Micropropagation of *Mimusops hexandra* (Roxb.) and its Phytochemical Studies

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CERTIFICATE

This is to certify that the thesis entitled **"Micropropagation of *Mimusops hexandra* (Roxb.) and its Phytochemical Studies"** submitted for the award of degree of Doctor of Philosophy in Botany is a bonafide work of Mrs. Neha Mishra and carried out under my supervision and guidance.

She has fulfilled the requirements for the degree of Doctor of Philosophy in Botany at Vardhman Mahaveer Open University, Kota regarding the nature and prescribed period of work. The thesis submitted by her incorporates work done by her and has not been submitted elsewhere for any degree or diploma.

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DECLARATION

This is to certify that the thesis entitled **"Micropropagation of *Mimusops hexandra* (Roxb.) and its Phytochemical studies"** submitted by me for the award of degree of Doctor of Philosophy in Botany is a bonafied work and carried out under the supervision of Prof. (Dr.) Arvind Pareek, Maharshi Dayanand Saraswati University, Ajmer.

The contents of this thesis, in full or parts have not been submitted in any other institute or University for the award of degree or diploma.

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LIST OF ABBREVIATIONS

The following table describes the significance of various abbreviations used throughout thesis.

Abbreviations	**Meaning**
PTC	Plant tissue culture
PGR	Plant growth regulators
WPM	Woody plant medium
MS	Murashige and Skoog's
NAA	Naphthalene acetic Acid
KN	Kinetin
IBA	Indole-3- butyric acid
IAA	Indole acetic acid
GA_3	Gibberellic acid
TDZ	Thidiazuron
BA	Benzyl adenine
ABA	Abscisic acid
2,4-D	2 4-dichlorophenoxyacetic acid
DPPH	2, 2–diphenyl picryl hydrazyl
EDTA	Ethylene diamine tetra acetic acid
IZEs	Immature zygotic embryos
TLC	Thin layer chromatography
ANOVA	The analysis of variance
DMRT	Duncan's multiple-range test
Rp	Least significant studentized range
Qα	Tabular value used at particular significance level
P	Number of groups compared
Y	Degrees of freedom for experimental error
MSE	Mean squire error

Abbreviations	**Meaning**
N	Number of observations
BSA	Bovine serum albumin
Hrs	Hours
Min	Minutes
mg	Mili gram
G	Gram
mL	Mili liter
µm	Micro meter
nm	Nano meter
v/v	Volume/volume
Lux	Unit of illumination
L	Liter
No.	Number
RT	Room Temperature
cm	Centimeter
pH	Potential of hydrogen
Rf	Retention Factor
lbs	Unit of pressure
i.e	That is
Fig.	Figure
%	Percentage
°C	Degree Celsius
N	Normality
P	Significance level
etc.	Etcetera
TNF	Tumor necrosis factor
NCS	Neuro Cardiogenic Syncope
GC-MS	Gas chromatography– mass spectrometry

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Chapter 1
Introduction

CHAPTER 1: INTRODUCTION

Medicinal plants have been used in healthcare since ancient time. Natural herbs work as a great link between the people and environment (Sofowora et al, 2013). Herbal drugs obtained from the plants are easily available, less expensive and efficient with nearly no side effects (Oyewole et al, 2018). The growing interest among people in production and utilization of herbal medicines provides great ways to discover new therapies. Sustainable human health can be achieved by using medicinal plants as they play vital role in field of medicine (Balick and Cox, 1997). A large number of plant extracts in form of decoctions or infusions are used by tribal people for treatment of several severe diseases (Petrovska, 2012). Medicinal plants also have been proved as the richest bio-resource of drugs for synthetic pharmaceutical entities (Ncube et al., 2008). Large number of research studies have been undergoing to verify efficiency of medicinal plants and to find new plant-based medicines. A detailed knowledge regarding the propagation and biochemical composition of the plant parts is needed to understand their potential completely.

The plant family Sapotaceae includes species of herbs, shrubs and trees containing potent drugs of therapeutic significance (Pennington, 1991; Tyagi, 2005). Various plants of this family such as *Madhuca longifolia, Mimusops elengi, Manilkara zapota, Pouturia lucuma* etc. are known to possess numerous biological properties and have huge history in the field of herbal treatments. The crude extracts obtained from *M. longifolia* and *M. elengi* lead to the isolation of drugs such as cocaine, codeine, digitoxin, quinine and morphine which are used to cure several diseases such as diabetes, ulcer, jaundice and digestive & respiratory disorders etc. (Pullaiah, 2002). A wide range of therapeutic constituents have been identified and isolated from plants of this family. *Mimusops hexandra* (Dubard) [Synonym: *Manilkara hexandra* (Roxb.)] is an another important medicinal plant belonging to this family. It is a socio-economic important specie of tropical deciduous forests of western and central India (Malik et al., 2012; Mishra and Pareek, 2015). It is a slow-growing evergreen tree that grows in tropical and temperate forests. It is a native of South Asian region and commonly known as Obtuse Leaved Mimusops in English; Rajadanah in Sanskrit; Khirni and Rayan in Hindi; Krini and Palamunpala in Malayalam & Ulakkaippalai in Tamil (Nambiar and Warrier, 1994; Sambamurty, 2005; Verma, 2011).

The plants of family Sapotaceae are often taxonomically characterized by presence of reddish-brown hairs on plant surfaces and milky sap. *M. hexandra* is an evergreen tall tree and has blackish gray bark, dark green and elliptic or oblong leaves, white bisexual flowers, 6-lobed calyx, 16 or 24-lobed corolla, 6 stamens, 12-celled hairy ovary; fruit is Berry, ovoid in shape, yellow and have one seed.

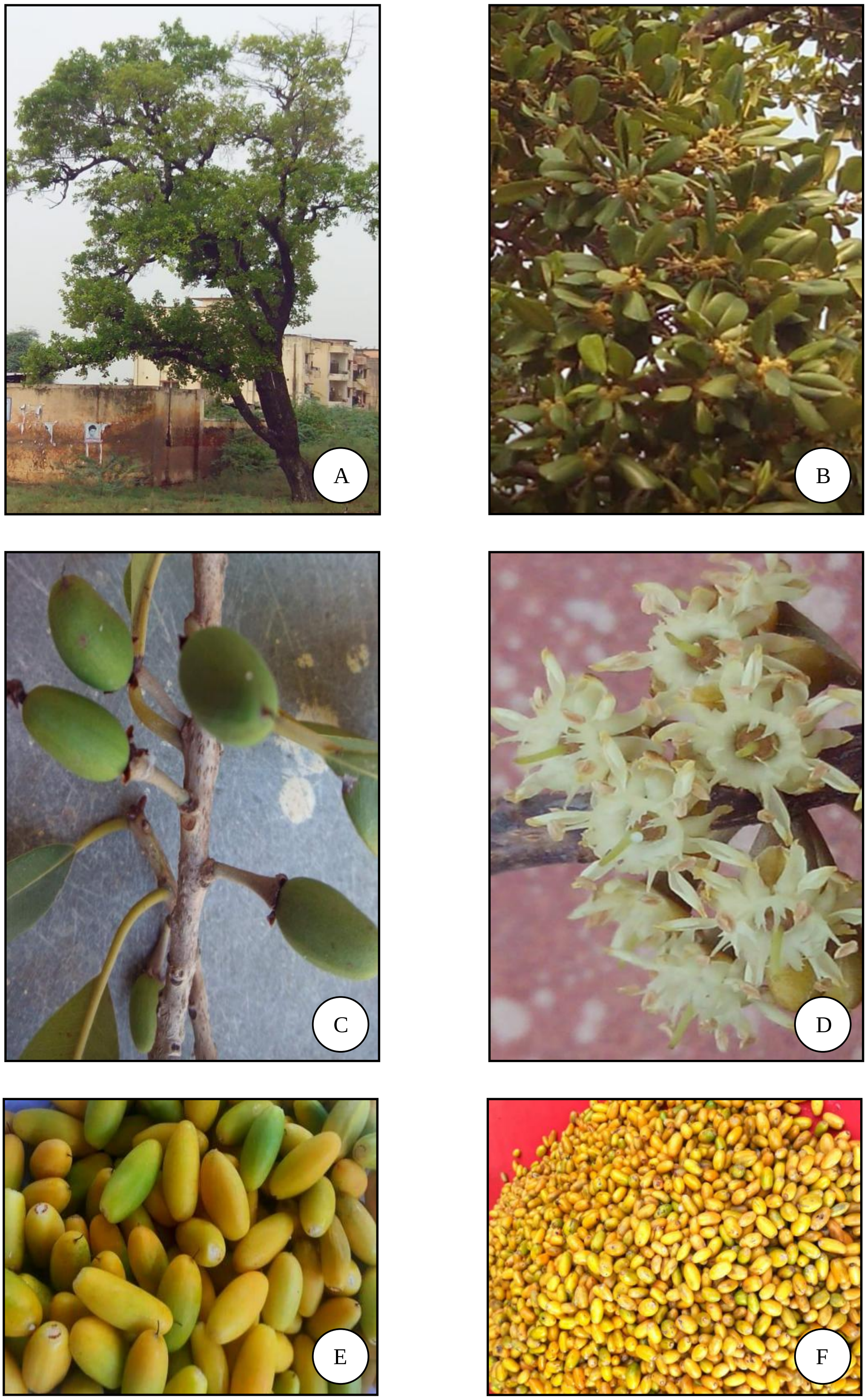

Fig. 1.1 (A) Natural wild population of *Mimusops hexandra;* (B) Elliptic and oblong leaves; (C) Unripe fruits; (D) Bisexual flowers; (E) Ripened fruit (Berry) (F) Fresh fruits sold in market.

Endospermic seeds covered with seed coat which are oily, hard and light brown to blackish in color. Sticky white latex is present in bark, leaves and fruits. Flowering occurs in the month of October to December and fruiting occurs during April–May (Dwivedi and Bajpai, 1974; Sethuraj and Raghavendra, 1987; Reddy et al, 2004).

M. hexandra is well known among tribal population for its numerous pharmacological activities. Extracts and metabolites of this plant such as phenolic compounds, flavonoid, carotenoid, terpenoid and triterpene possess huge biological importance (Sharma, 2009). A survey of the literature shows that whole plant parts have been used for various medicinal activities such as antiulcer, antidiabetic, antibacterial, anticonvulsant, anti-diarrheal, anthelmintic, anti-inflammatory, antimalarial, analgesic, ant nociceptive, antiviral, hypoglycemic activity and also for digestive, cardiovascular, respiratory, gynecological and sexual disorders (Sharma, 2009 and Khare, 2007). Plant has many therapeutic properties and used in the treatment of colic dyspepsia, helminthiasis, body ache, diarrhea, jaundice, hyper dyspepsia and burning sensation (Joshi, 2000). It works as blood purifier and used in treatment of abdominal colic, rheumatism and toxicosis (Rao, 1985). Acetone and ethyl acetate extract of bark of *M. hexandra* showed antiulcer properties against gastroduodenal ulcers. Ethyl acetate extract found most effective against ulcer by reducing the rate of acid secretion and induce mucosal protection processes (Modi et al., 2012; Shah et al., 2004). Gomathi (2012) indicated that polysaccharides extracted from *M. hexandra* bark significantly stimulate activity of macrophages which leads to stimulation of the immune system. Eskander et al (2013) found saponin in acetone fraction of *M. hexandra* which possess anti–inflammatory activity. Leaf extract of this plant used for treatment of asthma (Anjaneyulu and Sudarsanam, 2013). Fruit pulp is used to cure digestive disorder (Patil and Patil, 2012) also for arthritis & jaundice and heat burning & wormicide (Bakare, 2014). Latex obtained from *M. hexandra* is used to cure toothaches (Ragupathy and Newmaster, 2009). Root extract of *M. hexandra* is beneficial for relief from headache (Rao et al., 2010). The stem bark is used to cure body ache (Monisha and Vimla, 2018) and diarrhea (Raju and Reddy, 2005). Bharia and Gond Tribes of Madhya Pradesh takes stem bark of *M. hexandra* as tonic (Rai, 1987). The infusion of bark of *M. hexandra* is used as galactagauge (Padal et al., 2013). Methanol extracts of *M. hexandra* found very efficient for reduction in glucose level in blood (Nimbekar et al., 2013). Their study indicated that it can be use in the management or control of type II diabetes. Mashed fruits and bark decoctions are used in treatment of fever and hallucinations (Vinothkumar et al., 2011). Strong 2, 2–diphenyl picrylhydrazyl (DPPH) radical scavenging activity showed by methanolic leaf extracts of *M. hexandra (*Kumar et al., 2010). Parekh and Chanda (2007; 2010) tested antibacterial activity of aqueous and petroleum ether extracts of *M. hexandra* using the agar well diffusion method and found that extracts are active against various bacterial strains. Antibacterial activity of extract of *M. hexandra* against multi drug resistant bacteria causing enteric fever and respiratory disorders was also observed by Mahida and Mohan, (2007).

Different parts of the plant have been analyzed for isolation of various phytochemicals. Mishra and Pareek, 2018 observed that plant contain rich contents of carbohydrate and protein via quantitative analysis of leaf and bark extracts of *M. hexandra*. Madhak et al., (2013) observed the presence of sterols, volatile oil, tannin, cinnamic acid, hentriacontane, triterpene ketone, triterpenic hydrocarbon, taraxerol and quercitol in leaves of *M. hexandra* by biochemical testing of alcoholic extracts of leaves. The triterpene acid of the mesocarp has been identified as ursolic acid. The cinnamic acid ester of α- and β-amyrins with α-spinasterol, quercitol and taraxerol have been isolated by Misra and Mitra (1968) from the roots of *M. hexandra*. β-sitosterol and triterpenoid saponin, 1β 2α, 3β, 19α-tetrahydroxyursolic acid 28-O-β-D-glucopyranoside have been isolated from the stem bark of *Mimusops hexandra* and their structures elucidated on the basis of chemical and spectral evidence (Shrivastav and Singh, 1994). The unsaponifiable lipid constituents, hydrocarbons, triterpene alcohols and sterols were investigated from seed oils of *M. hexandra* by gas liquid chromatography. Some of the seed oils contained large quantities of α-amyrin, β-amyrin and cycloartenol (Saeecd et al., 2010). The chemical analysis of fresh fruits showed that it is a rich source of proteins, lipids and carbohydrates which are about 3.53%, 2.6%, 22% and 71.87% respectively (Daripkar and Jadhav, 2010). Presence of the fatty acid esters of triterpene alcohols was observed by Misra et al., (1974) in fruit pulps of *M. hexandra* which have been isolated for the first time from the family Sapotaceae. Three known phenolic compounds gallic acid, myrecetin, and quercetin were also isolated for the first time from *M. hexandra*. The chemical structures of the isolated saponin compounds were established by spectral techniques. The acetone fraction containing the crude saponin mixture possessed a significant anti-inflammatory effect (Eskander et al., 2013).

Plant parts such as fruits are sweet flavored and eaten fresh or dried. Fruits have high nutritional value and contain rich amount of vitamin A, sold in markets. Local tribal people and landless farmers of Rajasthan, Gujarat and Madhya Pradesh and Maharashtra gets socio-economic and livelihood support by selling the fruits of *M. hexandra*. Rayan oil (Pale yellow in color) is extracted from the seed kernels which have 30% oil of total dry mass. The bark is also used to slow down fermentation in sugar industries (Mishra and Pareek, 2014). Fruits, bark and seeds of *M. hexandra* has high market demand by various ethno-medicinal and economical means which creates pressure on natural wild population (Sarasan et al, 2006). Some other environmental factors such as climate change, deforestation, overexploitation and habitat loss are also cause for the average decreasing ratio of this valuable plant in environment. It may be direct cause of extinction and loss of biodiversity of this valuable plant (Malik et al, 2012).

Plant tissue culture techniques have been used since several decades for the conservation and preservation of threatened plants and also for production of pharmaceutically important drugs in huge amount (Wochok, 1981). Plant tissue culture (PTC) technology is widely used for propagation of medicinal plants. PTC techniques have major importance in the areas of plant propagation, crop improvement, elimination of diseases from plants and production of secondary products (Klerk et al, 2007). In addition, plant tissue culture is considered to be the most efficient technology for crop improvement. Explants can be used to produce hundreds and thousands of plants in a continuous process. Thousands of plants can be produce from single explant is very short time duration under controlled culture conditions (Bhojwani and Razdan, 1996; Sathyanarayana and Verghese, 2007). The micropropagation methods facilitates production of superior quality plants, isolation of useful variants in well-adapted high yielding genotypes with better disease resistance and stress tolerance capacities which leads to the development of commercially important improved varieties (Trigiano and Gray, 2000). Murashige and Skoog medium is most extensively used for the vegetative propagation of many plant species *in vitro*. Plant growth regulators also plays an essential role in determining the development pathway of plant cells and tissues in culture medium (Soh and Bhojwani, 2011). Plant tissue culture techniques can be used for controlled production of valuable therapeutic secondary metabolites (Meskaaoui, 2013). Various advances in the area of cell cultures make possible the production of wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids and amino acids (Mander and Liu, 2010). Advances in micropropagation techniques contribute to a considerable increase in plant cell cultures and also in production of compounds with a high therapeutic value (Kumar and Sopory, 2008).

Objectives of the research

This study was undertaken with the following objectives.

- To raise cultures of *M. hexandra* through plant tissue culture techniques.
- To establish *in vitro* cultures and acclimatization of plantlet in environment of Rajasthan.
- To prepare plant extracts using various solvents.
- Chemical characterization of various phytoconstituents.

World Health Organization (WHO) also suggest that medicinal plants can be used as best source for therapeutic drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Ibrahim et al, 2013). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Iwu and Wooten, 2002). The phytochemical and pharmacological investigations comprise only small proportion of information. When one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident. Present study aims to develop suitable protocol for *in vitro* cultivation and studies on phytochemical analysis of *M. hexandra* which may generate new information for conservation of this underutilized medicinal plant.

Chapter 2
Review of Literature

CHAPTER 2: REVIEW OF LITERATURE

Medicinal plants are the most important source of life saving drugs for the majority of the world's population (Khare, 2007). The wide use of traditional medicines leads to development and spread of modern medicines (Jeyaprakash et al, 2011). Medicinal plant such as *M. hexandra* has a long history in many indigenous communities for providing pharmaceutical drugs as useful tool to treat with various diseases. This plant is used by greater number of people seeking remedies and health approaches (Vardhana, 2008).

Plants of family Sapotaceae are a source of large number of drugs comprising to different medicinal properties (Takhtajan, 2009). Family Sapotaceae belongs to order Ericales and subdivided into five tribes with 53 genera and approximately 1250 species (Leyel, 2013; Pennington, 1991; Kramer et al, 1991). It consists of trees and shrubs with a world-wide distribution. The highest species diversity is found in the tropical and subtropical regions of South Asia (Swenson and Anderberg, 2005). Different pharmacological experiments in a number of *in vitro* and *in vivo* models have been carried out and numerous medicinally important phytoconstituents have been identified in the form of tinctures, teas, poultices, powders, and other herbal formulations from plants of this family (Balick and Cox, 1997; Meeta and Jindal, 1994).

National Review

Ethno-medicinal uses of *Mimusops hexandra*

M. hexandra is commonly known as Pedda pala, Kalleru in Konda Reddy and Koyas tribes in Khammam district of Andra Pradesh. Stem bark decoctions are used by local inhabitants in treatment of diarrhea and dysentery (Raju and Reddy, 2005). A decoction of bark and mashed fruits are used for fever and hallucinations (Vinothkumar et al, 2011). Leaf paste and bark powder of *M. hexandra* is obtained from temple trees of Tamilnadu and used for infertility and veterinary medicine (Gunasekaran and Balasubramanian, 2012).

Micropropagation

In-vitro regeneration techniques have great importance in production of herbal drugs from medicinal plants (Kishor, 1999; Jha and Ghosh, 2005). By micropropagation, the multiplication rate increases and leads to production of pathogen free plants also (Murashige, 1974). Mesocarp and endosperm of *A. sapota* was used for callus induction by Bapat and Narayanaswamy (1977). *In vitro* propagation was investigated in *Synsepalum dulcificum* through embryo and nodal explants using different levels and combinations of auxins and cytokinin in MS medium by Ogunsola and Ilori (2008). It works as blood purifier and has efficient effect to cure toxicosis, abdominal, rheumatism, colic swelling and gout (Nath and Khatri, 2010). Extract of stem bark is taken as tonic by Bharia and Gond Tribes of Tamiya and Petalkot of Madhya Pradesh (Rai, 1987). *M. hexandra*. Root extract of *M. hexandra* is used for relief from headache, bark for fever and tubers for piles (Rao et al., 2010).

Phytochemistry

Phytochemical analysis of the plant parts of *V. paradoxa* was done by Ndukwe et al, (2007) and the presence of carbohydrates, saponins, steroids and alkaloids was identified in rich amount. Phytochemical analysis of secondary metabolites from *Diploknema butyracea* has been carried out by Rashmi and Tyagi (2015) and presence of lipids, saponins, tannins, alkaloids, phenols, steroids and flavonoids was observed. Fruit extracts of *M. zapota* has been analyzed by Shafii et al (2017) & Jenitha and Bhuvaneshwari (2016) for identification of phenols, steroids, tannins, flavonoids, terpenoids, saponins, quinine and anthraquinine.

International Review

Ethno-medicinal uses of *Mimusops hexandra*

M. hexandra found as natural wild plant in the north and central India mostly in Maharashtra, Rajasthan, Madhya Pradesh and Gujarat (Malik et al. 2012). Mostly western and central part of India has a long history of traditional medicinal use of *M. hexandra* (Table 2.1). Fruits of *M. hexandra* have high economic value as mature fresh fruits are sweet and source of vitamin A, proteins, sugars, carbohydrates and minerals (Nautiyal, 2013).

Traditionally it is used in treatment of fever, odontopathy, jaundice, helminthiasis, hyper dyspepsia, colic dyspepsia and burning sensation (Goswami and Ram, 2017). It consists of bioactive compound possessing antipyretic, antimicrobial, antiulcer, anti-urolithiatic and anti–inflammatory activity (Khare, 2007).

Fruit pulp is used to cure digestive disorder (Patil and Patil, 2012), also for arthritis and jaundice, heat burning and wormicide (Bakare, 2014). Antiulcer effects of acetone extract and its different fractions namely diethyl ether, ethyl acetate and aqueous fractions of stem bark of *M. hexandra* have been tested by Modi et al., (2012) and Shah et al., (2004) for gastro–duodenal ulcers. The ethyl acetate fractions found most effective against ulcers and also induce mucosal protection process. Crude polysaccharides were extracted from *M. hexandra* bark by Gomathi (2012) to evaluate the stimulating effect of polysaccharides on immune system and it was found that the polysaccharides from *M. hexandra* bark significantly stimulate the immune activity by stimulating macrophage function. Treatment of macrophages with plant polysaccharides has also been reported to modulate expression of various cell surface receptors which recognize plant polysaccharides. Acute toxicity study regarding *M. hexandra* polysaccharides also showed that it is safe to animals. In the Dhar, Jhabua, Khargone and Ratlam distrcits of Madhya Pradesh and neighboring states of Maharashtra, Gujarat and Rajasthan, the Bhils are inhabitants with large number and they utilize bark of *M. hexandra* as herbal remedies in various diseases and ailments (Meeta and Jindal, 1994).

The stem bark boiled with water is used for bathing to cure body ache. Konda Dora Tribes in Vishakhapatanam district of Andra Pradesh uses the stem bark infusions of *M. hexandra* as Juice of stem bark is taken daily and also given to the lactating mothers (Padal et al, 2013). Bark decoction and fruit extract of *M. hexandra* is used to cure fever, jaundice, bronchitis, dysentery and alimentary disorders in western and central India (Malik et al. 2012). In the Rayalaseema region of Andhra Pradesh, the tribal people use leaf extract of *M. hexandra* for treatment of asthma. (Anjaneyulu and Sudarsanam, 2013). *M. hexandra* is commonly known as Pala maram in the Kodiakarai Reserve Forest (KRF). Latex of *M. hexandra* is used to cure toothaches (Ragupathy and Newmaster, 2009). Suranviduthi sacred groves of Pudukottai district in Tamilnadu is composed of dry evergreen species like *M. hexandra.*

Phytochemistry

Plant-derived substances have great importance due to their versatile applications. Various phytoconstituents have been identified in leaves, stem bark, roots, fruits and seeds of *M. hexandra* (Table 2.2). Alcoholic and chloroform extracts of bark of *M. hexandra* have been analyzed via TLC by Gopalkrishnan et al. (2014) and presence of starch, terpenoids, proteins, anthraquinone glycoside, cardiac glycoside, tannins and saponins have been identified. Shrivastav and Singh (1994) found the presence of β-sitosterol and a triterpenoid saponin (1β 2α, 3β, 19α-tetrahydroxyursolic acid 28-O-β-D-glucopyranoside) in stem bark of *M. hexandra*.

Carbohydrates, proteins, lipids and moisture content of fresh fruits of *M. hexandra* was estimated by Daripkar and Jadhav (2010) via biochemical analysis which is about 22%, 3.53%, 2.6% and 71.87% respectively. Presence of the fatty acid esters of common triterpene alcohols was also found by Misra et al. (1974) in fruit pulps of *M. hexandra.*

Bidesmosidic saponins (saponin 1, 2 and 3) and phenolic compounds such as myrecetin, quercetin and gallic acid were identified by Eskander et al. (2013) through chromatography of acetone fractions of seeds of *M. hexandra* which also possess anti-inflammatory activity. Unsaponifiable lipid constituents, tannins and saponins were isolated from seed oils of *M. hexandra* by Saeecd et al. (1991) through histochemical analysis and Thin Layer Chromatography (TLC) of chloroform and alcoholic extracts of *M. hexandra*. Sterol, tannin and volatile oil have been identified in leaves of *M. hexandra* by Madhak et al. (2013) through lead acetate tests of alcoholic extracts of leaves. Misra and Mitra (1968) found the presence of hentriacontane, taraxerol, quercitol and cinnamic acid in leaves of *M. hexandra***.**

Table 2.1: Ethno-medicinal uses of *M. hexandra* in India

Parts Used	Preparation	Ethno-medicinal Uses	Place, Country	Reference
Leaves	Decoction or Infusion	Asthma	Andra Pradesh, India	Anjaneyulu and Sudarsanam, 2013
Roots	Infusion or decoction	Headache	Andra Pradesh, India	Rao et al., 2010
Stem bark	Infusion	Dysentery and Diarrhea	Andra Pradesh, India	Raju and Reddy, 2005
Stem bark	Infusion	Galactagogue	Andra Pradesh, India	Padal et al., 2013
Stem bark	Decoction or Infusion	Tonic	Madhya Pradesh, India	Rai, 1987
Stem bark and Fruits	Decoction, Mashed	Bronchitis and Dysentery	Madhya Pradesh, India	Malik et al., 2012
Fruits	Mashed	Digestive disorder	Maharashtra, India	Patil and Patil, 2012
Fruits	Mashed	Arthritis, Blood purifier, Heat burning and Jaundice	Maharashtra, India	Bakare, 2014
Stem bark and Fruits	Decoction, mashed	Alimentary Disorder	Maharashtra and Gujarat, India	Malik et al., 2012
Stem bark and Fruits	Decoction, Mashed	Fever and Jaundice	Rajasthan, India	Malik et al., 2012
Stem bark and Fruits	Decoction, Mashed	Fever and Hallucinations	Tamilnadu, India	Vinothkumar et al., 2011
Stem bark and Leaves	Infusion	Infertility and Veterinary purposes	Tamilnadu, India	Gunasekaran and Balasubramanian, 2012
Latex	Applied directly	Toothache	Tamilnadu, India	Ragupathy and Newmaster, 2009
Stem bark and Fruits	Decoction	Jaundice and Biliousness	West Bengal, India	Sharma et al. 2014

Table 2.2: Phytoconstituents present in different parts of *M. hexandra.*

Phytoconstituents	Source	Reference
Sterols, Volatile oil and Tannins	Leaves	Madhak et al. 2013
Cinnamic acid, Hentriacontane, Taraxerol and Quercitol	Leaves	Misra and Mitra, 1968
Proteins, Lipids and Carbohydrates	Fruits	Daripkar and Jadhav, 2010
Triterpene alcohols	Fruits	Misra et al., 1974
Saponin (one, two and three), Myrecetin, Quercetin and Gallic acid	Seeds	Eskander et al., 2013
Sterols, Alcohols, Triterpene, Unsaponifiable lipids and Hydrocarbons	Seeds	Saeecd et al., 1991
Starch, Terpenoid, Proteins, Anthraquinone glycoside, Cardiac glycoside, Saponins and Tannins	Stem bark	Gopalkrishnan et al., 2014
Triterpenoid, saponin, β-sitosterol	Stem Bark	Shrivastava and Singh, 1994
α-spinasterol, taraxerol, α- and β-Amyrins	Roots	Misra and Mitra, 1968

Ethno-botanical Importance

It is also a commercial important tropical tree species and is a significant source of livelihood and nutritional support. *M. hexandra* plays very important role in the socio-economy and livelihood support for local tribal population. The seeds contain oil, which is used for cooking purpose in China (Shu, 1996). The wood of this tree is very hard, tough and durable and used for oil presses, house building and turnery. *M. hexandra* is commonly used in nurseries as commercial rootstock for sapota plants and its leaves also used as a fodder for cattle. *M. hexandra* is also utilized for manufacturing biofuel. The bark is often used to slow down toddy fermentation and tanning. It's hard, durable brown wood used in construction and for railway sleepers, ploughs, carts, piling, sugar mills and oil presses and tool handles (Malik et al, 2012).

Micropropagation also referred to as *in vitro*, axenic or sterile culture of cells, tissues and organs under adequate culture conditions, which is very necessary in field of applied studies (Dahiya et al, 2015; Debergh and Zimmerman, 1993). Various plants of Sapotaceae family have been cultured through micropropagation such as *Achras sapota* (Chikku), *Madhuca longifolia (Mahua)*, *Pouteria lucuma* (Lucuma) and *Mimusops elengi* (Maulsari) etc. These are tropical trees which has considerable nutritional and economical potential and also used as ideal fruit crop in dry areas.

Shoot buds and callus induction have been obtained from seedling and cotyledonary nodes of this plant by somatic embryogenesis (Purohit and Singhvi, 1998). Nodal segments, apical and axillary meristems of *M. longifolia* was used for *in vitro* bud break, multiplication and rooting by Rout and Das (1993). Micropropagation of *M. longifolia* (Mahua) was achieved by culturing excised nodes on Woody Plant Medium (WPM) supplemented with different plant growth regulators (Bansal and Chibbar, 2000).

Table 2.3: Ethno-botanical uses of *Mimusops hexandra* in India.

Region in India	Plant Part	Ethno-botanical Use	Reference
Rajasthan, India	Bark	Used for tanning	Malik et al, 2012
Madhya Pradesh, India	Bark	Used for production of natural dyes	Upadhyay and Choudhary, 2014
Central and western India	Fruits	Nutritive, sold in markets	Pareek et al. ,1998
Tamilnadu, India	Leaves	Used as fodder for cattle	Muruganandam et al., 2012
Madhya Pradesh, India	Wood	Used for oil presses house building and turnery	Malik et al., 2012

Jorden and Oyanedel (1992) achieved callusing and generation of adventitious shoots by organogenesis from shoot nodes, leaf, root, embryo and pericarp of *P. lucuma*. Shoots from zygotic embryos of *P. lucuma* with a portion of endosperm were established *in vitro* on Murashige and Skoog's medium by Padilla et al (2006). Axillary and apical buds and immature zygotic embryos (IZEs) were used as explants to initiate *in vitro* cultures of *M. elengi* (Gami et al., 2010; Bhore and Preveena, 2011). *In vitro* micropropagation protocol for *Vitellaria paradoxa* (Shea) via axillary shoot proliferation was also developed for plant regeneration (Lovett and Haq, 2013).

Similarly, phytochemical analysis of some important medicinal plants of this family suggest the presence of biologically important phytoconstituents in various parts of the plant. Fruit extracts of *M. zapota* has been analyzed by Shafii et al (2017) & Jenitha and Bhuvaneshwari (2016) for identification of phenols, steroids, tannins, flavonoids, terpenoids, saponins, quinine and anthraquinine. Fatty acids such as linoleic, palmitic and stearic acids have been isolated from seed oils of *Argania spinosa* by Mansour et al (2018). Pentacyclic terpenoids and phenolic compounds such as flavonols were identified in the leaf extracts of *A. spinosa* by Bonvicini et al (2016). Phytochemical analysis of leaf extracts of *M. longifolia* showed presence of carbohydrates, proteins, saponins, tannins, alkaloids triterpenoids, flavonoids and phytosterols (Kamal, 2014 and Annalakshmi et al, 2012). The presence of alkaloids, flavonoids, phenols, carbohydrates, proteins and amino acids were confirmed by Kalaiselvi et al, (2016) in various extracts of *M. elengi.* Saponin, flavonoids, tannin, alkaloids and cyanogenic glycoside have been isolated from fruit pulp of *S. dulcificum* by Chinelo et al, (2014). The biotechnological techniques are important to cultivate and conserve the medicinal plants. *In-vitro* regeneration has great potential for the production of herbal medicines (Chandra et al, 2013; Anis and Ahmad, 2016). Therefore, the current researches are emphasizing on evaluation and characterization of various plants against a number of diseases (Briskin., 2000; Arora, 2010; Shahid et al, 2013). Micropropagation has many advantages over conventional methods of vegetative propagation. Vegetative propagation methods have also been attempted using softwood grafting and veneer grafting for this plant (Pohare et al, 2016). The conservation and maintenance of plant biodiversity is an important issue relating to the global human population. Keeping in view it's medicinal and ethno-botanical applications, the typical culture environment should be developed for long term conservation of this plant (Malik et al, 2012). This can be achieved through different micropropagation methods which has many advantages over conventional methods of vegetative propagation (Chawla, 2009 and Cassells, 1997). The efficient systems are essential for the rapid chemical analysis of plant parts. The phytochemical analysis of the medicinal plants has great importance in therapeutics for the production of the new drugs for curing of various severe diseases (Arnason et al, 2013).

Chapter 3
Material and Methods

CHAPTER 3: MATERIAL AND METHODS

This study was conducted with the objective to develop a reliable and reproducible protocol for regeneration of *Mimusops hexandra* through tissue culture. The work mainly involves the study of the various combinations and concentrations of plant growth regulators influencing regeneration and optimization of various parameters that affect plant growth.

Source of explant

The explants of *M. hexandra* was obtained from its local habitat in Kota district of Rajasthan where this plant grows abundantly. Nodal segment and leaf was used as explants. Fresh disease free plant parts were collected during the month of September, 2014. Taxonomic identification of the plant was done by comparison with plant specimen (accession number: 20376) present in herbarium of Department of Botany, University of Rajasthan, Jaipur, Rajasthan and also with the help of “Indian Medicinal Plants: A Compendium of 500 Species” (Warrier and Nambia, 1993).

Preparation and sterilization of culture medium

Murashige and Skoog’s (1962) basal medium was used for plant regeneration. The medium contains various macro and micro nutrients, vitamins and amino acids required for the plant growth (Klerk et al, 2007; Bhojwani and Razdan, 1996; Sathyanarayana and Varghese, 2007; Murashige, 1974). As reviewed literature this media was found to be most suitable medium for regeneration in plants of this family (Swenson and Anderberg, 2005; Jordan and Oyanedel, 1992). It includes ammonium nitrate and potassium di phosphate which is used as nitrogen and phosphate source in the medium. Boron, molybdenum, copper and zinc etc. used in metabolic pathways of physiological reactions and vitamins are used as co-factor in various enzymatic reactions. Sucrose and glycine used as source of carbon and amino acids respectively (Bonga and Aderkas, 1992). The chemical composition of Murashige and Skoog's media used in this study is given in Table 3.1. Stock solutions of macro and micronutrients have been prepared in double distilled water for practical convenience and to maintain the concentration accuracy.

Stock solutions for growth regulators were also prepared and stored according to their optimum storage temperature. Growth regulators affect the growth and differentiation of plant tissue and helps in morphogenesis and regeneration of whole plant (Gaspar et al, 1996). Cytokinin's such as Benzyl Adenine (BA), Kinetin (KN) and Zeatin are known to differentiate shoot bud from shoot tip and nodal explants. BA is most commonly used cytokinin for shoot bud induction from nodal explants. Naphthalene acetic acid (NAA), Indole acetic acid (IAA), 2,4-Dichlorophenoxy acetic acid (2,4-D) and Indole-3- butyric acid (IBA) are most frequently used to induce root initiation (Davies, 1995; Aloni, 1980; Hall, 1976).

Table 3.1: Murashige and Skoog's media composition used for micropropagation of *M. hexandra* explants.

Stock Solution No.	Constituents used (Macronutrients)	Quantity used for One-liter medium	Used amount and strength in media preparation (one liter)	Actual amount in the medium (mg/lit.)
A	Ammonium nitrate (NH_4NO_3)	33 gm	50 ml (20X)	1650
	Potassium nitrate (KNO_3)	38 gm		1900
	Potassium bi phosphate (KH_2PO_4)	3.4 gm		170
	Boric acid (H_3BO_3)	0.124 gm		6.2
	Manganese sulphate heptahydrate ($MnSO_4 . 7 H_2O$)	0.446 gm		22.3
	Copper sulphate pentahydrate ($CuSO_4 . 5 H_2O$)	0.5 ml		0.025
	Sodium molybdate dihydrate ($Na_2MoO_4 . 2H_2O$)	0.5 ml		0.250
	Zinc sulphate heptahydrate ($ZnSO_4 .7 H_2O$)	0.172 gm		8.6
	Potassium iodide (KI)	0.0165 gm		0.825
	Cobalt chloride hexahydrate ($CoCl_2 .6H_2O$)	0.5 ml		0.025
B	(Calcium chloride dihydrate) $CaCl_2 .2H_2O$	11 gm	20 ml (50X)	440
C	Magnesium sulphate heptahydrate ($MgSO_4 .7H_2O$)	9.25 gm	20 ml (50X)	370
D	Ferrous sulphate heptahydrate ($FeSO_4 .7H_2O$)	2.780 gm	10 ml (100X)	27.8
	Ethylene diamine tetra acetic acid. Di sodium salt (Na_2-EDTA)	3.728 gm		37.28
E	Thiamine HCL	0.010 gm	10 ml (100X)	0.1
	Nicotinic Acid	0.050 gm		0.5
	Pyridoxine HCL	0.050 gm		0.5

Other supplements used with stock solutions-

- Glycine – 2.0 mg/lit.
- Myo-Inositol- 100 mg/lit.
- Sucrose- 300 mg/lit.
- Agar- 800 mg/lit.
- Proline- 0.2 mg/lit.

Abscisic acid (ABA), gibberellins and ethylene are also very important in regulation of plant growth. It was observed that the balance in the levels of cytokinin and auxin determines specific morphogenetic pattern (Dodds and Roberts, 1990; Bhojwani and Razdan, `1996; Klerk et al, 2007). The different types of plant growth regulators were weighed individually and dissolved in required volume of appropriate solvents. Then the solutions were stored as stocks at mg/ml concentration (Table 3.2 and 3.3). The quality of the stock solutions was maintained adequately for the desired culture growth. Other contents such as agar and myo-inositol wee added directly during preparation of the culture medium.

Preparation and sterilization of medium

Initially one-liter medium has been prepared by adding required volume of macronutrients, micronutrients and amino acids from the stock solutions in approximately 900 ml of double distilled water and kept under constant stirring. The plant growth regulators were added at required concentration either individually or in combinations. The organic supplements like sucrose (3%) and myo-inositol (100 mg/lit.) were also added and final volume of the medium was brought to one liter by adding double distilled water. The pH of the medium was maintained at 6.0 by using 0.1 N NaOH or 0.1N HCl. The medium was gelled with agar (0.8%) and the molten medium was dispensed into sterile culture flasks (40 ml each). These flasks autoclaved at 15 lbs. pressure and 121^{o} C temperature for15-20 minutes along with all required things needed for inoculation such as forceps and petri plates etc. for sterilization.

Sterilization of Explants

Young shoot nodes and leaves were washed in running tap water and then aseptically sterilized to remove surface particles, fungal spores, cuticle layers of plant tissues and to allow media uptake by the explants. The surface sterilization was done with 0.01% $HgCl_2$ solution in sterile condition at Laminar Air Flow Hood (LAF) for two minutes. The explants were washed several times with sterilized distilled water and used for inoculation in the culture flasks containing MS medium.

Initiation of the cultures

Cultures of *M. hexandra* were raised under aseptic conditions. LAF (0.3 µm filters) was used for aseptic inoculation of the explants to culture media. In initial cultures the MS basal medium supplemented with 30 gm/lit. sucrose, 8 gm/ lit. agar and cytokinin such as BA, KN at concentration of

0.5- 2.5 mg/lit. and Thidiazuron (TDZ) at concentration of 0.05- 0.5 mg/lit. was used for culture initiation and shoot induction. The shoot nodes of about 3-4 cm and leaf cuttings of about 2 cm in size was prepared using sterile surgical blade.

The explants were inoculated in manner to maintain the polarity as shoot nodes were inoculated vertically and leaves were inoculated horizontally. The culture bottles with inoculated explants were incubated in the culture room at 25± 2^{o} C and the relative humidity was maintained about 90%. The cultures were illuminated with white fluorescence lamps at 3000 lux intensities for a photo period of 15 hours per day. The observations regarding the plant growth were taken several times in between the time period of two months. The observations were recorded after 7^{th}, 15^{th}, 30^{th}, 45^{th} and 65^{th} day of inoculation with 85% of survival rate for the cultures. After 4^{th} day, presence of phenolic compounds was observed by browning of the plant tissue in explants. So, activated charcoal (2 %) was used with MS basal medium to reduce the browning. It can absorb toxic phenolic pigments, stabilizes pH and stimulate the growth of cultures. Proline at concentration of 0.2 gm /lit. was also used as it is known to induce the rate of culture initiation (Bhojwani and Dantu, 2013).

In further inoculations, phytohormones were used at different concentrations and combinations. BA (0.5 - 2.0 mg/lit.) with constant concentration of KN/ NAA/ IAA/2,4-D (0.1 mg/lit.) was used for shoot induction in the cultures.

Table 3.2: Preparation of stock solutions of growth regulators for micropropagation of *M. hexandra.*

Growth Regulator	Preparation of the solution					Typical working concentration used in the study (mg/lit.)
	Solvent	**Diluent**	**Powder Storage**	**Liquid Storage**	**Sterilization**	
Auxins						
IAA	Ethyl Alcohol	Water	Freezing	Freezing	Co- autoclaved	0.05-0.2
IBA	Ethyl Alcohol	Water	Refrigeration	Freezing	Co- autoclaved	0.05-0.2
2,4-D	-	Water	Room Temperature	Refrigeration	Co- autoclaved	0.05-0.2
NAA	Potassium Hydroxide	Water	Room Temperature	Refrigeration	Co- autoclaved	0.05-0.2
Cytokinin						
BA	Potassium Hydroxide	Water	Room Temperature	Refrigeration	Co- autoclaved	0.5- 2.5
KN	Potassium Hydroxide	Water	Freezing	Freezing	Co- autoclaved	0.5- 2.5
TDZ	Di-methyle sulphoxide	-	Room Temperature	Refrigeration	Co- autoclaved	0.05-0.5
Gibberellins						
GA_3	Ethyl Alcohol	-	Room Temperature	Refrigeration	Co- autoclaved	0.1

Table 3.3: Concentration range of growth regulators used in micropropagation of *M. hexandra*

Growth Regulator Used	Concentration of stock solution (mg/ml)	Molar Equivalence		Amount Used (ml)	Concentration of final Solution (mg/lit.)
		Molecular Weight	Concentration (μm for 1 mg/lit.)		
Auxins					
IAA	0.1	175.2	5.71	0.5-2.0	0.05-0.2
IBA	0.1	203.2	4.90	0.5-2.0	0.05-0.2
2,4-D	0.1	221.0	4.53	0.5-2.0	0.05-0.2
NAA	0.1	186.2	5.37	0.5-2.0	0.05-0.2
Cytokinin					
BA	1.0	225.3	4.44	0.5- 2.5	0.5- 2.5
KN	1.0	215.2	4.65	0.5- 2.5	0.5- 2.5
TDZ	0.1	220.2	4.5	0.5- 5.0	0.05-0.5
Gibberellins					
GA3	1.0	346.4	2.89	0.1	0.1

Increased rapid growth in the explants was observed when the growth regulators were used in combination with culture medium instead of alone hormone concentration used previously. It was observed that BA with KN and NAA is most effective for shoot induction in *M. hexandra* explants. IAA and 2,4-D also gave average response regarding shoot growth. Cell cultures were allowed to establish itself in the culture medium. Increased rate of growth in shoot elongation and number of shoots was observed. Whenever the culture medium got depleted, the cultures were subculture onto the same medium fortified with the same hormonal strength in which they were raised initially and the observations were recorded periodically for all cultures.

Rooting in the cultures

Proliferation in shoots were observed and shoots were excised from the culture bottles and cultured on half-strength MS and full-strength MS medium containing growth regulators responsible for rooting such as IAA, IBA, NAA and 2,4-D at different concentrations (0.05- 0.2 mg/lit.). Observations were taken after 3-9 weeks of the transfer. The rooting frequency, number of roots per shoot and length of roots were recorded after 65 days of culture and mean growth values were analyzed.

Statistical Analysis

To analyze the data obtained from the plant cultures statistical techniques have been used. It makes the calculation much easier and provides accurate results (Azen and Afifi, 1972). Independent cultures of particular hormonal concentrations each with 12 replicates were raised. The analysis of variance (ANOVA) was done via 'IBM SPSS Statistic Software version 25' to test the null hypotheses. Mean value, standard error, standard deviation was obtained via descriptive analysis at 95% confidence interval for means. Levene statistic was also obtained to test homogeneity of variance. ANOVA provides sum of squares, degrees of freedom and mean square error of within groups and between groups. The obtained F-ratio was too large it leads to rejection of the null hypotheses that all true means are equal (H_0: $M_1 = M_2 = M_3 = M_4$……. M_{12}).

To determine significant difference between means, post hoc comparisons was done. To maintain a low overall type, I error Duncan's multiple-range test was used. Initially mean value of all twelve replicates of each culture having same hormonal concentration and combination was calculated. Then all means were arranged in ascending order i.e. least to highest. Each pair of means was compared against a different critical value at specific significance level (0.05%). Critical value or least significant studentized range depends on the ranks of these means in the ordered array. The comparison of the sample means with a calculated least significant studentized range was done. The least significant studentized range denoted as Rp and depends upon the error degrees of freedom and the numbers of means in the subset. (n-1) Rp values were calculated by using following formula where n is the total number of groups (Duncan, 1955).

$$R_p = Q_\alpha (p, \upsilon) \frac{\sqrt{MSE}}{n}$$

Where,

Rp = Least significant studentized range

Qα = Tabular value used at particular significance level

P = Number of groups compared

υ = Degrees of freedom for experimental error

MSE = Mean square error (Within treatments)

n = Number of observations

If the mean difference is greater than corresponding Rp, the difference is considered as significant. If the mean differences are smaller than corresponding Rp, the difference is not considered as significant. The obtained result was also presented using alphabet notations. Treatment means followed by same letter within column are not significantly different from each other.

Phytochemical analysis

The phytochemical analysis of *M. hexandra* includes estimation of primary and secondary metabolites present in this plant, basically in leaf and bark. To ensure reproducible quality of herbal medicines correct identification of the plant phytoconstituents is very essential (Raaman, 2006).

The leaves were washed thoroughly 2-3 times with running tap water and air dried under shade. Shade dried plant leaves and bark was grinded in mixer and the powder was kept in small plastic bags with paper labelling. Crude plant extract was prepared by soxhlet extraction method. About 20 gm of powdered plant material was uniformly packed into a thimble and extracted with 250 ml of different solvents methanol, ethanol, and acetone separately for 24 hours. The extract was taken in a beaker and kept on hot plate and heated at 30-40^{o} C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4^{o} C for further use.

Qualitative estimation of primary metabolites

The qualitative estimation of primary and secondary metabolites was done via various biochemical testing procedures specific for particular compound to ensure the presence of these phytoconstituents (Table 3.4). Observed reaction responses such as precipitate formation and change in color of the solution etc. used for estimation of presence of these metabolites in leaf and bark extracts of *M. hexandra* (Serba, 1946; Saxena, 2006).

Quantitative estimation of primary metabolites

Plant extracts for estimation of total carbohydrate content has been prepared by method of Loomis and Shull (1939). 80 % ethanol has been used for homogenization of plant powders and proceeds for centrifugation at 1200 rpm for 15 min after 24 hours. The concentrated supernatants were used for quantitative analysis. 52% perchloric acid has been added to the residue for estimation of starch content (Mc Cready et al, 1950).

Phenol-sulphuric acid method was used for estimation of net content of carbohydrates (Dubois et al, 1951). 5% phenol and concentrated sulphuric acid was used for separation of total soluble sugars. Protein content was estimated by method of Lowry et al, 1951. Samples of leaf and bark was prepared via method of Osborne (1962) by using cold trichloric acid. Solution of 2% Na_2Co_3 and 0.5% $CuSO_4$ was also used with addition of diluted folin ciocalteau reagent for extraction of proteins. Further, the optical densities of used standard sugar (glucose) and protein (bovine serum albumin) with their respective samples was measured at 490 and 750 nm wavelength using spectrophotometer where distilled water was used as blank. Regression curve was prepared between the known concentration of glucose and BSA & their respective absorbance which followed the Beer-Lambert law (Swinehart, 1962). Net contents were calculated from regression curve by using Thorpe and Bray’s (1954) protocol was used by using ethanolic solution of gallic acid as standard. Folin ciocalteau reagent and Na_2Co_3 solution was added for extraction of phenolic contents (Singleton et al, 1999). The optical density of gallic acid and plant samples were observed at 750 nm and net phenolic contents were calculated as previous. Solution of chloroform and methanol (2:1, v/v) was used for estimation of net lipid content (Paquot, 1979). The lipids were separated with chloroform and collected in the pre-weight glass vials then weighed. The procedure repeated for three times and mean values were calculated.

Various biochemical test used to confirm the presence of secondary metabolites were also performed. Mayer’s, Wagner’s, Dragendorff’s and Hager’s test was used to identify presence of alkaloids, Shinoda test and Alkaline reagent test was used for flavonoids, Liebermann-Burchard's and Salkowaski test was used for sterols. Ferric chloride test was used for phenols (Acamovic and Brooker, 2005). Qualitative estimation of alkaloids was done via gravimetric method in which methanolic extracts of leaf and bark were prepared by soxhlet extraction and further extracted by chloroform. The free alkaloids were separated by ammonia (Woo et al, 1977). The extracts were further analyzed by Thin Layer Chromatography.

Flavonoids have been separated from powdered samples of leaf and bark with petroleum ether and 80% methanol via soxhlet extraction at 45-60°C. Then again fractioned by sequential extraction with petroleum ether, ethyl ether and ethyl acetate separately. Ethyl ether and ethyl acetate fractions were used for estimation of flavonoids (Marby et al, 1970). Identification of sterols have been done by using petroleum ether for separation of fats from dried plant samples. The dried preparation was again extracted with benzene and further proceeds for TLC (Hartmann and Benveniste, 1987).

Thin Layer Chromatography

Thin silica containing glass plates were used for chromatographic separation. The extracted samples were used for chromatographic separation and co-chromatographed with authentic alkaloids such as colchicine, flavonoid such as Kaempferol and sterol such as β-sitosterol in the chromatographic chamber saturated with solvent mixture of methanol and conc. ammonium hydroxide at ratio of 200: 3, n-butenol, acetic acid and water at ratio of 4:1:5 and hexane and acetone at the ratio of 8:2 for alkaloids, flavonoids and sterols respectively (Ciesla and Waksmundzka, 2009). The spots were identified coinciding with the colchicine, kaempferol and β- sitosterol marker. Ammonia fumes were used to darken the spots. The developed plates were air dried and visualized under ultra violet light. The retention factor (R_f Value) of each spot were calculated.

Further qualitative estimation of phytoconstituents of *M. hexandra* was done by GC-MS (Gas chromatography– mass spectrometry) analysis. As reviewed literature petroleum ether extracts of plant parts have been used preferably in GC-MS analysis for family Sapotaceae previously (Kumar et al, 2017; Souravi et al, 2015; Kumkum and Patni, 2017). Petroleum ether extracts also found more suitable instead of other solvents during the biochemical testing procedures, so petroleum ether extracts have been used for qualitative estimation of phytoconstituents in stem and flower extracts of *M. hexandra*. GC-MS analysis was done by using "Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole" instrument and the samples were injected into a 15 m × 0.25 mm i.d. × 0.25 µm system qualification column (SQC). It consists trace 1310 gas chromatograph (GC) coupled with mass selective detector (MSD). The temperature of injector and the detector was maintained at 260 °C and 290°C respectively. The temperature was maintained at 50 °C for two minutes and further raised from 50 °C to 290 °C with the constant increasing rate of 10 °C per minute. 1 µl of the sample was injected and the total run time was 48.36 minute (Azhagumurugan and Rajan, 2014). The identification of compounds was done by comparing with the data obtained from NIST Mass Spectral Library (Stein and Scott, 1994).

Table 3.4: Analytical testing of various primary and secondary metabolites present in the leaf and bark extracts of *M. hexandra*

Phytoconstituent	Analytical Test Performed	Treatment	Observations
Carbohydrates	Fehling's test	Fehling A and Fehling B reagents were mixed together in equal volume. 2 ml of this solution was added to crude extract and boiled.	Precipitate was form at the bottom of the test tube. The colour of the precipitate was observed.
	Benedict's test	Crude extract was mixed with 2 ml of Benedict's reagent and boiled.	Precipitate was form at the bottom of the test tube. The colour of the precipitate was observed.
	Molisch's test	Crude extract was mixed with 2 ml of Molisch's reagent and the mixture was shaken properly. In this solution concentrated H_2So_4 (2 ml) was added slightly to the side of the test tube.	A ring formed in the solution, the colour of the ring was observed.
	Iodine test	Crude extract was mixed with 2 ml of iodine solution	Colour of the solution was observed.
Proteins	Biuret test	Crude extract was treated with an equal volume of 1% potassium hydroxide. Few drops of aqueous copper (II) sulfate were added to the solution	Color of the solution was observed.
	Ninhidrin' s test	Crude extract was treated with 0.1 % Ninhydrin's solution. heated on burner for 10 minutes.	Color of the solution was observed
	Millon's test	Crude extract was mixed with 2 ml of Millon's reagent , Gentle heating was done	White precipitate was appeared in the solution. The color of the solution was observed
Lipid	Acrolein Test	Crude extracts were heated in the presence of potassium bi sulphate	Odor peculiar to burnt cooking grease was observed
	Sudan IV Test	Extracts were stained with Sudan IV dye	Color of the solution observed

Phytoconstituent	Analytical Test Performed	Treatment	Observations
Alkaloids	Mayer's Test	The sample extracts were treated with Mayer's reagent i.e. Potassium mercuric iodide solution.	A cream-colored precipitate was form
	Wagner's reagent	The sample extracts were treated with Wagner's reagent i.e. iodine solution in potassium iodide.	A red-brown colored precipitate was form
	Hager's reagent	The sample extracts were treated with Hager's reagent i.e. saturated solution of picric acid.	A yellow colored precipitate was form.
	Dragendorff's reagent	The sample extracts are treated with Dragendorff''s i.e. solution of potassium bismuth iodide.	An orange colored precipitate was form
Flavonoids	Shinoda test	Pieces of magnesium ribbon and concentrated HCl were mixed with crude plant extract	Pink colored scarlet appeared
	Alkaline reagent test	2 ml of 2% NaOH solution was mixed with plant crude extract, then 2 drops of diluted acid to solution was added	Intensive yellow color was formed which turned into colorless
Steroids	Liebermann-Burchard's test	Crude extract was dissolved in acetic anhydride then heated to boiling and then cooled. concentrated sulphuric acid (1 ml) was added slightly to the sides of the test tube.	The colour of the solution changed to green
	Salkowaski reaction	2 mg of dry extract was shaken with chloroform, to the chloroform layer conc. sulphuric acid was added slightly by the sides of test tube.	The colour of the solution changed to red.
Phenol and Tannins	Ferric Chloride Test	Crude plant extract was mixed with 2% $FeCl_3$ (2 ml) solution	The colour of the solution changed to green

Chapter 4
Results and Discussion

CHAPTER 4: RESULTS AND DISCUSSION

To study the possibility of *in vitro* clonal multiplication of *Mimusops hexandra,* this study was designed on Murashige and Skoog's (MS) basal medium supplemented with different concentration and combinations of growth hormones using young shoot tips, nodal segments and leaf cuttings as explants. The stem nodes were found more suitable explant instead of leaf meristem for optimum vegetative propagation of *M. hexandra.* Cytokinin, BA (0.50, 0.75 and 1.00 mg/lit.) with KN (0.1 mg/lit.) gives best results in multiple shoot induction as the average number of shoots per explant was obtained 6.8 ± 0.08 and the mean length of shoots was obtained approximately 7.4 ± 0.06 mm. It was observed that BA alone at concentration of 0.5 and 0.75 mg/lit. found most effective in shoot induction with mean length of shoots (5.5 ± 0.05 mm and 4.9 ± 0.14 mm) respectively. Kinetin alone at concentration of 0.5 and 1.00 mg/lit also found very effective with mean length of shoots (5.4 ± 0.06 and 5.1 ± 0.10 mm) respectively. Average growth initiation rate was found with higher concentrations of BA (1.00 - 2.00 mg/lt.) and KN (0.75 and 1.25 mg/lit.). No growth signs were found while using TDZ (0.05-0.5 mg/lit.) in the cultures for shoot induction.

During initiation of the cultures about ninety percent of the primary explants were found free of contamination after the disinfection procedures used in this study such as surface sterilization by 0.01 % $HgCl_2$ solution.

Cultures conditions were maintained as temperature of the culture room (25 ± 2^{o} C), relative humidity (90%), intensities of white fluorescence light (3000 lux) and photo period of 15 hours per day found optimum for the culture establishment of *M. hexandra* explants (Sathyanarayana and Varghese, 2007; Murashige, 1974; Dodds and Roberts, 1990).

Sprouting in the stem cuttings was observed within 6 days of inoculation. The observations were taken after 7th, 15th, 30th, 45th and 65th day of inoculation with 85% of survival rate for the cultures (Fig. 4.1). After 4 days browning in the cultures was observed due to phenolic compounds present in the plant. So, 2% activated charcoal was used with MS basal media, it reduces the browning of the explants but no effect was obtained in the culture growth (Bhojwani and Dantu, 2013) (Fig. 4.2). Proline at concentration of 0.2 gm /lit. with MS basal media also found beneficiary for culture initiation. Some cultures containing leaf cuttings survive for more than two months but no growth signs were found in the leaf cuttings during the complete study. The growth was observed and analyzed by "IBM SPSS Statistic Software version 25" to obtain mean number of shoots per explant and mean length of shoots. Significant difference in the growth among the explants cultured was analyzed by Duncan's Multiple Range Test (DMRT) which shows the significant difference among the results. Alphabetic notation is also used to show significance difference between the data in a column (Azen and Afifi, 1972; Duncan, 1955).

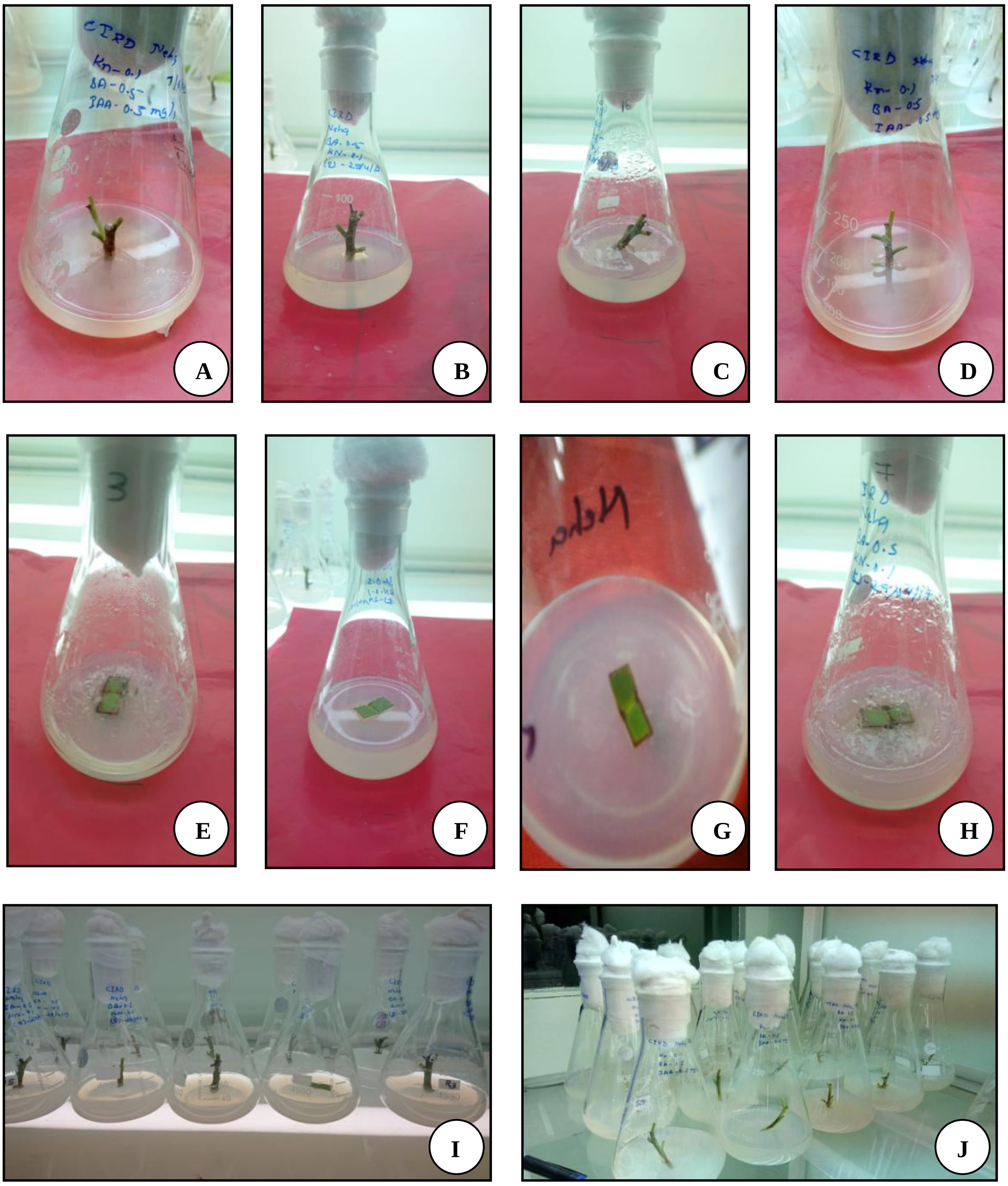

Fig. 4.1: (A-D) Inoculated shoot nodes; (E-H) leaf cuttings of *M. hexandra* on MS+ BA/ KN (0.5-2.5 mg/lit.) / TDZ (0.05-0.5 mg/lit.); (I-J) Establishment of cultures in optimum culture conditions.

Initially single hormone concentration was used for shoot induction in the explants and it was observed that BA is most effective for shoot induction at concentration of 0.5 and 0.75 mg/lit with mean length of shoots (5.5 ± 0.05 mm and 4.9 ± 0.14 mm) respectively. Kinetin at concentration of 0.5 and 1.00 mg/lit also found very effective with mean length of shoots (5.4 ± 0.06 and 5.1 ± 0.10 mm) respectively. Average growth initiation rate was found with higher concentrations of BA (1.00 - 2.00 mg/lt.).

When the concentration increased up to 2.25 and 2.50 mg/lit, no growth was obtained in the cultures. KN (0.75 and 1.25 mg/lit.) results in average growth (4.8 ± 0.12 and 2.6 ± 0.35 mm) initiation in cultures. Whereas no growth initiation was found in explants at higher concentrations of KN (1.50 - 2.50 mg/lit.) also. Average number of shoots per explant was also found 2.6 ± 0.09 from BA (0. 50 mg/lit) and 2.7 ± 0.04 from KN (0.50 mg/lit.). No growth signs were found while using TDZ (0.05-0.5 mg/lit.) in the cultures for shoot induction (Table 4.1).

Explants of *M. hexandra* were also cultured on MS basal media containing combinations of growth regulators majorly cytokinin for multiple shoot induction. Constant concentration (0.1 mg/lit.) of other hormones such as KN, NAA, IAA and 2,4-D was used with BA in combination and it was observed that BA (0.50, 0.75 and 1.00 mg/lit.) with KN (0.1 mg/lit.) gives best results in multiple shoot induction as the average number of shoots per explant reaches up to 5.75 ± 0.05 and the mean length of shoots was obtained approximately 12.6 ± 0.06 mm (Fig. 4.3).

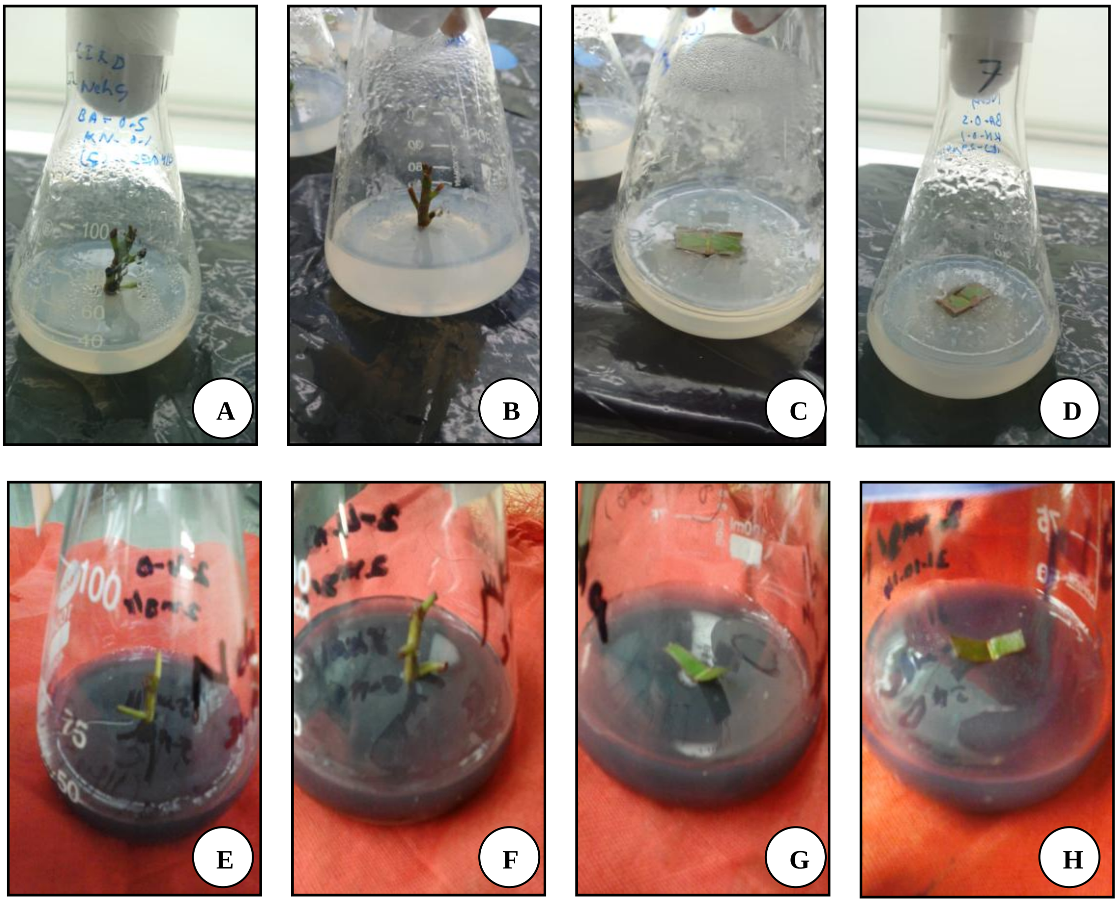

Fig. 4.2: Browning in the shoot nodes (A & B) and leaf cuttings (C & D) after 4Th day of inoculation. Inoculation of shoot nodes and leaves on MS + activated charcoal (2%) leads to reduction in the browning but no effect occurred on the culture growth (E-H).

Table 4.1: Effect of cytokinin on multiple shoot induction in shoot tip and nodal segments of *M. hexandra.*

Hormone concentration (mg/lit.)		Explants responded (%)	Average number of shoots/explant [a]	Mean length of shoots[a] (mm)
BA	0.50	58	2.6 ± 0.09 a	5.5 ± 0.05 a
	0.75	43	2.4 ± 0.07 a	4.9 ± 0.14 ab
	1.00	41	2.4 ± 0.13 a	3.8 ± 0.13 bc
	1.25	59	1.8 ± 0.15 b	2.9 ± 0.15 cd
	1.50	60	1.5 ± 0.07 b	2.1 ± 0.12 d
	1.75	30	1.6 ± 0.21 b	1.9 ± 0.12 d
	2.00	30	1.5 ± 0.06 b	2.2 ± 0.11 d
	2.25	-	-	-
	2.50	-	-	-
KN	0.50	30	2.7 ± 0.04 a	5.4 ± 0.06 a
	0.75	60	2.3 ± 0.12 a	4.8 ± 0.12 a
	1.00	58	1.6 ± 0.08 b	5.1 ± 0.10 a
	1.25	40	1.5 ± 0.15 b	2.6 ± 0.35 b
	1.50	-	-	-
	2.00	-	-	-
	2.25	-	-	-
	2.50	-	-	-
TDZ	0.05	-	-	-
	0.1	-	-	-
	0.2	-	-	-
	0.3	-	-	-
	0.4	-	-	-
	0.5	-	-	-

- No Response

a Values are mean ± standard error of three independent experiments each with 12 replicates. Observations were made after four weeks of inoculation. The means which have same letter within column are not significantly different from each other (P = 0.05); comparison between means was done by Duncan's multiple range test.

This medium was also used previously for propagation of plants belonging to family Sapotaceae. Micropropagation of *Madhuca longifolia* was achieved by culturing shoot nodes on MS medium supplemented with similar concentration of growth regulators by Bansal and Chibbar (2000). Maximum response rate regarding the culture growth was also obtained by using this hormone combination (Table 4.2).

Again, when the concentration of BA was increased up to 1.25- 2.00 mg/lit., the growth of explants declines and the lowest response rate of culture growth was obtained. NAA (0.1 mg/lit.) with BA (0.5- 1.00 mg/ lit) also gave good results with approximately 3.8 ± 0. 12 number of shoots per explant and 9.3 ± 0.06 mm mean shoot length. An increased response of explants was observed when the growth regulators were used in combination with culture media instead of alone hormone concentration used previously. Survivability of the explants was also found increased with culture media.

BA (0.50- 1.00 mg/ lit.) with IAA (0.1 mg/lit.) gave approximately 3.2 ± 0.22 number of shoots per explant and 5.6 ± 0.25 mm shoot length. 2,4-D (0.1 mg/lit.) also showed growth but in decreased proportion. Average number of shoots was found approximately 2.5 ± 0.07 shoots per explant whereas the mean shoot length was found approx. 4.6 ± 0.15 mm. No growth stimulus was observed in BA + IAA (2.00 + 0.1 mg/lit.) and BA + 2,4- D (1.75, 2.00 + 0.1 mg/ lit.) combination.

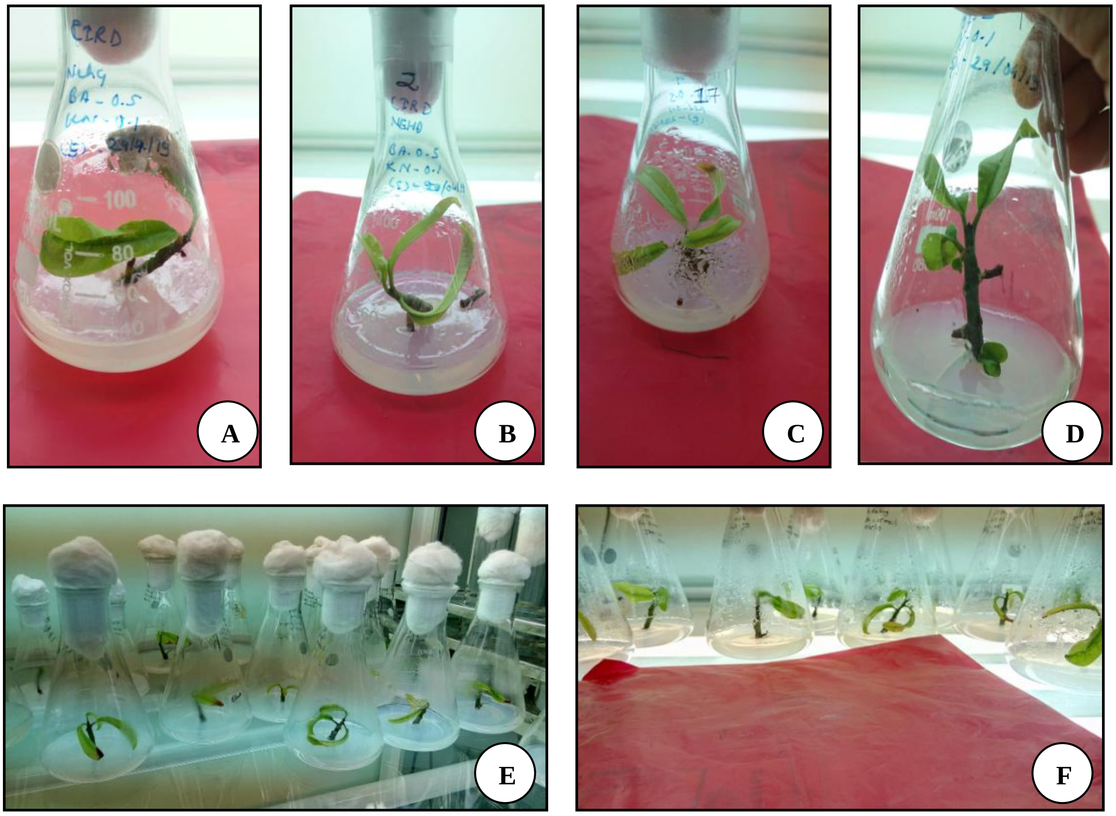

Fig. 4.3: (A-F) Sprouting and elongation in shoot nodes observed after 20 days of inoculation

Table 4.2: Effect of growth regulators in combination on multiple shoot induction using shoot tip explants of *M. hexandra*

Hormone concentration (mg/lit.)		**Explants responded (%)**	**Average number of shoots/explant [a]**	**Mean length of shoots (mm) [a]**
BA+ KN	0.50 + 0.1	83	5.75 ± 0.05 a	12.6 ± 0.06 a
	0.75 + 0.1	72	4.5 ± 0.14 a	10.3 ± 0.05 a
	1.00 +0.1	60	3.4 ± 0.09 b	8.5 ± 0.07 b
	1.25 +0.1	57	4.0 ± 0.05 a	5.4 ± 0.24 c
	1.50 +0.1	59	4.6 ± 0.07 a	4.7 ± 0.15 c
	1.75 +0.1	40	4.4 ± 0.08 b	3.7 ± 0.14 c
	2.00 +0.1	25	3.3 ± 0.05 c	2.8 ± 0.14 c
BA+ NAA	0.50 + 0.1	78	3.8 ± 0.12 a	9.3 ± 0.06 a
	0.75 +0.1	73	3.3 ± 0.07 a	8.5 ± 0.05 b
	1.00 +0.1	47	2.9 ± 0.27 b	8.3 ± 0.07 b
	1.25 +0.1	37	1.7 ± 0.07 c	5.2 ± 0.32 b
	1.50 +0.1	48	1.3 ± 0.07 d	4.5 ± 0.08 c
	1.75 +0.1	36	2.1 ± 0.13 c	3.1 ± 0.24 c
	2.00+ 0.1	37	1.7 ± 0.08 c	2.4 ± 0.08 c
BA + IAA	0.50 + 0.1	64	2.5 ± 0.09 b	5.6 ± 0.25 a
	0.75 +0.1	74	4.2 ± 0.22 a	4.8 ± 0.24 ab
	1.00 +0.1	73	2.6 ± 0.17 b	5.6 ± 0.61 a
	1.25 +0.1	49	2.3 ± 0.10 b	3.7 ± 0.37 b
	1.50 +0.1	58	1.6 ± 0.07 bc	3.2 ± 0.21 b
	1.75 +0.1	59	2.1 ± 0.15 c	2.5 ± 0.10 b
	2.00 +0.1	48	-	-
BA + 2,4-D	0.50 + 0.1	48	2.5 ± 0.07 b	4.6 ± 0.15 a
	0.75 +0.1	49	1.6 ± 0.12 c	3.5 ± 0.34 b
	1.00 +0.1	39	3.3 ± 0.12 a	2.9 ± 0.57 b
	1.25 +0.1	59	2.4 ± 0.10 b	1.6 ± 0.08 c
	1.50 +0.1	35	2.3 ± 0.17 b	2.3 ± 0.17 bc
	1.75 +0.1	-	-	-
	2.00 +0.1	-	-	-

- No Response
a Values are mean ± standard error of three independent experiments each with 12 replicates. Observations were made after eight weeks of inoculation. The means which have same letter within column are not significantly different from each other (P = 0.05); comparison between means was done by Duncan's multiple range test.

These results have shown that low levels of BA were necessary for maximum shoot proliferation. So, BA (0.5- 1.00 mg/lit.) was used with other hormones such as KN, NAA and GA_3 (0.1 mg/lit.) for further establishment, elongation and multiplication of cultures. A synergistic effect of growth regulators was noticed in combination with BA on promotion of shoot multiplication.

Table 4.3 shows the effect of various multiplication hormone combinations on shoot elongation. BA alone (0.5- 1.00 mg/lit.) shows a slow and steady growth on the cultures whereas the KN and NAA gives rapid and increased growth in the cultures. Survivability and response rate was also found increased in this combination. Highest shoot elongation and mean number of nodes per shoot was obtained with this combination.

This combination was also found effective in previous studies for other medicinal plant species of Sapotaceae such as *Mimusops elengi* (Bhore and Preveena, 2011) and *Madhuca longifolia* (Rout and Das, 1993). BA (0.1 mg/lit.) with GA_3 (0.1 mg/lit.) also showed rapid multiplication rate in the culture which is approx. 3.3 ± 0.12 mean number of nodes per shoot and approx. 2.5 ± 0.10 elongation in shoot length.

Proliferated shoots were excised from the culture bottles and cultured on half- strength MS and full-strength MS medium containing different concentrations of IAA, IBA and NAA for rooting.

Table 4.3: Effect of reduced concentration of growth regulators on shoot elongation in explants of *M. hexandra*.

Growth regulator (mg/lit.)		**Shoot elongation (mm)[a]**	**Mean no. of nodes per shoot [a]**
BA	0.50	2.6 ± 0.07 a	2.2 ± 0.04 a
	0.75	2.4 ± 0.08 a	1.5 ± 0.1 b
	1.00	2.2 ± 0.04 a	1.3 ± 0.03 b
BA + KN	0.50 + 0.1	3.1 ± 0.07 a	3.2 ± 0.08 a
	0.75+ 0.1	2.7 ± 0.10 a	2.7 ± 0.16 a
	1.00+ 0.1	3.2 ± 0.10 a	2.2 ± 0.17 a
BA + NAA	0.50 + 0.1	2.3 ± 0.06 a	2.3 ± 0.08 a
	0.75 + 0.1	1.5 ± 0.08 b	1.9 ± 0.15 a
	1.00 + 0.1	1.4 ± 0.04 b	1.6 ± 0.13 a
BA + GA_3	0.50 + 0.1	2.5 ± 0.10 a	1.4 ± 0.07 c
	0.75 + 0.1	2.4 ± 0.05 a	2.5 ± 0.25 b
	1.00 + 0.1	2.0 ± 0.13 a	3.3 ± 0.12 a

a Values are mean ± standard error of three independent experiments each with 12 replicates. Observations were made after eight weeks of inoculation. The means which have same letter within column are not significantly different from each other (P = 0.05); comparison between means was done by Duncan's multiple range test.

The rooting of the stem cuttings was delayed and only 3-5 roots were observed from the stem cuttings after 60 days. Moreover, the roots were short and the average root length was measured as 4-7 mm. The rooting frequency, number of roots per shoot and length of roots were recorded after 65 days of culture. The rooting response to different auxin treatments is shown in Table 4.4. IAA (0.1 and 0.2 mg/lit) with half strength MS basal media showed best rooting rate in the cultures i.e. approx. 10.2 ± 0.8 mm root length and maximum number of roots approx. 5.4 ± 0.08 per explant in the cultures

(Fig. 4.4). IAA (0.1 and 0.2 mg/lit.) also showed increased rooting frequency with full strength MS basal media. Average growth in rooting was observed when 0.5 mg/lit IAA was used with half strength MS media whereas no rooting was observed when it is used with full strength MS basal media. IBA also increased rotting when used at concentration of (0.05- 0.2 mg/lit.) with full strength MS media. No rooting was observed from IBA (0.05 and 0.1 mg/lit.) with half strength MS media, but IBA (0.2 mg/lit.) with half strength media showed rooting in the explants. NAA (0.05- 0.2 mg/lit.) with full strength MS media showed rooting with an average of 5.5 ± 0.08 mm root length. NAA (0.05-0.1 mg/lit.) did not produce any effect on rooting in the explants, but it showed rooting at concentration of 0.2 mg/lit with half strength MS media. 2,4- D did not show any effect on explant rooting at concentration of 0.05 and 0.1 mg/ lit. with full strength MS media and at 0.1 mg/lit with half strength MS basal media. 0.05 and 0.2 mg/lit. with half strength MS media and 0.2 mg/ lit. with full strength MS media showed rooting in the cultures.

No rooting was observed in auxin free half strength MS medium, but it was observed in auxin free full strength MS medium which is about 4.1 ± 0.22 mm root length and 4.2 ± 0.10 number of roots per explant. The cultured plants were subjected for sub culturing on half strength MS basal media.

Table 4.4: Effect of different auxins and strength of MS medium on *in vitro* rooting of micropropagated shoots of *M. hexandra*

Hormone used (mg/lit)	Medium used		Rooting response (%)	No. of roots/ explant [a]	Root length (mm)[a]
IAA	0.05	MS	-	-	-
IAA	0.1	MS	48	3.4 ± 0.07 a	8.2 ± 0.43 a
IAA	0.2	MS	56	2.7 ± 0.13 a	7.6 ± 0.50 a
IAA	0.05	½ MS	50	3.2 ± 0.04 b	5.3 ± 0.11 b
IAA	0.1	½ MS	68	5.4 ± 0.08 a	10.2 ± 0.7 a
IAA	0.2	½ MS	65	3.7 ± 0.22 b	10.2 ± 0.8 a
IBA	0.05	MS	69	2.4 ± 0.14 b	5.5 ± 0.08 a
IBA	0.1	MS	53	2.7 ± 0.11 b	4.8 ± 0.14 b
IBA	0.2	MS	62	3.4 ± 0.13 a	5.0 ± 0.07 ab
IBA	0.05	½ MS	-	-	-
IBA	0.1	½ MS	-	-	-
IBA	0.2	½ MS	36	2.6 ± 0.13	6.3 ± 0.22
NAA	0.05	MS	50	2.6 ± 0.04 b	5.5 ± 0.08 a
NAA	0.1	MS	48	3.2 ± 0.08 a	5.4 ± 0.07 a
NAA	0.2	MS	43	2.7 ± 0.07 b	5.4 ± 0.09 a
NAA	0.05	½ MS	-	-	-
NAA	0.1	½ MS	-	-	-
NAA	0.2	½ MS	67	3.1 ± 0.12	5.9 ± 0.22
2,4-D	0.05	MS	-	-	-
2,4-D	0.1	MS	-	-	-
2,4-D	0.2	MS	56	2.5 ± 0.09	5.08 ± 0.29
2,4-D	0.05	½ MS	48	3.0 ± 0.07 a	5.05 ± 0.30 a
2,4-D	0.1	½ MS	-	-	-
2,4-D	0.2	½ MS	68	3.6 ± 0.07 a	3.7 ± 0.17 b
0	-	MS	40	4.2 ± 0.10	4.1 ± 0.22
0	-	½ MS	-	-	-

-No Response
a Values are mean ± standard error of three independent experiments each with 12 replicates. Observations were made after eight weeks of inoculation. The means have same letter within column are not significantly different from each other (P = 0.05); comparison by Duncan's multiple range test

The callus induction was also seen in cultures of *M. hexandra* when 2, 4-D and KN both were used (0.2 mg/lit. for both) with MS basal media. This combination was also used previously by Purohit and Singhvi (1998) and it was also found suitable for shoot buds and callus induction from cotyledonary nodes of *Achras sapota*. The callus obtained was globular in nature and brown in color (Table 4.5).

The production of *in vitro* callus is may be the result of the interaction of environmental conditions and the equal concentration of auxin and cytokinin of the cultured plant cells (Fig. 4.4) Similar results have been observed by Bapat and Narayanaswamy (1977) during callus induction by using mesocarp and endosperm of *Achras sapota* at this concentration of 2,4-D and IAA. No callus growth was obtained from leaf cuttings.

Table 4.5: Effect of plant growth regulators on callus growth from shoot nodes of *M. hexandra*.

Medium Composition	**Texture of Callus**	**Color**
MS+ IAA (0.5-1.0 mg/lit.) + 2,4- D (0.5 mg/lit.)	Globular	Brown
MS+ IAA (0.5-1.0 mg/lit.) + KN (1.5 mg/lit.)	Globular	Brown

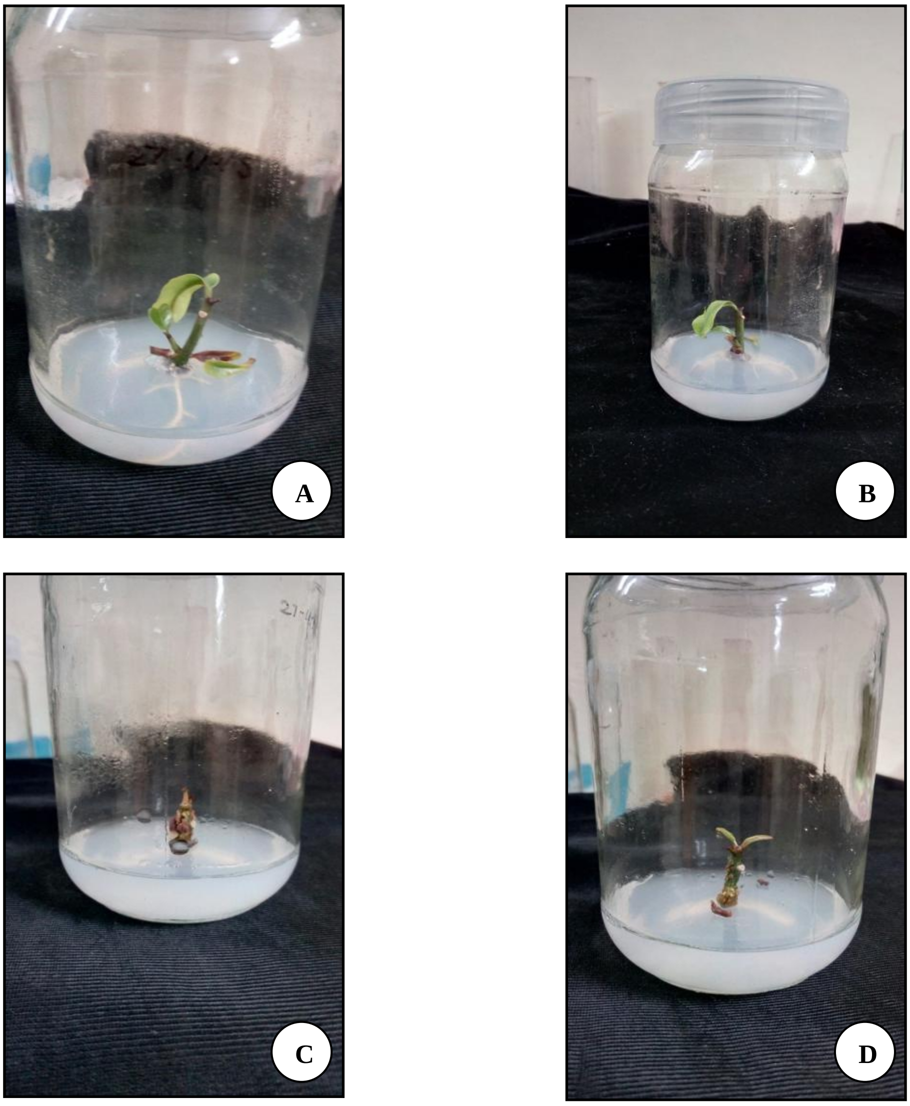

Fig. 4.4 Cultured explants on rooting media (A) IAA (0.1, 0.2 mg/lit.) + ½ MS; (B) IBA (0.2 mg/lit.) + ½ MS; (C-D) Induction of globular brown callus from shoot nodes of *M. hexandra* (C) MS+ IAA (0.5-1.0 mg/lit.) + 2,4-D (0.5 mg/lit.); (D) MS+ IAA (0.5-1.0 mg/lit.+ KN (0.5-1.5 mg/lit.).

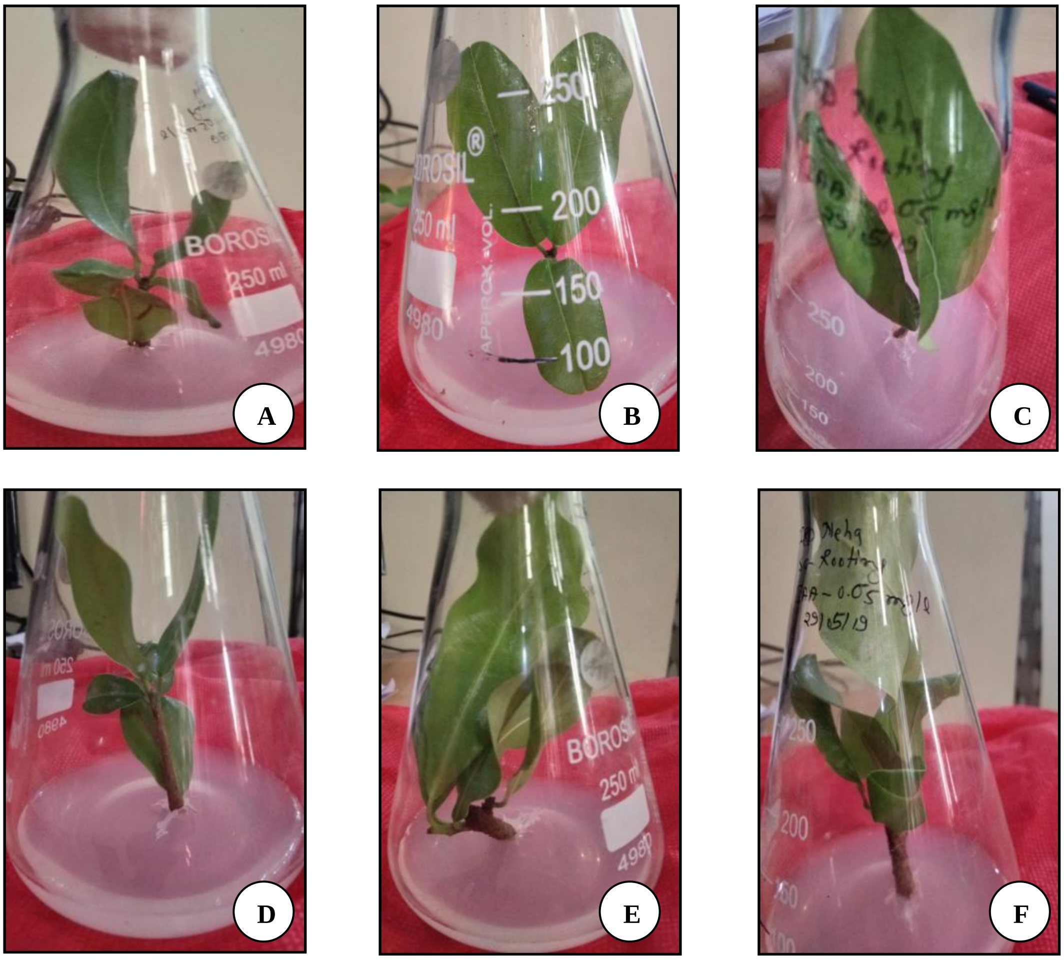

Fig. 4.5: (A-F) Development of shoot nodes of *M. hexandra* after 65 days of the inoculation.

Phytochemical Analysis

Chloroform, methanol and petroleum ether extracts of leaf and bark of *M. hexandra* were subjected to preliminary qualitative and quantitative phytochemical analysis. The various groups of phytochemical constituents found in these extracts are shown in Table 4.6. The following prominent observations were made from the phytochemical analysis.

Qualitative Estimation of Primary and Secondary Metabolites

Various biochemical tests have been performed to identify the presence of primary and secondary metabolites. The assessment was done on the basis of precipitate formation and changing of the color of the solution which was according to the nature of the identified compound. Table 4.6 represents all performed reactions and their respective responses specific to that compound.

Quantitative Estimation of Primary Metabolite

The quantitative estimation of primary metabolites specifically carbohydrates, proteins and lipids was done via respective methods. The assessment showed that plant contains rich amount of carbohydrates and proteins which makes it highly nutritive.

Carbohydrates and Starch

The ethanolic extract of leaf and bark were subjected to quantitative analysis. Both leaf and bark samples were analysis by the phenol-sulphuric acid method of Dubois et al. (1951).

Table 4.6: Estimation of the phytoconstituents present in leaf and bark extracts of *M. hexandra* by respective biochemical test

Phytoconstituent	Analytical test used	Response	Result
Carbohydrate	Fehling's Test	Formation of a brick red precipitate forms at the bottom edge of the test tube	Presence of carbohydrates
	Benedict's Test	Formation of a reddish-brown precipitate	Presence of carbohydrates
	Molisch's Test	Appearance of a violet ring at the middle of the test tube	Presence of carbohydrates
	Iodine Test	Formation of dark blue or purple colored solution	Presence of carbohydrates
Protein	Biuret Test	Formation of the purple colored solution	Presence of proteins
	Ninhidrin Test	The color of solution changes to violet	Presence of proteins
	Millon's Test	Formation of white precipitate, which turned red upon gentle heating	Presence of proteins
Lipid	Acrolein Test	The odor peculiar to burnt cooking grease was observed	Presence of lipids
	Sudan IV Test	The color of the solution changes to red- orange	Presence of lipids
Phenol	Ferric Chloride Test	The color of the solution changes to green	Presence of phenol
Alkaloids	Mayer's Test	Formation of cream colored precipitate	Presence of alkaloids.
	Wagner's reagent	Formation of red-brown colored precipitate	Presence of alkaloids
	Dragendorff's reagent	Formation of orange colored precipitate	Presence of alkaloids
	Hager's reagent	Formation of yellow colored precipitate	Presence of alkaloids
Flavonoids	Shinoda Test	Formation of pink colored scarlet	Presence of flavonoids
	Alkaline reagent test	Formation of yellow color, which turned into colorless	Presence of flavonoids
Steroids	Liebermann-Burchard's Test	The color of the solution changes to green	Presence of steroids
	Salkowaski Test	The color of the solution changes to red	Presence of steroids
Tannins	Ferric chloride test	The colors of the solution changes to green	Presence of tannins

Table 4.7 (a) Concentration of Glucose in the solution and respective optical density at 490 nm wavelength for estimation of total carbohydrate and starch content in leaf and bark extracts of *M. hexandra*

Concentration of standard sugar (Glucose) in the solution (mg/ml)	Absorbance at 490 nm wavelength
0.1	0.1298
0.2	0.1331
0.3	0.2051
0.4	0.2085
0.5	0.2148
0.6	0.2758
0.7	0.3287
0.8	0.3623
0.9	0.3921

(b) Absorbance of the carbohydrate and starch content present in leaf and bark samples of *M. hexandra*

Absorbance of the sample at 490 nm	
Carbohydrates	Leaf: 0.3431
	Bark: 0.2983
Starch	Leaf: 0.4831
	Bark: 0.3890

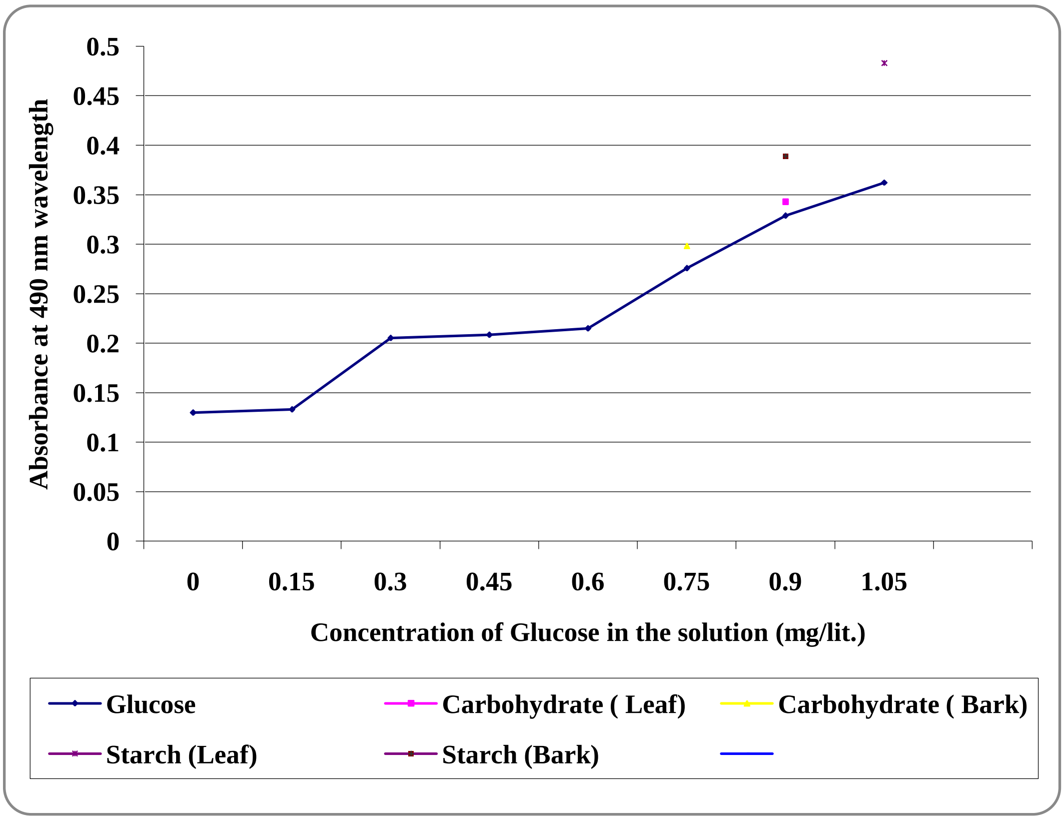

Fig. 4.6: Standard curve for total carbohydrate and starch estimation in leaf and bark extract of *M. hexandra* by Phenol –sulphuric acid method (Dubois et al, 1951).

The standard regression curve of glucose followed Beer's law (Fig. 4.6) and showed absorbance range between 0.1298 to 0.3921 at 490 nm wavelength. The absorbance of carbohydrates was measured 0.3431 for leaf and 0.2983 for bark (Table 4.7 a & b). The total concentration was measured via equation of standard line and it was found 39% and 32.35% for carbohydrates and total starch contents were found 44.95% and 38.1% of dry mass in leaf and bark respectively.

Proteins

Extraction and Quantification

The plant samples homogenised in TCA was used for quantitative estimation. Total protein content was estimated by the method of Lowry et al, (1951). The standard regression curve of Bovine serum albumin (BSA) also followed Beer's law (Fig. 4.7) and showed absorbance range between 0.1607 to 0.3645 at 750 nm wavelength. The absorbance of proteins was measured 0.1719 for leaf and 0.3441 for bark extracts. Total protein content was estimated via equation of standard line and it was found 5.03% in leaf and 32.9% in bark (Table 4.8 a & b).

Table. 4.8 (a) Concentration of BSA in solution and respective optical density at 750 nm wavelength for estimation of total protein content in leaf and bark extracts of *M. hexandra*

Concentration of BSA in the solution (mg/ml)	Absorbance at 750 nm wavelength
0.1	0.1607
0.2	0.1741
0.3	0.2027
0.4	0.2493
0.5	0.2668
0.6	0.2960
0.7	0.3031
0.8	0.3297
0.9	0.3645

(b)Absorbance of the protein content present in leaf and bark samples of *M. hexandra*

Absorbance of the sample at 750 nm wavelength	
Protein	Leaf: 0.1719 mg
	Bark: 0.3441 mg

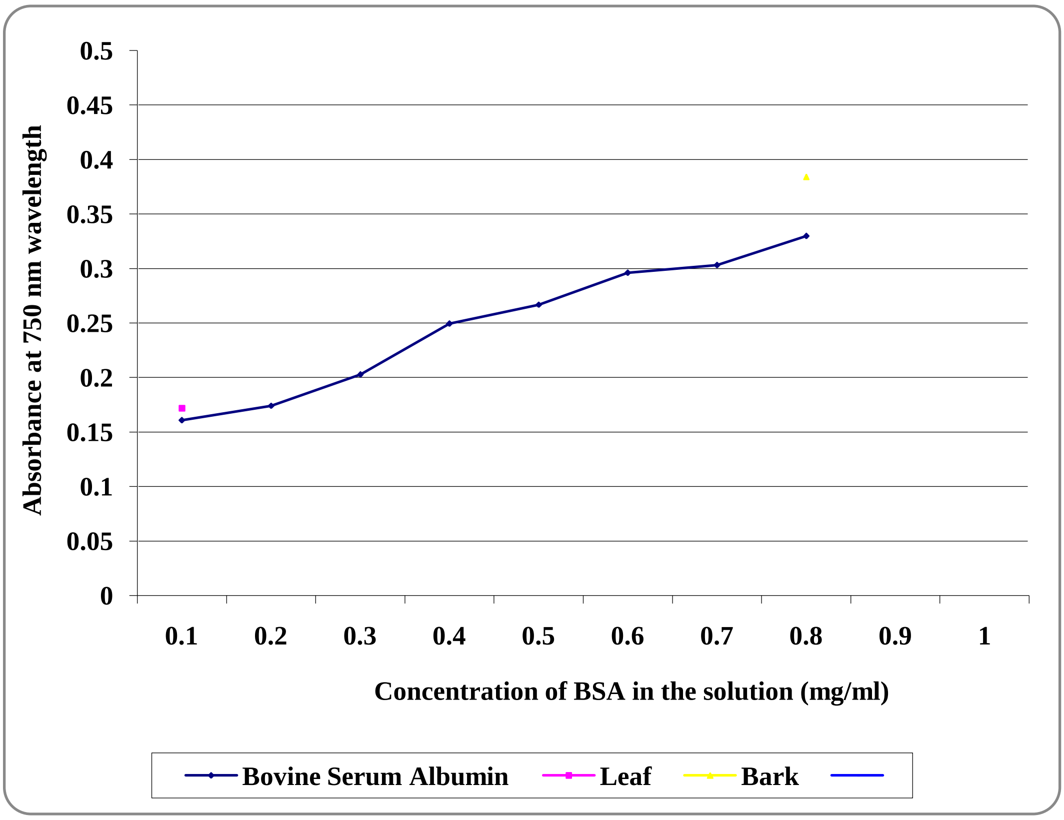

Fig. 4.7: Standard curve for total protein estimation by Folin- Lowry's method (1951).

Lipid

The chloroform and methanol extracts of dried powder of leaf and bark was centrifuged and the layer collected in pre -weight glass vials was weighted (Fig. 4.8). Table 4.9 represents measurements of three replicated experiments and the obtained mean value of concentration of lipids in leaf and bark extracts of *M. hexandra* (Table 4.9).

Table 4.9: Estimation of total lipid content in leaf and bark extracts of *M. hexandra*

Measurement	**Lipid Content in Leaf (mg/ 100 mg dry weight)**	**Lipid Content in Bark (mg/100 mg dry weight)**
First	0.08	0.07
Second	0.03	0.04
Third	0.04	0.05
Mean value	**0.05**	**0.05**

Phenol

Extraction and Quantification

Estimation of total phenol content was done by Bray and Thrope (1954) protocol in which standard curve of gallic acid was prepared (Table 4.10 a & b). The standard regression curve of Gallic acid also followed Beer's law (Fig. 4.9) and showed absorbance range between 0.1424 to 0.8311 at 750 nm wavelength. The absorbance of phenols was measured 0.2151 for leaf and 0.3661 for bark extracts. Total phenol content was found 0.52% in leaf and 1.46% of dry mass in bark.

Fig 4.8: The layer at the bottom showed lipid content in leaf and bark extracts of *M. hexandra*

Fig. 4.9: Leaf and Bark extracts of *M. hexandra* used for analysis of alkaloids via TLC

Table. 4.10 (a) Estimation of total phenol content in leaf and bark extracts of *M. hexandra*

Concentration of Gallic Acid in the solution (mg/ml)	Absorbance at 750 nm wavelength
0.1	0.1424
0.2	0.2629
0.3	0.3629
0.4	0.5325
0.5	0.6377
0.6	0.6581
0.7	0.6672
0.8	0.7031
0.9	0.8311

(b) Absorbance of the phenol content present in leaf and bark samples of *M. hexandra*

Content	Absorbance of the sample at 750 nm wave length
Phenol	Leaf: 0.2151
	Bark: 0.3661

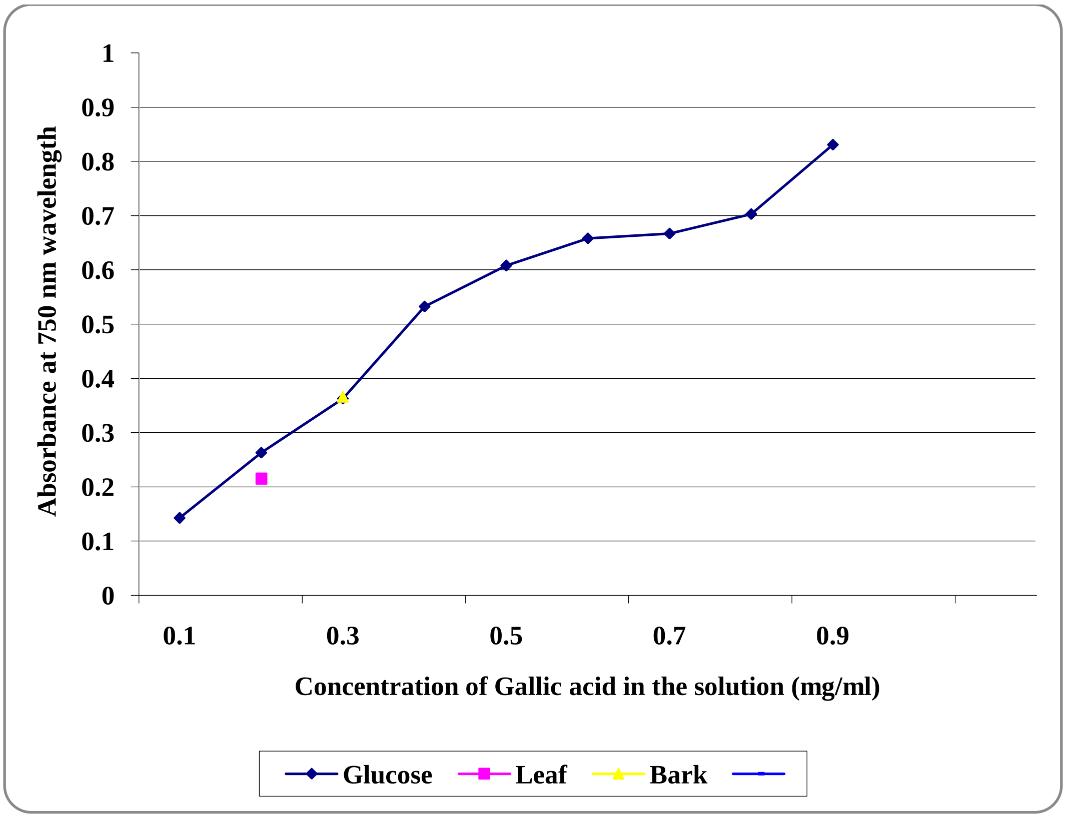

Fig. 4.10: Standard curve of estimation of phenol content in leaf and bark extracts of *M. hexandra* by Bray and Thrope method (1954).

Estimation of alkaloids, flavonoids and sterols by Thin Layer Chromatography (TLC)

The TLC studies showed that among the four solvents (petroleum ether, chloroform, ethyl acetate and methanol) used for extraction, the high polarity solvent methanol extracted higher quantity of secondary metabolites from the leaves and bark of *M. hexandra*. The corresponding R_f value of various secondary metabolites were recorded in Table 4.11.

Alkaloids

The prepared extracts (Fig. 4.10) were co-chromatographed with authentic alkaloid Colchicine as marker using TLC and the R_f value obtained was 0.50 for leaf and 0.61 for bark extracts. This value lies near the value of Colchicine which was obtained 0.63. So, it indicates the presence of alkaloids in leaf and bark extracts of *M. hexandra*.

Flavonoids

Each of the extract was co chromatographed with authentic samples of flavonoid Kaempherol using TLC and the R_f value obtained was 0.30 for leaf and 0.50 for bark extracts. This value lies near the value of Kaempherol which was obtained 0.45. It indicates the presence of alkaloids in leaf and bark extracts of *M. hexandra*. The spots also turn to more dark in response to reagent used for identification.

Sterols

Both leaf and bark extracts were co-chromatographed with β-Sitosterol using TLC. The R_f value obtained for leaf extract was 0.78 and 0.80 for bark extracts. The R_f value of β- Sitosterol was found 0.85 which indicates presence of sterols in the plant parts.

Table 4.11: Qualitative analysis of secondary metabolites present in leaf and bark extracts of *M. hexandra* by TLC

Phyto-constituent	Solvent System used	Used Ratio of Solvent	Plant Part	R_f Value (Extract)	R_f Value (Marker used)	Solution/ reagent used for identification
Alkaloids	Methanol and ammonium hydroxide	98.5:1.5	Leaf	0.50	0.63	Dragendorff's reagent
			Bark	0.61		
Flavonoids	n- Butanol, acetic acid and water	4:1:5	Leaf	0.30	0.45	1 % Aluminium chloride in methanol
			Bark	0.50		
Sterols	Hexane and ethyl acetate	1:1	Leaf	0.78	0.85	Liebermann-Burchard reagent
			Bark	0.80		

Previous TLC studies of another medicinal plants of this family also showed the presence of bioactive secondary metabolites. Vinay et al (2018) and selgal et al (2011) also observed the presence of flavonoid Quercetine in the leaf extracts of *Mimusops elengi.*

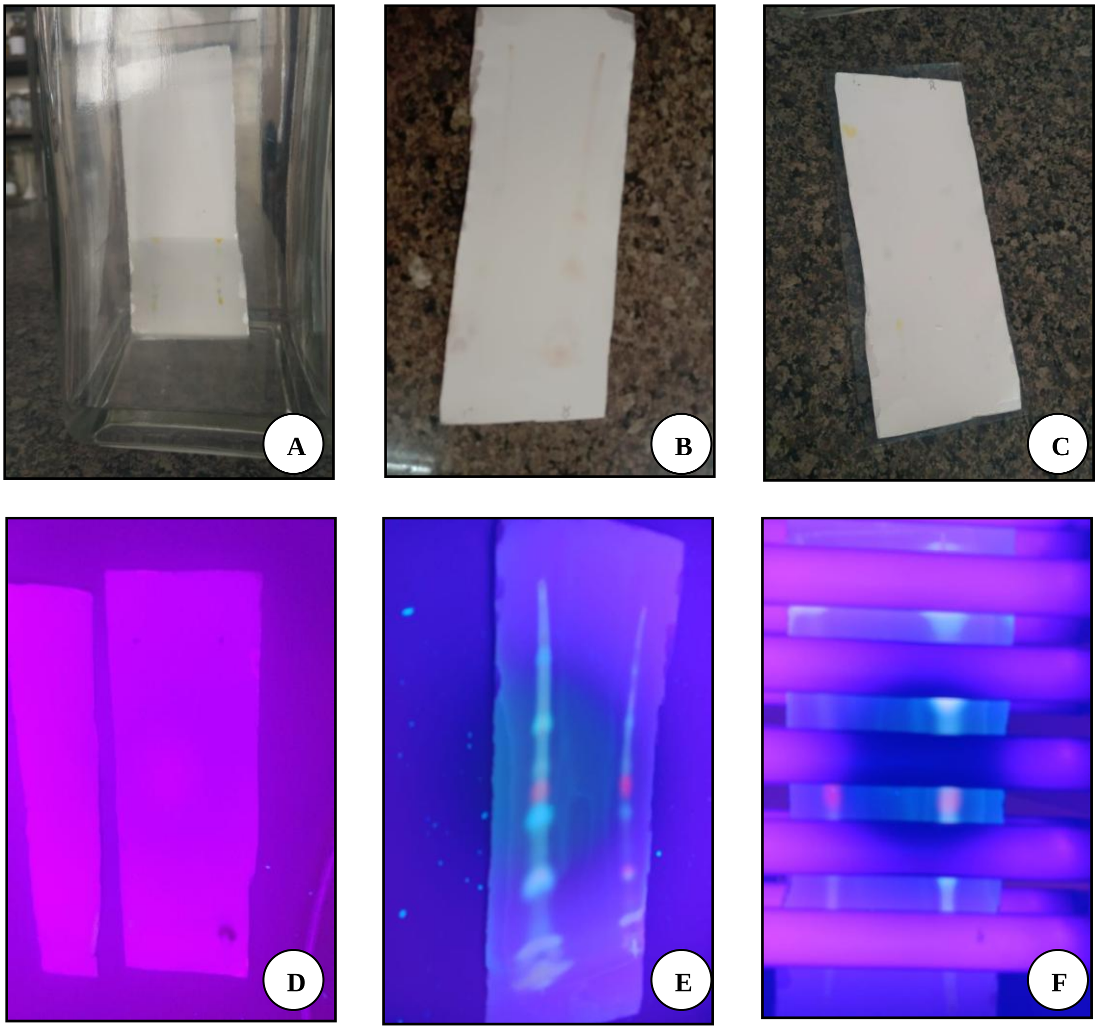

Fig. 4.11: (A-D) Thin Layer Chromatography of leaf and bark extracts of *M. hexandra.* (A) Marked silica plates in chromatographic chamber containing solvent; (B-C) Sample movement observed after chromatography; (D-F) Plates under ultraviolet light exposure and observed bands.

The results obtained from GC-MS analysis showed the presence of different phytoconstituents in the petroleum ether extract of *M. hexandra*. Total 57 and 48 compounds were identified in stem and flower extracts respectively presence of alkaloids, flavonoids, phenols, reducing sugars, steroids, tannins, terpenoids, carbohydrates etc. in petroleum ether extract of *M. hexandra* (Table 4.12 and 4.13). During the study the results obtained from stem and leaf extracts were found approximately similar so flower extracts were used for phytochemical estimation instead of leaf extracts. The results showed presence of various bioactive compounds possessing various biological properties such as antitumor, anaphylactic, encephalopathic, endocrino-protective, anti-amoebic, antidote, acidifier and acidulant property. Some identified compounds also possess inhibitory effect on the production of TNF (Tumor Necrosis factor) and NCS (Neuro-cardiogenic syncope) depressant activity, beta-galactosidase, alpha- amylase and Testosterone- 5-alpha- reductase inhibitory activity. These results showed similarity with the results obtained by Souravi et al (2015) via GC-MS analysis of stem and flower extracts of *Madhuca insignis* and by Kumari et al, 2018 via analysis of methanol extracts of leaf and flower extracts of *Maduka indica*. Azhagumurugan and Rajan, 2014 also identified the presence of similar bioactive compounds such as steric acid, esters and phenols in petroleum ether extracts of *Mimusos elengi*. The retention time was found in between 3.04- 50.65 for the stem extracts and 3.05- 49.34 for flower extracts. The results showed presence of various compounds at high peak area. Structure of the compound and the detailed GC-MS report has been given in the (appendix I and II).

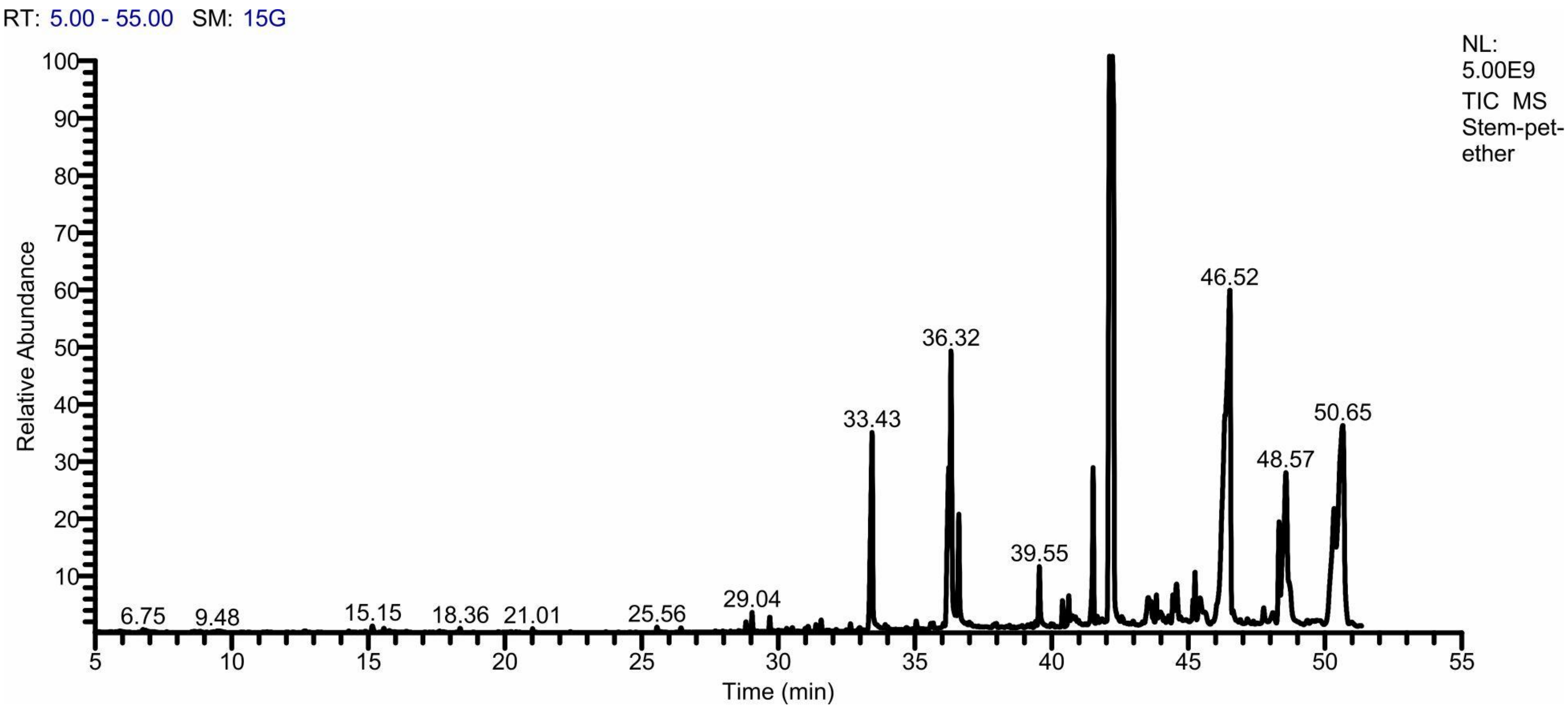

Fig.: 4.12(A) GC-MS Chromatogram of petroleum ether extracts of stem extracts of *Mimusops hexandra*.

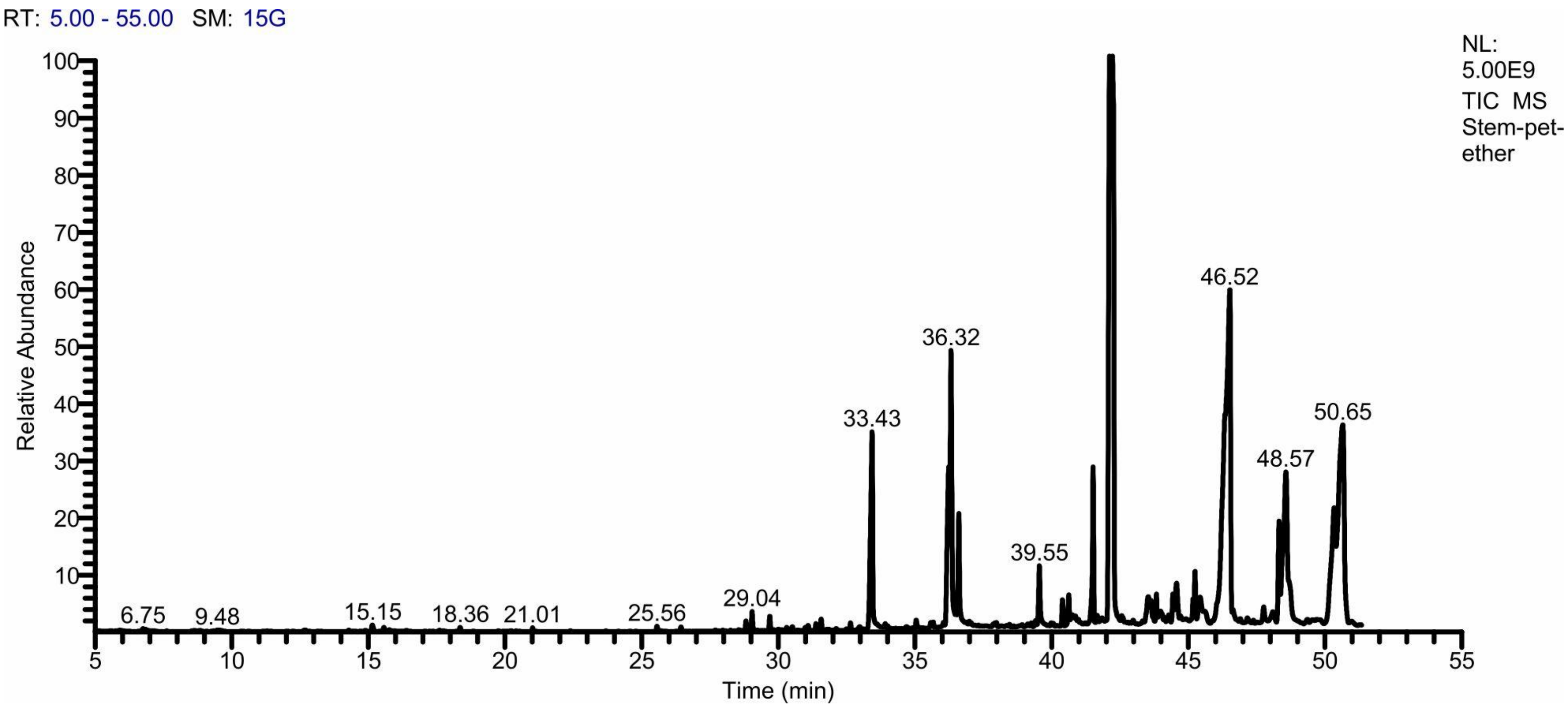

Fig.: 4.12 (B) GC-MS Chromatogram of petroleum ether extracts of flower extracts of *Mimusops hexandra.*

Table 4.12: Phytoconstituents identified via GC-MS analysis of stem extracts of *M. hexandra*

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological Properties**
3.04	9-Methyl-1-Decene	$C_{11}H_{22}$	154	6.42	Alkene	Methyl donor and Methyl Guanidine inhibitor
3.04	2-Ethyl oxetane	$C_5H_{10}O$	86	6.42	Branched chain alkane	No activity reported
3.04	3-Methylpentane	C_6H_{14}	86	6.42	Branched chain alkane	No activity reported
3.04	n-Hexane	C_6H_{14}	86	6.42	Alkane	Anaphylactic, antitumor, inhibit the production of TNF (Tumor Necrosis factor) and NCS (Neuro-cardiogenic syncope) depressant
3.34	2-Ethyl-4-methylpentanol	$C_8H_{18}O$	130	2.63	Polyols	No activity reported
33.43	n- Hexadecanoic acid	$C_{16}H_{32}OO_2$	256	3.90	Saturated fatty acid	Acidifier, urinary acidulant, anaphylactic and antitumor
36.24	Cis-9, Cis-1,2-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	2.96	Polyunsaturated fatty acid	Acidulant
36.24	Oxacyclononadec-10-en-2-one	$C_{18}H_{32}O_2$	280	2.96	Cyclic ether	Encephalopathic and endocrinoprotective

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological Properties**
36.24	Linoelaidic acid	$C_{18}H_{32}O_2$	282	2.96	Fatty acid	Urinary -acidulant
36.32	11-Octadecenoic acid	$C_{18}H_{34}O_2$	282	4.29	Ester	Acidifier
36.32	Oleic Acid	$C_{18}H_{34}O_2$	282	4.29	Fatty acid	Acidifier
39.55	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.94	Fatty acid	Inhibit production of uric acid
39.55	2,4-Bis (1-phenyl ethyl) phenol	$C_{22}H_{22}O$	302	0.94	Phenol	No activity reported
39.55	2-Benzoyl guaiazulene	$C_{22}H_{22}O$	302	0.94	Ketone	No activity reported
39.55	Palustric acid	$C_{20}H_{30}O_2$	302	0.94	Diterpenoid	Urine- acidifier
39.55	Phenyl methanone	$C_{22}H_{22}O$	302	0.94	Benzophenone	No activity reported
39.55	16-alpha-hydroxyandrostenedione	$C_{19}H_{26}O_3$	302	0.94	Ketosteroid	No activity reported
41.52	Phthalic acid	$C_{24}H_{38}O_4$	390	1.94	Benzoic acid	No activity reported
41.52	Di isooctyl phthalate	$C_{24}H_{38}O_4$	390	1.94	Benzoic acid ester	Antidote (Diazepam and Digitoxin), coronary dilator and diaphoretic
42.15	Bis (2 ethyl hexyl) phthalate	$C_{24}H_{38}O_4$	390	24.93	Benzoic acid ester	No activity reported
42.15	Cholesta-5, 24-dien-3. beta-ol	$C_{27}H_{44}O$		24.93	Steroid	Anti-amoebic and beta-galactosidase inhibitor

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological Properties**
42.15	Stigmasterol	$C_{29}H_{48}O$	412	24.93	Steroid	No activity reported
42.15	(22E) 4,4-Dimethyl cholesta 22, 24-dien-6-ol	$C_{29}H_{48}O$	412	24.93	Steroid	Anticancer and antidote (Emetine)
43.64	24,25-Dihydroxy cholecalciferol	$C_{27}H_{44}O_3$	416	0.36	Steroid	No activity reported
43.64	3-Ethyl-5 (2'ethylbutyl) octadecane	$C_{26}H_{54}O_3$	366	0.36	Ester	No activity reported
43.64	3- (octadecyloxy) propyl ester	$C_{39}H_{76}O_3$	592	0.36	Ester	No activity reported
43.83	2- Nonadecanone	$C_{19}H_{38}O$	282	0.31	Ketone	No activity reported
43.83	10- Octadecenal	$C_{18}H_{34}O$	266	0.31	Fatty aldehyde	No activity reported
43.83	2- Pentacosanone	$C_{25}H_{50}O$	366	0.31	Ketone	No activity reported
43.83	13-Methyl penta dec-14-ene1,13diol	$C_{16}H_{32}O_2$	256	0.31	Triacylglycerol	Decalcifier and Decongestant
43.99	Lupeol	$C_{30}H_{50}O$	426	0.39	Triterpenoid	No activity reported
43.99	Methyl 2,3-bis-O (trimethyl silyl)	$C_{17}H_{37}BO_6Si_2$	404	0.39	Trimethyl silyl ester	Anticancer and antidote (Organo-P)
44.44	Tetracosanoic acid	$C_{24}H_{48}O_2$	368	0.35	Saturated fatty acid	Acidulant
44.44	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.35	Saturated fatty acid	Antidepressant activity

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological Properties**
44.44	Docosanoic acid	$C_{22}H_{44}O_2$	340	0.35	Saturated fatty acid	Acidulant and acidifier
44.57	Alpha-amyrin	$C_{30}H_{50}O$	426	0.91	Triterpenoid	No activity reported
45.18	24-Noroleana-3,12-diene	$C_{29}H_{46}$	394	0.31	Triterpenoid	No activity reported
45.18	Squalene	$C_{30}H_{50}$	410	0.31	Triterpenoid	No activity reported
45.25	2,9-octadecenyloxyole	$C_{20}H_{40}O_2$	312	0.54	Phenolic glycosides	No activity reported
45.25	Z-14-Octadecen-1-ol acetate	$C_{20}H_{38}O_2$	310	0.54	Benzyl oxy carbonyls	Increases bioavailability of zinc
45.25	Hexacosanal	$C_{26}H_{52}O$	380	0.54	Fatty aldehyde	No activity reported
45.25	Tricosanal	$C_{23}H_{46}O$	338	0.54	Fatty aldehyde	No activity reported
45.25	Octacosanal	$C_{28}H_{56}O$	408	0.54	Fatty aldehyde	No activity reported
45.43	Lup20(29) en-3-one	$C_{30}H_{48}O$	424	0.80	Triterpenoid	Encephalopathic, endocrino-protective and entero-stimulant
45.43	13,27-Cycloursan-3-one	$C_{30}H_{48}O$	424	0.80	Fatty alcohol	No activity reported
s45.59	Olean-12-en-3-one	$C_{30}H_{48}O$	424	0.33	Triterpenoid	Endoanesthetic and endocrinoactive

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological Properties**
45.59	Alpha- Sitosterol	$C_{29}H_{50}O$	414	0.33	Stigmastanes	Alpha- amaylase inhibitor and Testosterone- 5-alpha-reductase inhibitor
45.49	Beta-Sitosterol	$C_{29}H_{50}O$	414	0.33	Stigmastanes	Antiamoebic
45.49	7,8-Epoxylanostan-11-ol	$C_{32}H_{54}O_4$	502	0.33	Pyrenes	No activity reported.
45.49	Ethyl isoallocholate	$C_{26}H_{44}O_5$	436	0.33	Gamma-Keto acid	No activity reported
46.52	Betulinaldehyde	$C_{30}H_{48}O_2$	440	17.35	Aldehyde	No activity reported
46.63	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.31	Fatty acid	No activity reported
47.76	Cyclohexanol	$C_{30}H_{52}O$	428	0.32	Cyclohexenol	No activity reported
47.76	1,6,10,14,18,22-Tetra cosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl	$C_{30}H_{50}O$	426	0.32	Retenoid	No activity reported
48.10	13,27-Cycloursan 3-ol acetate	$C_{32}H_{52}O_2$	468	0.39	Benzyloxycarbonyls	No activity reported
50.33	Olean 12-en-3-ol acetate	$C_{32}H_{52}O_2$	468	4.48	Retenoid	Endoanesthetic and enterorelaxant
50.65	Lupeol trifluoroacetate	$C_{32}H_{49}F_3O_2$	522	9.78	Triterpenoids	No activity reported

*Source: The Human Metabolome Database (online database)

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases (online database).

Table 4.13 Phytoconstituents identified via GC-MS analysis of flower extracts of *M. hexandra.*

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological properties**
3.05	2-Ethyloxetane	$C_5H_{10}O$	86	10.63	Branched chain alkene	No activity reported
3.05	n-Hexane	$C_5H_{10}O$	86	10.63	Alkane	Anaphylactic, Antitumor, Inhibit production of TNF (Tumor necrosis factor), and NCS depressant
3.05	2, 3- Dimethyl pentane	C_7H_{16}	100	10.63	Alkane	No activity reported
32.97	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	0.53	Fatty acid	Acidifier, Anaphylactic and antitumor
33.61	Methyl (2E)-2-hexadecenoate	$C_{17}H_{32}O_2$	268	0.75	Fatty acid ester	Anticancer and antidote (Emetine)
36.19	9,12- Octadecadienoic acid	$C_{18}H_{32}O_2$	280	0.75	Thio- carboxylic acid	Acidifier
36.19	Linoelaidic acid	$C_{18}H_{32}O_2$	280	4.13	Fatty acyle	Acidulant
36.29	Cis Vaccenic acid	$C_{18}H_{34}O_2$	282	7.70	Fatty acid	Urinary acidifier
36.58	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.00	Fatty acid	Acidulant
39.54	Eicosanoic acid	$C_{20}H_{40}O_2$	312	3.02	Saturated fatty acid	Acidulant

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological properties**
41.42	2,4- Bis (1-phenyl ethyl) phenol	$C_{22}H_{22}O$	302	0.58	Methoxyphenols	No activity reported
41.42	2- Benzoyl guaiazulene	$C_{22}H_{22}O$	302	0.58	Ketone	No activity reported
41.42	Palustric acid	$C_{20}H_{30}O_2$	302	0.58	Diterpenoid	Acidifier
41.42	6- Hydroxy androst-4-ene-3,17-dione	$C_{19}H_{26}O_3$	302	0.58	Androgens	Testosterone hydroxylase inducer
41.42	Phenyl methanone	$C_{22}H_{22}O$	302	0.58	Benzophenone	No activity reported
42.11	Bis (6-methylheptyl) phthalate	$C_{24}H_{38}O_4$	390	16.82	Benzoic acid ester	No activity reported
42.11	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	16.82	Tricarboxylic acid	Acidulant
42.11	Phthalic acid	$C_{24}H_{38}O_4$	390	16.82	Benzoic acid	Acidifier
42.26	Docosanoic acid	$C_{22}H_{44}O_2$	340	2.89	Saturated fatty acid	No activity reported
42.26	Hexadecanoic acid	$C_{35}H_{68}O_5$	568	0.40	Saturated fatty acid	Antitumor
42.26	Estra1-3,5 (10) trien-17-ol	$C_{18}H_{24}O$,	256	0.40	Acridones	Anti-carcinogenic
42.96	17-Pentatriacontene	$C_{35}H_{70}$	490	0.47	Glycerophosphocholines	No activity reported
42.96	Heptacosane	$C_{27}H_{56}$	380	0.47	Beta-Diketones	No activity reported
42.96	Octen triacontyl penta fluoro-	$C_{41}H_{77}F_5O$	696	0.47	Fatty Alcohols	No activity reported

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological properties**
	propionate	2				
42.96	Tetra pentacontane,	$C_{54}H_{108}Br_2$	914	0.47	Ergosterol	No activity reported
43.64	Nonacosane	$C_{29}H_{60}$	408,	0.77	Acyclic alkanes	No activity reported
43.64	Tetratetracontane	$C_{44}H_{90}$	618	0.77	Hydrolysable Tannins	No activity reported
43.64	Octacosane	$C_{28}H_{58}$	394	0.77	Acyclic alkanes	No activity reported
43.64	Pentacosane	$C_{25}H_{52}$	394	0.77	Acyclic Ketones	No activity reported
43.82	2-Heptadecanone	$C_{17}H_{34}O$	254	0.92	Acyclic Ketones	No activity reported
43.82	2-Nonadecanone	$C_{19}H_{38}O$	282	0.92	Acyclic Ketones	No activity reported
43.82	2-Pentacosanone	$C_{25}H_{50}O$	366	0.92	Acyclic Ketones	No activity reported
43.82	2-Pentadecanone	$C_{15}H_{30}O$	226	0.92	Acyclic Ketones	Anti-carcinogenic
43.99	13-Methylheptacosane	$C_{28}H_{58}$	394	1.69	Acyclic Ketones	Anti- depressant
43.99	Hentriacontane	$C_{31}H_{64}$	436	1.69	Acyclic alkanes	No activity reported
44.42	Glycidyl oleate	$C_{21}H_{38}O_3$	338	0.43	Fatty acid esters	No activity reported
44.42	10-Methoxycoryn-18-en-17acetate	$C_{22}H_{28}N_2O_3$	368	0.43	Benzyloxycarbonyls	Endorphinogenic, encephalopathic and endocrinoprotective

RT	Name of the compound	Molecular Formula	Molecular Weight (gm/mole)	Peak Area %	Compound Nature*	Pharmacological properties**
44.42	Dodecyl cis9,10-epoxy octadecanoate	$C_{30}H_{58}O_3$	466	0.43	Phenol	No activity reported
45.16	Squalene	$C_{30}H_{50}$	410	0.53	Triterpenoides	Squalene monooxygenase inhibitor
45.16	3-Ethy l5 (2'ethylbutyl) Octadecane	$C_{26}H_{54}$	366	0.53	1,3-diacylglycerols	Anticancer, Antidote (Lead and Lobelia), Antioxidant, Increase proliferation of T-lymphocytes
46.32	Stearic acid	$C_{39}H_{78}O_3$	594	0.52	Fatty acid	Diuretic
46.46	17-Pentatriacontene	$C_{35}H_{70}$	490	0.44	Glycero-phospho cholines	No activity reported
46.46	(6Z)5-Methyl 6-henicosen-11-one	$C_{22}H_{42}O$	322	0.44	Gamma butyrolactones	Increases zinc bioavailability and methyl donor
46.46	2-Octadecoxyethanol	$C_{20}H_{42}O_2$	314	0.44	Glycols	No activity reported
47.75	Oxirane	$C_{30}H_{50}O$	426	0.96	Epoxides	No activity reported
48.68	Tocopherol	$C_{28}H_{48}O_2$	416	1.47	Methylated phenol	No activity reported
49.34	Ethyl isoallocholate	$C_{26}H_{44}O_5$	436	0.45	Fatty acid ester	No activity reported

*Source: The Human Metabolome Database (online database)

**Source: Dr. Duke’s Phytochemical and Ethnobotanical Databases (online database)

Discussion

Micropropagation has been observed as efficient way in this study for mass multiplication of *Mimusops hexandra* which can be used to overcome from the overexploitation of this medicinal plant in environment. Adequate availability of the plant may provide sustainable financial and medicinal support to local inhabitants. Plant showed approximately the same culture requirements as another medicinal plants cultured previously from this family. The semisolid nature of Murashige and Skoog's media facilitates explants to establish itself in media and also continuous uptake of nutrients. The composition of culture media was found appropriate and contain approximately all the required nutrients required for cultivation of *M. hexandra*. Quiescent meristems of nodal segments respond to the culture media in a very short time period. Sprouting in explants in just after 5-6 days showed the adequate and quick uptake of media contents by shoot nodes. It also showed that normal environmental conditions used for *in vitro* cultures were suitable for the plant growth, there was no need to customize especially environmental factors that affect the growth of this plant. Disinfectants were capable to sterilize the explant from the surface with no toxic and adverse effects on the explant tissues. It takes very short time period and can be easily removed by washing with distilled water after the sterilization. Concentration of gelling agent (agar) found suitable to provide adequate viscosity to the medium. Slightly acidic medium (pH=6) makes easy for the plant to propagate and develop shoot buds.

The low concentrations of growth regulators such ad Benzyl Adenine (BA) and Kinetin (KN) were found very effective for shoot induction in this plant. Other hormones such as 2,4-Dichlorophenoxy acetic acid (2,4-D), Indole acetic acid (IAA) and Naphthalene acetic acid (NAA) were also played an efficient role as their results were slightly low from the maximum outputs. The cultures containing Thidiazuron (TDZ) did not show any growth, may be TDZ is not effective to initiate growth in the explants of *M. hexandra*. Gibberellic acid (GA_3) was also found efficient for establishment and elongation in cultures. The obtained results highly resemble with the results obtained by Bhore and Preveena (2011) during *in vitro* cultivation of *Mimusops elengi* and quite different from the results of cultivation of *Madhuca longifilia* where higher concentrations of the hormones were used by Bansal and Chibbar (2000). It was also observed that coordinate function of hormones used in combination leads to rapid proliferation in the shoot nodes. Quiescent meristems of nodal segments develop into complete plantlets during *in vitro* multiplication of *M. hexandra.* It promotes rapid increase in shoot length and also produced some signs of calls induction. Similar combinations were also used for cultivation of another plant of this family, *Synsepalum dulcificum* using different concentrations and combinations of auxins and cytokinins by Ogunsola and Ilori (2008). Micropropagated plantlets were true to type and showed the same agronomic characteristics as the wild population. No morphological variations between the micropropagated plants and the wild plants were observed. Developed roots were also capable to absorb the nutrients from the media and looks like it has similar working physiology like the roots of wild plants. Branching and increase in length was also occurred in roots. IAA at low concentrations (0.1- 0.2 mg/lit.) with half strength culture media found very effective in rooting instead of full strength culture media.

Sub-culturing of explants on fresh media having similar composition also induce the growth of the explants, but the chances of contamination were also found increased during the transfer of the explants. Sub-culturing of explants to half- strength culture media facilitate the plants to survive in comparatively low osmotic stress conditions which is very necessary before the transfer of the plants into the soil, because the soil has very low osmotic stress in comparison to Murashige and Skoog’s (Murashige and Skoog, 1962) media. It helps the plants to adapt itself for normal acclimatization in the soil.

Callus induction was found as a delayed and slow process in explants of *M. hexandra.* Only few globules like structures were developed on the shoot nodes after the time period of sixty days. The color of the callus was brown and fragile in nature which resemble with the callus developed from the explants of *M. elengi* (Gami et al, 2010). It also showed similar characteristics with callus developed from *Pouteria lucuma* explants by Jordan and Oyanedel (1992). It showed lack of suitable adequate concentration of hormones and moderation in other parameters for efficient formation of callus from the explants. It showed the possibility of further studies regarding the callus development from shoot nodes and leaves of *M. hexandra*.

Leaf cuttings of *M. hexandra* does not showed any growth in response to any concentration and combination of growth regulators. Even no effect of culture medium was observed on leaf cuttings during the complete study. Although leaves were survived for more than forty-five days, but no reaction and response was observed. It may be due to lack of quiescent meristematic tissue in the leaf cuttings. It was supposed during the inoculation that mid-rib or mid-conductive vein may develop in the culture media, but it did not show any growth. Approximately all auxins and cytokinins which are used basically in the *in vitro* cultures, have been tried for callus development from leaves in this study. Even as reviewed literature no other plant of this family has been propagated via leaf cuttings. It suggests that leaves may be not suitable explant for the cultivation of this plant or it needs a suitable micropropagation protocol for cultivation. This study may be helpful to fill the knowledge gap regarding *in vitro* cultivation of *M. hexandra* and may also provide new dimensions to pursue further research studies. Though very much encouraging growth were observed in *in vitro* conditions so nodal segment, there was very less callus formation was observed and developed plant have not much difference in term of their acclimatizing conditions as these developed plant also have a cuticle layer to protect a tissue from external environment that is lacking in plantlets developed from callus. That's why hardening of such developed plantlets need not feld.

Statistical analysis facilitates the analysis of growth obtained in the cultures. It was fousnd very helpful to measure the difference among the growth of different cultures. It was also helpful to differentiate between the cultures having same hormonal concentrations. Duncan's Multiple Range Test (DMRT) (Duncan, 1955) and alphabetic notation clearly differentiates between the significantly similar and different cultures. The data analysis was very convenient to analyse that how much an explant grows in response to particular hormonal concentration. It provides a mathematical explanation of effects of various growth regulators on the explant.

Phytochemical studies have been also found helpful to analyze biochemical composition of this medicinal plant, which is very necessary for further analytical researches for drug discovery. Extracts of leaf and bark showed the presence of various phytoconstituents such as ccarbohydrates (39%) and (32.35%) and proteins (5.03%) and (32.9%) were found in leaf and bark extracts respectively which explains about its high nutritional value. Low lipid content (0.05%) was found for both extracts. Total phenol content was found 0.52% and 1.46% in leaf and bark respectively. The standard protocols used for estimation of different compound were successfully performed and found suitable to analyze the presence and quantity of respective compound. It showed approximately same concentration of carbohydrates and proteins found in another species of this family such as *Manilkara zapota* (Shafii et al, 2017). The qualitative analysis of secondary metabolites such as alkaloids, flavonoids, phenols and sterols showed their presence in the leaf and bark extracts of *M. hexandra* same as already studied in another plants such as *Argania spinosa* (Mansour et al, 2018); *Diploknema butyracea* (Rashmi and Tyagi, 2015) and *Madhuca longifolia* (Kamal, 2014) etc.

The results obtained from spectrophotometry were beneficial to analyze the net content of carbohydrates, proteins and phenols in both leaf and bark extracts. All the solutions followed the beer-lambert law and expressed via the equation of standard line, which was helpful to estimate the net content of the compounds. The colorimetric methods found suitable for estimation of both carbohydrates and proteins. The Thin Layer Chromatography (TLC) of leaf and bark extracts was found very efficient to analyze the presence of secondary metabolites. The solvent system used for particular compound found suitable and gave expected results. The obtained R_f values of alkaloids, flavonoids and steroids indicates the presence of Colchicine, Kaempherol and β- sitosterol like compounds which can be used in formation of therapeutic drugs. Presence of these compounds was also observed in various extracts of *M. elengi by* Kalaiselvi et al, (2016) and *S. dulcificum* by Chinelo et al, (2014). Previous studies on *M. hexandra* also showed the presence of Saponines which has great therapeutic potential and currently used widely in formation of synthetic drugs (Eskander et al, 2013). GC- MS found very helpful in specific identification of the compounds. Various bioactive compounds such as 3-Ethy l5 (2'ethylbutyl) octadecane, n-hexane, stigma sterol, tannins and alpha & beta- sitosterol etc. have been identified in stem and flower extracts which explains about its wide therapeutic applications. The bioactive compounds usually found at high peak area of the GC-MS graph. Net quantity of these secondary metabolites can be further analyzed by High Performance Liquid Chromatography (HPLC). It indicates about wide opportunity to study about the qualitative and quantitative analysis of phytoconstituents present in *M. hexandra*.

Chapter 5
Summary

CHAPTER 5: SUMMARY

Medicinal plants have great importance in the environment due to their therapeutic potential. *Mimusops hexandra* belongs to family Sapotaceae and provide nutritional, medicinal and ethno- botanical support to local inhabitants of central India.

In vitro micropropagation protocol has been developed to cultivate *M. hexandra* by using Murashige and Skoog's medium composition with adequate culture conditions and different concentrations & combinations of growth hormones. It was observed that stem nodes are more suitable explant instead of leaf cuttings. Cytokinin such as Benzyl Adenine (0.50 mg/lit.) with Kinetin (0.1 mg/lit.) gave best results in multiple shoot induction as the average number of shoots per explant were obtained 5.75 ± 0.05 and the mean length of shoots were obtained approximately 12.5 ± 0.06 mm. Increased response of explants was observed when the growth regulators were used in combination with culture medium with approximately 85 % survival rate of the cultures. IAA (0.1 and 0.2 mg/lit) with half strength Murashige and Skoog's basal media shows best rooting rate in the cultures. Root length is found approximately 10.2 ± 0.8 mm and maximum number of roots is found 5.4 ± 0.08 per explant in the cultures. The callus induction was also seen in cultures by using 2, 4-D (0.5 mg/lit.) or KN (1.5 mg/lit.) with

IAA (0.5-1.0 mg/lit.) in culture media. The micro propagated plantlets were true to type and showed the same agronomic characteristics. This protocol of *in vitro* mass propagation may suggest a way for mass propagation and conservation of *M. hexandra*. It may be help in saving the labor and cost over raising the plantlets through traditional propagation practices. It can be further use to study another issues involving *in vitro* studies and other biotechnological issues.

The findings of phytochemical studies provided evidence that the stem bark and leaf of the plant possessed bio active compounds. Rich contents of carbohydrates (39%) and (32.35%) and proteins (5.03%) and (32.9%) were found in leaf and bark extracts respectively which explains about its high nutritional value. Low lipid content (0.05%) was found for both extracts. Total phenol content was found 0.52% and 1.46% in leaf and bark respectively. Presence of flavonoids, alkaloids and sterols were identified via thin layer chromatography. Various standard analytical tests were also showed the presence of both primary and secondary metabolites in leaf and bark extracts of *M. hexandra*. This study may be helpful to aid some information in further qualitative and quantitative analysis of phytoconstituents of *M. hexandra* and may be useful in discovery of novel drugs.

In conclusion, the developed protocol of *in vitro* micropropagation of *M. hexandra* assures a way for conservation and mass propagation of *M.*

hexandra. It may be helpful to overcome with the problems and issues observed during traditional propagation methods. It may also help in saving the labor and cost over raising the plantlets by another means. This study can be used as base study for further *in vitro* researches related to *M. hexandra* such as callus induction and organogenesis. The idea about appropriate concentration of growth regulators and physical conditions may be used for cultivation of another medicinal plants of this family. The results of micropropagation may be used for further analytical researches related to its morphology and physiological characteristics. It can be also used for crop improvement of this valuable plant. This study may also provide basic idea about primary and secondary metabolites present in leaf and bark extracts of *M. hexandra*. It may arise new quarries regarding the chemical composition of this plant. It also assures the possibility of occurrence of compounds which can be used in pharmaceutical industries. It may be also useful for further researches about the phytochemistry of this plant such as chemical characterization and structure elucidation of the compounds. This study may be useful to increase general knowledge of local inhabitants and researchers regarding the pharmaceutical and ethno-botanical applications of *M. hexandra* by which the plant can be utilized for various medicinal and economical means and definitely about the significance, importance and need of conservation of this novel medicinal plant in environment.

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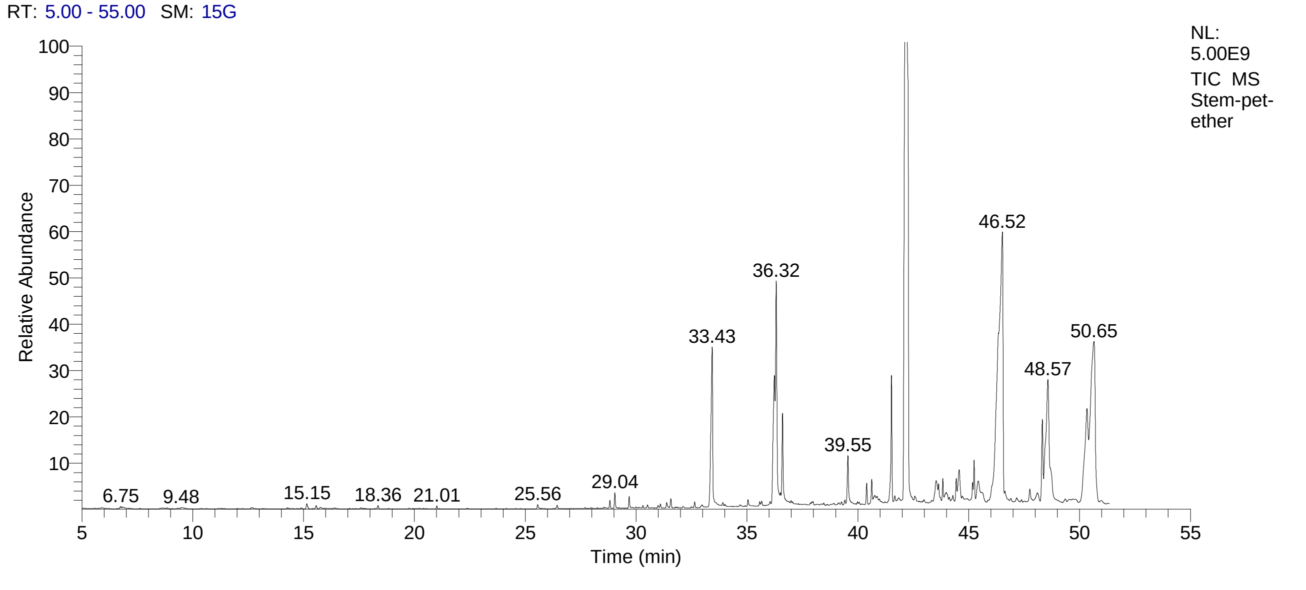
Appendix I

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

Instrument Model:	TSQ 8000	Instrument Serial Number:	1711009
Data File:	Stem-pet-ether	Original Data Path:	D:\Xcalibur\2019\May\Pest+Pyr+Safr\14-05-2019-scan\Data
Sample Type:	Unknown	Sample ID:	Stem pet ether
Sample Name:	Stem pet ether	Acquisition Date:	05/15/19 09:50:09 AM
Run Time(min):	48.34	Vial:	TrayHolder 2:Slot1:23
Scans:	14212	Instrument Name:CEGTH/INS/C/003	TSQ 8000



RT	Peak Area	Peak Height	Area %
3.04	16866058639.12	1539190190.68	6.42
3.25	6706120087.22	1380927673.90	2.55
3.34	6902048144.87	1327838409.93	2.63
3.43	5638545021.31	1230523930.80	2.15
33.43	10257301386.17	1730229132.53	3.90
36.24	7769255098.71	1407156949.49	2.96
36.32	11272748200.68	2424668518.85	4.29
36.60	4211685971.67	996997665.81	1.60
39.55	2468498772.00	533265773.85	0.94
40.63	963998983.91	273057792.61	0.37
41.52	5092409641.89	1385203594.10	1.94
42.15	65519083331.82	5766539243.72	24.93
43.53	1869600077.18	240557423.38	0.71
43.64	933754390.52	206523589.96	0.36
43.83	813694429.63	262968028.68	0.31
43.99	1027664766.41	112651700.23	0.39
44.44	911193390.39	260868677.01	0.35
44.57	2388439824.73	353552999.58	0.91
45.18	816109153.05	215227349.37	0.31
45.25	1418743616.93	454921047.99	0.54
45.43	2112079306.63	229696021.48	0.80
45.59	868874872.20	106335851.92	0.33
46.52	45587144146.88	2916889547.90	17.35
46.63	813715083.54	117587660.82	0.31
47.76	845851789.06	142263805.26	0.32
48.10	1026882436.55	101149129.62	0.39
48.32	3809078510.55	896006033.11	1.45
48.57	16398500024.88	1325391905.87	6.24
50.33	11777327712.13	1022876671.88	4.48
50.65	25689542174.61	1750778505.31	9.78

There is no signature data to report.

Library Search Report

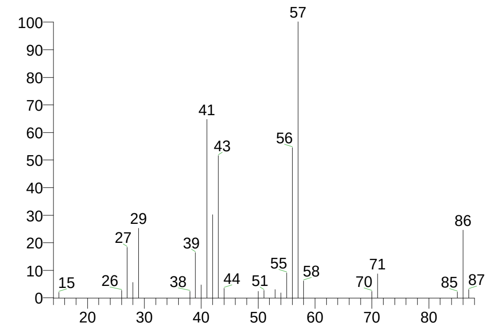
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

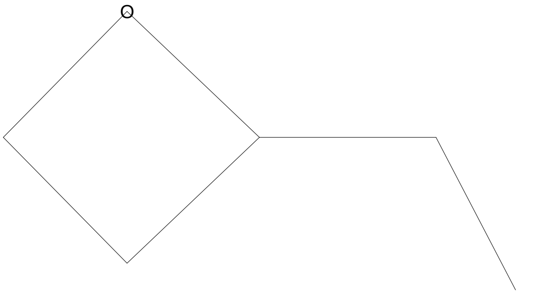
RT	Compound Name	Area	Area %
3.04	1-Decene, 9-methyl-	16866058639.12	6.42
3.04	2-Ethyl-oxetane	16866058639.12	6.42
3.04	Pentane, 3-methyl-	16866058639.12	6.42
3.04	n-Hexane	16866058639.12	6.42
3.04	n-Hexane	16866058639.12	6.42

Hit Spectrum	Compound Structure

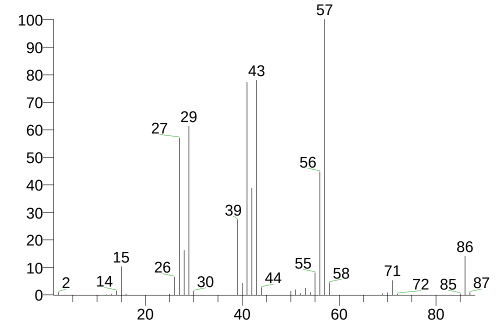
SI 767, RSI 877, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



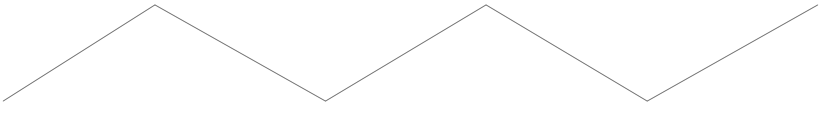
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N



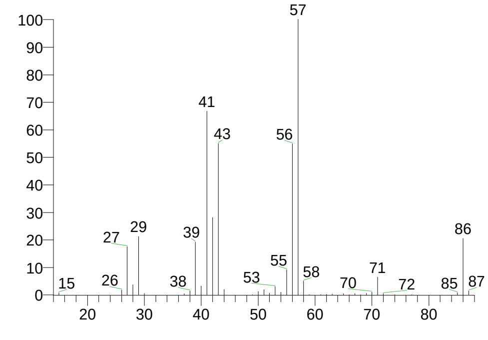
SI 767, RSI 828, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



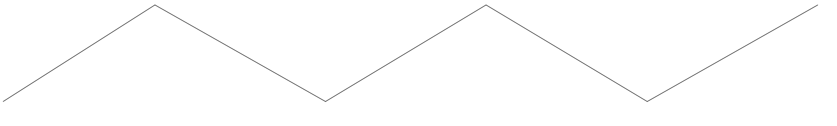
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane

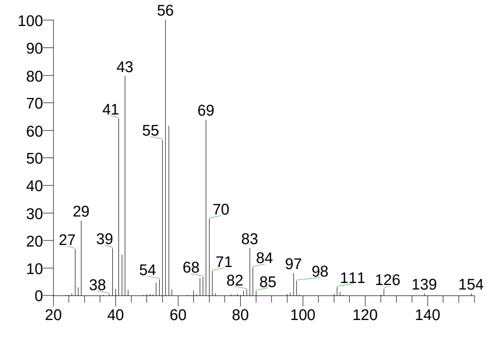


SI 761, RSI 797, replib, Entry# 5854, CAS# 110-54-3, n-Hexane

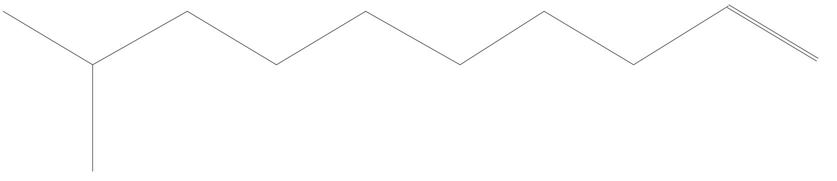


n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane





1-Decene, 9-methyl-
Formula C11H22, MW 154, CAS# 61142-78-7, Entry# 22158
9-Methyl-1-decene #



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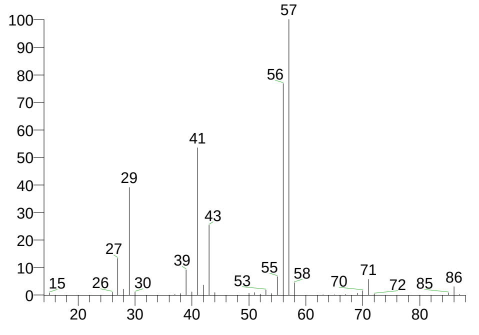
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

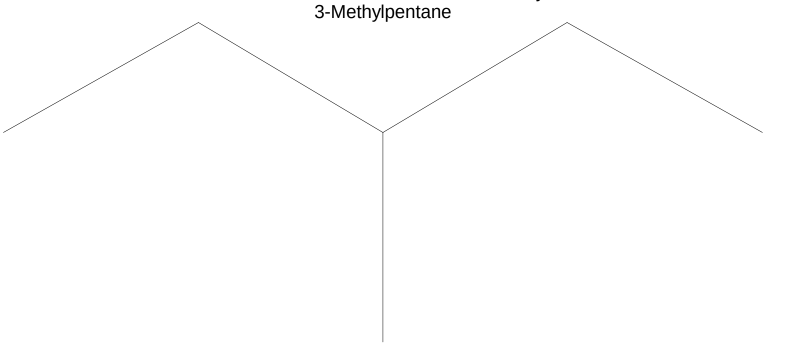
Stem pet ether

Hit Spectrum



Compound Structure

Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 1301
3-Methylpentane



There is no signature data to report.

Library Search Report

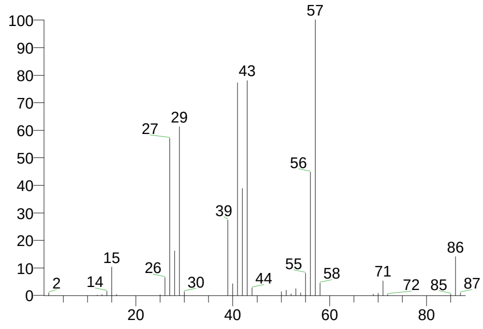
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

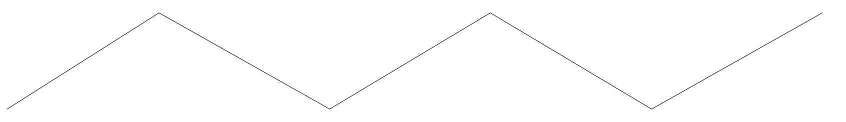
RT	Compound Name	Area	Area %
3.25	2-Ethyl-oxetane	6706120087.22	2.55
3.25	Pentane, 3-methyl-	6706120087.22	2.55
3.25	Pentane, 3-methyl-	6706120087.22	2.55
3.25	n-Hexane	6706120087.22	2.55
3.25	n-Hexane	6706120087.22	2.55

Hit Spectrum	Compound Structure

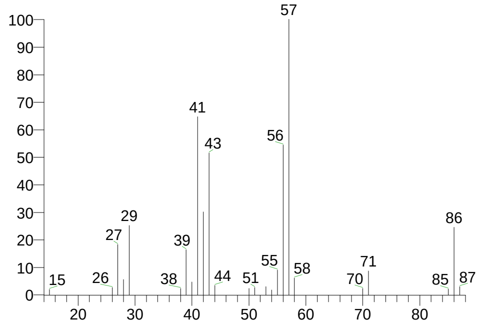
SI 778, RSI 833, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



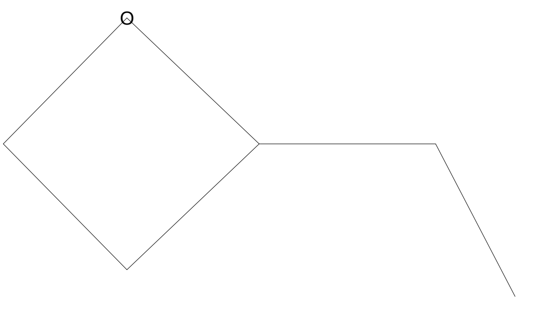
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane



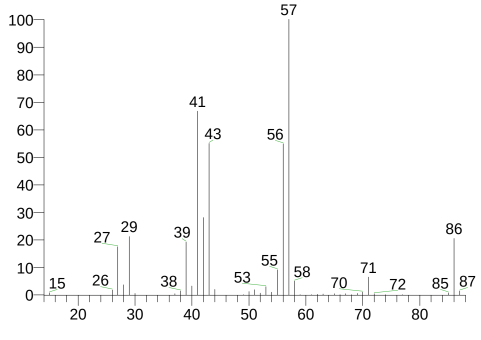
SI 777, RSI 876, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



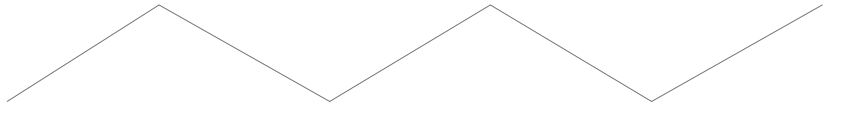
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N

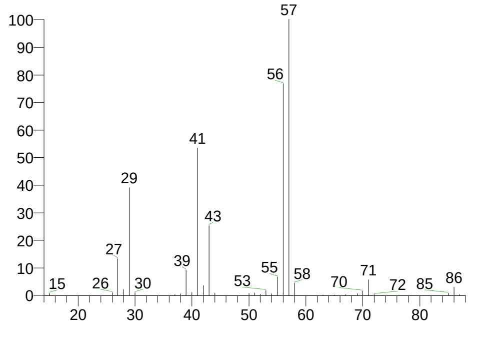


SI 771, RSI 802, replib, Entry# 5854, CAS# 110-54-3, n-Hexane

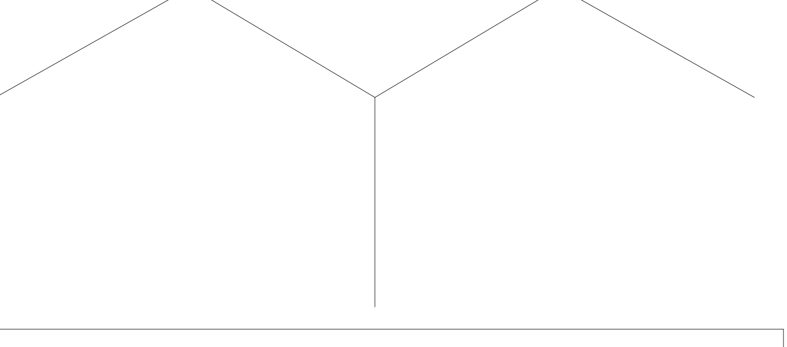


n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane





Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 1301
3-Methylpentane



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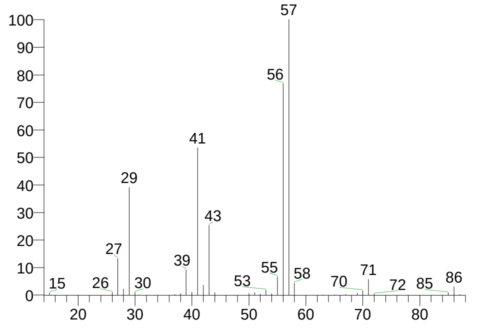
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

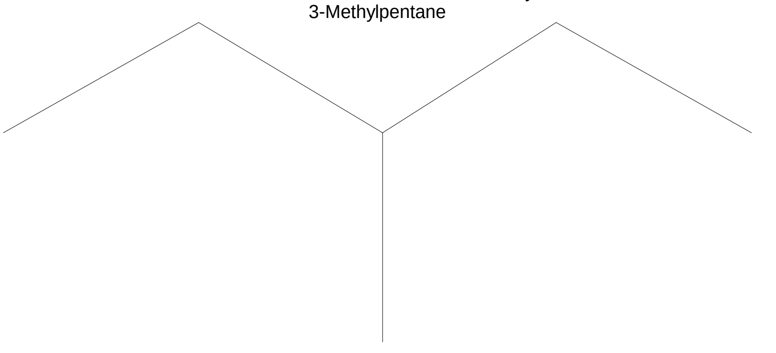
Hit Spectrum

SI 750, RSI 800, mainlib, Entry# 24856, CAS# 96-14-0, Pentane, 3-methyl-



Compound Structure

Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 24856
3-Methylpentane



There is no signature data to report.

Library Search Report

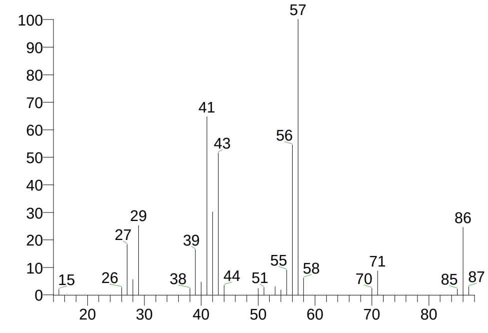
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

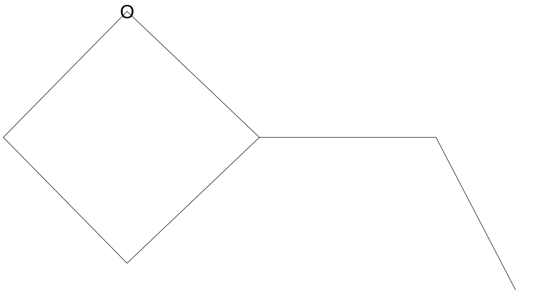
RT	Compound Name	Area	Area %
3.34	1-Pentanol, 2-ethyl-4-methyl-	6902048144.87	2.63
3.34	1-Pentanol, 2-ethyl-4-methyl-	6902048144.87	2.63
3.34	2-Ethyl-oxetane	6902048144.87	2.63
3.34	n-Hexane	6902048144.87	2.63
3.34	n-Hexane	6902048144.87	2.63

Hit Spectrum	Compound Structure

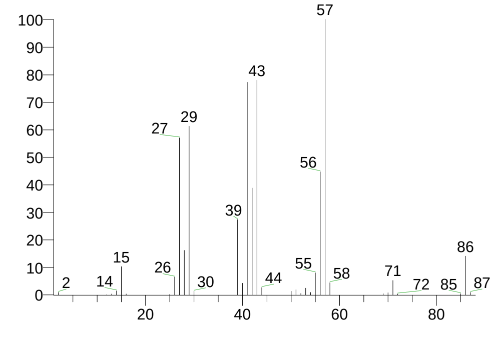
SI 778, RSI 877, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



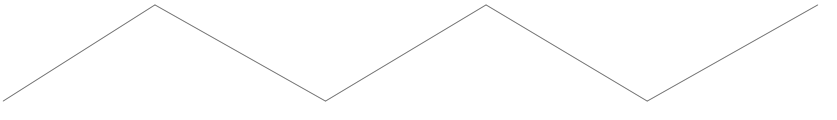
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N



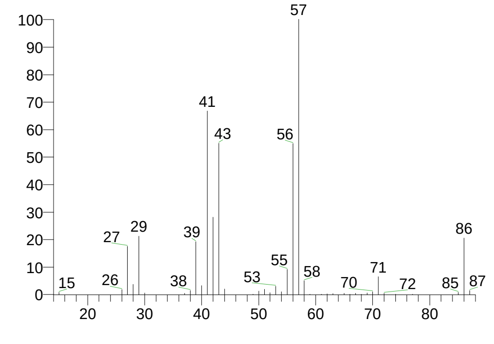
SI 771, RSI 829, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



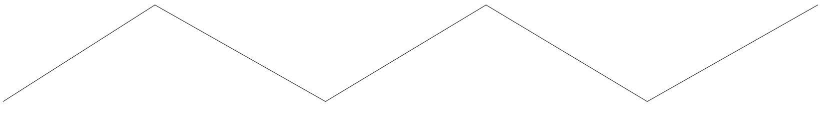
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane

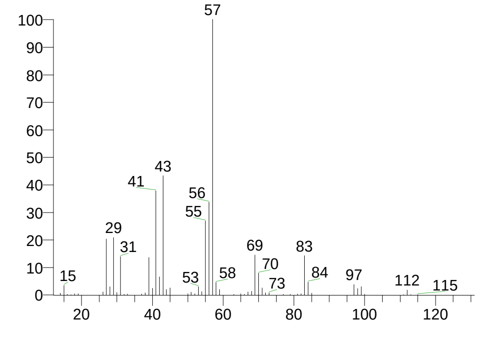


SI 766, RSI 799, replib, Entry# 5854, CAS# 110-54-3, n-Hexane

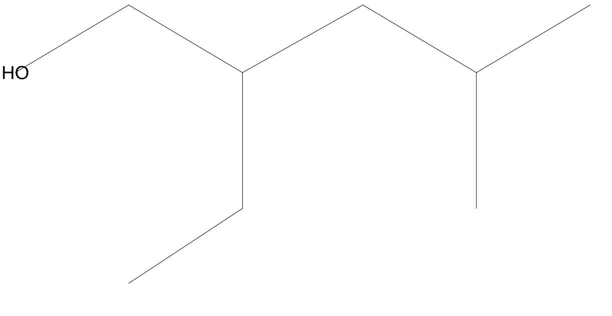


n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane





1-Pentanol, 2-ethyl-4-methyl-
Formula C8H18O, MW 130, CAS# 106-67-2, Entry# 1648
2-Ethyl-4-methylpentanol



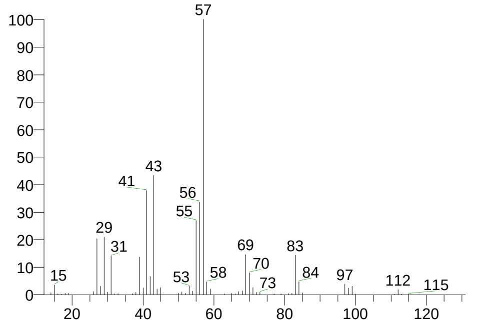
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

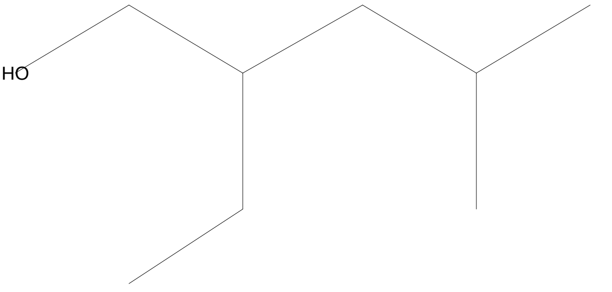
Stem pet ether

Hit Spectrum



Compound Structure

1-Pentanol, 2-ethyl-4-methyl-
Formula C8H18O, MW 130, CAS# 106-67-2, Entry# 24040
2-Ethyl-4-methylpentanol



There is no signature data to report.

Library Search Report

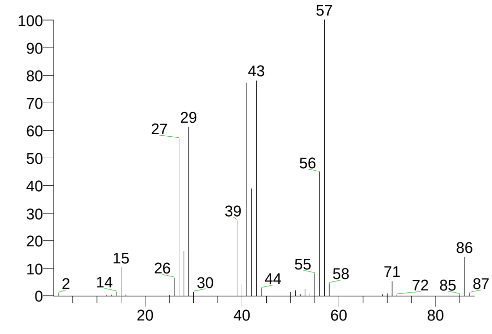
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

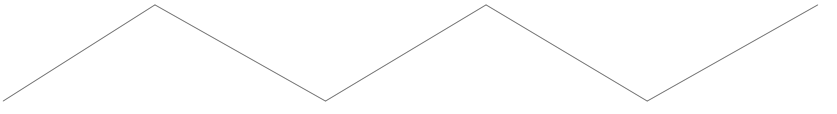
RT	Compound Name	Area	Area %
3.43	2-Ethyl-oxetane	5638545021.31	2.15
3.43	Pentane, 3-methyl-	5638545021.31	2.15
3.43	Pentane, 3-methyl-	5638545021.31	2.15
3.43	n-Hexane	5638545021.31	2.15
3.43	n-Hexane	5638545021.31	2.15

Hit Spectrum | Compound Structure

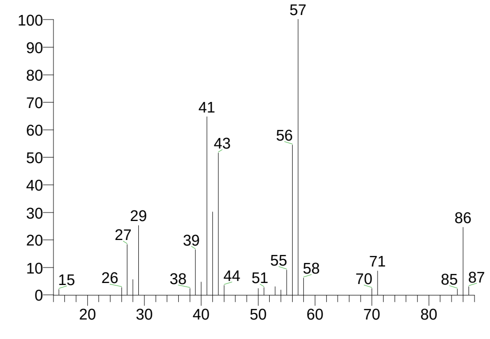
SI 776, RSI 833, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



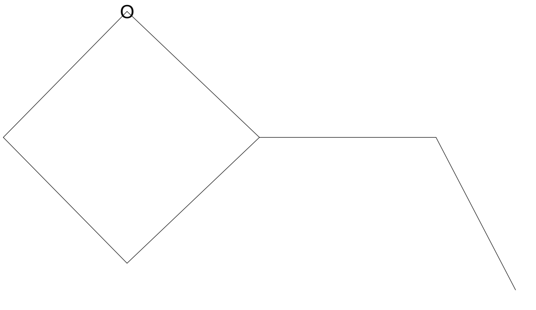
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane



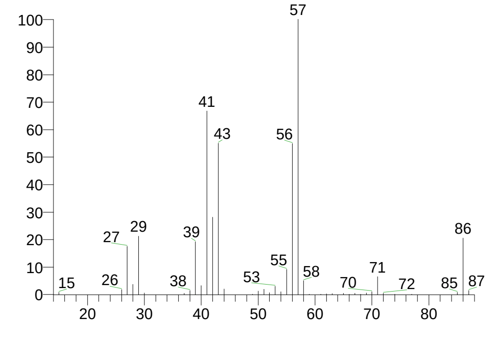
SI 771, RSI 871, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



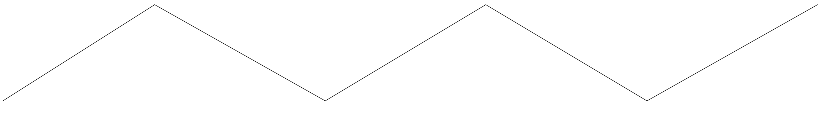
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N

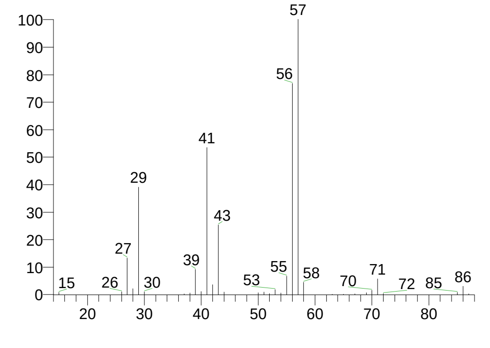


SI 771, RSI 805, replib, Entry# 5854, CAS# 110-54-3, n-Hexane

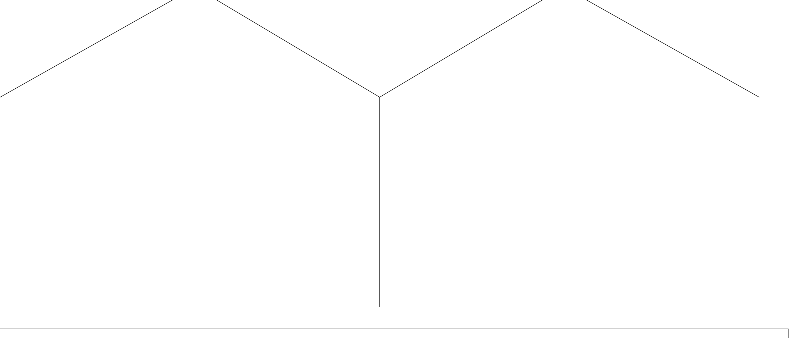


n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane





Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 1301
3-Methylpentane



There is no signature data to report.

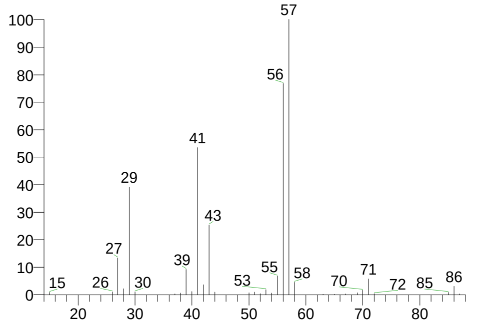
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

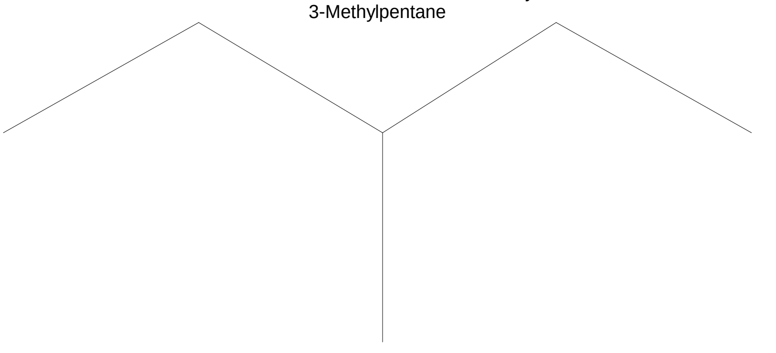
Hit Spectrum

SI 756, RSI 809, mainlib, Entry# 24856, CAS# 96-14-0, Pentane, 3-methyl-



Compound Structure

Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 24856
3-Methylpentane



There is no signature data to report.

Library Search Report

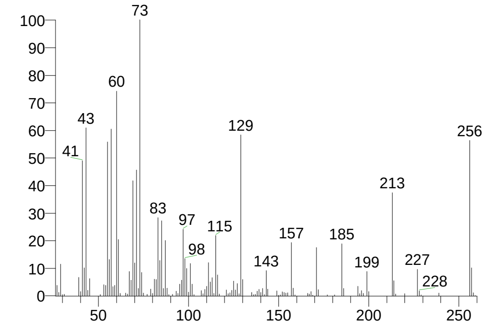
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

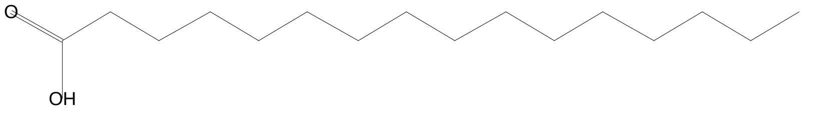
RT	Compound Name	Area	Area %
33.43	n-Hexadecanoic acid	10257301386.17	3.90
33.43	n-Hexadecanoic acid	10257301386.17	3.90
33.43	n-Hexadecanoic acid	10257301386.17	3.90
33.43	n-Hexadecanoic acid	10257301386.17	3.90
33.43	n-Hexadecanoic acid	10257301386.17	3.90

Hit Spectrum | Compound Structure

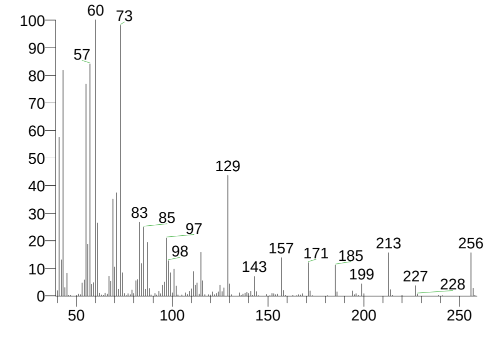
SI 928, RSI 933, replib, Entry# 9622, CAS# 57-10-3, n-Hexadecanoic acid



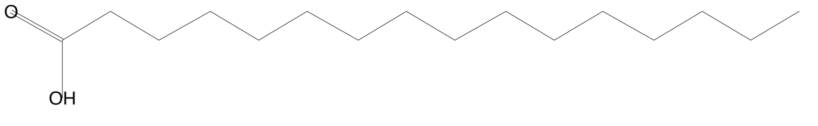
n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 9622
Hexadecanoic acid



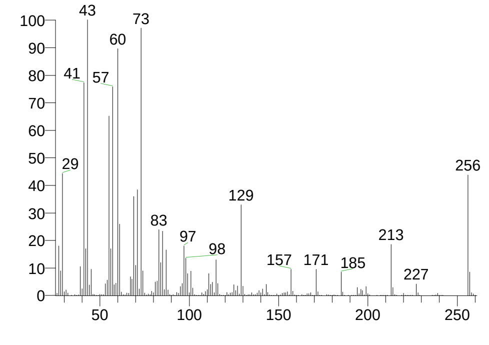
SI 925, RSI 928, replib, Entry# 7566, CAS# 57-10-3, n-Hexadecanoic acid



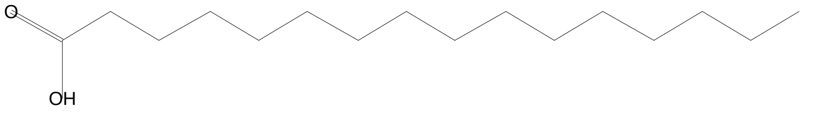
n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 7566
Hexadecanoic acid

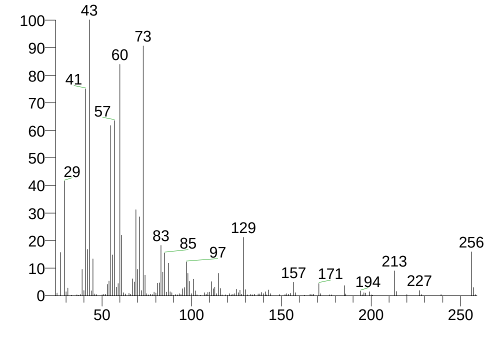


SI 875, RSI 875, replib, Entry# 2779, CAS# 57-10-3, n-Hexadecanoic acid

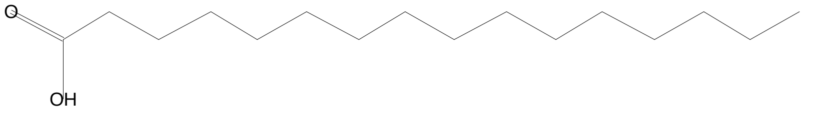


n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 2779
Hexadecanoic acid





n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 154
Hexadecanoic acid



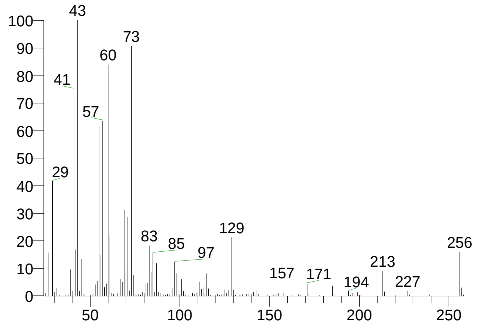
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

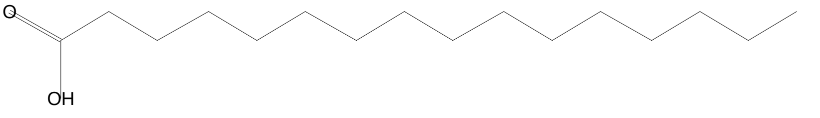
Stem pet ether

Hit Spectrum



Compound Structure

n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 9208
Hexadecanoic acid



There is no signature data to report.

Library Search Report

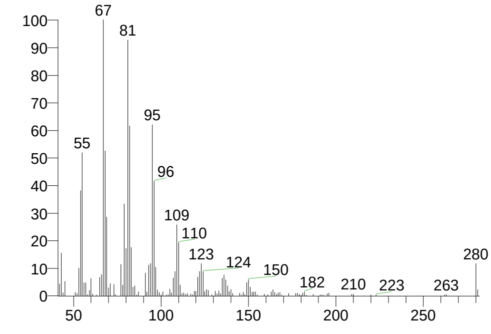
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

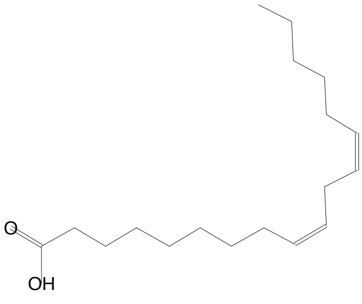
RT	Compound Name	Area	Area %
36.24	(Z)-18-Octadec-9-enolide	7769255098.71	2.96
36.24	9,12-Octadecadienoic acid (Z,Z)-	7769255098.71	2.96
36.24	9,12-Octadecadienoic acid (Z,Z)-	7769255098.71	2.96
36.24	9,12-Octadecadienoic acid (Z,Z)-	7769255098.71	2.96
36.24	Linoelaidic acid	7769255098.71	2.96

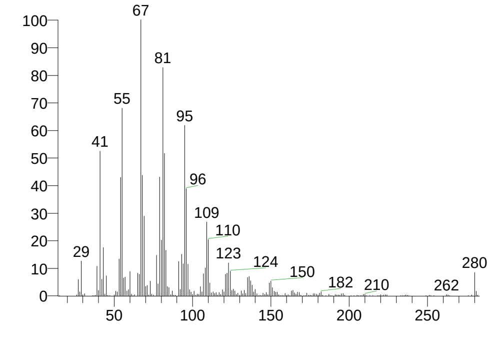
Hit Spectrum

Compound Structure

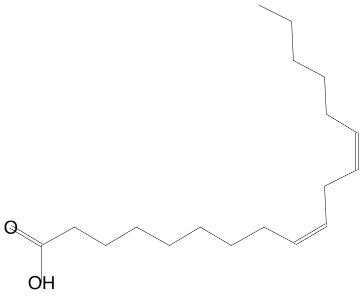


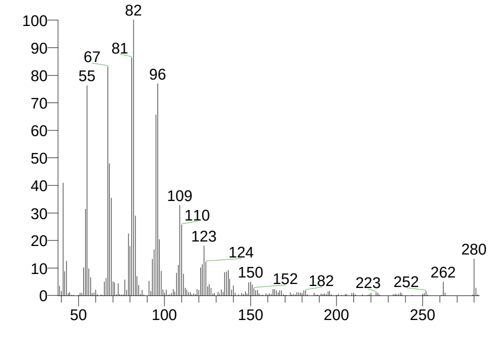
9,12-Octadecadienoic acid (Z,Z)-
Formula C18H32O2, MW 280, CAS# 60-33-3, Entry# 8129
cis-9,cis-12-Octadecadienoic acid



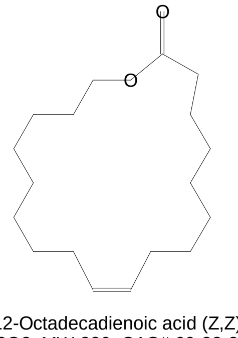


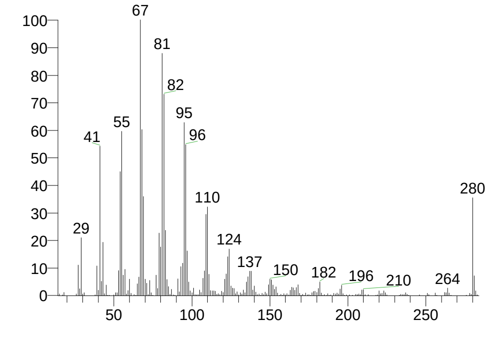
9,12-Octadecadienoic acid (Z,Z)-
Formula C18H32O2, MW 280, CAS# 60-33-3, Entry# 8112
cis-9,cis-12-Octadecadienoic acid



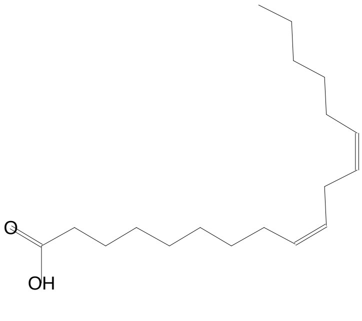


(Z)-18-Octadec-9-enolide
Formula C18H32O2, MW 280, CAS# 80060-76-0, Entry# 51421
Oxacyclononadec-10-en-2-one, (10Z)-





9,12-Octadecadienoic acid (Z,Z)-
Formula C18H32O2, MW 280, CAS# 60-33-3, Entry# 32846
cis-9,cis-12-Octadecadienoic acid



There is no signature data to report.

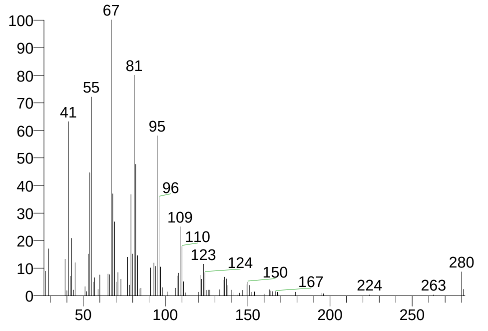
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

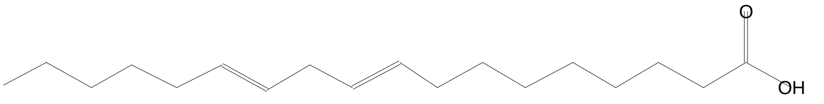
Hit Spectrum

SI 863, RSI 929, mainlib, Entry# 32814, CAS# 506-21-8, Linoelaidic acid



Compound Structure

Linoelaidic acid
Formula C18H32O2, MW 280, CAS# 506-21-8, Entry# 32814
$:28OYHQOLUKZRVURQ-AVQMFFATSA-N



There is no signature data to report.

Library Search Report

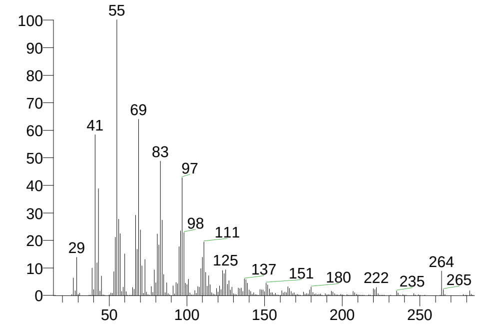
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

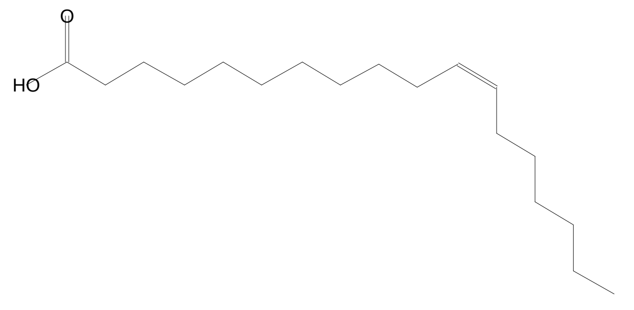
RT	Compound Name	Area	Area %
36.32	9-Octadecenoic acid, (E)-	11272748200.68	4.29
36.32	Oleic Acid	11272748200.68	4.29
36.32	cis-13-Octadecenoic acid	11272748200.68	4.29
36.32	cis-Vaccenic acid	11272748200.68	4.29
36.32	trans-13-Octadecenoic acid	11272748200.68	4.29

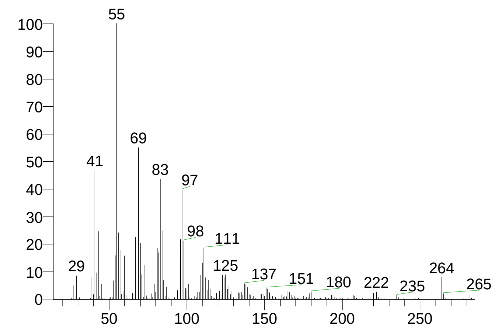
Hit Spectrum	Compound Structure

SI 904, RSI 905, mainlib, Entry# 20090, CAS# 506-17-2, cis-Vaccenic acid

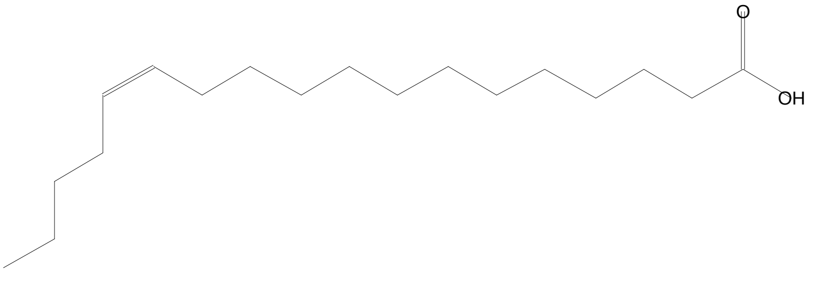


cis-Vaccenic acid
Formula C18H34O2, MW 282, CAS# 506-17-2, Entry# 20090
11-Octadecenoic acid, (Z)-

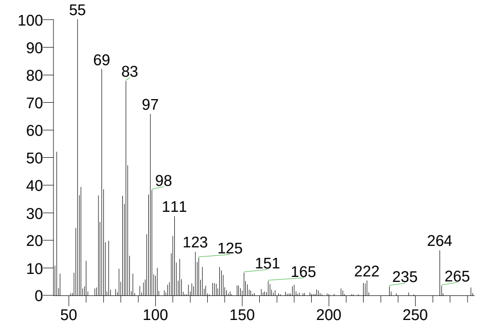




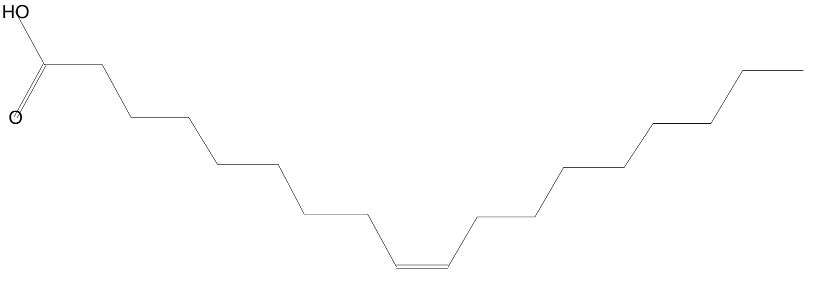
cis-13-Octadecenoic acid
Formula C18H34O2, MW 282, CAS# 13126-39-1, Entry# 20126
$:28BDLLSHRIFPDGQB-WAYWQWQTSA-N

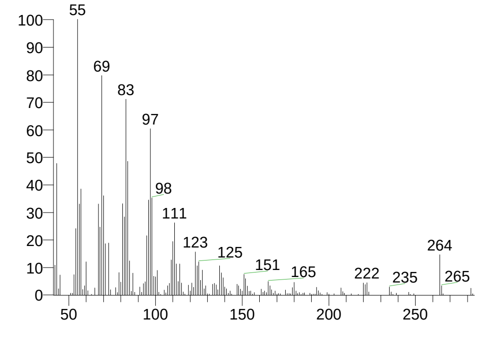


SI 900, RSI 909, replib, Entry# 5017, CAS# 112-80-1, Oleic Acid

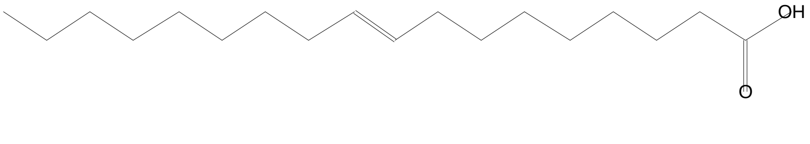


Oleic Acid
Formula C18H34O2, MW 282, CAS# 112-80-1, Entry# 5017
9-Octadecenoic acid (Z)-





9-Octadecenoic acid, (E)-
Formula C18H34O2, MW 282, CAS# 112-79-8, Entry# 5015
trans-ë(sup 9)-Octadecenoic acid



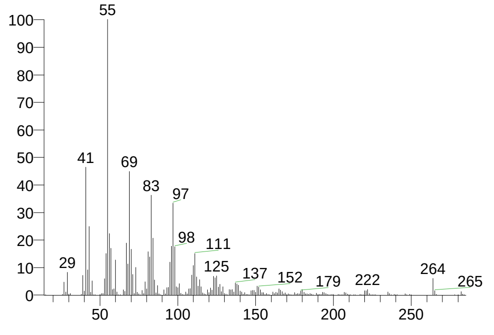
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

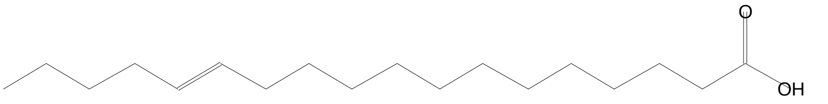
Stem pet ether

Hit Spectrum



Compound Structure

trans-13-Octadecenoic acid
Formula C18H34O2, MW 282, CAS# 693-71-0, Entry# 19306
$:28BDLLSHRIFPDGQB-AATRIKPKSA-N



There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

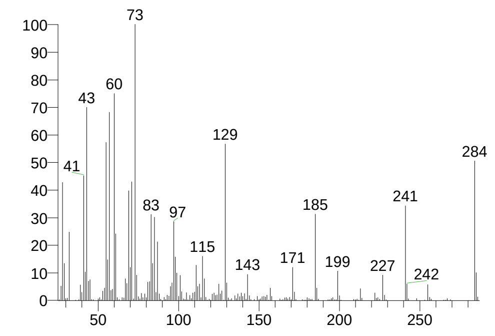
Stem pet ether

RT	Compound Name	Area	Area %
36.60	Octadecanoic acid	4211685971.67	1.60
36.60	Octadecanoic acid	4211685971.67	1.60
36.60	Octadecanoic acid	4211685971.67	1.60
36.60	Octadecanoic acid	4211685971.67	1.60
36.60	Octadecanoic acid	4211685971.67	1.60

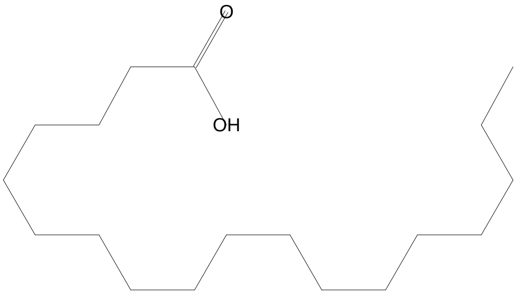
Hit Spectrum

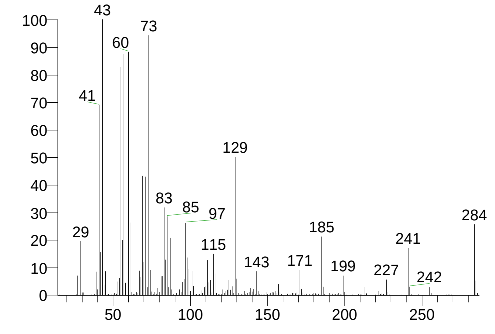
Compound Structure

SI 885, RSI 893, replib, Entry# 9623, CAS# 57-11-4, Octadecanoic acid

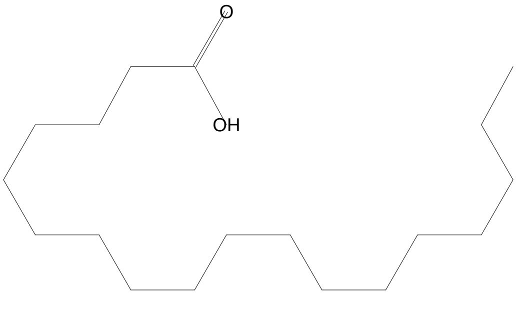


Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 9623
Stearic acid

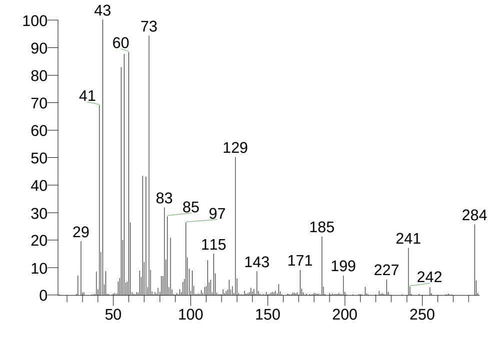




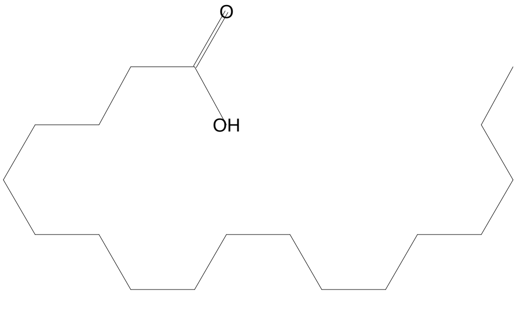
Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 266
Stearic acid



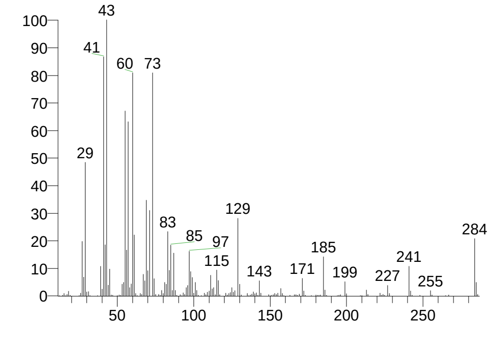
SI 885, RSI 890, mainlib, Entry# 9210, CAS# 57-11-4, Octadecanoic acid



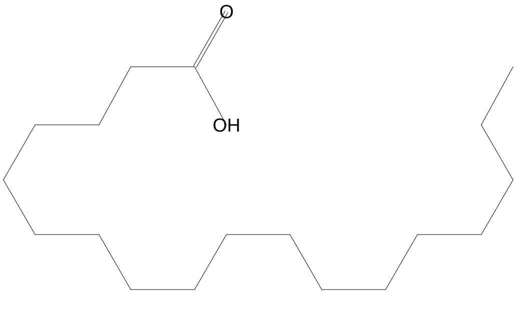
Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 9210
Stearic acid



SI 878, RSI 891, replib, Entry# 1866, CAS# 57-11-4, Octadecanoic acid



Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 1866
Stearic acid



There is no signature data to report.

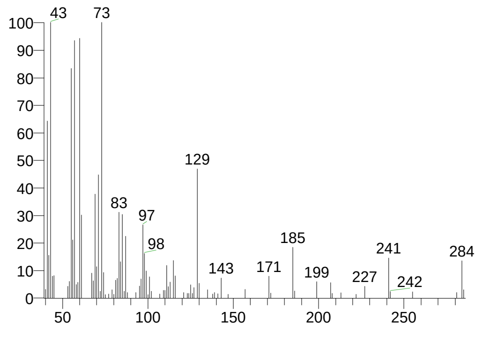
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

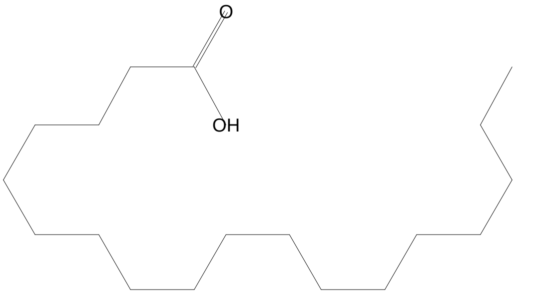
Hit Spectrum

SI 874, RSI 931, replib, Entry# 2781, CAS# 57-11-4, Octadecanoic acid



Compound Structure

Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 2781
Stearic acid



There is no signature data to report.

Library Search Report

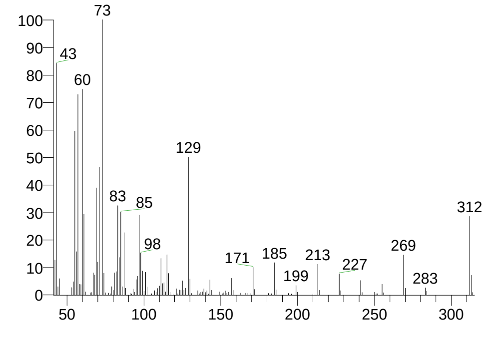
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

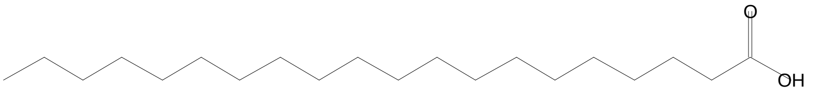
RT	Compound Name	Area	Area %
39.55	Eicosanoic acid	2468498772.00	0.94
39.55	Eicosanoic acid	2468498772.00	0.94
39.55	Eicosanoic acid	2468498772.00	0.94
39.55	Eicosanoic acid	2468498772.00	0.94
39.55	Eicosanoic acid	2468498772.00	0.94

Hit Spectrum | Compound Structure

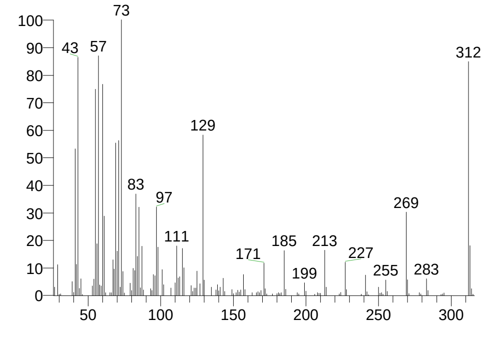
SI 902, RSI 940, replib, Entry# 9519, CAS# 506-30-9, Eicosanoic acid



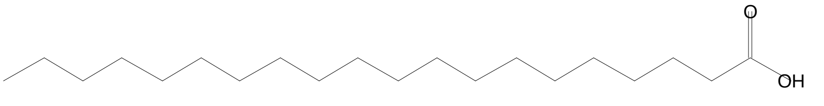
Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 9519
Arachic acid



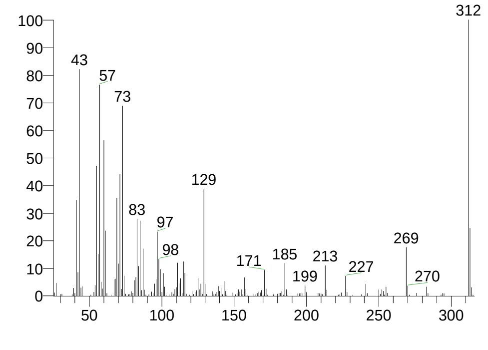
SI 868, RSI 915, replib, Entry# 9604, CAS# 506-30-9, Eicosanoic acid



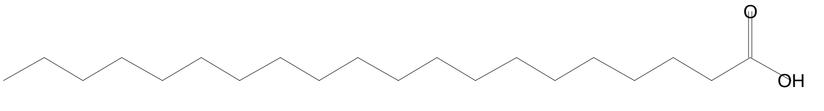
Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 9604
Arachic acid



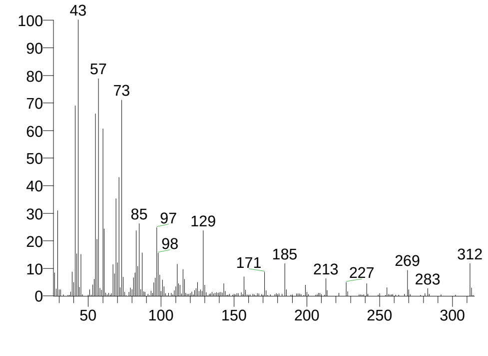
SI 848, RSI 868, replib, Entry# 32789, CAS# 506-30-9, Eicosanoic acid



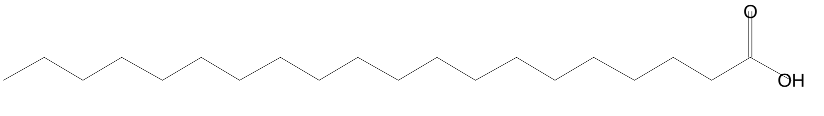
Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 32789
Arachic acid



SI 844, RSI 861, mainlib, Entry# 7899, CAS# 506-30-9, Eicosanoic acid



Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 7899
Arachic acid



There is no signature data to report.

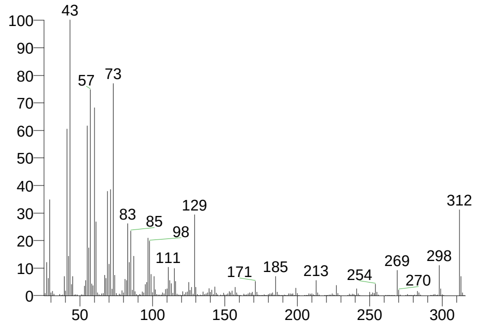
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

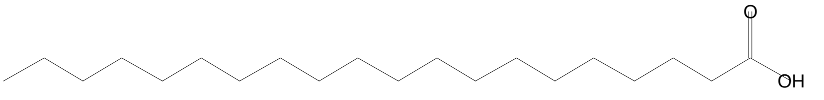
Hit Spectrum

SI 844, RSI 846, replib, Entry# 2778, CAS# 506-30-9, Eicosanoic acid



Compound Structure

Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 2778
Arachic acid



There is no signature data to report.

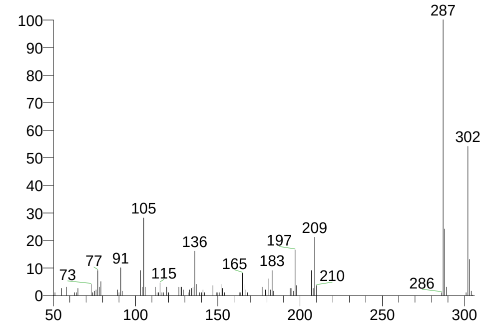
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

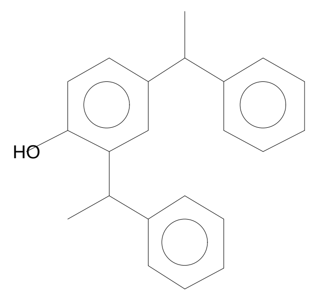
Stem pet ether

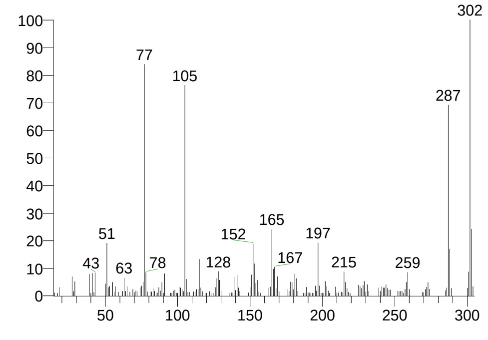
RT	Compound Name	Area	Area %
40.63	4-Androsten-6á-ol-3,17-dione	963998983.91	0.37
40.63	Methanone, [1,4-dimethyl-7-(1-methylethyl)-2-azulenyl]phenyl-	963998983.91	0.37
40.63	Methanone, [2,4-dimethyl-7-(1-methylethyl)azulenyl]phenyl-	963998983.91	0.37
40.63	Palustric acid	963998983.91	0.37
40.63	Phenol, 2,4-bis(1-phenylethyl)-	963998983.91	0.37

Hit Spectrum	Compound Structure

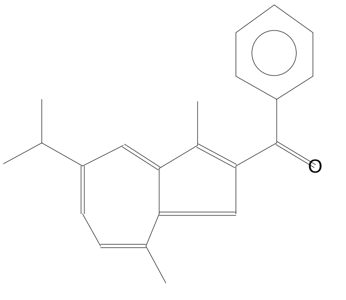


Phenol, 2,4-bis(1-phenylethyl)-
Formula C22H22O, MW 302, CAS# 2769-94-0, Entry# 221962
2,4-Bis(1-phenylethyl)phenol #

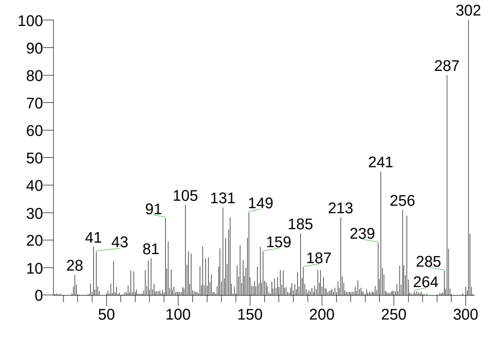




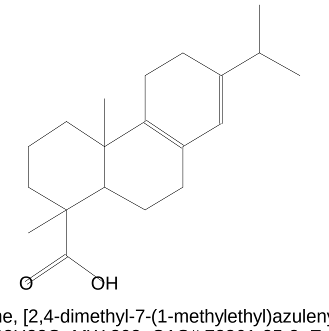
Methanone, [1,4-dimethyl-7-(1-methylethyl)-2-azulenyl]phenyl-
Formula C22H22O, MW 302, CAS# 39665-56-0, Entry# 225380
2-Benzoylguaiazulene

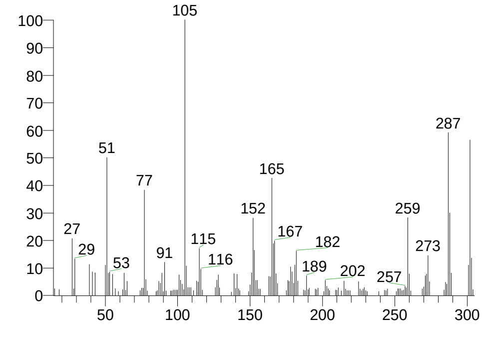


SI 638, RSI 640, mainlib, Entry# 225498, CAS# 1945-53-5, Palustric acid

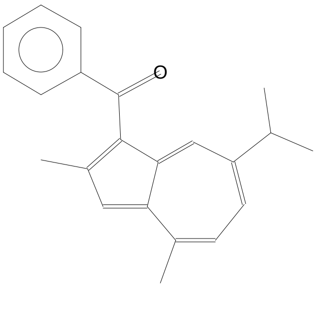


Palustric acid
Formula C20H30O2, MW 302, CAS# 1945-53-5, Entry# 225498
Podocarpa-8,13-dien-15-oic acid, 13-isopropyl-





Methanone, [2,4-dimethyl-7-(1-methylethyl)azulenyl]phenyl-
Formula C22H22O, MW 302, CAS# 72361-25-2, Entry# 82089
(7-Isopropyl-2,4-dimethyl-1-azulenyl)(phenyl)methanone #



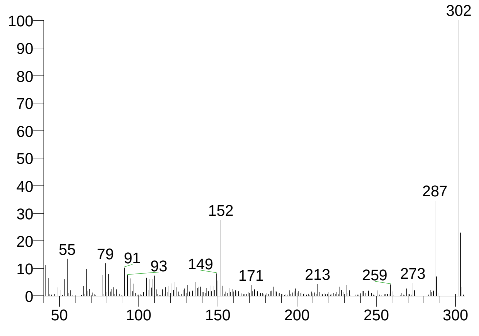
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

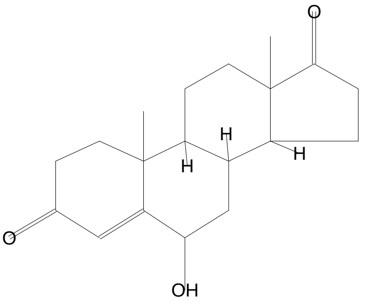
Stem pet ether

Hit Spectrum



Compound Structure

4-Androsten-6á-ol-3,17-dione
Formula C19H26O3, MW 302, CAS# 63-00-3, Entry# 225495
6-Hydroxyandrost-4-ene-3,17-dione #



There is no signature data to report.

Library Search Report

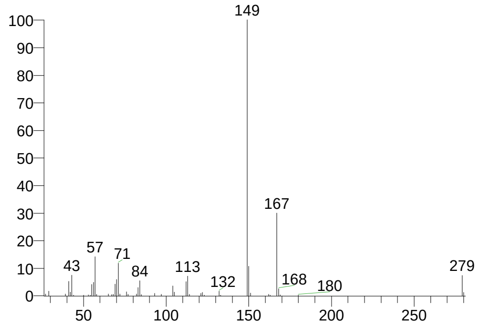
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

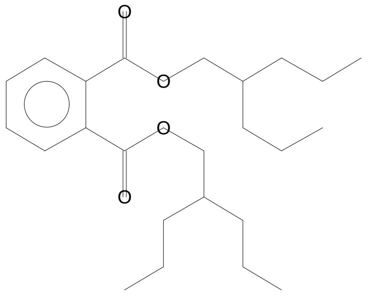
RT	Compound Name	Area	Area %
41.52	Bis(2-ethylhexyl) phthalate	5092409641.89	1.94
41.52	Bis(2-ethylhexyl) phthalate	5092409641.89	1.94
41.52	Diisooctyl phthalate	5092409641.89	1.94
41.52	Phthalic acid, di(2-propylpentyl) ester	5092409641.89	1.94
41.52	Phthalic acid, di(6-methylhept-2-yl) ester	5092409641.89	1.94

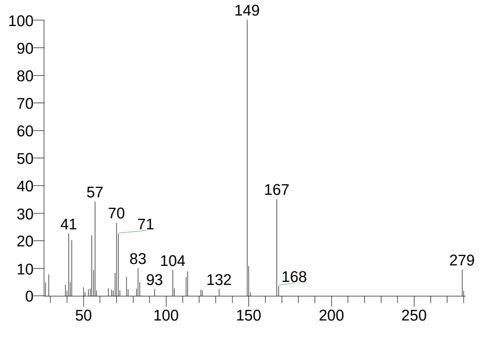
Hit Spectrum

Compound Structure

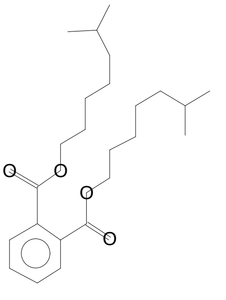


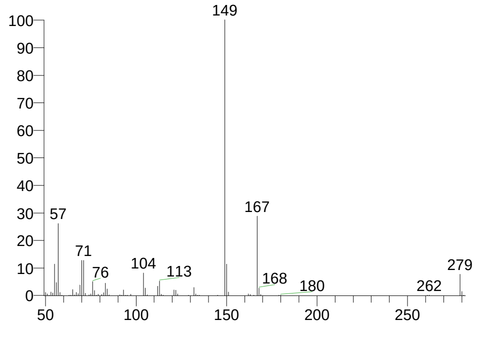
Phthalic acid, di(2-propylpentyl) ester
Formula C24H38O4, MW 390, CAS# NA, Entry# 138707
$:28KIYUVQCUDDMZRE-UHFFFAOYSA-N



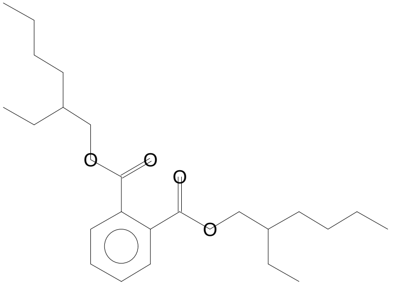


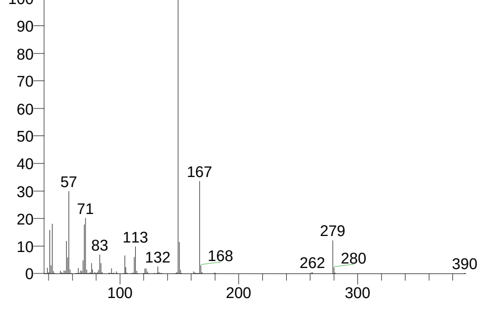
Diisooctyl phthalate
Formula C24H38O4, MW 390, CAS# 131-20-4, Entry# 23542
Bis(6-methylheptyl) phthalate



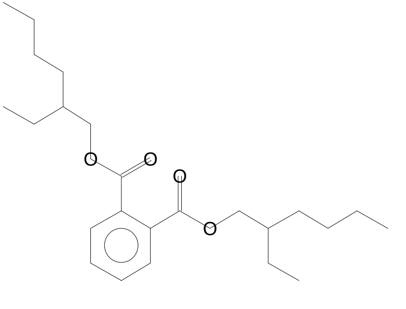


Bis(2-ethylhexyl) phthalate
Formula C24H38O4, MW 390, CAS# 117-81-7, Entry# 23540
Phthalic acid, bis(2-ethylhexyl) ester





Bis(2-ethylhexyl) phthalate
Formula C24H38O4, MW 390, CAS# 117-81-7, Entry# 23539
Phthalic acid, bis(2-ethylhexyl) ester



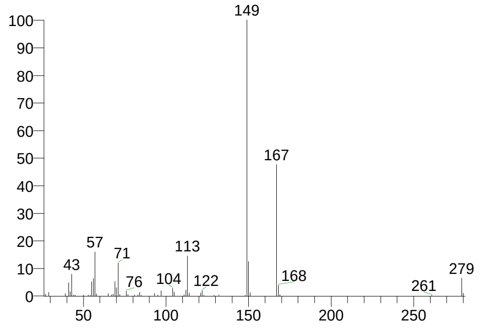
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

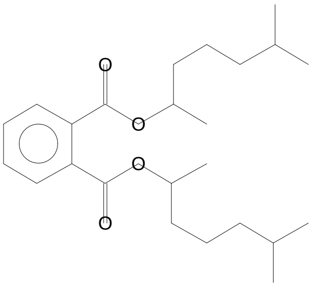
Stem pet ether

Hit Spectrum



Compound Structure

Phthalic acid, di(6-methylhept-2-yl) ester
Formula C24H38O4, MW 390, CAS# NA, Entry# 138710
$:28ASUMBDPCWUXZFX-UHFFFAOYSA-N



There is no signature data to report.

Library Search Report

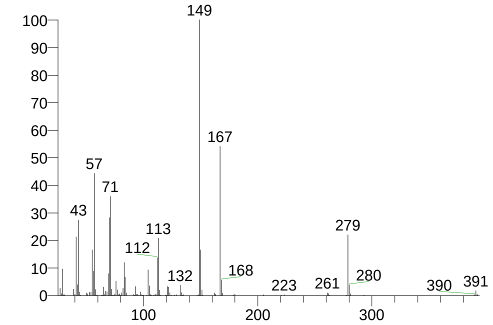
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

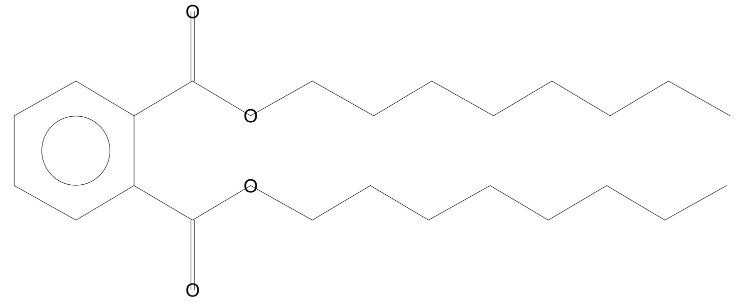
RT	Compound Name	Area	Area %
42.15	Bis(2-ethylhexyl) phthalate	65519083331.82	24.93
42.15	Bis(2-ethylhexyl) phthalate	65519083331.82	24.93
42.15	Bis(2-ethylhexyl) phthalate	65519083331.82	24.93
42.15	Di-n-octyl phthalate	65519083331.82	24.93
42.15	Diisooctyl phthalate	65519083331.82	24.93

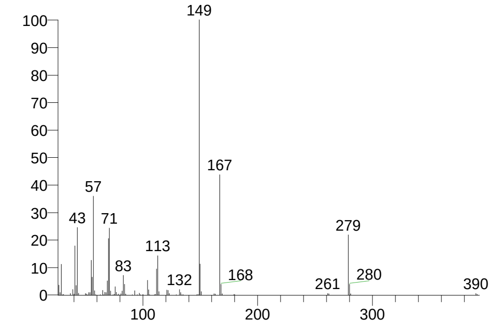
Hit Spectrum — Compound Structure

SI 936, RSI 936, replib, Entry# 23541, CAS# 117-84-0, Di-n-octyl phthalate

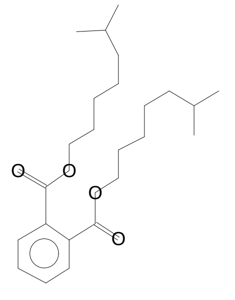


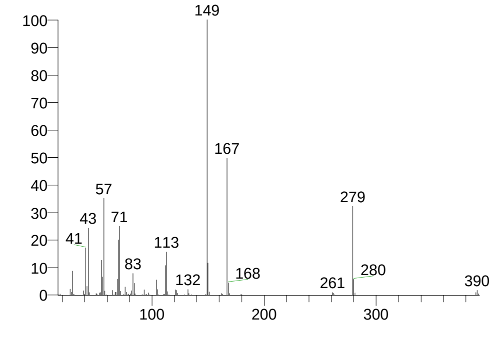
Di-n-octyl phthalate
Formula C24H38O4, MW 390, CAS# 117-84-0, Entry# 23541
1,2-Benzenedicarboxylic acid, dioctyl ester



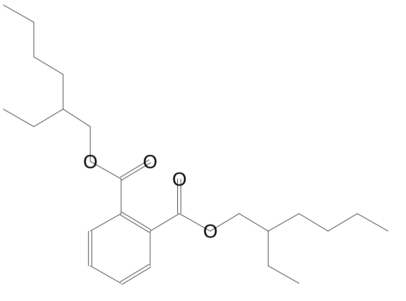


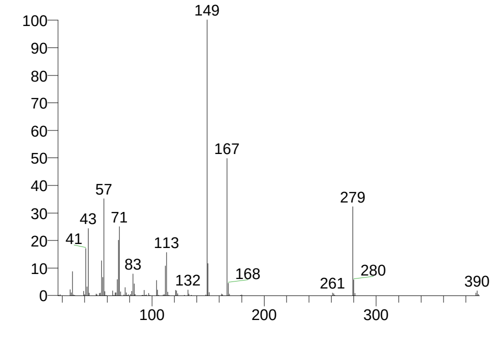
Diisooctyl phthalate
Formula C24H38O4, MW 390, CAS# 131-20-4, Entry# 138712
Bis(6-methylheptyl) phthalate



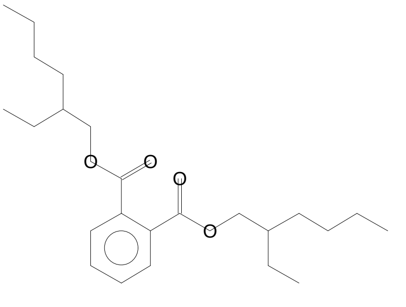


Bis(2-ethylhexyl) phthalate
Formula C24H38O4, MW 390, CAS# 117-81-7, Entry# 537
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester





Bis(2-ethylhexyl) phthalate
Formula C24H38O4, MW 390, CAS# 117-81-7, Entry# 138711
Phthalic acid, bis(2-ethylhexyl) ester



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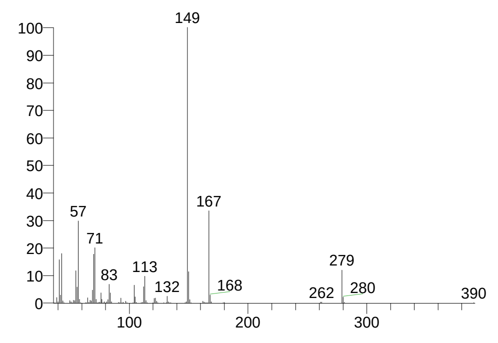
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

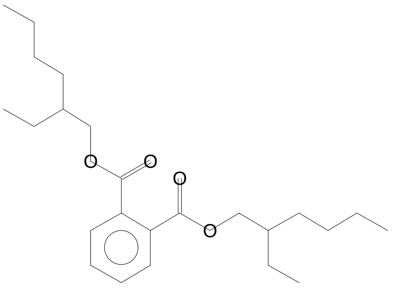
Stem pet ether

Hit Spectrum



Compound Structure

Bis(2-ethylhexyl) phthalate
Formula C24H38O4, MW 390, CAS# 117-81-7, Entry# 23539
Phthalic acid, bis(2-ethylhexyl) ester



There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

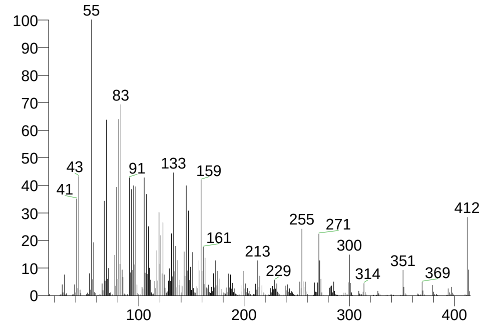
Stem pet ether

RT	Compound Name	Area	Area %
43.53	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	1869600077.18	0.71
43.53	Desmosterol	1869600077.18	0.71
43.53	Desmosterol	1869600077.18	0.71
43.53	Stigmasterol	1869600077.18	0.71
43.53	Stigmasterol	1869600077.18	0.71

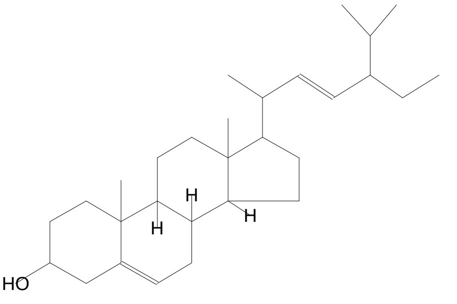
Hit Spectrum

Compound Structure

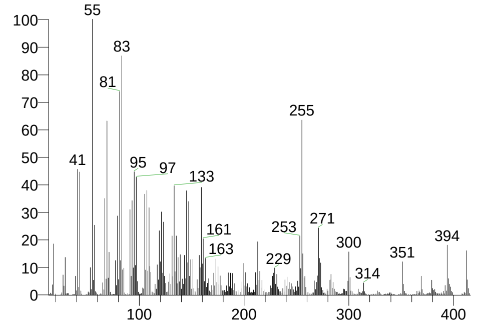
SI 826, RSI 844, mainlib, Entry# 20820, CAS# 83-48-7, Stigmasterol



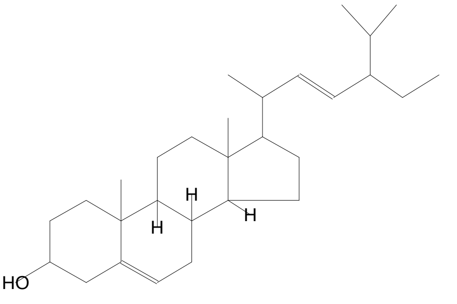
Stigmasterol
Formula C29H48O, MW 412, CAS# 83-48-7, Entry# 20820
Stigmasta-5,22-dien-3-ol, (3á,22E)-

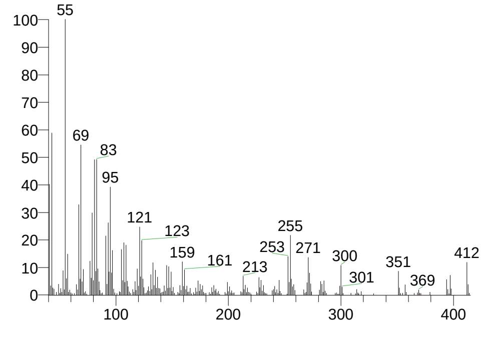


SI 823, RSI 825, replib, Entry# 5167, CAS# 83-48-7, Stigmasterol

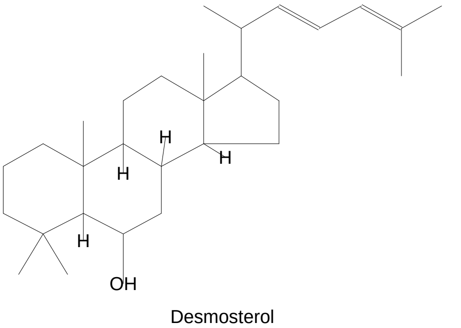


Stigmasterol
Formula C29H48O, MW 412, CAS# 83-48-7, Entry# 5167
Stigmasta-5,22-dien-3-ol, (3á,22E)-

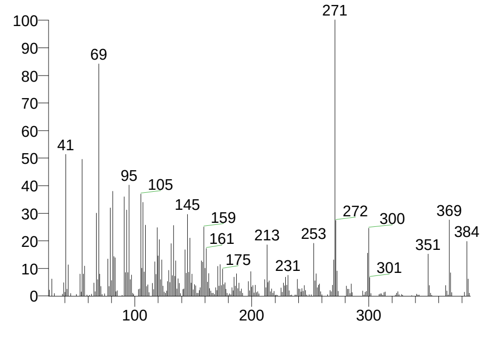




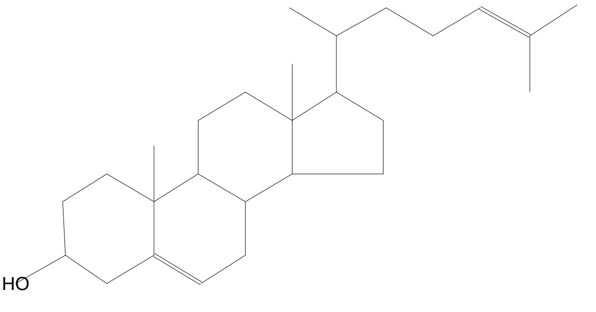
Cholesta-22,24-dien-5-ol, 4,4-dimethyl-
Formula C29H48O, MW 412, CAS# NA, Entry# 19679
(22E)-4,4-Dimethylcholesta-22,24-dien-6-ol #



SI 783, RSI 822, mainlib, Entry# 217090, CAS# 313-04-2, Desmosterol



Desmosterol
Formula C27H44O, MW 384, CAS# 313-04-2, Entry# 217090
Cholesta-5,24-dien-3-ol, (3á)-



There is no signature data to report.

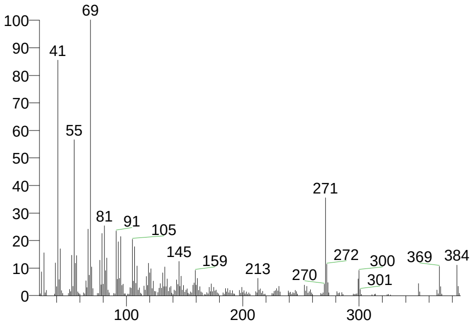
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

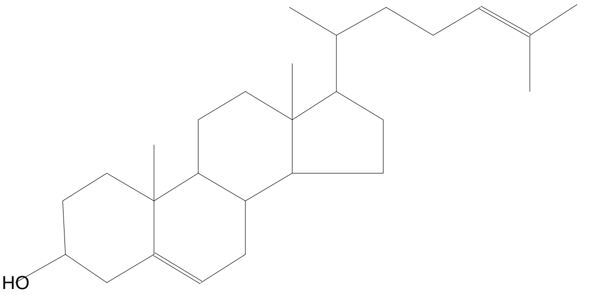
Hit Spectrum

SI 781, RSI 825, replib, Entry# 8466, CAS# 313-04-2, Desmosterol



Compound Structure

Desmosterol
Formula C27H44O, MW 384, CAS# 313-04-2, Entry# 8466
Cholesta-5,24-dien-3-ol, (3á)-



There is no signature data to report.

Library Search Report

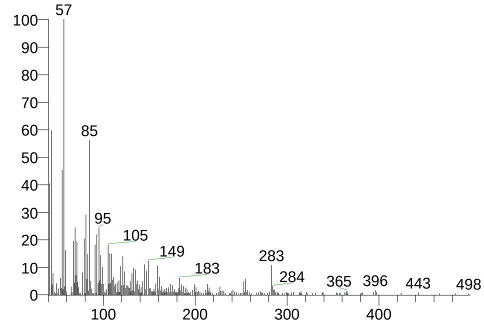
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

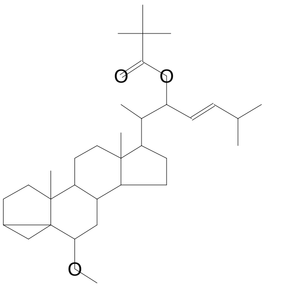
Stem pet ether

RT	Compound Name	Area	Area %
43.64	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	933754390.52	0.36
43.64	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	933754390.52	0.36
43.64	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	933754390.52	0.36
43.64	Oleic acid, 3-(octadecyloxy)propyl ester	933754390.52	0.36
43.64	Z-5-Methyl-6-heneicosen-11-one	933754390.52	0.36

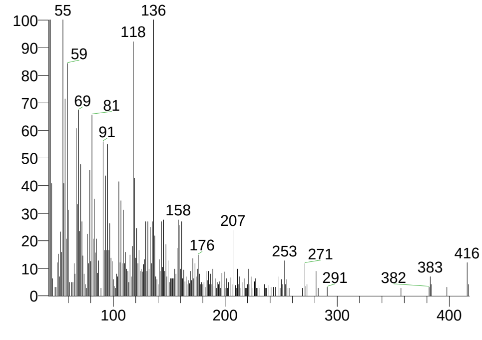
Hit Spectrum | Compound Structure

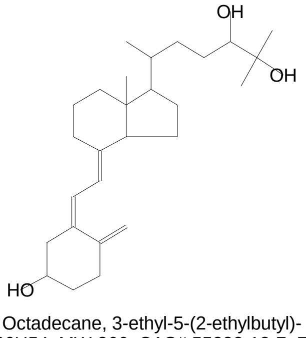
Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate
Formula C33H54O3, MW 498, CAS# NA, Entry# 24446
$:28TZJRPRMTYSBPHU-JLHYYAGUSA-N



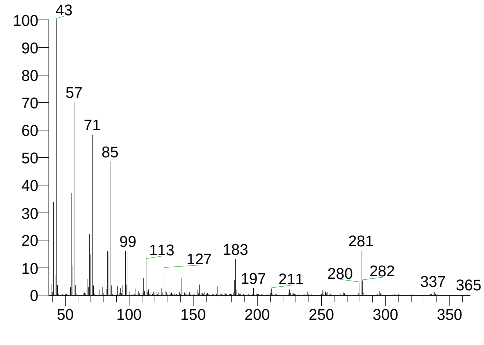


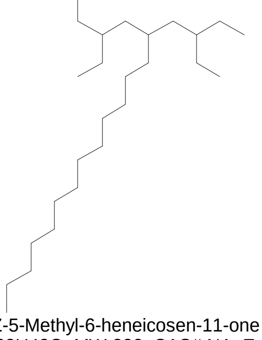
9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-
Formula C27H44O3, MW 416, CAS# 40013-87-4, Entry# 6730
24,25-Dihydroxycholecalciferol



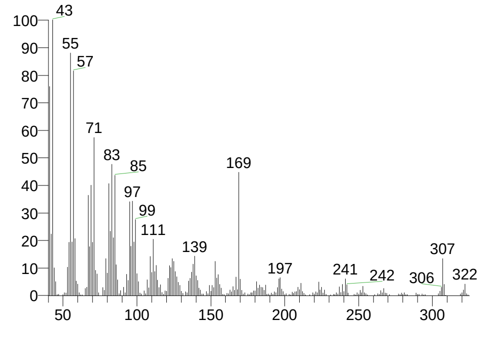


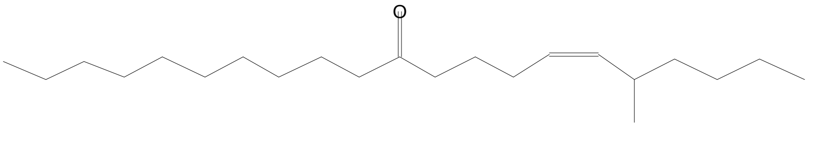
Octadecane, 3-ethyl-5-(2-ethylbutyl)-
Formula C26H54, MW 366, CAS# 55282-12-7, Entry# 2329
3-Ethyl-5-(2'-ethylbutyl)octadecane





Z-5-Methyl-6-heneicosen-11-one
Formula C22H42O, MW 322, CAS# NA, Entry# 7198
(6Z)-5-Methyl-6-henicosen-11-one #





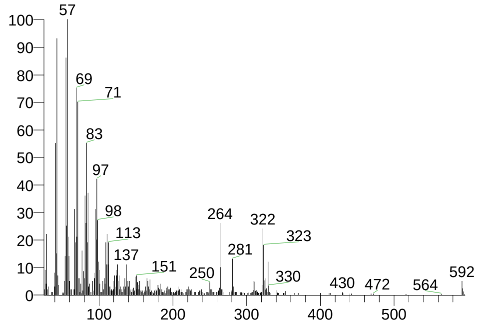
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

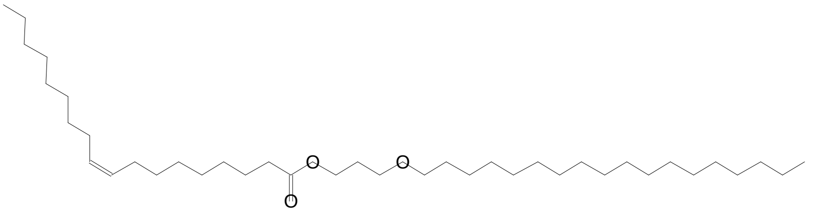
Stem pet ether

Hit Spectrum



Compound Structure

Oleic acid, 3-(octadecyloxy)propyl ester
Formula C39H76O3, MW 592, CAS# 17367-41-8, Entry# 24115
3-(Octadecyloxy)propyl (9E)-9-octadecenoate #



There is no signature data to report.

Library Search Report

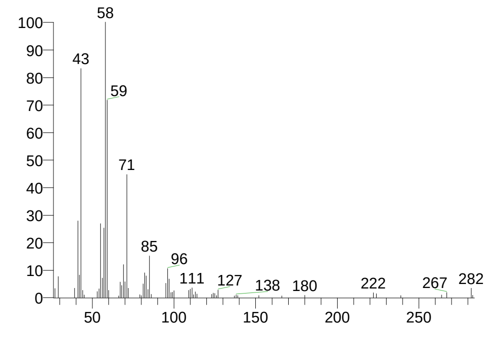
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

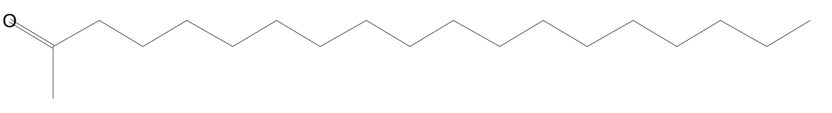
RT	Compound Name	Area	Area %
43.83	10-Octadecenal	813694429.63	0.31
43.83	13-Methylpentadec-14-ene-1,13-diol	813694429.63	0.31
43.83	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15á,16E)-	813694429.63	0.31
43.83	2-Nonadecanone	813694429.63	0.31
43.83	2-Pentacosanone	813694429.63	0.31

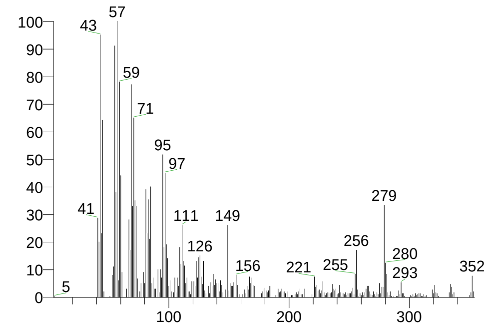
Hit Spectrum	Compound Structure

SI 691, RSI 905, mainlib, Entry# 27961, CAS# 629-66-3, 2-Nonadecanone

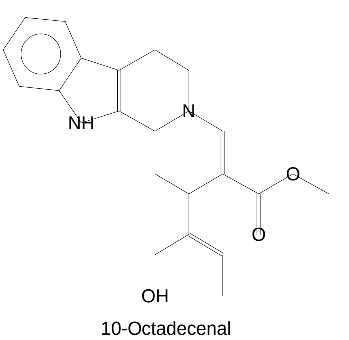


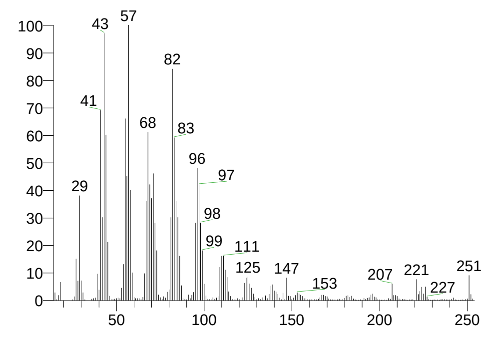
2-Nonadecanone
Formula C19H38O, MW 282, CAS# 629-66-3, Entry# 27961
Methyl heptadecyl ketone



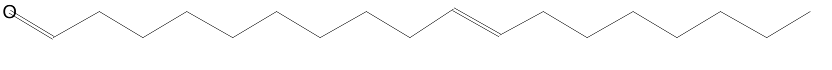


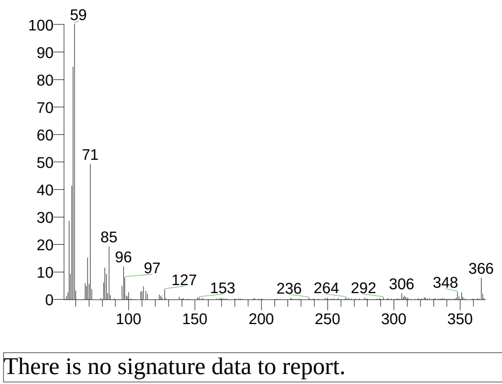
Formula C21H24N2O3, MW 352, CAS# 5523-49-9, Entry# 24103



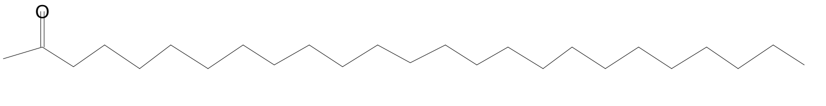


10-Octadecenal
Formula C18H34O, MW 266, CAS# 56554-92-8, Entry# 24429
(10E)-10-Octadecenal #





2-Pentacosanone
Formula C25H50O, MW 366, CAS# 75207-54-4, Entry# 30016
$:28CDTIEPBCRWXMBD-UHFFFAOYSA-N



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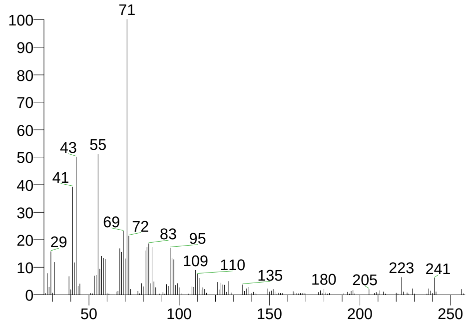
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

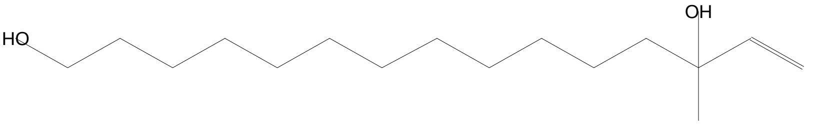
Stem pet ether

Hit Spectrum



Compound Structure

13-Methylpentadec-14-ene-1,13-diol
Formula C16H32O2, MW 256, CAS# NA, Entry# 38727
13-Methyl-14-pentadecene-1,13-diol #



There is no signature data to report.

Library Search Report

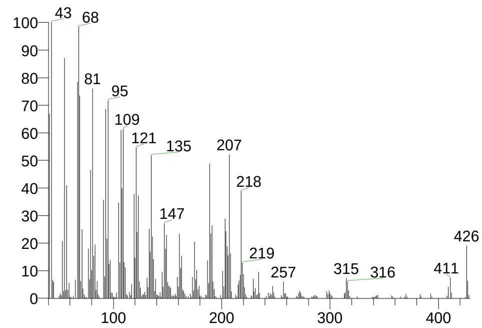
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

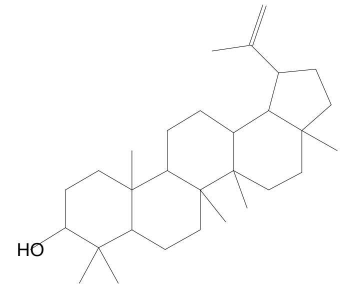
RT	Compound Name	Area	Area %
43.99	4,4,6a,6b,8a,11,12,14b-Octamethyl-docosahydropicene-3,13-diol	1027664766.41	0.39
43.99	Lup-20(29)-en-3-ol, acetate, (3á)-	1027664766.41	0.39
43.99	Lupeol	1027664766.41	0.39
43.99	Olean-12-ene-3,28-diol, (3á)-	1027664766.41	0.39
43.99	á-D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate	1027664766.41	0.39

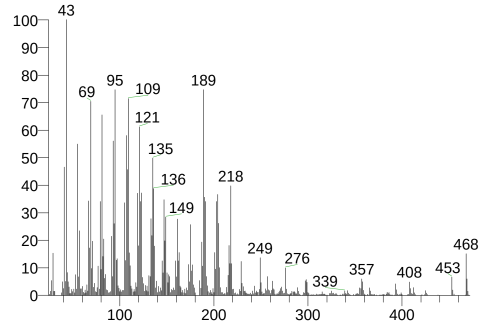
Hit Spectrum | Compound Structure

SI 708, RSI 758, replib, Entry# 2497, CAS# 545-47-1, Lupeol

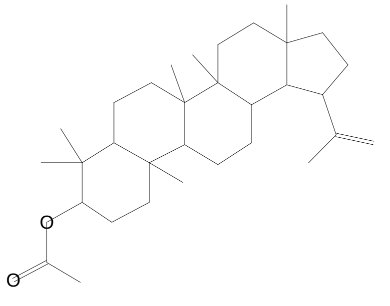


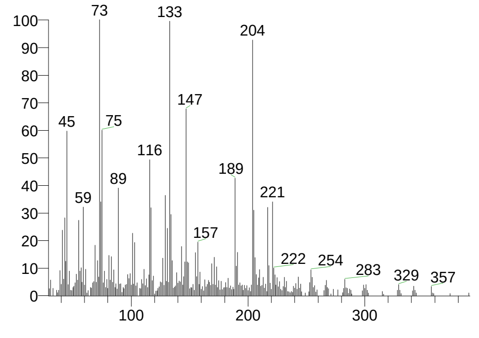
Lupeol
Formula $C_{30}H_{50}O$, MW 426, CAS# 545-47-1, Entry# 2497
Lup-20(29)-en-3-ol, (3á)-



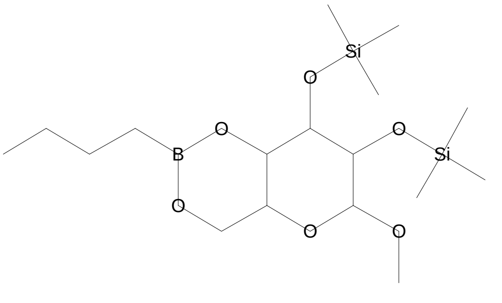


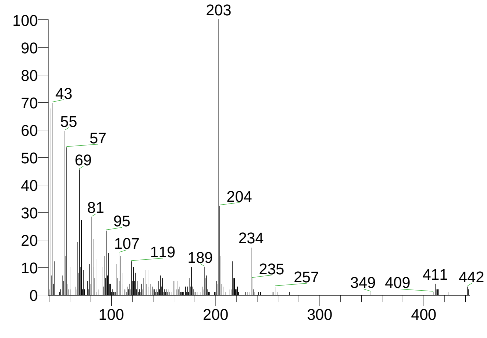
Lup-20(29)-en-3-ol, acetate, (3á)-
Formula $C_{32}H_{52}O_2$, MW 468, CAS# 1617-68-1, Entry# 13363
Lup-20(29)-en-3á-ol, acetate



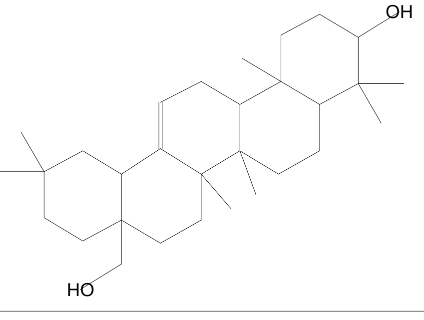


á-D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate
Formula $C_{17}H_{37}BO_6Si_2$, MW 404, CAS# 56211-10-0, Entry# 42381





Olean-12-ene-3,28-diol, (3á)-
Formula $C_{30}H_{50}O_2$, MW 442, CAS# 545-48-2, Entry# 185945
Olean-12-ene-3á,28-diol



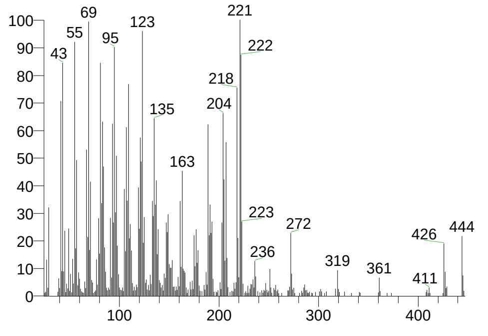
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

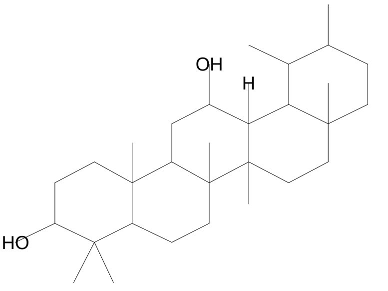
Stem pet ether

Hit Spectrum



Compound Structure

4,4,6a,6b,8a,11,12,14b-Octamethyl-docosahydropicene-3,13-diol
Formula C30H52O2, MW 444, CAS# NA, Entry# 196425
Ursane-3,12-diol #



There is no signature data to report.

Library Search Report

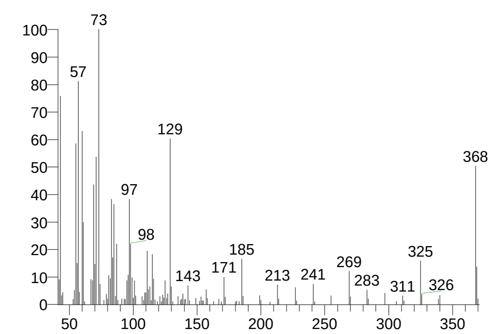
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

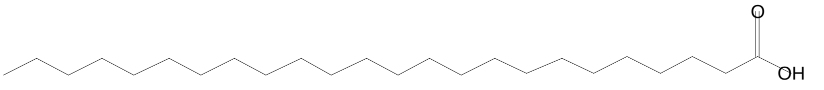
RT	Compound Name	Area	Area %
44.44	Docosanoic acid	911193390.39	0.35
44.44	Eicosanoic acid	911193390.39	0.35
44.44	Tetracosanoic acid	911193390.39	0.35
44.44	Tetracosanoic acid	911193390.39	0.35
44.44	Tetracosanoic acid, isopropyl ester	911193390.39	0.35

Hit Spectrum	Compound Structure

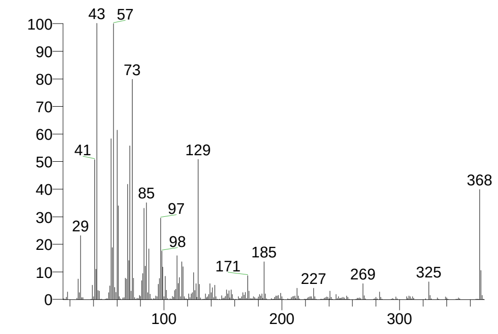
SI 790, RSI 906, replib, Entry# 9603, CAS# 557-59-5, Tetracosanoic acid



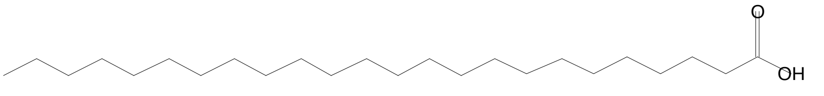
Tetracosanoic acid
Formula C24H48O2, MW 368, CAS# 557-59-5, Entry# 9603
Lignoceric acid



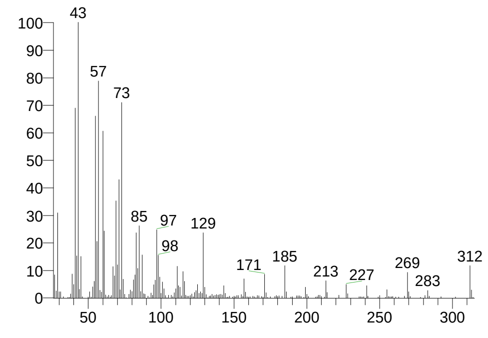
SI 773, RSI 798, mainlib, Entry# 7898, CAS# 557-59-5, Tetracosanoic acid



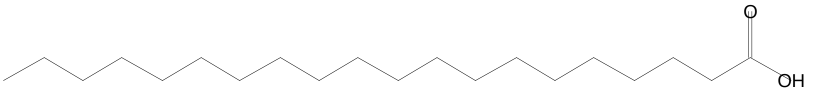
Tetracosanoic acid
Formula C24H48O2, MW 368, CAS# 557-59-5, Entry# 7898
Lignoceric acid

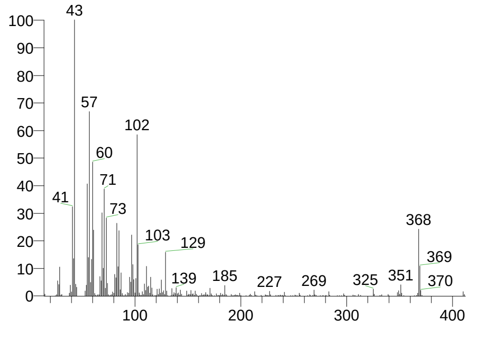


SI 728, RSI 822, mainlib, Entry# 7899, CAS# 506-30-9, Eicosanoic acid

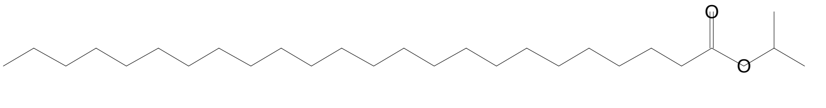


Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 7899
Arachic acid





Tetracosanoic acid, isopropyl ester
Formula C27H54O2, MW 410, CAS# NA, Entry# 7972
$:28GLLQWXVDRFCXDF-UHFFFAOYSA-N



There is no signature data to report.

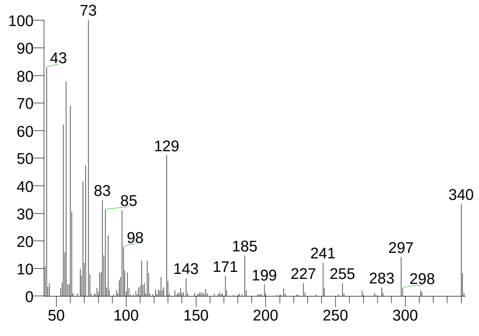
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

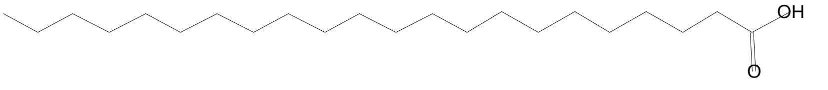
Hit Spectrum

SI 714, RSI 840, replib, Entry# 9514, CAS# 112-85-6, Docosanoic acid



Compound Structure

Docosanoic acid
Formula C22H44O2, MW 340, CAS# 112-85-6, Entry# 9514
n-Docosanoic acid



There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

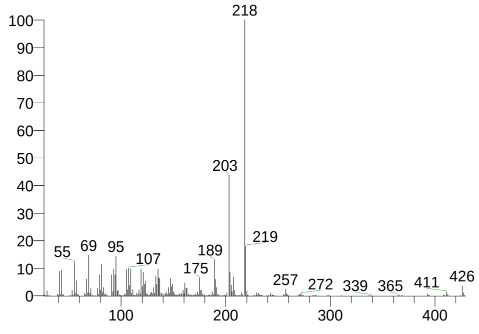
Stem pet ether

RT	Compound Name	Area	Area %
44.57	24-Noroleana-3,12-diene	2388439824.73	0.91
44.57	Olean-12-en-3-ol, acetate, (3á)-	2388439824.73	0.91
44.57	à-Amyrin	2388439824.73	0.91
44.57	à-Amyrin	2388439824.73	0.91
44.57	á-Amyrin	2388439824.73	0.91

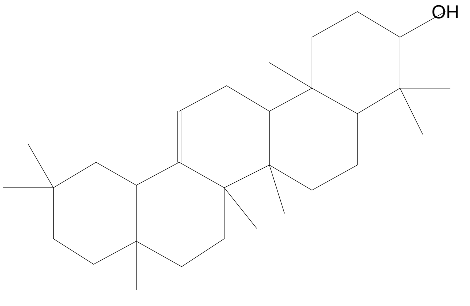
Hit Spectrum

Compound Structure

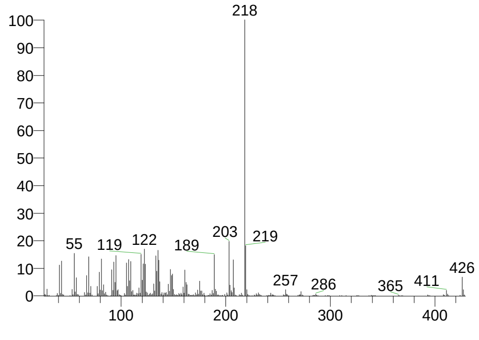
SI 885, RSI 899, mainlib, Entry# 195039, CAS# 559-70-6, á-Amyrin



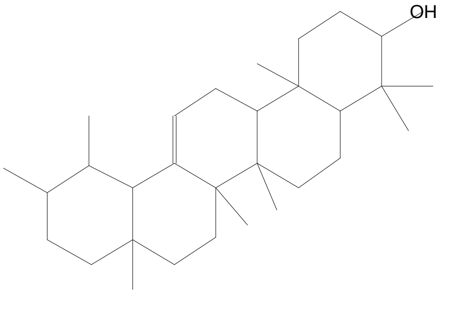
á-Amyrin
Formula C30H50O, MW 426, CAS# 559-70-6, Entry# 195039
Olean-12-en-3-ol, (3á)-

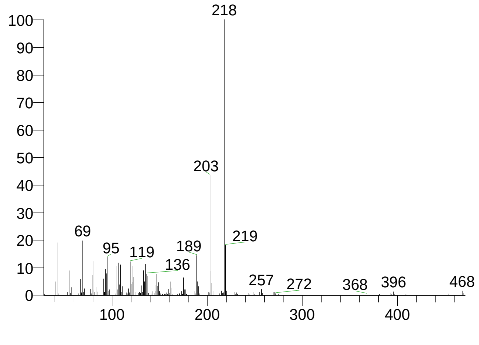


SI 849, RSI 863, mainlib, Entry# 195041, CAS# 638-95-9, à-Amyrin

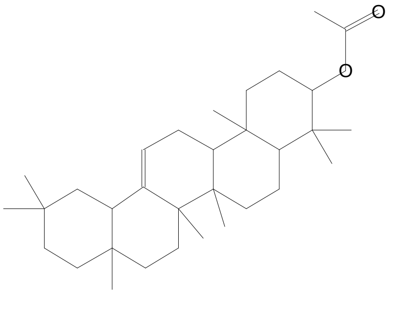


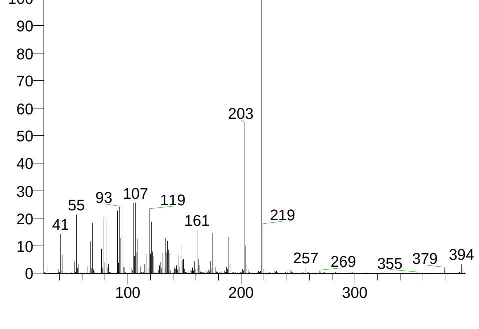
à-Amyrin
Formula C30H50O, MW 426, CAS# 638-95-9, Entry# 195041
Urs-12-en-3-ol, (3á)-



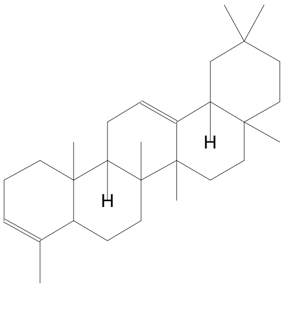


Olean-12-en-3-ol, acetate, (3á)-
Formula C32H52O2, MW 468, CAS# 1616-93-9, Entry# 195016
Olean-12-en-3á-ol, acetate





24-Noroleana-3,12-diene
Formula C29H46, MW 394, CAS# 201358-24-9, Entry# 195020



There is no signature data to report.

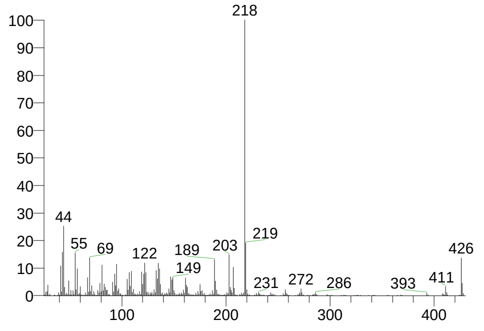
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

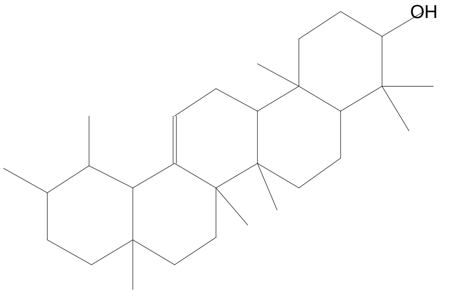
Hit Spectrum

SI 834, RSI 837, replib, Entry# 29720, CAS# 638-95-9, à-Amyrin



Compound Structure

à-Amyrin
Formula C30H50O, MW 426, CAS# 638-95-9, Entry# 29720
Urs-12-en-3-ol, (3á)-



There is no signature data to report.

Library Search Report

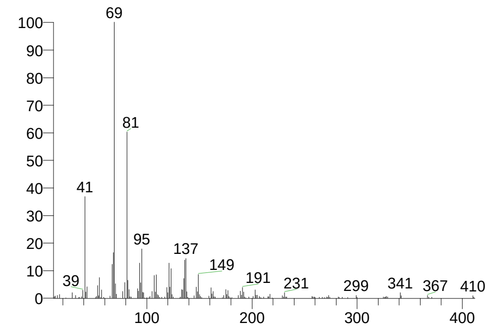
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

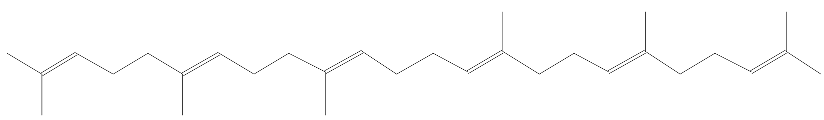
RT	Compound Name	Area	Area %
45.18	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	816109153.05	0.31
45.18	Squalene	816109153.05	0.31
45.18	Squalene	816109153.05	0.31
45.18	Squalene	816109153.05	0.31
45.18	Squalene	816109153.05	0.31

Hit Spectrum	Compound Structure

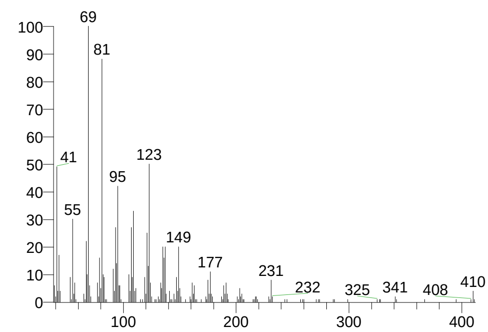
SI 830, RSI 897, replib, Entry# 8708, CAS# 111-02-4, Squalene



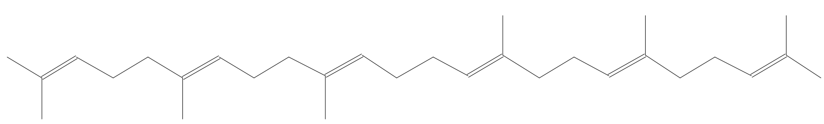
Squalene
Formula C30H50, MW 410, CAS# 111-02-4, Entry# 8708
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-



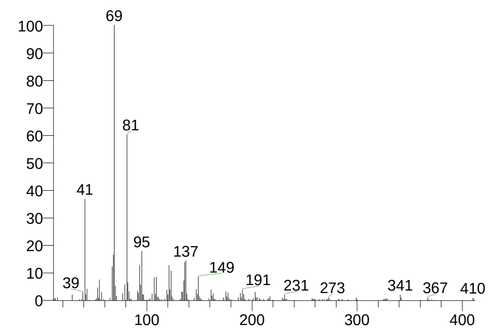
SI 827, RSI 886, replib, Entry# 8718, CAS# 111-02-4, Squalene



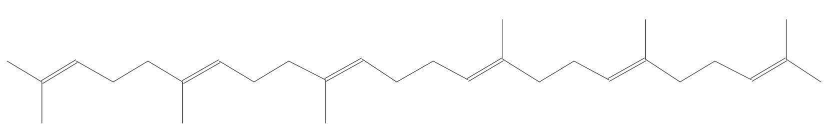
Squalene
Formula C30H50, MW 410, CAS# 111-02-4, Entry# 8718
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-



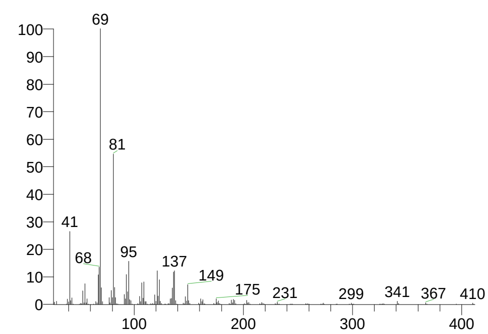
SI 826, RSI 892, replib, Entry# 8706, CAS# 111-02-4, Squalene



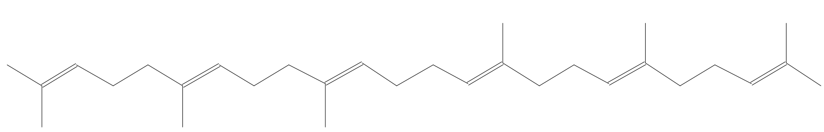
Squalene
Formula C30H50, MW 410, CAS# 111-02-4, Entry# 8706
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-



SI 818, RSI 882, mainlib, Entry# 35390, CAS# 111-02-4, Squalene



Squalene
Formula C30H50, MW 410, CAS# 111-02-4, Entry# 35390
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-



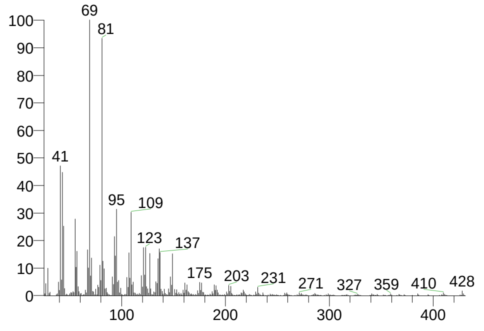
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

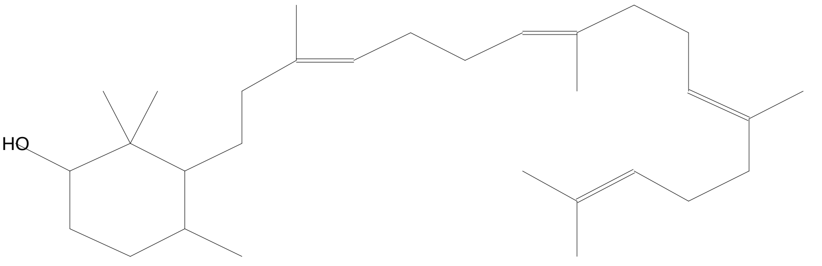
Stem pet ether

Hit Spectrum



Compound Structure

2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
Formula C30H52O, MW 428, CAS# NA, Entry# 35374
2,2,4-Trimethyl-3-[(3E,7E,11E)-3,8,12,16-tetramethyl-3,7,11,15-heptadecatetraenyl]cyclohexanol #



There is no signature data to report.

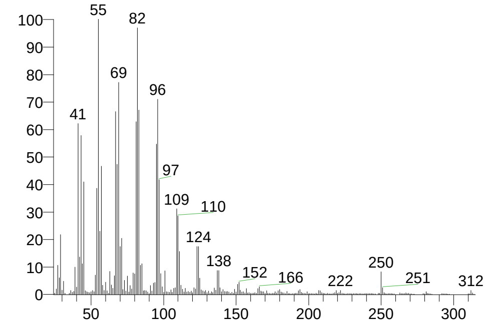
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

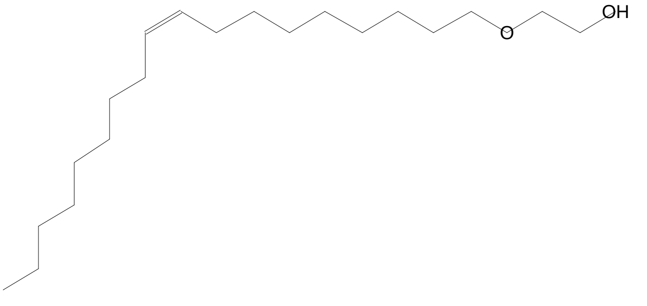
Stem pet ether

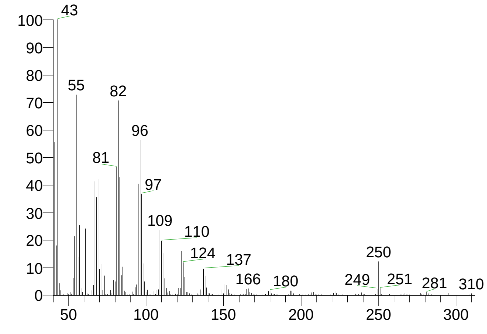
RT	Compound Name	Area	Area %
45.25	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	1418743616.93	0.54
45.25	Hexacosanal	1418743616.93	0.54
45.25	Octacosanal	1418743616.93	0.54
45.25	Tricosanal	1418743616.93	0.54
45.25	Z-14-Octadecen-1-ol acetate	1418743616.93	0.54

Hit Spectrum	Compound Structure

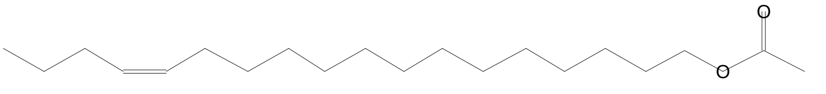


Ethanol, 2-(9-octadecenyloxy)-, (Z)-
Formula C20H40O2, MW 312, CAS# 5353-25-3, Entry# 20581
2-cis-9-Octadecenyloxyethanol

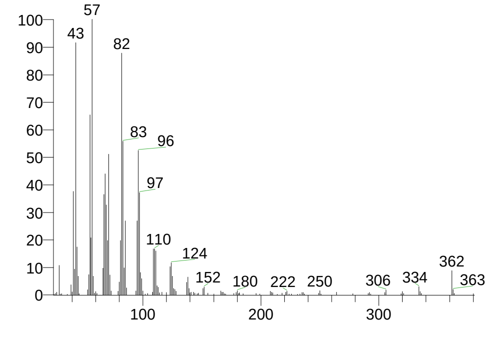




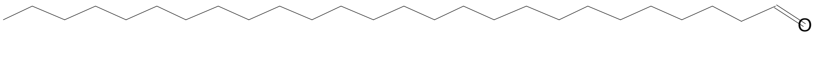
Z-14-Octadecen-1-ol acetate
Formula C20H38O2, MW 310, CAS# NA, Entry# 7356
(14Z)-14-Octadecenyl acetate #



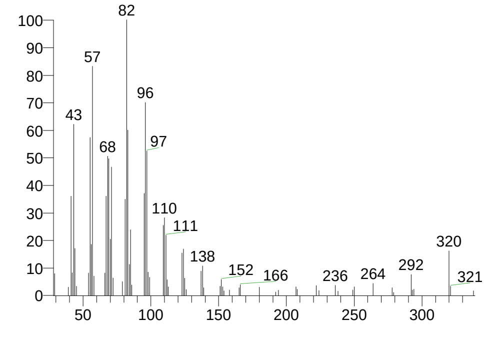
SI 787, RSI 883, mainlib, Entry# 24426, CAS# 26627-85-0, Hexacosanal



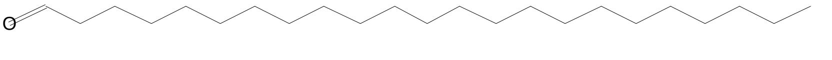
Hexacosanal
Formula C26H52O, MW 380, CAS# 26627-85-0, Entry# 24426
n-Hexacosanal



SI 784, RSI 914, mainlib, Entry# 51221, CAS# 72934-02-2, Tricosanal



Tricosanal
Formula C23H46O, MW 338, CAS# 72934-02-2, Entry# 51221
n-Tricosanal



There is no signature data to report.

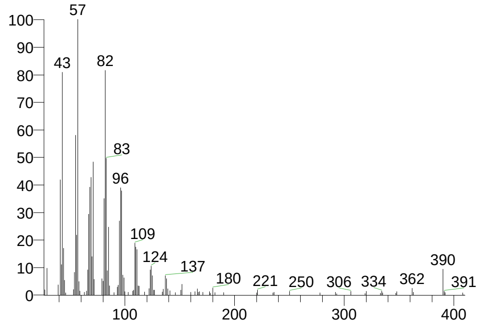
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

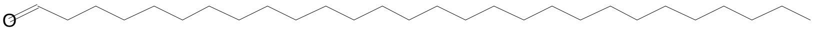
Hit Spectrum

SI 779, RSI 891, mainlib, Entry# 25706, CAS# 22725-64-0, Octacosanal



Compound Structure

Octacosanal
Formula C28H56O, MW 408, CAS# 22725-64-0, Entry# 25706
n-Octacosanal



There is no signature data to report.

Library Search Report

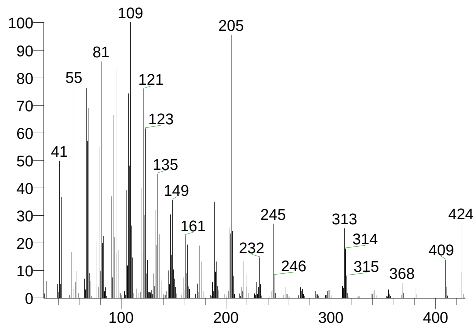
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

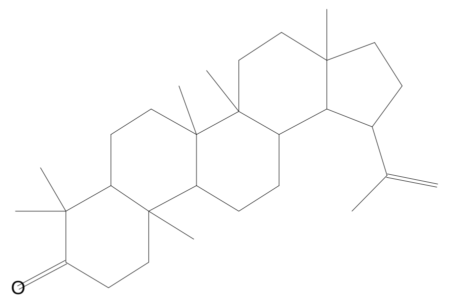
RT	Compound Name	Area	Area %
45.43	13,27-Cycloursan-3-one	2112079306.63	0.80
45.43	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	2112079306.63	0.80
45.43	Lup-20(29)-en-3-one	2112079306.63	0.80
45.43	Lup-20(29)-en-3-one	2112079306.63	0.80
45.43	Lup-20(29)-en-3-one	2112079306.63	0.80

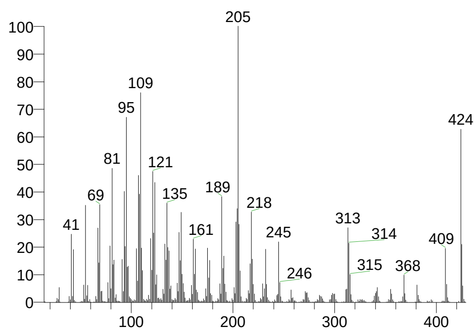
Hit Spectrum

Compound Structure

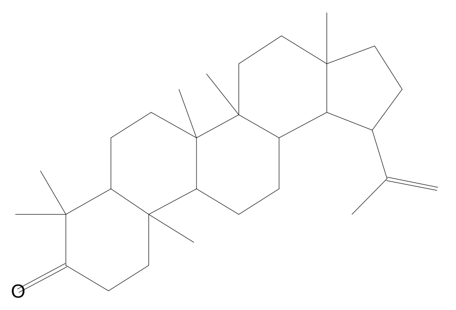


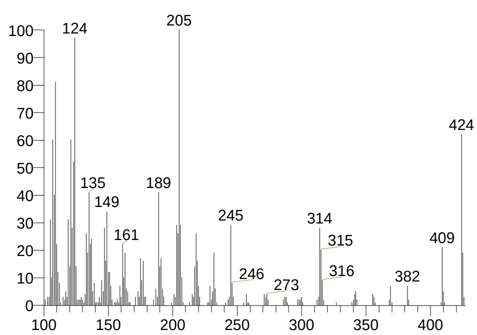
Lup-20(29)-en-3-one
Formula C30H48O, MW 424, CAS# 1617-70-5, Entry# 17408
Lup-20(30)-en-3-one



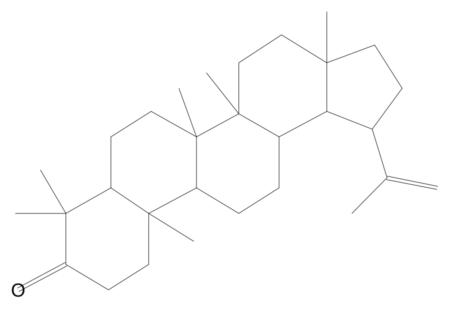


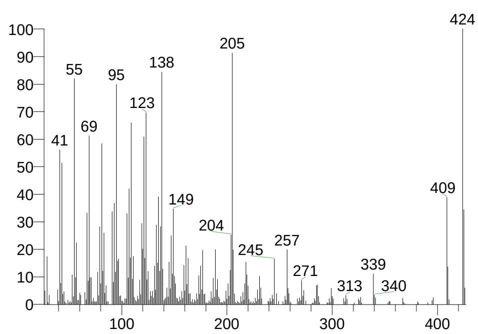
Lup-20(29)-en-3-one
Formula C30H48O, MW 424, CAS# 1617-70-5, Entry# 187299
Lup-20(30)-en-3-one



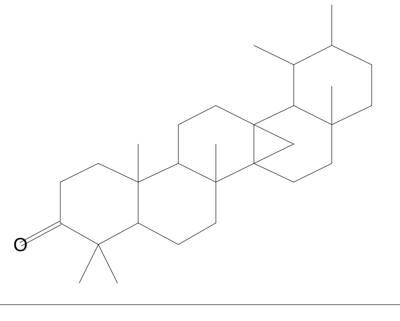


Lup-20(29)-en-3-one
Formula C30H48O, MW 424, CAS# 1617-70-5, Entry# 28970
Lup-20(30)-en-3-one





13,27-Cycloursan-3-one
Formula C30H48O, MW 424, CAS# NA, Entry# 239341
$:28VVSOUTKITCBWGW-UHFFFAOYSA-N



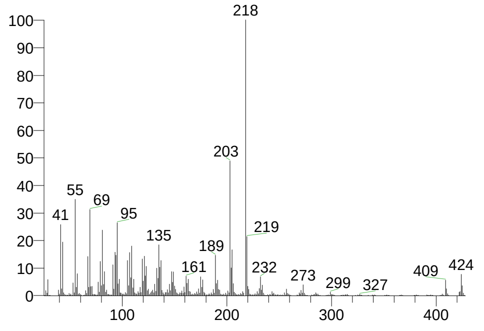
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

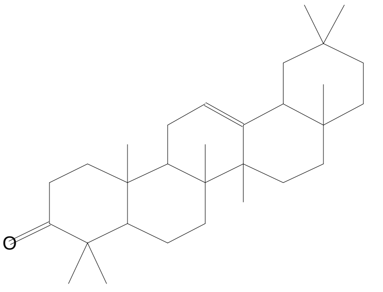
Stem pet ether

Hit Spectrum



Compound Structure

Formula C30H48O, MW 424, CAS# NA, Entry# 195013
Olean-12-en-3-one #



There is no signature data to report.

Library Search Report

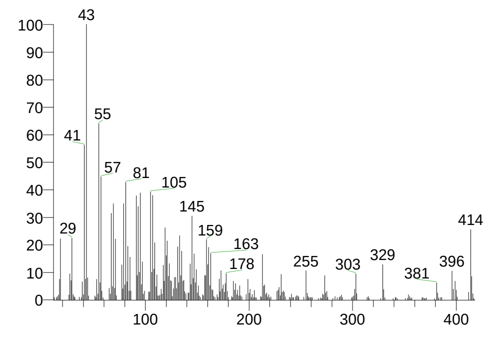
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

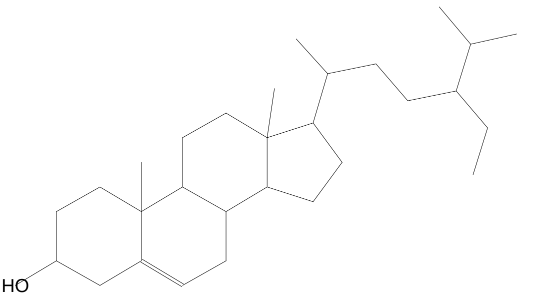
RT	Compound Name	Area	Area %
45.59	7,8-Epoxylanostan-11-ol, 3-acetoxy-	868874872.20	0.33
45.59	Ethyl iso-allocholate	868874872.20	0.33
45.59	Stigmasterol	868874872.20	0.33
45.59	á-Sitosterol	868874872.20	0.33
45.59	ç-Sitosterol	868874872.20	0.33

Hit Spectrum | Compound Structure

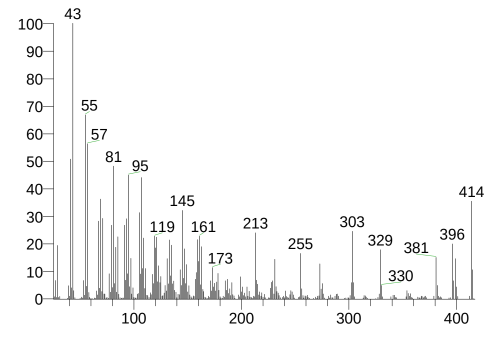
SI 759, RSI 813, replib, Entry# 2073, CAS# 83-46-5, á-Sitosterol



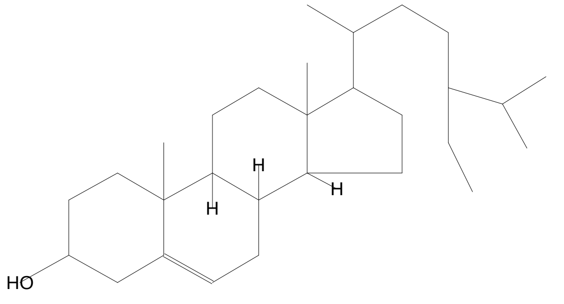
á-Sitosterol
Formula C29H50O, MW 414, CAS# 83-46-5, Entry# 2073
Stigmast-5-en-3-ol, (3á)-



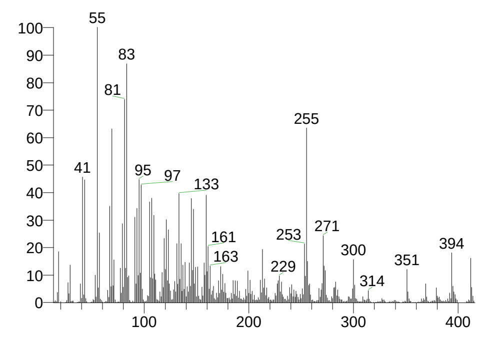
SI 736, RSI 764, mainlib, Entry# 7212, CAS# 83-47-6, ç-Sitosterol



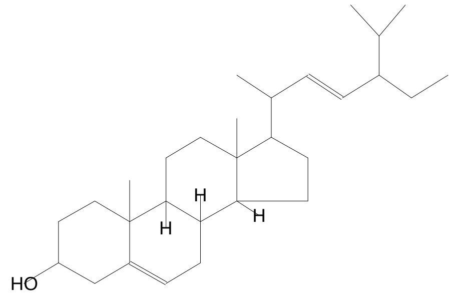
ç-Sitosterol
Formula C29H50O, MW 414, CAS# 83-47-6, Entry# 7212
Stigmast-5-en-3-ol, (3á,24S)-

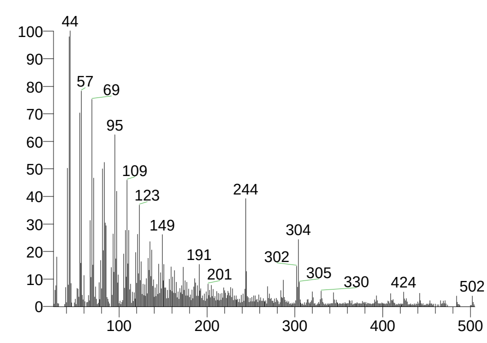


SI 723, RSI 740, replib, Entry# 5167, CAS# 83-48-7, Stigmasterol

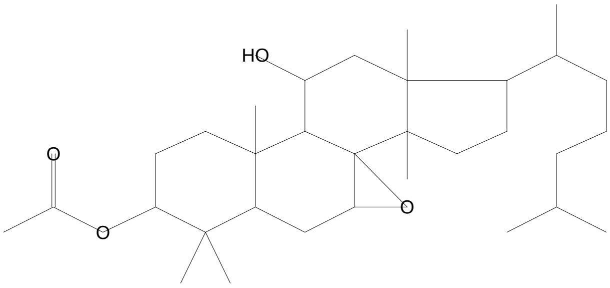


Stigmasterol
Formula C29H48O, MW 412, CAS# 83-48-7, Entry# 5167
Stigmasta-5,22-dien-3-ol, (3á,22E)-





7,8-Epoxylanostan-11-ol, 3-acetoxy-
Formula C32H54O4, MW 502, CAS# NA, Entry# 15359



There is no signature data to report.

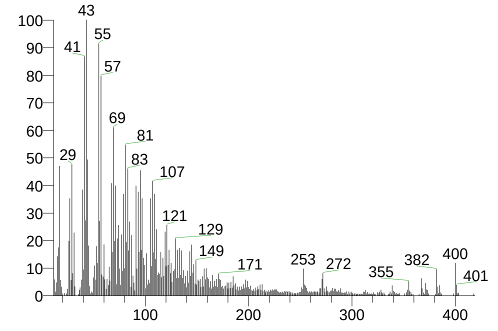
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

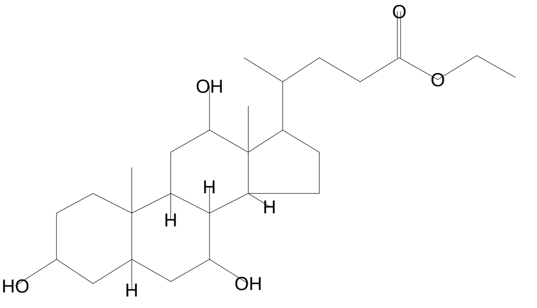
Hit Spectrum

SI 722, RSI 754, mainlib, Entry# 7020, CAS# NA, Ethyl iso-allocholate



Compound Structure

Ethyl iso-allocholate
Formula C26H44O5, MW 436, CAS# NA, Entry# 7020
Ethyl 3,7,12-trihydroxycholan-24-oate #



There is no signature data to report.

Library Search Report

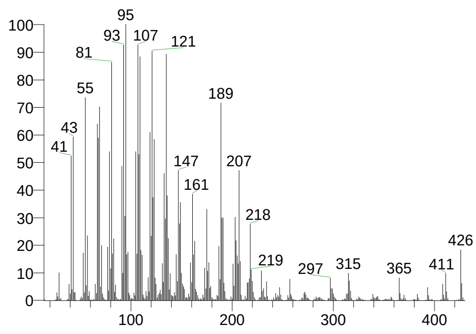
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

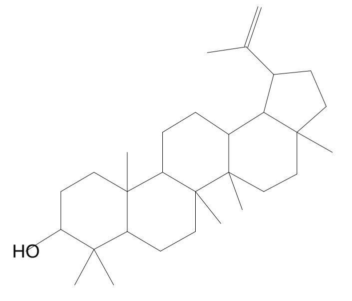
RT	Compound Name	Area	Area %
46.52	Betulin	45587144146.88	17.35
46.52	Betulin	45587144146.88	17.35
46.52	Betulinaldehyde	45587144146.88	17.35
46.52	Lupeol	45587144146.88	17.35
46.52	Lupeol	45587144146.88	17.35

Hit Spectrum	Compound Structure

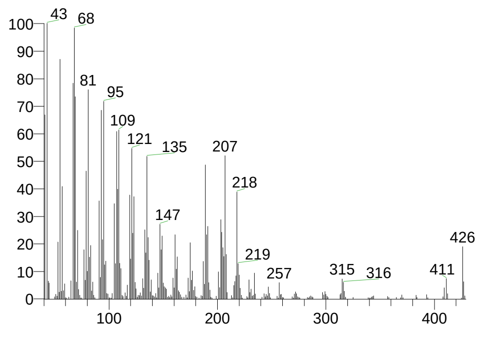
SI 924, RSI 924, mainlib, Entry# 68267, CAS# 545-47-1, Lupeol



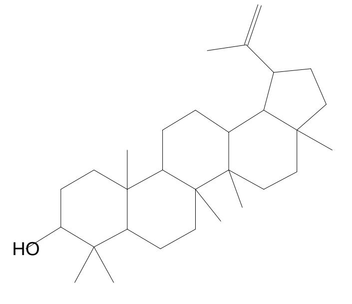
Lupeol
Formula C30H50O, MW 426, CAS# 545-47-1, Entry# 68267
Lup-20(29)-en-3-ol, (3á)-

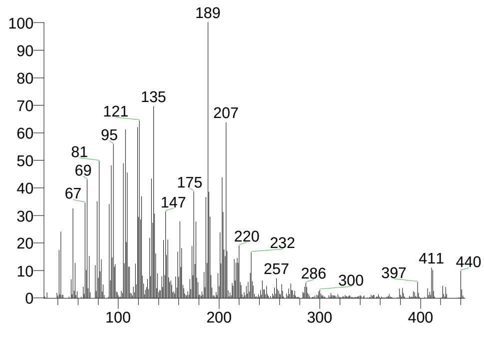


SI 923, RSI 930, replib, Entry# 2497, CAS# 545-47-1, Lupeol

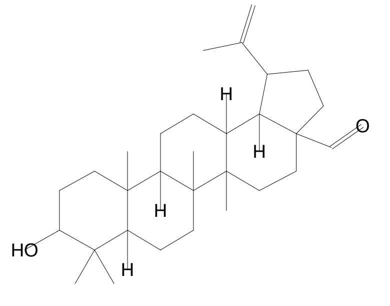


Lupeol
Formula C30H50O, MW 426, CAS# 545-47-1, Entry# 2497
Lup-20(29)-en-3-ol, (3á)-

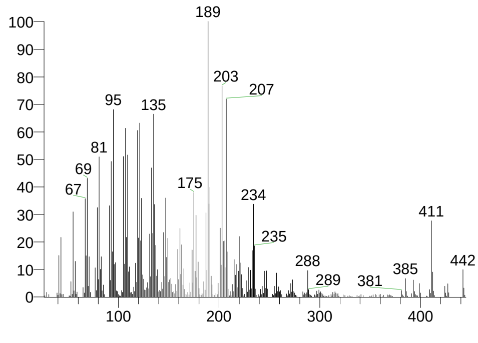




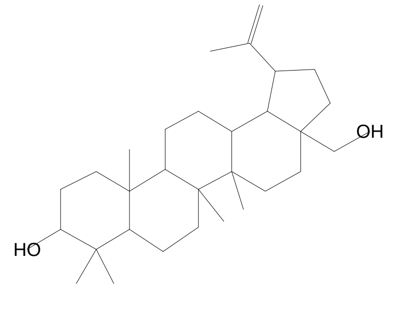
Betulinaldehyde
Formula C30H48O2, MW 440, CAS# 13159-28-9, Entry# 175929
$:28FELCJAPFJOPHSD-UHFFFAOYSA-N



SI 820, RSI 825, replib, Entry# 27797, CAS# 473-98-3, Betulin



Betulin
Formula C30H50O2, MW 442, CAS# 473-98-3, Entry# 27797
Lup-20(29)-ene-3,28-diol, (3á)-



There is no signature data to report.

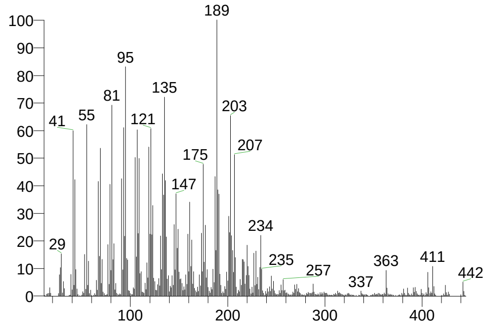
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

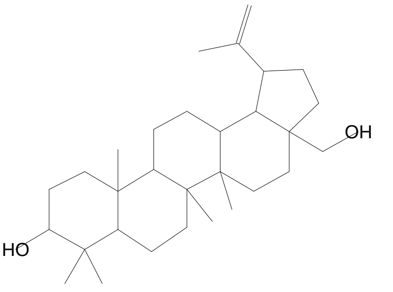
Hit Spectrum

SI 799, RSI 799, replib, Entry# 27738, CAS# 473-98-3, Betulin



Compound Structure

Betulin
Formula C30H50O2, MW 442, CAS# 473-98-3, Entry# 27738
Lup-20(29)-ene-3,28-diol, (3á)-



There is no signature data to report.

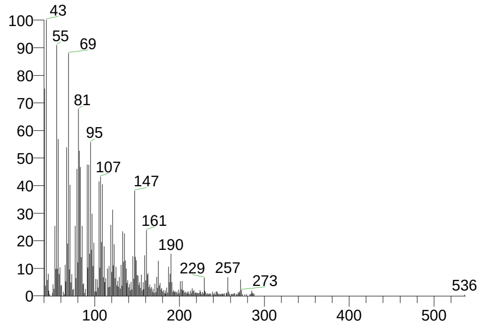
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

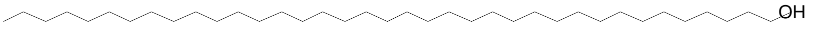
Stem pet ether

RT	Compound Name	Area	Area %
46.63	1-Heptatriacotanol	813715083.54	0.31
46.63	Betulin	813715083.54	0.31
46.63	Lupeol	813715083.54	0.31
46.63	Lupeol	813715083.54	0.31
46.63	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	813715083.54	0.31

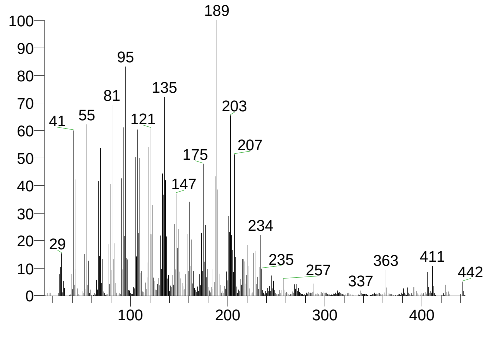
Hit Spectrum	Compound Structure



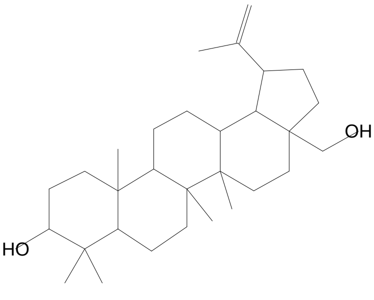
1-Heptatriacotanol
Formula C37H76O, MW 536, CAS# 105794-58-9, Entry# 7279
1-Heptatriacontanol #



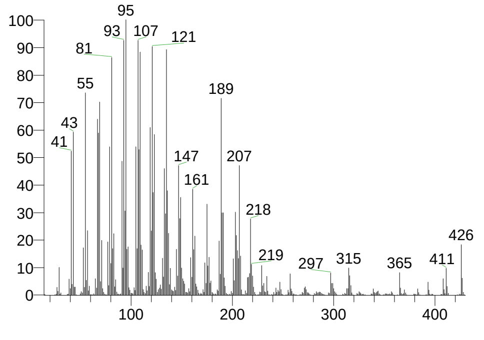
SI 752, RSI 753, replib, Entry# 27738, CAS# 473-98-3, Betulin



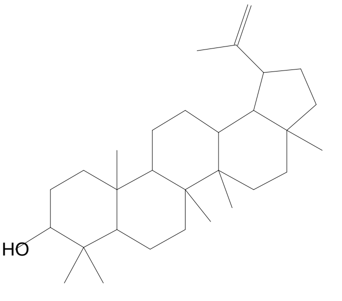
Betulin
Formula C30H50O2, MW 442, CAS# 473-98-3, Entry# 27738
Lup-20(29)-ene-3,28-diol, (3á)-

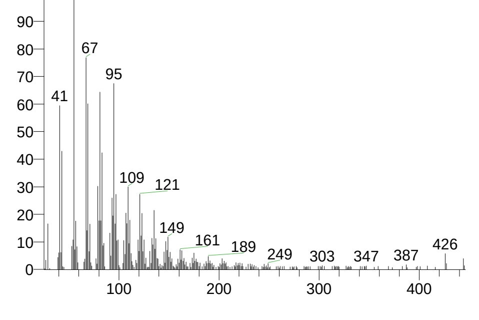


SI 751, RSI 761, mainlib, Entry# 68267, CAS# 545-47-1, Lupeol

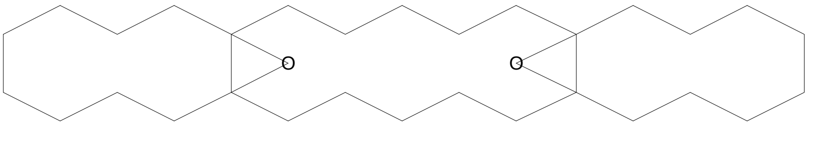


Lupeol
Formula C30H50O, MW 426, CAS# 545-47-1, Entry# 68267
Lup-20(29)-en-3-ol, (3á)-





Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-
Formula C30H52O2, MW 444, CAS# NA, Entry# 20028
$:28XVGPDAFFXRGERF-UHFFFAOYSA-N



There is no signature data to report.

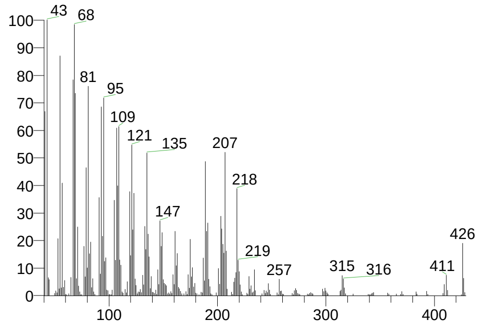
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

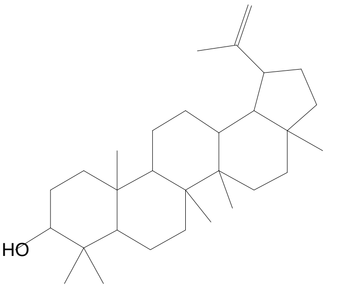
Hit Spectrum

SI 748, RSI 790, replib, Entry# 2497, CAS# 545-47-1, Lupeol



Compound Structure

Lupeol
Formula C30H50O, MW 426, CAS# 545-47-1, Entry# 2497
Lup-20(29)-en-3-ol, (3á)-



There is no signature data to report.

Library Search Report

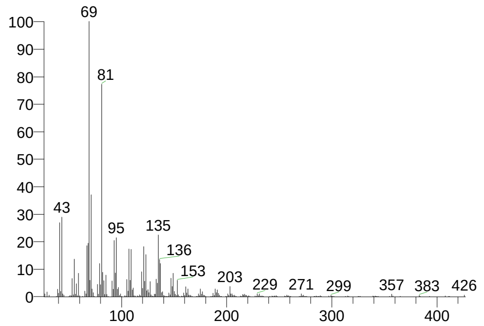
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

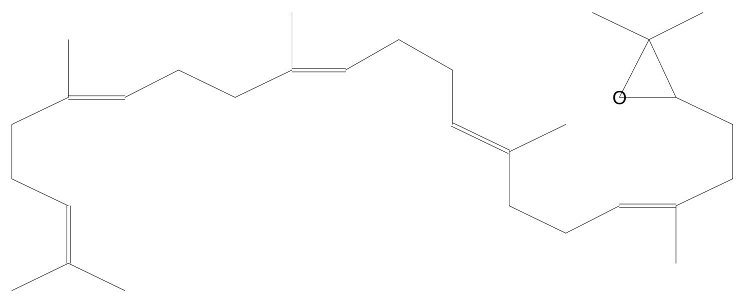
Stem pet ether

RT	Compound Name	Area	Area %
47.76	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	845851789.06	0.32
47.76	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(ñ)-	845851789.06	0.32
47.76	1-Heptatriacotanol	845851789.06	0.32
47.76	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	845851789.06	0.32
47.76	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	845851789.06	0.32

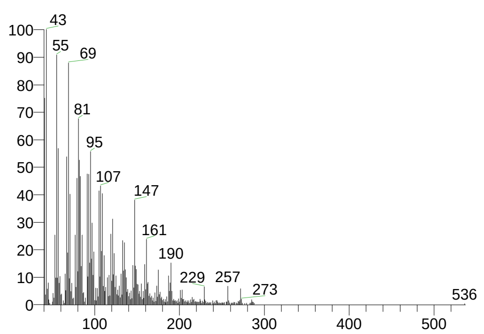
Hit Spectrum | Compound Structure

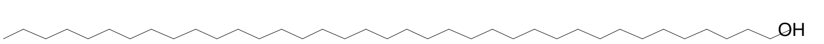
Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-
Formula C30H50O, MW 426, CAS# 7200-26-2, Entry# 8712



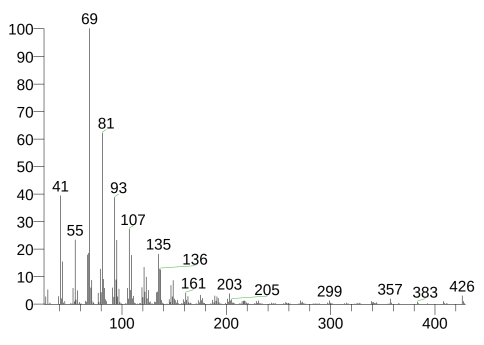


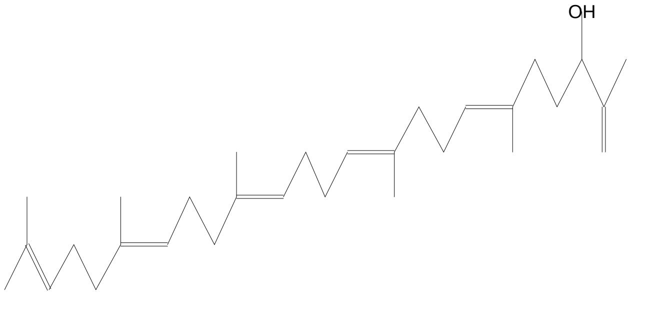
1-Heptatriacotanol
Formula C37H76O, MW 536, CAS# 105794-58-9, Entry# 7279
1-Heptatriacontanol #





1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-
Formula C30H50O, MW 426, CAS# 54159-46-5, Entry# 35385
(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol #





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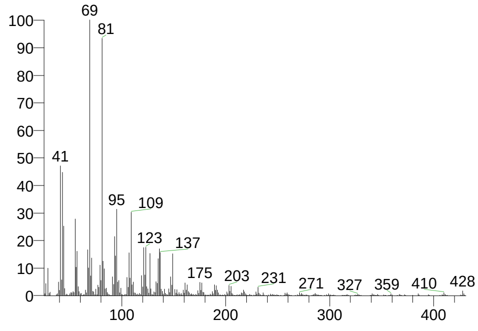
Library Search Report

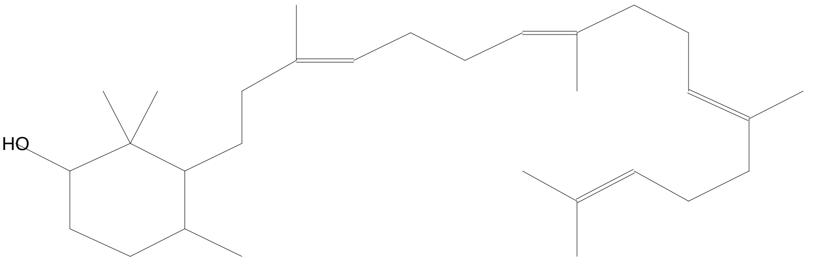
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

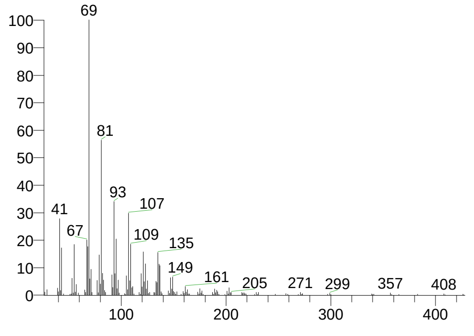
Stem pet ether

Hit Spectrum | Compound Structure

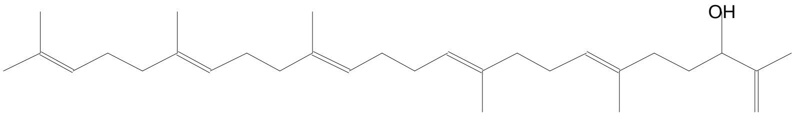


2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
Formula C30H52O, MW 428, CAS# NA, Entry# 35374
2,2,4-Trimethyl-3-[(3E,7E,11E)-3,8,12,16-tetramethyl-3,7,11,15-heptadecatetraenyl]cyclohexanol #





1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(ñ)-
Formula C30H50O, MW 426, CAS# 97232-74-1, Entry# 35419
$:28JLUBMMAQMKVTGL-RLROCYJYSA-N



There is no signature data to report.

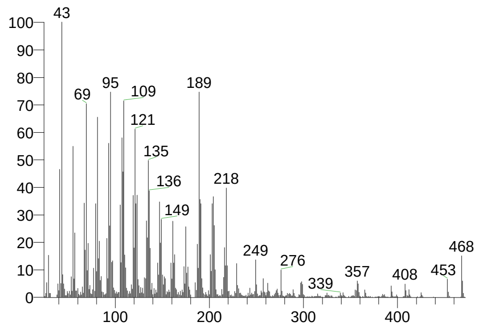
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

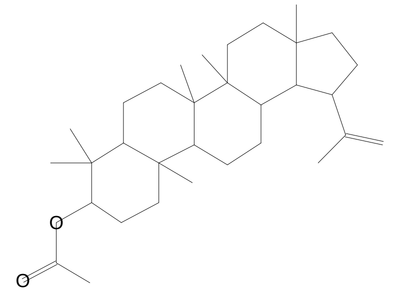
Stem pet ether

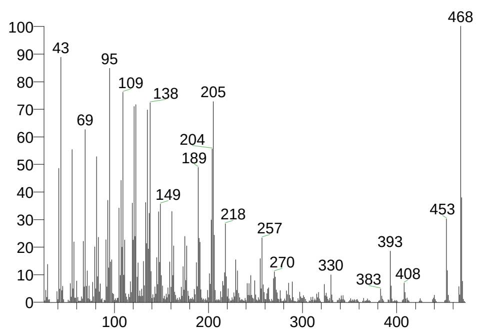
RT	Compound Name	Area	Area %
48.10	13,27-Cycloursan-3-ol, acetate, (3á,13á,14á)-	1026882436.55	0.39
48.10	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	1026882436.55	0.39
48.10	Lup-20(29)-en-3-ol, acetate, (3á)-	1026882436.55	0.39
48.10	Lup-20(29)-en-3-ol, acetate, (3á)-	1026882436.55	0.39
48.10	Lupeol	1026882436.55	0.39

Hit Spectrum	Compound Structure

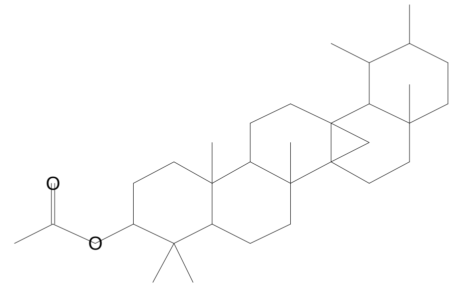


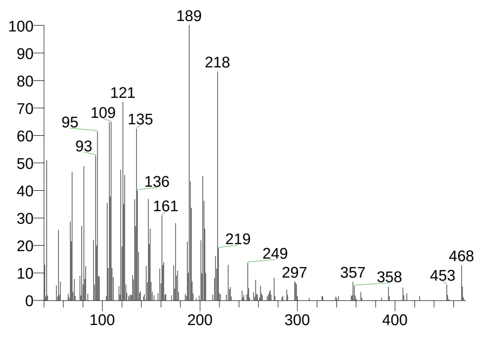
Lup-20(29)-en-3-ol, acetate, (3á)-
Formula C32H52O2, MW 468, CAS# 1617-68-1, Entry# 13363
Lup-20(29)-en-3á-ol, acetate



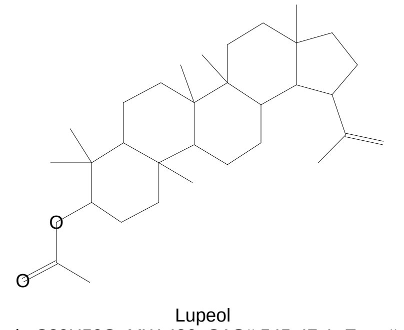


13,27-Cycloursan-3-ol, acetate, (3á,13á,14á)-
Formula C32H52O2, MW 468, CAS# 100324-79-6, Entry# 240766
$:28JLNPCDQETWAXSL-UHFFFAOYSA-N

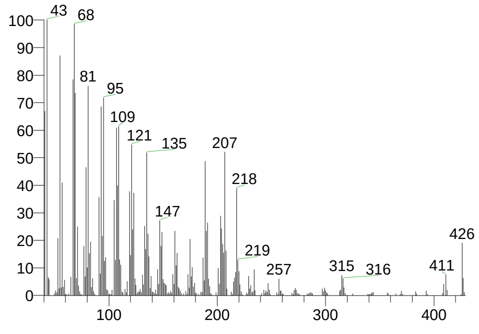




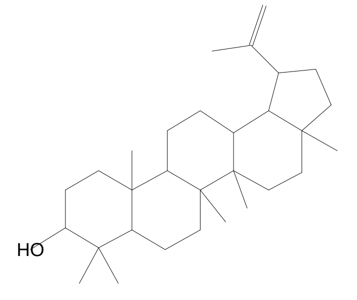
Lup-20(29)-en-3-ol, acetate, (3á)-
Formula C32H52O2, MW 468, CAS# 1617-68-1, Entry# 27803
Lup-20(29)-en-3á-ol, acetate



SI 739, RSI 782, replib, Entry# 2497, CAS# 545-47-1, Lupeol



Lupeol
Formula C30H50O, MW 426, CAS# 545-47-1, Entry# 2497
Lup-20(29)-en-3-ol, (3á)-



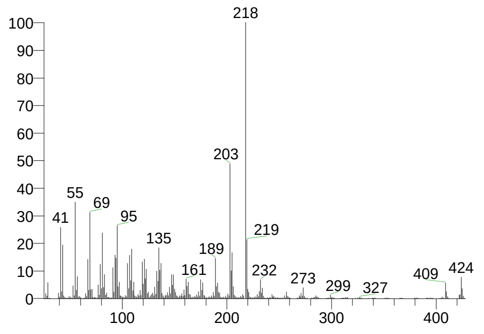
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

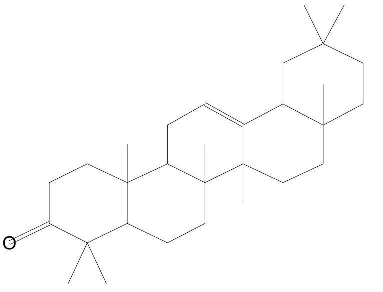
Stem pet ether

Hit Spectrum



Compound Structure

Formula C30H48O, MW 424, CAS# NA, Entry# 195013
Olean-12-en-3-one #



There is no signature data to report.

Library Search Report

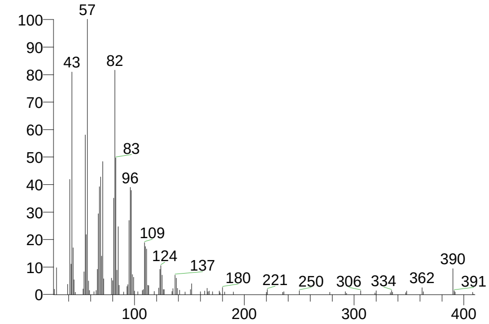
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

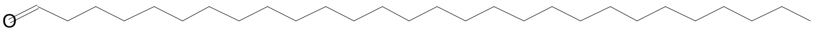
RT	Compound Name	Area	Area %
48.32	Docosanal	3809078510.55	1.45
48.32	Henicosanal	3809078510.55	1.45
48.32	Hexacosanal	3809078510.55	1.45
48.32	Octacosanal	3809078510.55	1.45
48.32	Tricosanal	3809078510.55	1.45

Hit Spectrum	Compound Structure

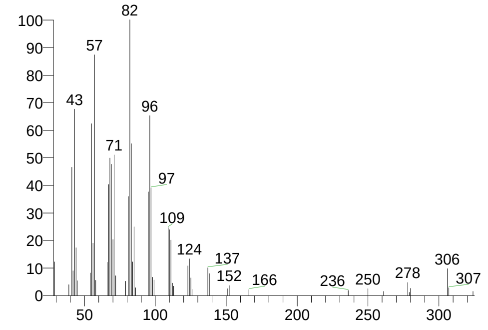
SI 808, RSI 902, mainlib, Entry# 25706, CAS# 22725-64-0, Octacosanal



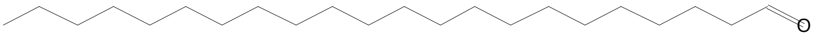
Octacosanal
Formula C28H56O, MW 408, CAS# 22725-64-0, Entry# 25706
n-Octacosanal



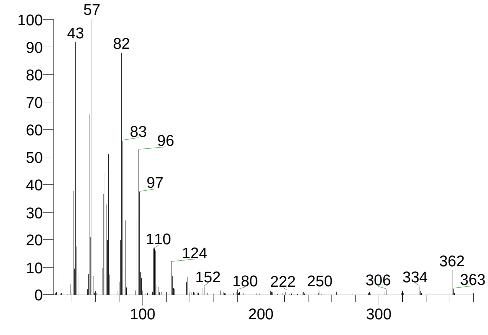
SI 797, RSI 941, mainlib, Entry# 51214, CAS# 57402-36-5, Docosanal



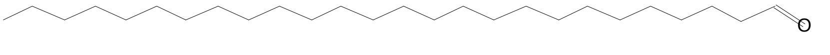
Docosanal
Formula C22H44O, MW 324, CAS# 57402-36-5, Entry# 51214
1-Docosanal



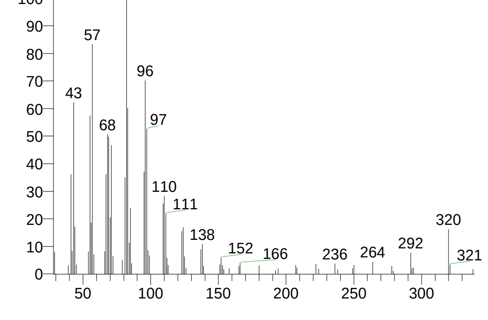
SI 795, RSI 877, mainlib, Entry# 24426, CAS# 26627-85-0, Hexacosanal



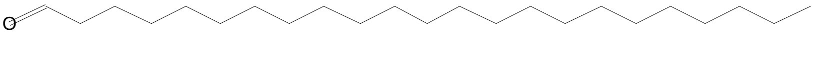
Hexacosanal
Formula C26H52O, MW 380, CAS# 26627-85-0, Entry# 24426
n-Hexacosanal



SI 793, RSI 909, mainlib, Entry# 51221, CAS# 72934-02-2, Tricosanal



Tricosanal
Formula C23H46O, MW 338, CAS# 72934-02-2, Entry# 51221
n-Tricosanal



There is no signature data to report.

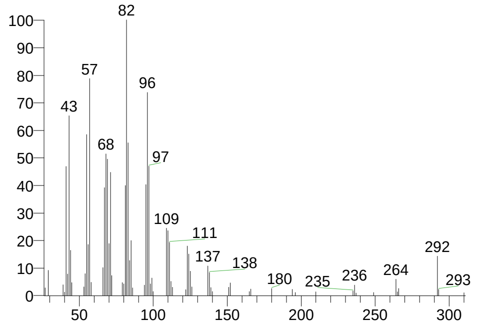
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

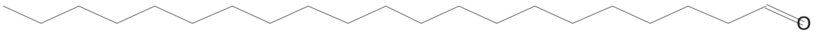
Hit Spectrum

SI 790, RSI 911, mainlib, Entry# 51223, CAS# 51227-32-8, Henicosanal



Compound Structure

Henicosanal
Formula C21H42O, MW 310, CAS# 51227-32-8, Entry# 51223
Heneicosanal



There is no signature data to report.

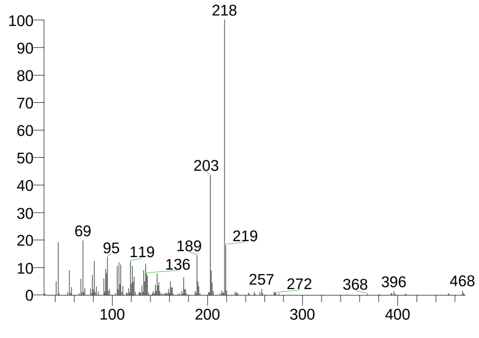
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

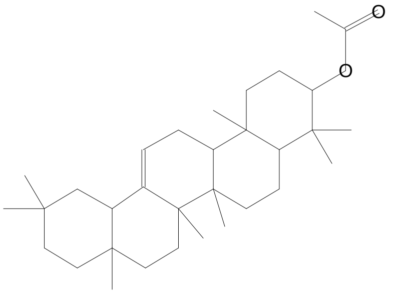
Stem pet ether

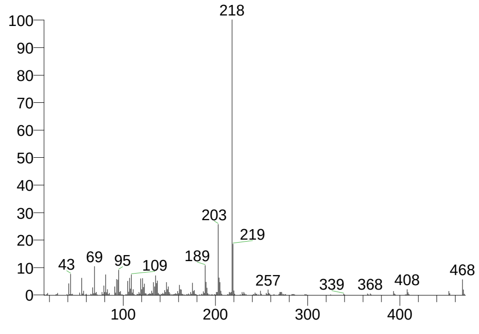
RT	Compound Name	Area	Area %
48.57	12-Oleanen-3-yl acetate, (3à)-	16398500024.88	6.24
48.57	24-Noroleana-3,12-diene	16398500024.88	6.24
48.57	Olean-12-en-3-ol, acetate, (3á)-	16398500024.88	6.24
48.57	á-Amyrin	16398500024.88	6.24
48.57	á-Amyrone	16398500024.88	6.24

Hit Spectrum | Compound Structure

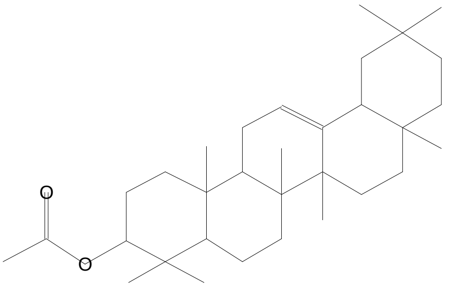


Olean-12-en-3-ol, acetate, (3á)-
Formula C32H52O2, MW 468, CAS# 1616-93-9, Entry# 195016
Olean-12-en-3á-ol, acetate

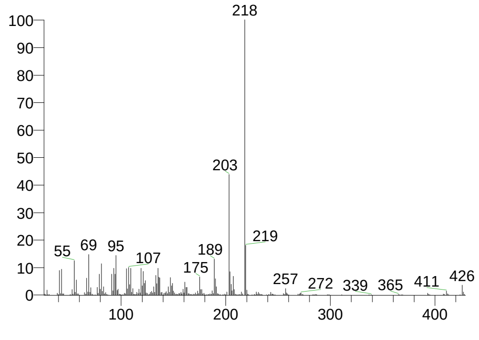




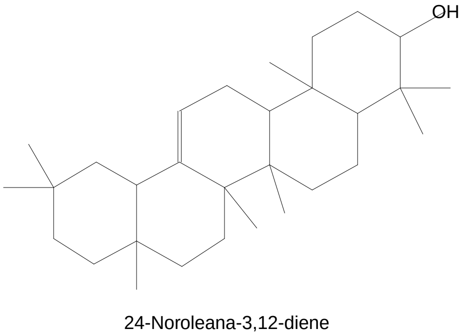
12-Oleanen-3-yl acetate, (3à)-
Formula C32H52O2, MW 468, CAS# 33055-28-6, Entry# 195038
Olean-12-en-3-yl acetate #

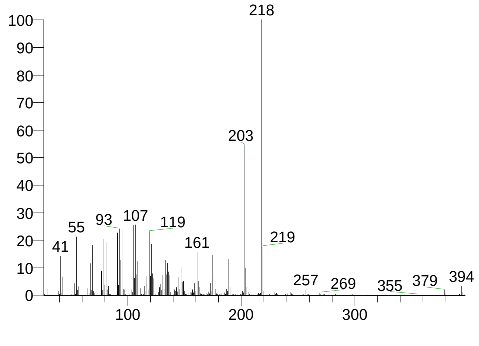


SI 887, RSI 894, mainlib, Entry# 195039, CAS# 559-70-6, á-Amyrin

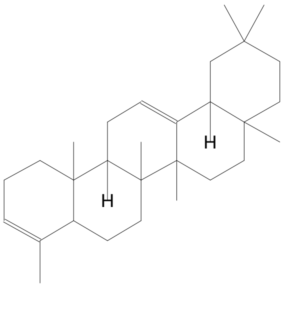


á-Amyrin
Formula C30H50O, MW 426, CAS# 559-70-6, Entry# 195039
Olean-12-en-3-ol, (3á)-





24-Noroleana-3,12-diene
Formula C29H46, MW 394, CAS# 201358-24-9, Entry# 195020



There is no signature data to report.

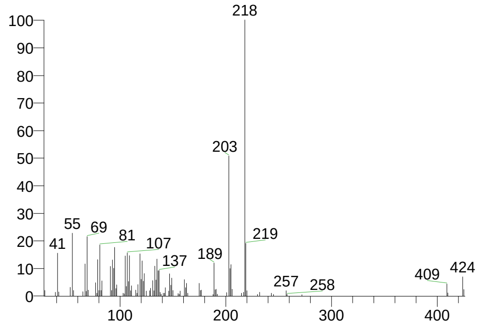
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

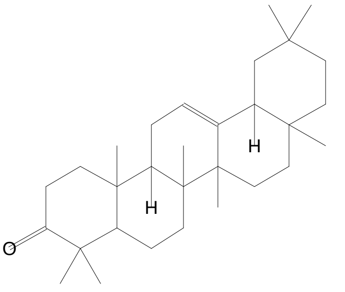
Hit Spectrum

SI 843, RSI 894, mainlib, Entry# 195015, CAS# 638-97-1, á-Amyrone



Compound Structure

á-Amyrone
Formula C30H48O, MW 424, CAS# 638-97-1, Entry# 195015



There is no signature data to report.

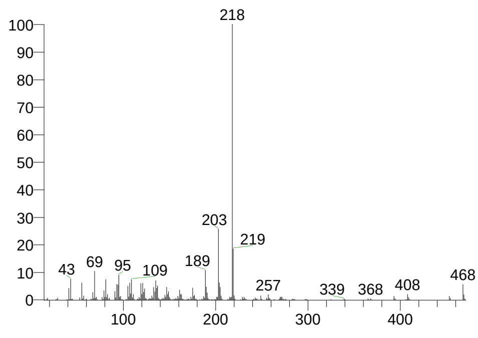
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

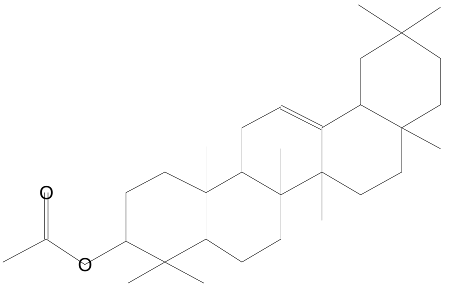
Stem pet ether

RT	Compound Name	Area	Area %
50.33	12-Oleanen-3-yl acetate, (3à)-	11777327712.13	4.48
50.33	24-Norursa-3,12-diene	11777327712.13	4.48
50.33	Olean-12-en-3-ol, acetate, (3á)-	11777327712.13	4.48
50.33	à-Amyrin	11777327712.13	4.48
50.33	á-Amyrin	11777327712.13	4.48

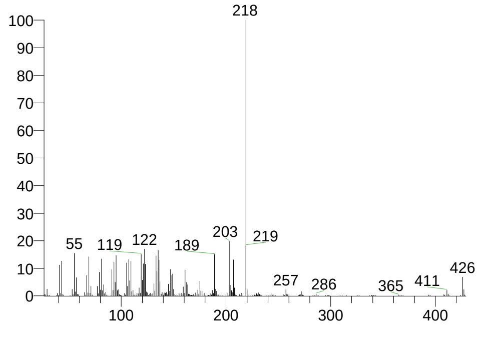
Hit Spectrum | Compound Structure



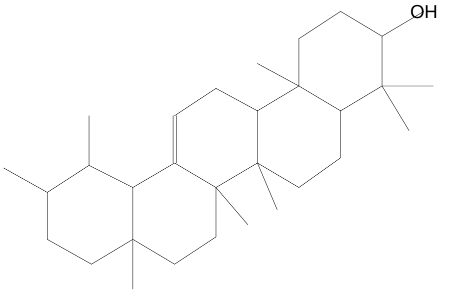
12-Oleanen-3-yl acetate, (3à)-
Formula C32H52O2, MW 468, CAS# 33055-28-6, Entry# 195038
Olean-12-en-3-yl acetate #

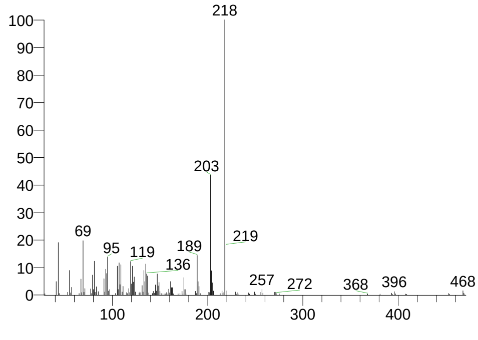


SI 799, RSI 831, mainlib, Entry# 195041, CAS# 638-95-9, à-Amyrin

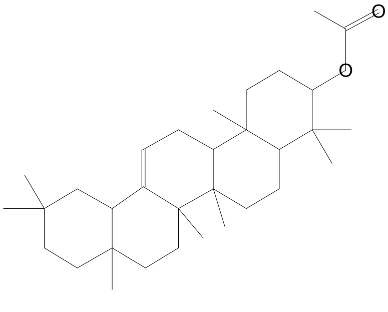


à-Amyrin
Formula C30H50O, MW 426, CAS# 638-95-9, Entry# 195041
Urs-12-en-3-ol, (3á)-

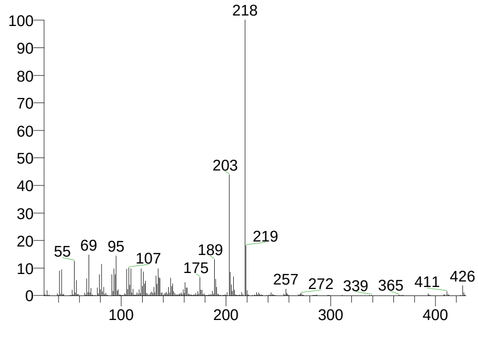




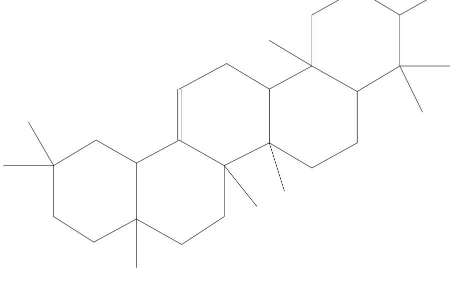
Olean-12-en-3-ol, acetate, (3á)-
Formula C32H52O2, MW 468, CAS# 1616-93-9, Entry# 195016
Olean-12-en-3á-ol, acetate



SI 798, RSI 830, mainlib, Entry# 195039, CAS# 559-70-6, á-Amyrin



á-Amyrin
Formula C30H50O, MW 426, CAS# 559-70-6, Entry# 195039
Olean-12-en-3-ol, (3á)-



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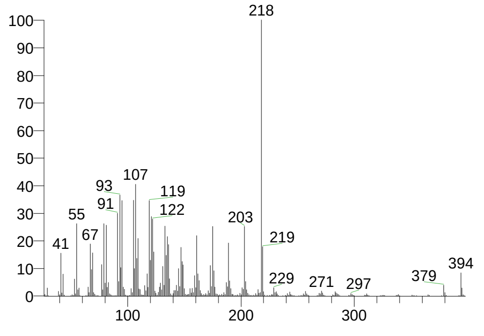
Library Search Report

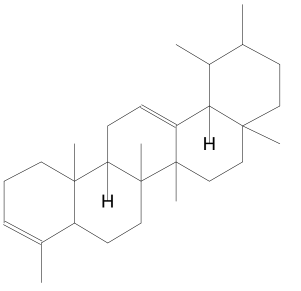
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

Hit Spectrum | Compound Structure



24-Norursa-3,12-diene
Formula C29H46, MW 394, CAS# 201358-25-0, Entry# 194805



There is no signature data to report.

Library Search Report

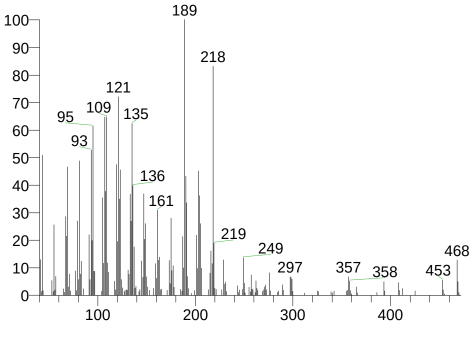
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Stem pet ether

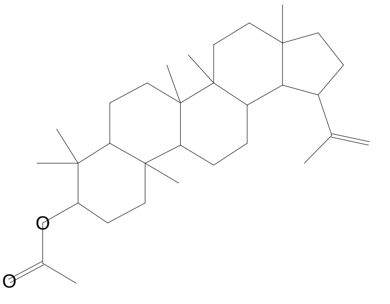
RT	Compound Name	Area	Area %
50.65	Acetic acid, 10-acetoxy-1,6a,6b,9,9,12a-hexamethyl-2-methylen-eicosahydro-picen-4a-ylm ethyl ester	25689542174.61	9.78
50.65	Lup-20(29)-en-3-ol, acetate, (3á)-	25689542174.61	9.78
50.65	Lup-20(29)-en-3-ol, acetate, (3á)-	25689542174.61	9.78
50.65	Lupeol	25689542174.61	9.78
50.65	Lupeol, trifluoroacetate	25689542174.61	9.78

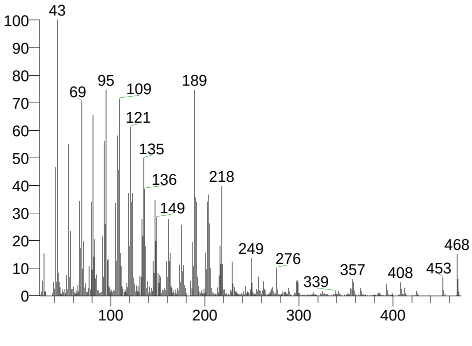
Hit Spectrum

Compound Structure

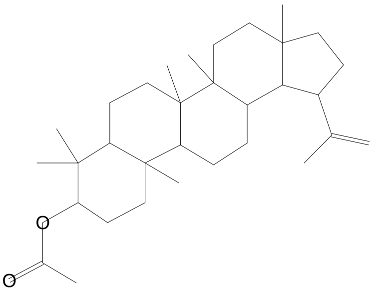


Lup-20(29)-en-3-ol, acetate, (3á)-
Formula C32H52O2, MW 468, CAS# 1617-68-1, Entry# 27803
Lup-20(29)-en-3á-ol, acetate

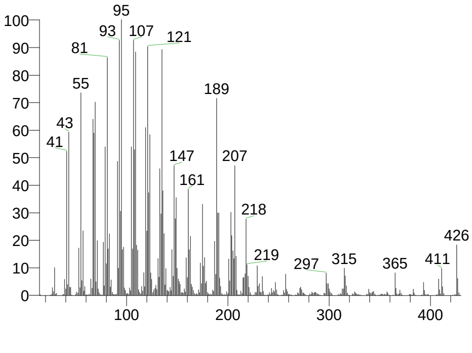




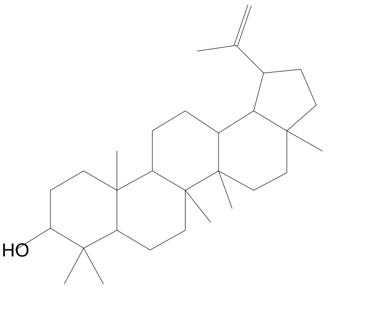
Lup-20(29)-en-3-ol, acetate, (3á)-
Formula C32H52O2, MW 468, CAS# 1617-68-1, Entry# 13363
Lup-20(29)-en-3á-ol, acetate



SI 814, RSI 824, mainlib, Entry# 68267, CAS# 545-47-1, Lupeol



Lupeol
Formula C30H50O, MW 426, CAS# 545-47-1, Entry# 68267
Lup-20(29)-en-3-ol, (3á)-



There is no signature data to report.

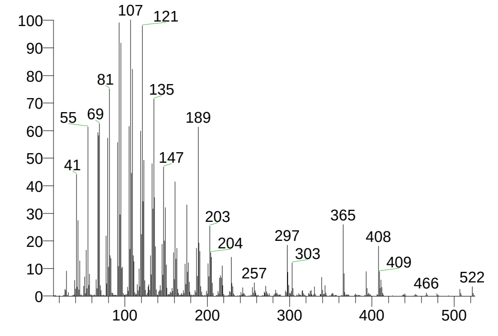
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

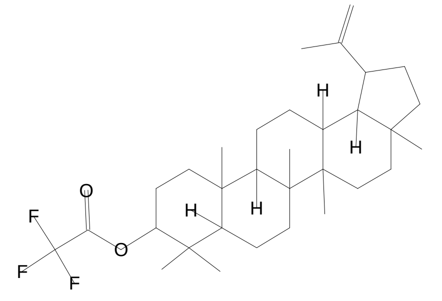
Stem pet ether

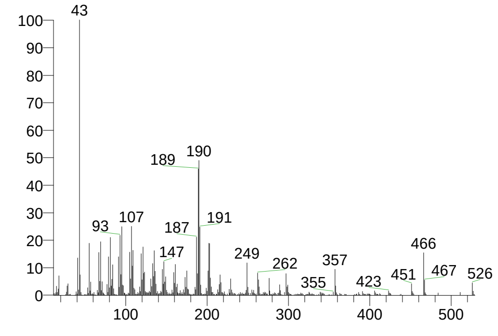
Hit Spectrum | Compound Structure

SI 794, RSI 811, mainlib, Entry# 83820, CAS# NA, Lupeol, trifluoroacetate

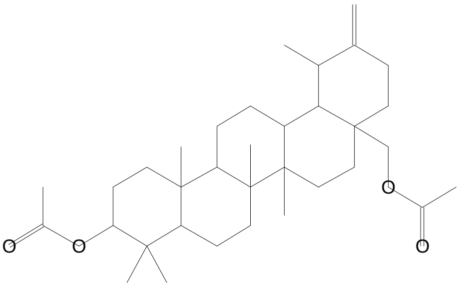


Lupeol, trifluoroacetate
Formula C32H49F3O2, MW 522, CAS# NA, Entry# 83820
$:28BOPCUTUKBZHEOW-UHFFFAOYSA-N





Formula C34H54O4, MW 526, CAS# NA, Entry# 13388
28-(Acetyloxy)urs-20(30)-en-3-yl acetate #



There is no signature data to report.

Appendix II

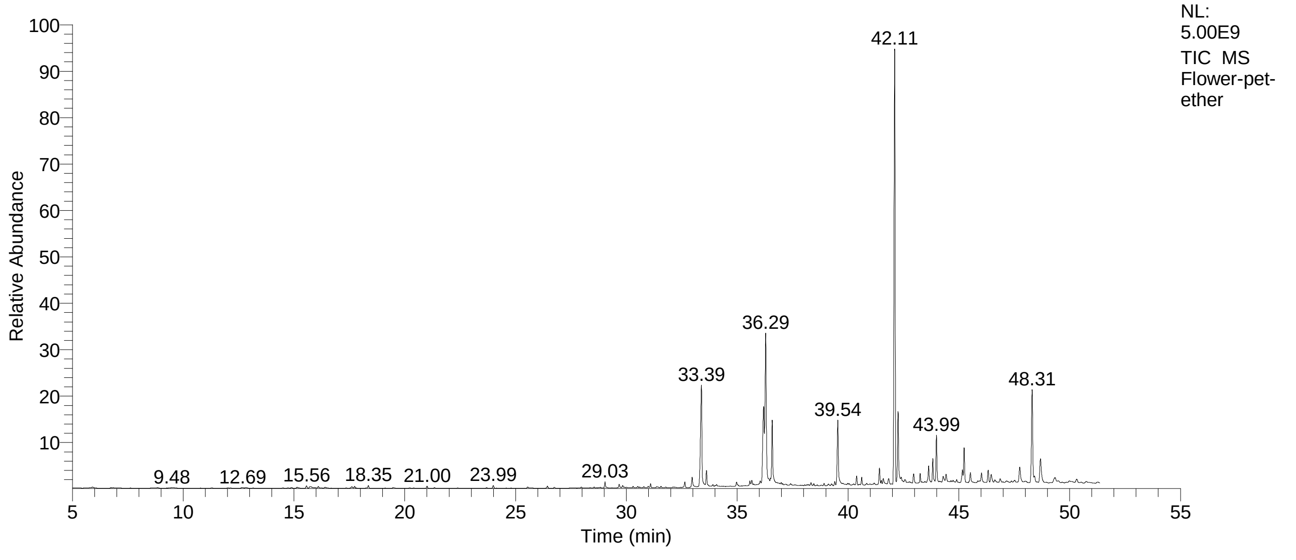
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

Instrument Model:	TSQ 8000	Instrument Serial Number:	1711009
Data File:	Flower-pet-ether	Original Data Path:	D:\Xcalibur\2019\May\Pest+Pyr+Safr\14-05-2019-scan\Data
Sample Type:	Unknown	Sample ID:	Flower pet ether
Sample Name:	Flower pet ether	Acquisition Date:	05/15/19 08:51:35 AM
Run Time(min):	48.36	Vial:	TrayHolder 2:Slot1:22
Scans:	14219	Instrument Name:CEGTH/INS/C/003	TSQ 8000

RT: 5.00 - 55.00 SM: 15G



RT	Peak Area	Peak Height	Area %
3.05	10287185889.68	1681182254.91	10.63
3.14	8647420779.30	1516798243.26	8.94
3.25	4494331440.59	1500990962.66	4.64
3.29	15049277192.11	1482688476.90	15.55
32.97	517355767.39	111258207.82	0.53
33.39	5226496172.97	1102170955.41	5.40
33.61	722142594.50	180882036.67	0.75
36.19	4000762666.36	853253163.26	4.13
36.29	7451090263.34	1644059392.92	7.70
36.58	2905408819.79	709134140.54	3.00
39.54	2920176160.79	708329032.80	3.02
41.42	558448836.68	180519183.37	0.58
42.11	16274256128.93	4688930463.23	16.82
42.26	2792730214.96	783440323.87	2.89
42.40	390905733.86	73655602.75	0.40
42.96	450137300.52	108175519.88	0.47
43.64	740318312.77	187999691.44	0.77
43.82	893079627.98	267004758.26	0.92
43.99	1636286733.14	517829163.03	1.69
44.42	418355774.28	94671840.17	0.43
45.16	511669171.33	139881382.70	0.53
45.24	1169354000.38	376977641.13	1.21
45.52	460917741.17	108470372.41	0.48
46.02	453910061.03	107811223.58	0.47
46.32	503388222.12	138201619.12	0.52
46.46	425499630.94	91616414.96	0.44
47.75	927975968.38	171315454.45	0.96
48.31	4078185904.50	1003429168.54	4.21
48.68	1421055160.50	259760768.48	1.47
49.34	439935189.67	58474431.29	0.45

There is no signature data to report.

Library Search Report

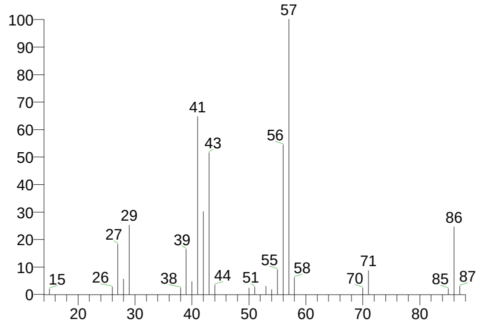
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

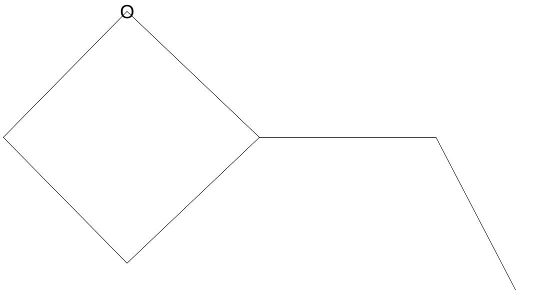
RT	Compound Name	Area	Area %
3.05	2-Ethyl-oxetane	10287185889.68	10.63
3.05	Pentane, 2,3-dimethyl-	10287185889.68	10.63
3.05	n-Hexane	10287185889.68	10.63
3.05	n-Hexane	10287185889.68	10.63
3.05	n-Hexane	10287185889.68	10.63

Hit Spectrum	Compound Structure

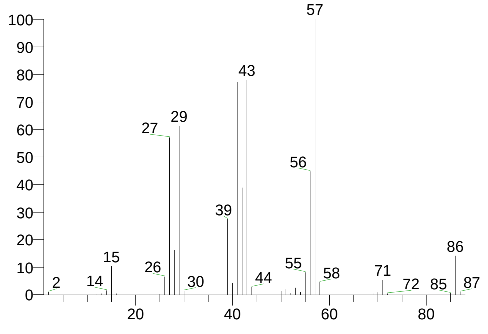
SI 797, RSI 889, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



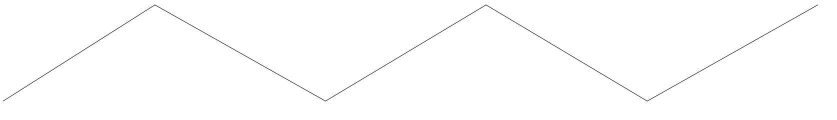
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N



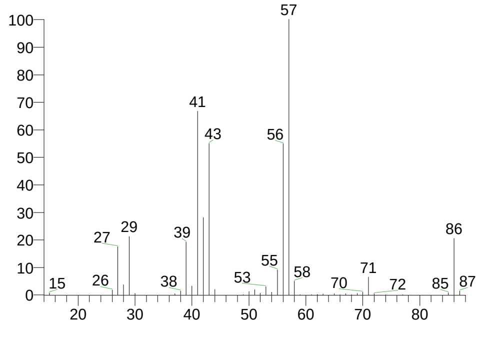
SI 796, RSI 854, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



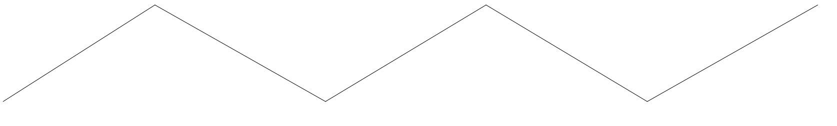
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane

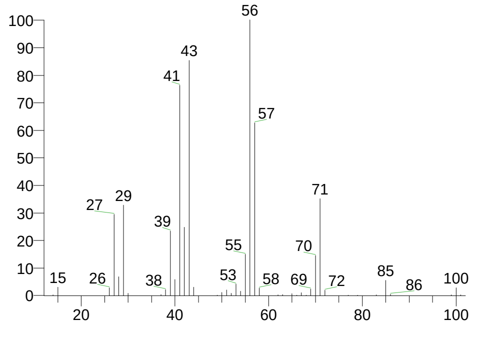


SI 790, RSI 822, replib, Entry# 5854, CAS# 110-54-3, n-Hexane

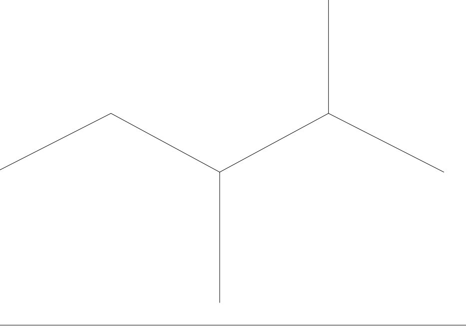


n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane





Pentane, 2,3-dimethyl-
Formula C7H16, MW 100, CAS# 565-59-3, Entry# 5451
2,3-Dimethylpentane



There is no signature data to report.

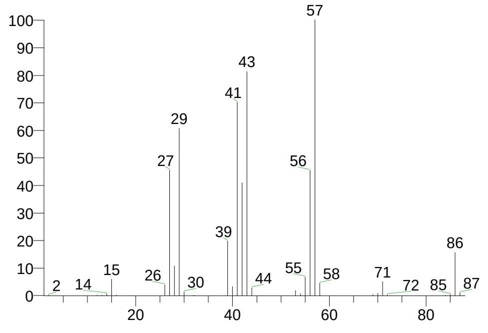
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

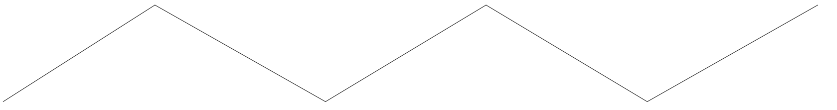
Hit Spectrum

SI 761, RSI 815, mainlib, Entry# 24052, CAS# 110-54-3, n-Hexane



Compound Structure

n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 24052
Hexane



There is no signature data to report.

Library Search Report

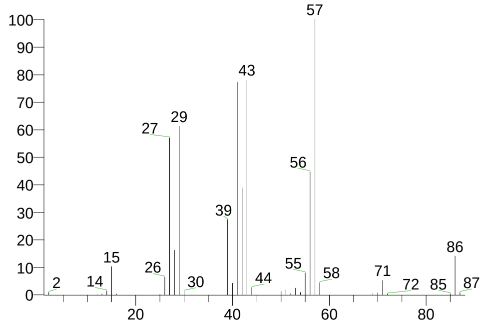
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

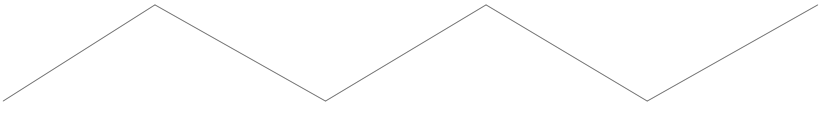
RT	Compound Name	Area	Area %
3.14	2-Ethyl-oxetane	8647420779.30	8.94
3.14	Pentane, 3-methyl-	8647420779.30	8.94
3.14	Pentane, 3-methyl-	8647420779.30	8.94
3.14	n-Hexane	8647420779.30	8.94
3.14	n-Hexane	8647420779.30	8.94

Hit Spectrum	Compound Structure

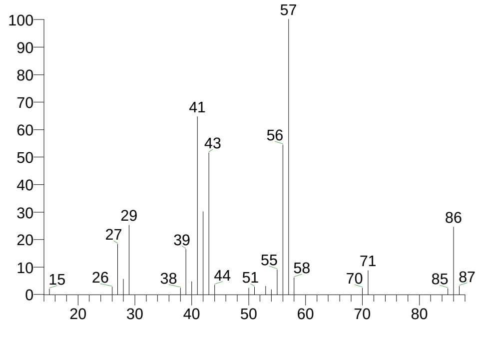
SI 801, RSI 856, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



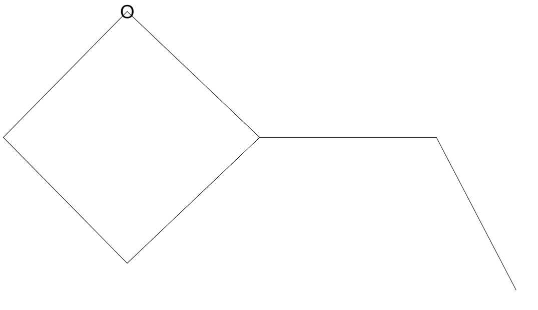
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane



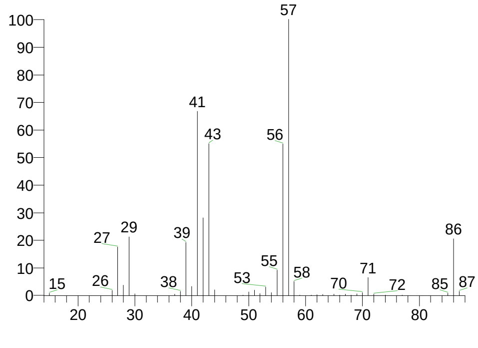
SI 799, RSI 889, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



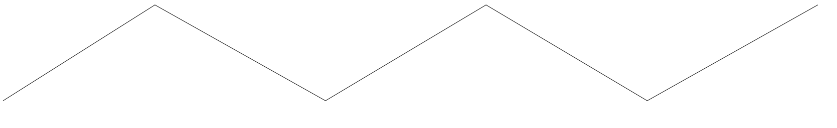
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N

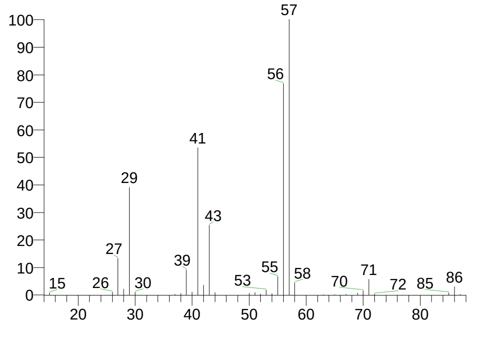


SI 788, RSI 818, replib, Entry# 5854, CAS# 110-54-3, n-Hexane

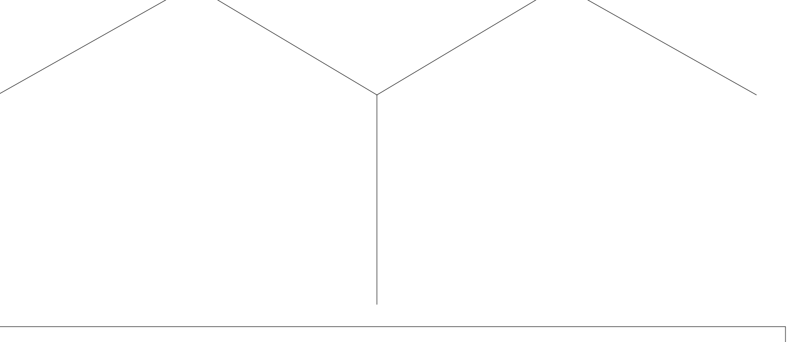


n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane





Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 1301
3-Methylpentane



There is no signature data to report.

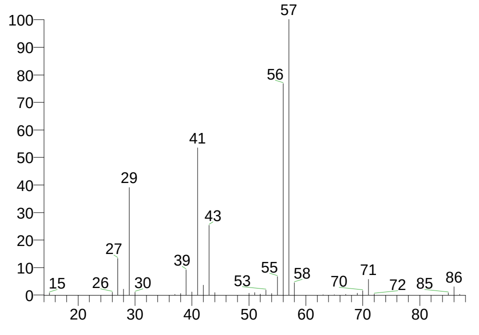
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

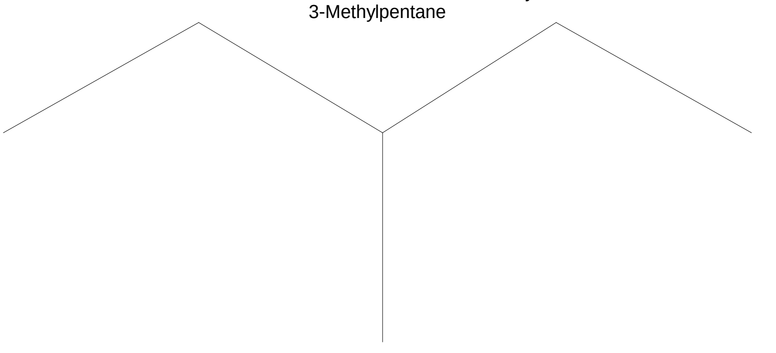
Hit Spectrum

SI 769, RSI 818, mainlib, Entry# 24856, CAS# 96-14-0, Pentane, 3-methyl-



Compound Structure

Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 24856
3-Methylpentane



There is no signature data to report.

Library Search Report

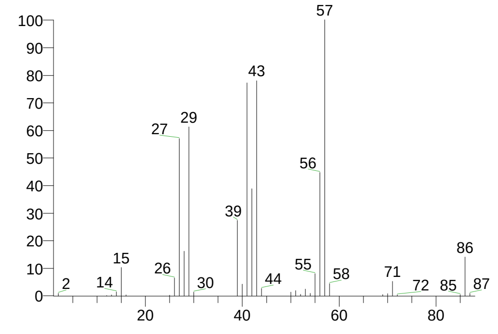
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

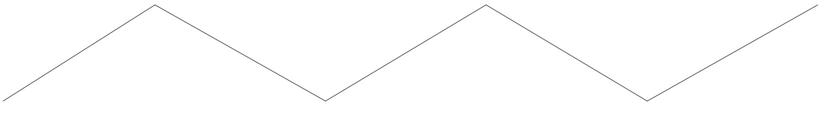
RT	Compound Name	Area	Area %
3.25	2-Ethyl-oxetane	4494331440.59	4.64
3.25	Pentane, 3-methyl-	4494331440.59	4.64
3.25	Pentane, 3-methyl-	4494331440.59	4.64
3.25	n-Hexane	4494331440.59	4.64
3.25	n-Hexane	4494331440.59	4.64

Hit Spectrum | Compound Structure

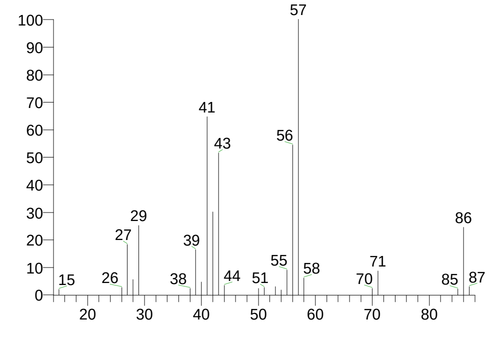
SI 812, RSI 864, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



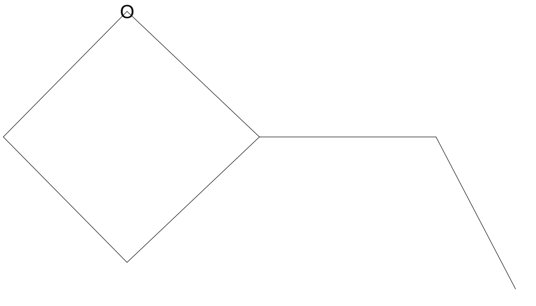
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane



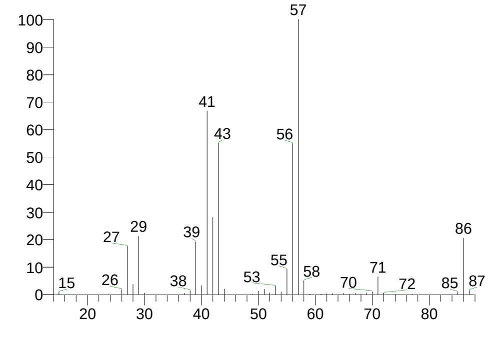
SI 796, RSI 881, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



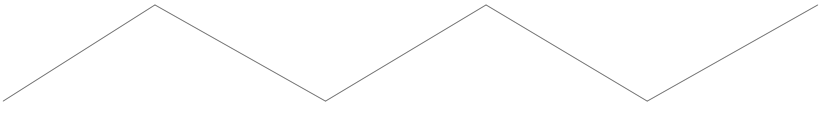
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N

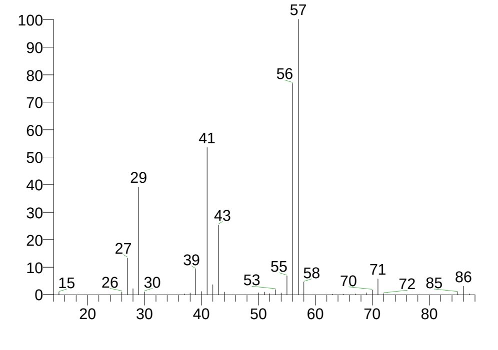


SI 793, RSI 822, replib, Entry# 5854, CAS# 110-54-3, n-Hexane

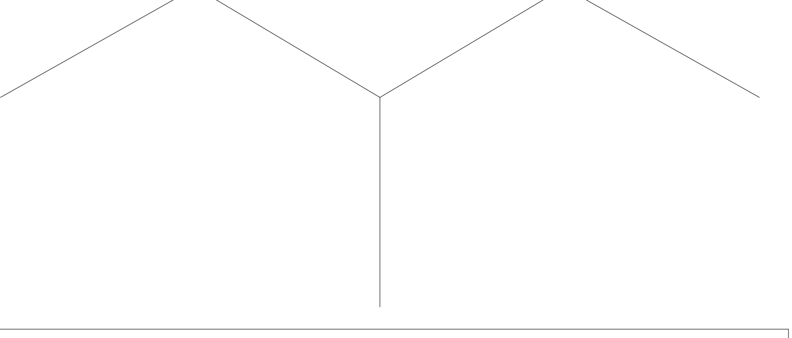


n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane





Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 1301
3-Methylpentane



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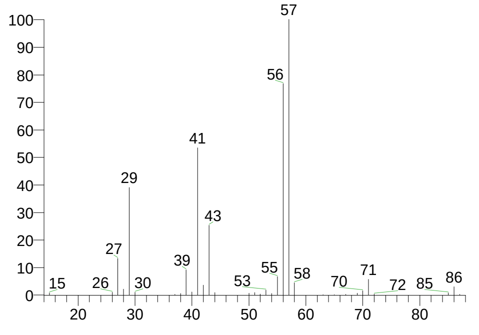
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

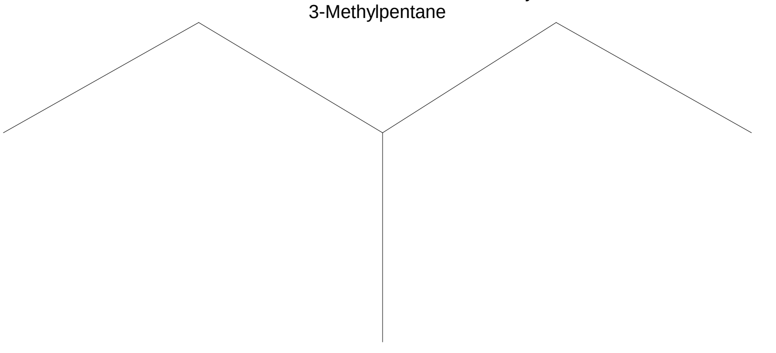
Hit Spectrum

SI 775, RSI 822, mainlib, Entry# 24856, CAS# 96-14-0, Pentane, 3-methyl-



Compound Structure

Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 24856
3-Methylpentane



There is no signature data to report.

Library Search Report

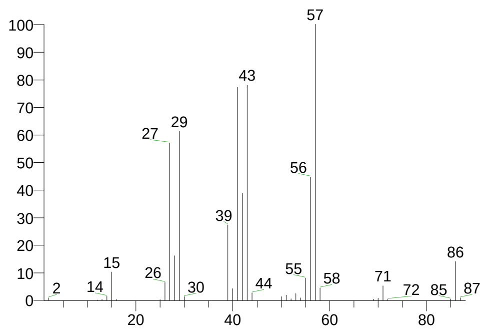
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

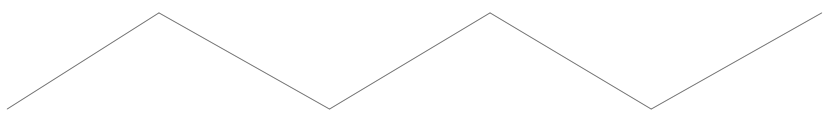
RT	Compound Name	Area	Area %
3.29	2-Ethyl-oxetane	15049277192.11	15.55
3.29	Pentane, 3-methyl-	15049277192.11	15.55
3.29	n-Hexane	15049277192.11	15.55
3.29	n-Hexane	15049277192.11	15.55
3.29	n-Hexane	15049277192.11	15.55

Hit Spectrum	Compound Structure

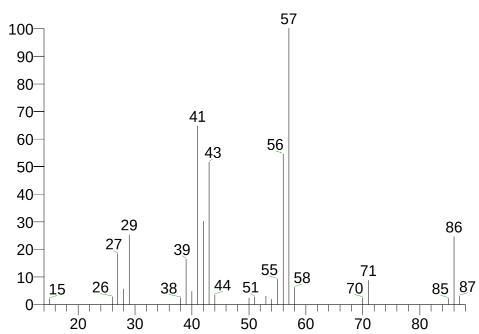
SI 808, RSI 860, replib, Entry# 6014, CAS# 110-54-3, n-Hexane



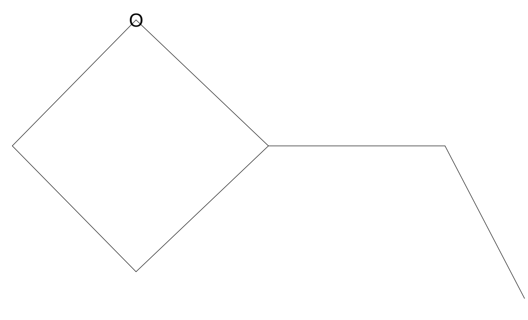
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 6014
Hexane



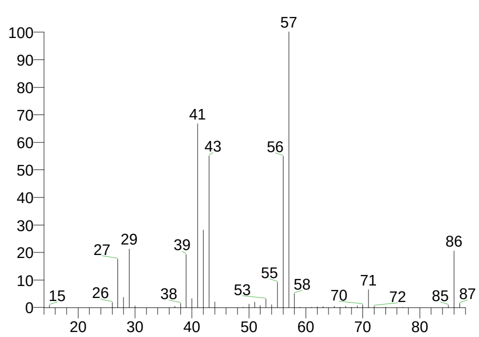
SI 804, RSI 888, mainlib, Entry# 23551, CAS# NA, 2-Ethyl-oxetane



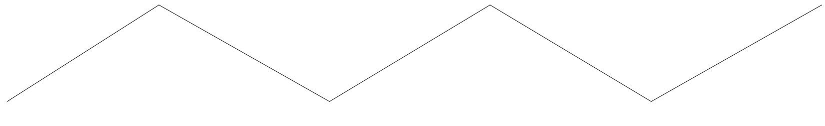
2-Ethyl-oxetane
Formula C5H10O, MW 86, CAS# NA, Entry# 23551
$:28GDHROTCPZLVPJT-UHFFFAOYSA-N



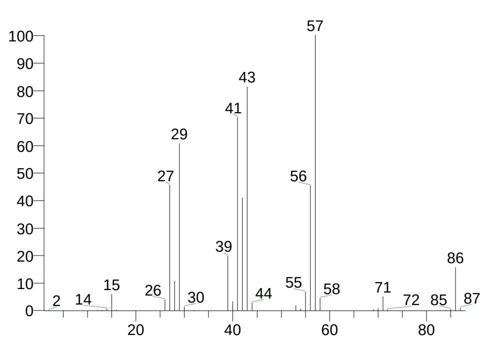
SI 787, RSI 816, replib, Entry# 5854, CAS# 110-54-3, n-Hexane



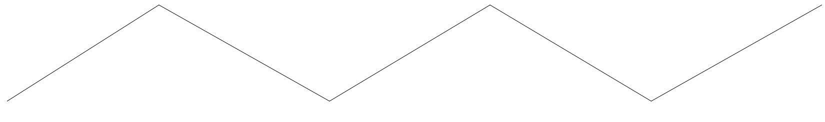
n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 5854
Hexane



SI 769, RSI 818, mainlib, Entry# 24052, CAS# 110-54-3, n-Hexane



n-Hexane
Formula C6H14, MW 86, CAS# 110-54-3, Entry# 24052
Hexane



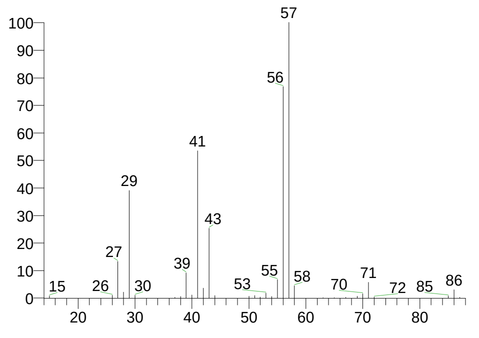
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

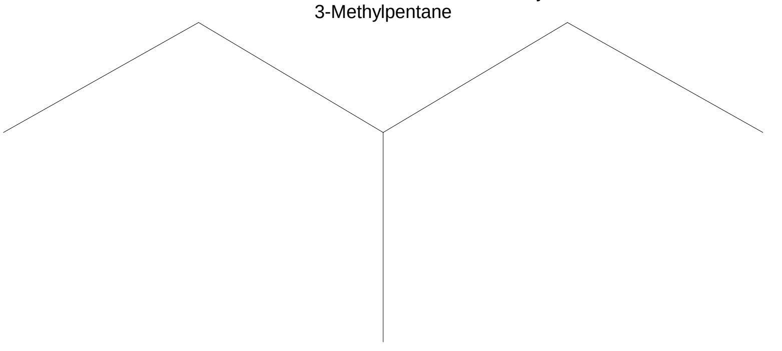
Flower pet ether

Hit Spectrum



Compound Structure

Pentane, 3-methyl-
Formula C6H14, MW 86, CAS# 96-14-0, Entry# 1301
3-Methylpentane



There is no signature data to report.

Library Search Report

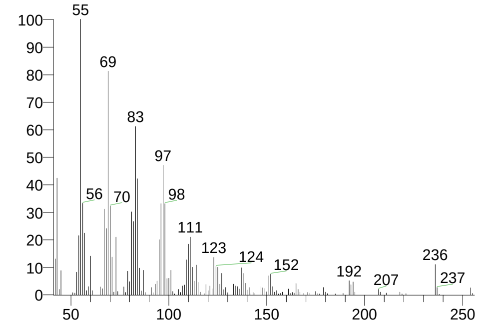
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

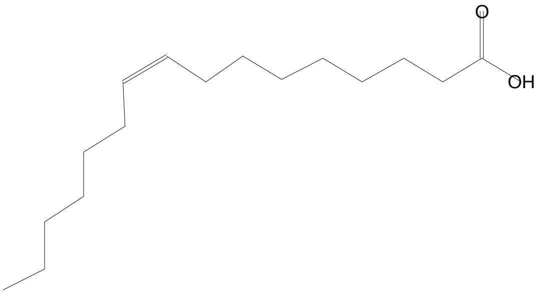
RT	Compound Name	Area	Area %
32.97	9-Hexadecenoic acid	517355767.39	0.53
32.97	Hexadecenoic acid, Z-11-	517355767.39	0.53
32.97	Hexadecenoic acid, Z-11-	517355767.39	0.53
32.97	Palmitoleic acid	517355767.39	0.53
32.97	Palmitoleic acid	517355767.39	0.53

Hit Spectrum | Compound Structure

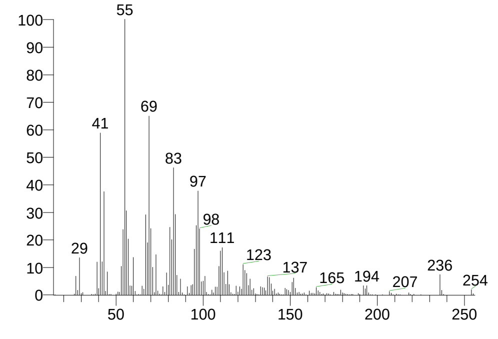
SI 914, RSI 932, replib, Entry# 5020, CAS# 373-49-9, Palmitoleic acid



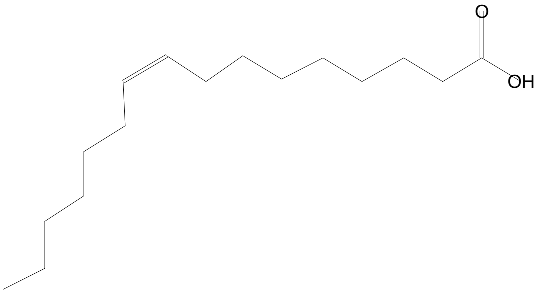
Palmitoleic acid
Formula C16H30O2, MW 254, CAS# 373-49-9, Entry# 5020
cis-9-Hexadecenoic acid

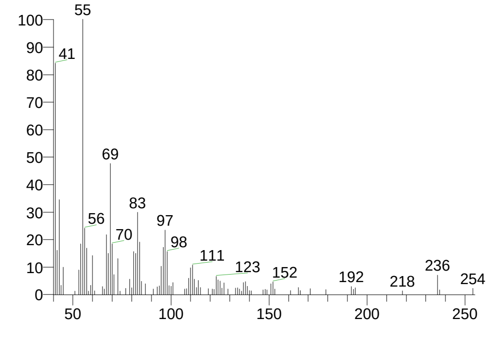


SI 908, RSI 916, mainlib, Entry# 20127, CAS# 373-49-9, Palmitoleic acid

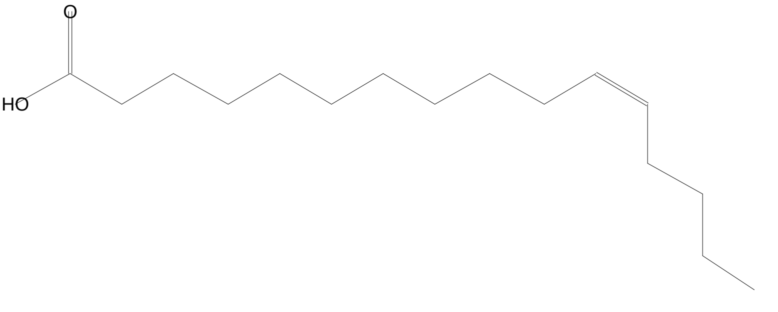


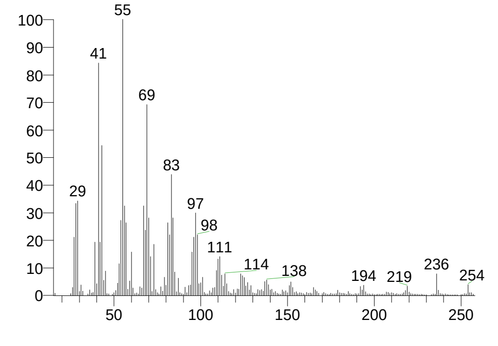
Palmitoleic acid
Formula C16H30O2, MW 254, CAS# 373-49-9, Entry# 20127
cis-9-Hexadecenoic acid



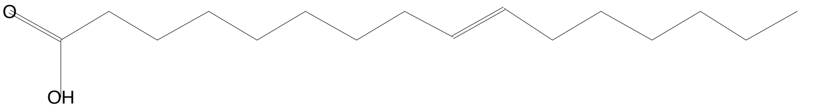


Hexadecenoic acid, Z-11-
Formula C16H30O2, MW 254, CAS# 2416-20-8, Entry# 4717
Z-11-Hexadecenoic acid





9-Hexadecenoic acid
Formula C16H30O2, MW 254, CAS# 2091-29-4, Entry# 19244
(9E)-9-Hexadecenoic acid #



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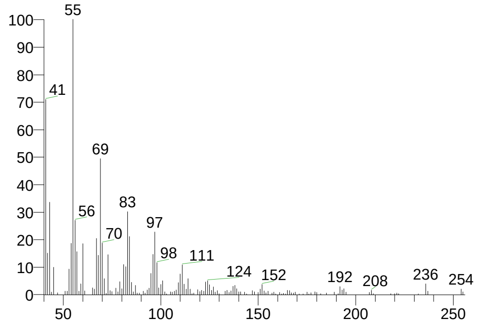
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

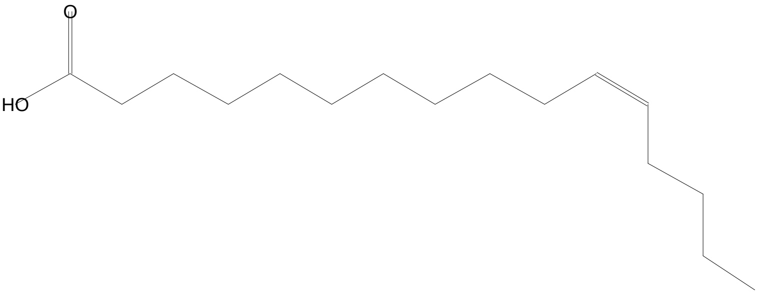
Flower pet ether

Hit Spectrum



Compound Structure

Hexadecenoic acid, Z-11-
Formula C16H30O2, MW 254, CAS# 2416-20-8, Entry# 19262
Z-11-Hexadecenoic acid



There is no signature data to report.

Library Search Report

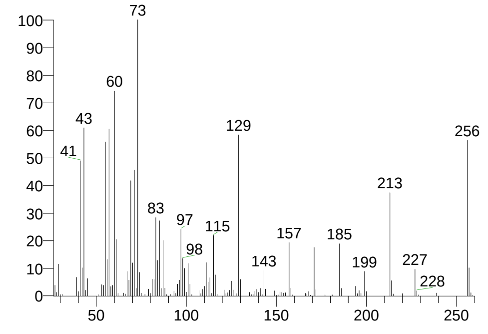
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

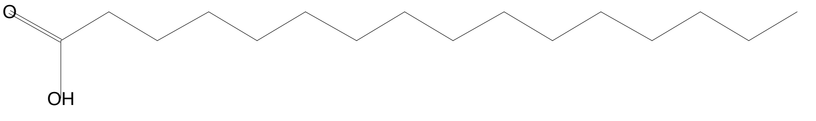
RT	Compound Name	Area	Area %
33.39	n-Hexadecanoic acid	5226496172.97	5.40
33.39	n-Hexadecanoic acid	5226496172.97	5.40
33.39	n-Hexadecanoic acid	5226496172.97	5.40
33.39	n-Hexadecanoic acid	5226496172.97	5.40
33.39	n-Hexadecanoic acid	5226496172.97	5.40

Hit Spectrum | Compound Structure

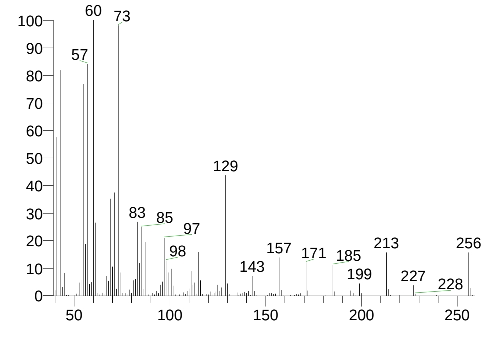
SI 933, RSI 937, replib, Entry# 9622, CAS# 57-10-3, n-Hexadecanoic acid



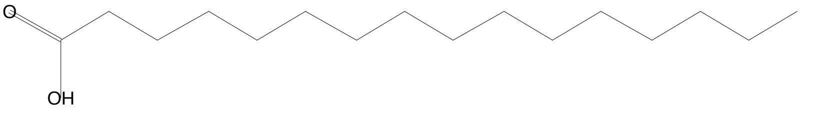
n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 9622
Hexadecanoic acid



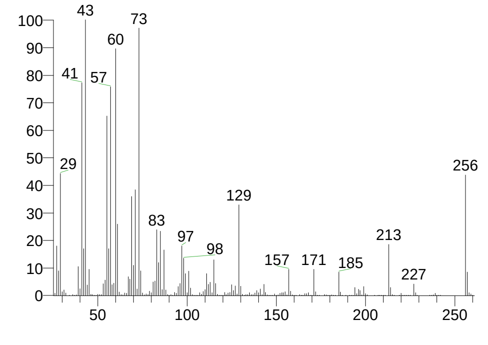
SI 928, RSI 931, replib, Entry# 7566, CAS# 57-10-3, n-Hexadecanoic acid



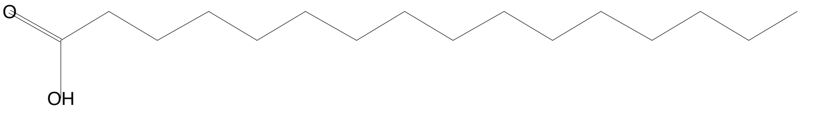
n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 7566
Hexadecanoic acid

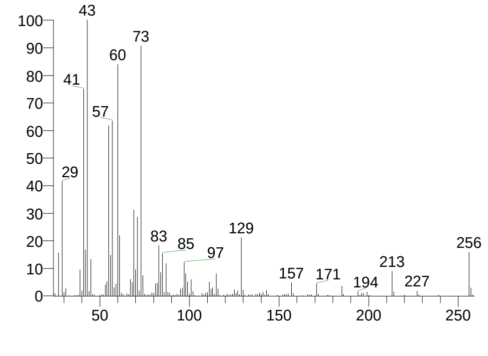


SI 879, RSI 879, replib, Entry# 2779, CAS# 57-10-3, n-Hexadecanoic acid

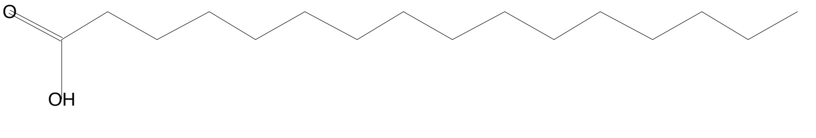


n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 2779
Hexadecanoic acid





n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 154
Hexadecanoic acid



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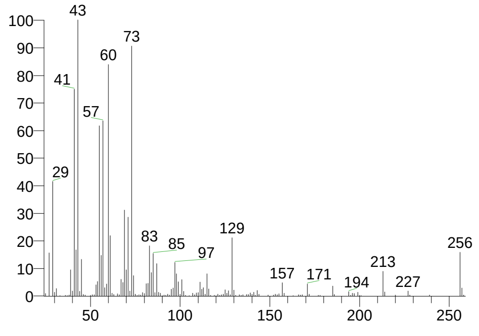
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

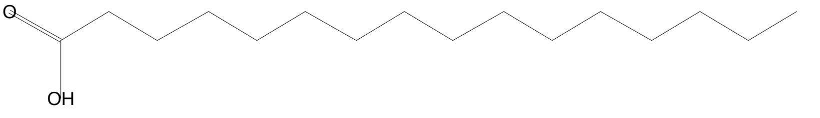
Flower pet ether

Hit Spectrum



Compound Structure

n-Hexadecanoic acid
Formula C16H32O2, MW 256, CAS# 57-10-3, Entry# 9208
Hexadecanoic acid



There is no signature data to report.

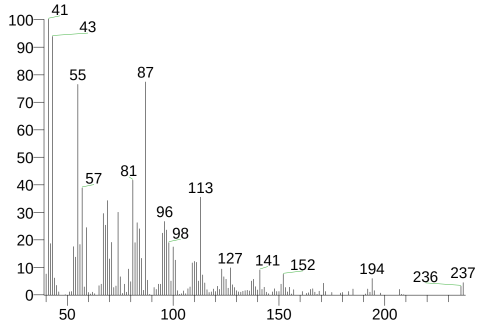
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

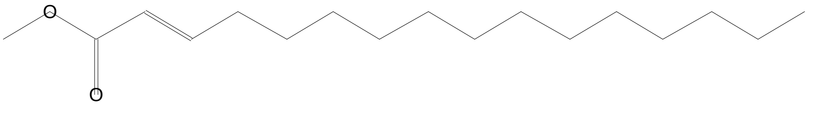
Flower pet ether

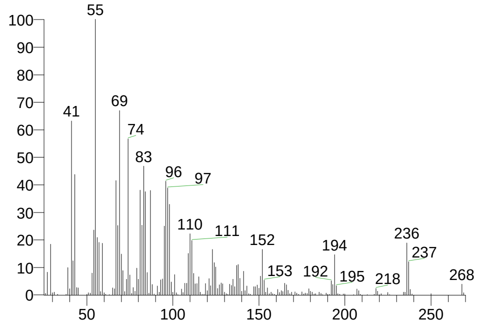
RT	Compound Name	Area	Area %
33.61	2-Hexadecenoic acid, methyl ester, (E)-	722142594.50	0.75
33.61	7-Hexadecenoic acid, methyl ester, (Z)-	722142594.50	0.75
33.61	9-Hexadecenoic acid, methyl ester, (Z)-	722142594.50	0.75
33.61	9-Hexadecenoic acid, methyl ester, (Z)-	722142594.50	0.75
33.61	Methyl hexadec-9-enoate	722142594.50	0.75

Hit Spectrum	Compound Structure

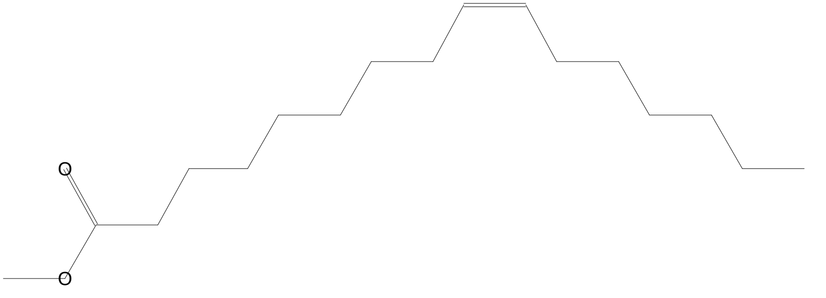


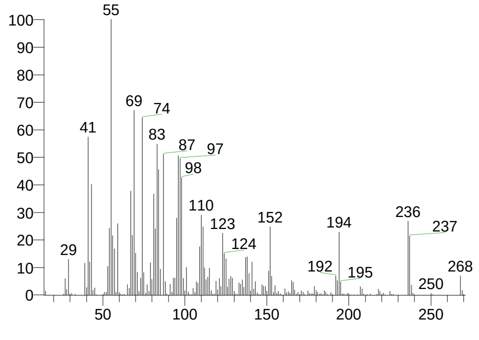
2-Hexadecenoic acid, methyl ester, (E)-
Formula C17H32O2, MW 268, CAS# 2825-81-2, Entry# 2523
Methyl (2E)-2-hexadecenoate #



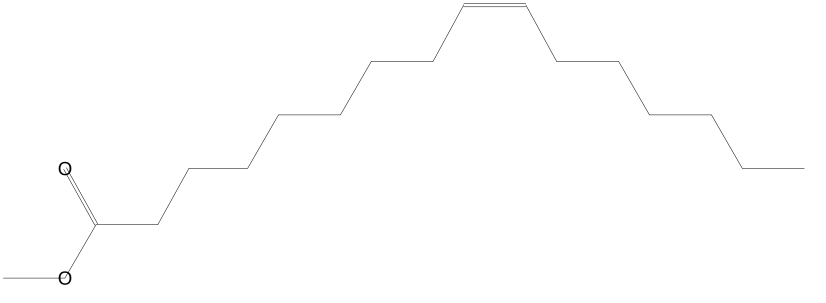


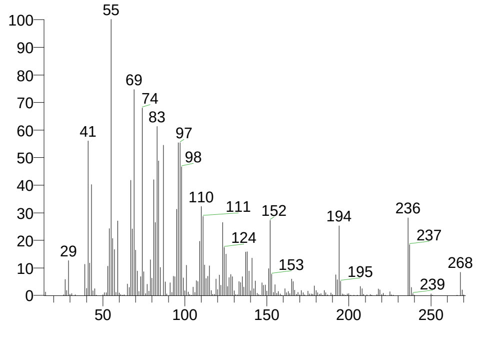
9-Hexadecenoic acid, methyl ester, (Z)-
Formula C17H32O2, MW 268, CAS# 1120-25-8, Entry# 4955
Methyl palmitoleate



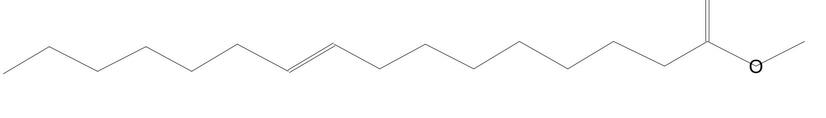


9-Hexadecenoic acid, methyl ester, (Z)-
Formula C17H32O2, MW 268, CAS# 1120-25-8, Entry# 20213
Methyl palmitoleate





Methyl hexadec-9-enoate
Formula C17H32O2, MW 268, CAS# 10030-74-7, Entry# 20216
Methyl palmitelaidate



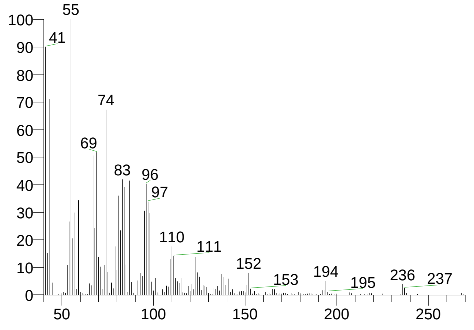
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

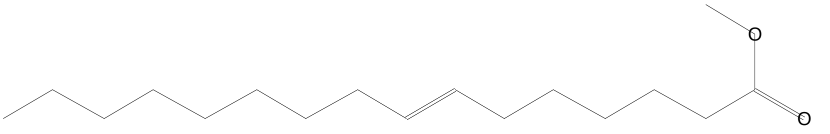
Flower pet ether

Hit Spectrum



Compound Structure

7-Hexadecenoic acid, methyl ester, (Z)-
Formula C17H32O2, MW 268, CAS# 56875-67-3, Entry# 4629
Methyl (7E)-7-hexadecenoate #



There is no signature data to report.

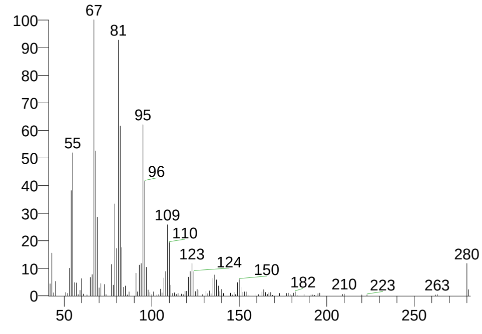
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

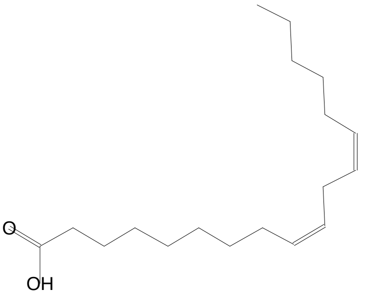
Flower pet ether

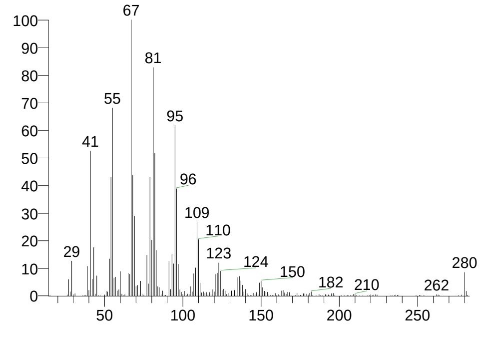
RT	Compound Name	Area	Area %
36.19	(Z)-18-Octadec-9-enolide	4000762666.36	4.13
36.19	9,12-Octadecadienoic acid (Z,Z)-	4000762666.36	4.13
36.19	9,12-Octadecadienoic acid (Z,Z)-	4000762666.36	4.13
36.19	9,12-Octadecadienoic acid (Z,Z)-	4000762666.36	4.13
36.19	Linoelaidic acid	4000762666.36	4.13

Hit Spectrum | Compound Structure

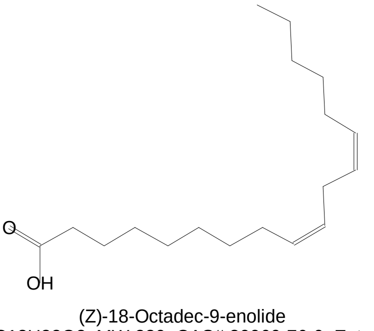


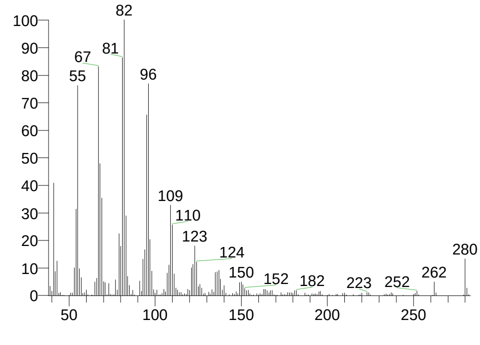
9,12-Octadecadienoic acid (Z,Z)-
Formula C18H32O2, MW 280, CAS# 60-33-3, Entry# 8129
cis-9,cis-12-Octadecadienoic acid



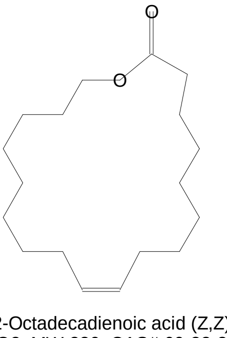


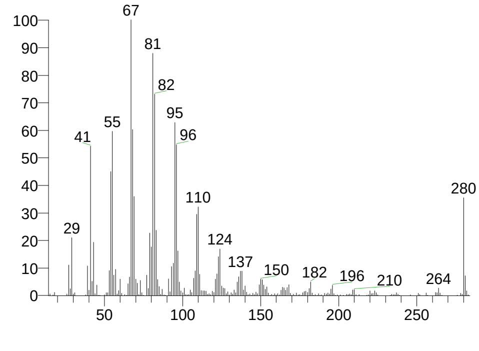
9,12-Octadecadienoic acid (Z,Z)-
Formula C18H32O2, MW 280, CAS# 60-33-3, Entry# 8112
cis-9,cis-12-Octadecadienoic acid



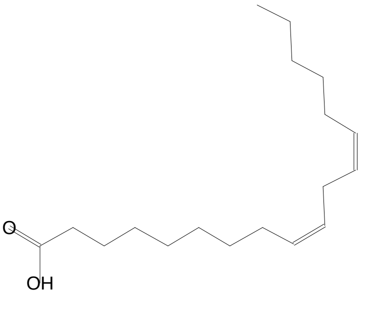


(Z)-18-Octadec-9-enolide
Formula C18H32O2, MW 280, CAS# 80060-76-0, Entry# 51421
Oxacyclononadec-10-en-2-one, (10Z)-





9,12-Octadecadienoic acid (Z,Z)-
Formula C18H32O2, MW 280, CAS# 60-33-3, Entry# 32846
cis-9,cis-12-Octadecadienoic acid



There is no signature data to report.

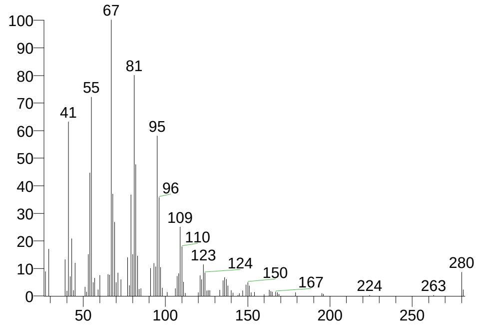
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

Hit Spectrum

SI 874, RSI 931, mainlib, Entry# 32814, CAS# 506-21-8, Linoelaidic acid

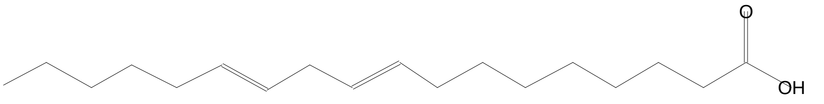


Compound Structure

Linoelaidic acid

Formula C18H32O2, MW 280, CAS# 506-21-8, Entry# 32814

$:28OYHQOLUKZRVURQ-AVQMFFATSA-N



There is no signature data to report.

Library Search Report

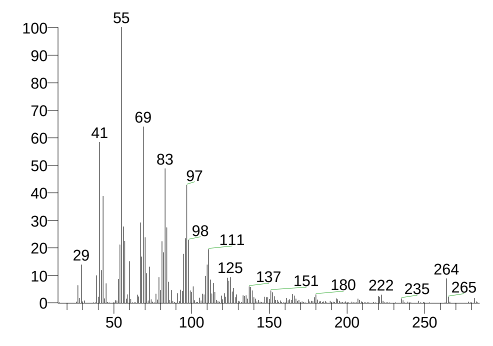
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

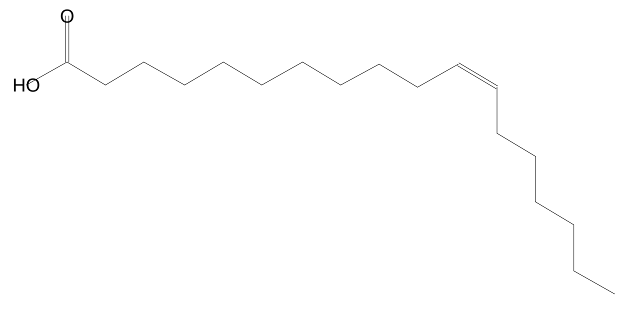
RT	Compound Name	Area	Area %
36.29	9-Octadecenoic acid, (E)-	7451090263.34	7.70
36.29	Oleic Acid	7451090263.34	7.70
36.29	cis-13-Octadecenoic acid	7451090263.34	7.70
36.29	cis-Vaccenic acid	7451090263.34	7.70
36.29	trans-13-Octadecenoic acid	7451090263.34	7.70

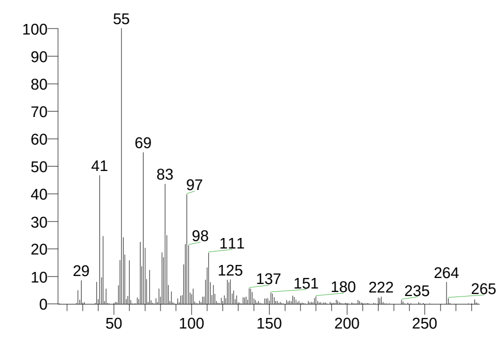
Hit Spectrum | Compound Structure

SI 913, RSI 913, mainlib, Entry# 20090, CAS# 506-17-2, cis-Vaccenic acid

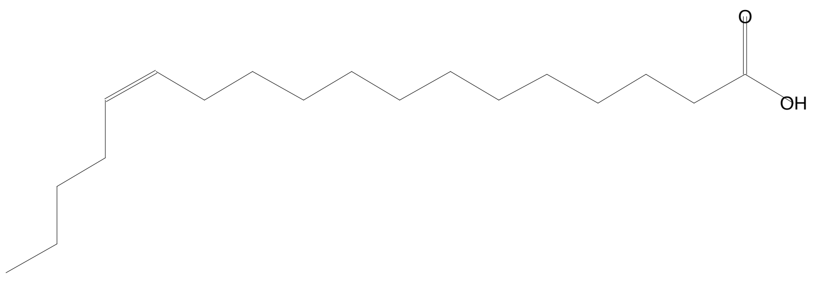


cis-Vaccenic acid
Formula C18H34O2, MW 282, CAS# 506-17-2, Entry# 20090
11-Octadecenoic acid, (Z)-

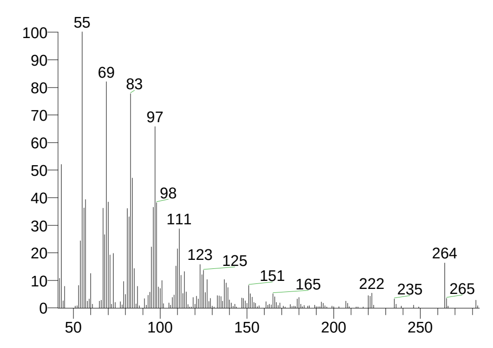




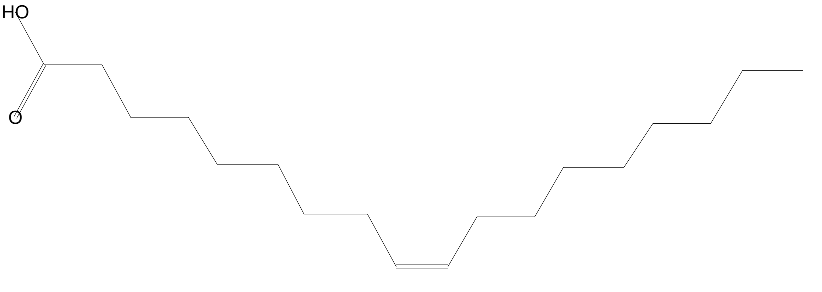
cis-13-Octadecenoic acid
Formula C18H34O2, MW 282, CAS# 13126-39-1, Entry# 20126
$:28BDLLSHRIFPDGQB-WAYWQWQTSA-N

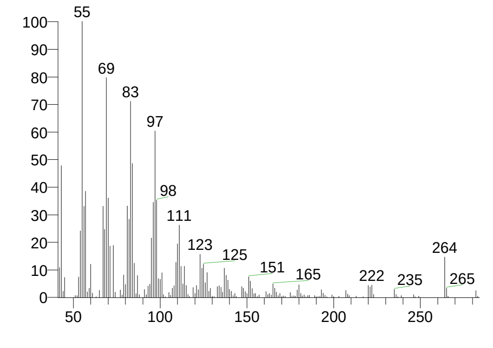


SI 909, RSI 917, replib, Entry# 5017, CAS# 112-80-1, Oleic Acid

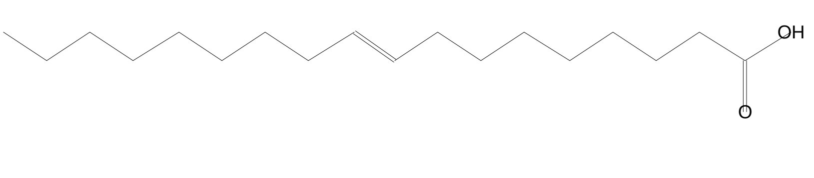


Oleic Acid
Formula C18H34O2, MW 282, CAS# 112-80-1, Entry# 5017
9-Octadecenoic acid (Z)-





9-Octadecenoic acid, (E)-
Formula C18H34O2, MW 282, CAS# 112-79-8, Entry# 5015
trans-ë(sup 9)-Octadecenoic acid



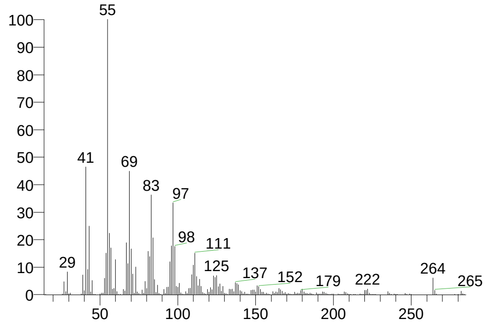
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

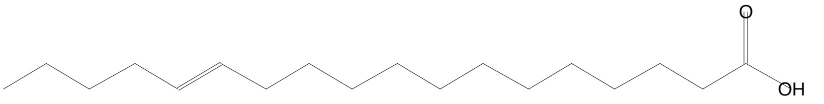
Flower pet ether

Hit Spectrum



Compound Structure

trans-13-Octadecenoic acid
Formula C18H34O2, MW 282, CAS# 693-71-0, Entry# 19306
$:28BDLLSHRIFPDGQB-AATRIKPKSA-N



There is no signature data to report.

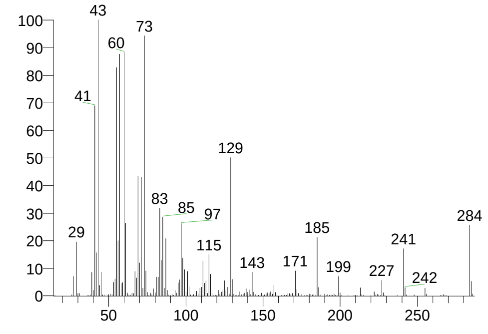
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

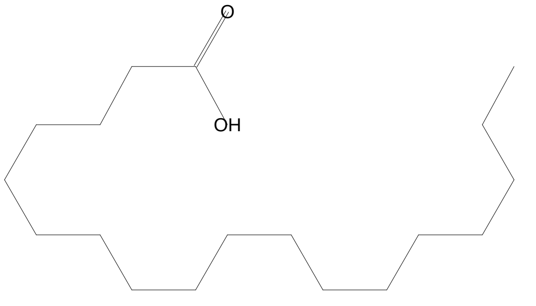
Flower pet ether

RT	Compound Name	Area	Area %
36.58	Octadecanoic acid	2905408819.79	3.00
36.58	Octadecanoic acid	2905408819.79	3.00
36.58	Octadecanoic acid	2905408819.79	3.00
36.58	Octadecanoic acid	2905408819.79	3.00
36.58	Octadecanoic acid	2905408819.79	3.00

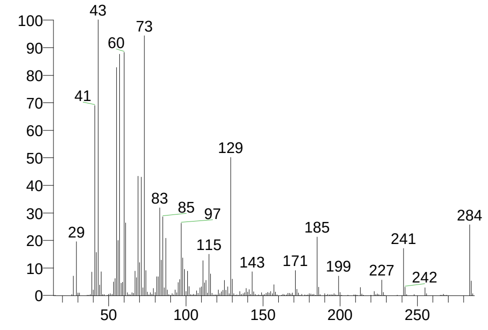
Hit Spectrum	Compound Structure



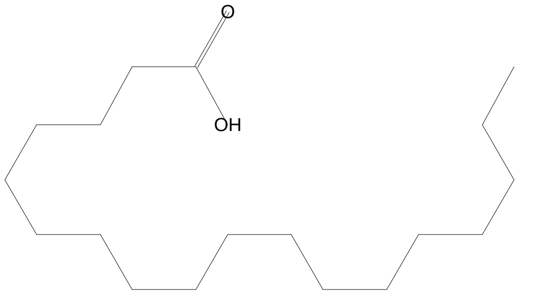
Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 266
Stearic acid



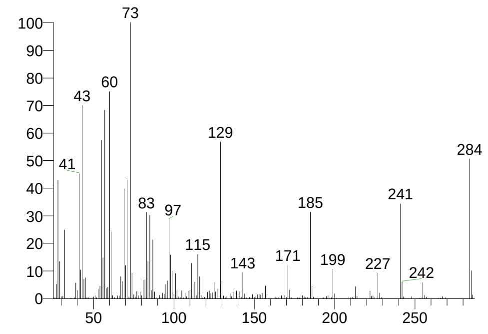
SI 887, RSI 891, mainlib, Entry# 9210, CAS# 57-11-4, Octadecanoic acid



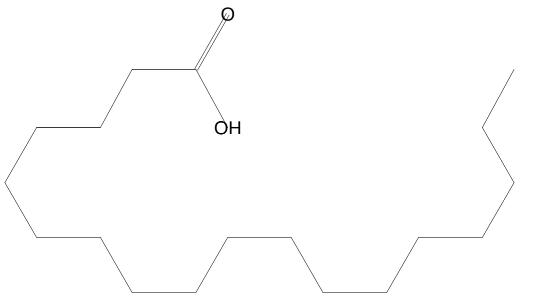
Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 9210
Stearic acid



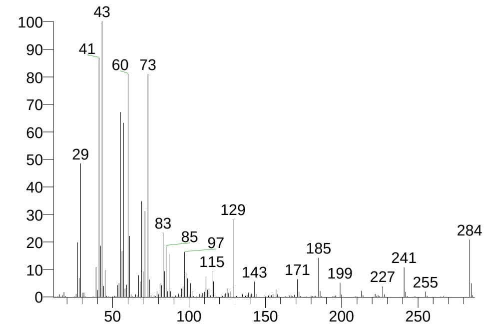
SI 886, RSI 894, replib, Entry# 9623, CAS# 57-11-4, Octadecanoic acid



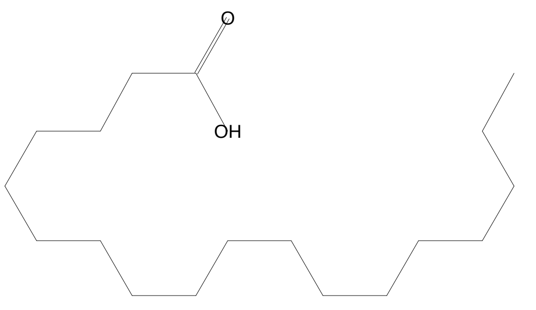
Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 9623
Stearic acid



SI 880, RSI 894, replib, Entry# 1866, CAS# 57-11-4, Octadecanoic acid



Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 1866
Stearic acid



There is no signature data to report.

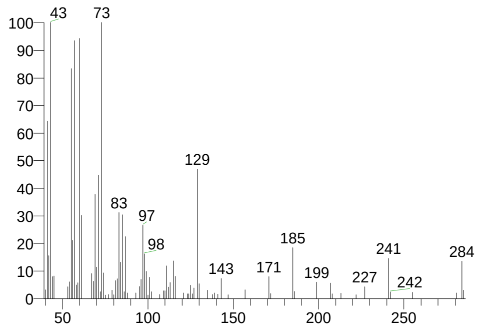
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

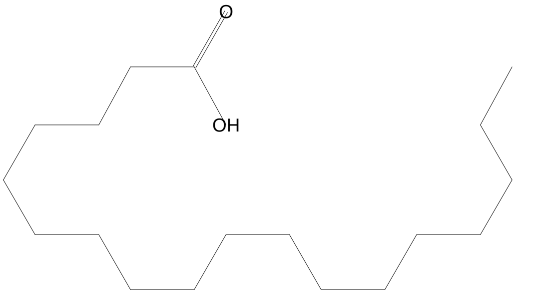
Hit Spectrum

SI 873, RSI 931, replib, Entry# 2781, CAS# 57-11-4, Octadecanoic acid



Compound Structure

Octadecanoic acid
Formula C18H36O2, MW 284, CAS# 57-11-4, Entry# 2781
Stearic acid



There is no signature data to report.

Library Search Report

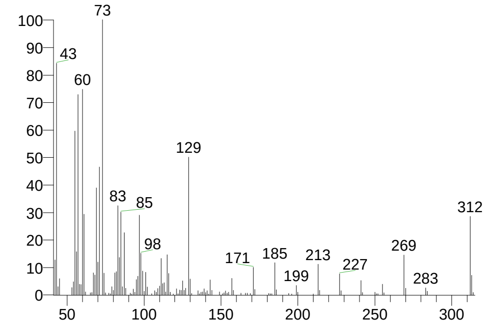
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

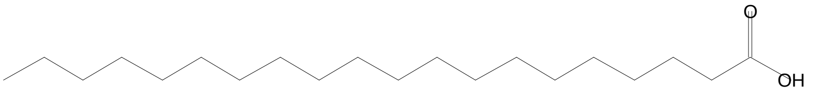
RT	Compound Name	Area	Area %
39.54	Eicosanoic acid	2920176160.79	3.02
39.54	Eicosanoic acid	2920176160.79	3.02
39.54	Eicosanoic acid	2920176160.79	3.02
39.54	Eicosanoic acid	2920176160.79	3.02
39.54	Eicosanoic acid	2920176160.79	3.02

Hit Spectrum | Compound Structure

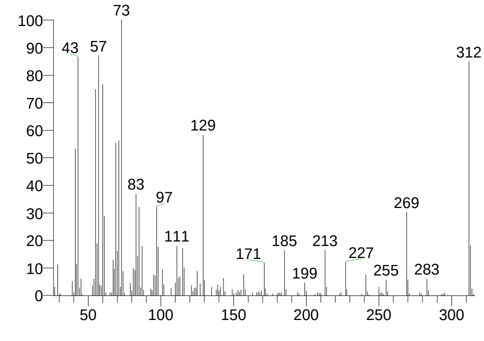
SI 913, RSI 945, replib, Entry# 9519, CAS# 506-30-9, Eicosanoic acid



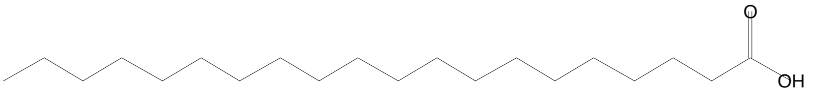
Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 9519
Arachic acid



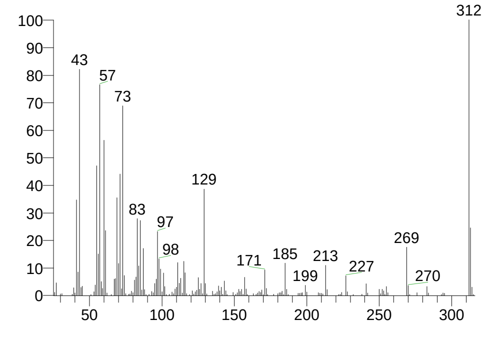
SI 885, RSI 927, replib, Entry# 9604, CAS# 506-30-9, Eicosanoic acid



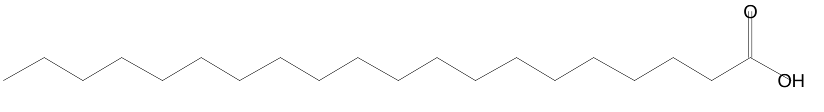
Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 9604
Arachic acid



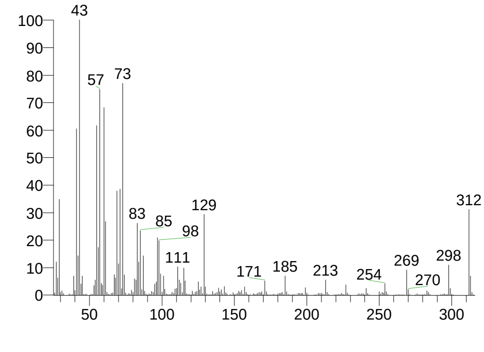
SI 858, RSI 875, replib, Entry# 32789, CAS# 506-30-9, Eicosanoic acid



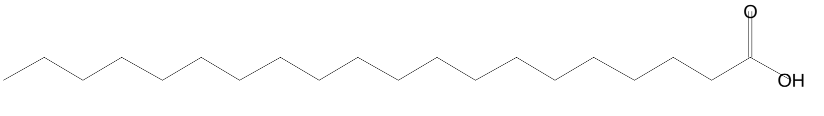
Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 32789
Arachic acid



SI 843, RSI 845, replib, Entry# 2778, CAS# 506-30-9, Eicosanoic acid



Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 2778
Arachic acid



There is no signature data to report.

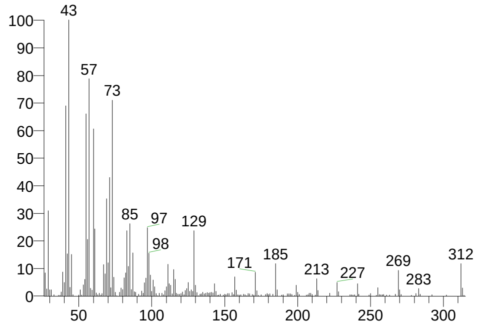
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

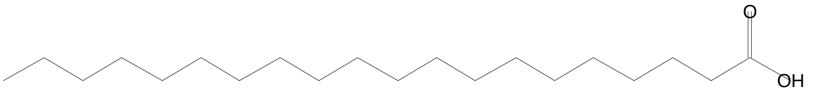
Hit Spectrum

SI 837, RSI 852, mainlib, Entry# 7899, CAS# 506-30-9, Eicosanoic acid



Compound Structure

Eicosanoic acid
Formula C20H40O2, MW 312, CAS# 506-30-9, Entry# 7899
Arachic acid



There is no signature data to report.

Library Search Report

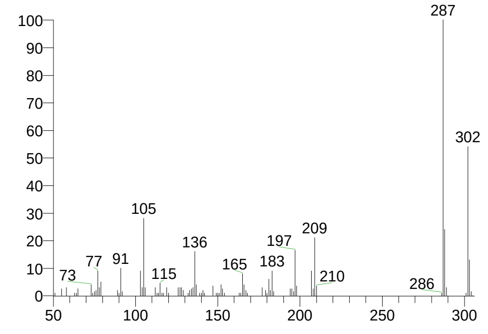
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

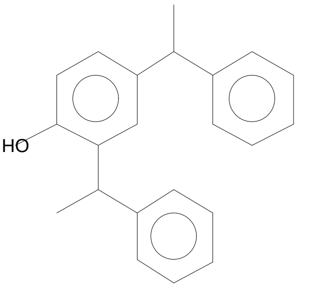
Flower pet ether

RT	Compound Name	Area	Area %
41.42	4-Androsten-6á-ol-3,17-dione	558448836.68	0.58
41.42	Methanone, [1,4-dimethyl-7-(1-methylethyl)-2-azulenyl]phenyl-	558448836.68	0.58
41.42	Methanone, [2,4-dimethyl-7-(1-methylethyl)azulenyl]phenyl-	558448836.68	0.58
41.42	Palustric acid	558448836.68	0.58
41.42	Phenol, 2,4-bis(1-phenylethyl)-	558448836.68	0.58

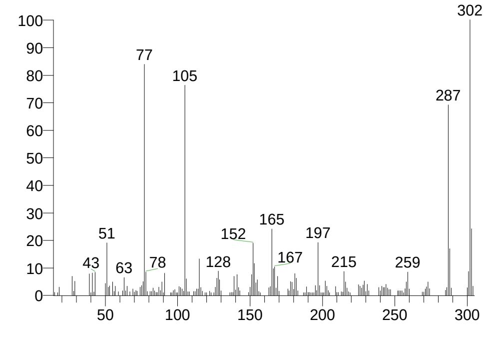
Hit Spectrum | Compound Structure

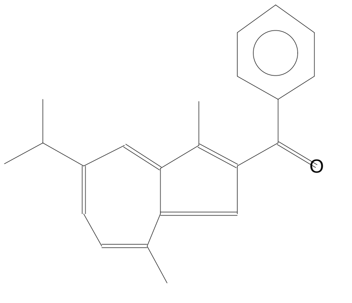
Phenol, 2,4-bis(1-phenylethyl)-
Formula C22H22O, MW 302, CAS# 2769-94-0, Entry# 221962
2,4-Bis(1-phenylethyl)phenol #





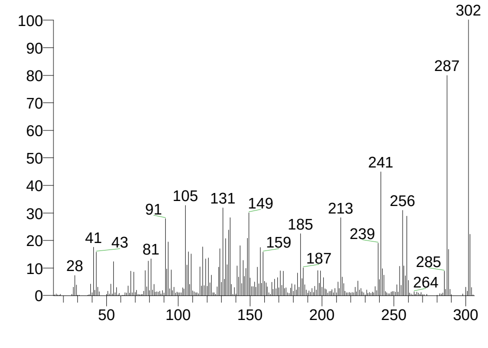
Methanone, [1,4-dimethyl-7-(1-methylethyl)-2-azulenyl]phenyl-
Formula C22H22O, MW 302, CAS# 39665-56-0, Entry# 225380
2-Benzoylguaiazulene

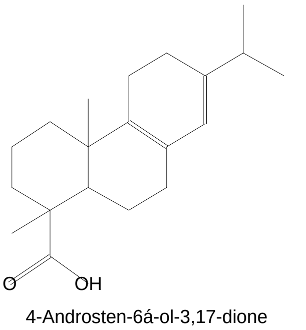




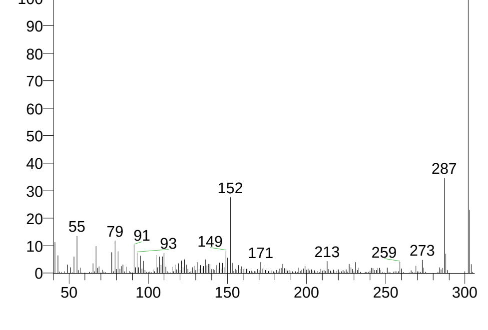
SI 649, RSI 655, mainlib, Entry# 225498, CAS# 1945-53-5, Palustric acid

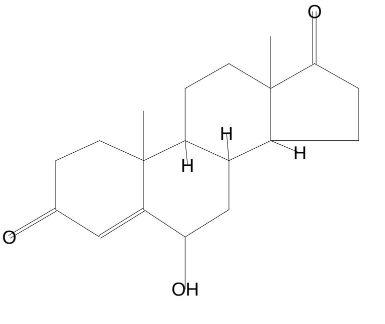
Palustric acid
Formula C20H30O2, MW 302, CAS# 1945-53-5, Entry# 225498
Podocarpa-8,13-dien-15-oic acid, 13-isopropyl-





4-Androsten-6á-ol-3,17-dione
Formula C19H26O3, MW 302, CAS# 63-00-3, Entry# 225495
6-Hydroxyandrost-4-ene-3,17-dione #





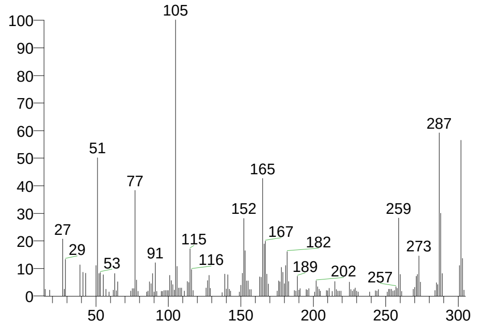
There is no signature data to report.

Library Search Report

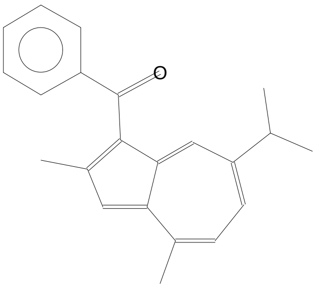
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

Hit Spectrum | Compound Structure



Methanone, [2,4-dimethyl-7-(1-methylethyl)azulenyl]phenyl-
Formula C22H22O, MW 302, CAS# 72361-25-2, Entry# 82089
(7-Isopropyl-2,4-dimethyl-1-azulenyl)(phenyl)methanone #



There is no signature data to report.

Library Search Report

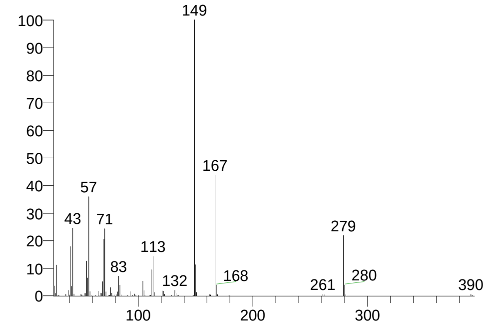
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

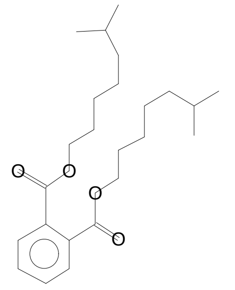
Flower pet ether

RT	Compound Name	Area	Area %
42.11	Bis(2-ethylhexyl) phthalate	16274256128.93	16.82
42.11	Bis(2-ethylhexyl) phthalate	16274256128.93	16.82
42.11	Bis(2-ethylhexyl) phthalate	16274256128.93	16.82
42.11	Di-n-octyl phthalate	16274256128.93	16.82
42.11	Diisooctyl phthalate	16274256128.93	16.82

Hit Spectrum | Compound Structure

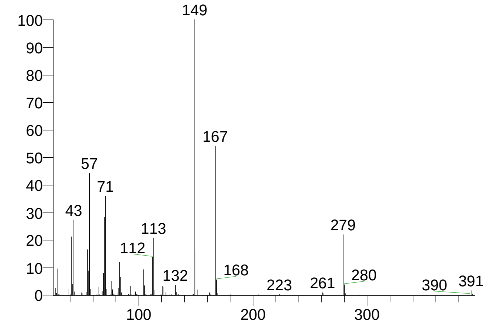
Diisooctyl phthalate
Formula C24H38O4, MW 390, CAS# 131-20-4, Entry# 138712
Bis(6-methylheptyl) phthalate

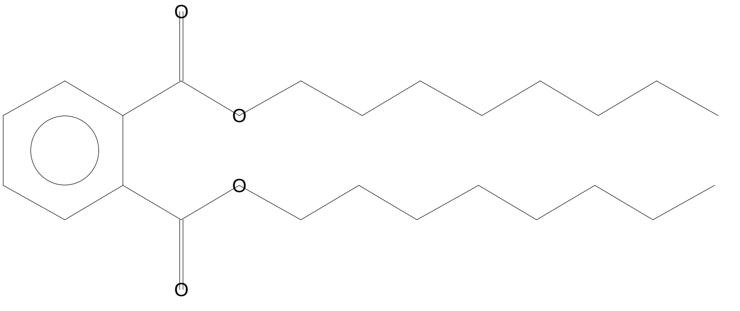




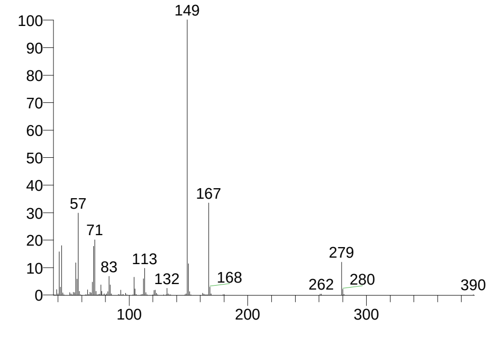
SI 931, RSI 931, replib, Entry# 23541, CAS# 117-84-0, Di-n-octyl phthalate

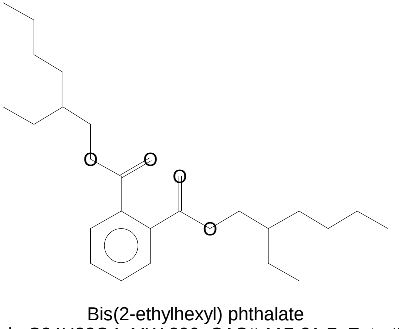
Di-n-octyl phthalate
Formula C24H38O4, MW 390, CAS# 117-84-0, Entry# 23541
1,2-Benzenedicarboxylic acid, dioctyl ester



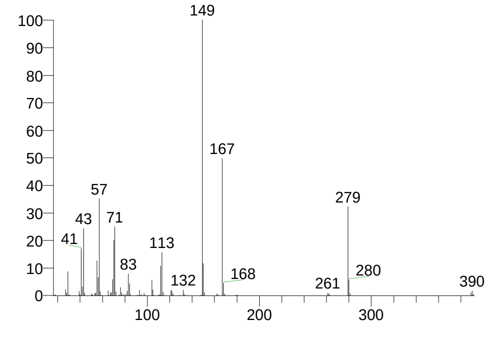


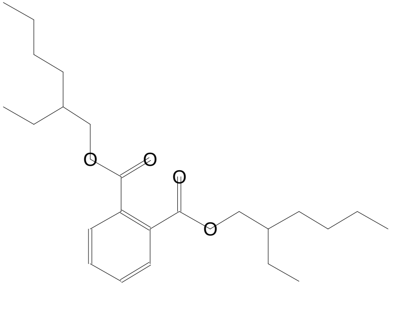
Bis(2-ethylhexyl) phthalate
Formula C24H38O4, MW 390, CAS# 117-81-7, Entry# 23539
Phthalic acid, bis(2-ethylhexyl) ester





Bis(2-ethylhexyl) phthalate
Formula C24H38O4, MW 390, CAS# 117-81-7, Entry# 537
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester





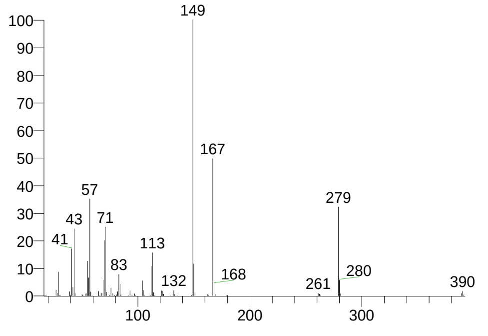
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

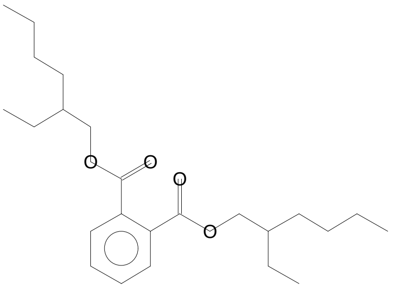
Flower pet ether

Hit Spectrum



Compound Structure

Bis(2-ethylhexyl) phthalate
Formula $C_{24}H_{38}O_4$, MW 390, CAS# 117-81-7, Entry# 138711
Phthalic acid, bis(2-ethylhexyl) ester



There is no signature data to report.

Library Search Report

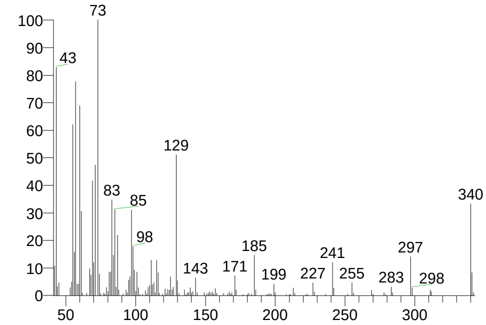
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

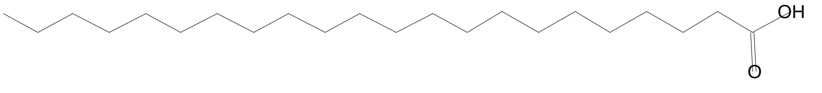
RT	Compound Name	Area	Area %
42.26	Docosanoic acid	2792730214.96	2.89
42.26	Docosanoic acid	2792730214.96	2.89
42.26	Docosanoic acid	2792730214.96	2.89
42.26	Docosanoic acid	2792730214.96	2.89
42.26	Docosanoic anhydride	2792730214.96	2.89

Hit Spectrum	Compound Structure

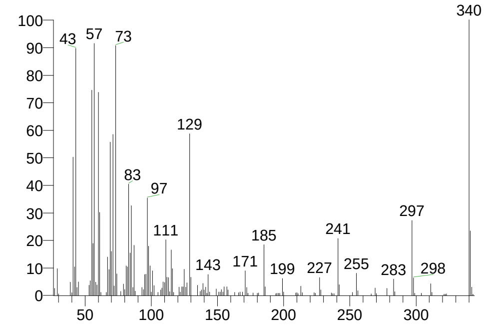
SI 905, RSI 937, replib, Entry# 9514, CAS# 112-85-6, Docosanoic acid



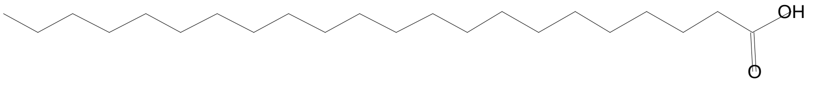
Docosanoic acid
Formula C22H44O2, MW 340, CAS# 112-85-6, Entry# 9514
n-Docosanoic acid



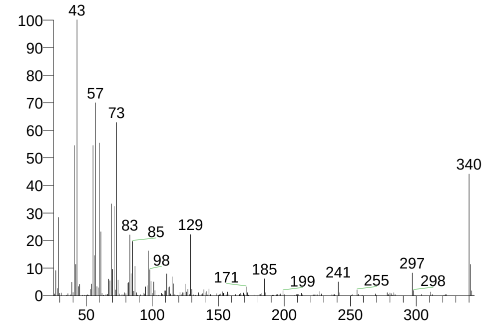
SI 881, RSI 915, replib, Entry# 33157, CAS# 112-85-6, Docosanoic acid



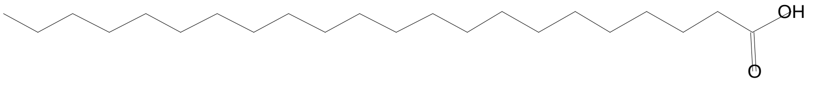
Docosanoic acid
Formula C22H44O2, MW 340, CAS# 112-85-6, Entry# 33157
n-Docosanoic acid



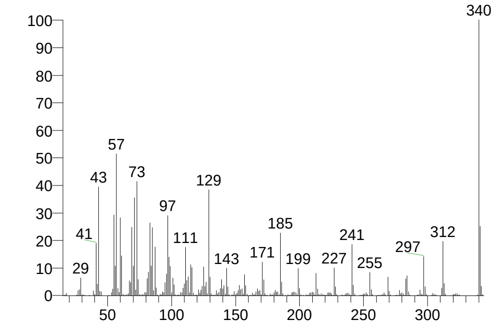
SI 833, RSI 849, replib, Entry# 2342, CAS# 112-85-6, Docosanoic acid



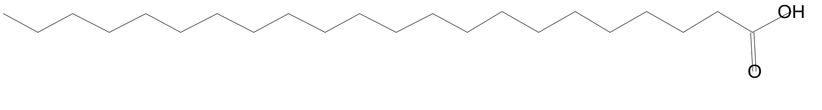
Docosanoic acid
Formula C22H44O2, MW 340, CAS# 112-85-6, Entry# 2342
n-Docosanoic acid



SI 829, RSI 830, mainlib, Entry# 232352, CAS# 112-85-6, Docosanoic acid



Docosanoic acid
Formula C22H44O2, MW 340, CAS# 112-85-6, Entry# 232352
n-Docosanoic acid



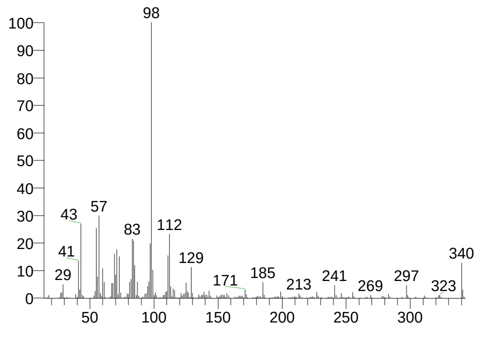
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

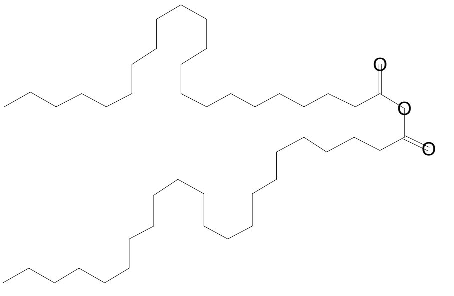
Flower pet ether

Hit Spectrum



Compound Structure

Docosanoic anhydride
Formula C44H86O3, MW 662, CAS# 55726-23-3, Entry# 71054
$:28IJVLPVWXJLKHID-UHFFFAOYSA-N



There is no signature data to report.

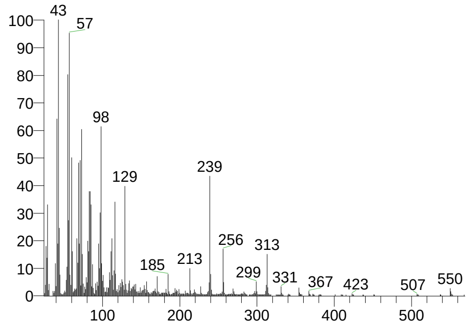
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

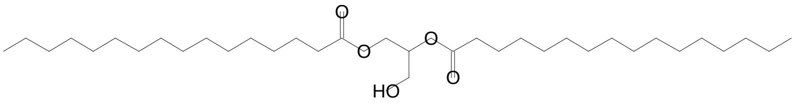
Flower pet ether

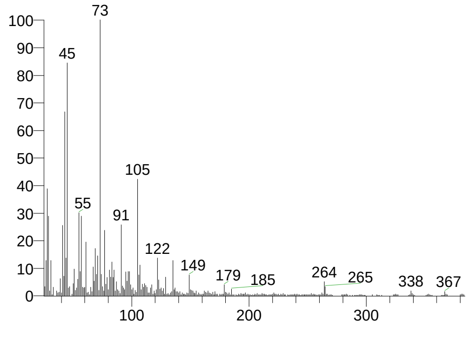
RT	Compound Name	Area	Area %
42.40	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	390905733.86	0.40
42.40	Docosanoic acid	390905733.86	0.40
42.40	Estra-1,3,5(10)-trien-17á-ol	390905733.86	0.40
42.40	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	390905733.86	0.40
42.40	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	390905733.86	0.40

Hit Spectrum | Compound Structure

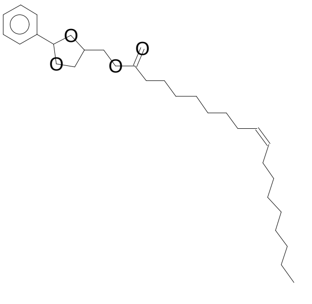


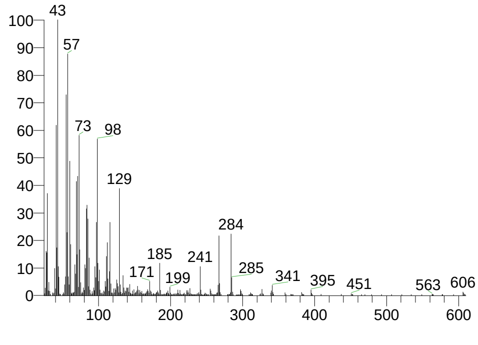
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester
Formula C35H68O5, MW 568, CAS# 761-35-3, Entry# 7720
Palmitin, 1,2-di-



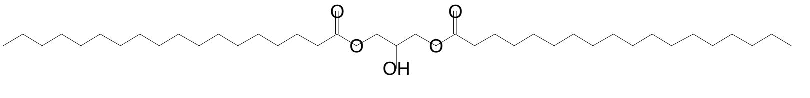


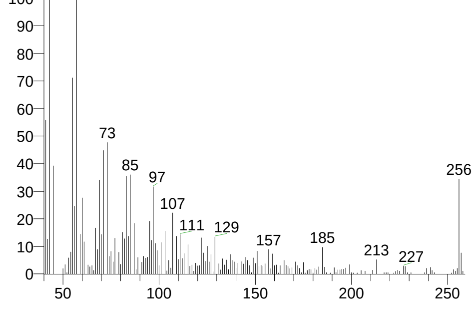
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-
Formula C28H44O4, MW 444, CAS# 56599-45-2, Entry# 40756
(2-Phenyl-1,3-dioxolan-4-yl)methyl 9-octadecenoate, cis-



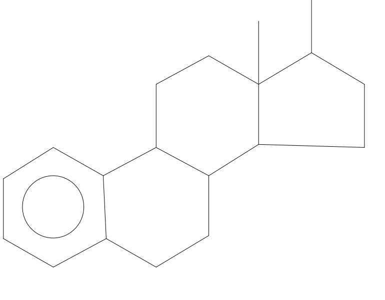


Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester
Formula C39H76O5, MW 624, CAS# 504-40-5, Entry# 2272
Stearin, 1,3-di-





Estra-1,3,5(10)-trien-17á-ol
Formula C18H24O, MW 256, CAS# 2529-64-8, Entry# 7736
Estra-1,3,5(10)-trien-17-ol, (17á)-



There is no signature data to report.

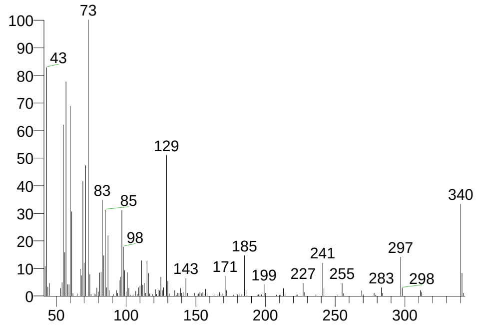
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

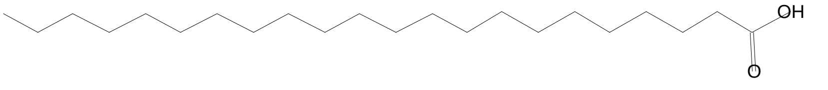
Hit Spectrum

SI 667, RSI 778, replib, Entry# 9514, CAS# 112-85-6, Docosanoic acid



Compound Structure

Docosanoic acid
Formula C22H44O2, MW 340, CAS# 112-85-6, Entry# 9514
n-Docosanoic acid



There is no signature data to report.

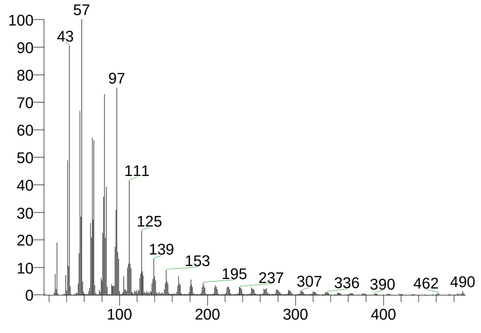
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

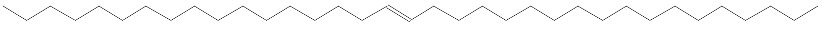
Flower pet ether

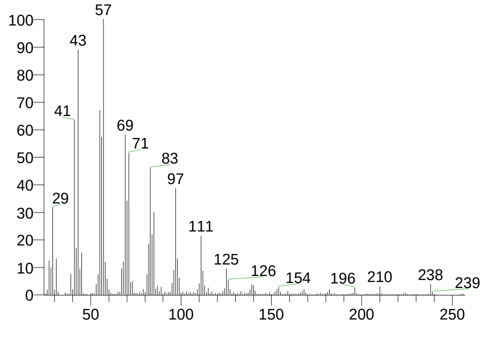
RT	Compound Name	Area	Area %
42.96	1-Hexadecanol, 2-methyl-	450137300.52	0.47
42.96	17-Pentatriacontene	450137300.52	0.47
42.96	Heptacosane	450137300.52	0.47
42.96	Octatriacontyl pentafluoropropionate	450137300.52	0.47
42.96	Tetrapentacontane, 1,54-dibromo-	450137300.52	0.47

Hit Spectrum | Compound Structure

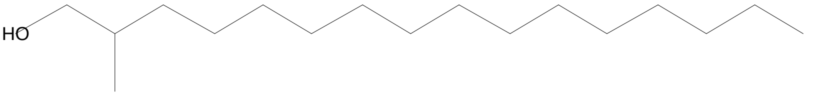


17-Pentatriacontene
Formula C35H70, MW 490, CAS# 6971-40-0, Entry# 24490
(17E)-17-Pentatriacontene #

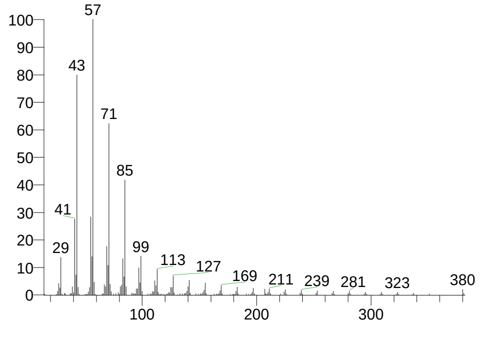




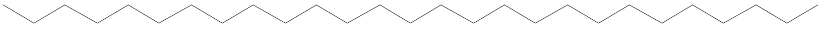
1-Hexadecanol, 2-methyl-
Formula C17H36O, MW 256, CAS# 2490-48-4, Entry# 24113
2-Methylhexadecan-1-ol

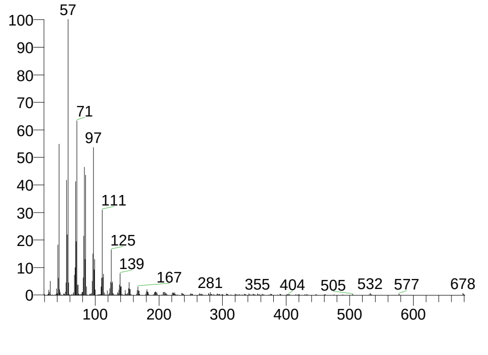


SI 739, RSI 813, replib, Entry# 6113, CAS# 593-49-7, Heptacosane

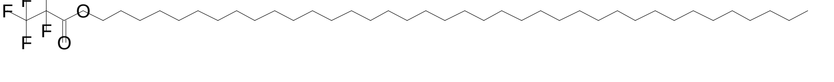


Heptacosane
Formula C27H56, MW 380, CAS# 593-49-7, Entry# 6113
n-Heptacosane





Octatriacontyl pentafluoropropionate
Formula C41H77F5O2, MW 696, CAS# NA, Entry# 25256
Octatriacontyl 2,2,3,3,3-pentafluoropropanoate



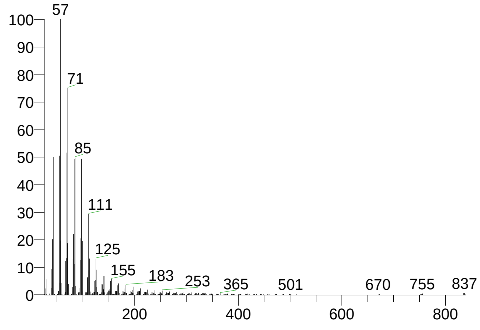
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

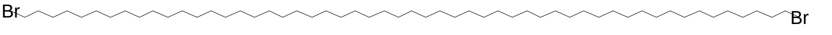
Flower pet ether

Hit Spectrum



Compound Structure

Tetrapentacontane, 1,54-dibromo-
Formula C54H108Br2, MW 914, CAS# NA, Entry# 25430
1,54-Dibromotetrapentacontane #



There is no signature data to report.

Library Search Report

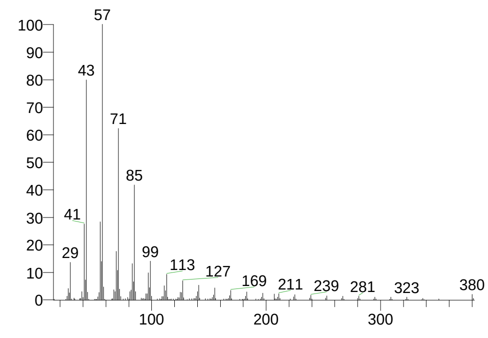
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

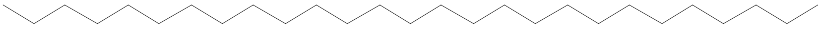
RT	Compound Name	Area	Area %
43.64	Heptacosane	740318312.77	0.77
43.64	Nonacosane	740318312.77	0.77
43.64	Octacosane	740318312.77	0.77
43.64	Pentacosane	740318312.77	0.77
43.64	Tetratetracontane	740318312.77	0.77

Hit Spectrum	Compound Structure

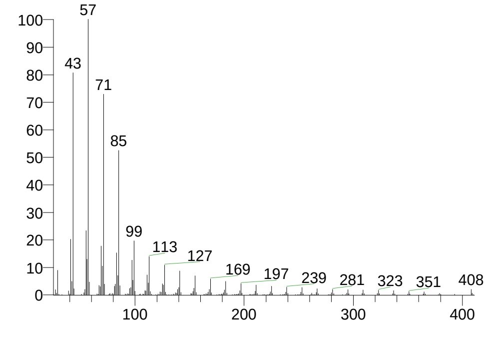
SI 817, RSI 879, replib, Entry# 6113, CAS# 593-49-7, Heptacosane



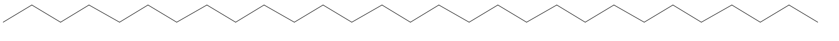
Heptacosane
Formula C27H56, MW 380, CAS# 593-49-7, Entry# 6113
n-Heptacosane



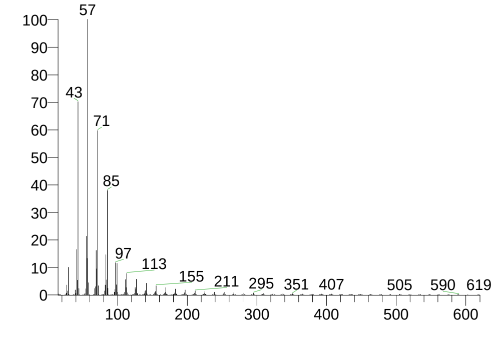
SI 772, RSI 830, replib, Entry# 6082, CAS# 630-03-5, Nonacosane



Nonacosane
Formula C29H60, MW 408, CAS# 630-03-5, Entry# 6082
n-Nonacosane

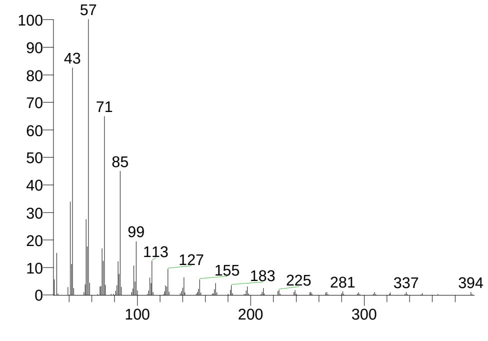


SI 770, RSI 818, replib, Entry# 6126, CAS# 7098-22-8, Tetratetracontane



Tetratetracontane
Formula C44H90, MW 618, CAS# 7098-22-8, Entry# 6126
n-Tetratetracontane

SI 765, RSI 902, replib, Entry# 6074, CAS# 630-02-4, Octacosane



Octacosane
Formula C28H58, MW 394, CAS# 630-02-4, Entry# 6074
n-Octacosane

There is no signature data to report.

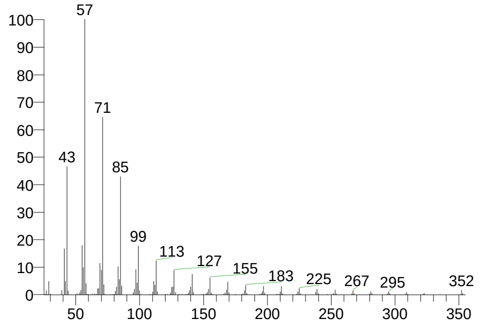
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

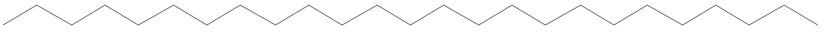
Hit Spectrum

SI 760, RSI 853, replib, Entry# 6410, CAS# 629-99-2, Pentacosane



Compound Structure

Pentacosane
Formula C25H52, MW 352, CAS# 629-99-2, Entry# 6410
n-Pentacosane



There is no signature data to report.

Library Search Report

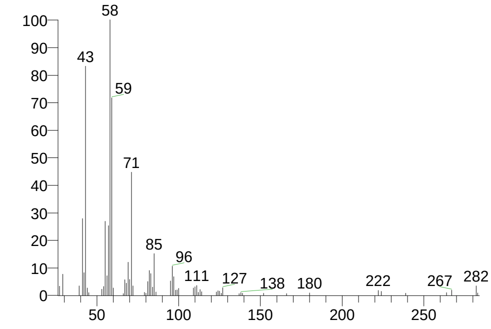
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

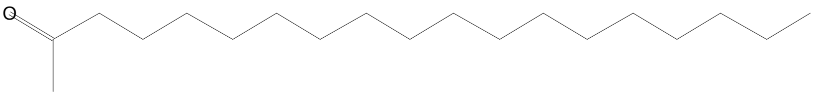
RT	Compound Name	Area	Area %
43.82	2-Heptadecanone	893079627.98	0.92
43.82	2-Nonadecanone	893079627.98	0.92
43.82	2-Pentacosanone	893079627.98	0.92
43.82	2-Pentadecanone	893079627.98	0.92
43.82	2-Pentadecanone, 6,10,14-trimethyl-	893079627.98	0.92

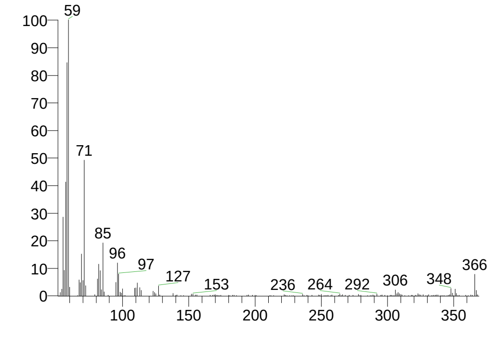
Hit Spectrum | Compound Structure

SI 734, RSI 915, mainlib, Entry# 27961, CAS# 629-66-3, 2-Nonadecanone

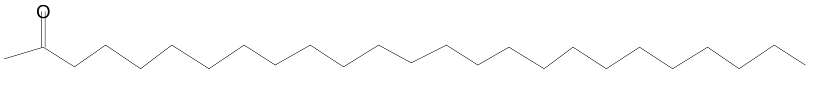


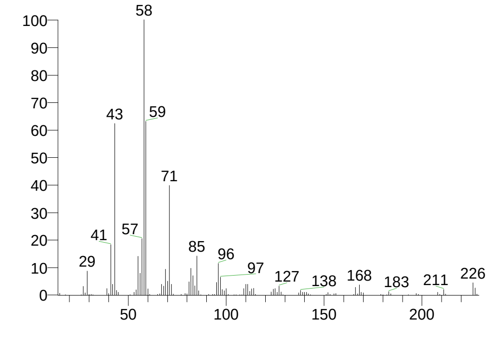
2-Nonadecanone
Formula C19H38O, MW 282, CAS# 629-66-3, Entry# 27961
Methyl heptadecyl ketone



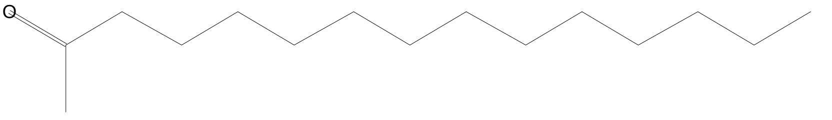


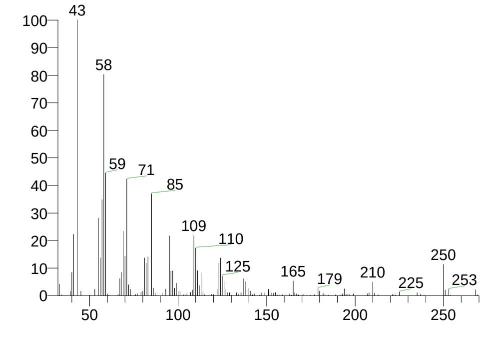
2-Pentacosanone
Formula C25H50O, MW 366, CAS# 75207-54-4, Entry# 30016
$:28CDTIEPBCRWXMBD-UHFFFAOYSA-N



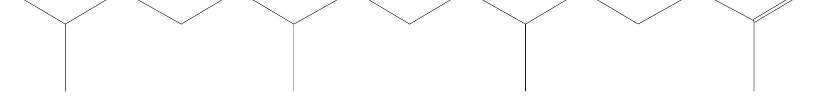


2-Pentadecanone
Formula C15H30O, MW 226, CAS# 2345-28-0, Entry# 28246
Methyl tridecyl ketone





2-Pentadecanone, 6,10,14-trimethyl-
Formula C18H36O, MW 268, CAS# 502-69-2, Entry# 2408
Hexahydrofarnesyl acetone



There is no signature data to report.

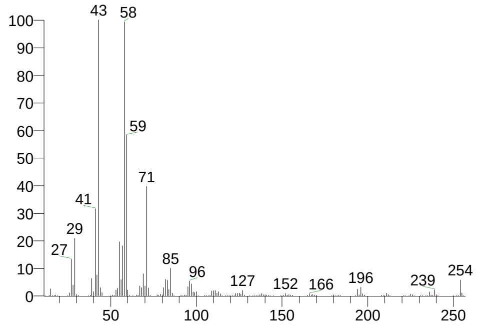
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

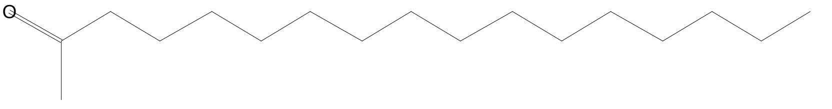
Hit Spectrum

SI 679, RSI 762, replib, Entry# 2404, CAS# 2922-51-2, 2-Heptadecanone



Compound Structure

2-Heptadecanone
Formula C17H34O, MW 254, CAS# 2922-51-2, Entry# 2404
Heptadecan-2-one



There is no signature data to report.

Library Search Report

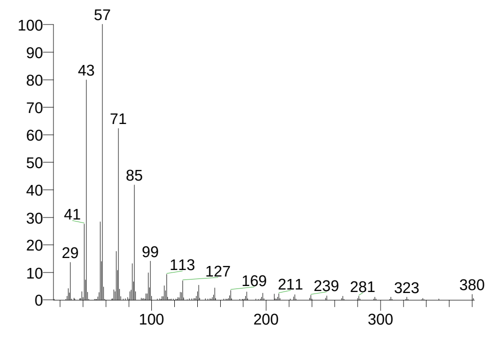
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

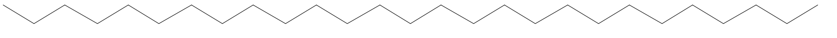
RT	Compound Name	Area	Area %
43.99	13-Methylheptacosane	1636286733.14	1.69
43.99	Hentriacontane	1636286733.14	1.69
43.99	Heptacosane	1636286733.14	1.69
43.99	Octacosane	1636286733.14	1.69
43.99	Tetratetracontane	1636286733.14	1.69

Hit Spectrum | Compound Structure

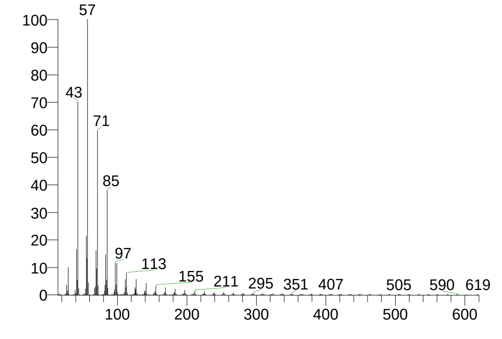
SI 821, RSI 848, replib, Entry# 6113, CAS# 593-49-7, Heptacosane



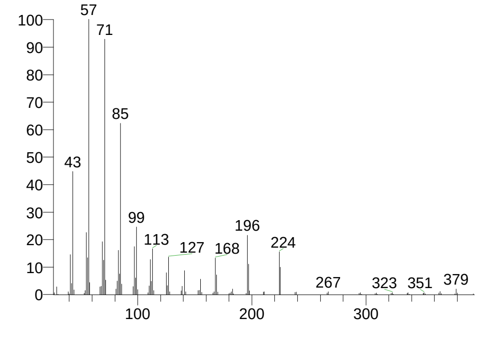
Heptacosane
Formula C27H56, MW 380, CAS# 593-49-7, Entry# 6113
n-Heptacosane



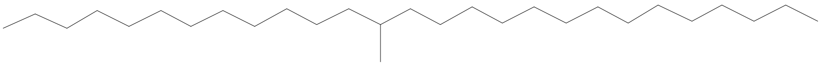
SI 811, RSI 821, replib, Entry# 6126, CAS# 7098-22-8, Tetratetracontane



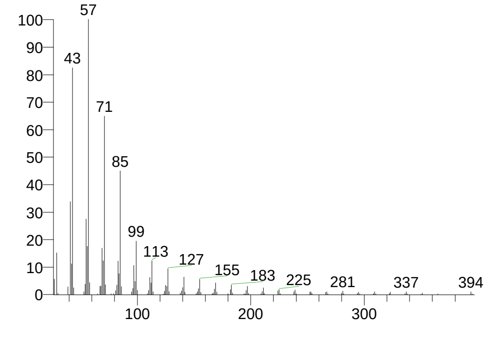
Tetratetracontane
Formula C44H90, MW 618, CAS# 7098-22-8, Entry# 6126
n-Tetratetracontane



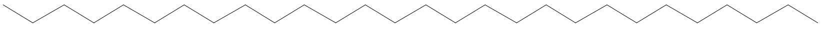
13-Methylheptacosane
Formula C28H58, MW 394, CAS# 15689-72-2, Entry# 25535
Heptacosane, 13-methyl



SI 810, RSI 863, replib, Entry# 6074, CAS# 630-02-4, Octacosane



Octacosane
Formula C28H58, MW 394, CAS# 630-02-4, Entry# 6074
n-Octacosane



There is no signature data to report.

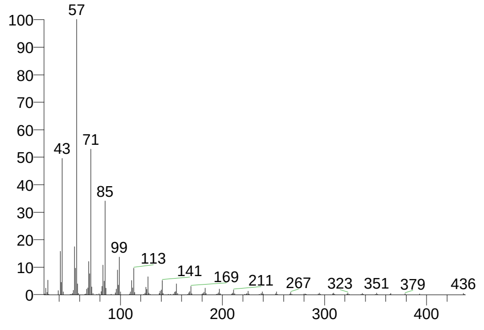
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

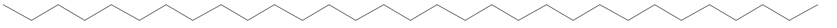
Hit Spectrum

SI 809, RSI 852, replib, Entry# 6374, CAS# 630-04-6, Hentriacontane



Compound Structure

Hentriacontane
Formula C31H64, MW 436, CAS# 630-04-6, Entry# 6374
n-Hentriacontane



There is no signature data to report.

Library Search Report

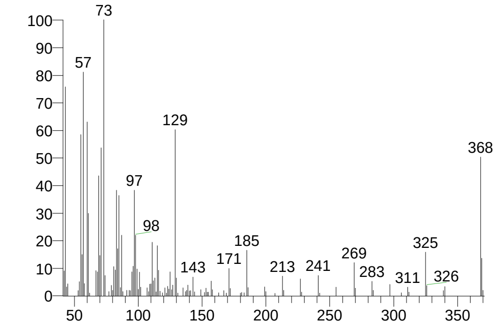
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

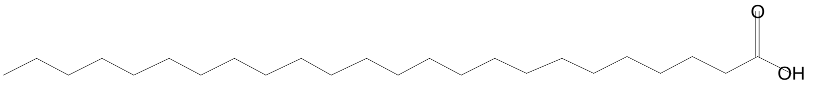
RT	Compound Name	Area	Area %
44.42	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	418355774.28	0.43
44.42	Dodecyl cis-9,10-epoxyoctadecanoate	418355774.28	0.43
44.42	Glycidyl oleate	418355774.28	0.43
44.42	Tetracosanoic acid	418355774.28	0.43
44.42	Tetracosanoic acid	418355774.28	0.43

Hit Spectrum | Compound Structure

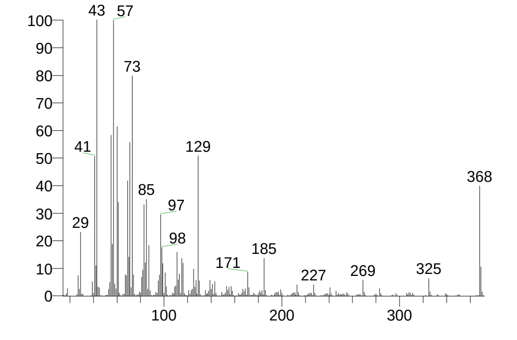
SI 699, RSI 849, replib, Entry# 9603, CAS# 557-59-5, Tetracosanoic acid



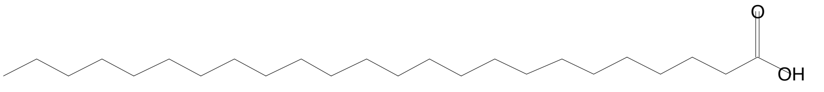
Tetracosanoic acid
Formula C24H48O2, MW 368, CAS# 557-59-5, Entry# 9603
Lignoceric acid



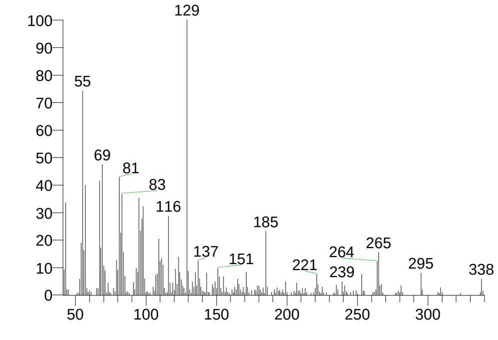
SI 698, RSI 723, mainlib, Entry# 7898, CAS# 557-59-5, Tetracosanoic acid



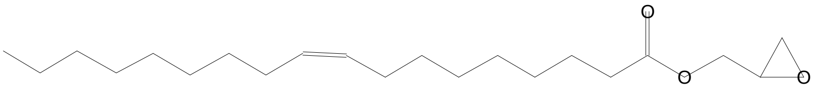
Tetracosanoic acid
Formula C24H48O2, MW 368, CAS# 557-59-5, Entry# 7898
Lignoceric acid

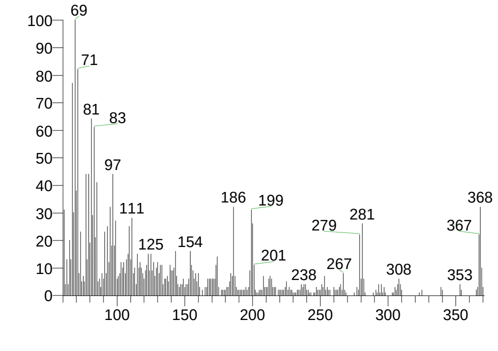


SI 693, RSI 763, mainlib, Entry# 112970, CAS# NA, Glycidyl oleate

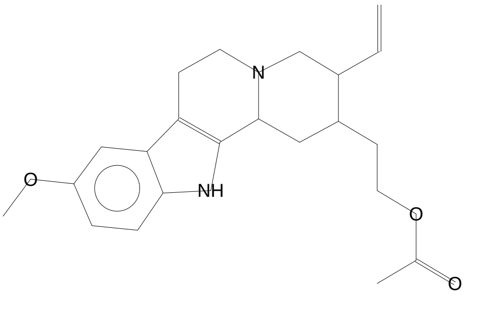


Glycidyl oleate
Formula C21H38O3, MW 338, CAS# NA, Entry# 112970
$:28VWYIWOYBERNXLX-KTKRTIGZSA-N





Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)
Formula C22H28N2O3, MW 368, CAS# 56053-13-5, Entry# 35310
10-Methoxycoryn-18-en-17-yl acetate #



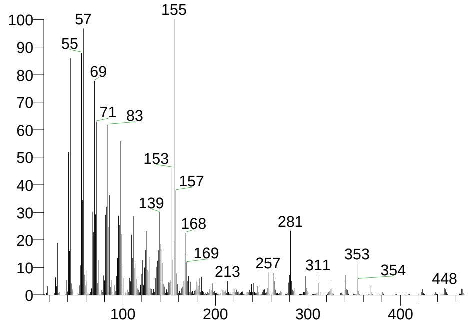
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

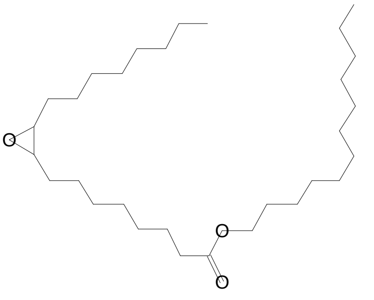
Flower pet ether

Hit Spectrum



Compound Structure

Dodecyl cis-9,10-epoxyoctadecanoate
Formula C30H58O3, MW 466, CAS# 92332-53-1, Entry# 144303
Dodecyl 8-(3-octyl-2-oxiranyl)octanoate #



There is no signature data to report.

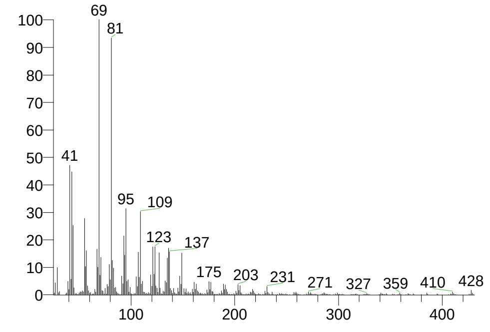
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

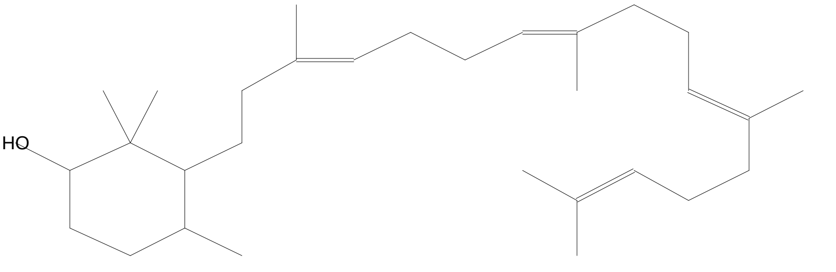
Flower pet ether

RT	Compound Name	Area	Area %
45.16	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	511669171.33	0.53
45.16	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	511669171.33	0.53
45.16	Squalene	511669171.33	0.53
45.16	Squalene	511669171.33	0.53
45.16	Squalene	511669171.33	0.53

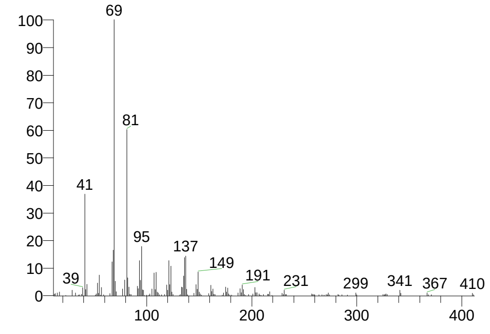
Hit Spectrum | Compound Structure



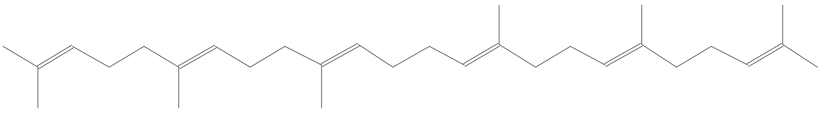
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
Formula C30H52O, MW 428, CAS# NA, Entry# 35374
2,2,4-Trimethyl-3-[(3E,7E,11E)-3,8,12,16-tetramethyl-3,7,11,15-heptadecatetraenyl]cyclohexanol #



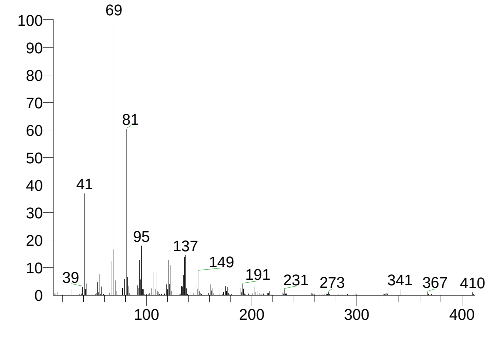
SI 776, RSI 861, replib, Entry# 8708, CAS# 111-02-4, Squalene



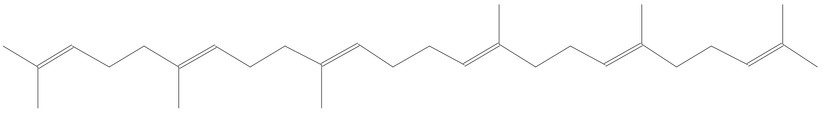
Squalene
Formula C30H50, MW 410, CAS# 111-02-4, Entry# 8708
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-



SI 776, RSI 860, replib, Entry# 8706, CAS# 111-02-4, Squalene



Squalene
Formula C30H50, MW 410, CAS# 111-02-4, Entry# 8706
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-



There is no signature data to report.

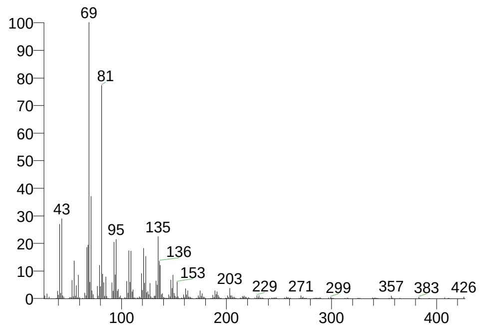
Library Search Report

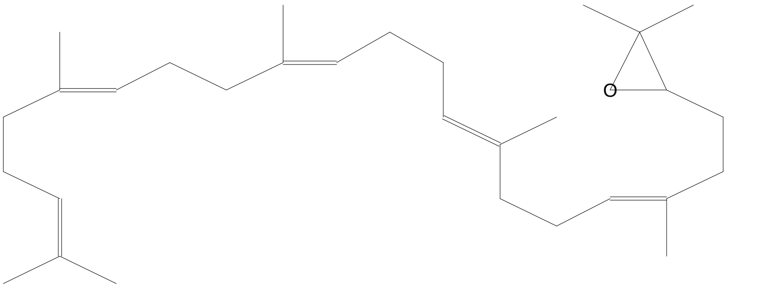
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

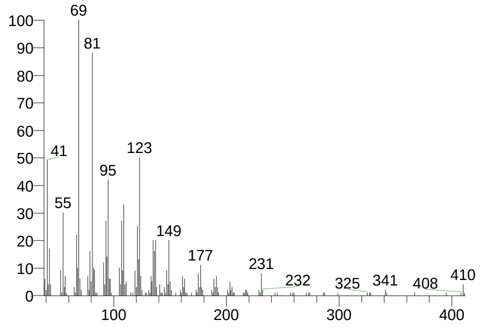
Hit Spectrum	Compound Structure

Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-
Formula C30H50O, MW 426, CAS# 7200-26-2, Entry# 8712

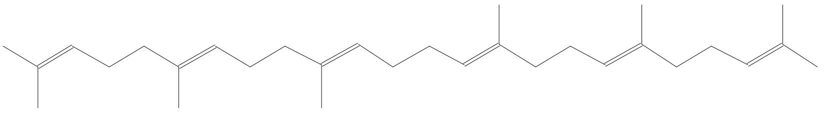




SI 767, RSI 852, replib, Entry# 8718, CAS# 111-02-4, Squalene



Squalene
Formula C30H50, MW 410, CAS# 111-02-4, Entry# 8718
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-



There is no signature data to report.

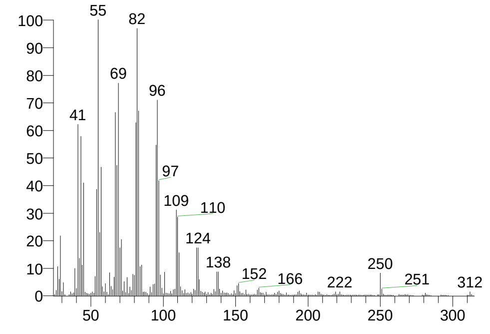
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

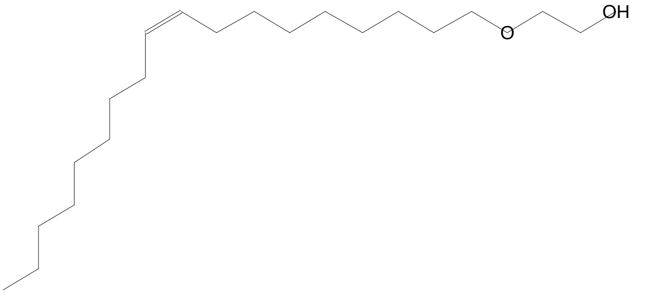
Flower pet ether

RT	Compound Name	Area	Area %
45.24	Docosanal	1169354000.38	1.21
45.24	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	1169354000.38	1.21
45.24	Hexacosanal	1169354000.38	1.21
45.24	Tricosanal	1169354000.38	1.21
45.24	Z-14-Octadecen-1-ol acetate	1169354000.38	1.21

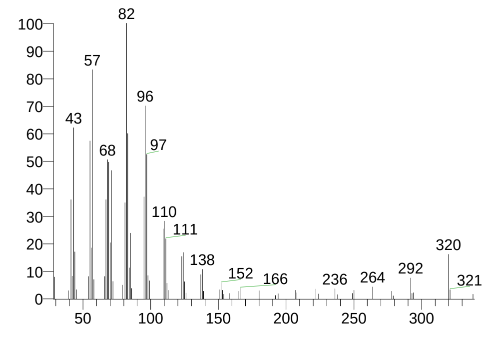
Hit Spectrum | Compound Structure



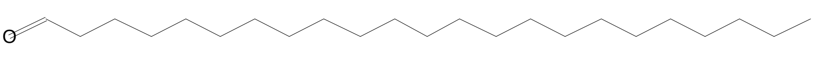
Ethanol, 2-(9-octadecenyloxy)-, (Z)-
Formula C20H40O2, MW 312, CAS# 5353-25-3, Entry# 20581
2-cis-9-Octadecenyloxyethanol

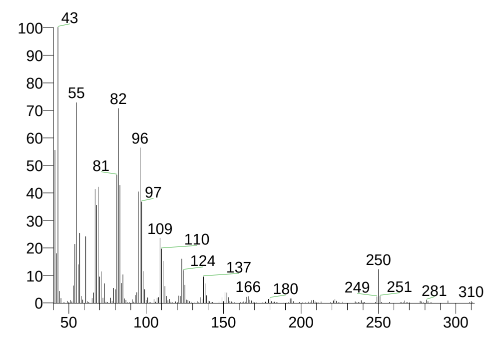


SI 794, RSI 917, mainlib, Entry# 51221, CAS# 72934-02-2, Tricosanal

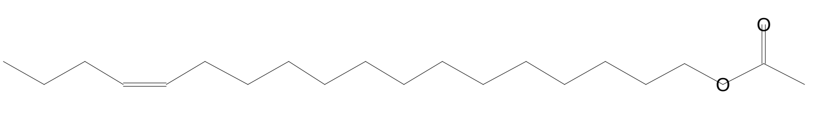


Tricosanal
Formula C23H46O, MW 338, CAS# 72934-02-2, Entry# 51221
n-Tricosanal

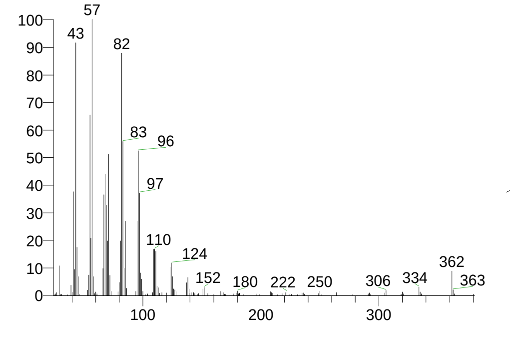




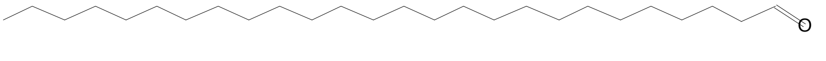
Z-14-Octadecen-1-ol acetate
Formula C20H38O2, MW 310, CAS# NA, Entry# 7356
(14Z)-14-Octadecenyl acetate #



SI 790, RSI 884, mainlib, Entry# 24426, CAS# 26627-85-0, Hexacosanal



Hexacosanal
Formula C26H52O, MW 380, CAS# 26627-85-0, Entry# 24426
n-Hexacosanal



There is no signature data to report.

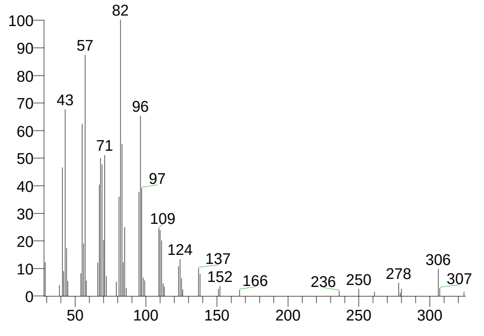
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

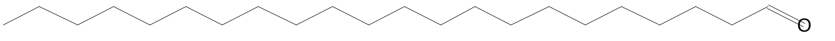
Hit Spectrum

SI 774, RSI 933, mainlib, Entry# 51214, CAS# 57402-36-5, Docosanal



Compound Structure

Docosanal
Formula C22H44O, MW 324, CAS# 57402-36-5, Entry# 51214
1-Docosanal



There is no signature data to report.

Library Search Report

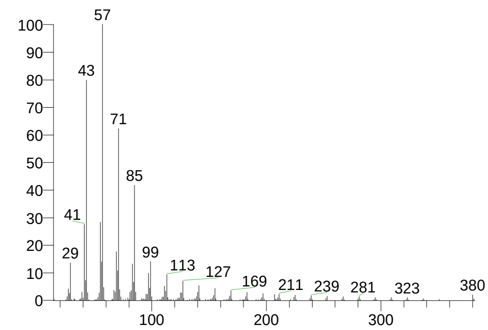
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

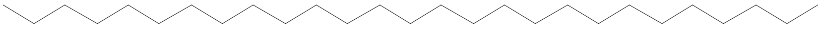
RT	Compound Name	Area	Area %
45.52	Heptacosane	460917741.17	0.48
45.52	Hexacosane, 9-octyl-	460917741.17	0.48
45.52	Nonacosane	460917741.17	0.48
45.52	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	460917741.17	0.48
45.52	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	460917741.17	0.48

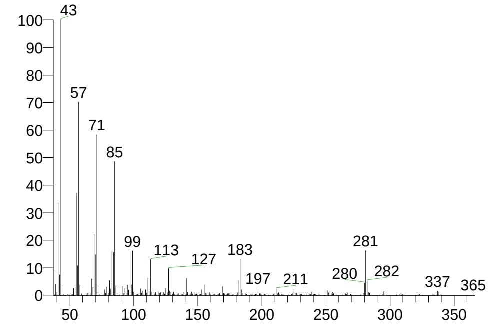
Hit Spectrum | Compound Structure

SI 745, RSI 828, replib, Entry# 6113, CAS# 593-49-7, Heptacosane

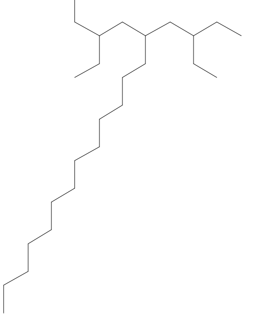


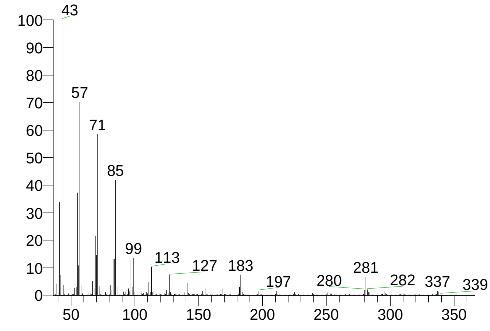
Heptacosane
Formula C27H56, MW 380, CAS# 593-49-7, Entry# 6113
n-Heptacosane



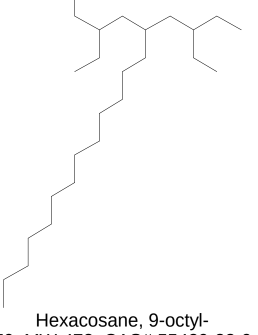


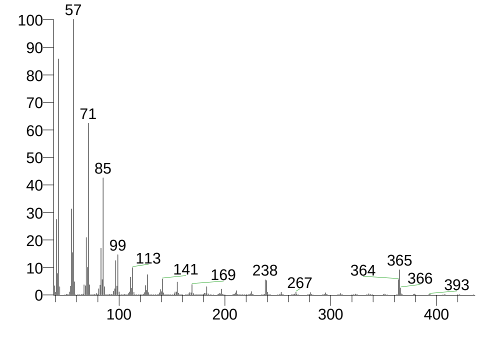
Octadecane, 3-ethyl-5-(2-ethylbutyl)-
Formula C26H54, MW 366, CAS# 55282-12-7, Entry# 2329
3-Ethyl-5-(2'-ethylbutyl)octadecane



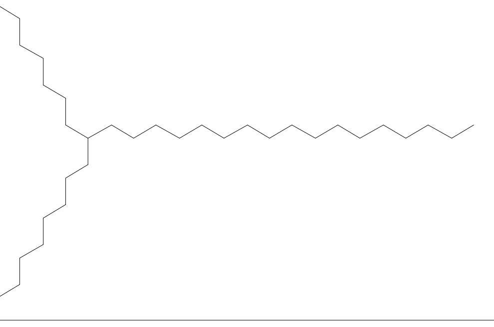


Octadecane, 3-ethyl-5-(2-ethylbutyl)-
Formula C26H54, MW 366, CAS# 55282-12-7, Entry# 7878
3-Ethyl-5-(2'-ethylbutyl)octadecane





Hexacosane, 9-octyl-
Formula C34H70, MW 478, CAS# 55429-83-9, Entry# 24361
9-n-Octylhexacosane



There is no signature data to report.

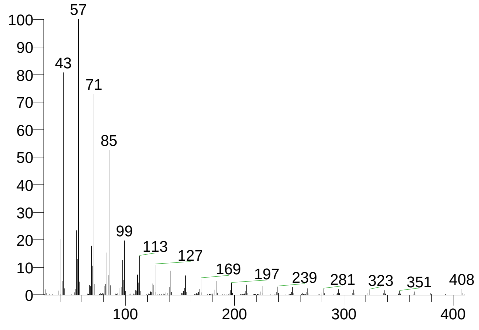
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

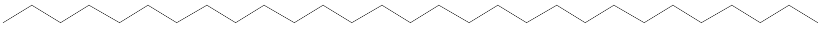
Hit Spectrum

SI 734, RSI 788, replib, Entry# 6082, CAS# 630-03-5, Nonacosane



Compound Structure

Nonacosane
Formula C29H60, MW 408, CAS# 630-03-5, Entry# 6082
n-Nonacosane



There is no signature data to report.

Library Search Report

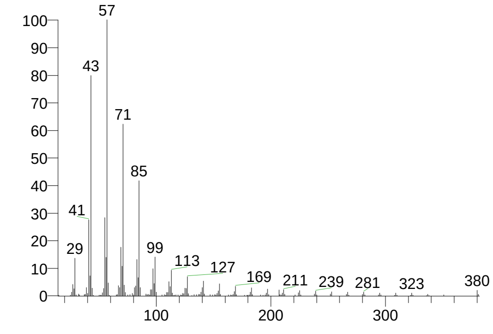
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

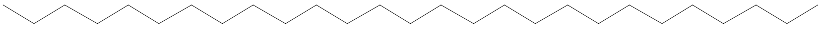
RT	Compound Name	Area	Area %
46.02	Heptacosane	453910061.03	0.47
46.02	Nonacosane	453910061.03	0.47
46.02	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	453910061.03	0.47
46.02	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	453910061.03	0.47
46.02	Tetrapentacontane, 1,54-dibromo-	453910061.03	0.47

Hit Spectrum	Compound Structure

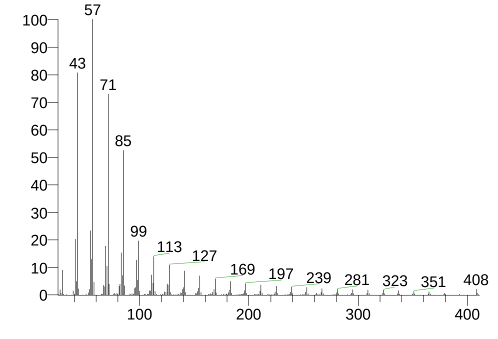
SI 769, RSI 842, replib, Entry# 6113, CAS# 593-49-7, Heptacosane



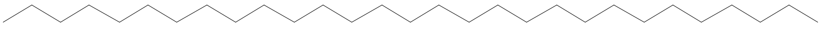
Heptacosane
Formula C27H56, MW 380, CAS# 593-49-7, Entry# 6113
n-Heptacosane

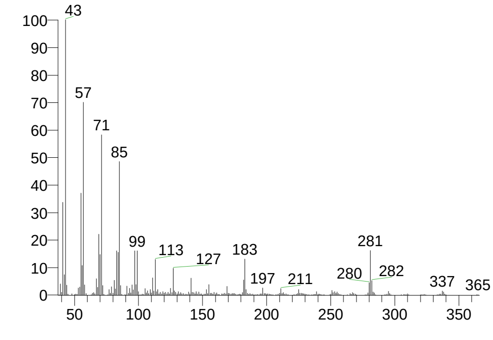


SI 746, RSI 800, replib, Entry# 6082, CAS# 630-03-5, Nonacosane

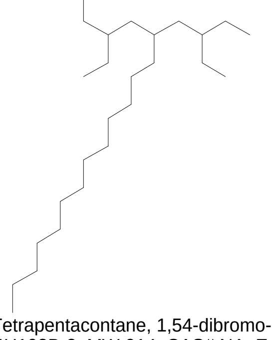


Nonacosane
Formula C29H60, MW 408, CAS# 630-03-5, Entry# 6082
n-Nonacosane

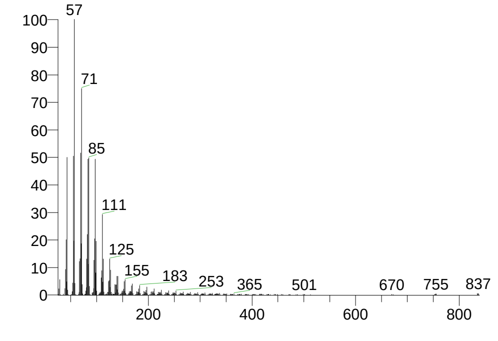


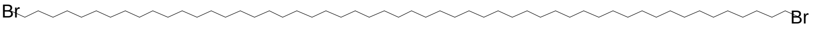


Octadecane, 3-ethyl-5-(2-ethylbutyl)-
Formula C26H54, MW 366, CAS# 55282-12-7, Entry# 2329
3-Ethyl-5-(2'-ethylbutyl)octadecane



Tetrapentacontane, 1,54-dibromo-
Formula C54H108Br2, MW 914, CAS# NA, Entry# 25430
1,54-Dibromotetrapentacontane #





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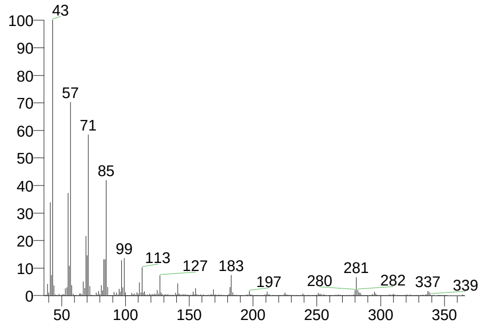
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

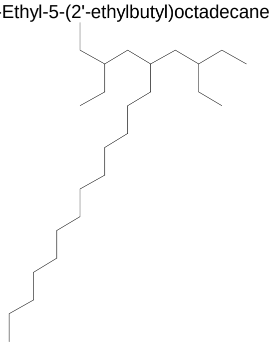
Flower pet ether

Hit Spectrum



Compound Structure

Octadecane, 3-ethyl-5-(2-ethylbutyl)-
Formula C26H54, MW 366, CAS# 55282-12-7, Entry# 7878
3-Ethyl-5-(2'-ethylbutyl)octadecane



There is no signature data to report.

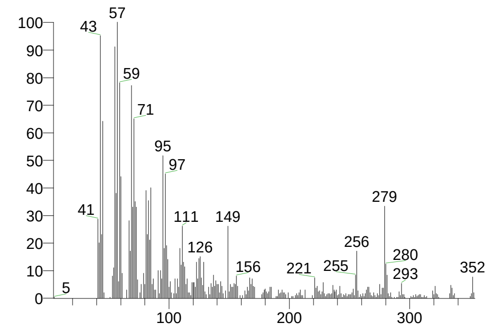
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

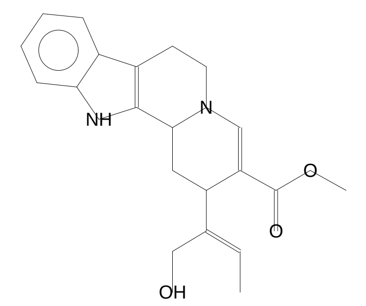
Flower pet ether

RT	Compound Name	Area	Area %
46.32	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15á,16E)-	503388222.12	0.52
46.32	2-Heptacosanone	503388222.12	0.52
46.32	2-Nonadecanone	503388222.12	0.52
46.32	Hexacosa-2,25-dione	503388222.12	0.52
46.32	Stearic acid, 3-(octadecyloxy)propyl ester	503388222.12	0.52

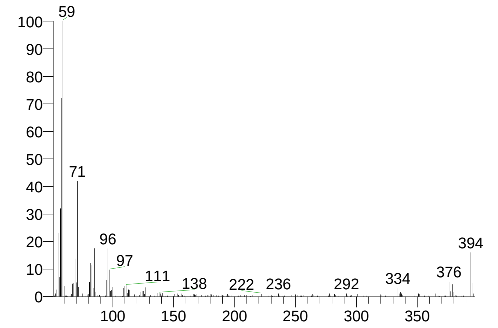
Hit Spectrum	Compound Structure

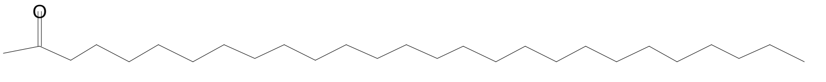


Formula C21H24N2O3, MW 352, CAS# 5523-49-9, Entry# 24103



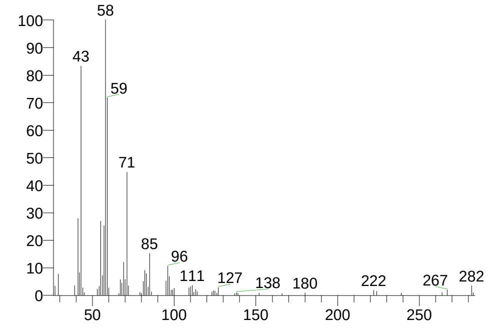
2-Heptacosanone
Formula C27H54O, MW 394, CAS# 7796-19-2, Entry# 30017
$:28MJEFMWGOVVCQIK-UHFFFAOYSA-N

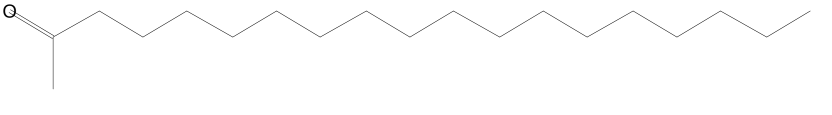




SI 667, RSI 901, mainlib, Entry# 27961, CAS# 629-66-3, 2-Nonadecanone

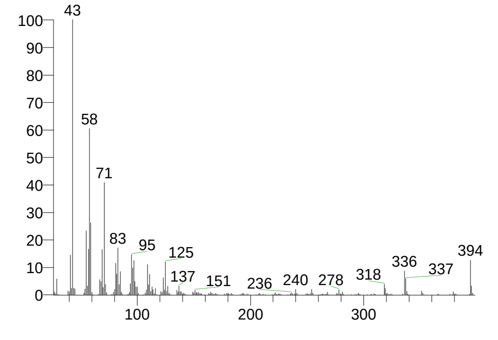
2-Nonadecanone
Formula C19H38O, MW 282, CAS# 629-66-3, Entry# 27961
Methyl heptadecyl ketone

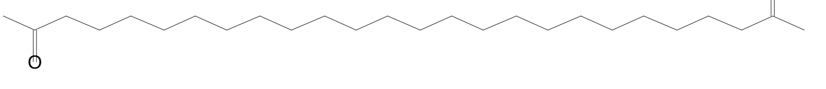




SI 664, RSI 701, mainlib, Entry# 8102, CAS# NA, Hexacosa-2,25-dione

Hexacosa-2,25-dione
Formula C26H50O2, MW 394, CAS# NA, Entry# 8102
2,25-Hexacosanedione #





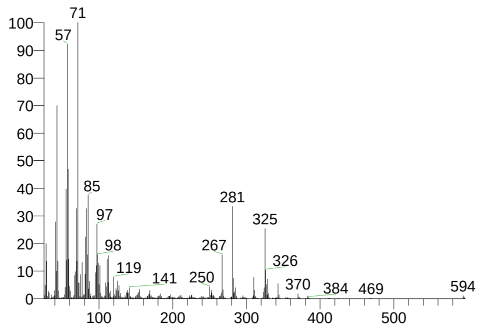
There is no signature data to report.

Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

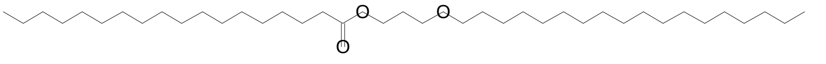
Flower pet ether

Hit Spectrum



Compound Structure

Stearic acid, 3-(octadecyloxy)propyl ester
Formula C39H78O3, MW 594, CAS# 17367-40-7, Entry# 38787
3-Octadecyloxy-1-O-octadecanoylpropanol



There is no signature data to report.

Library Search Report

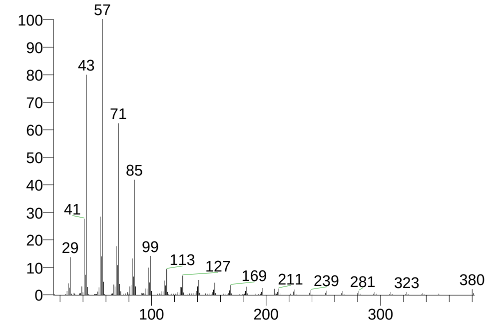
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

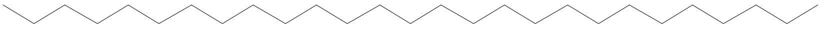
RT	Compound Name	Area	Area %
46.46	17-Pentatriacontene	425499630.94	0.44
46.46	Ethanol, 2-(octadecyloxy)-	425499630.94	0.44
46.46	Heptacosane	425499630.94	0.44
46.46	Tetrapentacontane, 1,54-dibromo-	425499630.94	0.44
46.46	Z-5-Methyl-6-heneicosen-11-one	425499630.94	0.44

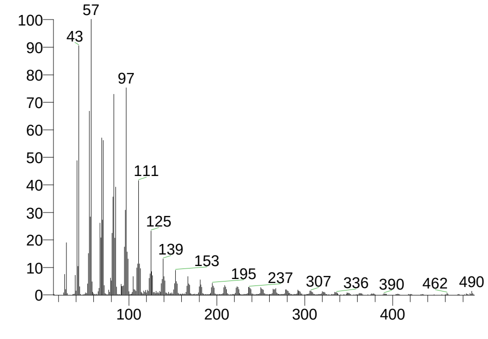
Hit Spectrum | Compound Structure

SI 732, RSI 803, replib, Entry# 6113, CAS# 593-49-7, Heptacosane

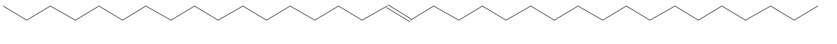


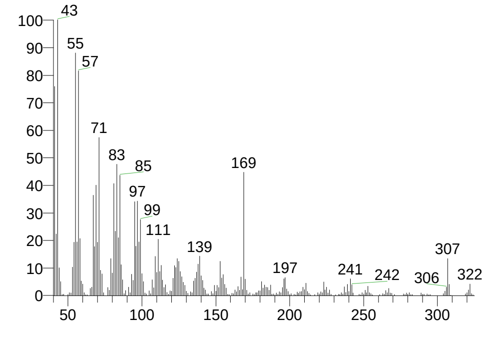
Heptacosane
Formula C27H56, MW 380, CAS# 593-49-7, Entry# 6113
n-Heptacosane



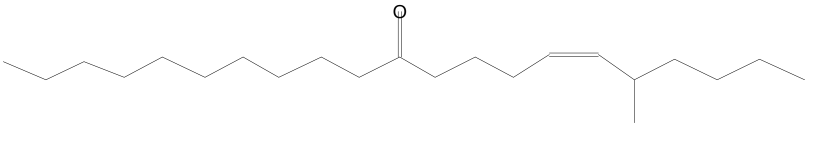


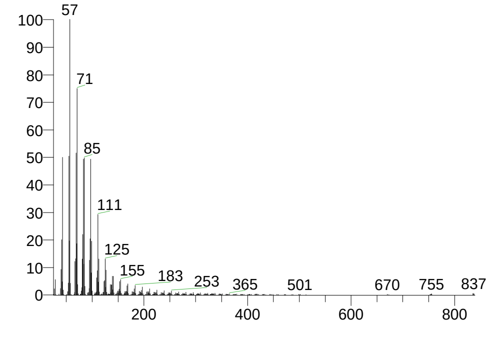
17-Pentatriacontene
Formula C35H70, MW 490, CAS# 6971-40-0, Entry# 24490
(17E)-17-Pentatriacontene #



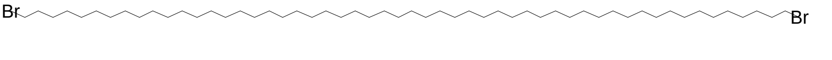


Z-5-Methyl-6-heneicosen-11-one
Formula C22H42O, MW 322, CAS# NA, Entry# 7198
(6Z)-5-Methyl-6-henicosen-11-one #





Tetrapentacontane, 1,54-dibromo-
Formula C54H108Br2, MW 914, CAS# NA, Entry# 25430
1,54-Dibromotetrapentacontane #



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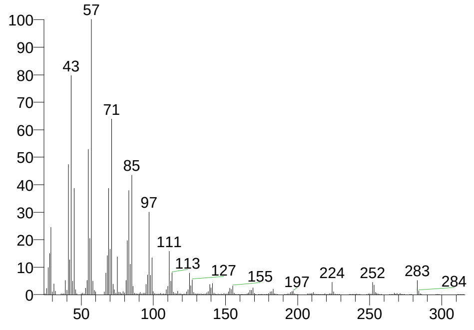
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

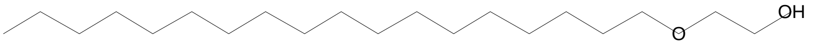
Flower pet ether

Hit Spectrum



Compound Structure

Ethanol, 2-(octadecyloxy)-
Formula C20H42O2, MW 314, CAS# 2136-72-3, Entry# 24333
2-Octadecoxyethanol



There is no signature data to report.

Library Search Report

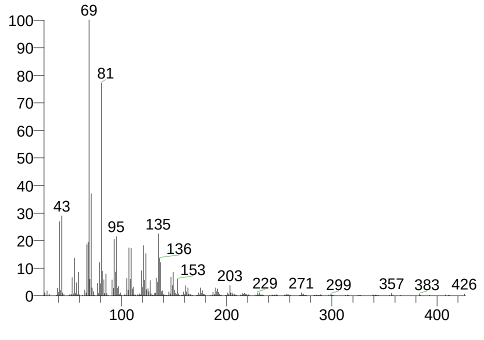
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

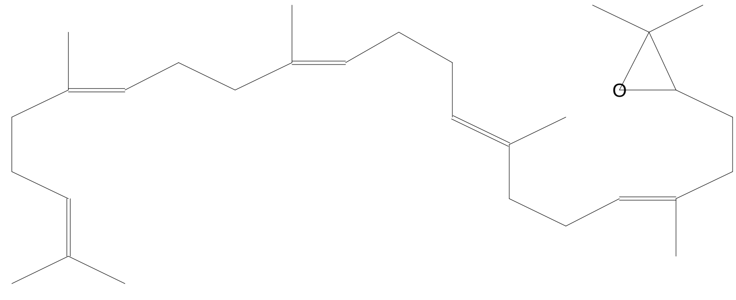
RT	Compound Name	Area	Area %
47.75	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	927975968.38	0.96
47.75	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(ñ)-	927975968.38	0.96
47.75	1-Heptatriacotanol	927975968.38	0.96
47.75	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	927975968.38	0.96
47.75	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	927975968.38	0.96

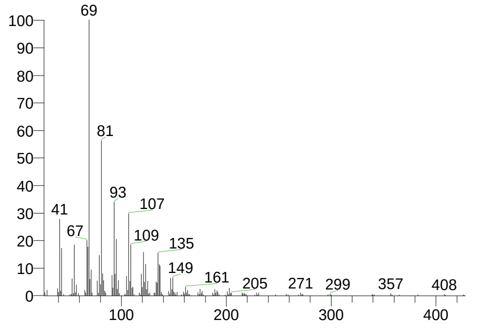
Hit Spectrum

Compound Structure

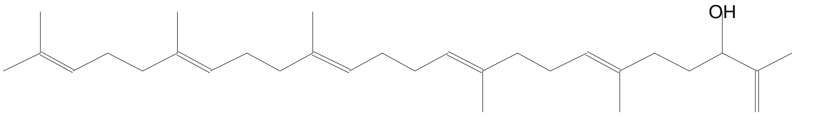


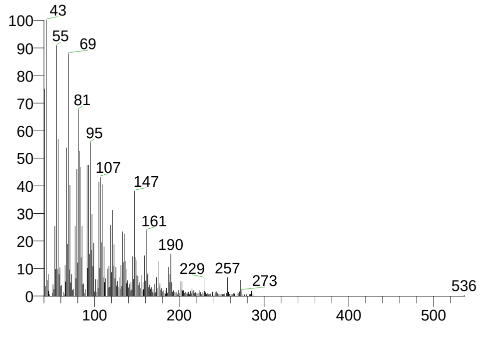
Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-
Formula C30H50O, MW 426, CAS# 7200-26-2, Entry# 8712



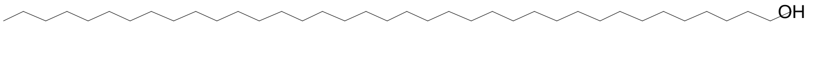


1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(ñ)-
Formula C30H50O, MW 426, CAS# 97232-74-1, Entry# 35419
$:28JLUBMMAQMKVTGL-RLROCYJYSA-N





1-Heptatriacotanol
Formula C37H76O, MW 536, CAS# 105794-58-9, Entry# 7279
1-Heptatriacontanol #



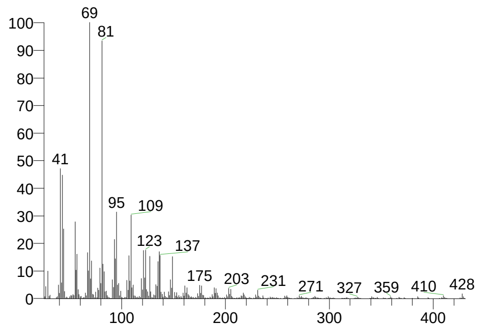
There is no signature data to report.

Library Search Report

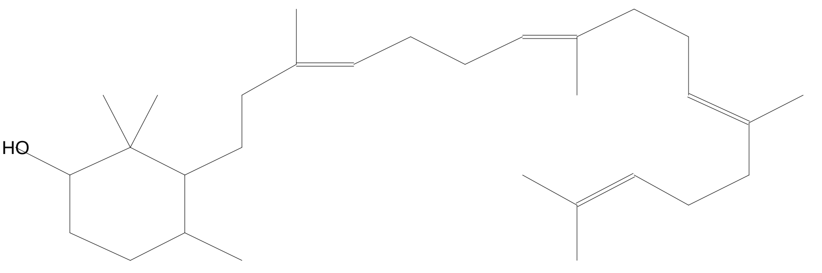
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

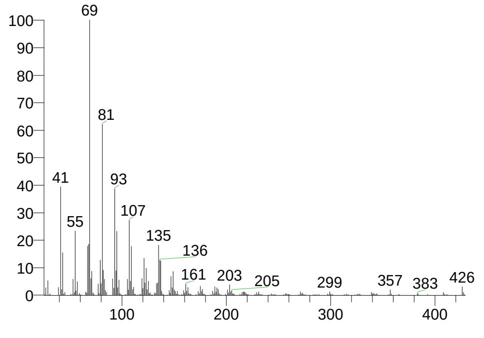
Flower pet ether

Hit Spectrum | Compound Structure



2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
Formula C30H52O, MW 428, CAS# NA, Entry# 35374
2,2,4-Trimethyl-3-[(3E,7E,11E)-3,8,12,16-tetramethyl-3,7,11,15-heptadecatetraenyl]cyclohexanol #





1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-
Formula C30H50O, MW 426, CAS# 54159-46-5, Entry# 35385
(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol #

There is no signature data to report.

Library Search Report

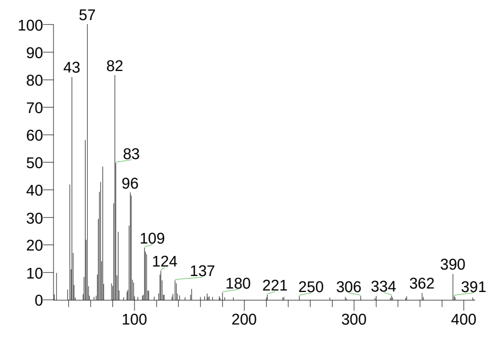
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

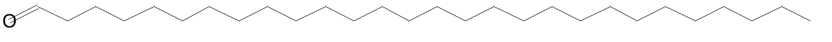
RT	Compound Name	Area	Area %
48.31	Docosanal	4078185904.50	4.21
48.31	Henicosanal	4078185904.50	4.21
48.31	Hexacosanal	4078185904.50	4.21
48.31	Octacosanal	4078185904.50	4.21
48.31	Tricosanal	4078185904.50	4.21

Hit Spectrum | Compound Structure

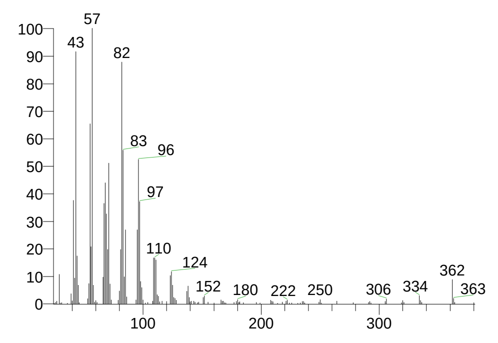
SI 832, RSI 894, mainlib, Entry# 25706, CAS# 22725-64-0, Octacosanal



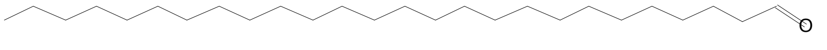
Octacosanal
Formula C28H56O, MW 408, CAS# 22725-64-0, Entry# 25706
n-Octacosanal



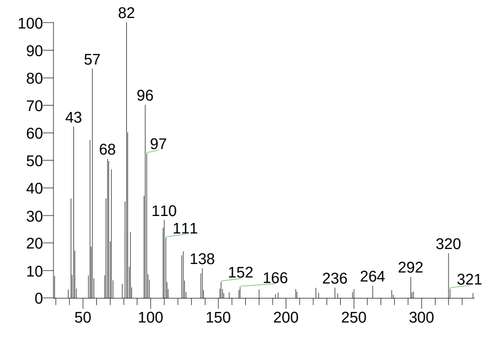
SI 829, RSI 887, mainlib, Entry# 24426, CAS# 26627-85-0, Hexacosanal



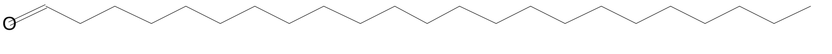
Hexacosanal
Formula C26H52O, MW 380, CAS# 26627-85-0, Entry# 24426
n-Hexacosanal



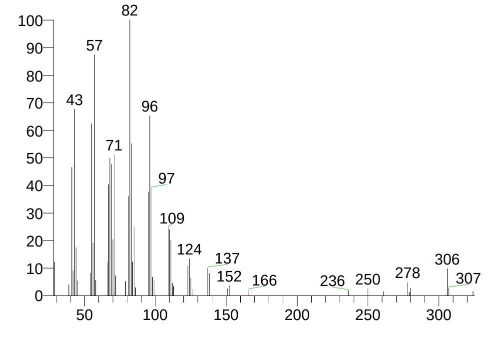
SI 828, RSI 910, mainlib, Entry# 51221, CAS# 72934-02-2, Tricosanal



Tricosanal
Formula C23H46O, MW 338, CAS# 72934-02-2, Entry# 51221
n-Tricosanal



SI 824, RSI 935, mainlib, Entry# 51214, CAS# 57402-36-5, Docosanal



Docosanal
Formula C22H44O, MW 324, CAS# 57402-36-5, Entry# 51214
1-Docosanal

There is no signature data to report.

Library Search Report

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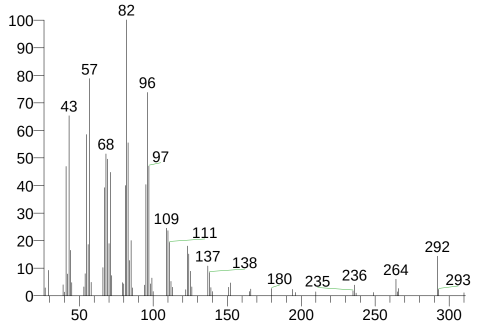
Flower pet ether

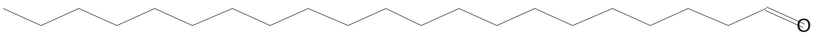
Hit Spectrum

SI 823, RSI 912, mainlib, Entry# 51223, CAS# 51227-32-8, Henicosanal



Compound Structure

Henicosanal
Formula C21H42O, MW 310, CAS# 51227-32-8, Entry# 51223
Heneicosanal



There is no signature data to report.

Library Search Report

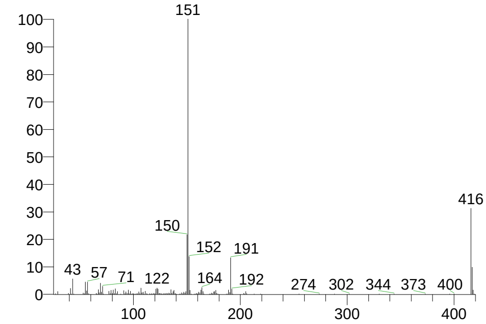
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

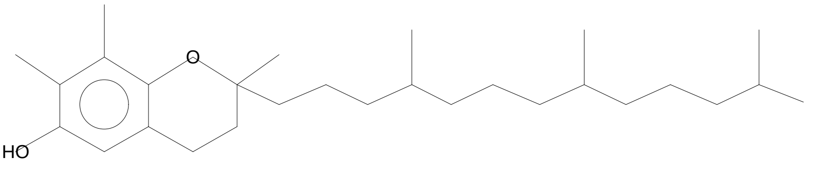
RT	Compound Name	Area	Area %
48.68	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman	1421055160.50	1.47
48.68	3,4-Dihydro-3,5,8-trimethyl-3-(4,8,12-trimethyltridecyl)-(2H)1-benzopyran-6 -acetate	1421055160.50	1.47
48.68	ç-Tocopherol	1421055160.50	1.47
48.68	ç-Tocopherol	1421055160.50	1.47
48.68	ç-Tocopherol	1421055160.50	1.47

Hit Spectrum	Compound Structure

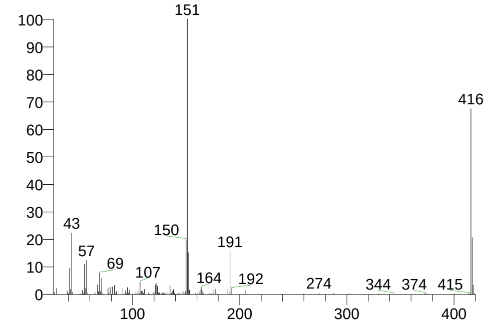
SI 825, RSI 884, replib, Entry# 23865, CAS# 7616-22-0, ç-Tocopherol



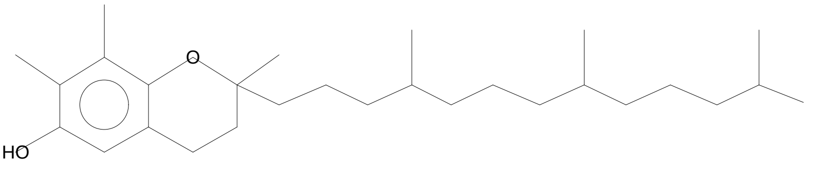
ç-Tocopherol
Formula C28H48O2, MW 416, CAS# 7616-22-0, Entry# 23865
2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-



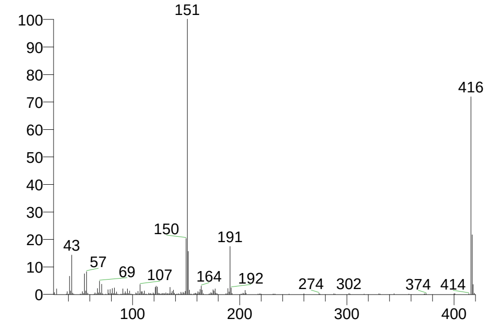
SI 819, RSI 891, replib, Entry# 23863, CAS# 7616-22-0, ç-Tocopherol



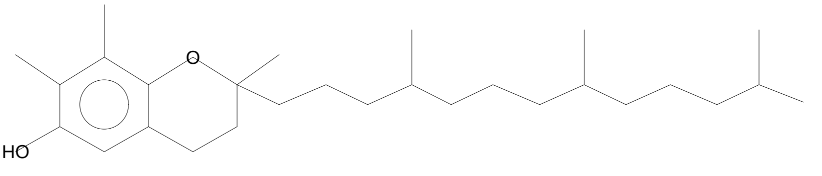
ç-Tocopherol
Formula C28H48O2, MW 416, CAS# 7616-22-0, Entry# 23863
2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-

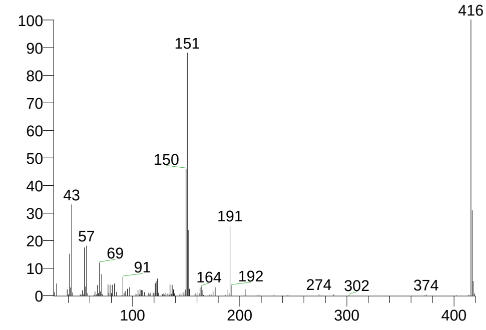


SI 809, RSI 860, mainlib, Entry# 141945, CAS# 7616-22-0, ç-Tocopherol

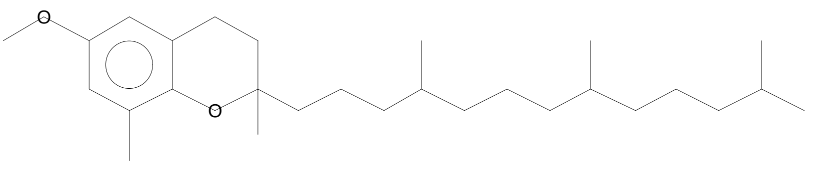


ç-Tocopherol
Formula C28H48O2, MW 416, CAS# 7616-22-0, Entry# 141945
2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-





(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman
Formula C28H48O2, MW 416, CAS# 1116113-36-0, Entry# 239082
2H-1-Benzopyran, 3,4-dihydro-6-methoxy-2,8-dimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-, (2R)-



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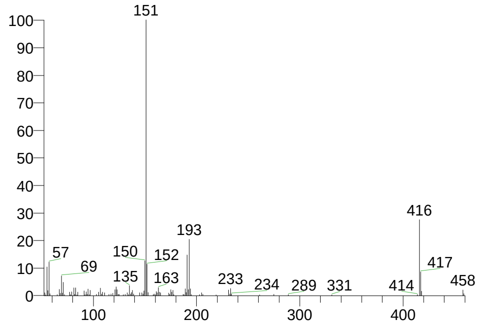
Library Search Report

CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

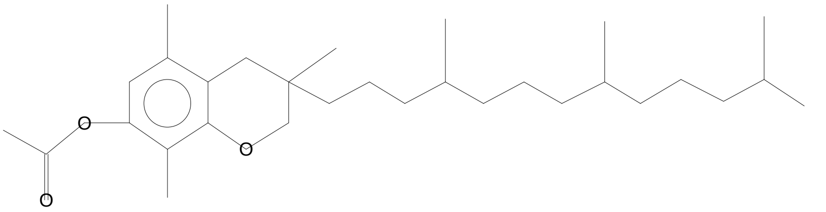
Flower pet ether

Hit Spectrum



Compound Structure

3,4-Dihydro-3,5,8-trimethyl-3-(4,8,12-trimethyltridecyl)-(2H)1-benzopyran-6-acetate
Formula C30H50O3, MW 458, CAS# NA, Entry# 141944
3,5,8-Trimethyl-3-(4,8,12-trimethyltridecyl)-3,4-dihydro-2H-chromen-7-yl acetate #



There is no signature data to report.

Library Search Report

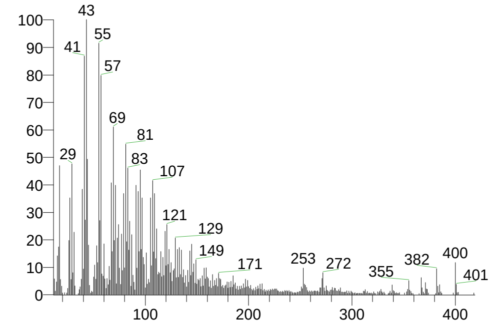
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

Flower pet ether

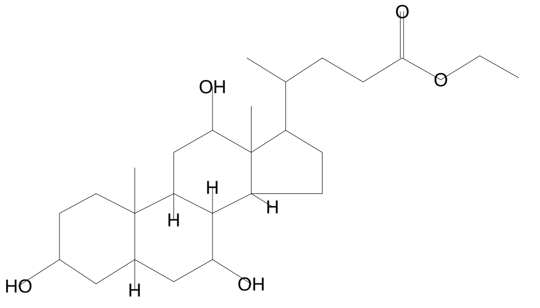
RT	Compound Name	Area	Area %
49.34	1-Heptatriacotanol	439935189.67	0.45
49.34	10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-2H-picene-4a-carboxylic acid, methyl ester	439935189.67	0.45
49.34	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	439935189.67	0.45
49.34	Ethyl iso-allocholate	439935189.67	0.45
49.34	Oleic acid, 3-(octadecyloxy)propyl ester	439935189.67	0.45

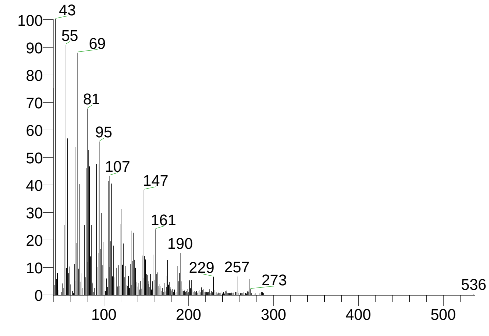
Hit Spectrum	Compound Structure

SI 736, RSI 745, mainlib, Entry# 7020, CAS# NA, Ethyl iso-allocholate

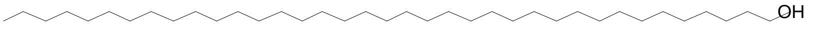


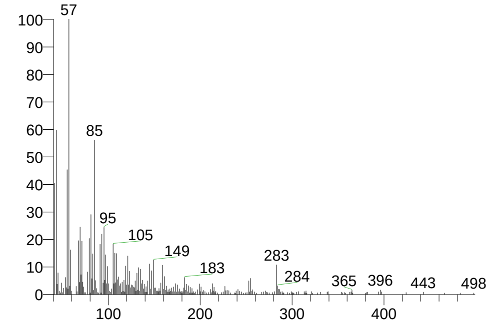
Ethyl iso-allocholate
Formula C26H44O5, MW 436, CAS# NA, Entry# 7020
Ethyl 3,7,12-trihydroxycholan-24-oate #



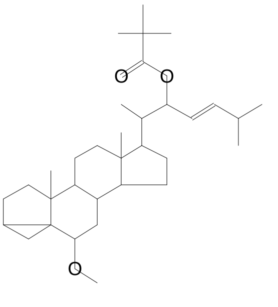


1-Heptatriacotanol
Formula C37H76O, MW 536, CAS# 105794-58-9, Entry# 7279
1-Heptatriacontanol #





Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate
Formula C33H54O3, MW 498, CAS# NA, Entry# 24446
$:28TZJRPRMTYSBPHU-JLHYYAGUSA-N



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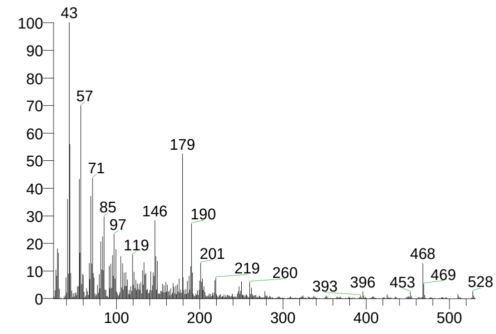
Library Search Report

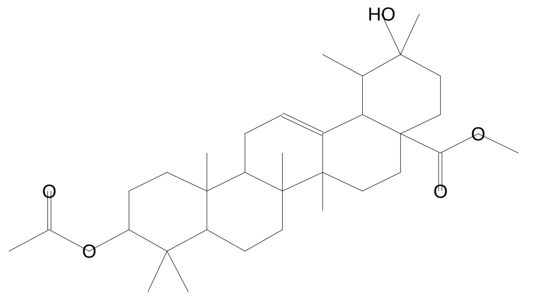
CEG TEST HOUSE AND RESEARCH CENTRE PVT.LTD. JAIPUR

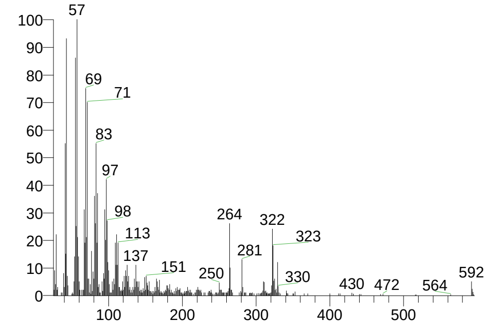
Flower pet ether

Hit Spectrum | Compound Structure

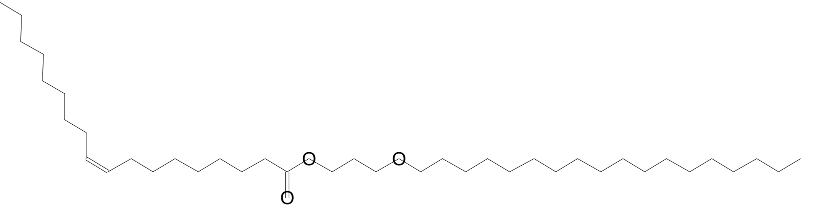


Formula C33H52O5, MW 528, CAS# 14356-56-0, Entry# 7689
Methyl 3-(acetyloxy)-20-hydroxyurs-12-en-28-oate #





Oleic acid, 3-(octadecyloxy)propyl ester
Formula C39H76O3, MW 592, CAS# 17367-41-8, Entry# 24115
3-(Octadecyloxy)propyl (9E)-9-octadecenoate #



There is no signature data to report.

Appendix III

PUBLICATIONS

Research Paper Published

Mishra, N. & Pareek, A. (2014). Traditional Uses, Phytochemistry and Pharmacology of *Mimusops hexandra* Roxb. *Advances in Pharmaceutical and Ethnomedicines*, *2* (2), 32 – 35.

Mishra, N. & Pareek, A. (2015). Floristic Diversity of Angiosperms with special reference to their medicinal properties from Kota district of Rajasthan, India. *International Journal of Advanced Research*, *3*(12), 994 – 1007.

Mishra, N. & Pareek, A. (2018) phytochemical analysis of leaf and bark extracts of *M. hexandra* (Roxb.)- a valuable medicinal plant. *Journal of Phytological Research*, *31* (1-2), 17-22.

Conference Presentations

Poster Presented

Poster titled "Ethno-botanical Knowledge of Saharia Tribe about *Mimusops hexandra* (Roxb.)" has been presented in National conference on "Biodiversity: Harmonizing conservation with life and landscape of arid and semiarid areas" held on 29-30 October, 2014 organized by IIS University, Jaipur, Rajasthan.

Oral Presentations

Oral presentation titled "Phytochemical analysis of leaf and bark extracts of *M. hexandra* (Roxb.)- A valuable medicinal plant" has been presented in International Conference on Recent Advances at Interfaces of Physical and Life Sciences held on 28-30 January, 2019 organized by Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan.

Oral presentation titled "Micropropagation and comparative analysis of primary metabolites from leaf and bark extracts of *Mimusops hexandra* (Roxb.)" has been presented in award category in International Conference on Photobiology, Phytochemistry and Plant Biotechnology held on 8-9 May, 2019 organized by Department of Botany, Mahrshi Dayanand Saraswati University, Ajmer, Rajasthan.

Appendix IV

Advances in Pharmaceutical and Ethnomedicines 2 (2): 32 – 35
http://dx.doi.org/10.14737/journal.ape/2014/2.2.32.35

Mini Review Article

Traditional Uses, Phytochemistry and Pharmacology of *Mimusops hexandra* Roxb

Neha Mishra, Arvind Pareek

School of Science and Technology, Vardhaman Mahaveer Open University, Kota, Rajasthan, India
*Corresponding author: arvindvmou@gmail.com

ARTICLE HISTORY

Received: 2014–09–21
Revised: 2014–10–12
Accepted: 2014–10–14

Key Words: *Mimusops hexandra*, Sapotaceae, Medicinal properties, Phytochemical constituents, Pharmacological actions

ABSTRACT

Mimusops hexandra (Roxb.) is an evergreen tree species with a long history of traditional medicinal uses in south Asia especially in western and central India, belongs to family Sapotaceae. The plant has been known for its curative properties and has been utilized for treatment of various diseases such as ulcer, bronchitis, jaundice, ulitis, fever, hyper dyspepsia, arthritis and alimentary disorders. A survey of the literature shows extracts and metabolites from this plant possess pharmacological properties such as anti–inflammatory, antiulcer, aphrodisiac, alexipharmic, anthelmintic, antibacterial, and free radical scavenging activity. Beside medicinal uses, plant has high economic value due to its edible and nutritive fruit, useful wood, latex and bark and provides substantial livelihood support to local inhabitants. A wide range of chemical compounds including sterols, starch, terpenoids, anthraquinone glycoside, cardiac glycoside, saponinand tannins etc. have been isolated from this species. The presented review summarizes the information concerning the traditional uses, phytochemistry and biological activity of *Mimusops hexandra*.

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ARTICLE CITATION: Mishra N, Pareek A (2014). Traditional uses, phytochemistry and pharmacology of *Mimusops hexandra* roxb. Adv. Pharm. Ethnomed. 2 (2): 32 – 35.

Mimusops hexandra (Roxb.)[Synonym: *Manilkara hexandra* (Roxb.) Dubard] is ethno medicinally important species of tropical deciduous forests of western and central India. It belongs to family Sapotaceae and it is native of South Asian region (Balfour, 1873; Vincken et al., 2007). *M. hexandra* grows in natural wild conditions and mainly propagated through seeds.Its usage has been reported mostly in traditional medicinal system of India. Traditionally it isused in medicinal herbal drugs to cure various diseases such as jaundice, ulitis, odontopathy, fever, colic dyspepsia, helminthiasis, hyper dyspepsia and burning sensation(Joshi, 2000). It purifies the blood and beneficial in swelling, abdominal colic, gout, rheumatism and toxicosis (Rao, 1985).It contains a variety of components which possess various biological activities such asanti–inflammatory, diuretic, antiurolithiatic, analgesic, antipyretic and antimicrobial activity (Khare, 2007).

M. hexandra is commonly known as Obtuse Leaved Mimusopsin English; Khirni and Rayan in Hindi;Rajadanah in Sanskrit; Ulakkaippalai and Palai in Tamil; Patla, Pola and Kirni in Telgu; Krini and Palamunpala in Malayalam and Hale and Hannu in Kannada (Warrier, 1995). It is evergreen tree, 50–60 ft. tall, with blackish gray and deeply furrowed bark; leaves are 7–10 cm long, elliptic, obovate or oblong, flowers are bisexual, white, calyx 6–lobed, corolla 16 or 24–lobed, stamens 6, ovary is hairy and multi–locular with axile placentation, fruit is berry, one seeded shining yellow with ovoid shape and seeds are endospermic and oily (Dwivedi and Bajpai, 1974). *M. hexandra* yield edible fruit, useful wood and latex which are significant source of nutritional and livelihood support for tribal population(Peter,1999). The bark of this plant species is astringent, refrigerant, febrifuge, sweet, tonic and is used traditionally to treat a wide range of gastrointestinal disorders (Shah et al., 2004). Seed oil of *M. hexandra* is demulcent and emollient (Anjaria, 1997). The purpose of the present review is highlighting the various traditional uses, phytochemistry and pharmacological reports on *Mimusopshexandra.*

Ethnopharmacological studies show that *M. hexandra* is used in many parts of India for the treatment of number of diseases (Table 1). Mostly western and central part of India (Andra Pradesh, Madhya Pradesh, Maharashtra, Rajasthan and Tamil Nadu) has a long history of traditional medicinal use of *M. hexandra*. Some of these uses are outlined here.

A survey from Jalgaon district of North Maharashtra shows that its fruits are used to relieve digestive disorder (Patil and Patil, 2012). Mashed fruits of *M. hexandra* are taken to cure diseases like arthritis and jaundice, also used for heat burning, wormicide, and to purify blood by local population of Nawargaon village (Bakare, 2014) and Bhadrawati tehsil of Chandrapur District, Maharashtra (Harney, 2013). Tribal people of Rayalaseema region of Andhra Pradesh use *M. hexandra* leaf extract for treatment of asthma (Anjaneyulu and Sudarsanam, 2013).The Irulas medicinal utility of the flora in the Kodiakarai Reserve Forest (KRF) shows that the latex of *M. hexandra* is applied on teeth and gums for toothaches (Ragupathy and Newmaster, 2009). According to Paderu division of Eastern Ghats of Andhra Pradesh root

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Table 1: Ethno medical uses of *Mimusops hexandra* in India

Place, Country	Parts used	Ethno medical use	Preparation (s)	Reference (s)
Maharashtra	Fruit	Arthritis, Blood purifier, Heat Burning, Jaundice	Mashed	Bakare , 2014
West Bengal	Bark, Fruits	Jaundice, Biliousness	Decoction	Sharma et al. 2014
Andra Pradesh	Leaves	Asthma	Decoction or Infusion	Anjaneyulu and Sudarsanam, 2013
Andra Pradesh	Stem Bark	Galactagauge	Infusion	Padal et al., 2013
Maharashtra	Fruit	Digestive disorder	Mashed	Patil and Patil, 2012
Tamilnadu	Stem Bark, Leaves	Infertility, Veterinary	Infusion	Gunasekaran and Balasubramanian, 2012
Rajasthan	Stem Bark, Fruit	Fever, jaundice,	Decoction, Mashed	Malik et al., 2012
Madhya Pradesh	Stem Bark, Fruit	Bronchitis, Dysentery	Decoction, Mashed	Malik et al., 2012
Maharashtra and Gujarat	Stem Bark, Fruit	Alimentary Disorders	Decoction, Mashed	Malik et al., 2012
Tamilnadu	Stem Bark, Fruit	Fever, Hallucination	Decoction, Mashed	Vinothkumar et al., 2011
Andra Pradesh	Root	Headache	Infusion or Decoction	Rao et al., 2010
Kodiakarai	Latex	Toothache	Applied Directly	Ragupathy and Newmaster, 2009
Andra Pradesh	Stem Bark	Dysentery and Diarrhea	Decoction	Raju and Reddy, 2005
Madhya Pradesh	Stem Bark	Tonic	Decoction or Infusion	Rai , 1987
Madhya Pradesh	Stem Bark	Bodyache	Boiled	Maheshwary et al., 1985

Table 2: Ethno botanical uses of *Mimusops hexandra*

Place, Country	Plant Part	Ethno botanical use	Reference(s)
Madhya Pradesh, India	Bark	used to produce natural dyes 2014 nutritive, sold in markets	Upadhyay and Choudhary, 2014
Rajasthan, India	Bark	used for tanning	Malik et al, 2012
Tamil Nadu, India	Leaves	used for oil presses,	Muruganandam et al., 2012
Madhya Pradesh , India	Wood	house building and turnery	Malik et al., 2012
Central and Western India	Fruits	used as fodder for cattle	Pareek et al. ,1998

Table 3: Phytoconstituents of *Mimusops hexandra*

Phytoconstituent (s)	Source	Reference (s)
Triterpenoidsaponin, β–sitosterol 1994	Stem bark	Gopalkrishnan et al., 2014
Sterols, Volatile oil and Tannis	Leaves	Madhak et al. 2013
Saponin 1, 2 and 3, Gallic acid, Myrecetin, and Quercetin	Seeds	Eskander et al., 2013
Proteins, Lipids and Carbohydrates	Fruits	Daripkar and Jadhav, 2010
Unsaponifiable lipids, Alcohols, Hydrocarbons, Triterpene and Sterols	Seeds	Saeecd et al., 1991
Triterpene alcohols	Fruits	Misra et al., 1974
Cinnamic acid, Hentriacontane Taraxerol and Quercitol	Leaves	Misra and Mitra, 1968
α– and β– Amyrins, Taraxerol α–spinasterol	Roots	Misra and Mitra, 1968

extract of *M. hexandra* is beneficial for relief from headache (Rao et al., 2010). The folk use of *M. hexandra* has been documented in Konda Reddy, Koyas tribes in Khammam district of Andra Pradesh, a decoction of the stem bark is used to cure dysentery and diarrhea (Raju and Reddy, 2005). The stem bark boiled with water is used for bathing to cure body ache by bhil tribe of Jhabua district, Madhya Pradesh (Maheshwary et al., 1985). Extract of stem bark is taken as tonic by Bharia and Gond Tribes of Tamiya and Petalkot of Madhya Pradesh (Rai, 1987).The stem barks infusion of *M. hexandra* is widely used in Konda Dora Tribes in Vishakhapatnam district of Andra Pradesh as galactagauge (Padal et al., 2013). A decoction of bark and mashed fruits are used in sacred groves in Pudukottai district Tamil Nadu for fever and hallucinations (Vinothkumar et al., 2011).

Several phytochemicals have been isolated and identified from different parts of *M. hexandra*. Summary of the related literature have been discussed in Table 3.

Madhak et al., (2013) observed the presence of sterols and volatile oil in leaves of *M. hexandra* by phytochemical analysis and appropriate chemical tests of aqueous and alcoholic extracts of leaves and lead acetate test of leaf extracts also shows the presence of tannin. Misraand Mitra (1968) isolated cinnamic acid, hentriacontane, taraxerol and quercitol from leaves of *M. hexandra.*

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Daripkar and Jadhav (2010) evaluated the proteins, lipids, carbohydrates and moisture content of fresh fruits of *M. hexandra* through chemical analysis which is about 3.53%, 2.6%, 22% and 71.87% respectively. A study carried out by Misra et al., (1974) show the presence of the fatty acid esters of common triterpene alcohols from fruit pulps of *M. hexandra*.

Three bidesmosidic saponins namely saponin 1, 2 and 3 possessing protobassic acid and 16–ahydroxyprotobassic acid as aglycons and also three phenolic compounds such as gallic acid, myrecetin, and quercetin were isolated by Eskander et al., (2013) through chromatographic separation of acetone precipitate of seeds of *M. hexandra*. Saeecd et al., (1991) isolated the unsaponifiable lipid constituents.
Saponinsand tannins in bark of *M. hexandra* through physiochemical, histochemical analysis and Thin Layer Chromatography (TLC) of alcoholic, chloroform and water extracts of *M. hexandra* bark. Atriterpenoidsaponin, 1β 2α, 3β, 19α–tetrahydroxyursolic acid 28–O–β–D–glucopyranosideand β–sitosterol have been isolated from the stem bark of *M. hexandra*by Shrivastav and Singh (1994).

Misra and Mitra (1968) have been isolated the cinnamic acid ester of α– and β–amyrins, taraxerol, α–spinasterol and quercitol from the roots of *M. hexandra*.

Gopalkrishnan et al., (2014) found the presence of starch, terpenoids, proteins, anthraquinoneglycoside, cardiac glycoside, saponinsand tannins in bark of *M. hexandra* through physiochemical, histochemical analysis and Thin Layer Chromatography (TLC) of alcoholic, chloroform and water extracts of *M. hexandra* bark. A triterpenoidsaponin, 1β 2α, 3β, 19α–tetrahydroxyursolic acid 28–O–β–D–glucopyranoside and β–sitosterol have been isolated from the stem bark of *M. hexandra*by Shrivastav and Singh (1994)

Several pharmacological activities and medicinal applications of *M. hexandra* are widely known. Whole plant parts have been used for various medicinal purposes. A summary of the biological studies on *M. hexandra* is presented below.

Antiulcer effects of acetone extract and its different fractions namely diethyl ether, ethyl acetate and aqueous fractions of stem bark of *M. hexandra* have been tested by Modi et al., (2012) and Shah et al., (2004) for the presence of preliminary phytoconstituents and were screened for their antiulcer potential against experimental gastro–duodenal ulcers. The antiulcer activity was –shown by ethyl acetate extract as it decreases gastric acidsecretary activity along with strengthening of mucosal defensive mechanisms.

Gomathi(2012) indicated that polysaccharides extracted from *M. hexandra*bark significantly stimulate the immune system by stimulating macrophage function. Eskander et al.(2013) suggested that acetone fraction of *M. hexandra* containing the crude saponin mixture possessed significant anti–inflammatory activity.

Nimbekar et al., (2013) observed that methanolic extract of *M. hexandra* reduces theblood glucose level and shows significant hypoglycemic effect. Their study indicates that it can be use in the management or control of type II diabetes.

A study conducted by Kumar et al., (2010) shows that methanol leafextracts of *M. hexandra* showed strong 2, 2–diphenyl picrylhydrazyl (DPPH) radical scavenging activity.

Antibacterial activity of aqueous, petroleum and alcoholic extracts of *M. hexandra* was tested by Parekh and Chanda (2007; 2010) using the agar disc diffusion and agar well diffusion methods and found that ethanol or methanol extracts are active against six bacterial strains belonging to *Enterobacteriaceae* and various infectious diseases. The antimicrobial activities of root extracts of *M. hexandra* prepared in different solvents were screened by Bharwad et al., (2011) through agar well diffusion method, zone of inhibition was measured as a property of antimicrobial activity and it was observed that methanol root extracts of *M. hexandra* exhibited good antibacterial activity against *Staphylococcus aureus, Micrococcus leutius, Salmonella paratyphi*, *Serratia marcescens and Klebsiella pneumonia.* Mahidaet al., (2007) also indicates that extract of *M. hexandra* shows antibacterial activity against multi drug resistant bacteria species i.e.*Salmonella typhy, S. paratyphe, Staphylococcus aurius and S. epidermis* which are associated with skin, respiratory diseases and enteric fever.

CONCLUSION

Mimusops hexandra is a well–known medicinal and commercial important tree species and widely used as herbal drug and as a source of livelihood support by local tribal population.

The phytochemical studies conducted on *M. hexandra* indicate presence of various phytoconstituents such as sterols, tannin, saponins, unsaponifialble lipids,triterpene alcohols, terpenoids and phenolic compounds such as gallic acid, myrecetin, and quercetin etc.in different parts of the plant. Plant extracts of *M. hexandra* exhibit diverse categories of pharmacological activities such as antiinflammatory, antiulcer, antidiabetic, antibacterial and free radical scavenging activity etc.
However, only a small proportion has been investigated both phytochemically and pharmacologically. It is important to investigate the gaps in the studies, which may be further bridged in order to exploit the full medicinal potential of *M. hexandra*, as this plant has widespread use also with extraordinary medicinal potential which should be better explored to find new biological properties which may increase its importance as efficient medicinal plant in biodiversity.

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PHYTOCHEMICAL ANALYSIS OF LEAF AND BARK EXTRACTS OF *M. HEXANDRA (ROXB.)*- A VALUABLE MEDICINAL PLANT

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Phytoconstituents from leaf and bark extracts of *M. hexandra* have been analyzed quantitatively and qualitatively by respective standard procedures. Plant contain rich contents of carbohydrate and protein which was estimated by phenol- sulphuric acid method and Lowry's method respectively. Total carbohydrate contents were found 39% and 32.35% and total starch contents were found 44.95% and 38.1% of dry mass in leaf and bark respectively. Total protein content was estimated 5.03% in leaf and 32.9% in bark. Total lipid content was found low 0.05 % for both extracts. Total phenol content was estimated via folin ciocalteau method and it was found 0.52% in leaf and 1.46% of dry mass in bark. Soxhlet extraction procedure was used to prepare methanol, petroleum ether and chloroform extracts for estimation of presence of various secondary metabolites such as alkaloids, flavonoids and sterols and their presence were identified via Thin layer chromatography. Various standard analytical tests were also performed for detection of both primary and secondary metabolites in leaf and bark extracts of *M. hexandra.* The findings of the study provided evidence that the stem bark and leaf of the plant possessed bio active compounds. It justifies their use in the traditional medicines for the treatment of different diseases.

Keywords: *M. hexandra,* Phytochemistry, Primary and Secondary metabolites.

Introduction

Mimusops hexandra (Roxb.) is a medicinal and socio-economic plant species of tropical deciduous forests of western and central India, belongs to family Sapotaceae[1]. Bark, fresh fruits and seeds of *M. hexandra* have high economic value due to its nutritional and medicinal applications[2]. Plant used as a significant source of livelihood and nutritional support for tribal people. Extracts and metabolites of this plant possess huge biological importance[3]. Stem bark possess medicinal properties such as antiulcer, anti-inflammatory antidiabetic and antibacterial activity[4,5,6]. Decoctions of stem bark widely used to cure dysentery, diarrhea, body ache, fever and hallucinations[7,8,9], whereas the infusion used as galactagauge[10]. Leaves of *M. hexandra* are traditionally used as anti-inflammatory, anti-urolithiatic, anti-pyretic, anti-microbial, analgesic and diuretic activities. Leaf extract used in treatment of asthma[11]. Madhak et al[12] observed the presence of sterols volatile oil, tannin via phytochemical analysis of alcoholic extracts of leaves. Misra and Mitra[13] observed the presence of cinnamic acid, hentriacontane, taraxerol and quercitol in aqueous and alcoholic extracts of leaves. Triterpenoid saponin and B-sitosterol have been isolated from the stem bark of *M. hexandra* and their structures elucidated on the basis of chemical and spectral evidence[14]. However, only a small proportion has been investigated phytochemically. Present study aims to analyze phytoconstituents of *M. hexandra* via standard procedures which may generate new information for drug discovery from this valuable medicinal plant.

Material & Methods

The air- dried and grinded powder of leaves and bark used for qualitative analysis. Biochemical tests specific for particular metabolite have been performed and the reaction responses were noted to ensure the presence of particular compound. Carbohydrates were tested by Fehling's, Benedict's, Molisch's and Iodine test, whereas for proteins Biurate, Ninhydrin and Millon' s test were used. Lipids were tested by Acrolein and Sudan IV test[15,16]. Plant sample for quantitative estimation of carbohydrates were prepared by method of Loomis and Shull[17]. Starch samples were prepared from the residue of total extracted sugar samples by method of McCready et al[18]. Phenol-sulphuric acid method was used for estimation of net content of carbohydrates[19]. 5% phenol and concentrated sulphuric acid was used for separation of total soluble sugars. Protein content was estimated by method of Lowry et al[20]. Samples of leaf and bark was prepared via method of Osborne by using cold trichloric acid[21]. Solution of 2% Na_2Co_3 and 0.5% $CuSO_4$ was also used with addition of diluted folin ciocalteau reagent for extraction of proteins.

Further, the optical densities of used standard sugar (glucose) and protein (bovine serum albumin) with their respective samples was measured at 490 and 750 nm wavelength using spectrophotometer where distilled water was used as blank. Regression curve was prepared between the known concentration of glucose and BSA & their respective absorbance which followed and Beer- Lambert law[22]. Net contents were calculated from regression curve by using equation of standard line. Similarly, for the estimation of total phenolic contents, Thorpe and Bray's[23] protocol was used by using ethanolic solution of gallic acid as standard. Folin ciocalteau reagent and Na_2Co_3 solution was added for extraction of phenolic contents[24]. The optical density of gallic acid and plant samples were observed at 750 nm and net phenolic contents were calculated as previous. Solution of chloroform and methanol (2:1, v/v) was used for estimation of net lipid content[25]. The lipids were separated with chloroform and collected in the pre-weight glass vials then weighed. The procedure repeated for three times and mean values were calculated.

Various biochemical test used in estimation of presence of secondary metabolites were also performed. Mayer's, Wagner's, Dragendorff's and Hager's test was used to identify presence of alkaloids, Shinoda test and Alkaline reagent test was used for flavonoids, Liebermann-Burchard's and Salkowaski test was used for sterols. Ferric chloride test was used for phenols[26]. Qualitative estimation of alkaloids was done via gravimetric method in which methanolic extracts of leaf and bark were prepared by soxhlet extraction and further extracted by chloroform. The free alkaloids were separated by ammonia[27]. The extracts were further analyzed by Thin Layer Chromatography. Flavonoids have been separated from powdered samples of leaf and bark with petroleum ether and 80%

methanol via soxhlet extraction at 45-60 ºC. Then again fractioned by sequential extraction with petroleum ether, ethyl ether and ethyl acetate separately. Ethyl ether and ethyl acetate fractions were used for estimation of flavonoids[28]. Identification of sterols have been done by using petroleum ether for separation of fats from dried plant samples. The dried preparation was again extracted with benzene and further proceeds for TLC[29].

Thin Layer Chromatography

Thin silica containing glass plates were used for chromatographic separation. The extracted samples were used for chromatographic separation and co-chromatographed with authentic alkaloids such as colchicine, flavonoid such as Kaempferol and sterol such as B-sitosterol in the chromatographic chamber saturated with solvent mixture of methanol and conc. ammonium hydroxide at ratio of 200:3, n-butenol, acetic acid and water at ratio of 4:1:5 and hexane and acetone at the ratio of 8:2 for alkaloids, flavonoids and sterols respectively[30]. The spots were identified coinciding with the colchicine, kaempferol and B- sitosterol marker. Ammonia fumes was used to darken the spots. The developed plated were air The dried and visualized under ultra violet light. The retention factor (R_f Value) of each spot were calculated.

Results and Discussion

The biochemical tests show positive responses in all reactions. Observed responses such as formed precipitate and change in color of the solution indicates the presence of particular primary and secondary metabolite. Net contents of carbohydrate, starch, protein and phenols analyzed by quantitative estimation has shown in table 1.

Presence of various secondary metabolites such as alkaloids, flavonoids and sterols were also identified by Thin layer chromatography using respective standards. They appear as a single spot and have the same color and retention factor (R_f) value nearly equivalent to their standards on the TLC plate. The R_f values of these compounds in the different solvent systems were calculated as in table 2.

Infusions and decoctions of *M. hexandra* have been used to cure most common to severe diseases by local inhabitants since long time period. Bioactive compounds such as taraxerol, quercitol and B-sitosterol etc. have been identified and isolated from different parts of the plant previously.

Table 1: Net content of various phytoconstituents found in leaf and bark extracts of *M. hexandra*.

Phytoconstituent	Plant part	Net content found(mg/ml)	% Dry weight
Carbohydrates	Leaf	0.780	39%
	Bark	0.647	32.35%
Starch	Leaf	0.899	44.95%
	Bark	0.762	38.1%
Protein	Leaf	0.151	5.03%
	Bark	0.988	32.9%
Lipid	Leaf	0.05	0.05%
	Bark	0.05	0.05%
Phenol	Leaf	0.104	0.52%
	Bark	0.292	1.46%

Table 2 Estimation of the presence of various secondary metabolites in leaf and bark extracts of *M. hexandra* using Thin layer chromatography.

Phytoconstituent	Solvent system	Plant part	R_f value
Alkaloids	Methanol and conc. ammonium hydroxide (200:3)	Leaf	0.50
		Bark	0.61
Flavonoids	n-Butenol, acetic acid and water (4:1:5)	Leaf	0.30
		Bark	0.50
Sterols	Hexane and acetone (8:2)	Leaf	0.78
		Bark	0.80

In this study secondary metabolites such as alkaloids, flavonoids, phenols and sterols have been identified in leaf and bark extracts of *M. hexandra*. This work may aid in further qualitative and quantitative characterization of secondary metabolites which may be help in discovery of novel drugs.

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Appendix V

International Conference
on
Recent Advances at Interfaces of Physical and Life Sciences

RAIPLS-2019

January 28-30, 2019

CERTIFICATE

This is to certify that Prof./Dr./Mr./Ms. ......Neha Mishra.......

from ......Vardhman Mahaveer Open University, Kota....... ~~*delivered invited talk/chaired session*~~*/presented paper (Oral/Poster) entitled* ....

....

in International Conference on Recent Advances at Interfaces of Physical and Life Sciences (RAIPLS-2019) organized by Department of Chemistry, University of Rajasthan, Jaipur held on January 28-30, 2019.

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ICPPP-2019

International Conference on Photobiology, Phytochemistry & Plant Biotechnology

Plant Bioenergetics & Biotechnology Laboratory

Dept. of Botany, MLSU, Udaipur, India presents this certificate to

NEHA MISHRA,
Vardhman Mahaveer Open University, Kota.

for participation in the

International Conference on

Photobiology, Phytochemistry & Plant Biotechnology

held on 8th - 9th May, 2019. And presented a paper.

Dr. Vineet Soni
Organizing Secretary

Prof. Kanika Sharma
Convener

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Bioenergetics & Biotechnology Laboratory