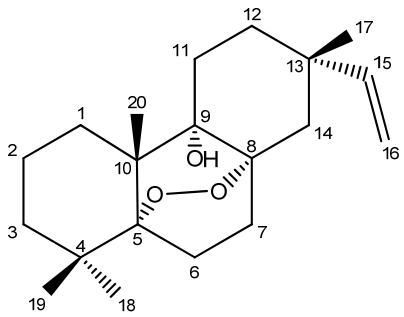


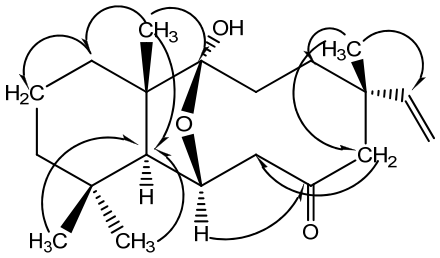
Figure 15: Structure of compound **4**

Compound 3:

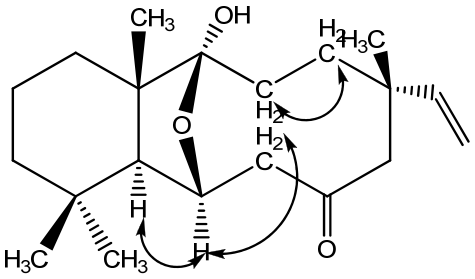
Compound **3** was obtained as a white amorphous solid. The ESIMS of **3** showed an $[M + 1]^+$ at *m*/*z* 321, $[M + Na]^+$ at *m*/*z* 343 suggesting the molecular formula $C_{20}H_{32}O_3$ with five degrees of unsaturation.

^{13}C NMR and DEPT spectra of **3** revealed presence of 4 methyls, 8 methylene, 3 methine and 5 quaternary carbons. Vinylic carbons at δ110.44 (=*C*H_2), and 145.29 (=*C*H) revealed this compound to be pimarane class of diterpene (Table 3). Presence of methine carbon at δ73.84 indicated compound **3** to be similar to compound **5**. Its location at 6 was fixed by observation COSY correlation between δ 4.35 (H-6) and 1.88 (H-5), later being fixed by observation of C-5 HMBC correlation with H-18, H-19 and H-20. Quaternary carbon at δ 107.66 was placed at position 9 by observation of its HMBC correlation with H-20 methyl at δ1.11. Highly downfield shifted C-9 indicated attachment of two oxygen functions with one as hydroxyl group and another probably forming 1, 4-epoxy bridge with C-6. This necessitates cleaving of 8-9 bond.

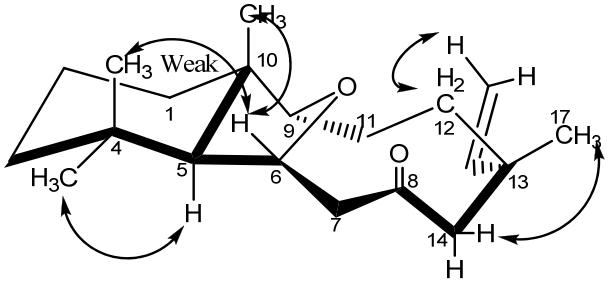
COSY correlation of H-5 with δ3.3 and 2.33 (attached to δ49.30 methylene) protons fixed later at 7. A three bond HMBC correlation between C-14 proton at δ3.05 and C-7 confirmed C5-C6-C7 linkage. This also excluded possibility of δ73.84 to be placed at C7 and epoxy bridge at C9-C7. Carbonyl carbon at δ213.09 was placed at 8 by observation of its HMBC correlations with H-6. This also confirmed 8, 9 bond cleavage and hence 1, 4-epoxy bridge at C-6 and C-9. Figure 16a shows these key correlations as well other important correlations that helped in assigning the given structure. Figure 16b depicts COSY correlations that revealed isolated 5-6-7 and 11-12 linkages. NOE peak of H-6 with H-19 and H-20 methyls confirmed β orientation of epoxy bridge while position of H-17 methyl as β was confirmed by observation of its NOE (weak) with both protons at δ2.21 and 3.05 (H-14) as well as with H-12 protons. Rest important NOESY correlations are shown in Figure 16c. From these spectral studies compound **3** was identified as a new isopimarane type of diterpene, 8,9-secoisipimara-6β,9β-epoxy-9α-hydroxy-8-one. This is probably first report of 8,9-secoisipimara skeleton.



16a. HMBC correlations

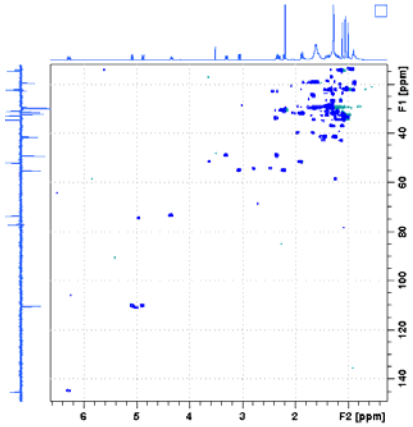


16b. COSY correlations

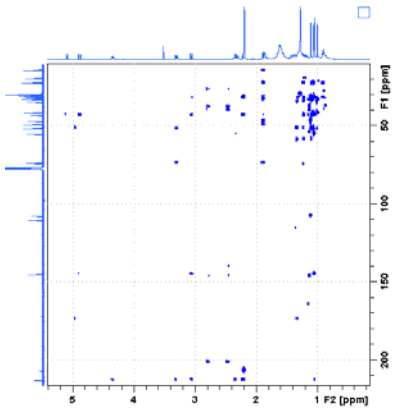


16c. NOESY correlations

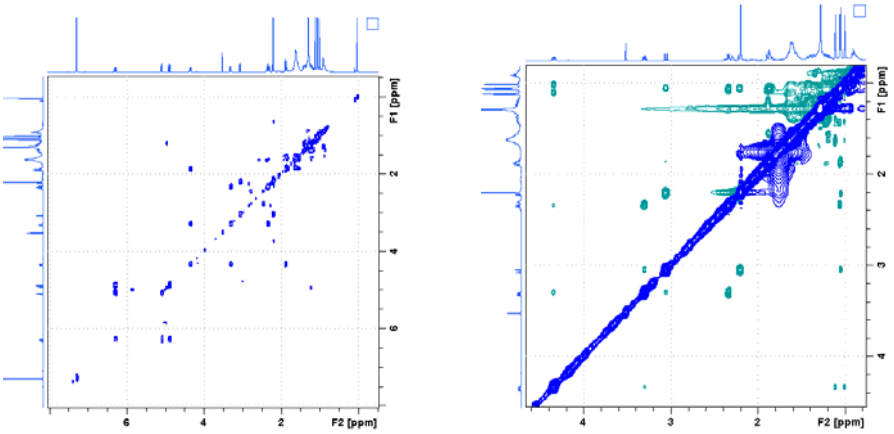
Figure 16: 2D correlations for compound **3**



17a. HSQC spectrum

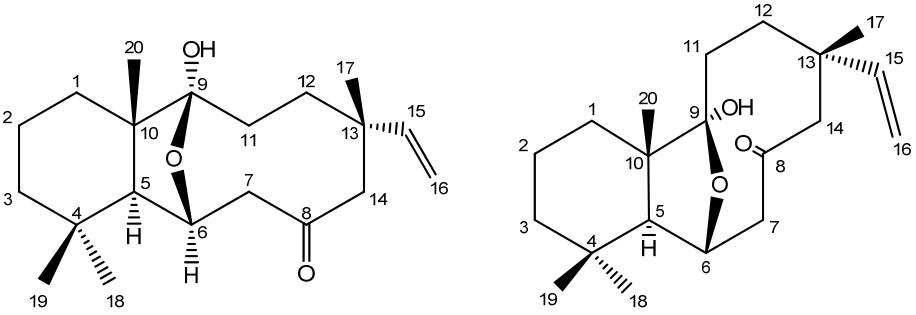


17b. HMBC spectrum



17c. COSY spectrum **17d.** NOESY spectrum

Figure 17: 2D spectra of compound **3**



a **b**

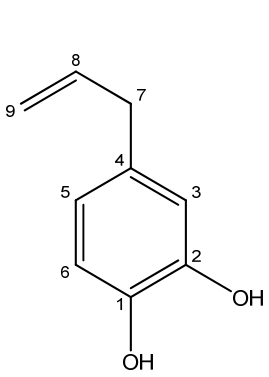
Figure 18: a. Compound **3** **b.** Different perspective

Compound 7:

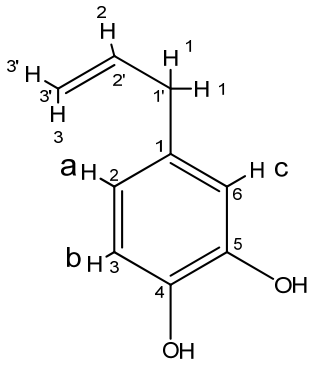
Compound **7** was obtained as a greenish low melting crystalline material. The ESIMS of **7** showed an $[M + 1]^+$ at *m/z* 151, $[M + Na]^+$ at *m/z* 173 suggesting the molecular formula $C_9H_{10}O_2$ with five degrees of unsaturation. The IR spectrum showed a stretching frequency of hydroxyl group (3430cm^{-1}) and aromatic region (1635, 1605, 1525, 1444, 1281, 1114cm^{-1}).

^{13}C NMR spectrum of **7** revealed 9 carbons with 8 in aromatic region, two resonances at δ 143.42 and 141.64 indicating two hydroxyl groups. DEPT spectrum showed two methylenes, one attached to aromatic ring (at δ39.47) and another in olefinic region (δ 115.56) indicative of terminal olefinic group (Table 5). Thus compound was identified as belonging to phenyl propanoid class, specifically 3-phenyl propene with two hydroxyl substituent on ring. Analysis of

^{1}H NMR revealed, 1, 2, 4-substitued pattern with two ortho coupled protons at δ 6.81 and 6.65 (both d, 8Hz) and a broad singlet at δ 6.74. Thus the compound was identified as hydroxychavicol which was also confirmed by comparison of its NMR data with the reported ^{13}C and ^{1}H NMR values of hydroxychavicol [6]. Hydroxychavicol has been isolated from family Piperaceae and this is the first report of its isolation from family Lamiaceae.



7 numbering



literature numbering

Figure19: Compound **7**

5.5: Experimental:

A. Collection and processing:

A. heyneana, whole plant, in flowering, was collected and processed for preparation of acetone extract as described earlier.

B. Extraction and Isolation:

Extraction and isolation of compounds from acetone extract is described earlier.

Compound **1**:

White amorphous powder (4mg, 0.00035% based on dry weight of plant), ESIMS at *m/z* 479[M + Na]$^+$ and at *m/z* 495[M + K]$^+$; IR (ATR) 1688, cm^{-1}; ^{1}H NMR, 0.91(3H, s, H-25), 0.97 (3H, d, 6Hz, H-30), 1.05(3H, s, H-26), 1.08(3H, s, H-24), 1.025 (3H, d, 6Hz, H-29), 1.24(3H, s, H-23), 1.27 (3H, s, H-27), 5.51(1H, m, H-12); for ^{13}C NMR, see Table 1.

Compound **2**:

White amorphous powder (12mg, 0.00105%), ESIMS m/z 446[M+ NH_4]$^+$, 467[M+ Na]$^+$; IR ($CHCl_3$) 3409 (s), 1622 (m), 1457 (m) cm^{-1}; ^{1}H NMR, 0.81(3H, s), 0.82 (3H, s), 1.05(3H, s), 1.08(3H, s, H-24), 1.02 (3H, s), 1.03(3H, s), 1.27 (3H, s, H-27), 3.24(1H, dd, 10.76, 5.13Hz), 5.14(1H, d, 3.42Hz)- Note: only clearly identifiable peaks are mentioned; for ^{13}C NMR spectral data, see Table 2.

Compound **3**:

Yellowish amorphous powder (6 mg, 0.000525%); ESIMS m/z 321 [M+ 1]$^+$, 343[M+ Na]$^+$; ^{1}H and ^{13}C NMR spectral data, see Table 3.

Compound **4**:

White amorphous powder (7mg, 0.00062%); ESIMS m/z 321 [M+ 1]$^+$343[M+ Na]$^+$; $[\alpha]_D^{25}$-1.71 (*c* 0.4, acetone); ^{1}H and ^{13}C NMR spectral data, see Table 3.

Compound **5**:

White crystals (12mg, 0.00105%); ESIMS m/z 323[M+ 1]$^+$, 345 [M+ Na]$^+$; $[\alpha]_D^{25}$6.86 (*c* 0.81, acetone); ^{1}H and ^{13}C NMR spectral data, see Table 3.

Compound **6**:

White crystals (120mg, 0.0105%), mp 200.7^{0}C; ESIMS m/z 339[M+ 1]$^+$, 361[M+ Na]$^+$; $[\alpha]_D^{23.2}$ -27.93 (*c* 1.00, Acetone); IR (chloroform) 3419 (s), 1634 (m), 1453 (m) cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Table 3,

X-ray crystallography:

Crystals of the compound were grown by slow evaporation of the solution in 10% acetone: petroleum ether. Randomly collected colourless crystal of approximate size 0.39 x 0.07 x 0.01mm^3 was used for data collection on *Bruker SMART APEX* CCD diffractometer using Mo K_α radiation. Exposure / frame = 20.0 sec / frame, Crystals belong to Monoclinic, space group P21, a = 10.9350(8), *b* = 7.4522(6), *c* = 12.3825(9) Å, *V* = 915.24(12) Å^3, *Z* = 2, D_c = 1.228 g/cc, μ (MoK_α) = 0.71073 Å, *T* = 296 K, 8640 reflections measured, R value 0.0438, wR2 = 0.1005. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL)[7] was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement as per the riding model. Data collection and refinement parameters are listed in Tables 4a-4d.

Compound **7**:

Greenish low melting crystaline solid (10mg, 0.000885%); ESIMS m/z 173 [M+ Na]$^+$ 189[M+ K]$^+$;IR ($CHCl_3$) 3430 (s), 1635(m), 1605(m), 1525(m), 1444(m) , 1281(m), 1114(m) cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Table 5.

5.6. Tables:

Table 1: ^{13}C NMR Data for compound **1**

	1 (Pyridine-d_5)	Ursolic acid* (Pyridine-d_5)
1	39.10	39.2
2	28.21	28.2
3	78.15	78.2
4	39.51	39.6
5	55.84	55.91
6	18.80	18.8
7	33.60	33.7
8	39.98	40.1
9	48.07	48.1
10	37.47	37.5
11	23.71	23.7
12	125.67	125.7
13	139.29	139.3
14	42.52	42.6
15	28.78	28.8
16	25.00	25.0
17	48.07	48.1
18	53.57	53.6
19	39.51	39.5
20	39.43	39.4
21	31.08	31.1
22	37.48	37.4
23	28.84	28.8
24	16.62	16.5
25	15.70	15.7
26	17.55	17.5
27	23.94	24.0
28	179.93	179.7
29	17.48	17.5
30	21.45	21.4

* as given in Literature [3]

Table 2: Tabulation of ^{13}C NMR peaks of compound **2** and multiplicities from DEPT.

No.	Peaks	DEPT	No.	peaks	DEPT
1	15.61	CH_3	16	32.92	CH_2
2	15.67	CH_3	17	33.74	C
3	16.85	CH_3	18	36.88	C
4	17.46	CH_3	19	38.76	CH_2
5	18.33	CH_2	20	39.60	CH
6	21.39	CH_3	21	39.64	CH
7	23.25	CH_3	22	40.00	C
8	23.35	CH_2	23	41.51	CH_2
9	26.60	CH_2	24	42.06	C
10	27.26	CH_2	25	47.69	CH
11	28.08	CH_2	26	55.15	CH
12	28.10	CH_3	27	59.05	CH
13	28.74	CH_3	28	79.05	CH
14	29.69	CH_2	29	124.40	CH
15	31.24	CH_2	30	139.57	C

Table 3: Compounds **3**, **4**, **5** and **6**

no	**6**		**4**		**5**		**3**	
	δ_C[1]	δ_H[2]	δ_C[1]	δ_H[2]	δ_C[1]	δ_H[2]	δ_C[1]	δ_H[2]
1	28.29	2.02 (m)β, 1.19 (m)	25.64	2.11, 1.33	34.34	1.42 (m)	31.32	1.38 (m), 1.18 (m)
2	17.64	1.85 (m)β, 1.55 (m) *	17.52	1.57, 1.48	17.58	1.84(m),1.47 (m)	19.54	1.62 (m)
3	37.65	1.83 (m), 1.08 (m)	37.12	1.77, 1.07	37.74	1.60, 1.15	41.54	1.42 (m), 1.23 (m)
4	39.64	-	37.91	-	38.89	-	32.79	-
5	79.79	-	85.25	-	77.23	-	51.89	1.88 (d, 10.62Hz)
6	73.70	4.31 (bt, 2.82Hz)	29.67	1.27 (m)	72.73	4.11 (bt, 3.02 Hz)	73.84	4.35 (dd, 10.65, 6.54)
7	38.54	2.37 (m), 1.6 (dd, 15.22, 2.77 Hz)	50.97	1.91, 3.69	43.57	2.14 (dd, 14.28, 3.43Hz), 1.73(m)	49.30	3.30 (dd, 10.65, 6.54), 2.33 (d, 10.62Hz)
8	77.21	-	81.01	-	74.65	-	213.09	-
9	78.71	-	78.93	-	49.02	1.73 (m)	107.66	-
10	43.23	-	48.40	-	40.12	-	46.77	-
11	23.39	2.29 (m), 1.43	23.79	2.29, 1.47	17.04	1.73 (m), 1.40(m)	29.85	1.63 (m)
12	31.43	1.78(dd, 13.82, 3.87 Hz), 1.29 (d, 13.82 Hz)	31.25	1.80, 1.38	37.78	1.59, 1.40	31.96	1.85 (m)
13	35.36	-	35.64	-	36.32	-	43.05	-
14	46.57	1.95 (d, 14.14 Hz), 1.15 (dd, 14. 14, 2.05 Hz)	46.76	2.16 (m), 1.06 (m)	51.09	1.43, 1.39	55.25	2.21 (d, 14.21) ,3.05 (d, 14.21)
15	151.04	5.79 (dd, 10.65, 17.65)	150.49	5.78 (dd, 10.82, 17.42)	151.12	5.74 (dd, 11.06, 17.65)	145.29	6.29 (dd, 11.03, 17.65)
16	108.90	4.92 (dd, 17.65, 1.01 Hz), 4.87(dd, 10.65, 1.01 Hz)	109.24	4.93 (d, 10.82), 4.88 (d, 17.42)	108.90	4.9 (dd, 17.65, 1.08 Hz), 4.85 (dd, 11.06, 1.08 Hz)	110.44	5.09 (d, 11.03), 4.89 (d, 17.65)
17	24.96	1.28 (s)	25.02	1.26 (s)	24.02	1.22 (s)	32.79	1.047 (s)
18	27.37	0.99 (s)	27.94	0.99 (s)	27.66	1.00 (s)	34.63	1.06 (s)
19	25.91	1.47 (s)	24.54	1.40 (s)	25.26	1.44 (s)	22.30	1.00 (s)
20	19.68	1.54 (s)	19.48	1.23 (s)	18.07	1.42 (s)	14.57	1.11 (s)

* Multiplicity in bracket

Table 4a: Crystal data and structure refinement for compound **6**.

Parameter	Value
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 10.9350(8) Å α= 90°. b = 7.4522(6) Å β= 114.9020(10)°. c = 12.3825(9) Å γ = 90°.
Volume	915.24(12) $Å^3$
Z	2
Density (calculated)	1.228 Mg/m^3
Crystal size	0.39 x 0.07 x 0.01mm^3
Reflections collected	8640
Data / restraints / parameters	3202 / 1 / 225
Final R indices [I>2sigma(I)]	R1 = 0.0438, wR2 = 0.1005

Table 4b. Bond lengths [°] for compound **6**.

Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]
C(1)-C(2)	1.528(3)
C(1)-C(10)	1.547(3)
C(2)-C(3)	1.520(4)
C(3)-C(4)	1.544(3)
C(4)-C(19)	1.544(3)
C(4)-C(18)	1.551(4)
C(4)-C(5)	1.570(3)
C(5)-C(6)	1.548(3)
C(5)-C(10)	1.590(3)
C(6)-C(7)	1.527(3)
C(7)-C(8)	1.525(3)
C(8)-C(14)	1.524(3)
C(8)-C(9)	1.566(3)
C(9)-C(11)	1.537(3)
C(9)-C(10)	1.587(3)
C(10)-C(20)	1.552(3)
C(11)-C(12)	1.533(3)
C(12)-C(13)	1.536(3)
C(13)-C(15)	1.517(3)
C(13)-C(14)	1.539(3)
C(13)-C(17)	1.548(3)
C(15)-C(16)	1.275(4)

Table 4c. Bond angles [°] for compound **6**.

Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]	Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]
C(2)-C(1)-C(10)	113.85(18)	C(11)-C(9)-C(10)	115.40(18)
C(3)-C(2)-C(1)	112.5(2)	C(8)-C(9)-C(10)	113.42(16)
C(2)-C(3)-C(4)	113.4(2)	C(1)-C(10)-C(20)	106.17(18)
C(19)-C(4)-C(3)	108.6(2)	C(1)-C(10)-C(9)	110.10(17)
C(19)-C(4)-C(18)	105.4(2)	C(20)-C(10)-C(9)	109.84(17)
C(3)-C(4)-C(18)	108.4(2)	C(1)-C(10)-C(5)	107.29(17)
C(19)-C(4)-C(5)	117.03(19)	C(20)-C(10)-C(5)	113.75(18)
C(3)-C(4)-C(5)	107.87(19)	C(9)-C(10)-C(5)	109.57(17)
C(18)-C(4)-C(5)	109.3(2)	C(12)-C(11)-C(9)	112.23(19)
C(6)-C(5)-C(4)	115.13(19)	C(11)-C(12)-C(13)	114.03(19)
O(1)-C(5)-C(10)	107.68(16)	C(15)-C(13)-C(12)	111.2(2)
C(6)-C(5)-C(10)	112.91(18)	C(15)-C(13)-C(14)	107.3(2)
C(4)-C(5)-C(10)	114.21(18)	C(12)-C(13)-C(14)	109.92(19)
C(7)-C(6)-C(5)	111.38(18)	C(15)-C(13)-C(17)	105.6(2)
C(8)-C(7)-C(6)	113.31(18)	C(12)-C(13)-C(17)	111.2(2)
C(14)-C(8)-C(7)	109.48(18)	C(14)-C(13)-C(17)	111.5(2)
C(14)-C(8)-C(9)	110.27(18)	C(8)-C(14)-C(13)	117.31(19)
C(7)-C(8)-C(9)	111.28(18)	C(16)-C(15)-C(13)	131.2(3)
C(11)-C(9)-C(8)	107.17(17)		

Table 4d. Torsion angles [°] for compound **6**.

Connectivity	angles [°]	Connectivity	angles [°]
C(10)-C(1)-C(2)-C(3)	-54.6(3)	C(11)-C(9)-C(10)-C(20)	-48.6(2)
C(1)-C(2)-C(3)-C(4)	54.8(3)	C(8)-C(9)-C(10)-C(20)	75.6(2)
C(2)-C(3)-C(4)-C(19)	73.4(3)	C(11)-C(9)-C(10)-C(5)	-174.20(17)
C(2)-C(3)-C(4)-C(18)	-172.6(2)	C(8)-C(9)-C(10)-C(5)	-50.0(2)
C(2)-C(3)-C(4)-C(5)	-54.3(3)	C(6)-C(5)-C(10)-C(1)	170.69(18)
C(19)-C(4)-C(5)-C(6)	66.3(3)	C(4)-C(5)-C(10)-C(1)	-55.2(2)
C(3)-C(4)-C(5)-C(6)	-171.01(19)	C(6)-C(5)-C(10)-C(20)	-72.2(2)
C(18)-C(4)-C(5)-C(6)	-53.3(3)	C(4)-C(5)-C(10)-C(20)	61.9(2)
C(19)-C(4)-C(5)-C(10)	-66.7(3)	C(6)-C(5)-C(10)-C(9)	51.2(2)
C(3)-C(4)-C(5)-C(10)	56.0(2)	C(4)-C(5)-C(10)-C(9)	-174.76(16)
C(18)-C(4)-C(5)-C(10)	173.63(18)	C(8)-C(9)-C(11)-C(12)	60.5(2)
C(4)-C(5)-C(6)-C(7)	171.75(19)	C(10)-C(9)-C(11)-C(12)	-172.12(17)
C(10)-C(5)-C(6)-C(7)	-54.6(2)	C(9)-C(11)-C(12)-C(13)	-56.6(3)
C(5)-C(6)-C(7)-C(8)	56.4(2)	C(11)-C(12)-C(13)-C(15)	164.4(2)
C(6)-C(7)-C(8)-C(14)	-177.04(17)	C(11)-C(12)-C(13)-C(14)	45.7(3)
C(6)-C(7)-C(8)-C(9)	-54.9(2)	C(11)-C(12)-C(13)-C(17)	-78.3(3)
C(14)-C(8)-C(9)-C(11)	-57.5(2)	C(7)-C(8)-C(14)-C(13)	175.84(18)
C(7)-C(8)-C(9)-C(11)	-179.18(17)	C(9)-C(8)-C(14)-C(13)	53.1(2)
C(14)-C(8)-C(9)-C(10)	173.98(18)	C(15)-C(13)-C(14)-C(8)	-166.7(2)
C(7)-C(8)-C(9)-C(10)	52.3(2)	C(12)-C(13)-C(14)-C(8)	-45.6(3)
C(2)-C(1)-C(10)-C(20)	-69.0(2)	C(17)-C(13)-C(14)-C(8)	78.2(3)
C(2)-C(1)-C(10)-C(9)	172.19(19)	C(12)-C(13)-C(15)-C(16)	5.0(4)
C(2)-C(1)-C(10)-C(5)	53.0(2)	C(14)-C(13)-C(15)-C(16)	125.2(4)
C(11)-C(9)-C(10)-C(1)	68.0(2)	C(17)-C(13)-C(15)-C(16)	-115.7(4)
C(8)-C(9)-C(10)-C(1)	-167.80(17)		

Table 5: ^{13}C NMR and ^{1}H NMR data of compound **7**.

	7 [c]			Hydroxychavicol*	
No.[a]	$^{13}C(\delta)$	^{1}H (δ)	No[b].	$^{13}C(\delta)$ in $CDCl_3$	^{1}H (δ) in CD_3OD
1	141.64	-	1	115.53	-
2	143.42	-	2	121.01	6.77(d, 8Hz)*
3	115.67	6.74	3	133.27	6.60 (dd, 8, 2Hz)*
4	133.20	-	4	141.67	-
5	120.98	6.65(d, 8Hz)	5	143.46	-
6	115.32	6.81(d, 8Hz)	6	137.60	6.69 (d, 2 Hz)
7	39.47	3.295(d, 6.53Hz)	1’	39.45	3.37 (d, 6.7 Hz)
8	137.60	5.95m	2’	115.70	5.90
9	115.56	5.08	3’	115.37	5.01, 5.04
OH	-	5.40bs	-	-	-
OH	-	5.40bs	-	-	-

[a] numbering as given in 7, [b] literature numbering, [c] both spectra in $CDCl_3$,

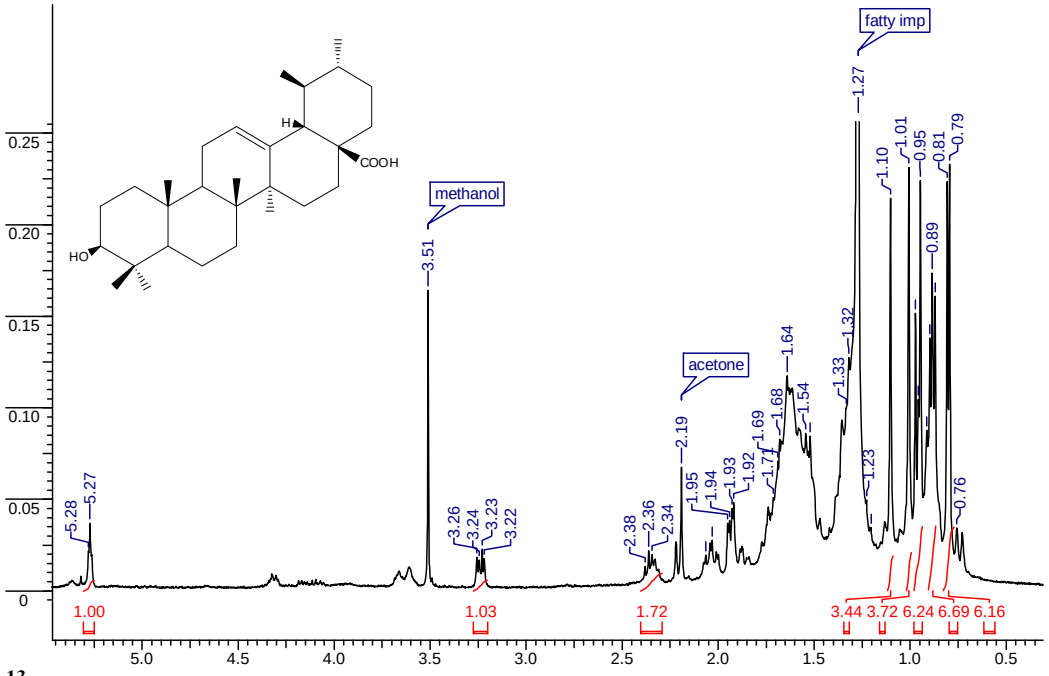
* as given in literature [6]

5.7. Spectral data:

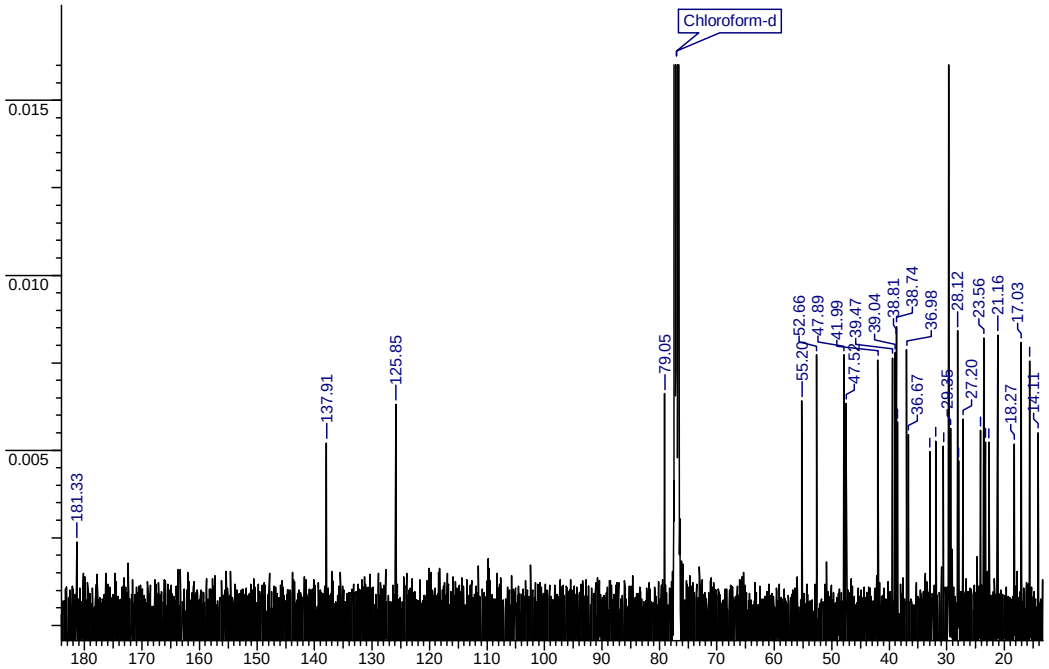
Compound **1**:

i. Spectra in $CDCl_3$: CD_3OD (3:1)

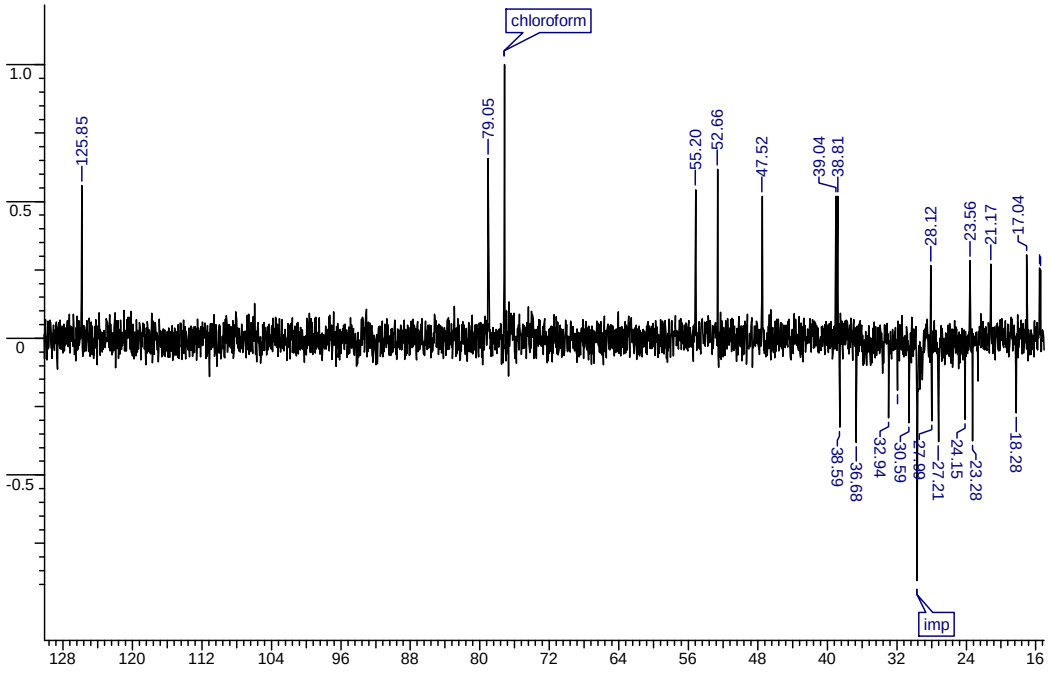
^{1}H NMR



^{13}C NMR



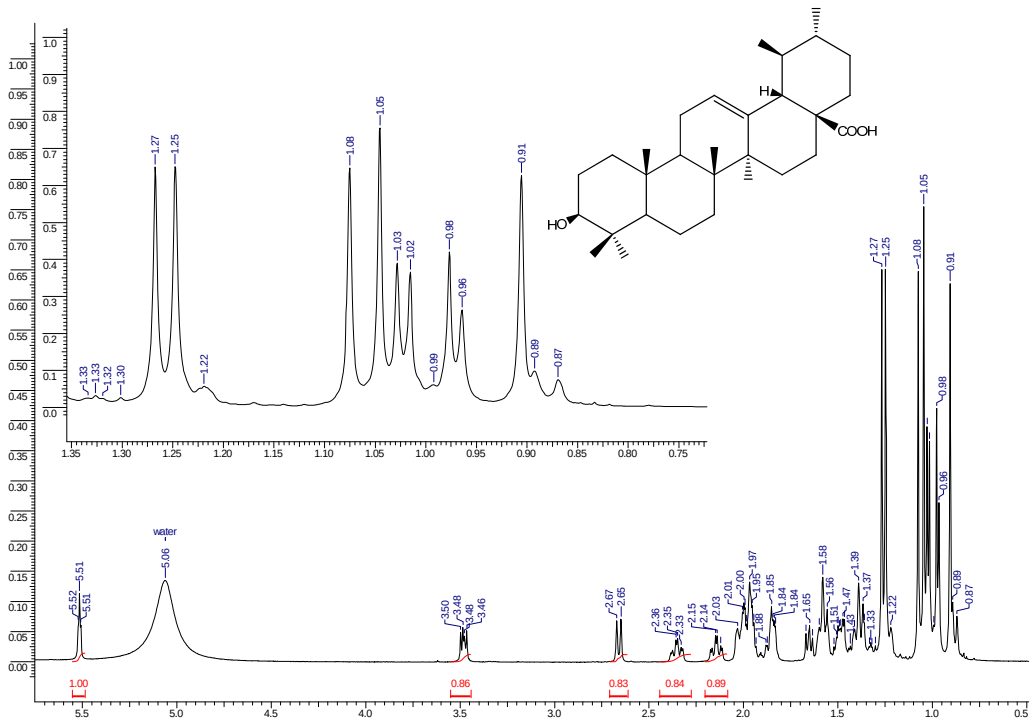
DEPT



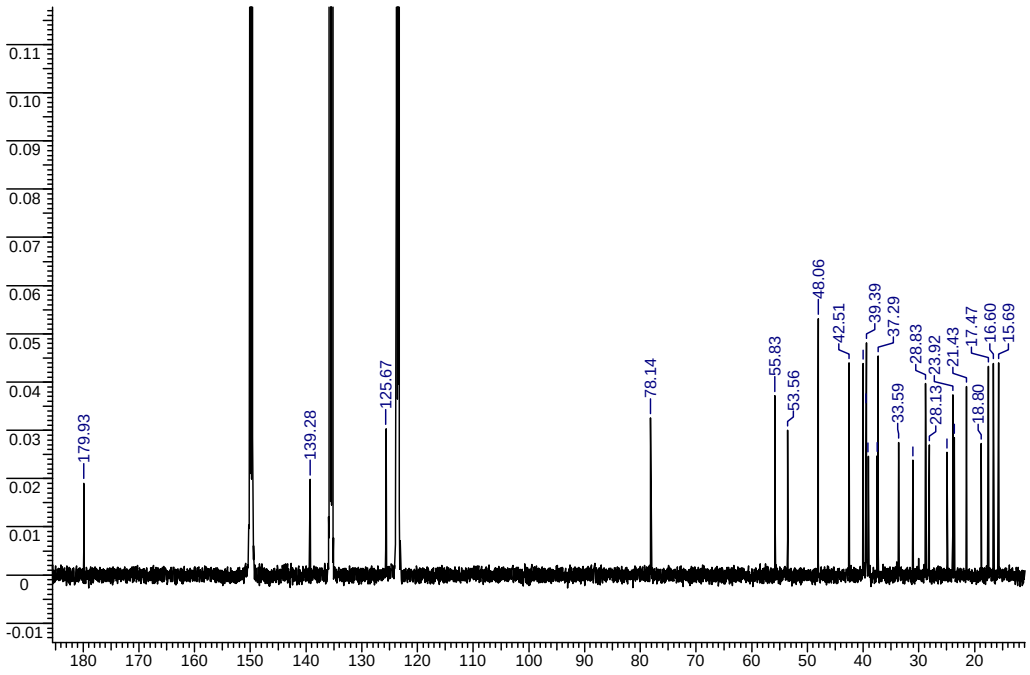
Compound **1**:

ii. Spectra in Pyridine-d_5

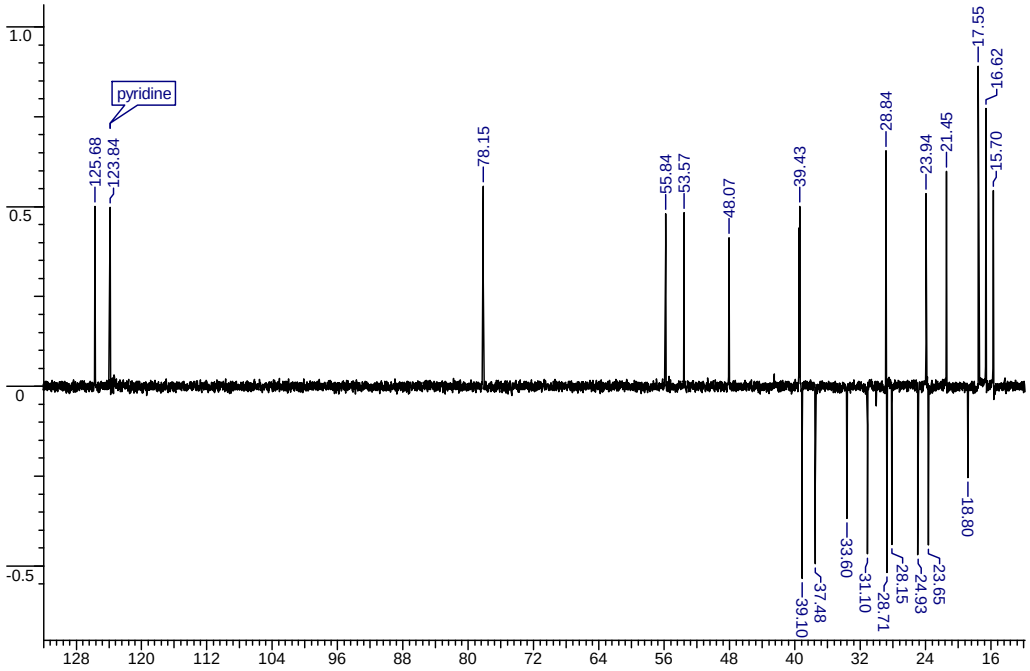
^{1}H NMR



^{13}C NMR

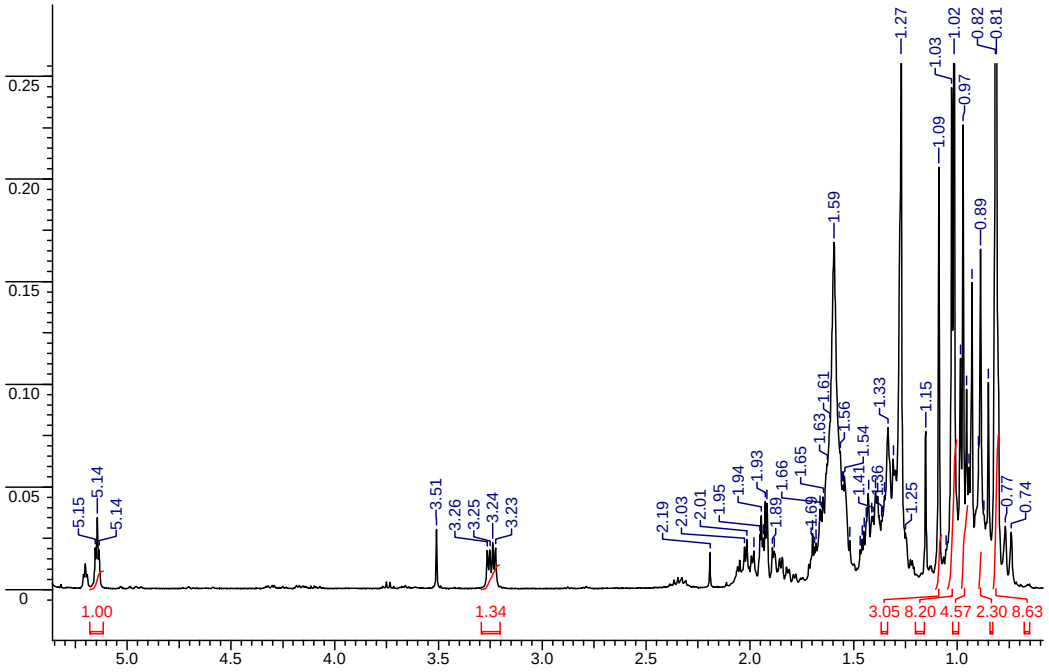

179.93
139.28
125.67
78.14
55.83
53.56
48.06
42.51
39.39
37.29
33.59
28.83
28.13
23.92
21.43
18.80
17.47
16.60
15.69

DEPT

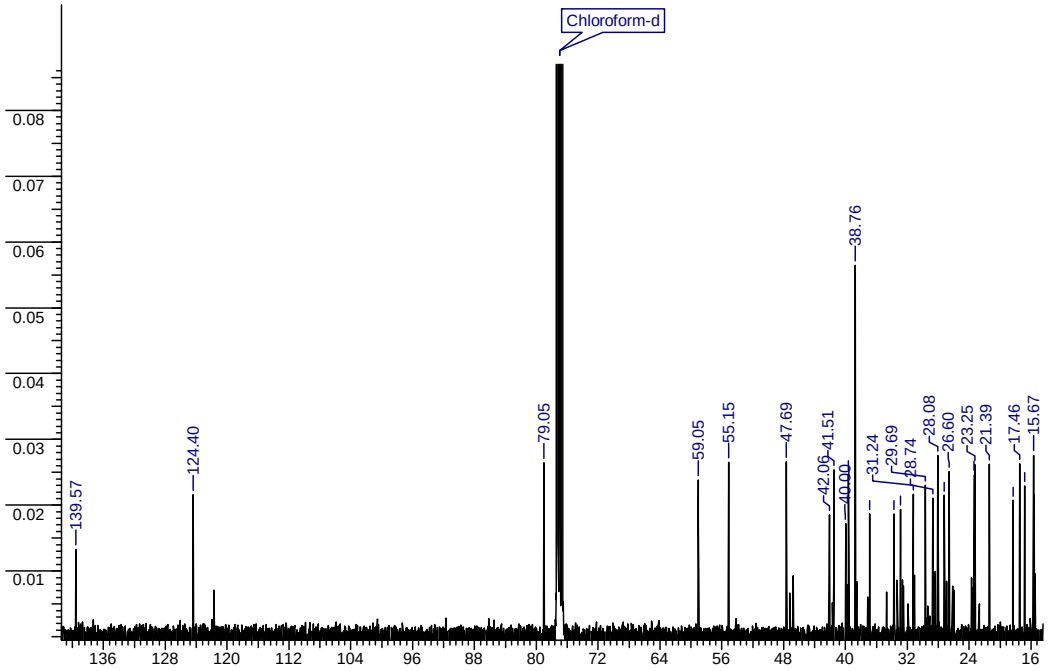

pyridine
125.68
123.84
78.15
55.84
53.57
48.07
39.43
28.84
23.94
21.45
17.55
16.62
15.70
18.80
33.60
31.10
28.71
28.15
24.93
23.65
39.10
37.48

Compound **2**:

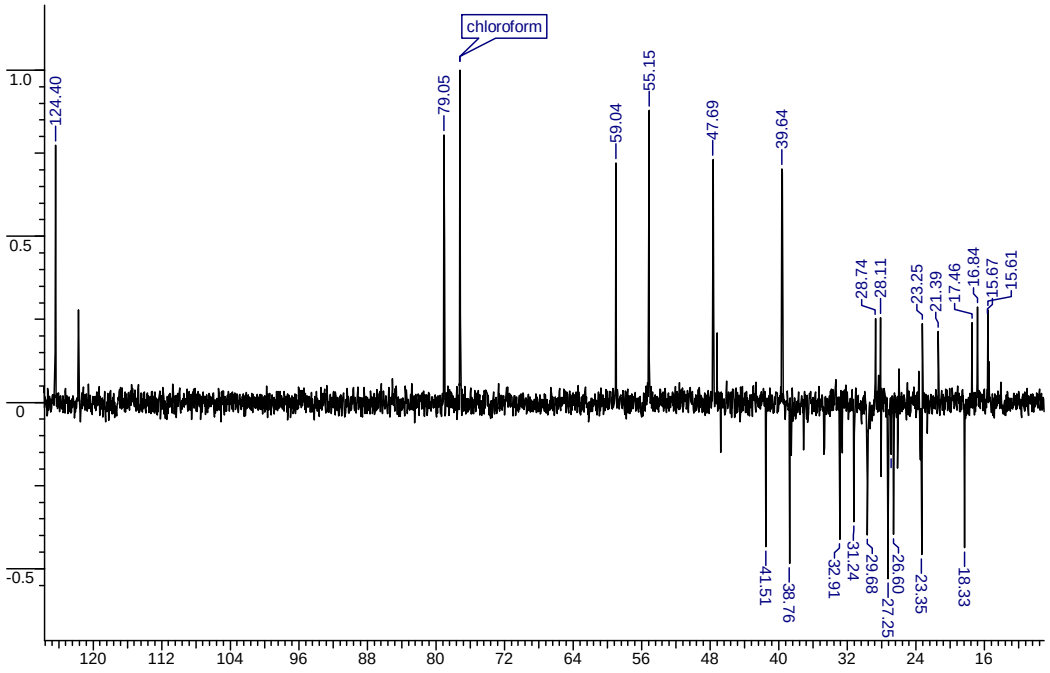
^{1}H NMR



^{13}C NMR:

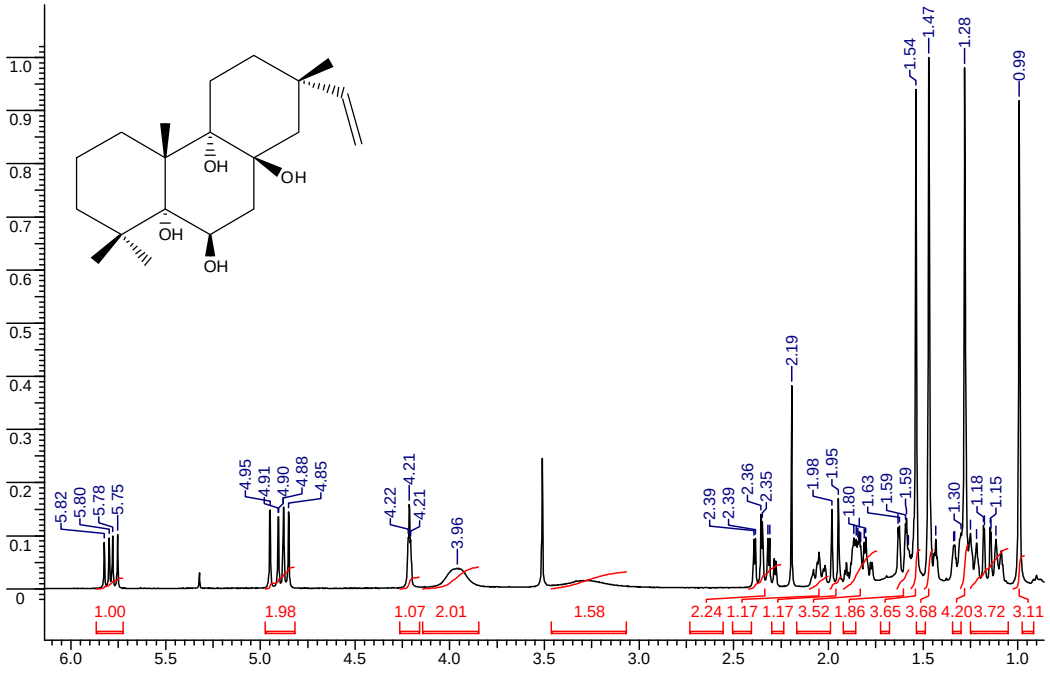


DEPT

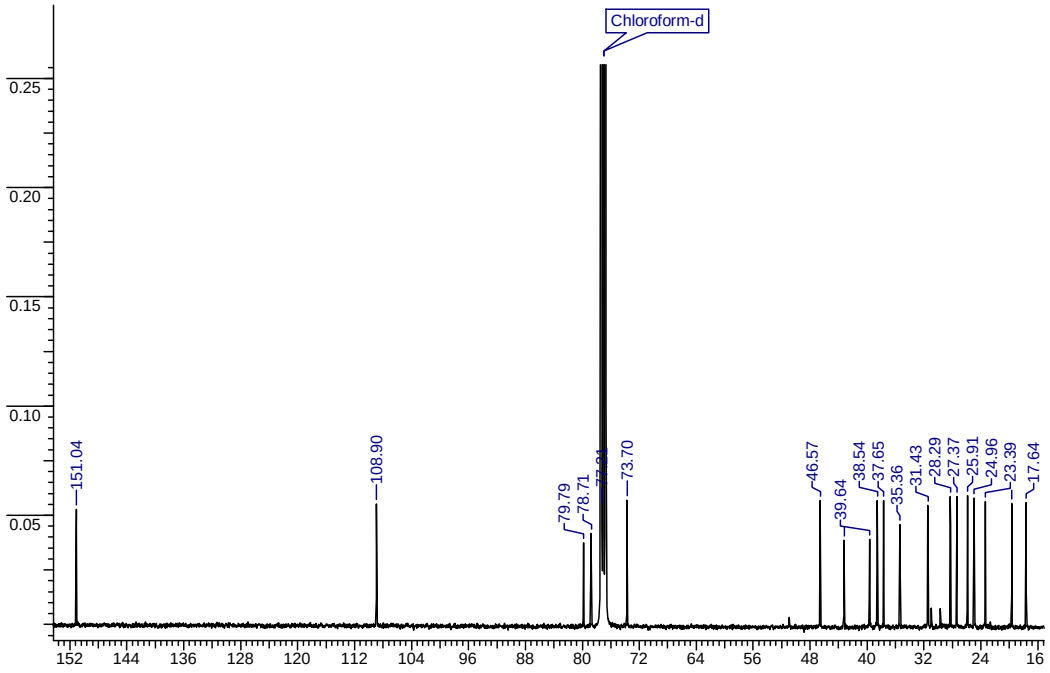


Compound **6**:

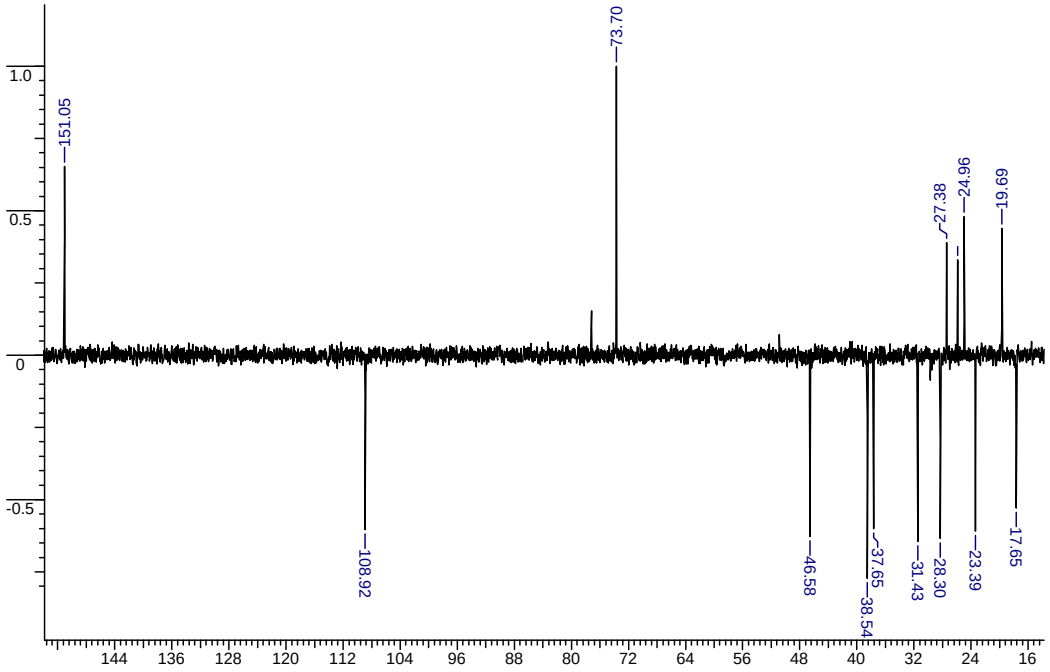
^{1}H NMR



^{13}C NMR

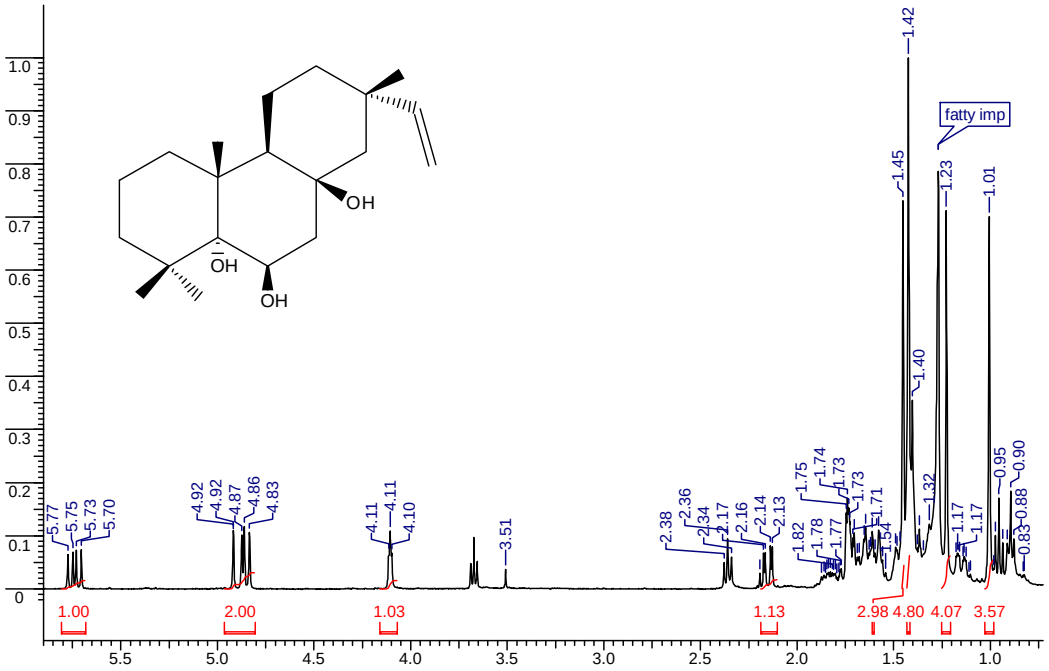

Chloroform-d
151.04
108.90
79.79
78.71
77.01
73.70
46.57
39.64
38.54
37.65
35.36
31.43
28.29
27.37
25.91
24.96
23.39
17.64

DEPT

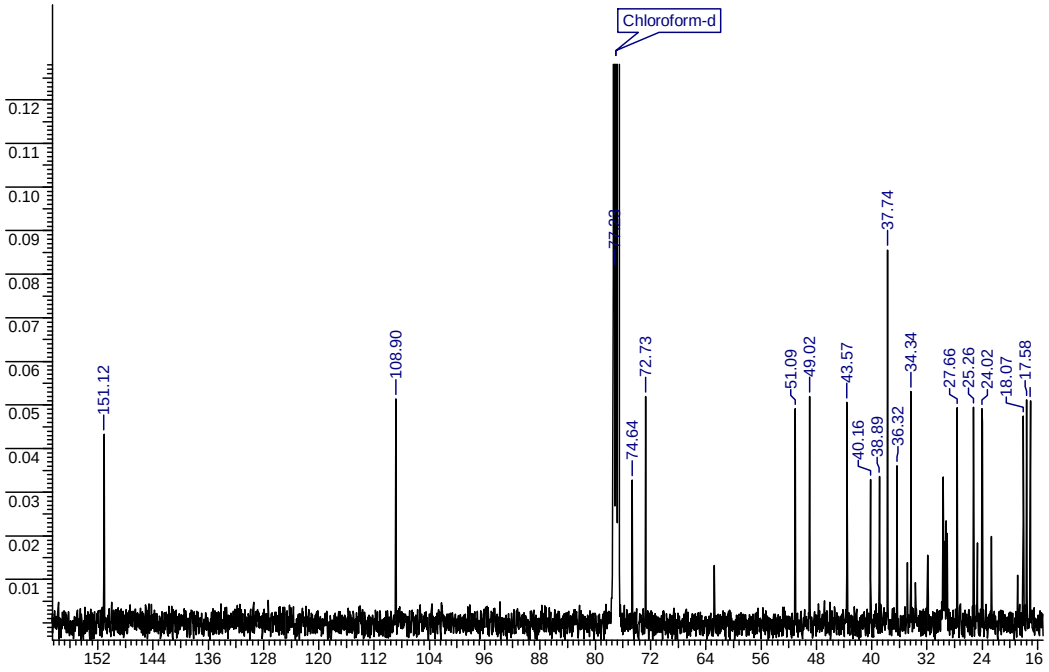

73.70
151.05
27.38
24.96
19.69
108.92
46.58
37.65
38.54
31.43
28.30
23.39
17.65

Compound **5**:

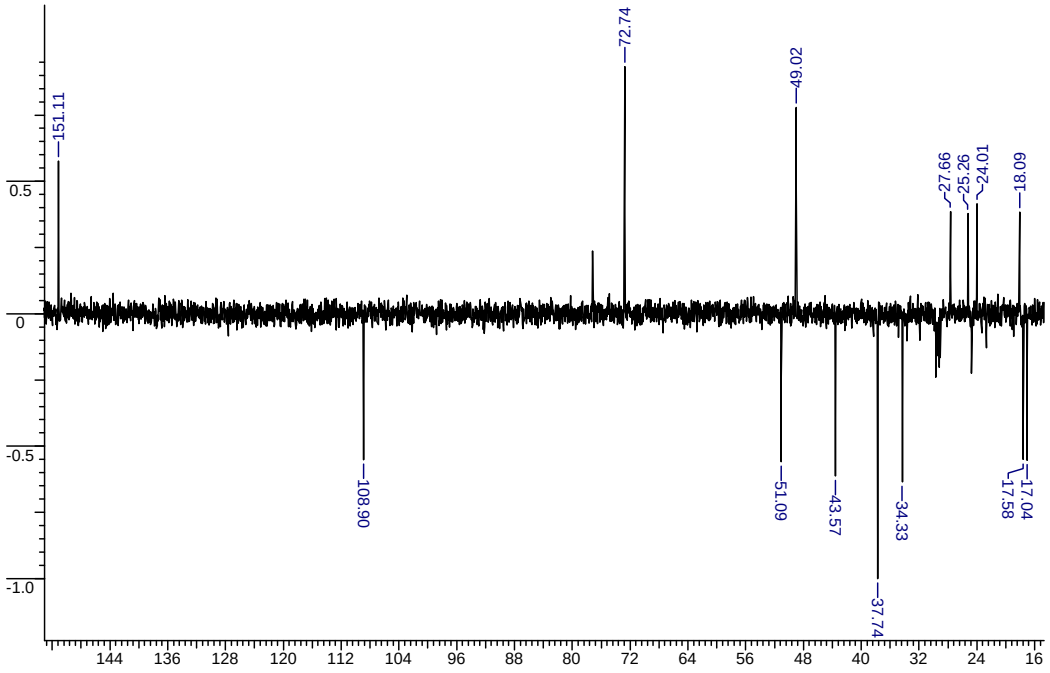
^{1}H NMR



^{13}C NMR

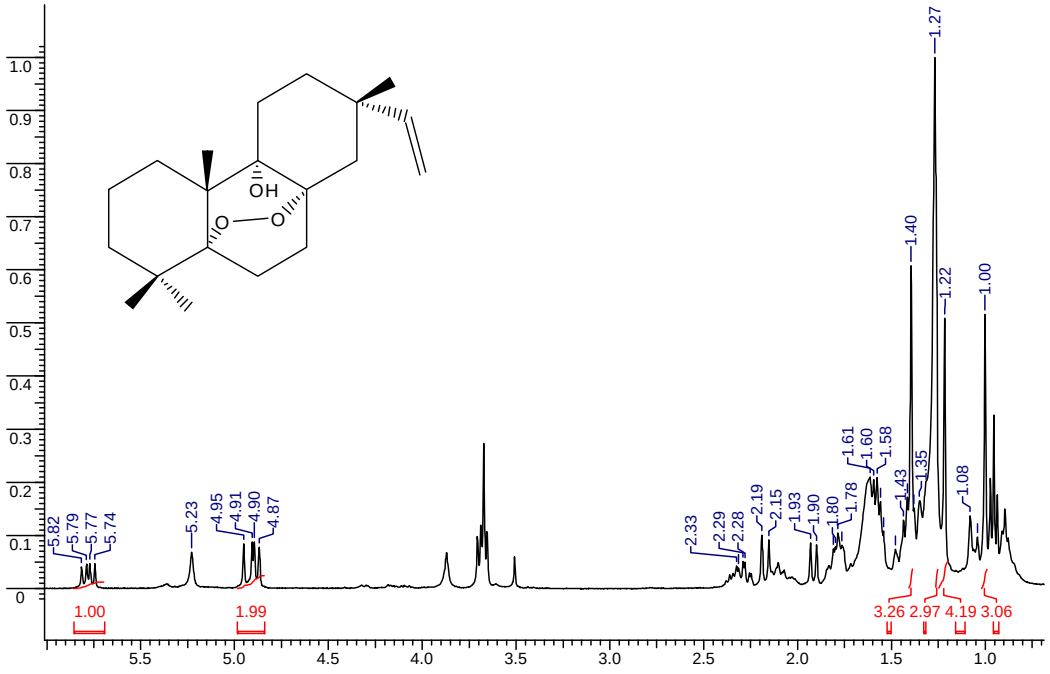


DEPT

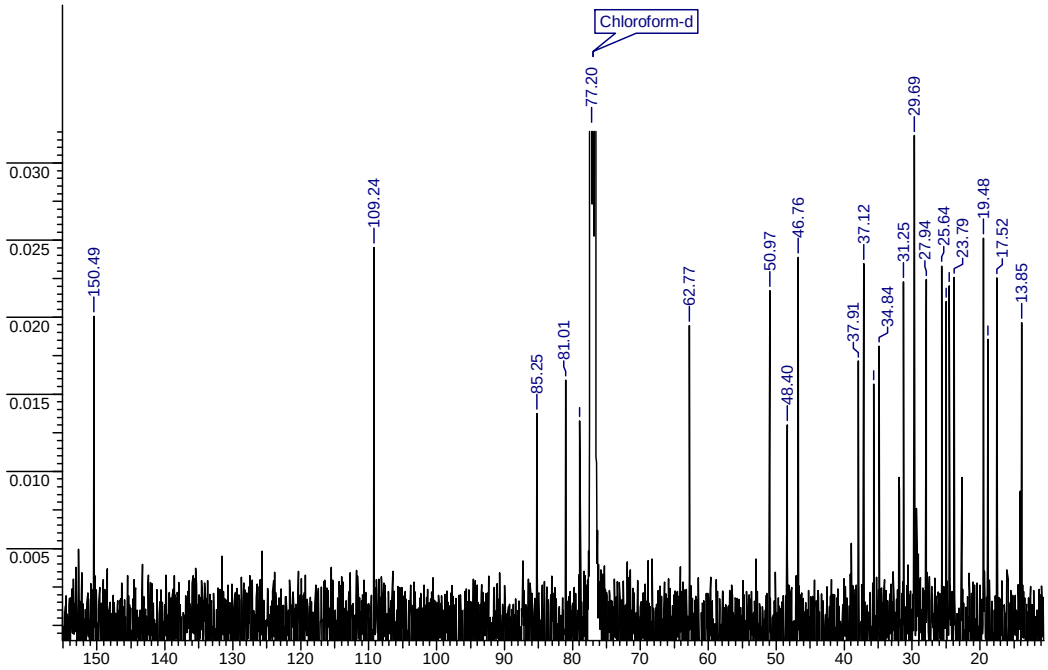


Compound **4**:

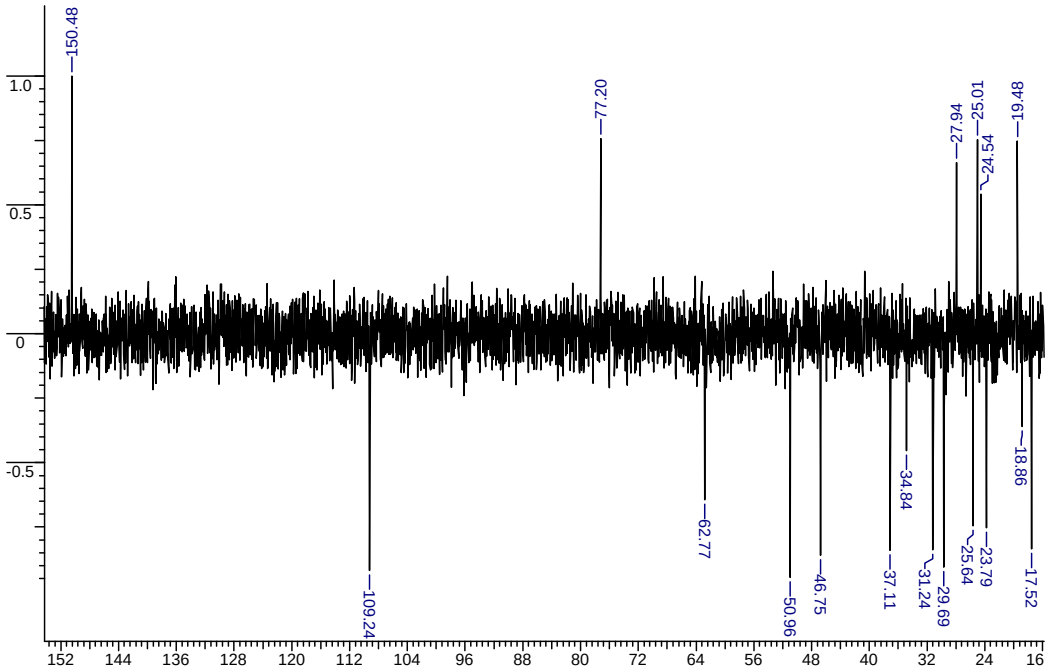
^{1}H NMR



^{13}C NMR

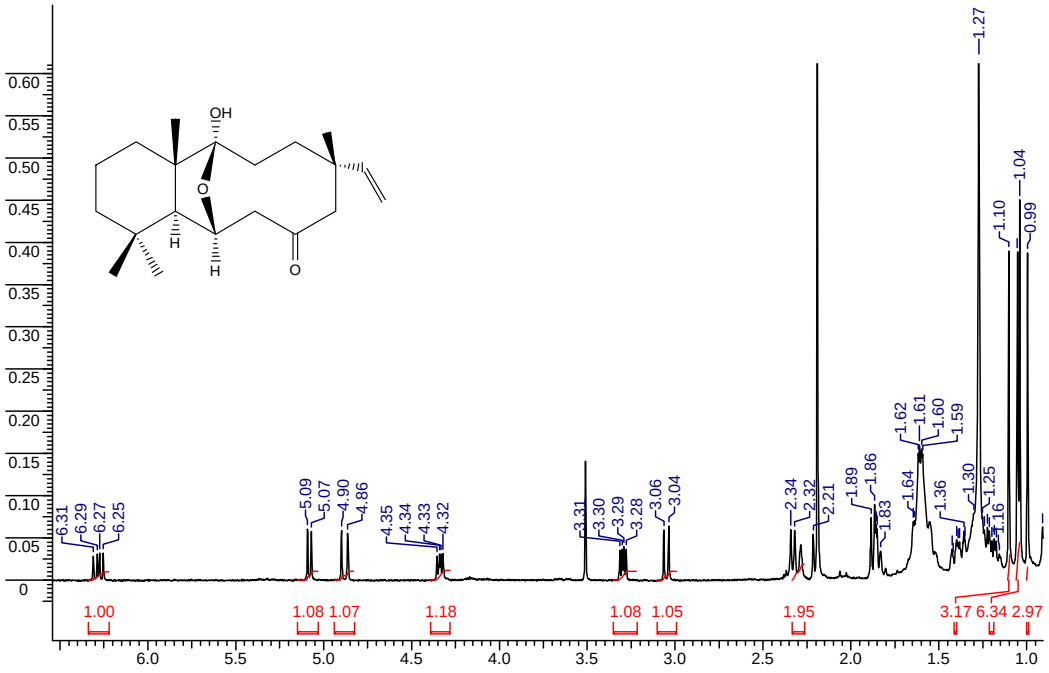

Chloroform-d
77.20
150.49
109.24
85.25
81.01
62.77
50.97
48.40
46.76
37.91
37.12
34.84
31.25
29.69
27.94
25.64
23.79
19.48
17.52
13.85
0.030
0.025
0.020
0.015
0.010
0.005
150
140
130
120
110
100
90
80
70
60
50
40
30
20

DEPT

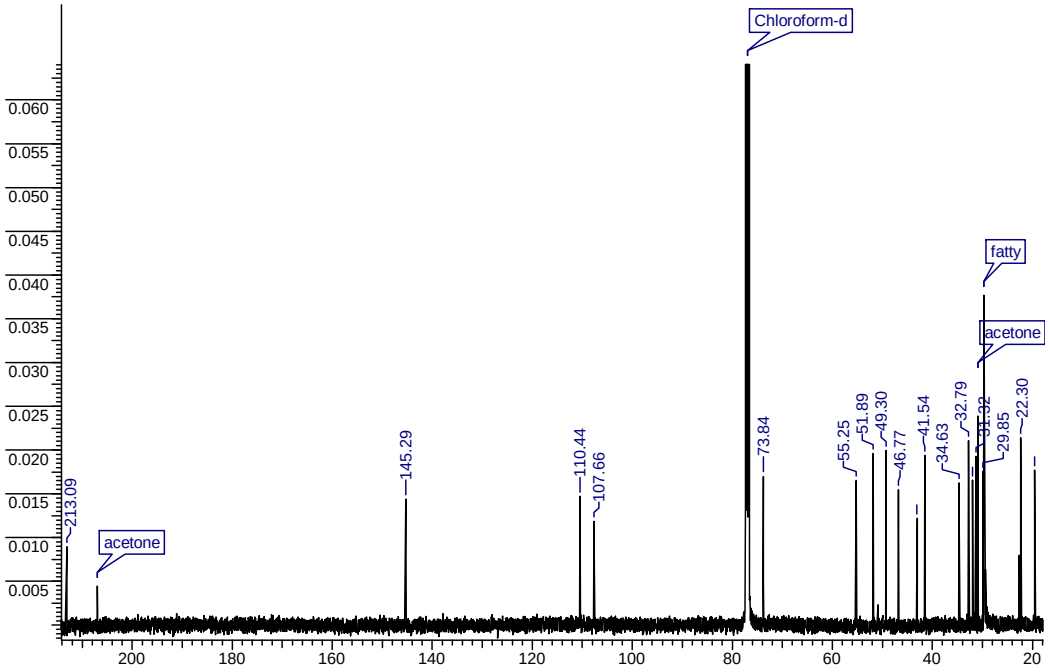

150.48
77.20
27.94
25.01
24.54
19.48
18.86
34.84
62.77
109.24
50.96
46.75
37.11
31.24
29.69
25.64
23.79
17.52
1.0
0.5
0
-0.5
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16

Compound **3**:

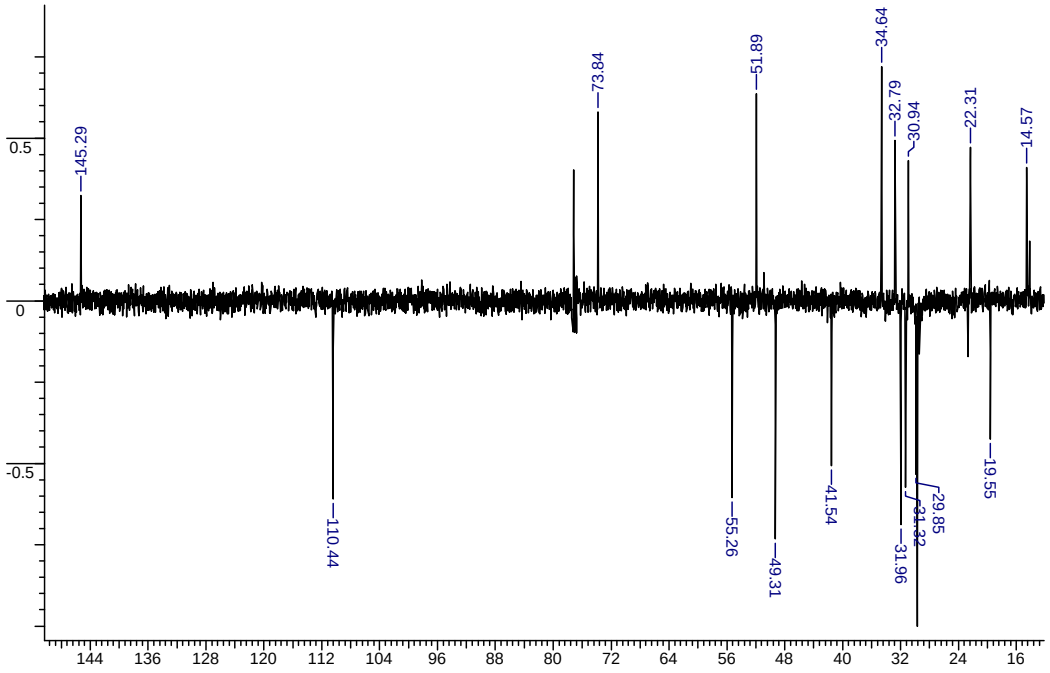
^{1}H NMR



^{13}C NMR

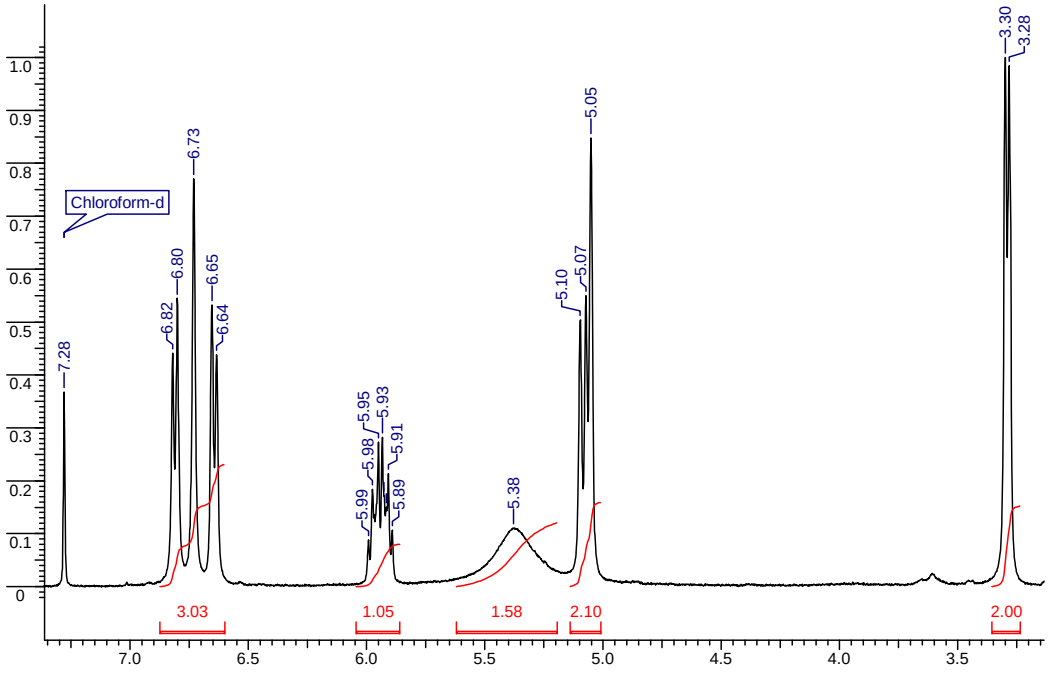


DEPT

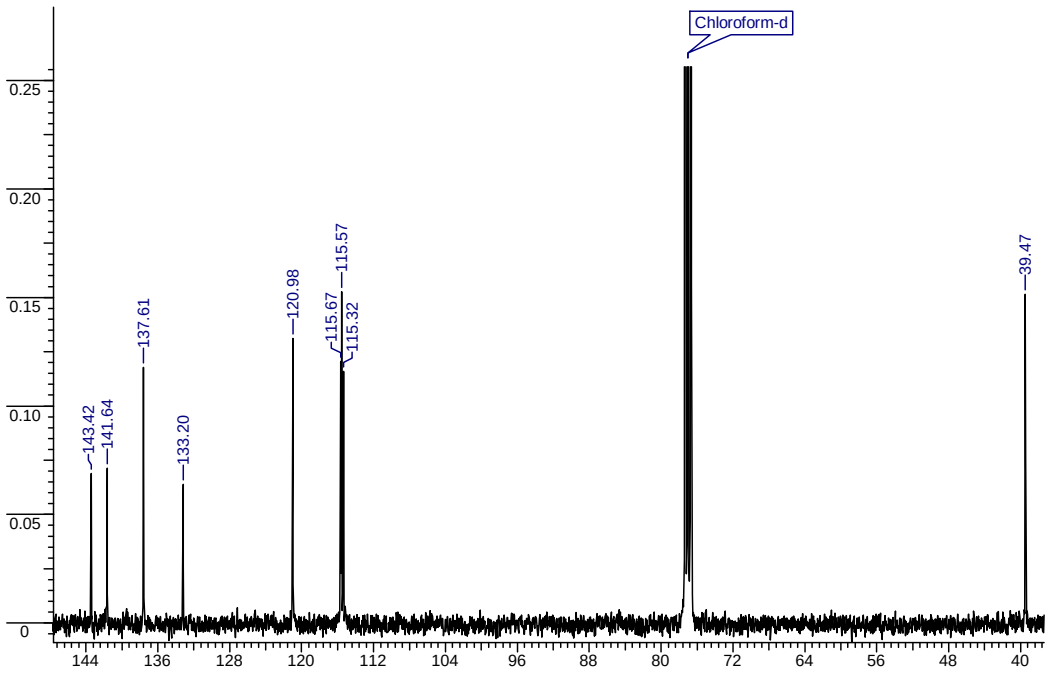


Compound **7**:

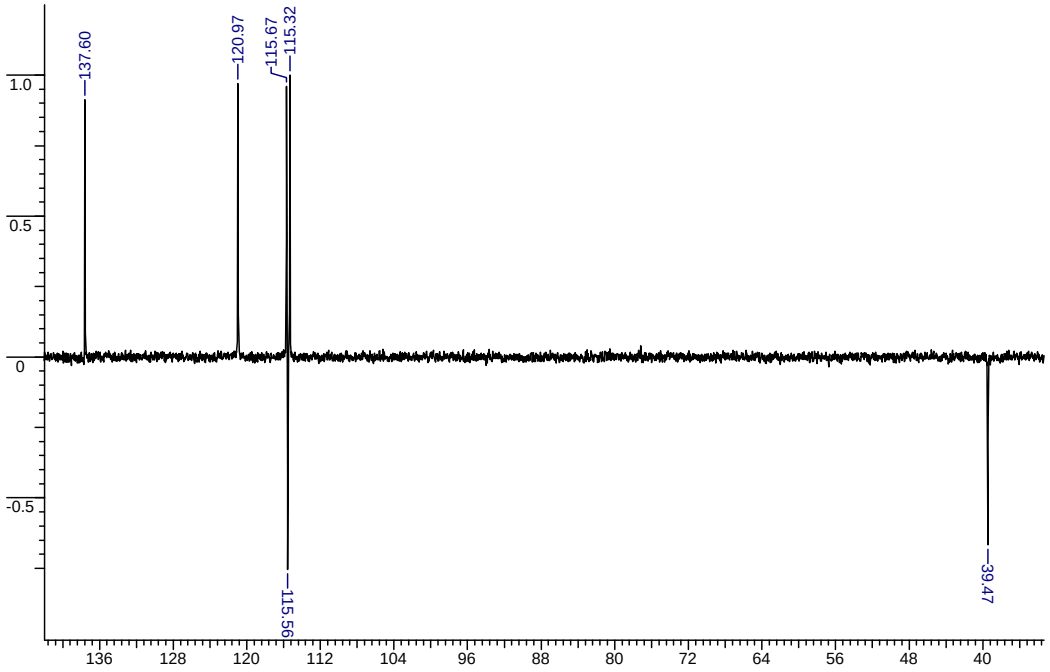
^{1}H NMR



^{13}C NMR


Chloroform-d
143.42
141.64
137.61
133.20
120.98
115.67
115.57
115.32
39.47
0.25
0.20
0.15
0.10
0.05
0
144
136
128
120
112
104
96
88
80
72
64
56
48
40

DEPT


137.60
120.97
115.67
115.32
115.56
39.47
1.0
0.5
0
-0.5
136
128
120
112
104
96
88
80
72
64
56
48
40

5.8. Reference:

[1] S. S. A. Paton, *Kew Bulletin*, 2009, **64**, 235.

[2] *Flora of Maharashtra State – Dicotyledons* (Flora of India series 2), ed. N.P. Singh, P. Lakshminarasimhan, S. Karthikeyan and P. V. Prasanna; published by, The Director, Botanical Survey of India, Culcatta, 2001, Vol **2**, pg713.

[3] W. Seebacher, N. Simic, R. Weis, R. Saf and O. Kunert, *Magn. Reson. Chem.* 2003, **41**, 636.

[4] R. Xu, G. C. Fazio, S. P.T. Matsuda, Phytochemistry, 2004, **65**, 261.

[5] T. Anthonsen, T. Bruun, E. Hemmer, D. Holme, A. Lamvik, E. Sunde and N. Sorensen, *Acta Chim. Scand.*, 1979, **24**(7), 2479.

[6] E. Shimoni, T. Baasov, U. Ravid and Y, Shoham, *Journal of Biotechnology*, 2003, **105**, 61.

[7] G. M. Sheldrick, SHELX-97 program for crystal structure solution and refinement, University of Gottingen, Germany, 1997.

Chapter 6

Phytochemical investigation on

Plectranthus mollis (Aiton) Spreng.

Figure 1: ***Plectranthus mollis*** **(Aiton) Spreng.**.; ***P. incanus*** L.

6.1. Introduction:

Plectranthus is a genus of perennial or annual, sometimes succulent, subshrubs or herbs or geoxylic herbs, usually aromatic and is represented by around three hundred species in the Old World tropics [1]. Genus *Plectranthus* is represented in Maharashtra by fourteen species [2]. *Plectranthus mollis* (=*P. incanus*) called Soft-stem mintleaf or Lal aghada in Marathi, is a small, erect, fleshy, annual herb, growing up to 30-50 cm tall. Oppositely arranged leaves are broadly ovate, 5-12 cm long, pointed, heart-shaped at base, with a toothed margin. Leaf stalks are 3-7 cm long. Flowers (July-August) are borne in branched racemes, 7-20 cm long and at the end of branches. Flowers are pale blue, carried on 3-4 cm long stalk. Sepal cup is 2-lipped. Fruits are round, 2-3 mm, brown, dotted with purple. [3]

In India, *P. mollis* leaves are cooked as vegetable [4]. *P. mollis* is used as febrifuge, insect repellent and also in treatment of rheumatism, as cardiac depressant, as a cure for haemorrhage, in treatment of mental retardation, snakebites and as tonic. *P. mollis* is also reported to exhibit relaxant activity on smooth and skeletal muscles [4]. It also has tumour inhibiting property. In India, Kenya and Tanzania, it is used to drive evil spirits away [4]. Earlier chemical investigations on *P. mollis* have reported isolation of fatty acids [5], while analysis of essential oil has revealed piperitone and piperitone oxide as some of the major constituents [5, 6].

6.2. Collection and processing:

P. mollis, whole plants, in flowering, were collected along roadside from Kas area, District Satara, in September, 2010. A herbarium is deposited in Botanical Survey of India, Western Circle, Pune (No. SPJ11). Roots were separated and aerial parts were cleaned off adhering dust and unwanted plant material. Cleaned aerial parts were divided into two parts. One part was cut into small pieces and processed for obtaining essential oil. Second part was dried in shade, cut and pulverized.

6.3. Extraction and Isolation:

i) Distillation of essential oil:

Oil was isolated from fresh aerial parts by hydro-distillation using *Clevenger*-type apparatus with 0.034% yield on fresh plant material basis.

ii) Extraction and isolation of compounds from acetone extract:

Pulverized aerial parts (2.0kg) were extracted with acetone (3L ×3 × 14h) at room temperature. The acetone solubles were filtered and concentrated under reduced pressure to provide a greenish acetone extract (50.0g, 2.5% based on dry plant weight). Acetone extract, 48g, was separated by column chromatography (CC) in acetone: petroleum ether gradient to collect 20 fractions (PM1-PM20).

From fraction PM3, **1** (20mg), was isolated as white precipitate. PM8 (990mg) was subjected to CC in 10% acetone in pet-ether to collect 8 fractions (PM8a-PM8h). Fraction PM9 (1.6g) was subjected to CC in acetone: petroleum ether gradient from 10-25% to collect nine fractions (PM9a-PM9i). Fractions, PM8f, PM8g and PM9f were combined and compound **2** (12mg) was isolated from it by CC in 10% acetone in petroleum ether. Compound **2** was purified by crystallization from acetone. From combined fractions, PM9g, PM9h and PM9i, compound **3** (30mg) was isolated by CC using elution system 5% methanol in chloroform and purifying by washing with chloroform. Fractions PM16 and PM17 had compound **4**. It was isolated by CC of these fractions separately in 5% methanol in chloroform. From fractions PM16a and PM17a, compound **4** (35mg) was obtained as white precipitate. It was purified by washing the precipitate successively with chloroform and acetone. Fractions PM18 (1.0g) and PM19 (1.1g) were separately subjected to CC in chloroform with gradient of methanol from 1-5% to collect seven and six fractions (PM18a-PM18f and PM19a-PM19f) respectively. From fractions PM18a and PM18b, compound **5** was obtained as yellow precipitate (40mg) which was purified further by washing with acetone. From PM19b and PM19c, compound **6** was obtained as yellow precipitate (20mg) which was washed successively with chloroform and acetone and finally purified by preparative TLC using 30% ethylacetate in benzene as developing system.

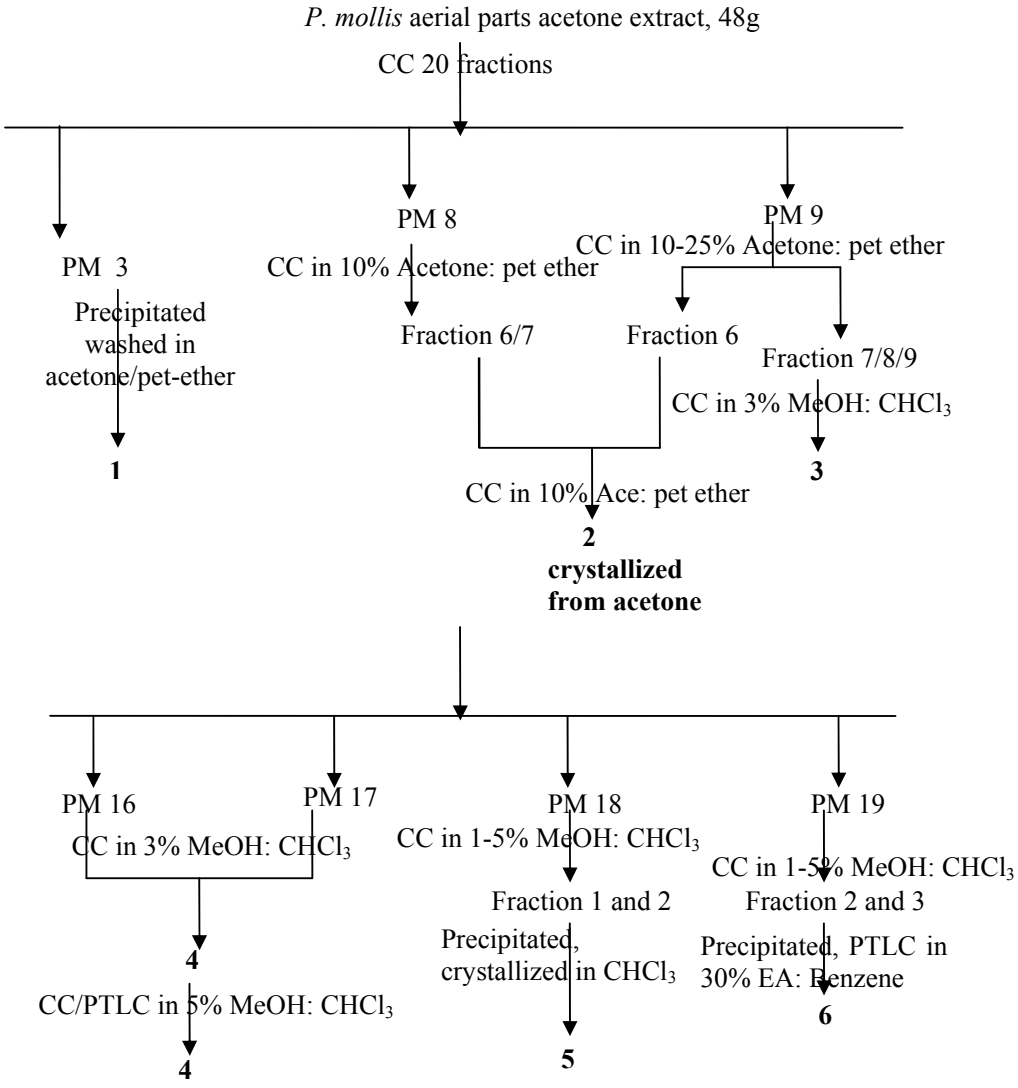


Chart 1: Chromatographic separation of *P. mollis*.

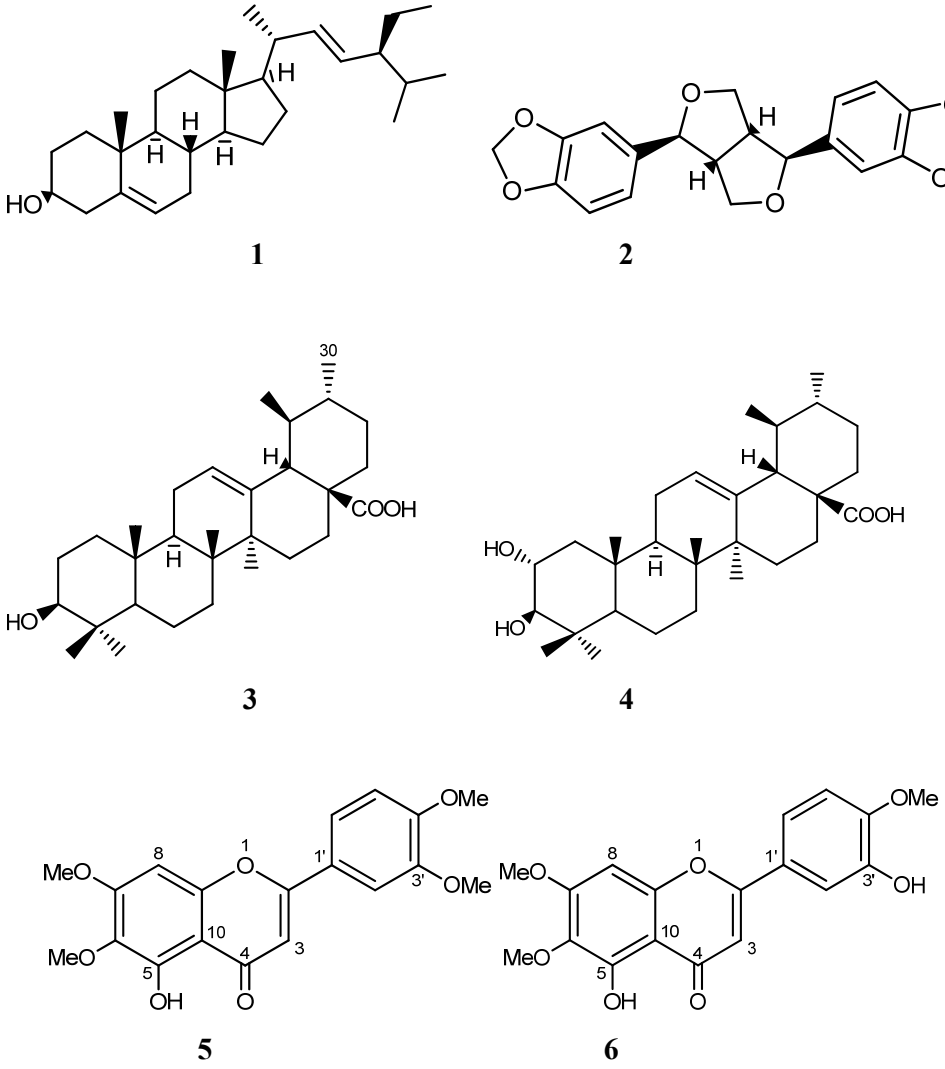


Figure 2: Compounds isolated from *P. mollis*

6.4. Structure elucidation:

Compound **1**:

Compound **1** was isolated as white needles. It was found to be a phytosterol mixture with stigmasterol as major constituent. It was identified by comparison of its TLC pattern (R_f value, charring pattern) with authentic samples and literature comparison of its ^{1}H and ^{13}C NMR data [7].

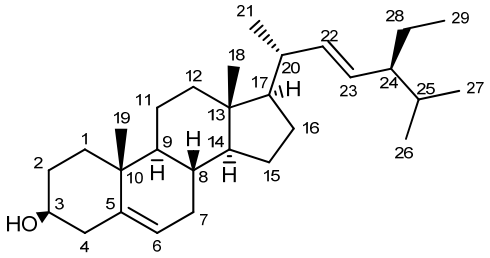


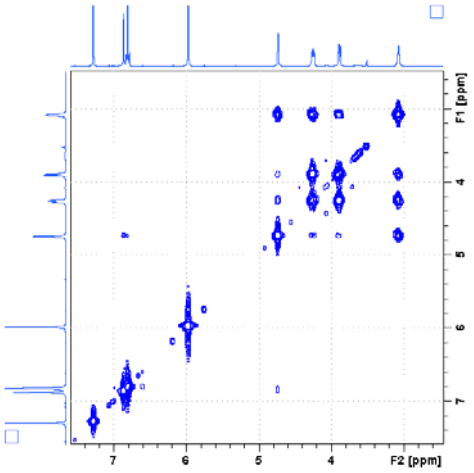
Figure 3: Structure of compound **1**.

Compound **2**

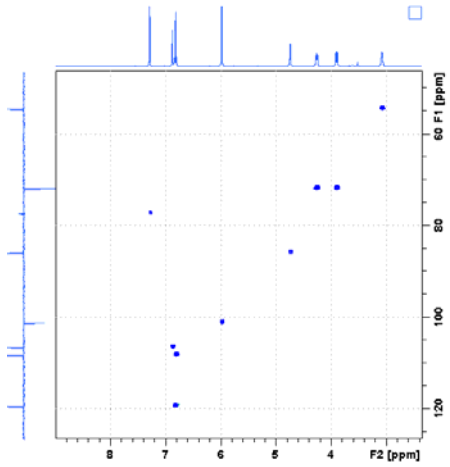
Compound **2** was obtained as brown needles. The ESIMS of **2** showed an $[M + 1]^+$ at *m/z* 355, $[M + Na]^+$ at *m/z* 377 suggesting the molecular formula $C_{20}H_{18}O_6$ with twelve degrees of unsaturation. The IR spectrum showed a stretching frequency of aromatic rings (1443cm^{-1}) and ether linkages (1245, 1037cm^{-1}).

^{13}C NMR revealed 10 resonance signals indicating compound **2** to be an symmetric dimer (Table 1). Presence of methylene carbon at δ101.07 and two carbons at δ147.09 and 147.95 indicated methylene dioxy fused with benzene ring. ^{1}H NMR revealed 1, 2, 4-trisubstitution pattern with signals at δ 6.87 (bs), 6.82 (dd, 8, 2Hz) and 6.79 (d, 8Hz). Signal of methine at δ 85.78 was assigned for 3 and 3'. This was supported by its HMBC correlation with protons at δ 6.87, 6.82. HMBC of proton at δ 3.07 (methine at δ 54.30) with carbon at δ135.02 (positions 4/4') confirmed location of δ 54.30 methine at 2/2'. From these spectral characteristics, compound **2** was identified as sesamin [8]. Compound **2** had mp 119.5^{0}C and optical rotation of $[\alpha]_D^{24.9}$ + 47 (c, 1.15 $CHCl_3$). The reported values were in accordance with reported values of [+]sesamin (reported: mp 119.5^{0}C [10]; $[\alpha]_D^{20}$ + 64.5 (c, 1.75 $CHCl_3$ [10]). This structure was also confirmed by single crystal X-ray crystallography of crystal grown in acetone (Figure 5, Tables 2a-2d).

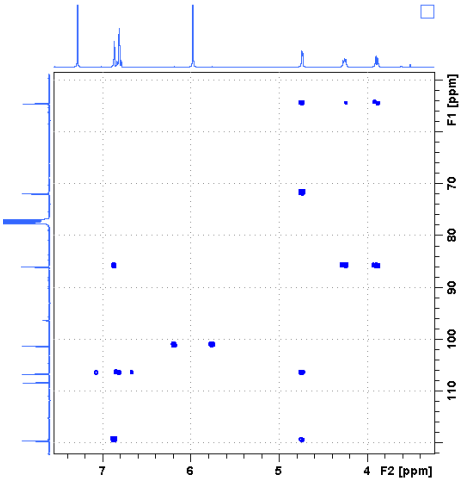
This is the first report of isolation of [+] sesamin from genus *Plectranthus*. Sesamin is mainly reported from diverse families such as Asteraceae, Brassicaceae, Rutaceae as well as from few Lamiaceae species [11, 12].



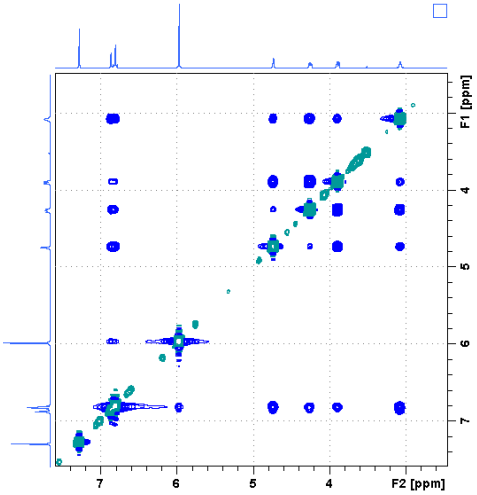
4a. COSY spectrum of **2**



4 b. HSQC spectrum of **2**



4c. HMBC spectrum of **2**



4d. NOESY spectrum of **2**

Figure 4: 2D spectra of compound **2**

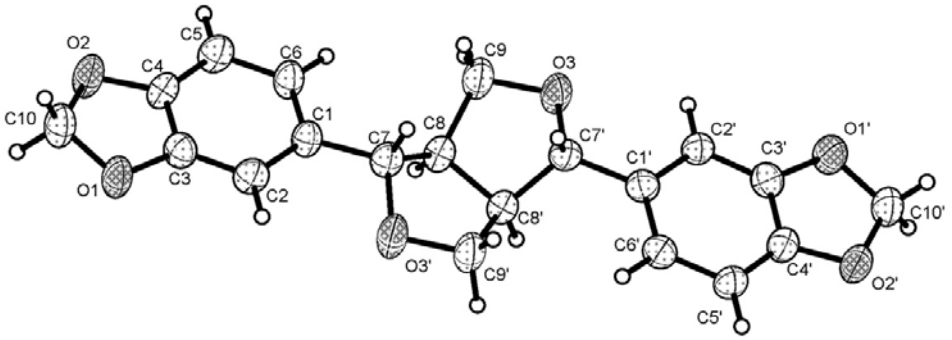


Figure 5: ORTEP diagram for compound **2**. Ellipsoids are drawn at 50% probability.

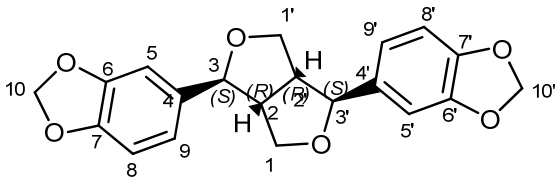


Figure 6: Structure of compound **2**.

Compound **3**:

Compound **3** was obtained as white amorphous powder. The ESIMS of **3** exhibited an $[M + 1]^+$ at *m*/*z* 455, $[M + Na]^+$ at *m*/*z* 479 suggesting the molecular formula $C_{30}H_{48}O_3$ with seven degrees of unsaturation. Carbonyl resonance at δ 180.01, olefinic carbons at δ139.36 and 125.75 along with methine at δ78.22 in its ^{13}C NMR (Table 3) indicated it to be a pentacyclic triterpene acid. Presence of 7 quaternary carbons and two doublet methyl groups at δ 1.025 and 0.97 indicated it to be Ursolic acid. Comparison of its NMR data with that reported in the literature confirmed this assignment [13]. Ursolic acid reported earlier from *P. rugosus* and *P. strigosus* [5]. This is the first report of its isolation from *P. mollis*.It has also been reported from *A. heyneana* as described in Chapter 5. Here ^{13}C NMR values are given in Table 3 for comparison with compound **4** while spectral compilation is omitted.

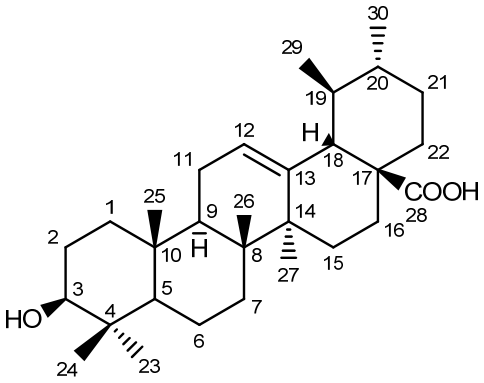


Figure 7: Structure of compound **3**.

Compound **4**:

Compound **4** was obtained as white amorphous powder. The ESIMS of **4** showed an $[M + 1]^+$ at *m/z* 473, $[M + Na]^+$ at *m/z* 495 suggesting the molecular formula $C_{30}H_{48}O_4$ with seven degrees of unsaturation. Carbonyl resonance at δ180.01, olefinic carbons at δ139.36 and 125.64 in its ^{13}C NMR (Table 3) indicated it to be a pentacyclic triterpenes acid. Presence of 7 quaternary carbons and two doublet methyl groups at δ1.025 and 0.97 indicated it to be ursolic acid derivative, with one additional hydroxyl group. Methines at δ68.69 and 83.90 are typical of 2α, 3β dihydroxy pattern. Comparison of its NMR data with that reported in literature confirmed this assignment and compound **4** was identified as corosolic acid [14]. This is the first report of corosolic acid isolation from *P. mollis*.

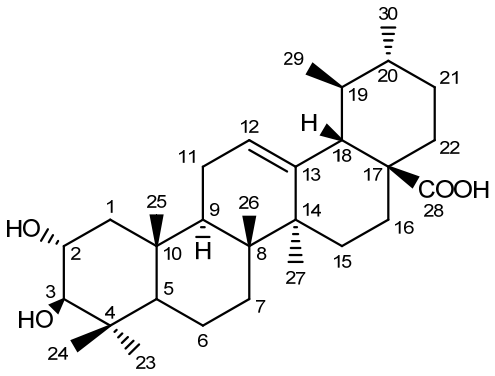


Figure 8: Structure of compound **4**.

Compound **5**:

Compound **5** was obtained as yellow crystals. The ESIMS of **5** showed an $[M + 1]^+$ at *m/z* 359, $[M + Na]^+$ at *m/z* 381 suggesting the molecular formula $C_{19}H_{18}O_7$ with eleven degrees of unsaturation. From the above data, compound **6** was found to be a flavonoid. Presence of singlet at δ12.75 and carbonyl carbon at δ182.46 indicated it to be 5-hydroxyflavone (Table 4). Presence of four methoxyl groups in spectrum and a singlet proton at δ6.54 along with protons at δ 7.48 (dd, 8, 2Hz), 7.295 (d, 2Hz) and 6.95 (d, 8Hz) indicated it to be a 6,7,3',4'-tetramethoxy or 7,8,3',4'-tetramethoxy derivative of 5–hydroxyflavone. Presence of one methoxyl resonance at around δ60 (here δ 60.72) is characteristic of methoxyl at position 6. Thus the compound **5** was identified as 6,7,3',4'-tetramethoxy-5–hydroxyflavone (3'-O-methyleupatorin). This was confirmed by comparison of its spectral characteristics with that reported in the literature [15] (Table 4). This assignment was additionally confirmed by analysis of its UV spectral data. Large bathochromic shift in Band II from 241 to 290nm on addition of NaOMe indicated hydroxyl group at 5 position. Similar bathochromic shift in Band II from 241 to 288 on addition of $AlCl_3$ which remained unchanged on addition of HCl, also supported presence 5-hydroxyl group. This is the first report of 3'-O-methyleupatorin isolation from *P. mollis*.

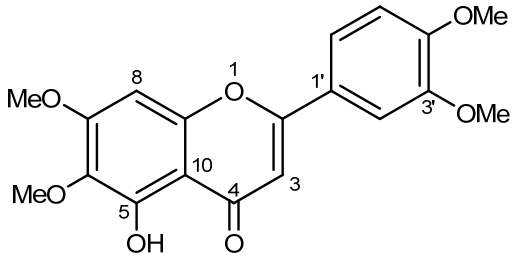


Figure 9: Structure of compound **5**.

Compound **6**:

Compound **6** was obtained as yellow amorphous powder. The ESIMS of **6** showed an $[M + 1]^+$ at *m/z* 345, $[M + Na]^+$ at *m/z* 367 suggesting the molecular formula $C_{19}H_{16}O_7$ with eleven degrees of unsaturation. Presence of singlet at δ12.77 and carbonyl carbon at δ 182.68 indicated it to be a 5-hydroxyflavone (Table 5). Presence of three methoxyl groups in the spectrum and a singlet proton at δ6.56 along with protons at δ7.47 (dd, 8, 2Hz), 7.49 (d, 2Hz) and 6.97 (d, 8Hz)

indicated it to be a 6,7,3',-trimethoxy (cirsilineol) or 6,7,4'-trimethoxy (eupatorin) derivative of 5–hydroxyflavone. Presence of one methoxyl resonance at around δ 60 (here δ 60.86) is characteristic of methoxyl at position 6. By comparison with literature data, compound **6** was identified as eupatorin (6, 7, 4', -trimethoxy-5, 3'–dihydroxyflavone) [16a] (Table 5). This assignment was confirmed by analysis of its UV spectral data. Large bathochromic shift in Band II from 241 to 293nm on addition of AlCl3 which remained unchanged on addition of HCl indicated hydroxyl group at 5 position while absence of shift in Band I at 347nm on addition of NaOMe indicated absence of hydroxyl at position 4'. Eupatorin is earlier reported from *P. fruticosus* [17]. This is the first report of eupatorin isolation from *P. mollis*.

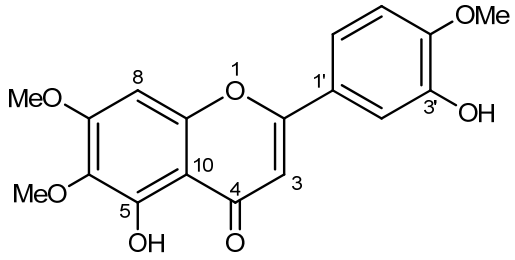


Figure 10: Structure of compound **6**.

6.5. Experimental:

A. Collection and processing:

Plectranthus mollis, whole plant, in flowering, was collected and processed for preparation of acetone extract as described earlier.

B. Extraction and Isolation:

Extraction and isolation of compounds from acetone extract and separation of essential oil from fresh aerial parts is described earlier.

Compound **1**:

White amorphous powder (20mg, 0.001% based on dry plant weight); IR (chloroform)3421, 1641, 1093cm^{-1}; ESIMS at *m/z* 435[M + Na]$^{+}$ and at *m/z* 451 [M + K]$^{+}$; ^{1}H NMR (δ); 0.70 (3H, s, H-18), 0.79 (3H, d, 6.5Hz, H-26), 0.80 (3H, t, 7.5Hz, H-29), 0.84 (3H, d, 6.5Hz, H-27), 1.02 (3H, d, 7.5Hz, H-21), 3.52 (1H, m, H-6), 5.14 (1H, m, H-23), 5.36 (1H, bs, H-6); ^{13}C NMR (δ); 12.08 (C-29), 12.31 (C-18), 19.02 (C-27), 19.44 (C-19), 21.10 (C-11), 21.2 (C-21), 21.26 (C-26), 24.41 (C-15), 25.46 (C-28), 28.4 (C-16), 31.65(C-2), 31.91(C-8), 31.91(C-25), 31.92 (C-7),36.53 (C-10), 37.28 (C-1), 39.70 (C-12), 40.56 (C-20), 42.24 (C-13), 42.29 (C-4), 50.15 (C-9), 51.27 (C-24), 55.91 (C-17), 56.89 (C-14), 71.6 (C-3), 121.73 (C-6), 129.28 (C-23), 138.37 (C-22), 140.79 (C-5).

Compound **2**:

Brown crystals (12mg, 0.0006%); mp 119.5^{o}C, ESIMS at *m/z* 377[M + Na]$^{+}$ and at *m/z* 393[M + K]$^{+}$; $[\alpha]_D^{24..3}$ +40 (1.15% chloroform); IR (chloroform) 1503, 1443, 1245, 1037cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1.

X-ray crystallography:

Single crystals of the compound were grown by slow evaporation of the solution in acetone. Crystal of approximate size 0.14 x 0.13 x 0.05 mm^3 was used for data collection on *Bruker SMART APEX* CCD diffractometer using Mo K_α radiation. Exposure / frame = 10.0 sec / frame. Crystals belong to Monoclinic, space group P21, a =9.8827 (8), *b* = 6.9772(5), *c* = 11.8465(9) Å, *V* = 815.47(11) Å^3, *Z* = 2, D_c = 1.443 g /cc, μ (Mo K_α) = 0.71073 Å, *T* = 200(2) K, 7946 reflections measured, R value 0.0431, wR2 = 0.1021. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (ShelxTL)[18] was used for structure solution and full matrix least squares refinement on F^2. Hydrogen atoms

were included in the refinement as per the riding model. Data collection and refinement parameters as well as compound bond angles, bond lengths and torsion angles are listed in Tables 2a-2d.

X-ray analysis revealed the relative conformation of the molecule at C2/2' and C3/3 as R and S configurations.

Compound **3**:

White amorphous powder (30mg, 0.0015%); ESIMS at *m/z* 479$[M + Na]^+$ and at *m/z* 495$[M + K]^+$; IR (ATR) 1688, cm^{-1}; for ^{13}C NMR, see Table 3.

Compound **4**:

White amorphous powder (35mg, 0.00175%); ESIMS at *m/z* 495$[M + Na]^+$ and at *m/z* 511$[M + K]^+$; ^1H NMR, 0.97 (3H, d, J = 5.8 Hz, H-29), 0.99(3H, s, H-26), 1.01 (3H, d, J = 6.6 Hz, H-30), 1.06 (3H, s, H-25), 1.09 (3H, s, H-24), 1.23 (3H, s, H-27), 1.29 (3H, s, H-23), 2.64 (1H, d, J =11.1 Hz, H-18), 3.42 (1H, d, J = 9.16 Hz, H-3), 4.12 (1H, ddd, J = 10.98, 9.3,4.27 Hz, H-2),5.48 (1H, t 3.36Hz, H-12); for ^{13}C NMR, see Table 3.

Compound **5**:

Yellow crystals (40 mg, 0.002%), mp 193.8^oC; ESIMS at *m/z* 359 $[M + 1]^+$, *m/z* and 381$[M + Na]^+$; UV(MeOH), 241, 278, 339nm; UV(NaOMe), 290, 298, 310nm; UV($AlCl_3$), 262, 288, 348, 368nm; UV($AlCl_3$-HCl), 257, 287, 348nm; IR (chloroform)3421, 1658, 1602, 1515, 1496, 1456, 1121cm^{-1}; for ^1H NMR and ^{13}C NMR , see Table 4.

Compound **6**:

Yellow amorphous powder (20 mg, 0.001%); ESIMS at 345 $[M + 1]^+$, *m/z* and 367$[M + Na]^+$; UV(MeOH), 281, 346nm; UV(NaOMe), 280, 347, 368nm; UV($AlCl_3$), 293, 347, 368nm; UV($AlCl_3$-HCl), 282, 347, 363nm; UV(NaOAc), 276, 344. 633; IR (ATR) 1649, 1596, 1453cm^{-1}; for ^1H and ^{13}C NMR, see Table 5.

6.6. Analysis of essential oil of *P. mollis*:

Distillation of essential oil:

It is described earlier.

GC analysis of essential oil:

The GC-FID analyses of the essential oils was carried out with an Varian CP 3800 apparatus equipped with FID and a GsBP5 capillary column (30m length, 0.25mm i.d., film thickness 0.25mm). Oven temperature was programmed rising from 50 to 260^0 at 3^0/min hold at 260^0C for 5 min; injector temperature, 250^0C; detector temperature, 300^0C, carrier gas, He (1.0 ml/min); injection volume, 1µl; split ratio, 6:4. The Linear Retention Indices (LRIs) of the constituents (Table 6) were determined relative to the retention times of a series of n-alkanes (C9–C38), and the relative percentages of the individual components of the oils were obtained from the GC-FID peak-area percentages after applying correction factors.

GC/MS Analysis of essential oil:

The GC/MS analyses of essential oil was performed with an Perkin Elmer Clarus 500 gas chromatograph coupled to a Perkin Elmer Clarus 500 quadruple mass spectrometer equipped with a GsBP5 capillary column (30m length, 0.25mm i.d., film thickness0.25 mm). Oven temperature was programmed rising from 50 to 280^0C at 5^0C /min with hold at 50^0C for 1min and at 280^0C for 10min.; injector temp., 280^0C; detector temperature, 300^0C; carrier gas, He (1.0 ml/min); injection volume, 1 µl; mass spectra, positive electron impact mode at 70 eV.

Identification of oil constituents:

The identification of the individual constituents of the oils was based on the comparison of their LRI and mass spectra with those of authentic compounds by means of the NBS and NIST databases and published data on http://www.webbook.nist.gov/ (Table 6).

Mosquito larvicidal assay of essential oil and acetone extract:

Standard WHO method of testing the susceptibility of mosquito larvae to insecticides [19] was followed in all the experiments with slight modification. Larvicidal assay was carried on larvae of three species of mosquitoes viz. *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*. Test samples were

dissolved in analar grade acetone to prepare stock solution. Ten early 4th instar mosquito larvae were introduced in 100 ml glass beaker containing 50 ml water. A known volume of stock solution was added in beaker to get various concentrations of oil/extract. Acetone control was run simultaneously. For each concentration and control, 5 replicates were used and each test was repeated three times. The beakers were kept at 26 $\pm 2^0$C. The corrected mortality was analyzed using Abbott's formula wherever required. Results are given in Tables 7 and 8. The mortality were analyzed by log probit method and Lethal Concentrations (LC_{50} & LC_{95}) were calculated (Table 9).

Mosquito repellency assay:

Mosquito repellent activity was assessed on the basis of the protection period offered by repellent test sample [20]. For the study, 4-6 days old, blood starved, sucrose fed (0.5M solution) females of *A. aegypti* were taken.

Human hand covered with snugly fitting glove was introduced in the cage containing 100 hungry females. Mosquitoes were allowed to bite on the back of the hand through muslin cloth screen stuck over a small window (2cm X 2cm) cut out in the glove. Test sample was loaded on the muslin cloth screen at concentrations, 0.5, 1.0 and 2.0mg/cm^2. Control muslin cloth screen was treated with solvent alone. After introduction of the hand covered with the glove with treated muslin screen into the mosquito cage, number of bites received in subsequent 5min was counted. In the event of no bites in the initial 5min exposure, the test hand was exposed repeatedly after every consecutive 30min for 5min test till the time a confirmed bite was received. Protection period was recorded as the time elapsed between repellent application and the time at which a confirmed bite was observed. A control arm was placed in the cage randomly before or after the treated arm to asses mosquitoes bite (Table 10).

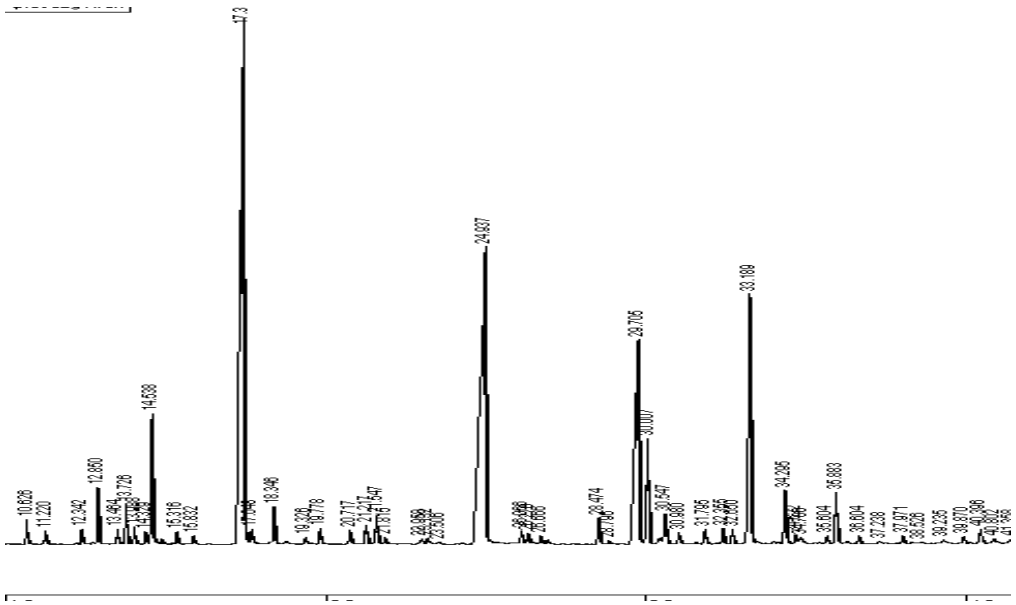


Figure 11: GC-FID analysis of essential oil.

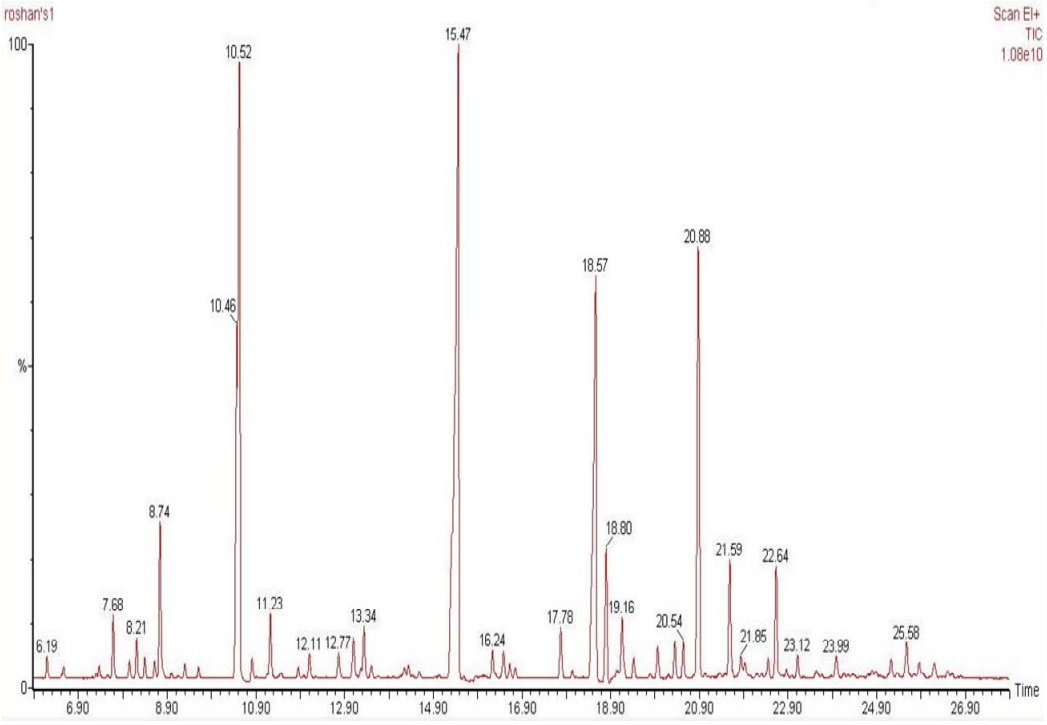


Figure 12: GC-MS analysis of essential oil.

Results and Discussion:

Analysis of Essential Oil: The oil was obtained by hydrodistillation and characterized using GC-FID and GC/MS analyses. The identified oil components with their relative contents are reported in Table 6. The essential oil contained

piperitone oxide (23.76%), fenchone (19.19%) and β-caryophyllene (10.39%) as main components and to a lesser extent limonene (2.83%), copaene (2.90%), germacrene D (2.58%), δ-cadinene (2.42%). The oil had significant percentage of sesquetrpenes and also less percentage of aromatic compounds.

Mosquito larvicidal activity: Eseential oil was the most potent with 100% mortality at > 125pmm against *A. stephensi*, 100ppm against *A. aegypti* and 125ppm against *C. quinquefasciatus* (Table 7) with LC_{50} in the range of 25 to 31ppm (Table 9). The acetone extract exhibited 100% mortality at 500 ppm (Table 8) with LC_{50} values in the range of 195-215ppm (Table 9).

Mosquito repellent activity: The essential oil was evaluated for repellent activity against *A. aegypti* at 0.5, 1.0 and 2.0mg/cm^2 concentrations (Table 5). The oil did not show any significant repellency.

Conclusion: The results demonstrated that essential oil of *P. mollis* was more potent than acetone extract in mosquito larvicidal assay. Also the oil did not show significant repellency for *A. aegypti*. These results were roughly reversed in case of essential oil of *L. gibsoni*, later oil being potent repellent and moderated larvicidal. Certain observations may be noted that could correlate observed activity pattern with chemical compositions of the oils. Thus *P. mollis* oil was characterized by less percentage of aromatic monoterpenes and high percentage of lesser volatile sesqueterpenes while significant percentage of *L. gibsoni* oil is composed of aromatic monoterpenes and very less percentage of sesqueterpenes.

6.7. Tables:

Table 1: ^{1}H NMR and ^{13}C NMR data for Compound **2**

No.	**2** (in $CDCl_3$)		Literature (in $CDCl_3$)*	
	^{13}C (δ)	^{1}H (δ)	^{13}Ca (δ)	^{1}Hb (δ)
1/1’	71.69	4.255, 3.885	71.7	4.23, 3.84 (both m)
2/2’	54.30	3.07	54.4	3.04 (m)
3/3’	85.78	4.735	85.8	4.71(m)
4/4’	135.02	-	135.1	-
5/5’	106.48	6.79-6.83(m)	106.5	6.76-6.84 (m)
6/6’	147.95	-	148.0	-
7/7’	147.09	-	147.1	-
8/8’	108.18	6.87 (bs)	108.2	6.76-6.84 (m)
9/9’	119.35	6.79-6.83(m)	119.4	6.76-6.84 (m)
10/10’	101.06	5.97	101.1	5.92 (s)

* As reported in the literatures [8]

Table 2a. Crystal data and structure refinement for compound **2**.

Parameter	Value
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 9.8827(8) A α = 90 deg. b =6.9772(5) A β = 93.3380(10) deg. c = 11.8465(9) A γ = 90 deg.
Volume	815.47(11) $Å^3$
Z	2
Density (calculated)	1.443 mg/m^3
Crystal size	0.14 x 0.13 x 0.05 mm^3
Reflections collected	7946
Data / restraints / parameters	2878 / 1 / 235
Final R indices [I>2sigma(I)]	R1 = 0.0431, wR2 = 0.1021

Table 2b. Bond lengths for compound **2**

Carbon (no.)-Carbon (no.)	Bond lengths [Å]
C(1)-C(6)	1.385(3)
C(1)-C(2)	1.405(3)
C(1)-C(7)	1.509(3)
C(2)-C(3)	1.367(4)
C(3)-C(4)	1.374(3)
C(4)-C(5)	1.367(3)
C(5)-C(6)	1.399(4)
C(7)-C(8)	1.517(3)
C(8)-C(9)	1.541(4)
C(8)-C(8')	1.542(3)
C(1')-C(6')	1.388(3)
C(1')-C(2')	1.401(3)
C(1')-C(7')	1.504(4)
C(2')-C(3')	1.373(4)
C(3')-C(4')	1.374(3)
C(4')-C(5')	1.362(3)
C(5')-C(6')	1.395(4)
C(7')-C(8')	1.516(3)
C(8')-C(9')	1.519(3)

Table 2c. Bond angles for compound **2**

Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]
C(6)-C(1)-C(2)	120.1(2)
C(6)-C(1)-C(7)	120.0(2)
C(2)-C(1)-C(7)	119.7(2)
C(3)-C(2)-C(1)	117.0(2)
C(2)-C(3)-C(4)	122.5(2)
C(5)-C(4)-C(3)	121.9(2)
C(4)-C(5)-C(6)	116.6(2)
C(1)-C(6)-C(5)	121.9(2)
C(1)-C(7)-C(8)	118.20(19)
C(7)-C(8)-C(9)	115.4(2)
C(7)-C(8)-C(8')	102.68(18)
C(9)-C(8)-C(8')	104.18(19)
C(6')-C(1')-C(2')	119.5(2)
C(6')-C(1')-C(7')	119.2(2)
C(2')-C(1')-C(7')	121.3(2)
C(3')-C(2')-C(1')	117.2(2)
C(2')-C(3')-C(4')	122.2(2)
C(5')-C(4')-C(3')	122.1(2)
C(4')-C(5')-C(6')	116.3(2)
C(1')-C(6')-C(5')	122.7(2)
C(1')-C(7')-C(8')	116.2(2)
C(7')-C(8')-C(9')	116.8(2)
C(7')-C(8')-C(8)	102.96(19)
C(9')-C(8')-C(8)	103.65(19)

Table 2d. Torsion angles for compound **2**

Carbon (no.)-Carbon (no.)-Carbon (no.)-Carbon (no.)	angles [°]
C(6)-C(1)-C(2)-C(3)	-1.0(4)
C(7)-C(1)-C(2)-C(3)	174.4(2)
C(1)-C(2)-C(3)-C(4)	-0.1(4)
C(2)-C(3)-C(4)-C(5)	1.1(4)
C(3)-C(4)-C(5)-C(6)	-0.8(4)
C(2)-C(1)-C(6)-C(5)	1.3(4)
C(7)-C(1)-C(6)-C(5)	-174.1(2)
C(4)-C(5)-C(6)-C(1)	-0.4(4)
C(6)-C(1)-C(7)-C(8)	-37.7(3)
C(2)-C(1)-C(7)-C(8)	146.8(2)
C(1)-C(7)-C(8)-C(9)	89.6(3)
C(1)-C(7)-C(8)-C(8')	- 157.8(2)
C(6')-C(1')-C(2')-C(3')	-0.4(4)
C(7')-C(1')-C(2')-C(3')	178.0(2)
C(1')-C(2')-C(3')-C(4')	-0.4(4)
C(2')-C(3')-C(4')-C(5')	0.7(4)
C(3')-C(4')-C(5')-C(6')	-0.1(4)
C(2')-C(1')-C(6')-C(5')	1.0(4)
C(7')-C(1')-C(6')-C(5')	-177.4(2)
C(4')-C(5')-C(6')-C(1')	-0.7(4)
C(6')-C(1')-C(7')-C(8')	-53.1(3)
C(2')-C(1')-C(7')-C(8')	128.5(2)
C(1')-C(7')-C(8')-C(9')	91.4(3)
C(1')-C(7')-C(8')-C(8)	-155.8(2)
C(7)-C(8)-C(8')-C(7')	-106.9(2)
C(9)-C(8)-C(8')-C(7')	13.8(2)
C(7)-C(8)-C(8')-C(9')	15.2(3)
C(9)-C(8)-C(8')-C(9')	135.9(3)

Table 3: ^{13}C NMR Data for compounds **3** and **4*****

	3 (Pyridine-d_5)	Ursolic acid* (Pyridine-d_5)	**4** (Pyridine-d_5)	Corosolic acid** (Pyridine-d_5)
1	39.10	39.2	48.08	48.4
2	28.21	28.2	68.69	69.0
3	78.15	78.2	83.9	84.2
4	39.51	39.6	39.95	40.2
5	55.84	55.91	55.99	56.3
6	18.80	18.8	18.94	19.2
7	33.60	33.7	33.60	33.9
8	39.98	40.1	40.12	40.4
9	48.07	48.1	48.18	48.5
10	37.47	37.5	38.52	38.8
11	23.71	23.7	24.99	25.3
12	125.67	125.7	125.65	125. 9
13	139.29	139.3	139.39	139.7
14	42.52	42.6	42.63	42.9
15	28.78	28.8	28.73	29.0
16	25.00	25.0	23.83	24.1
17	48.07	48.1	48.12	48.5
18	53.57	53.6	53.60	53.9
19	39.51	39.5	39.50	39.9
20	39.43	39.4	39.57	39.8
21	31.08	31.1	31.17	31.5
22	37.48	37.4	37.54	37.8
23	28.84	28.8	29.47	29.8
24	16.62	16.5	17.07	17.2
25	15.70	15.7	17.57	17.9
26	17.55	17.5	17.82	18.1
27	23.94	24.0	24.01	24.3
28	179.93	179.7	180.01	180.3
29	17.48	17.5	17.62	17.4
30	21.45	21.4	21.52	21.8

* as reported in the literatures [13], ** as reported in the literatures [14],

*** ursolic acid values given for comparison

Table 4: ^{1}H NMR and ^{13}C NMR data for compound **5**.

	5 ($CDCl_3$)		3'-O-Methyl eupatorin($CDCl_3$)*	
	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
2	163.78	-	163.85	-
3	104.20	6.54	104.21	6.55
4	182.46	-	182.46	-
5	153.04	-	153.07	-
6	132.43	-	132.55	-
7	158.60	-	158.66	-
8	90.47	6.51	90.54	6.51
9	152.86	-	152.90	-
10	105.93	-	105.98	-
1'	123.52	-	123.58	-
2'	108.51	7.29 (d, 2Hz)	108.71	7.29 (d, 2.1Hz)
3'	149.14	-	149.25	
4'	152.13	-	152.26	
5'	110.98	6.94(d, 8Hz)	111.11	6.94(d, 8.5Hz)
6'	119.92	7.48 (dd, 8, 2Hz)	119.98	7.48 (dd, 8.5, 2.1 Hz)
6-OMe	60.72	3.97	60.72	3.95
7- OMe	56.22	3.97	56.25	3.94
3'- OMe	55.98	3.95	56.04	3.93
4'- OMe	55.98	3.91	56.04	3.90
5-OH	-	12.75	-	12.30

* As reported in the literatures [15]

Table 5: ^{1}H NMR and ^{13}C NMR data for compound **6**

	6 ($CDCl_3$)		Eupatorin ($CDCl_3$)*		Cirsilineol($CDCl_3$)*	
	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
2	163.80		163.9	-	165.1	-
3	104.50	6.60	104.8	6.59	104.1	6.55
4	182.68		182.8	-	183.4	
5	153.23		153.3	-	153.9	-
6	132.61		132.9	-	133.4	-
7	158.75		158.9	-	160.0	-
8	90.56	6.56	90.7	6.56	91.9	6.58
9	153.03		153.4	-	154.0	-
10	106.16		106.4	-	106.4	-
1’	124.48		124.8	-	121.3	-
2’	110.68	7.49 (1H, d, 2.3,Hz)	110.9	7.49 (1H, d, 2.3Hz)	110.5	7. 33 (1H, d, *J*=2.0 Hz,)
3’	146.03		146.3	-	148.7	-
4’	149.57		149.7	-	151.4	-
5’	112.32	6.975 (d, 8.5Hz)	112.6	6.97 (d, 8.5Hz)	123.5	7. 04 (, d, 8.4 Hz,)
6’	119.11	7.45 (dd, 8.5, 2.3 Hz)	119.2	7.45 (dd, 8.5, 2.3 Hz)	116.3	7. 50 (dd, 8.4 2.0 Hz,)
6-OMe	60.86	3.94	61.0	3.94	60.5	3.93
7- OMe	56.30	3.99	56.4	3.98	56.7	3.98
3’- OMe					56.5	4.01
4’- OMe	56.14	4.00	56.3	4.00		
5-O*H*		12.77		12.75 bs		12.81
3’-O*H*		5.79		5.75 bs		-

* as reported in the literatures [16a, 16b]

Table 6: Chemical composition of essential oil of aerial parts of *P. mollis*

Compound	%	LRI
α-Pinene	0.36	936
1-Octen-3-ol	0.16	980
β-Myrcene	1.06	988
α-Phellandrene	0.31	1008
3-Carene	0.74	1013
α-Terpinene	0.37	1019
Limonene	2.83	1032
Fenchone	19.19	1096
Fenchol alpha/exo	1.35	1118
Camphor	0.49	1149
Borneol	0.54	1170
p-Cymenol	1.22	1189
Piperitone oxide	23.76	1265
Bornyl acetate	0.62	1290
Copaene	2.90	1383
β-Caryophyllene	10.39	1411
Germacrene D	2.58	1488
δ-Cadinene	2.42	1528
γ- Eudesmol	0.87	1649
Total	72.16	

Table 7: Mosquito larvicidal activities for *P. mollis* essential oil.

Concentration*	*A. stephensi***	*A. aegypti***	*C. quinquefasciatus***
125	95.33 ± 1.33	100 ± 0	100 ± 0
100	83.33 ± 1.26	100 ± 0	90.61 ± 1.53
50	71.33 ± 1.65	91.33 ± 2.15	82.66 ± 1.18
30	61.33 ± 1.65	70.66 ± 1.81	64.0 ± 1.31
25	30.66 ± 0.66	40.66 ± 1.53	35.33 ± 1.33
15	10.66 ± 0.66	15.33 ± 1.33	11.33 ± 0.90

*ppm; ** %mortality ± S. D.

Table 8: Mosquito larvicidal activities for *P. mollis* aerial acetone extract.

Concentration*	*A. stephensi***	*A. aegypti***	*C. quinquefasciatus***
500	100 ± 0	100 ± 0	100 ± 0
300	82.0 ± 1.74	89.33 ± 1.81	84.61 ± 1.34
250	69.33 ± 1.81	74.66 ± 12.15	71.33 ± 1.65
200	48.66 ± 1.92	56.0 ± 1.31	51.33 ± 1.65
150	10.0 ± 1.38	21.33 ± 0.90	11.33 ± 0.90

*ppm; ** %mortality ± S. D.

Table 9: LC_{50} and LC_{95} values of *P. mollis* essential oil and acetone extract.

	A. stephensi	*A. aegypty*	*C. quinquefasciatus*
Essential oil			
Regression equation	Y= 2.34X + 1.52	Y= 4.76X -1.69	Y= 3.34X + 9.50
LC_{50}	30.68± 0.80 (18.03 – 52.20)[b]	25.37 ± 0.86[a] (23.68 – 25.37)	29.48 ± 0.84 (20.25 – 29.48)
LC_{95}	155.32 (55.17-437.26)	56.19 (48.27-65.41)	91.71 (37.69-223.17)
Acetone extract			
Regression equation	Y= 7.18X - 11.76	Y=6.69X-10.33	Y=7.35X-12.06
LC_{50}	215.01 ± 1.34 (205.45 – 225.02)	195.19 ± 1.29 (185.53 – 205.35)	209.49 ± 1.33 (200.30-219.10)
LC_{95}	364.28 (328.47-403.98)	343.73 (309.62-381.59)	350.79 (318.56-386.28)

[a] ±SE, [b]Fiducial Limits

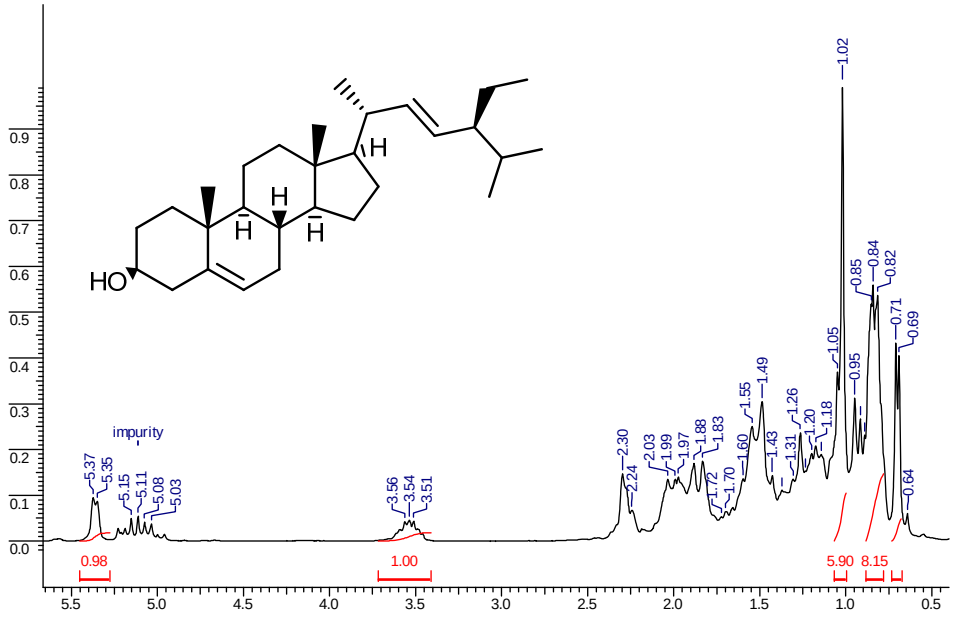
Table 10: Repellent activity of essential oil against *A. aegypty*

Dose (mg/cm^2)	Protection time offered (min)
0.5	10
1	40
2	120

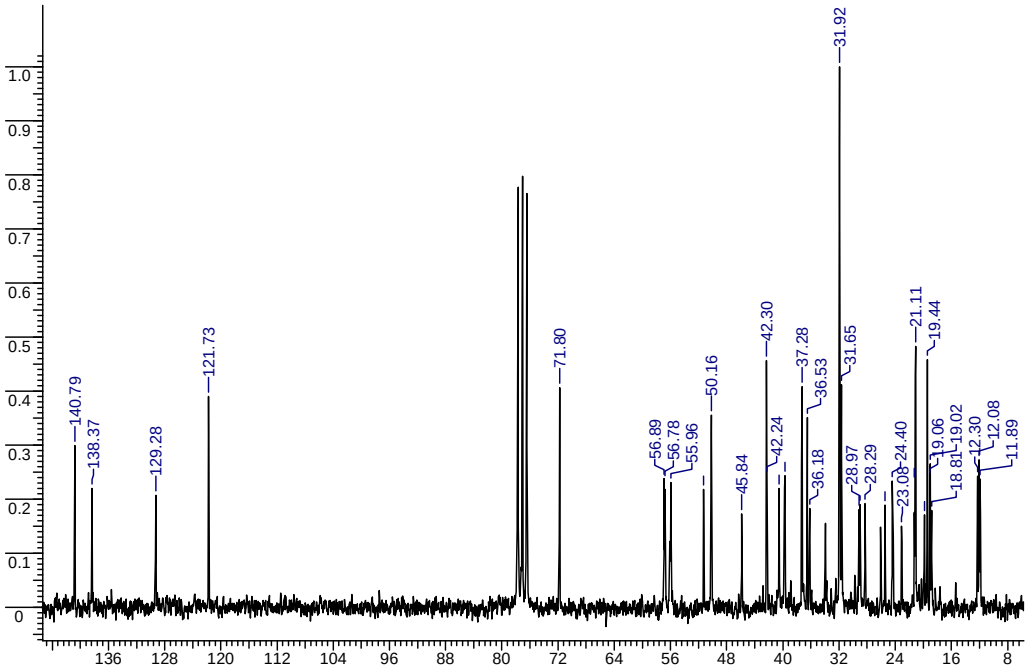
6.8 NMR data:

Compound **1**:

^{1}H NMR:



^{13}C NMR:



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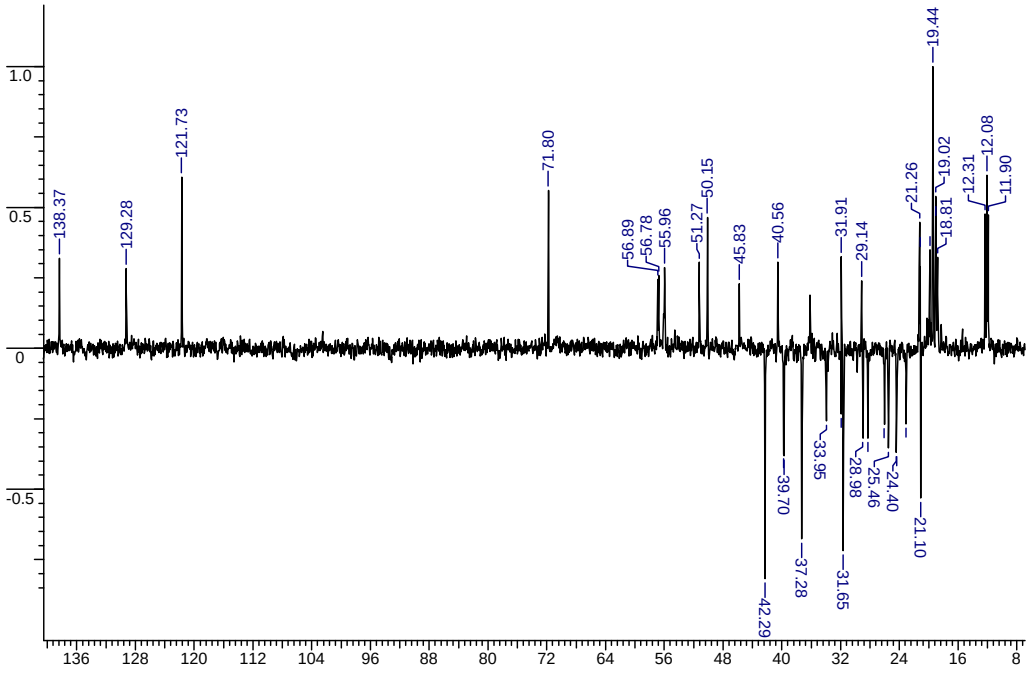

Compound **2**:

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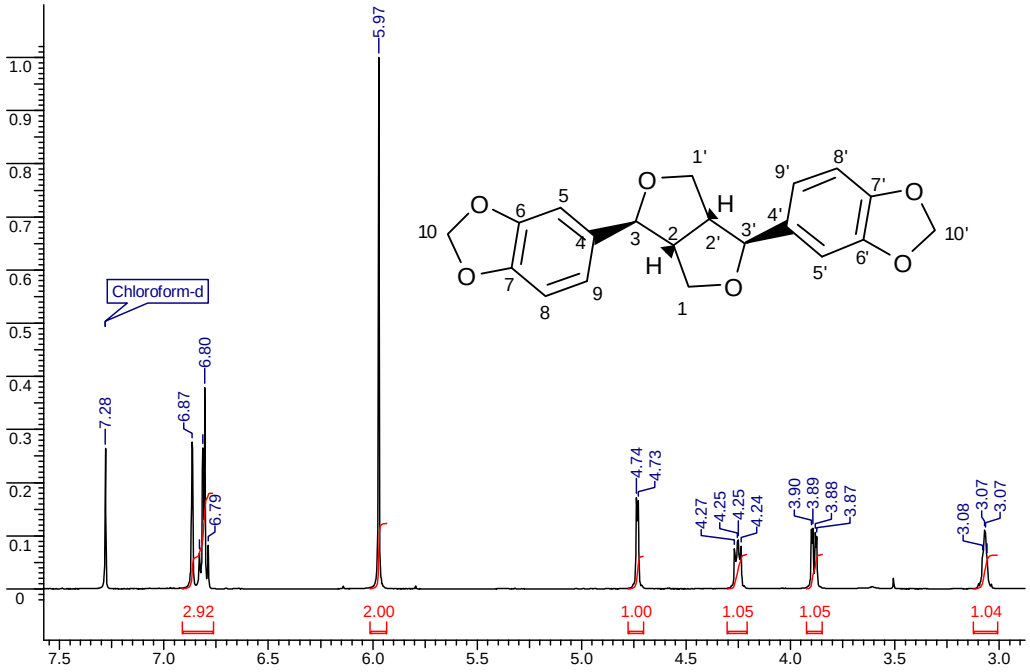
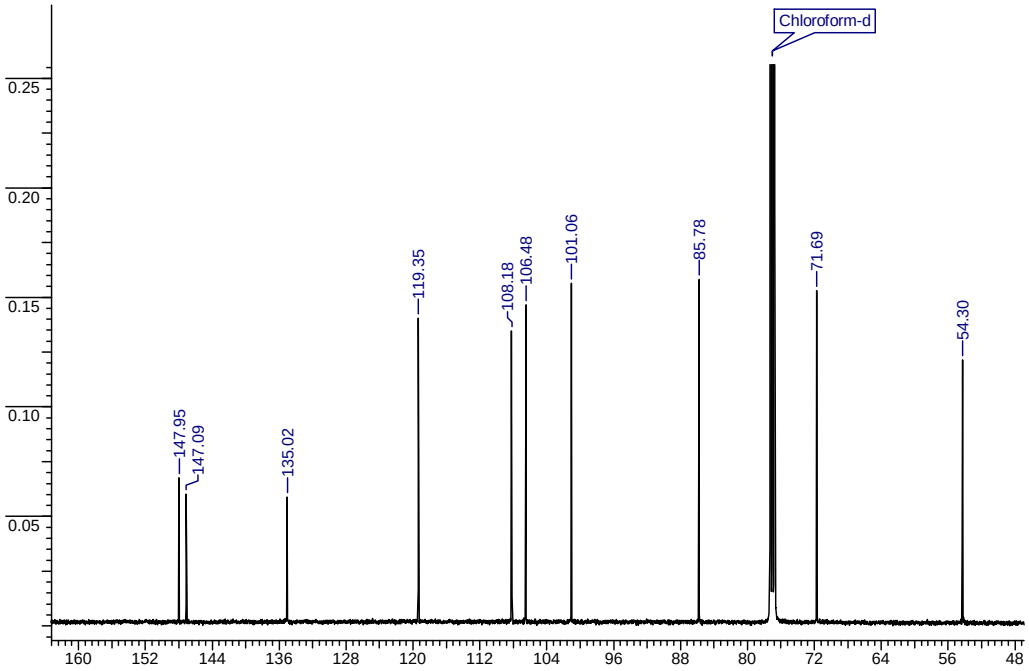


^{13}C NMR:


Chloroform-d
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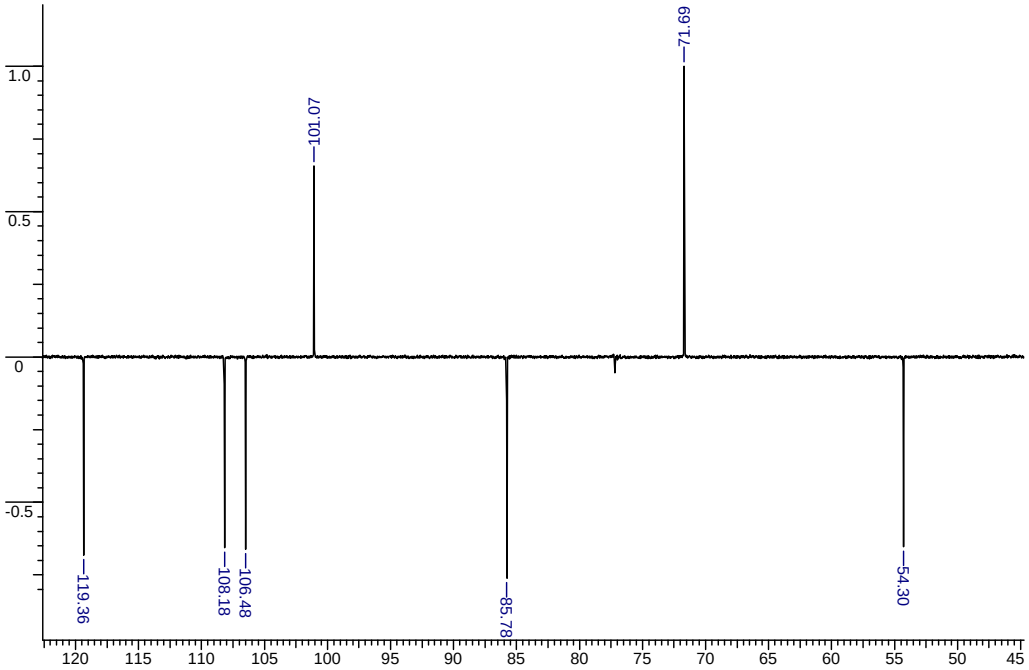
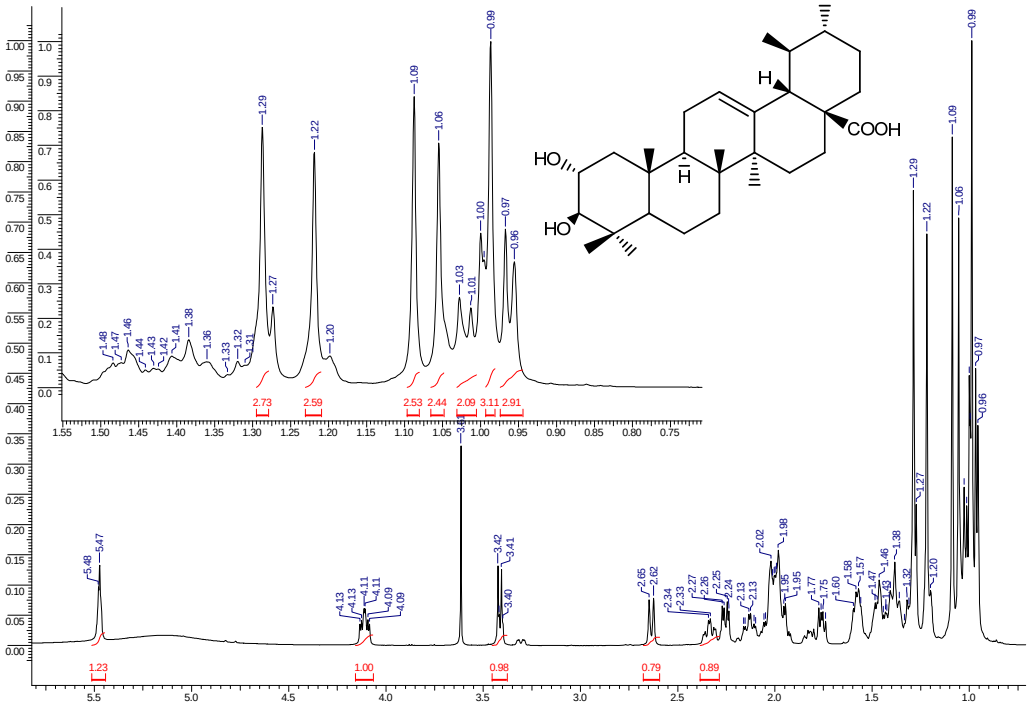
135.02
119.35
108.18
106.48
101.06
85.78
71.69
54.30

DEPT:

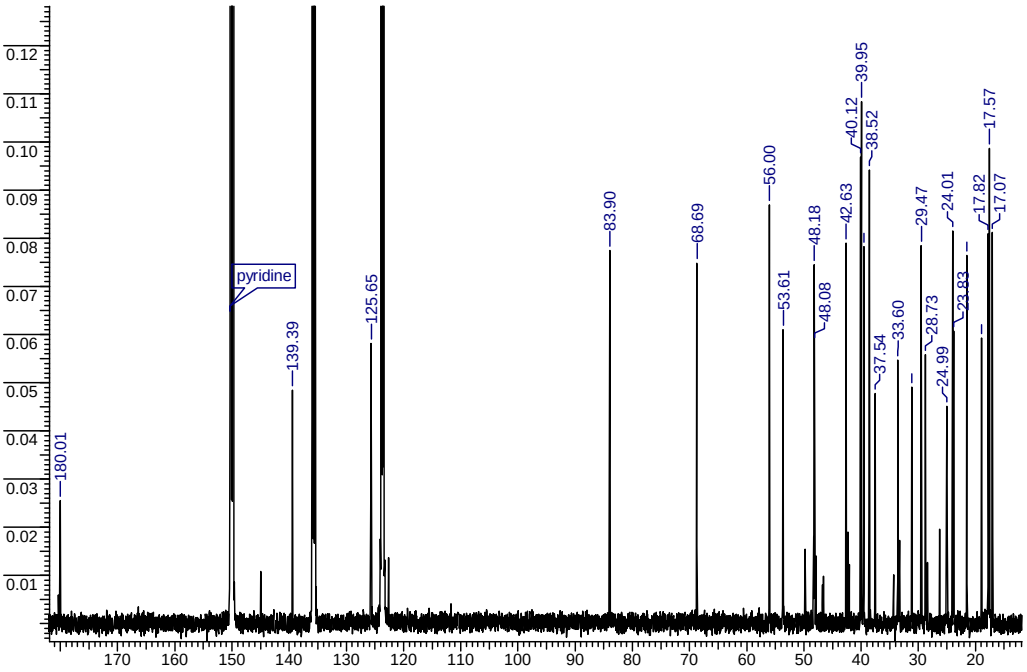

71.69
101.07
119.36
108.18
106.48
85.78
54.30

Compound **4**:

^{1}H NMR:



^{13}C NMR:



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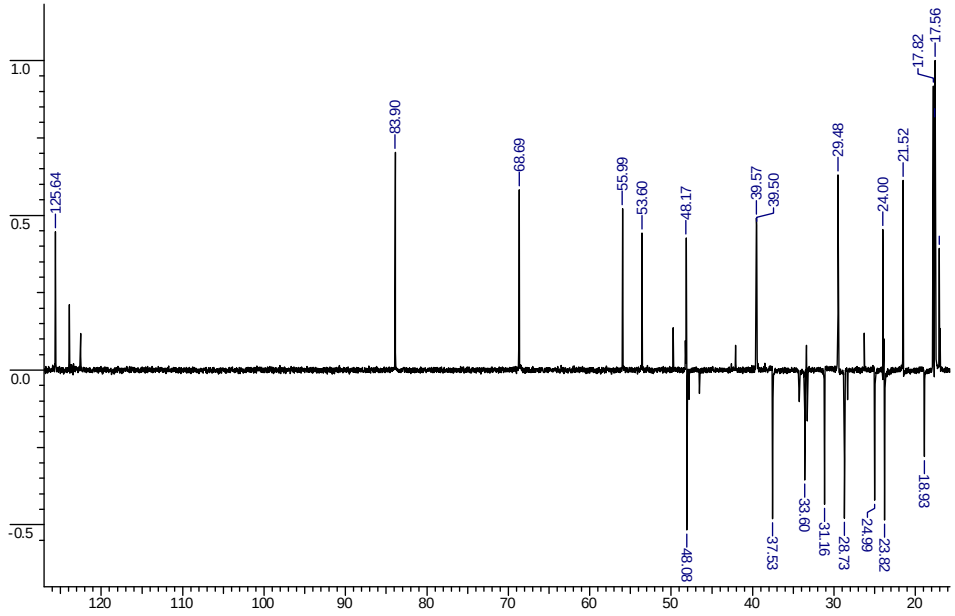
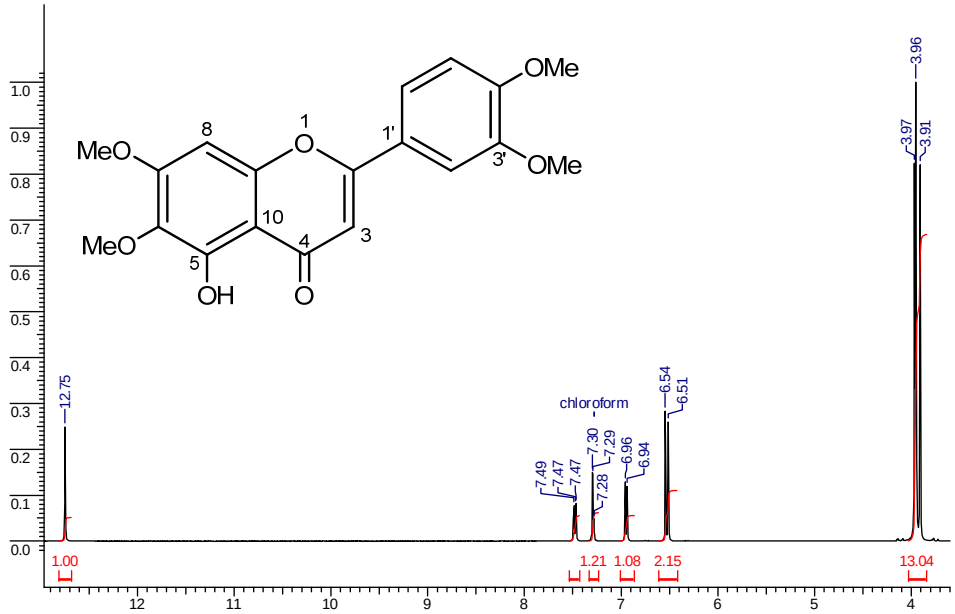
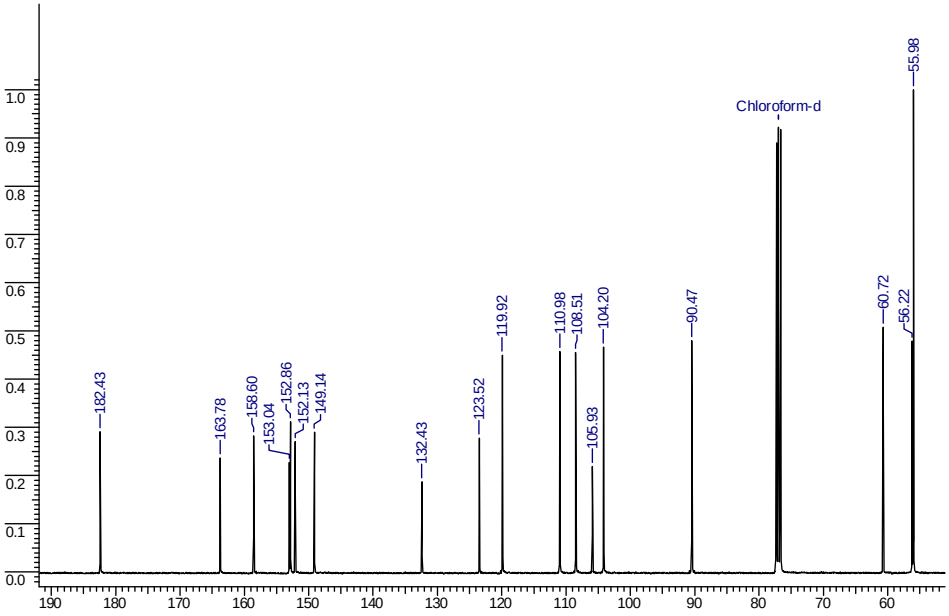


Compound **5**:

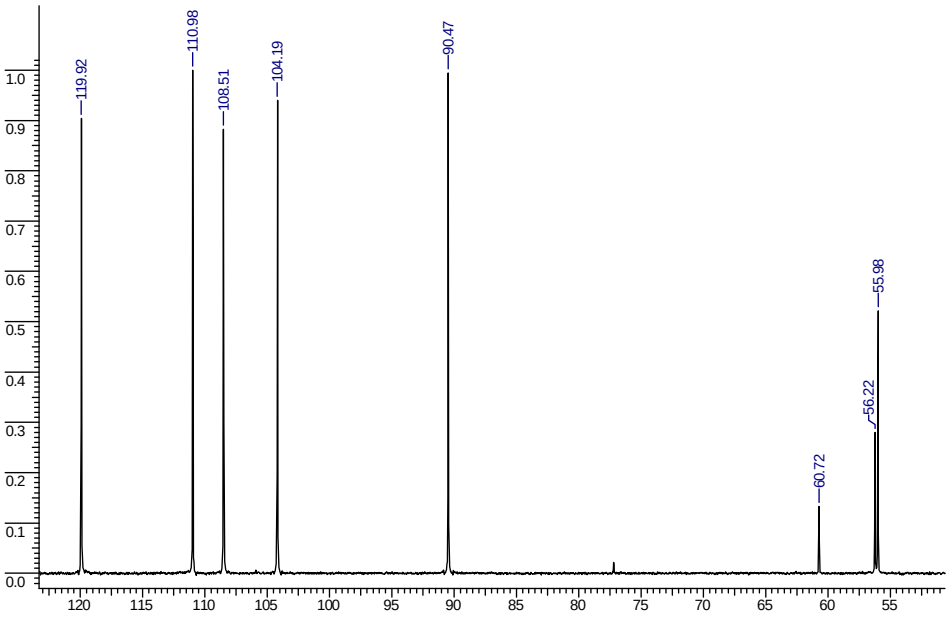
^{1}H NMR:



^{13}C NMR:


182.43
163.78
158.60
153.04
152.86
152.13
149.14
132.43
123.52
119.92
110.98
108.51
105.93
104.20
90.47
Chloroform-d
60.72
56.22
55.98

DEPT:


119.92
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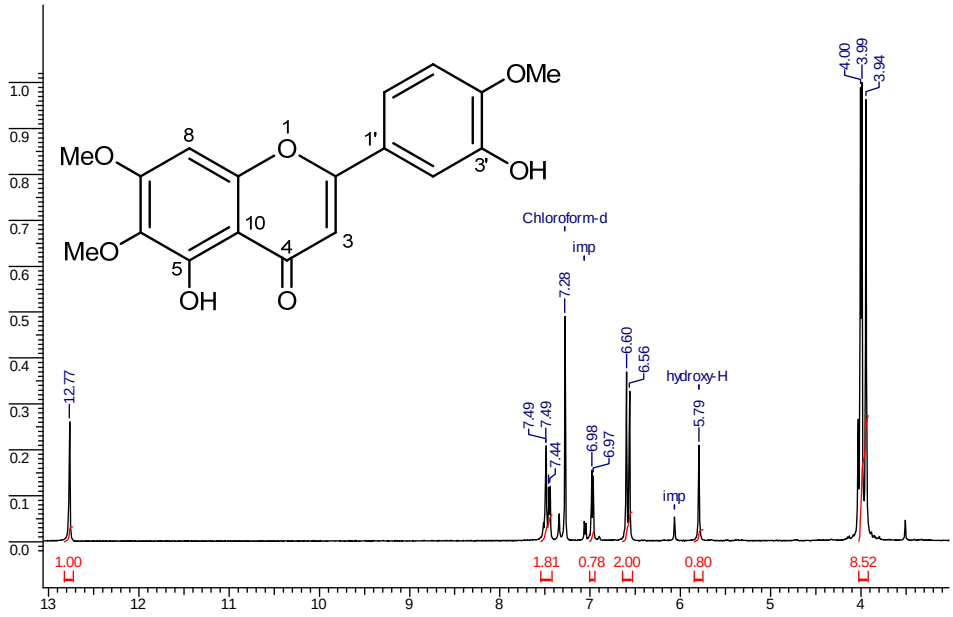
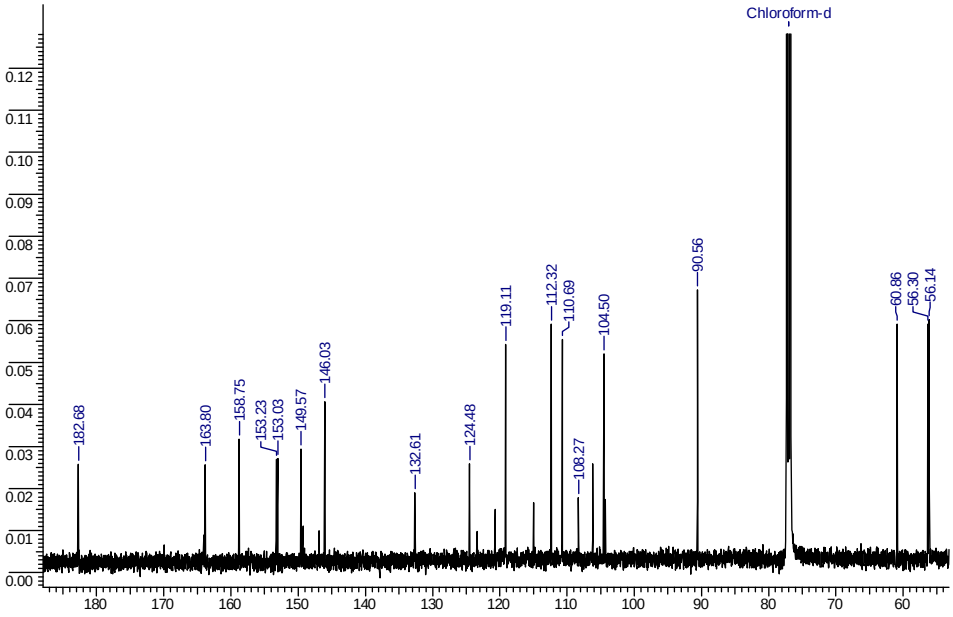
104.19
90.47
60.72
56.22
55.98

Compound **6**:

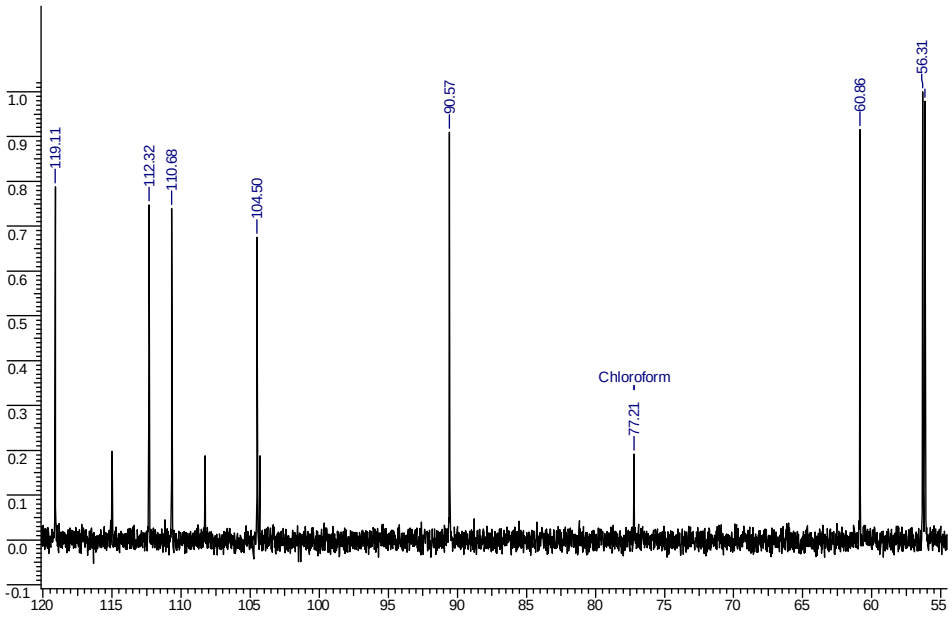
^{1}H NMR:



^{13}C NMR:



DEPT:



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Patents:

1. PP No. 2221DEL2011 filed on 5-8-11.
   Title: “Anti-tubercular activity of compounds from *Plectranthus mollis*”.
   Inventors: S. P. Joshi, **R. R. Kulkarni**, D. Sarkar, S. Sarkar, K. Shurpali
2. PP No. 2222DEL2011 filed on 5-8-11.
   Title:"Useful compounds from *Anisomeles*".
   Inventors: S. P. Joshi, **R. R. Kulkarni**
3. PP No. 2223DEL2011 filed on 5-8-11.
   Title:"Bioactivity of *Plectranthus mollis* essential oil".
   Inventors: S. P. Joshi, **R. R. Kulkarni**, A. Sen, P. V. Pawar, M. Joseph
4. PP No. 2224DEL2011 dated, 5-8-11.
   Title: "Natural selective inhibitors of Mycobacterium tuberculosis from *Leucas stelligera*".
   Inventors: S. P. Joshi, **R. R. Kulkarni**, D. Sarkar, S. Sarkar, K. Shurpali
5. PP No. 2225DEL2011 filed on 5-8-11.
   Title:"Bioactivity of *Lavandula gibsoni* essential oil".
   Inventors: A. Sen, P. V. Pawar, M. Joseph
6. PP No. 2226DEL2011 filed on, 5-8-11.
   Title:"Pimarane diterpenes from *Anisochilus verticillatus*".
   Inventors: S. P. Joshi, **R. R. Kulkarni**, D. Sarkar, S. Sarkar, K. Shurpali
7. PP No. 0680DEL2010 filed on 22-03-2010
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   Inventors: D. Sarkar, S. P. Joshi., U. Singh, K. Shurpali., **R. Kulkarni**

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1. **R. R. Kulkarni**, A. D. Virkar, and Priscilla D'mello, “Antioxidant and Anti-inflammatory Activity of *Vitex negundo*”, *Indian J Pharm Sci*., 2008, **70**(6), 838–840.

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1. Screening of indigenous plant products as potential candidate microbicides.

Navin Pathare, Swati Joshi, Nutan Jadhav, Sunayna Shelar, Arati Mane, **Roshan Kulkarni**, Arun Risbud and Smita Kulkarni

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2. Isobutrin a new Natural Sensitizer for Dye Sensitized Solar Cells.

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1. "Anti-Oxidant studies on the leaves of *Vitex negundo*", at '4th International Seminar on Ayurvedic Education, Research and Drug Standardization-A global Perspective', Gujarat Ayurved University, Jamnagar, January, 2003.