CHEMICAL INVESTIGATION OF SOME INDIGENOUS PLANTS

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CERTIFICATE

This is to certify that this thesis entitled "CHEMICAL INVESTIGATION OF SOME INDIGENOUS PLANTS" is an authentic record of the research work carried out by Sri. Saidutty A., in the Department of Chemistry, University of Calicut, under my supervision in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry, under the Faculty of Science of the University of Calicut and that no part thereof has been presented earlier for any other degree.

C. U. Campus
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DECLARATION

I, Saidutty A., hereby declare that this thesis is an authentic record of original research work carried out by me under the guidance and supervision of Dr. P. Mohamed Shafi, M.Sc., Ph.D., Professor, Department of Chemistry, University of Calicut. No part of this thesis has previously formed the basis for the award of any degree or diploma as stipulated in the statutes of Calicut University.

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PREFACE

Plant Kingdom, nature's gift to man, has attended to all his basic needs namely, food, clothing, shelter and also medicine. Phytochemistry deals with the study of secondary metabolites isolated from plant kingdom, their structures, medicinal properties, antimicrobial activities etc. As we approach the close of twentieth century, we are highly benefited by the sophisticated spectroscopic and chromatographic methods in the isolation and characterisation of a vast number of plant ingredients. Phytochemical studies have become important in solving many problems in disciplines like plant systematics, pathology, ecology, plaeobotany and the like.

Plants are sources of innumerable organic compounds of diverse nature. These plant derived products are used as medicines, pesticides, perfumes, fragrances etc. There are synthetic chemicals which fulfil all the above needs. However, their side effects are of major concern in the modern world. Moreover many of the microbes and insects have become resistant to most of these chemicals. The nature of chemicals employed in the future has to be more selective and environment friendly. Hence we look to nature as an ally and resource in finding new strategies to combat diseases of man and his animals and also of plants.

Ayurveda, the Indian system of medicine which makes use of plant extractives, is worth-mentioning in this context. *Ayurveda* was developed more than three thousand years ago. *Ayurveda* defines life as the union of body, senses, mind and soul. In *Ayurveda* human beings, animals and plants represent a microcosmos in a macrocosmos and the microcosmos consists of same elements as the macrocosmos including fire (Tejas), sky (Akasa), soil (Prthivi), water (Jala) and air (Vayu). According to *Ayurveda*, soma (consisting of above elements) is closely related and governed by psychis (Mana). Diagnosis and treatment of diseases follow the philosophy of the so-called tridoshas 'vata', 'pitta' and 'kapha'. Phytochemical studies have increased the credibility of *Ayurvedic* system of treatment. *Ayurveda* had blossomed into a science when most other branches of modern science were still dormant. But foreign invasions and mystic practices that existed in the system reduced it into a folk-lore. However, the discovery of reserpine once again attracted the attention of outside world into this traditional system of medicine. Now research works are going on to isolate plant ingredients which can cure various human ailments. Scientists are now in search of drugs which can cure cancer and AIDS, the dreaded diseases of the present.

This thesis presents chemical and olfactoric analyses of seven essential oils from four different plants and the phytochemical analysis of the stem bark of *Commiphora caudata.*

Chemical and olfactoric analyses of the essential oils of leaves and roots of *Annona reticulata* are given in chapter I. GC-FID and GC-

MS analyses made it possible to identify seventy three compounds each from both the leaf oil and root oil. Both oils were olfactorically evaluated and compounds responsible for specific odour notes were characterised. The composition and odour characteristics of the oils suggest that for fine perfumery applications there may be found a new source for products with tobacco-like, leather-like, moody-earthy and smoky notes. Based on this work a paper has been published with title "Analysis of the essential oils of leaves and roots of *Annona reticulata* (Annonaceae) from South India" in the journal, *Nutrition*, 1, **1998**, 9.

Chapter II is about the analyses of the volatiles of *Zanthoxylum rhetsa* seeds and leaves. GC, GC-MS studies revealed the presence of one hundred and twenty compounds from the leaf oil and seventy seven compounds from the seed oil. Olfactometric study enabled the identification of compounds responsible for different odours exhibited by this seed oil. This work has been accepted for publication as a paper entitled "Volatile constituents of *Zanthoxylum rhetsa* leaves and seeds" in *Journal of Essential Oil Research.*

Analyses of the essential oils of leaves and root bark of the plant, *Clerodendrum infortunatum* are given in chapter III. In the folk medicinal system different parts of the plant are used for various applications. GC, GC-MS and olfactoric studies revealed the presence of sixty seven compounds in the leaf oil and sixty six compounds in the root bark oil. The compounds identified in the oils account for many of the reported medicinal applications of the plant. Also, the olfactoric charecteristics of the oils are correlated with identified compounds.

Based on this work a paper entitled "Essential oil analysis of the leaves and root bark of the plant *Clerodendrum infortunatum* used in Indian (*Ayurvedic*) medicine" has been accepted for publication in the journal, *Herba Polonica.*

In chapter IV the analysis of essential oil of *Coleus zeylanicus* is given. Since ancient times, preparations of *Coleus* species have been used for medical treatment in traditional medicine. The major uses are for heart diseases, abdominal colic, respiratory disorders, painful micturation, insomnia and convulsions. GC, GC-MS and olfactoric evaluation of the leaf oil of *Coleus zeylanicus* showed the presence of sixty four compounds. The biological activities of various identified compounds are given. The identified compounds account for many of the reported folk medicinal applications of the plant. Olfactometric study enabled the correlation of odour profile of the oil with identified compounds. A paper on this work entitled "Analyses of the essential oil of the leaves of the medicinal plant *Coleus zeylanicus* (Benth.) *Cramer* from India" has been published in the journal, *Scientia Pharmaceutica,* 66, **1998**, 223.

Chapter V describes the phytochemical analysis of stem bark of *Commophora caudata.* The identification of twenty six compounds including β-sitosterol was possible from this plant material.

ANALYSIS OF THE ESSENTIAL OILS OF LEAVES AND ROOTS OF ANNONA RETICULATA (ANNONACEAE)

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CHAPTER I

ANALYSIS OF THE ESSENTIAL OILS OF LEAVES AND ROOTS OF *ANNONA RETICULATA (ANNONACEAE)*

Section 1: General Introduction

In our daily life we see that the fruits, flowers, leaves, stems, barks and roots of nearly all plants have some pleasant smell. It has been observed that this pleasant smell is actually due to the presence of certain highly volatile oils known as essential oils. They bear the name because they were thought to represent the very essence of flavour and frangrance. Younger plants produce more oils than older ones, but old plants are richer in more resinous and darker oil because of the continuing evaporation of lighter fractions of the oil. The oils are stored as microdroplets in glands of plants. After diffusing through the walls of the glands, the droplets spread over the surface of the plant before evaporating and filling the air with perfume. The most odoriferous plants are found in the tropics, where solar energy is greatest.

The essential oils are complex mixtures of hydrocarbons and their oxygenated derivatives. The main constituents of the essential oils are the terpenoids having carbon atoms up to C_{15} (i.e., mono and sesquiterpenes) and their oxygenated derivatives such as alcohols, aldehydes, ketones etc. Essential oils, due to their pleasant smell, are commercially important, especially in perfumery. Moreover, various essential oils show biological activity such as insecticidal, anthelmintic or antiseptic action, so they are also useful in pharmacy[1].

I.1.1 **Isolation**

Essential oils[2] frequently occur as a very small percentage by weight of the original plant material, so the processing of large quantities is often required to obtain considerable amount of oil. In general four methods are used for the isolation of essential oils. They are steam distillation, expression, solvent extraction and enfleurage (extraction by using fat). Of these, steam distillation is the most commonly used method. At first the plant material is crushed or ground to reduce the particle size and to rupture some of the cell-walls of oil bearing glands. The crushed or ground mass is then steam distilled when the essential oils go into distillate from which they are extracted using pure organic solvents like diethylether. Steam distillation may change or modify the components of essential oils. Liquid carbon dioxide extraction[3] retains the true to nature characteristics of the essential oils.

The function of the essential oil in a plant is not well understood. Odours of flowers probably aid in natural selection by acting as attractants for certain insects. Leaf oils, wood oils and root oils may serve to protect against plant parasites or depredations by animals. Oleoresinous exudations that appear when the trunk of a tree is injured prevent loss of sap and act as protective seal against parasites and disease organisms. Many components of essential oils are chemically active and thus could participate readily in metabolic reactions. They are sources of plant metabolic energy, although some investigators maintain that these materials are simply waste products of plant biosynthesis.

Essential oils contain a large number of volatile components made up of low molecular mass organic molecules of carbon, hydrogen, oxygen and occasionally nitrogen and sulphur. They are mainly terpenoids belonging to mono and sesquiterpenoid groups. Terpenoids are made up of isoprene units joined in a head-to-tail fashion, though there are some exceptions. The monoterpenoids are the simplest among the naturally occurring isoprenoid compounds and form the important constituents of essential oils. They are composed of two isoprene units. They may be aliphatic, alicyclic, or bi- or tricyclic with varying degree of unsaturation. Sesquiterpenes contain three isoprene units, diterpenes four, triterpenes six etc. The same species of plant grown in different parts of the world usually contains the same chemical constituents, but the relative percentages may be different. Climatic and topographical conditions affect plant chemistry and can alter the essential oil content qualitatively and quantitatively.

I.1.2 Analytical methods

The complexity of an essential oil is in principle, unlimited; what can be identified depends on the resolution power of our present analytical tools. In many instances the human nose is still much more sensitive than our analytical detection level. The techniques such as odour or colour comparison was the early methods used for the determination of essential oils. Later analytical techniques such as specific gravity, refractive index, distillation range, iodine number determination and gas-liquid chromatography etc., are employed for the determination of volatile components of the oil.

The techniques such as capillary gas chromatography [with the more sensitive flame ionisation detector (FID)], gas chromatography-mass spectrometry (GC-MS) and gas chromatography-infrared spectroscopy-mass spectrometry (GC-IR-MS) are the modern analytical methods used for the separation and identification of components of essential oils. Using the above methods, separation of even trace components have become possible. The Fourier-transform GC-IR, high resolution GC-MS and chemical ionisation GC-MS are more powerful and selective characterisation tools for the structure elucidation of components of oils. The combination GC-FTIR-MS is potentially more powerful and it is possible to operate these in series, but chromatographic resolution may deteriorate as the effluences passes along the light pipe in FTIR instrument enroute to the MS. Therefore the GC effluent is usually split so that 2% goes directly to the more sensitive MS instrument, and the remaining 98% goes to FTIR instrument. Eventhough the informations from IR and MS are complimentary to each other, their combined ability to identify components in GC is great indeed.

Gas chromatography[4] is essentially an analytical technique commonly used for qualitative analysis by comparing the retention data of the analyte with those of the compound which it is thought to be. Simple retention times are not very reproducible and it is better to use relative retentions or retention indices. The most useful system of retention indices is the one due to Kovats. It takes advantage of the linear relation between the logarithms of the adjusted retention times of a homologous series (the n-alkanes) and the number of carbon atoms in the molecules. The n-alkanes are used as the reference compounds because of their stability, ready availability, cheapness, and wide range of boiling points. The retention of any analyte is compared with the two n-alkanes which elute nearest to it. The adjusted retention time of the analyte is measured at the same time as those of n-alkanes which elute in front and behind it (containing 'Z' and 'Z+1" carbon atoms respectively) and the retention index of the analyte, I is then defined by

$$I = 100 \ \mathrm{X} \left[\frac{\log t'_R\,(\mathrm{subst}) - \log t'_R\,(n\text{-}C_z)}{\log t'_R\,(n\text{-}C_{z+1}) - \log t'_R(n\text{-}C_z)} \quad + Z \right]$$

For n-alkanes the term $\log t'_R\,(\mathrm{subst}) - \log t'_R\,(n\text{-}C_z)$ reduces to zero and they have retention indices equal to the number of carbon atoms in the molecule multiplied by one hundred.

Sometimes a component with critical odour properties may be present in the oil at ultra trace level for which a discrete GLC peak cannot be readily assigned. In this case the use of olfactory detection involving GLC sniff runs is employed to pinpoint the elution time of the trace constituent.

I.1.3 Gas chromatography-Olfactometry

Aroma chemicals have two sensory odour properties namely intensity and quality[5]. They are usually hydrophobic organic compounds containing a limited number of functional groups. However the presence of functional group is not a pre-requisite for odour. The two odour properties are very difficult to measure objectively with physical instruments. Olfactometers simply generate and deliver an odorous air sample of known concentration to a human subject for assessment. The technique gas chromatography-olfactometry, more commonly referred as GC sniffing is a more useful method to check the olfactoric purity of a sample. Provided that the GC conditions adequately separate the components of a mixture, each component can be smelt at the exit of the port of a GC column in a olfactorily pure state. Repeated analysis of the same sample at successively high dilutions is a methodology commonly used to identify the components which contributed towards the overall odour of that sample. An advantage of the method is its ability to analyse minute quantities of a sample and to assess pure components. Due to the absence of a universal odour language and the subjective nature of odour perception, sometimes the odour description from two laboratories may be slightly different.

Gel Permeation Chromatography[6] (GPC) is a useful supplementary method for the separation, identification and carbon skeleton determination of mono and sequiterpenes of essential oils having same molecular formula but differing carbon skeletons on the basis of their molecular size.

I.1.4 Economic Importance

Essential oils have extensive applications in the field of flavour and fragrance industry. Examples are mint and cinnamon used in toothpaste, mouth wash or lozenges. Some combination of essential oils can be found in soaps, detergents, room freshners, paper, printing ink, paint, candles, condiments, floor-polishes etc. Flavour essential oils are used in baked foods, snacks, soft drinks, liquors, sauces, gravies, salad dressings and other food products[7].

I.1.5 Biological Activity of Essential Oils and their possible applications

Since time immemorial, essential oils have been known in many different applications[8]. Many of them from ancient times were used as medicaments, disinfectants, insect repellents, fragrances etc. Most commonly they were known for their multiple therapeutic properties. From ancient times to the most modern aromatherapy and pharmacy, essentials oils are recognised as medicaments or para-pharmaceuticals and are widely applied by most advanced and most traditional doctors. Because of their well-known disinfectant properties they were considered excellent remedies against infections and epidemic diseases. Sometimes the oils were believed to be remedy for nearly all health problems. Fragrances in our environments have multiple effects on our life. They are not only for pleasure and seduction, healing and magic products but also for insecticides, agriculture, auxiliary agents and as mood stimulants. However, little is known about the mechanism of essential oils. Their lipoid solubility and therefore their possibility to panetrate into the cells may give rise to influence on the metabolism of the micro-organism and thus give an explanation of the effect. It has been observed that the antiseptic activity of many compounds is based on their lipoid solubility. The antibacterial activity of essential oils has been expressed in their phenol-coefficients. It tells us how many times stronger or weaker is the action of the essential oil in question, compared to phenol[8] (Phenol has the factor one).

I.1.6 Essential oils as therapeutics

The knowledge of therapeutic properties of essential oils is as old as mankind's use of plants as medicaments. Essential oils are complex mixtures which can act in different complicated ways. From experience we know that a certain oil is useful in a specific case, but a similar illness in another patient may not be cured with the same oil. These effects are different from those achieved with individual chemicals which are much more convenient for evaluation and use in chemotherapy. Therefore individual chemicals isolated from essential oils are more often used than the oils. There are however more and more research works on the therapeutic properties of essential oils treated as standard medicaments and subjected to full pharmacological evaluation. The most thoroughly examined are antimicrobial properties of essential oils which in many ways are better than antibiotics, due to their wider spectrum of activity. There is also very interesting phenomenon of synergic activity of two or more essential oils against bacteria in which the addition of one oil to another will increase the original bactericidal activity of individual oils. Even more interesting is the synergic activity of essential oils with antibiotics. It was found that the addition of a small quantity of oil to some antibiotic would increase the activity of the antibiotic several times. Bactericide activity of essential oils can also be applied in food preservation. Apart from the above few examples of uses of essential oils, there are so many preparations using essential oils as their constituents. Examples are ointments, syrups, pills for remedies of pains, infections, eczema, bronchitis, skin diseases and many other problems.

I.1.7 Essential oils in the world of insects

It was known from experience that certain plants growing at proper places can save other plants from dangerous insects. ‘Zdrawetz’ planted around rose bushes will prevent lice from attacking the rose buds and also hemps (*Cannabis sativa*) planted around vegetable gardens act as repellents against a variety of worms and insects.

Essential oils also attract insects. They can be used in mixtures with insecticides to increase their effect by attracting the insect to the insectcide. In the field of bio-activity of essential oils also synergism can be observed. For example a mixture of basil and eucalyptus oils will kill 100% of mosquito larvae at a concentration two to six percent lower than individual oils. Artemisa oil will kill larvae of anophele at a concentration of 0.016%; while the same effect can be obtained with 0.014% DDT. A mixture of peppermint (50%), camphor (25%) and coumarin (25%) is used as a very effective home fumigant. The oil of *Chrysantemum balsamita* is a very effective insecticide against lice. Rose oil in 1:1000 dilution will kill earth worms and leeches within 30 to 60 minutes. It is interesting to know that calamus oil will sterilize males of housefly, so most probably an old custom of decorating houses in spring with calamus leaves is based on this property of the oil. The few examples given above show the importance of essential oils in the control of insects with safe natural products. Careful research and observations of insect bahaviour in the presence of essential oils and their constituents can save nature from the use of efficient but dangerous chemicals.

I.1.8 Essential oils in Agriculture

Allelopathy is a phenomenon which deals with chemical interactions of plants with each other. It was well-known that some plants grow in perfect harmony with others where as some others perish without any good reason. There are many reasons for such effects including metabolites exchange in roots, organic and inorganic products washed from leaves into the soil, interactions between plants and soil microflora, and the presence of volatile plant products in the air and soil. Essential oils can dissolve in leaves of the plants and migrate into their roots. There are some positive interactions and negative interactions. Certain plants produce oils as toxins against their diseases. Tobacco disease *Pseudomonas solancearum* will induce the synthesis of mixture of sesquiterpenes in tobacco leaves. Another interesting example is allelopathic toxic activity of essential oils of sage and artemisia against grasses. Allelopathy of oilbearing plants is still an under-investigated area with enormous potential. Possible uses of plants or oils as safe natural herbicides, growth boosters and other agents in agriculture are still open to research scientists.

I.1.9 Essential oils in Human Psychology

Volatile components of essential oils act on human body and mind creating good feeling, calm or arch stimulating. In Europe, hop pillows were used in folk medicine as sedatives. Head space analysis of dried hops showed the presence of dimethylvinyl carbinol (0.15%) which was proved to have a distinct sedative-hypnotic effect. Cleopatra used pillows with rose petals. The electroencephalographic studies of healthy subjects and psychotic patients showed that rose oil stimulated the central nervous system. The dreams become more frequent, brighter and longer lasting. Also rose oil increased the ability of concentration of healthy subjects, accelerated the working rate and improved capacity to do work. Thus modern scientific methods proved that old tricks were very well justified and all psychological effects of essential oils, their components and mixtures known from ancient times can be used in modern medicine.

Essential oils are generally expensive. The high price of natural oil coupled with their limited availability has encouraged a search for substitutes. Great progress has been made in the synthesis of individual components such as geraniol, citral, linalyl acetate and the like. These synthetics have been combined with natural oils to extend supplies, and they have also been blended together in an attempt to duplicate the oils themselves. Such reconstituted oils usually lack certain of the odour-notes of natural products, because of the absence of trace ingredients, often unidentified, that may be present in natural oils. They also tend to have a more 'chemical' odour, because of trace impurities in the synthetics that are different from the components of natural oils.

Section 2: Studies on the essential oils of leaves and roots of *Annona reticulata*

I.2.1 Introduction

Annona reticulata L (custard apple or bullock's heart) belongs to the genus *Annona* of *Annonaceae* family (subfamily *Annonoideae*). The plant comes under the tribe *Unoneae*. The *Anonaceae* family comprises 120 genera and more than 2000 species[9]. The family consists of trees, shrubs and woody climbers found mainly in tropics, although a few species extend to temperate region. It is economically important as a source of edible fruits (soursop, bullock's heart), spices ('false nutmeg', 'aethiopian pepper') and raw material for perfumery (ylang-ylang)[10]. Oils from the seeds of species like *A.muricata*, *A.senegalensis* may be used for the production of soap and as edible oils[11]. Further, many species of the family are used in folk medicine. For example, a decoction of the leaves of *A.muricata* is used as soothing and sudorific remedy for fever, while the pounded fresh leaves are applied to cicatrize wounds. In general the leaves are considered a useful treatment for fever and dysentery[12]. In India, the root bark is given in ptomain poisoning[13]. An extract of the leaves and stems has a passing depressive effect on the blood pressure which has been attributed to γ-aminobutyric acid[14]. Intraperitoneal administration of this acid (3g/kg) has been shown to protect animals against the convulsive action of brucine if it is given three days before-hand[15]. Intraceribroventricular administration (25 Mg) of (+)-coclaurine, the alkaloid isolated from various *Annona* species in mice suppresses or prevents the locomotor activity induced by dopaminergic stimulating agents[16]. An insecticidal principle, resistant to heat but not to saponification has been detected in the seeds of *A.muricata*. It was effective against a great number of insects[17]. The screening of different extracts of the plant as to anticancer action gave no significant results[18].

In Northern America and in Gambia the leaves of *A.squamosa* are used as insecticide and to eliminate bed-bugs[12]. In Indonesia seeds of the plant are used against head-lice[19]. The aporphine alkaloids isolated from the bark[20] were found to have significant antineoplastic activity[21,22].

The people of Northern Nigeria use the leaves of *A.senegalensis* mixed with cereal cakes or in the form of large bolus mixed with soda and bran, as an anthelmintic for horses. Also the plant *A.arenaria* is used as a remedy for epilepsy[23].

The plant under investigation *A.reticulata*, is a small tree native to tropical America but brought to Asia several centuries ago. In India the plant is often cultivated around villages or gardens in towns. The fruits of *A.reticulata*, also called bullock's heart[24] is a type of apple (castard apple) with a smooth skin which becomes dull red when the fruit is ripe. Its leaves are alternate and flowers are usually bisexual. It flowers in April-June. The fruit is an aggregate of distinct fruit-lets(berries). An aqueous solution of the seeds of the plant kills most wood-bugs. Insecticidal activity has been reported for the roots, stems and seeds of the plant[19]. The leaves of the plant are reported to be anthelmintic, insecticidal, the fruit antidiarrhoeic and the bark astringent[25]. The constituents of *A.reticulata* explain the cardiotonic activity of the plant which is inotropic, chronotropic and also spasmolytic[26].

I.2.2 Secondary metabolites reported from *Annona* species:

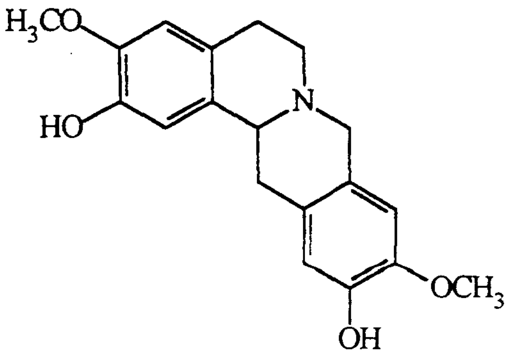
Durand and coworkers[14] in the year 1962 were able to isolate γ-aminobutyric acid from the fruits and stems of *Annona muricata*. In its leaves two phytosterol glycosides, namely upuranol and anonol, have been found to be present by Merck [27]. Two alkaloids, muricine and muricinine have been isolated[28,29]. The main alkaloids in the roots of *A.muricata* from Guyana are (+)-reticuline and (+) coclaurine. Three minor alkaloids of isoquinoline type, coreximine, anomurine, anomuricine have also been reported. Hydrocyanic acid has been found in various amounts in the roots, leaves and mainly in the bark[28].

Leboeuf and co-workers[31] have isolated a benzyltetrahydroisoquinoline alkaloid, higemanine from the leaves and stems of *A.squamosa*. Further, aporphine alkaloids (anonaine, roemerine, norcorydine, corydine, nor isocorydine and glaucin) have been isolated from the bark, roots, stems and seed of *A.squamosa* by Bhakuni and co-workers[20].

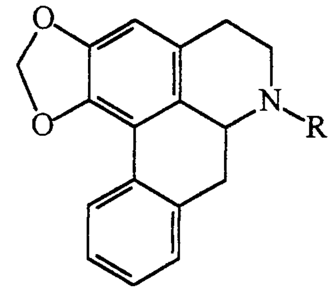
The leaves of *A.senegalensis* is found to contain rutin, quercetin and quercitrin[21]. Its stem bark gives positive reaction for alkaloids, saponins and tannins[22]. The leaf contains a hard and soft wax, both of which contain higher saturated fatty acids. From the hard wax, primary alcohols and palmitone (hentriacontanone) and from the softwax an yellow sesquiterpene oil has been obtained.

Phytochemical screening tests have revealed the presence of many triterpenes and sterols in many *Annonaceae* members. β-Sitosterol has been isolated from species like *A.muricata* and *A.squamosa*[32,33]. Sometimes β-sitosterol is found to co-exist with stigmasterol, campesterol and cholesterol.

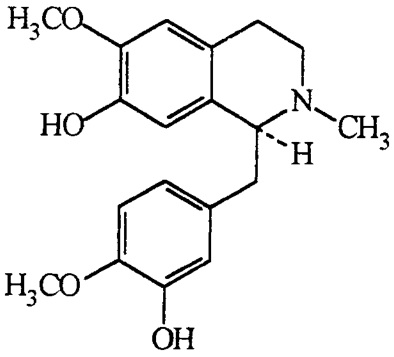
Guinaudeau and co-workers[34] isolated anonaine, roemerine, corydine, isocorydine and many other aporphine alkaloids from the leaves and stems of *A.reticulata*. In addition L-dopamine, salsinol(1-methyl-6,7-dihydroxy-1, 2,3,4-tetrahydroquinoline) and coclaurine have been identified by thin layer and gas chromatography by Forgaes and coworkers[26]. Later studies have shown the presence of alkaloids, flavonoids and acetogenins from different parts of the plant[35-41]. The acetogenins isolated from *Annonaceae* species are referred to as annonaceous acetogenins[42]. These compounds show cytotoxic and bactericidal activities and were therefore tested by various groups to find out the mechanism of the above mentioned effects.



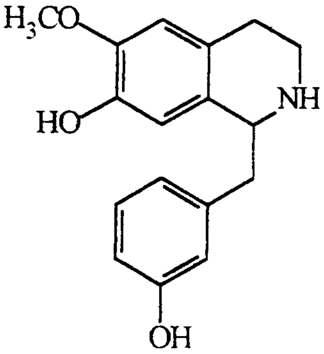
Coreximine



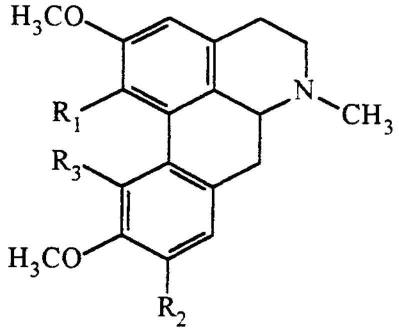
R = H = Anonaine
R = CH_3 = Roemerine



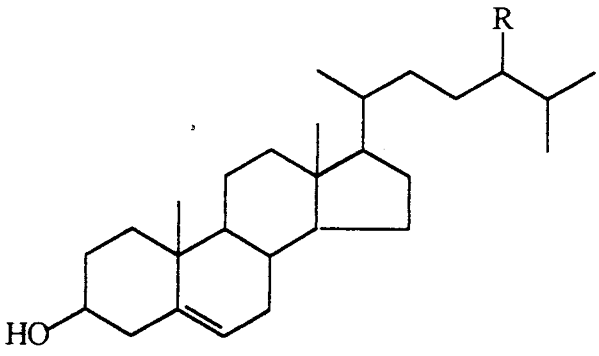
Reticuline



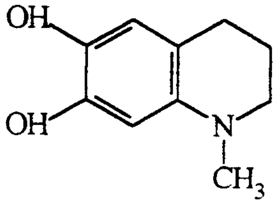
Coclaurine



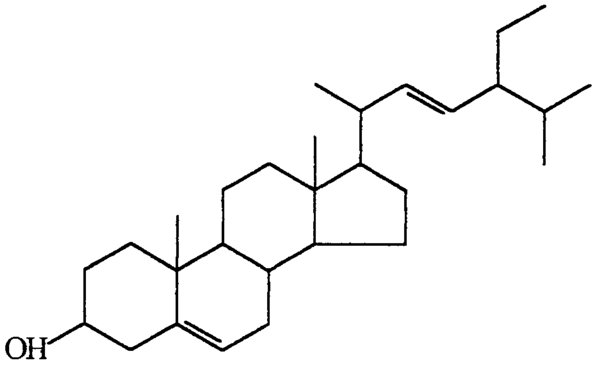
Glaucine $R_1 = R_2 = OCH_3$ $R_3 = H$
Corydine $R_1 = OH$ $R_2 = H$ $R_3 = OMe$
Isocorydine $R_1 = OMe$ $R_2 = H$ $R_3 = OH$



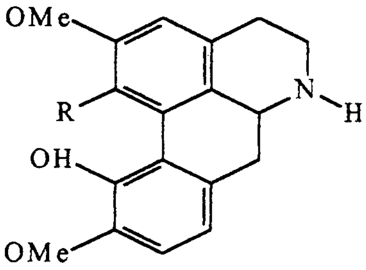
$R = H$ Cholesterol
$R = CH_3$ Campesterol
$R = C_2H_5$ β-sitosterol



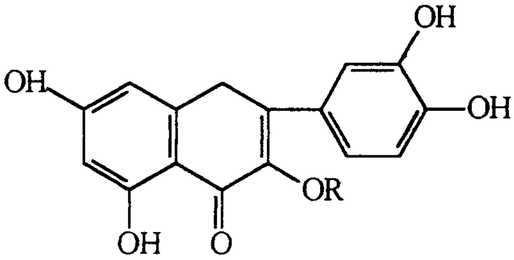
Salsinol



Stigmasterol



R = OMe Muricine
R = OH Muricinine



R = H Quercetin
R = $C_6H_{11}O_4$ Quercitrin
R = $C_{12}H_{21}O_9$ Rutin

I.2.3 Essential oils of *Annonaceae* family

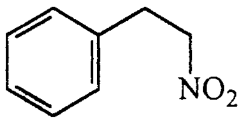
Composition of essential oils of various genera of *Annonaceae* family have been studied. Fournier and Co-workers[43-46] analysed various *Xylopia* species essential oils and found to be rich in p-cymene, β-caryophyllene, α-phellandrene, limonene, α-cubebene, elemol, cadinene, γ-muurolene, cubenol, α-gurjunene, spathulenol and trans-pinocarveol.

Essential oil from the fruits of *Anaxagorea dolichopra* has been found to contain mainly δ-cadinol, caryophyllence oxide, δ-cadalene, α-copaene and γ-muurolene by the same group of scientists[47]. Volatiles of various *Artabotrys* speceis were found to be abundant in camphene, α-copaen-11-ol, cyprene, β-caryophyllene, ledol, palustrol, caryophyllenol, spathulenol, caryophyllenc oxide, 1,5-epoxy-salvial-4(14)-ene, widdrol, δ-cadinol, cadalene and cyprenone[48].

Fournier and co-workers [49] analysed the root bark oil and fruits oil of *Mananthotaxis diclina* and found verbenone, caryophyllene oxide, β-humulene, α-humulene and β-caryophyllene in the fruits oil and trans-pinocarveol, spathulenol and cedrol in the root bark oil as the major components.

Essential oils of *Guatteria* species have been found to contain caryophyllene oxide, α-cadinol, trans-carveol, spathulenol, γ-terpinene, p-cymene, β-caryophyllene, borneol, δ-3-carene, limonene and α-humulene[50].

Seeds of *Dennotia tripetala* is found to contain a rare compound, 2-phenylnitroethane(1)[51]. The essential oil of *Uvaria chamae* is found to contain a novel C-benzylated monoterpene, chamanen(2) together with thymoquinoldimethylether(3) in its root bark[52,53].



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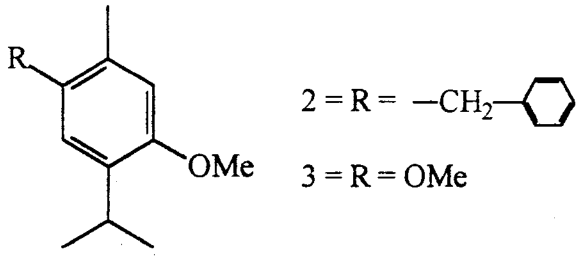


The volatiles of *Uvaria narum* leaf has been found to be rich in β-caryophyllene, benzoic acid, benzyl benzoate, α-bulnesene, β-elemene, α-copaene while patchoulenone, bornyl acetate, borneol and camphene were the major components in the root bark oil[54].

The fruits oil of *A.muricata* has been subjected to several studies; it contains mainly aliphatic esters among which methyl hexanoate, methyl(E)-2-but-enoate and methyl(E)-hex-2-enoate predominate[55,56]. The leaf essential oil obtained from the species native to Ivory Coast was rich in sesquitepenes (76%) of which β-caryophyllene, elemenes and cadalene were the most abundant[57]. Boyam and co-workers[58] analysed the leaf essential oil and found to be rich in β-caryophyllenc (40%) β-elemene (14.4%), α-santalene (9.5%) and (Z)-hex-3-enol (5.2%). The same group analysed the fruit and leaf oils of *A.senegalensis* and found to contain α-phellandrene, myrcene, (Z)-β-ocimene, (E)-β-ocimene, α-pinene and 1,8-cineole as the major components.

I.2.4 Antimicrobial activity reported from *Annona* Species

Annonane separated from various *annona* species has been found to possess antimicrobial properties against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Mycobacterium smegmatis* and *Canadida albicans* at 100 µ.g /mL level[59]. Corydine was reported to have anticancer activity[20]. An ether extract of the seeds *A.squamosa* has been found to act as a stomach poison in the larvae and eggs of *Bombyx mori* and to be moderately toxic to *Musa nebulo* and *Triboleum castanum* adults. When petroleum ether was added to the extract an insoluble resin is formed and this treatment has been found to increase the toxity gainst *M.nebulo* by a factor of six[60].

I.2.5 Present work

The volatiles of the fruits of *Annona reticulata* are discussed only by two groups of scientists[23,26], while little information is given on volatiles of the leaves[36] and no data is available on the essential oil of roots. Therefore both leaf and root oils were analysed chemically, to identify as many compounds as possible, and olfactorically. The component-olfactoric properties were correlated and discussed.

I.2.6 Plant material

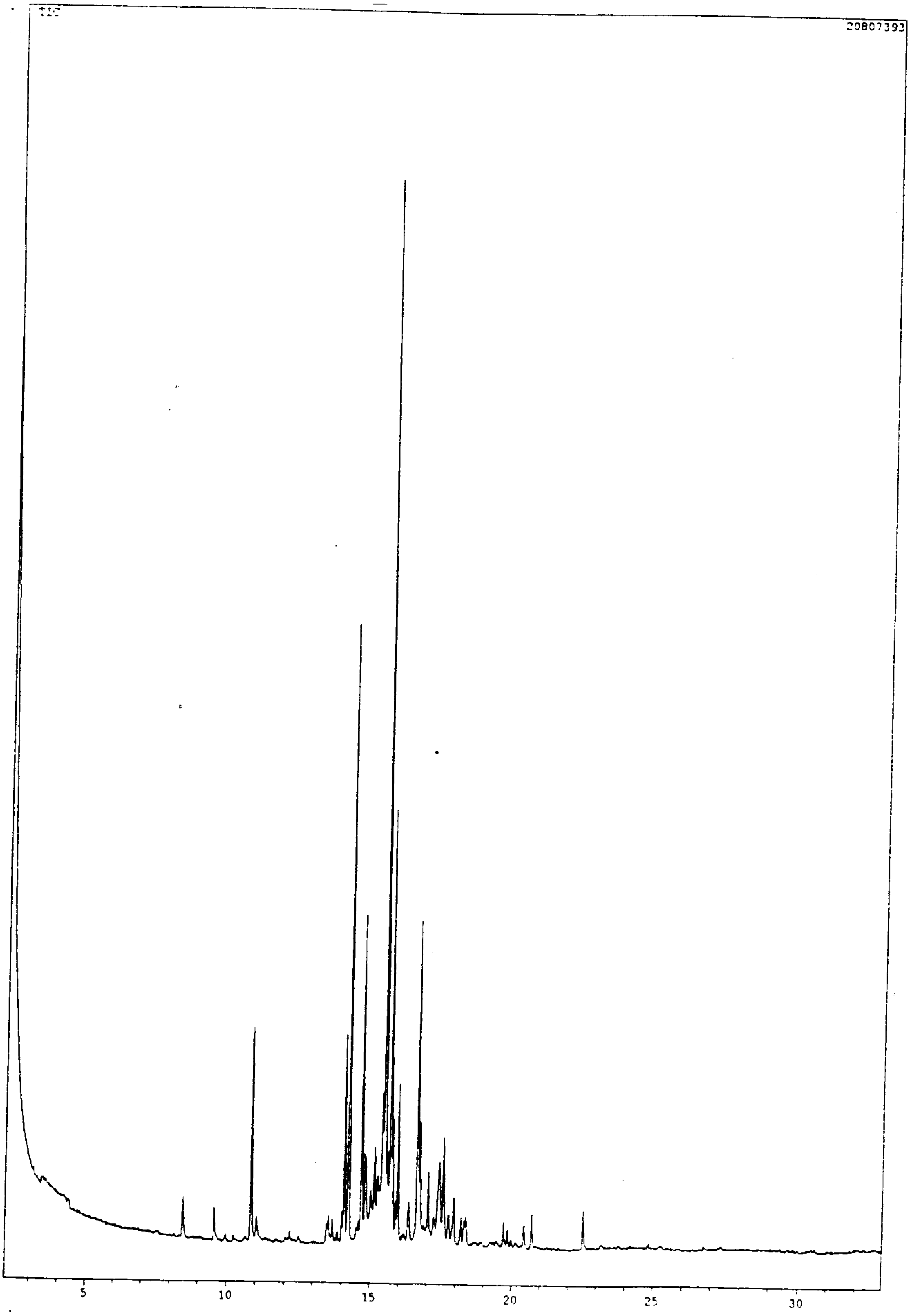
The leaves and roots of *A.reticulata* were collected from Thrissur district of Kerala state and the plant was identified by Dr.A.K.Pradeep, Department of Botany, Calicut University. Herbarium specimens are deposited in the Department of Chemistry, University of Calicut.

The essential oil of leaves was obtained by steam distillation of 500g of dry powdered leaves for 5 hours. The distillate was extracted with diethyl ether (3x50mL) and dried with anhydrous sodium sulphate. The dry extract on evaporation yielded 0.2g(0.04%) of essential oil.

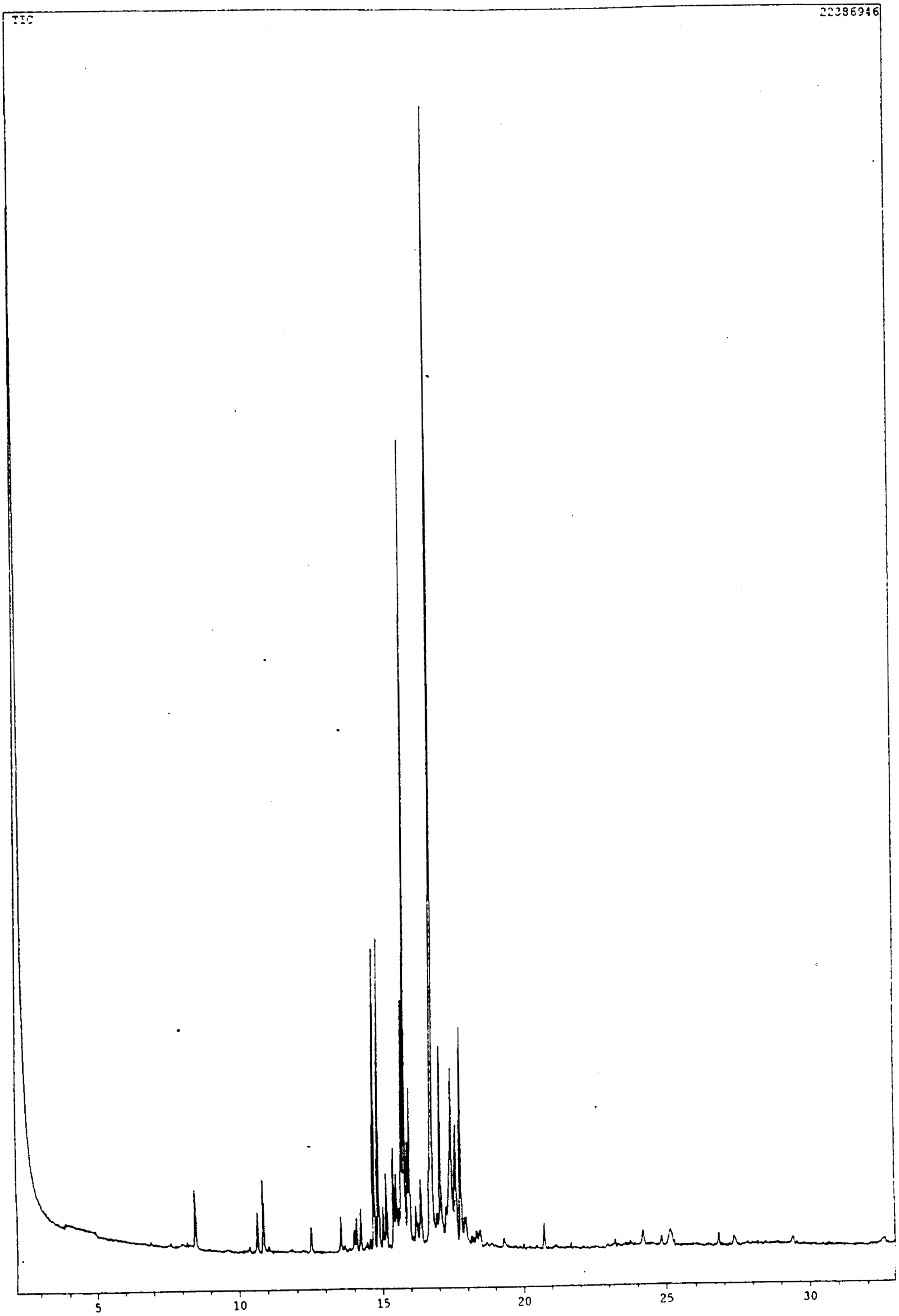
Totally 800g of dry powdered, root bark were taken for the essential oil extraction. It was steam distilled for 4 hours, the distillate extracted with diethyl ether and dried using anhydrous sodium sulphate. The yield of essential oil after the removal of ether was 0.3g (ca.04%)

1.2.7 Instrumentation

Gas chromatographic analysis of the volatiles were carried out using a Shimadzu 14A-GC fitted with FID and a Shimadzu C-R6A-Chromatopac integrator and a Varian 3700-GC with FID and a Shimadzu C-RIB-Chromatopac integrator using hydrogen as carrier gas. GC-MS determination was carried out on a Shimadzu GC-17A with QP5000 and on a Hewlett-Packard GC-5890 with HP-5970-MSD using helium as carrier gas. The mass spectra was recorded in the 41-550 amu scan range (EI mode, 70 eV). The columns used for both the analyses were 30m x 0.32 mm bonded FSOT-RSL-200 fused silica (film thickness 0.25 micron, Biorad Co.) and 30m x 0.32 mm bonded Stabilwax (film thicknes, 0.50 micron, Restek Co.). The temperature programme for GC and GC-MS were the same. The sample was kept at 40^{0}C for 5 minutes and was heated to 280^{0}C at a rate of 6^{0}C per minute. The compound identification was possible by co-injection of pure compounds and by comparison of GC and GC-MS data with those in the computer database of the instruments. The retention indices of the identified compounds matched very well with published retention indices data. Quantification of the compounds was made by %-peak-area-calculations.



Gas Chromatogram of *Annona reticulata* leaf essential oil



Gas Chromatogram of *Annona reticulata* root essential oil

I.2.8 Results and Discussions

I.2.8.a. Identification of components.

Using GC/FID and GC/MS seventy three compounds each could be identified from both the leaf and root oils (Table I.1). The main compounds of the leaf essential oil of *Annona reticulata* were the sesquiterpenes, spathulenol (7.42%), delta-cadinene(6.45%), elemol (4.56%), beta-caryophyllene (4.02%), alpha-copaene (3.97%) and alpha-eudesmol (3.02%) in combination with the monoterpenes, terpinen-4-ol (4.04%) and alpha-terpineol(3.76%).

The essential oil of root of *A.reticulata* was found to be rich in sesquiterpenoids like spathulenol (11.89%), delta-cadinene (9.29%), alpha-muurolene (6.79%), beta-bisabolene (5.17%) and alpha-bergamotene (3.12%).

In both leaf and root oil terpenes form the major class of compounds. In the leaf essential oil terpenes constituted 74% of the total composition (monoterpenes 17.25%, sesquiterpenes 52.61%, diterpenes 4.14%). while in the root essential oil terpene content was 75.54% (monoterpene 9.14%, sesquiterpene 64.69%, diterpene 1.71%). Further, the terpene hydrocarbon- its oxygenated derivatives division in the leaf oil was 38.75%-45.24% and that in the root essential oil was 48.88% -26.66%.

Table I.1

Compounds identified in the leaf and root essential oil of *Annona reticulata* in % peak area GC/FID (Stabilwax)

	Compound	Leaf oil	Root oil
Monoterpenes			
1.	Borneol	1.44	0.71
2.	Carvacrol	1.23	0.89
3.	p-Cymene	0.73	0.08
4.	Limonene	1.64	0.28
5.	Linalool	0.73	0.52
6.	(E)-Linalool oxide	0.06	t
7.	(Z) –Linalool oxide	0.02	t
8.	Linalyl acetate	0.21	0.15
9.	α-Phellandrene	0.09	0.39
10.	β- Phellandrene	0.64	0.18
11.	α-Pinene	0.12	1.01
12.	β-Pinene	0.74	1.44
13.	Sabinene	0.31	t
14.	Terpinen-4-ol	4.04	0.88
15.	α-Terpineol	3.76	1.42
16.	α-Thujene	0.82	t
17.	α-Thymol	0.28	1.15
18.	Tricyclene	0.04	0.01
19.	β-ionone	0.35	0.04

	Compound	Leaf oil	Root oil
Sesquiterpenes			
1.	Aromadendrene	0.14	2.42
2.	α- Bergamotene	1.42	3.12
3.	β-Bisabolene	0.92	5.17
4.	β-Bisabolol	0.11	0.41
5.	δ-Cadinene	6.45	9.29
6.	γ-Cadinene	1.38	0.72
7.	δ -Cadinol	2.72	0.91
8.	β-Caryophyllene	4.02	2.33
9.	β-Caryophyllene oxide	0.99	0.26
10.	α-Copaene	3.97	2.63
11.	α-Copaen-8-ol	0.34	0.17
12.	β-Cubebene	2.11	2.62
13.	α-Elemene	1.69	1.54
14.	β-Elemene	2.37	1.76
15.	Elemol	4.56	1.89
16.	α-Eudesmol	3.02	2.07
17.	β-Farnesene	0.33	0.16
18.	Farnesol	1.21	0.92
19.	Farnesyl acetate	0.89	0.22
20.	Germacrene-D	0.13	1.79
21.	α-Murrolene	1.82	4.79
22.	(E)-Nerolidol	0.85	0.96

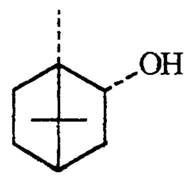
	Compound	Leaf oil	Root oil
23.	γ-Selinene	1.27	1.69
24.	Spathulenol	7.42	11.89
25.	Valencene	2.31	0.87
26.	Ylangene	0.17	1.09
Diterpenes			
1.	8-β–13- β-Kaur-16-ene	3.12	1.5
2.	Kauran alcohol*	1.02	0.21
Aromatic compounds			
1.	(E)-β-Asarone	0.31	T
2.	Benzaldehyde	0.67	0.10
3.	5-Benzofuranoacetic acid	0.21	0.62
4.	Benzoic acid	1.03	1.34
5.	Benzyl alcohol	2.14	0.11
6.	2,3-Dihydrobenzofuran	0.42	0.22
7.	Eugenol	1.71	0.83
8.	Eugenyl acetate	0.94	0.25
9.	Indole	0.01	0.26
10.	2-Phenylethanol	0.32	0.04
11.	Vinylphenylether	t	0.06
Fattyacids and esters			
1.	Myristic acid	1.4	0.98
2.	Myristic acidethyl ester	0.3	0.12
3.	Palmitic acid	0.31	0.50

Miscellaneous Compounds			
	Compound	Leaf oil	Root oil
1.	Acetic acid	0.22	0.13
2.	Ambrettolide	0.47	0.61
3.	Decanoic acid	0.65	2.88
4.	Guajacol	2.28	1.17
5.	Hexadecanoic acid	0.36	2.44
6.	Hexanol	1.16	0.10
7.	1-Hexen-3-ol	1.77	0.02
8.	3-Hexenol	2.00	0.11
9.	Nonanal	0.46	0.06
10.	Octanol	t	0.34
11.	1-Octen-3-ol	t	0.55
12.	Propenylguajacol	0.18	0.76
	Unknown	Ca.5	Ca.8

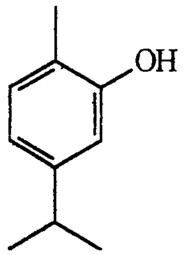
t=trace compound

*=Position of-OH not identified.

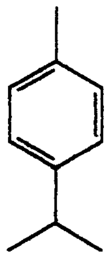
Structures of compounds identified in the essential oils of *Annona reticulata*



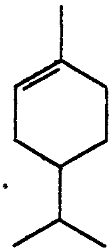
Borneol



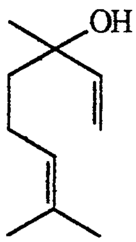
Carvacrol



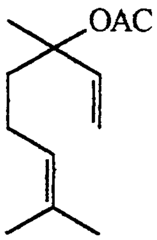
p-Cymene



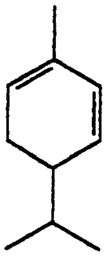
Limonene



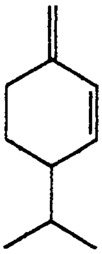
Linalool



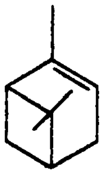
Linalyl acetate



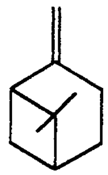
α-Phellandrene



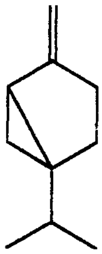
β-Phellandrene



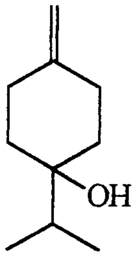
α-Pinene



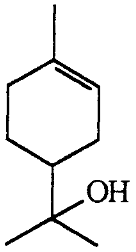
β-Pinene



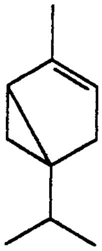
Sabinene



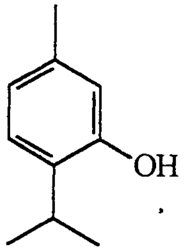
Terpinen-4-ol



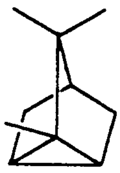
α-Terpineol



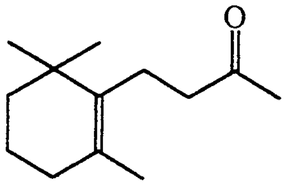
α-Thujene



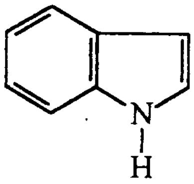
Thymol



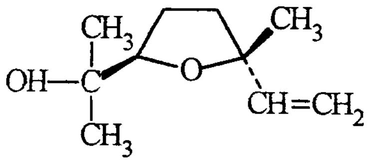
Tricyclene



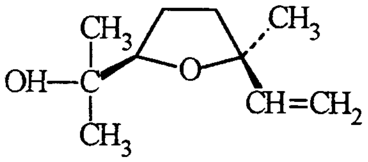
β-Ionone



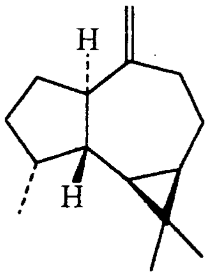
Indole



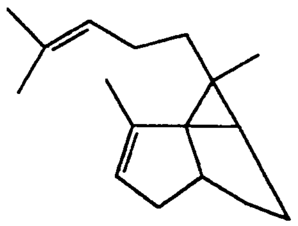
(E)-Linalool oxide



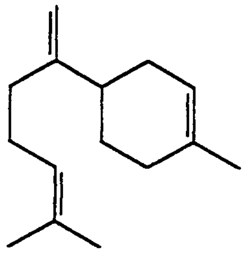
(Z)-Linalool oxide



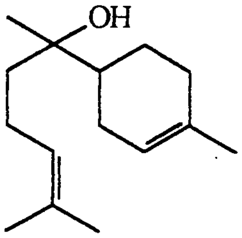
Aromadendrene



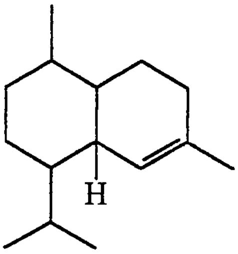
α-Bergamotene



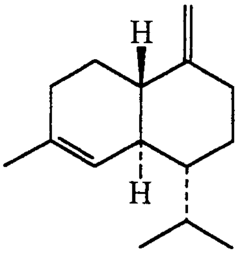
β-Bisabolene



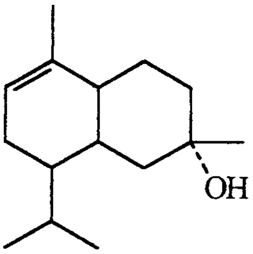
β-Bisabolol



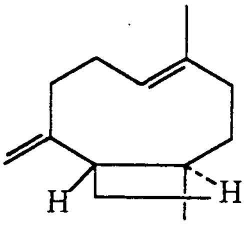
δ-Cadinene



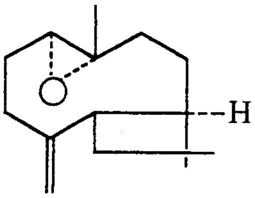
γ-Cadinene



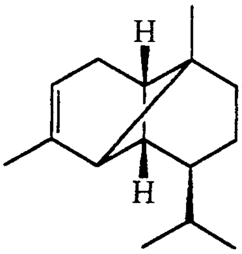
δ-Cadinol



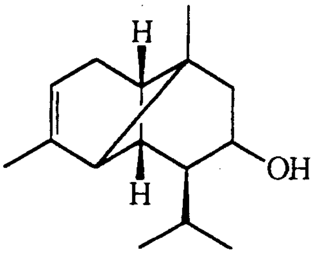
β-Caryophyllene



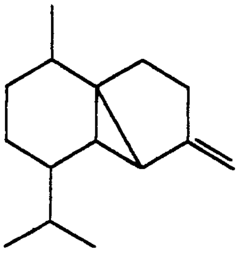
β-Caryophyllene oxide



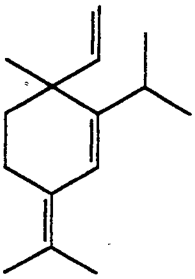
α-Copaene



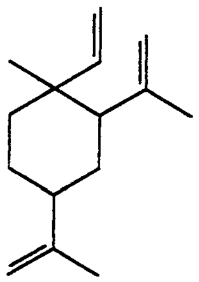
α-Copen-8-ol



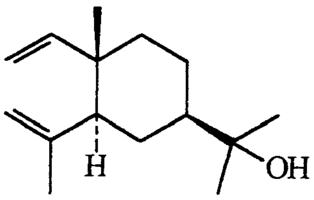
β-Cubebene



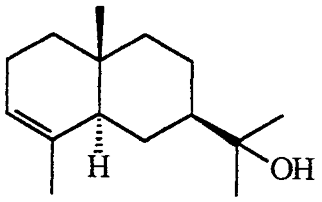
α-Elemene



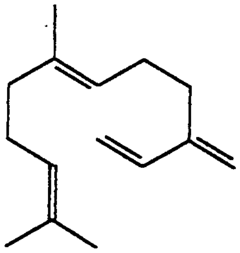
β–Elemene



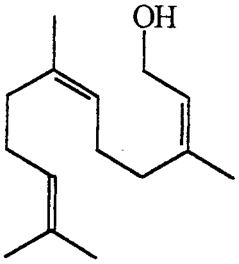
Elemol



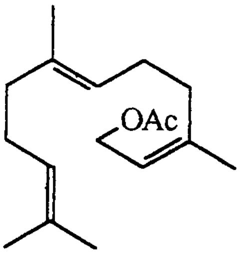
α-Eudesmol



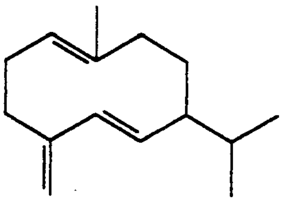
β-Farnesene



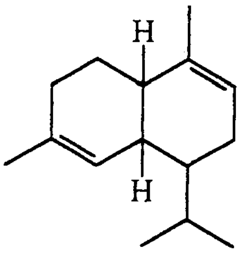
β-Farnesol



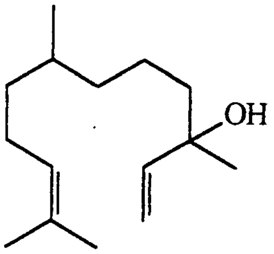
Farnesyl acetate



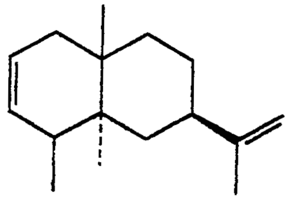
Germacrene-D



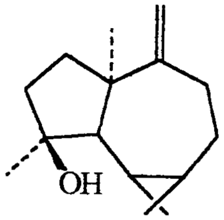
α-Muurolene



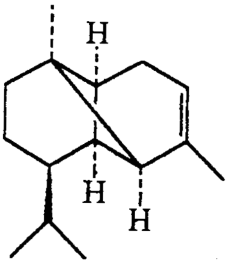
(E)-Nerolidol



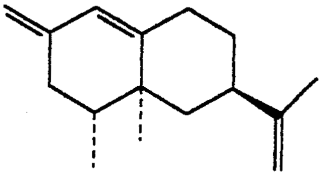
γ-Selinene



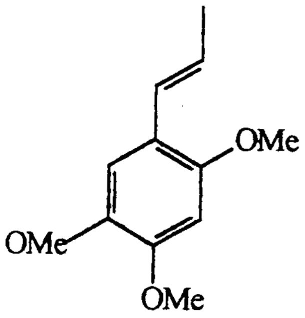
Spathulenol



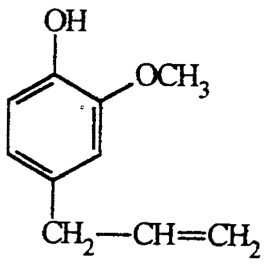
Ylangene



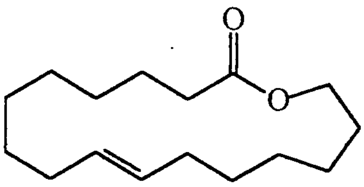
Valencene



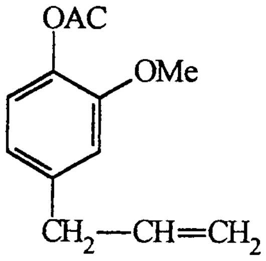
(E) β-Asarone



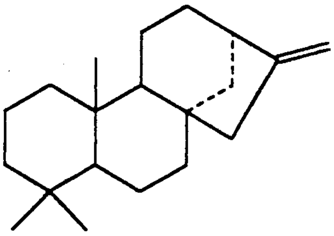
Eugenol



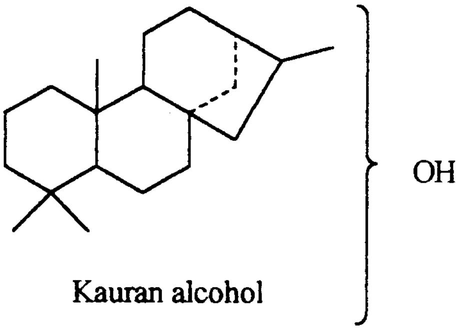
Ambrettolide



Eugenyl acetate



8-β, 13-β-kaur-16-ene



Kauran alcohol

I.2.8.b. Olfactoric properties

The essential oils were olfactorically evaluated. The leaf oil was found to be green-herbal-spicy, in the direction of pepper and thyme, and weak floral, guajacol-like, tobacco-like, smoky in the background of fatty and animalic odour. The odour of root oil was described as spicy, nutty, smoky, tobacco-like, guajacol-like, woody-moody-earthy in the back ground.

The green odour denotes an odour reminiscent of green foliage such as leaves, stalks, green vegetables and the smell of freshly cut grass[61]. The 'green' odour is contributed by hexane derivatives especially (Z)-3-hexenol ("leaf alcohol") and (E)-2-hexenal ("leaf aldehyde"). The leaf alcohol is produced as a result of mechanical injury, such as that caused when a lawn is mowed[62,63]. Further, there is appreciable difference in the odour between the cis and trans forms of 3-hexenol, the cis form being consistently sharper and greener while the trans form more fatty[64]. The leaf oil of *A.reticulata* was found to contain about 5% hexane derivatives including 2% 3-hexenol which along with monoterpens like pinenes, phellandrenes, thymol explains the green odour of the leaf oil.

Alpha-bisabolol, (+)-β-bisabolol, bisabolenes together with cadinenes and caryophyllenes are reported to have a pleasant, herbal spicy flavour[65]. All these compounds except alpha-bisabolol were found to be present in the leaf oil which accounts for the herbal spicy odour of the same.

Macrocyclic lactones form an important group of aroma compounds[66]. Ambrettolide, cis-7-hexadecenolide, is a natural musk of vegetable origin, first isolated from ambrette seed oil, which along with β-ionone, indole, nonanal, fatty acids and sesquiterpenes like elemenes, valencene, ylangene etc contributes to the complex tobacco-like, smoky, fatty and animalic odour impressions of the leaf oil. Benzyl alcohol, beta-ionone, linalool derivatives, (E)-nerolidol and 2-phenylethanol account for the weak floral odour. These compounds are among the constituents which contribute to the floral odour of rose, jasmine and neroli oil[71]. Guajacol and propenyl guajacol account for the guajacol like odour of the leaf oil.

The woody odour describes the odour of not only the essential oils derived from various trees such as cedar wood and sandal wood but also some oils derived from leaves like patchouli and grasses like vetiver[67]. Several authors have independently reported that the diastereoisomers of linalool oxides together with linalool make a sweet-woody note[68]. Further, Caryophyllene oxide together with β-caryophyllene has a marked woody note[69]. The above mentioned compounds were found to be present in the *A.reticulata* root oil and account for the woody-moody-earthy flavour.

The nor-sesquiterpenoid, β-ionone has a particularly high aroma value due to its characteristic odour qualities and its low threshold value. The ketone is assumed to contribute to the flavour of tobacco[70]. It along with ambrettolide, sesquiterpenes, indole, nonanal and fatty acids accounts for the tobacco like, smoky, leather-like odour profile of *A.reticulata* root oil.

The compositions of these oils allow the conclusion that for fine perfumery applications there may be found a new source for products with tobacco-like, leather-like, moody-earthy and smoky notes. The flavouring of food can be discussed only after serious investigations of the relatively high concentrated sesquiterpenes on nutrition products as well as on customers.

VOLATILE CONSTITUENTS OF ZANTHOXYLUM RHETSA LEAVES AND SEEDS

Saidutty A "Chemical investigation of some indigenous plants" Thesis.
Department of Chemistry, University of Calicut, 1999

CHAPTER II

VOLATILE CONSTITUENTS OF *ZANTHOXYLUM RHETSA* LEAVES AND SEEDS

II. 1 Introduction

Zanthoxylum rhetsa D.C.Syn. *Zanthoxylum budrunga* Wall and *Z.limonella* (Dennst.) Alston is a lofty deciduous tree up to 30m tall indigenous to evergreen forest of Assam, Meghalaya and Eastern and Western Ghats of Peninsular India [72,73]. This tree belongs to the family *Rutaceae*. Its stems and branches are covered with spines or prickles of 2 to 3cm length. It is called Ashwagra in Sanskrit, Badrang in Hindi, Triphal (Trifla) in Marathi, Munnina in Kannada and Mullilam in Malayalam [74].

The tree is, essentially of spice value and its dried fruits and fresh leaves are used as a condiment in both sweet and savoury preparations, especially in the preparation of fish curries. The dried fruits are used as an ingredient in the preparation of spice blends like 'garam masala' powder [75]. The fruits are digestive and appetizing. The pericarp of unripe fruit is pleasantly aromatic and tastes like orange rind. The seeds are hard, black in colour with a soft kernel and resemble black pepper in pungency[76]. The dried fruits contain an essential oil called Mullilam oil, which is obtained by steam distillation of the powdered fruits. The oil has a pleasant odour resembling that of sweet orange or tangerine [72,75]. The oil is manufactured on a commercial scale but its uses are not well known. However, the fruits are claimed to be useful in asthma, bronchitis, heart troubles, toothache and rheumatism [77]. The essential oil, obtained from the fruits (3.7%), the main constituent of which is (-)sabinene (30-90%) is used in the treatment of cholera in the indigenous system of medicine. Its effectiveness in inflammatory dermatitis on topical application has been chemically proved [78]. Local anaesthetic activity has also been proved[79] (maximum potency in 2% concentration). The drug possess a wide margin of safety [79] (LD_{50} 1000mg/kg)

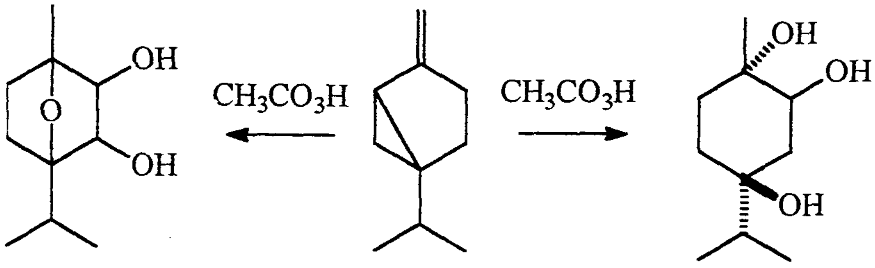
II. 2 Previous studies on the essential oil of *Zanthoxylum rhetsa*

There are a number of scientific publications on this plant describing the medicinal and essential oil aspects of the plant. Earlier independent works on the chemical composition of the essential oil by various groups of scientists led by Rao, Naves and Mathur [76, 80-82] revealed the presence of alpha and beta-pinenes, (-) sabinene, alpha-terpinene, beta-phellandrene, (+)- terpinen-4-ol, mullilam diol, 1,4-cineole, cuminic aldehyde, octanal, decanal, (-) cryptone and phloroacetophenone dimethyl ether.

Joy and co-workers [83] analyzed the essential oil from the carpels of air dried fruit by GC and GC-MS. They were able to detect 32 components from the laboratory sample and 31 compounds from commercial sample. Thirteen of the 21 compounds identified are new components, reported for the first time from *Z.rhetsa*. Sabinene constitutes the major portion of the oil (approx.50%). Terpinen-4-ol (14.1%), alpha-pinene (10.0%) and beta-pinene (7.5%) are the other major components.

Kalyani and co-workers [84] studied the *invitrio* anthelmintic activity of the fruit oil against tapeworms (*Toenia solium),* roundworms *(Ascaridia galli)* and earthworms *(Pheritima postuma)* using piperazine phosphate as reference substance [85]. The anthelmintic activity is assessed following the method of Gaind and Budhiraj [86]. They found that the oil is more active on earthworms than on both types of intestinal worms. For tapeworms and round- worms, the correlation between the death rate and concentration is exponential upto a concentration of 4% and linear beyond this concentration. Further, the activity of essential oil on all types of worms is higher than that of reference substance, piperazine phosphate.

Paknikar and Kamat [87] have revised the structure of so-called 'mullilam diol', a crystalline compound obtained from *Zanthoxylum rhetsa* fruit oil, which was formerly formulated as p-menthene-2,3-dihydroxy-1,4-oxide (I) and assigned the new structure, (±)-p-menthan- 1α, 2β, 4β-triol (II). Mullilam diol has been isolated from the fruit oil along with the major constituent sabinene by Mathur and co-workers [82]. It is found to be identical with the compound prepared by Henderson and Robertson [88] by the action of peracetic acid on sabinene. Hikino [89] re-investigated the reaction and conclusively proved the structure (II) instead of struture (I) for the Henderson and Robertson compound. This is further verified by the work of Halsall and co-workers [90]. Pakniker and Kamat [87] isolated the crystalline compound (m.p. 171° C) from mullilam oil and established its identity with an authentic sample of mullilam diol isolated by Mathur[82]. They chemically and spectroscopically verified that the structure of mullilam diol is (II) with molecular formula, $C_{10}H_{20}O_3$ and not (I) having molecular formula $C_{10}H_{18}O_3$. They also suggested to replace the name mullilam diol by the name of structure (II) from literature to avoid confusion.



I Sabinene II

Shankaracharya and co-workers [91] analysed the dried fruits of five commercial samples for their physical and chemical composition. The colour of the fruit varies from dull brown to dark brown, number of fruits per 100g varies from 700 to 825, essential oil content obtained by Clevenger method varies from 1.14% to 4.05% while that obtained by steam distillation method varies from 1.0% to 3.0%. The reducing sugar content before inversion varies from 1.09% to 4.6% while the same, after inversion varies from 2.19% to 8.63%. The seeds are found to contain a small quantity of essential oil (0.35%) and the carpels are found rich in essential oils. As the seeds are poor in essential oil and rich in crude fibre (ca. 50%) they may be separated from their husks and discarded if the bulk is not required for the preparation of final product like spice powders or essential oil.

The essential oil is found to be laevo-rotatory and poor in aldehyde content. The oil has high specific gravity and high evaporation residue indicating that the oil does not evaporate easily when exposed to air. Also the oil does not dissolve easily in 90% alcohol indicating that the oil is mainly composed of terpenoid hydrocarbons, sabinene being the major component. Its composition varies from 35.73% to 67.73%. The other major compounds are β-pinene, myrcene, gamma-terpineol, alpha-terpinene and terpinene-4-ol. The analysis shows that there are differences in the composition of Clevenger (hydro) and steam distilled oils. The steam distilled oils have more amount of sabinene and alpha-pinene compared to Clevenger distilled oils [91].

II.3 Present work

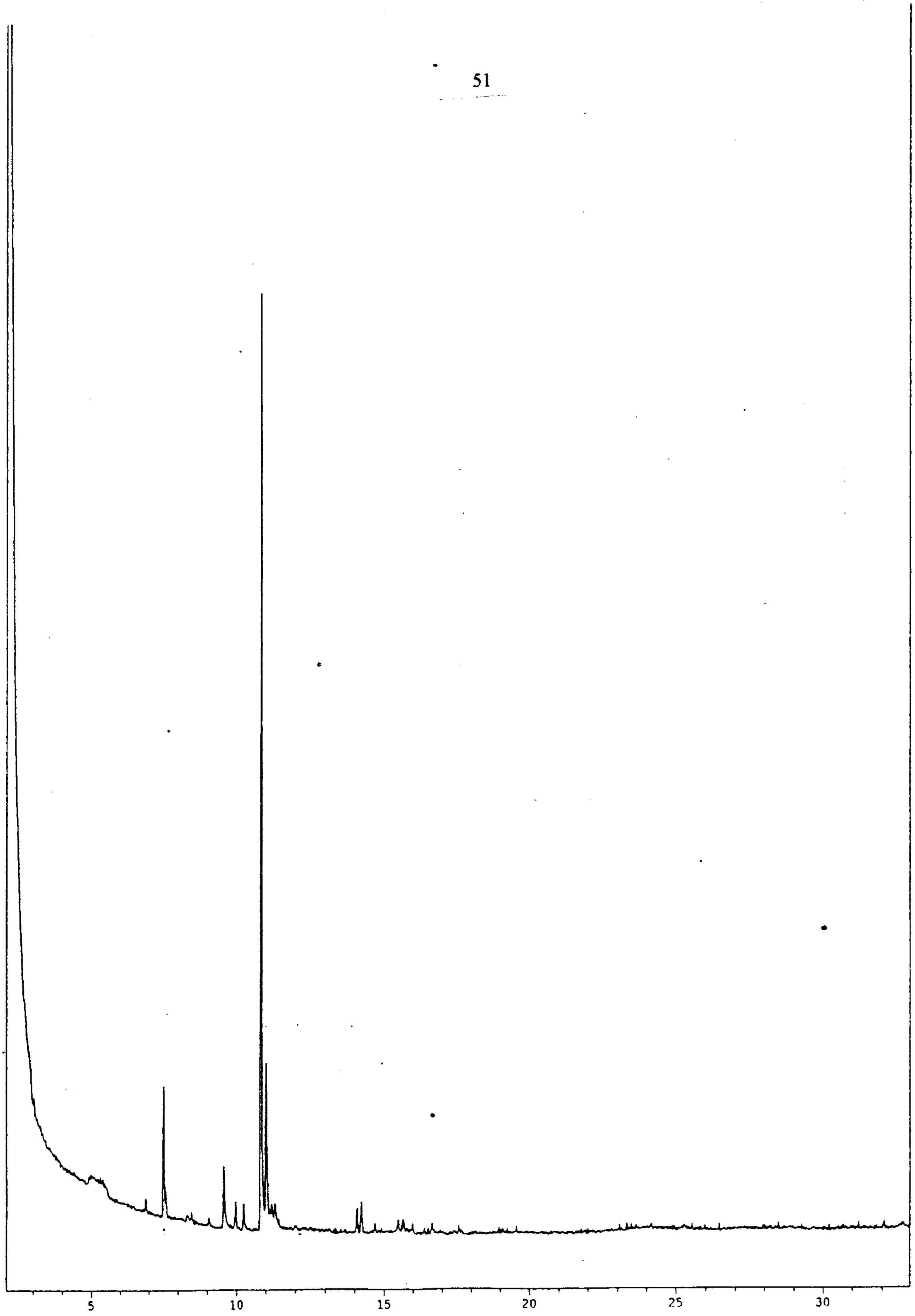
The earlier works on *Zanthoxylum rhetsa* seed essential oil showed that it is rich in monoterpenes, especially sabinene [76,80-84,87]. No work on the leaf essential oil has so far been reported. So the present work aimed to analyse the aroma compounds of leaf-essential oil and compared it to the seed oil. Also olfactoric properies of the seed oil were studied and the odour -compound relations were studied and discussed.

II.4 Materials and Methods

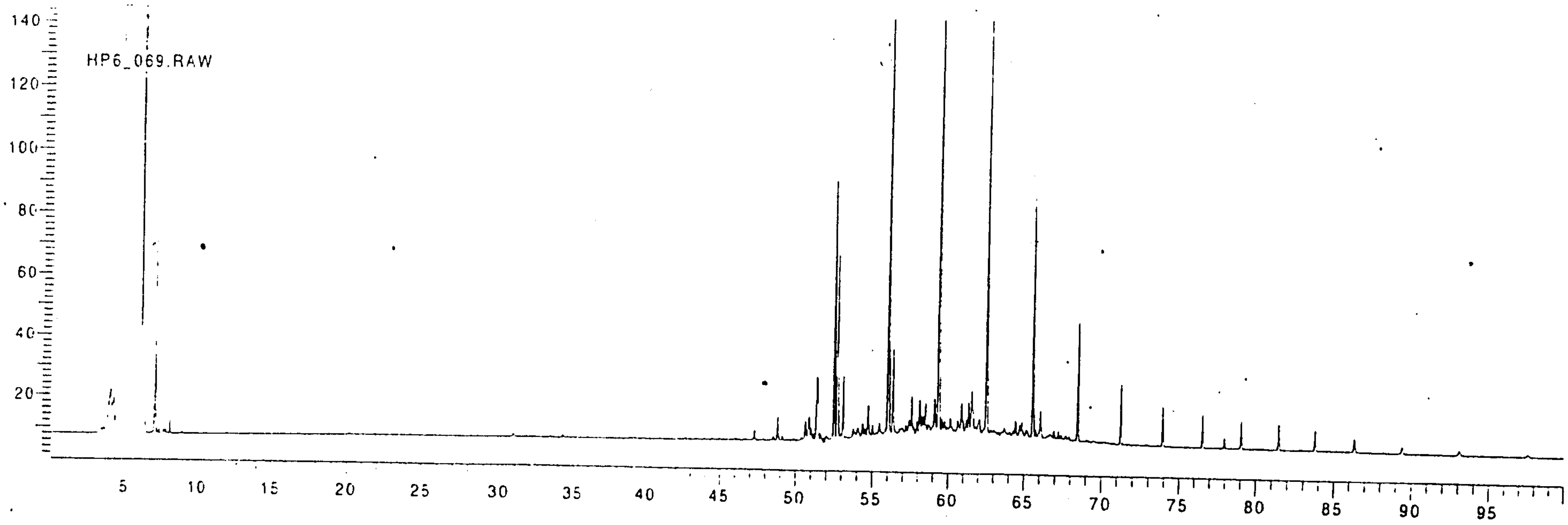
Fresh green leaves and greenish black seeds of *Z. Rhetsa* were collected from Malappuram district of Kerala in December. Fresh leaves (750g) were cut into pieces, ground into a paste using an electric mixer grinder and steam distilled for 5 hours. The distillate was extracted with solvent ether (3x100mL). The ether portions thus obtained were pooled together and dried with anhydrous sodium sulphate. On evaporation dry ether extract after removal of sodium sulphate, over a water-bath yielded 0.25g (0.03% of fresh weight) of the essential oil.

The seeds (650g) were steam distilled for 4 hours without powdering or maceration. The distillate was extracted with diethyl ether (2.5x 100mL) and dried using anhydrous sodium sulphate. The solvent on evaporation gave 4.5g (0.69% of fresh weight) of the seed oil.

GC-MS analysis was carried out using a Varian 3400GC fitted with an OPTIC® injector and coupled to a Finnigan ITS 40 ion trap mass spectrometer using helium as carrier gas at a rate of 1.6mL per minute. The mass spectrum was recorded in the scan range 35-450 amu (Eimode 70 eV). The column used was a HP ultra 2 (Hewlett Packard), 50m x0.2mm (film thickness 0.33mm). The sample was kept at 50° C and heated to 270° C at a rate of 2° c per minute. Quantification and Kovats determination was carried out using a Hewlett Packard 5890 GC fitted with and OPTIC® injector and FID detector using nitrogen as carrier gas at a rate of 0.3mL per minute. The column used was HP5 (Hewlett Packard) 25m x0.2mm (film thickness 0.33mm). The sample was kept at 30° C and heated to 280° C at a rate of 3 per minute.



Gas Chromatogram of *Zanthoxylum rhetsa* seed oil



Gas Chromatogram of *Zanthoxylum rhetsa* leaf oil

II.5 Results and Discussions

II.5.a Identification of components

The leaf essential oil was waxy in appearance while the seed oil was a pale yellow liquid. One hundred and twenty compounds were identified from the leaf oil of which monoterpenes (1.9%), sesquiterpenes (39.4%), aliphatic compounds (25.6%) and aromatic compounds (2.6%) were the major classes of compounds. Seventy-seven compounds were identified from the seed oil of which monoterpenes (91.7%) and sesquiterpenes (1.2%) were the major classes of compounds. The compounds were identified by comparison of mass spectral data with those in the computer database of the instrument. The retention indices of the identified compounds matched very well with the published data. The compounds and their percentages from both the oils are listed in table II.1.

Twenty-four monoterpenes were found to be present in the leaf oil while thirty-eight monoterpenes were identified in the seed oil. The concentration of individual monoterpenes in the leaf oil was very low, that of geranyl acetone being the highest with 1.2%. However, the percentage of monoterpenes in the seed oil was as high as 91.7% in which sabinene (66.3%), alpha-pinene (6.6%), beta-pinene (6.4%), terpinen-4-ol (3.5%) were the major constituents.

There were nineteen sesquiterpenes in both the oils though only a few were common. The major sesquiterpenes in the leaf oil were caryophyllene oxide (12.7%), beta-caryophyllene (9.6%), beta-copaene and spalhulenol (3.3%). In the seed oil the concentration of individual sesquiterpenenes were very low.

In the leaf essential oil the higher hydrocarbon content was very high (19.3%), but in the seed oil there was only trace quantities of the same. The high alkane content in the leaf oil is justifiable as they act as safe guards against microbial attack on leaves [92].

The conversion of sabinene to terpinen-4-ol during steam distillation is well known [93]. In the essential oil [94] of the seeds of *Z. rhetsa* collected from Thrissur district in April, the sabinene content is 47% and that of terpinen-4-ol is 6.6%. In the present work the sabinene content was found to be as high as 66.3% while that of terpinen-4-ol was only 3.5%. In this connection it is also of interest to note the Briggs and Sutherland's [95] findings that sabinene is found in all the oils in which terpinen-4-ol occurs. However, Koedam and Co-workers [93] are of the view that it is probably more realistic to formulate the reverse. Conversion of sabinene and sabinene hydrate to alpha and gamma terpinene, terpinolene and terpinen-4-ol were brought about by the acidity of water rather than by high temperature conditions, as the experiments were performed with aqueous solutions. The rearrangements of the above type will be catalysed by the organic acids present in the plant material. The observed decrease in sabinene content in the seed essential oil extracted in April may be due to the high level of organic acids during that period. Maarse and Nijsen[96] has observed a decrease of sabinene and sabinene hydrate where as the concentration of alpha and gamma terpinene, terpinolene and terpinen-4-ol showed an increase during heat sterilisation of black pepper.

Table II.1 **Compounds identified in the essential oils of leaves and seeds of *Zanthoxylum rhetsa***

Compounds	**Retention Index (RI) on SE54**	**Leaf oil % RPA**	**Seed oil % RPA**
Monoterpenes			
1 Tricyclene	927	nd	t
2 α-Thujene	929	nd	0.5
3 α-Pinene	938	t	6.6
4 Camphene	954	nd	t
5 Sabinene	977	t	66.3
6 β-Pinene	982	nd	6.4
7 2,3-dehydro-1, 8-cineole	990	t	nd
8 Myrcene	992	nd	1.4
9 α-Phellandrene	1009	nd	0.1
10 δ-3-Carene	1013	nd	t
11 α-Terpinene	1021	t	0.4
12 p-Cymene	1029	t	0.5
13 p-Cymenene	1129	t	nd
14 Limonene	1033	t	0.2
15 β-Phellandrene	1035	0.1	1.4
16 1,8-Cineole	1037	nd	0.1
17 (Z)- β-Ocimene	1039	nd	t
18 (E)- β-Ocimene	1049	nd	0.2

Compounds	Retention Index (RI) on SE54	Leaf oil % RPA	Seed oil % RPA
19. γ-Terpinene	1063	nd	1.0
20. cis-Linalooloxide	1076	t	nd
21. Terpinolene	1091	nd	0.3
22. Linalool	1101	0.1	1.2
23. α-Thujone	1113	nd	t
24. α-Cyclocitral		t	nd
25. β-Thujone	1123	nd	t
26. cis-2,8-para-Mentha-dien-1-ol	1126	t	nd
27. p-Menth-2-enol*	1130	nd	0.2
28. Pinocarveol	1148	nd	0.1
29. p-Menthdienol		nd	t
30. Terpinen-4-ol	1184	nd	3.5
31. p-Cymen-8-ol	1190	nd	t
32. α-Terpineol	1197	t	1.3
33. Myrtenol	1203	nd	0.1
34. Safranal	1206	0.2	nd
35. trans-Piperitol	1220	nd	0.1
36. β-Cyclocitral	1228	0.1	nd
37. Nerol	1232	nd	t
38. Cuminaldehyde	1249	t	t

Compounds	**Retention Index (RI) on SE54**	**Leaf oil % RPA**	**Seed oil % RPA**
39. Carvone	1250	t	nd
40. Geraniol	1256	t	t
41. Linalyl acetate	1257	nd	t
42. Piperitone	1264	0.1	t
43. β-Homocyclocitral	1268	t	nd
44. Geranial	1276	t	nd
45. Bornyl acetate	1292	nd	t
46. Isobornyl acetate	1296	nd	t
47. Cuminyl alcohol	1297	nd	t
48. Carvacrol	1305	nd	t
49. α-Ionone	1441	0.1	nd
50. Geranyl acetaone	1457	1.2	nd
51. β-Ionone	1500	t	nd
Sesquiterpenes			
1. α-Cubebene	1359	0.3	t
2. α-Copaene	1385	1.0	0.2
3. β-Damascenone	1395	0.1	nd
4. β-Copaene	1403	5.3	0.2
5. β-Elemene	1403	0.4	0.3
6. Bourbonene	1403	0.2	nd
7. α-Gurjunene	1419	nd	t

Compounds	**Retention Index (RI) on SE54**	**Leaf oil % RPA**	**Seed oil % RPA**
8. β-Caryophyllene	1431	9.6	t
9. Aromadendrene	1450	0.2	t
10. α-Humulene	1472	1.4	t
11. γ-Muurolene	1487	0.3	t
12. β-selinene	1497	nd	t
13. Germacrene D	1499	0.8	0.2
14. α-Selinene	1505	nd	0.1
15. Valencene	1512	nd	t
16. δ-Cadinene	1536	1.3	0.1
17. Nerolidol*	1568	0.4	nd
18. Spathulenol	1593	3.3	t
19. Globulol	1549	0.2	t
20. Caryophyllene oxide	1604	12.7	0.1
21. Ledol	1627	t	nd
22. Caryophyllene oxide isomer*	1631	1.1	nd
23.Cubenol	1645	0.8	t
24. Farnesol	1740	nd	0.1
Higher alkanes			
1. Pentadecane	1500	0.9	nd
2. Hexadecane	1600	1.0	nd
3. Heptadecane	1700	2.9	t

Compounds	**Retention Index (RI) on SE54**	**Leaf oil % RPA**	**Seed oil % RPA**
4. Octadecane	1800	2.9	nd
5. Nonadecane	1900	2.5	nd
6. Eicosane	2000	1.6	nd
7. Heneicosane	2100	0.9	nd
8. Docosane	2200	0.5	nd
9. Tricosane	2300	0.3	nd
10. Tetracosane	2400	0.2	nd
11. Pentacosane	2500	0.3	nd
12. Hexacosane	2600	0.5	nd
13. Heptacosane	2700	0.7	nd
14. Octacosane	2800	1.1	nd
15. Nonacosane	2900	1.5	nd
16. Triacontane	3000	1.5	nd
Lower alkanes and their derivatives			
1. Isoamyl alcohol	729	t	nd
2. 2-Methylbutanol	733	0.2	nd
3. Amyl alcohol	740	t	nd
4. 2-Methylbut-2-enol	772	t	nd
5. Hexanal	800	t	nd
6. (E)-3-Hexanol	849	0.1	nd
7. (E)-2-Hexenal	851	t	nd
8. (Z)-3-Hexanol	852	0.1	nd
9. (E)-2-Hexenol	863	t	nd

Compounds	**Retention Index (RI) on SE54**	**Leaf oil % RPA**	**Seed oil % RPA**
10. Hexanol	865	t	nd
11. Hexanoic acid	976	t	nd
12. Hexyl acetate	1012	nd	t
13. Heptane	700	3.5	nd
14. 2-Heptanone	891	t	t
15. (Z)-4-Heptenol	900	t	nd
16. Heptanal	901	t	t
17. Heptanol	969	t	nd
18.6-methyl-5-hepten-2-one	986	0.3	t
19. 6-methyl-5-hepten-2-ol	993	t	nd
20. 6-methyl hepta-3,5-dien- 2-one	1107	0.2	t
21. Octane	800	t	nd
22. Octenol	984	0.7	nd
23. Octanal	1003	t	1.9
24. Octanol	1069	0.1	0.72
25. 3,5-Octadien-2-one		t	nd
26. Octanoic acid	1169	nd	t
27. Nonane	900	nd	t
28. 2-Nananone	1093	nd	t
29. Nonanal	1104	nd	t

Compounds	Retention Index (RI) on SE54	Leaf oil % RPA	Seed oil % RPA
30. Nonadienal	1155	t	nd
31. Nonanol	1172	nd	0.1
32. Decanal	1206	nd	2.3
33. 2-Decenol	1265	nd	0.1
34. Decanol	1272	nd	0.2
35. 2-Undecanone	1295	t	t
36. Undecanal	1308	nd	t
37. Dodecanal	1410	nd	0.3
38. Dodecenal	1471	nd	t
39. Dodecanol	1472	nd	t
40. Dodecanoic acid	1558	1.1	nd
41. Tetradecanal	1614	nd	0.1
Aromatic compounds			
1. Benzaldehyde	964	t	nd
2. Benzyl alcohol	1037	t	nd
3. o-Cresol	1054	0.2	nd
4. Styrallyl alcohol	1064	t	nd
5. Acetophenone	1072	t	nd
6. p-Cresol+m-Cresol	1074	0.2	nd
7. p-Tolyl aldehyde	1083	t	nd
8. Guaiacol	1091	0.1	nd
9. 2-Phenylethanol	1120	0.1	nd
10. Isophorone	1127	0.1	nd

Compounds	**Retention Index (RI) on SE54**	**Leaf oil % RPA**	**Seed oil % RPA**
11. p-Tolyl alcohol	1136	t	nd
12. 2-Ethylphenol	1140	t	nd
13. Benzyl cyanide	1145	t	nd
14. o-Dimethoxybenzene	1149	0.1	nd
15. 2,5-Dimethylphenol	1152	0.1	nd
16. Benzoic acid	1164	0.1	nd
17. 4-Ethyl phenol	1167	0.1	nd
18. Cresol*	1196	0.1	nd
19. Methyl salicylate	1203	nd	0.1
20. 4-vinyl phenol	1218	0.5	nd
21. 4-Ethyl guiacol	1286	0.1	nd
22. Indole	1304	t	nd
23. 2-Methoxy-4-vinyl-phenol	1321	0.4	nd
24. Eugenol	1367	0.2	nd
25. Dihydroeugenol	1375	t	nd
26. 1,2,4-Trimethoxy benzene	1375	t	nd
27. Methyl cinnamate	1394	t	nd
28. Vanillin	1405	t	nd
29. Ethylvanillin	1463	0.2	nd

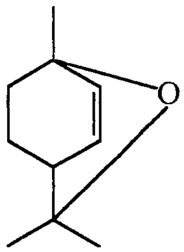
Compounds	**Retention Index (RI) on SE54**	**Leaf oil % RPA**	**Seed oil % RPA**
Others			
1. 2,4,4-Trimethyl-2-cyclohexen-1-ol	1060	t	nd
2. 4,4,6-Trimethyl-2-cyclohexen-1-one	1084	t	nd
3. 6-Hydroxy-2,2,6-trimethyl-cyclohexanone		t	nd
4. 2-Acetonyl-4-methyl tetra hydropyran	1162	t	nd
5. γ-Nonalctone	1368	t	nd
6. Dihydro actinidiolide	1547	0.2	nd

nd-Not detected in the oil

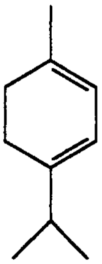
t-Trace amount (<0.1% RPA)

*-Correct isomer not characterized.

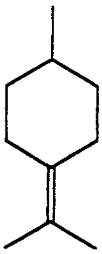
Structures of the compounds identified in the essential oils of *Zanthoxylum rhetsa* (structures given in other chapters are omitted)



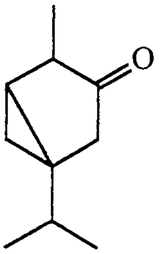
2,3-Dehydro-1,8-cineol



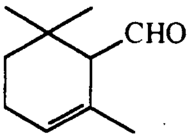
α-Terpinene



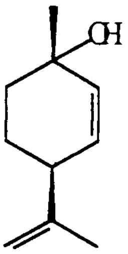
Terpinolene



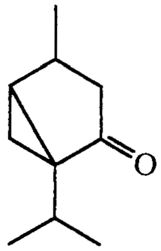
α-Thujone



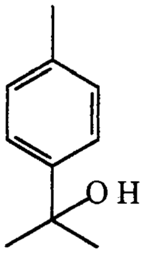
α-Cyclocitral



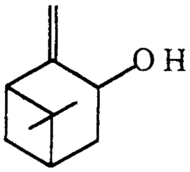
cis-2,8-p-Menthadien-1-ol



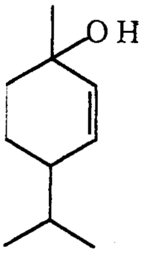
β-Thujone



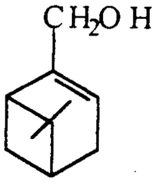
p-Cymen-8-ol



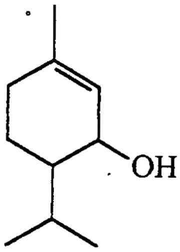
Pinocarveol



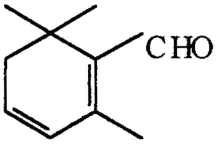
p-Menth-2-enol



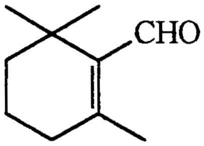
Myrtenol



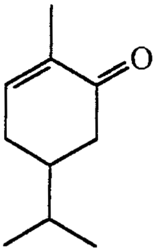
Piperitol



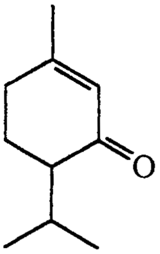
Safranal



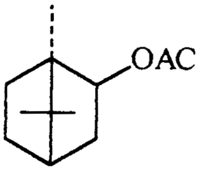
β-Cyclocitral



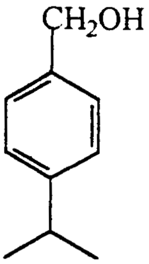
Carvone



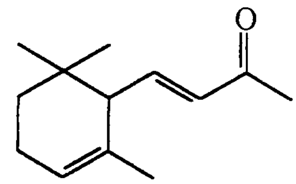
Piperitone



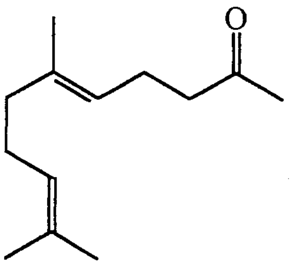
Isobornyl acetate



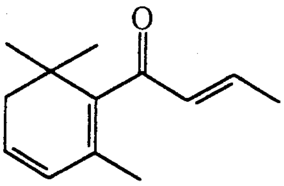
Cuminyl alcohol



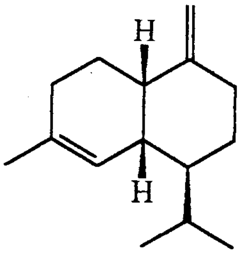
α-ionone



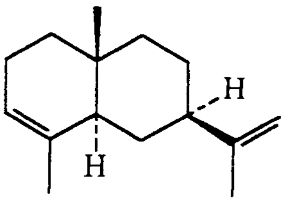
Geranylacetone



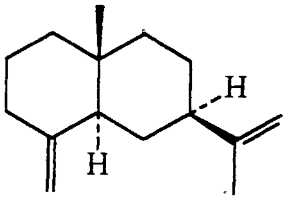
β– Damascenone



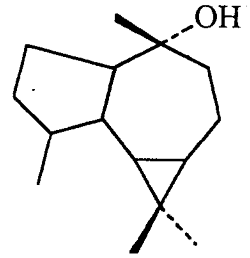
γ- muurolene



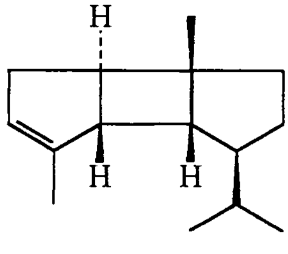
α-Selinene



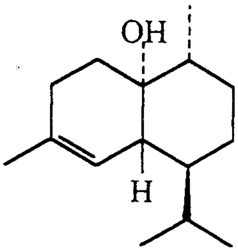
β-Selinene



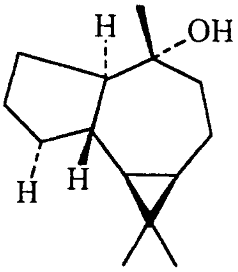
Ledol



Bourbonene



Cubenol



Globulol

b. Olfactoric studies.

The seed essential oil of *Z.rhetsa* was olfactorically evaluated. As the very faint odour of the leaf oil was of little significance, its olfactoric studies were not carried out. The odour of the essential oil of seeds of *Z.rhetsa* was described by professional perfumers as spicy in the direction of pepper, herbal-earthy, weak fruity like lemon and sweet orange, weak floral, pinene and terpenine like with woody-smokey notes in the back ground.

The chemical analysis of the seed oil was compatible with the findings of the perfumers. The spicy odour similar to that of black pepper was caused by monoterpene hydrocarbons like sabinene, pinenes and delta-3-cerene as well as by monoterpene alcohols, terpinene-4-ol, alpha-terpineoland linalool.. These compounds dominate in the essential oil of *Piper nigrum*[97].

The ester functionality is not necessarily a criterion for fruity odour.. Out of 85 fruity odourants studied by McGugan[98] 41% are esters, 24% ketones, 9% aldehydes, 7% lactones and the remaining miscellaneous. The fruity lemon-like, sweet orange-like odour of *Z.rhetsa* seed oil was due to the presence of limonene, alpha-terpinene, linalool, β-pinene, gamma-terpinene, linalyl acetate and valencene which are among the constituents of lime oil[99,100], orange juice and orange peel oil[101].

The weak floral odour of the seed oil was caused by linalool, alpha-terpineol, linalyl acetate, farnesol, nonanal, octanal etc. The floral odour is the combined contribution of monoterpenes and sespuiterpenes. Of the monoterpenes linalool, which is a constituent of rose, jasmine and neroli oils[102,103] imparts a floral odour. Alpha-pinene, beta-pinene, gamma-terpene and terpinolene accounted for the pinene- and terpinene-notes of the seed oil.

The herbal-earthy and woody-smokey notes of the oil was due to the presence of sabinene, terpinen-4-ol, selinene and especially by delta-cadinene which adds the smokey, tarry tonality to the odour bouquet[104]. In conclusion, it is possible to report that the essential oil of seeds of mullilam may be an interesting alternative to traditional pepper oil, for flavouring food as the oils of *Z.rhetsa* and *P.nigrum* seeds have a similar, but not identical, chemical composition.

ESSENTIAL OIL ANALYSIS OF THE LEAVES AND ROOT BARK OF THE PLANT CLERODENDRUM INFORTUNATUM USED IN AYURVEDZC MEDICINE.

Saidutty A "Chemical investigation of some indigenous plants" Thesis.
Department of Chemistry, University of Calicut, 1999

CHAPTER III

ESSENTIAL OIL ANALYSIS OF THE LEAVES AND ROOT BARK OF THE PLANT *CLERODENDRUM INFORTUNATUM* USED IN *AYURVEDIC* MEDICINE.

III. 1 Introduction

The plant, *Clerodendrum infortunatum* L (syn.*C.viscosum*) belongs to the family *Verbenaceae*. The family comprises of ca. seventy five genera and three thousand species, which are predominantly tropical and subtropical in distribution. A few members are native to temperate regions. In India the family is represented by ca. 17 genera and 198 species, of which 6 species are endemic to Peninsular India[105]. The genus *Clerodendrum* belongs to the subfamily *Viticoideae*. It is the largest genus of the *Verbenaceae* family and comprises ca.560 species and varieties according to Moldenke[106].

The species, *C.infortunatum* grows throughout India as undergrowth in forests up to 1800m and as weed along road sides and in wastelands. It is a large gregarious tawny-villous shrub, 0.9 to 2.4m high with bluntly quadrangular branchlets. Its leaves are round, ovate to ablong, hairy on both sides, more strongly so on the nerves beneath, up to 10 inch long and 8 inch broad. The flowers are white, tinged with pink in terminal panicles; fruits are some what globose drupes,. seated on the enlarged pink calyx containing 1-4 pyrenes [107,108]. The plant is called Peruku in Malayalam, Karukanni or Perugilai in Tamil, Bhant or Bhat in Hindi and Barhibarha or Barhichuda in Sanskrit [108].

In the traditional folk medicinal system Ayurveda, the different plant parts of *C.infortunatum* are used for various applications [109,110]. The plant has a bitter, pungent taste with a flavour. It is a bitter tonic, aphrodisiac, antipyretic, anthelmintic; useful in biliousness, "kapha", "tridosha", leucoderma, thirst, burning sensations, foul odours and diseases of blood. The leaves and roots are employed externally for tumours and certain skin diseases. The leaves are a cheap and efficient substitute for Chiretta (*Swertia chirayitta* Buch-Ham.) as a tonic and antiperiodic. The fresh juice is employed as a vermifuge, and also as a bitter tonic and febrifuge in malarious fevers, especially in those of children. Fresh leaf juice is used as an enema into rectum against ascarids. The leaves enter into the composition of pills used by Mundas of Chota Nagpur in chest complaint with cough and difficult expectoration. The sprouts are recommended in the treatment of snake-bite (Charaka, Sushruta); the leaves and flowers are prescribed in scorpion-sting (Charaka, Sushruda); but they are not an antidote to either snake-venom or scorpion-venom [108]. Further, the leaves are acrid, thermogenic, laxative, cholagogue, antiseptic, demulcent, anti-inflammatory, depurative, vermifuge, expectorant and are useful in vitiated conditions of kapha, helminthiasis, abscesses, leprosy, skin diseases, indolent ulcers, cough, bronchitis, inflammations, intermittent fevers, general debility and proctoptosis[111]. The roots of *C.infortunatum* relieve congestion and torpidity of bowels; extracts can inhibit the angiotensin-converting enzyme and is effective against hair loss disorders and shows insecticidal and fungicidal effects.

III. 2 Pharmacological actions of other *Clerodendrum* species.

The roots of *C.indicum* are used in the treatment of asthma, cough, scrofulous affections and its leaves are used as a vermifuge. The leaves of *C.inerme* are used as a febrifuge, contain an amorphous bitter principle resembling that found in Chiretta (*Swertia chirayita* Buch- Ham.). Poultice of leaves used to resolve buboes. The roots of *C.phlomidis* are aromatic and astringent, decoction used as a demulcent in gonorrhoea. The roots of *C.serratum* are used in rheumatism and dyspepsia. Its seeds are aperient, used in dropsy. Leaves are used as a febrifuge, also employed in external application for cephalagia and ophthalmia [112]. Further, alcoholic extract of leaves of *C.inerme* stimulated pregnant uterus, raised blood pressure and increased intestinal movements in rats. Aqueous extract of plant stimulated uterine motility at different stages of sex cycle in isolated rat uterus. Plant did not produce toxic effects with doses as high as 8g/kg [113]. Also alcoholic extract and saponin isolated from root bark of *C.serratum* caused release of histamine from lung tissues.

III. 3 Phytochemical studies reported on *Clerodendrum* species.

Jack and Rimpler[114] studied the distribution of iridoid glycosides in

Clerodendrum species. Twelve *Clerodendrum* species namely, *C.ugandense*, *C.serratum*, *C.indicum*, *C.thomsonae*, *C.inerme*, *C.tomentosum*, *C.trichotomum*, *C.colebrokianum*, *C.splendens*, *C.paniculatum*, *C.buchanani* and *C.bungei* have been examined for iridoids. The first eight species contain iridoids, while iridoids are absent in the last four species. Two species namely, *C.ugandense* and *C.serratum*, which belong to the section *Cyclonema*, contain exclusively iridoid glycosides with C-4 formyl group which have not been found previously in *Verbenaceae*. The other 6 species contain harpagide-and / or aucubin-type iridoides. Various iridoid glycosides isolated from *Clerodendrum* species are given in the table III.1

Table III-1

Occurrence of iridoid glycosides in *Clerodendrum* species

Species	Iridoid glycosides	Concn. %
1. *C. ugandense*	Ugandonside	0.07
Leaves	Euphoroside	0.1
2. *C.serratum* Var.	Plantarenaloside	0.05
dentatum Leaves	Euphoroside	0.03
3. *C.inerme*	Melittoside	0.1
Leaves		
4. *C.thomsonae*	Melittoside	0.18
Leaves	Aucubin	0.1
	8-O-Acetylharpagide	0.1
	Ajugoside	0.05
	8-O-Acetylmioporoside	0.05
	Reptoside	0.1
5. *C.tomentosum*	Harpagide	0.1
Leaves		
6. *C.trichotomum*		
Leaves	Harpagide	0.01
Fruits	Harpagide	0.05
	Melittoside	0.19
7. *C.colebrokianum*		
Leaves	Melittoside	0.1
Fruits	Harpagide	0.02
	Melittoside	0.2
8. *C.indicum*		
Leaves	Harpagide	0.02

Akihisa and coworkers[115] found that plant species belonging to the genus *Clerodendrum* of the family *Verbenaceae* are known to contain 24-β-ethyl sterols possessing a Δ^{25} bond i.e., 24 β-ethylcholesta-5,25-dien-3-β-ol (clerosterol) and its 22E-dehydro derivative, 24-β-ethylcholesta-5,22E,25-trien-3β-ol (22E-dehydro-clerosterol) as the dominant sterols. This is the characteristic feature of *Clerodendrum* species since the great majority of higher plants contain 24 α- alkyl sterols which lack a Δ^{25}- bond, as the major sterols such as sitosterol and stigmasterol. Recent studies by the same group on the sterol constituents of two *Clerdendrum* species, *C.fragrans*[116] and *C.infortunatum*[117] have shown that these plants contain clerosterol and its 22E-dehydro derivative as the major sterols along with cholesterol, 24-methylcholestrol, sitosterol and stigmasterol as the minor sterol constituents. This work shows the co-occurrence of 24α- and 24β-alkyl sterols in *C.fragrans* and *C.infortunatum.*

Leaves and flowers of *C.infortunatum* contain (24S)-ethylcholesta-5, 22,25-trien-3β-ol. Hispidulin-7-O-glucuronide and scutellarein-7-O-glucuronide are also present in leaves. Clerodin, hentriacontane, fumaric acid, ethyl and methyl esters of caffeic acid, β-sitosterol and its glucoside and a new flavone glycoside, acacetin-7-O-methylglucuronate are reported from the flowers. Further an acteoside isolated from flowers is identified as 2-(3′,4′-dihydroxyphenyl)ethanol-1-O-α-L-rhamnopyranosyl-(1→3)- β-D-

(4″-O-caffeyl)glucopyranoside. Seed fat of the plant is found to be rich in palmitic acid (12.6%), oleic acid (74%) and linoleic (7.4%) acids. Studies on roots show the presence of lupeol and β-sitosterol [120].

Roots and stems of *C.fragrans* are found to contain (24S)-ethylcholesta-5,22,25-trien-3β-ol, clerosterol, clerodolone, α-amyrin, β-sitosterol and its glucoside and triacontane. Leaves of *C.multiflorum* contain scutellarein, pectolinarigenin, (24S)-ethylcholesta-5,22,25-trien-3β-ol. Pectolinarigenin, hispidulin, apigenin and luteolin are found to be present in leaves while ceryl alchol, clerodin, clerosterol and clerodendrin are reported from roots[113,118].

(24S)-ethylcholesta-5,22,25- trien- 3β-ol, hispidulin, hispidulin-7-O-glucuronide and scutellarein-7-O-glucuronide are found to be present in the leaves of *C.indicum*. Chemical analysis of stems shows the presence of β-sitosterol and (24S)-ethylcholesta-5,22,25-trien-3β-ol. Scutellarein-4′-L-arabinoside, acacetin-7-glucoside and (24S)-ethylcholesta-5,22,25-trien-3β-ol are found to be present in the leaves of *C.neriifolium* [113,118].

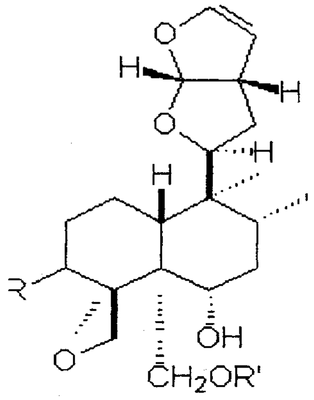
Clerodin, clerodolone, (24S)-ethylcholesta-5,22,25-trien-3β-ol, α-amyrin, β-sitosterol and triacontane are found to be present in the roots of *C.colebrookianum* while hentriacontane, octacosanol, (24S)-ethylcholesta-5,22,25-trien-3β-ol, clerodolone, clerosterol, 5-hydroxy-4′-7-dimethoxy flavone from leaves and α-amyrin, (24S)-ethylcholesta-5,22,25-triene and oleanolic acid are reported from stems of *C.tomentosum*[118,119].

Chemical analysis of the root bark of *C.serratum* shows the presence of glucose, D-mannitol and a crude saponin which on hydrolysis yielded oleanolic acid, queretaroic acid and a new serratagenic acid [119]. The chemical analysis of various parts of the plant, *C.inerme* shows the following results. (24S)-Ethylcholesta-5,22,25-trien-3β-ol, scutellarein-7-O-glucuronide and apigenin-7-O-glucuronide are present in the leaves. Clerodin, caryoptin, 3-epicaryoptin and neolignans I, II & III, are isolated from seeds where as royleanone, dehydroroyleanone, α-amyrin, β-sitosterol, betulin and (24S)-ethylcholesta-5,22,25-trien-3β-ol are found to be present in the stem [113,118].

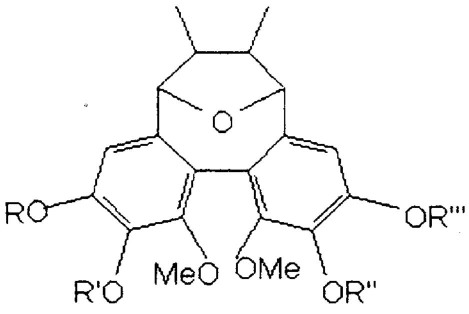
III.4 Present work

Phytochemical studies on the various parts of *C.infortunatum* were carried out [114-117,120-131] in detail, but no information on the essential oil was given until now. So the present work was aimed to identify as many compounds as possible in the essential oils of leaves and root bark using gas·chromatographic- spectroscopic methods (GC and GC-MS) in combination with the olfactoric evaluation and to correlate these data with the corresponding olfactoric and possible medicinal effects for this wellknown Ayurvedic drug.

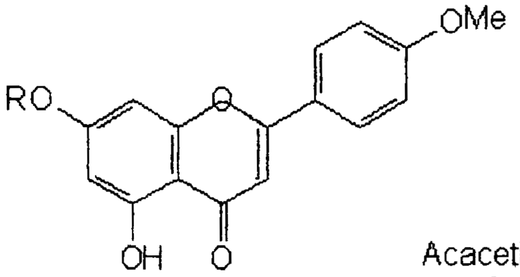
New compounds reported from *Clerodendrum* species



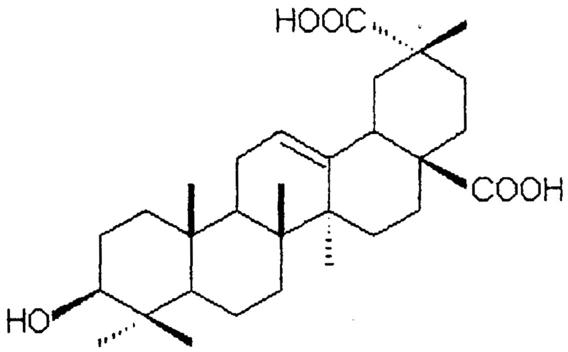
Clerodin
R=H, R'=H
Caryoptin
R=   , OAc, R'=Ac
3-Epicaryoptin
R=b-OAc, R'=Ac



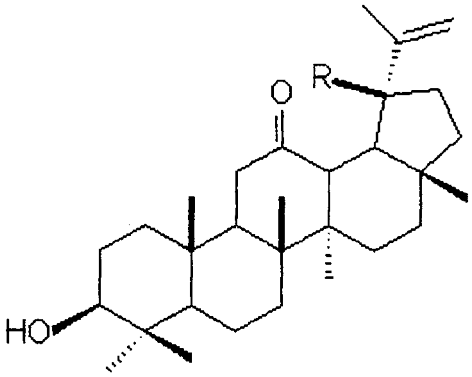
Neolignan **I**
R R', R'' R'''. = $-CH_2-$
Neolignan **II**
R R' = $-CH_2-$, R'' ,R'''= Me
Neolignan **III**
R,R' = Me , R'' R'''. = $-CH_2-$



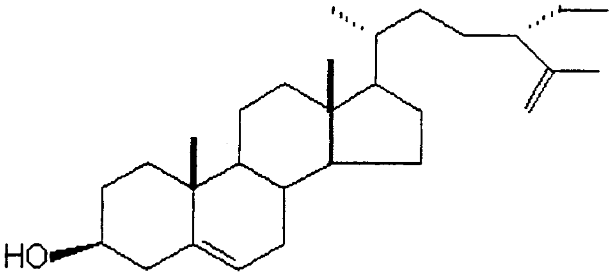
Acacetin 7-O-methylglucuronate
R = Gluc.acid(6-OMe)



Serratagenic acid



Clerodone R = H
Clerodolone R = b-OH



Clerosterol

III. 5 Plant Material

Five hundred gram of fresh leaves and three hundred gram of fresh root bark of *Clerodendrum infortunatum* were collected in April 1998 from Calicut University Campus. The plant was identified by Dr.A.K.Pradeep, Department of Botany, Calicut University and voucher specimen was deposited in the Herbarium of Chemistry Department of the University.

The leaves as well as the root bark were ground into a paste with 500mL of water and steam distilled for three hours each. The distillates were extracted with solvent ether and dried using anhydrous sodium sulphate. After removing the ether, a yeild of 15mg essential oil of the leaves and 25mg of root bark essential oil were obtained.

III. 6 Instrumentation

Gas chromatographic analysis of the volatiles of the essential oils of the leaves and the root bark of *C.infortunatum* were carried out using a Shimadzu 14A-FID (Japan) and a Varian GC-3700-FID (Germany) gas chromatographs, using hydrogen as carrier gas. The instruments were fitted with Shimadzu C-R6A-Chromatopac and Shimadzu C-R1B-Chromatopac integrator systems respectively. GC-MS analysis were carried out on a Shimadzu GC-17A-QP-5000 and on a Hewlett-Packard GC-HP 5890 with an HP-5970-MSD using helium as carrier gas. The mass spectra was recorded in the 41-550 amu scan-range (EI-mode 70eV). Columns used for both analyses were 30mx0.32mm bonded nonpolar FSOT-RSL-200 fused silica (film-thickness 0.25µm; Biorad Co; Belgium) and 30mx0.32mm bonded polar stabilwax (film-thickness 0.50µm, Restek Co; USA). The samples were kept at 40°C for 5minutes and heated to 280°C at a rate of 8°C/minute.

The olfactoric analyses were done by diluting the oils with dichloromethane and some drops of these solutions (1%) were placed on a commercial (Dragoco Company, Germany) odourstrip. After evaporation of the solvent, the odour zone was olfractorically evaluated by professional perfumers.

III.7 Results and Discussions

a. Identification of components.

Gas chromatographic-spectroscopic (GC and GC-MS) analyses of the volatiles of the leaf oil and root bark oil revealed the presence 71 compounds in the leaf oil and 70 compounds in the root bark oil respectively. The combined chromatographic, spectroscopic and olfactoric data interpretation lead to identification of 67 components in the leaf oil and 66 components in the root bark oil. Four compounds each remained unidentified in both the oils. The analysis showed exceptionally high amount of fatty acids and their esters in both the oils which is rather rare in essential oils.

The essential oil of leaves of *C.infortunatum* was found to contain 65.84% fatty acids and their esters, 14.1% monoterpenes, 12.1% sesquiterpenes, 5.9% aliphatic compounds and 1% aromatic compounds. The composition of root bark oil was as follows. Fatty acids and their esters (60.8%), monoterpenes (8.9%), sesquiterpenes (21.2%), aliphatic compounds (4.9%) and aromatic compounds (2.6%). Palmitic acid was the main component in both the oils. Limonene, α-pinene, β-pinene, p-cymene and myrcene were the major monoterpenes and β-caryophyllene and β-eudesmol were the dominant sesquiterpenes in both the oils. The compounds identified their percentages, methods of identification and Kovat's indices are listed in table III.2

The results of the analysis showed that the components of the essential oils of leaves and root bark of *C.infortunatum* are more common to fatty oils rather than to essential oils. The folk medicinal use of this *Clerodendrum* species may be due to the identified essential oil constituents along with the less-or non-volatile compounds identified in the previous studies[114-117,120-131]. The fatty acids and their esters can take part in the complex effects of a laxative like it is known from *Rhicinus* oils. Also a support of dermal application in the case of skin diseases can be assumed for fatty acids and their esters as well as higher hydrocarbons because of the skin penetration effects of these compounds.

On the identified mono-and sesquiterpenes, following effects in correlation to the mentioned applications of *Clerodendrum infortunatum* have been reported. Myrcene is known as a monoterpene with antitumour[132-135] and cercaricidal[136] activity. Linalool[137,138] shows insecticidal and molluscicidal properties. Monoterpenes like geraniol, linalool, pinenes and terpinenes and sesquiterpenes like cadinene and farnesene are reported to have bactericidal and disinfecting effects[139-141]. Further, the monoterpene alcohol linalool and the sesquiterpene alcohol α-bisabolol show spasmolytic[137] activites, which may play a role in the treatment of problems with the bowels.

Although the essential oil content in the leaves and the root bark of C.*infortunatum* is not so high as in many other essential oils used in medicine, fragrance compounds are known to show significant biological activities also in very low concentrations[142,143]. Therefore the above mentioned oil constituents with discussed pharmacological properties will show additional or synergic effects in correlation to the biological activities of less-or non-volatile compounds identified in the previous studies.

Table III.2. Composition of the essential oil of the leaves [A] and the root bark [B] of *Clerodendrum infortunatum*

Compound	[A]	[B]	Identification	RI$^+$
Fatty acids and their esters				
1. Myristic acid	12.4	4.3	GC,GC-MS	
2. Palmitic acid	45.6	32.7	GC,GC-MS	
3. Palmitic acid ethylester	2.24	1.7	GC,GC-MS	
4. Stearic acid	4.8	2.0	GC,GC-MS	
5. Stearic acid ethyl ester	0.5	0.3	GC,GC-MS	
6. Oleic acid	0.3	19.8	GC,GC-MS	
Monoterpenes				
1. α-Thujene	0.3	0.1	GC,GC-MS	935
2. α-Pinene	1.8	1.4	GC,GC-MS	955
3. Camphene	0.5	tr*	GC,GC-MS	958
4. Sabinene	0.7	0.1	GC,GC-MS	977
5. β- Pinene	1.2	1.7	GC,GC-MS	980
6. Myrcene	1.3	0.4	GC,GC-MS	987
7. α-Phellandrene	0.6	0.3	GC,GC-MS	1002
8. p-Cymene	0.7	1.1	GC,GC-MS	1018
9. cis-Ocimene	0.3	0.2	GC,GC-MS	1022
10. β-Phellandrene	0.6	0.1	GC,GC-MS	1025
11. 1,8-Cineole	0.4	tr	GC,GC-MS	1028
12. Limonene	3.2	2.7	GC,GC-MS	1031
13. trans-Ocimene	0.2	tr	GC,GC-MS	1038
14. Linalool	0.9	0.3	GC,GC-MS	1091
15. Terpinen-4-ol	0.6	0.2	GC,GC-MS	1170
16. α-Terpineol	0.4	tr	GC,GC-MS	1173
17. Geraniol	0.3	tr	GC,GC-MS	1239
18. p-Cymenene	0.1	0.3	GC,GC-MS	1277

Compound	[A]	[B]	Identification	RI^{+}
Sesquiterpenes				
1. α-Copaene	0.1	0.7	GC,GC-MS	1365
2. β-Cubebene	tr	0.6	GC,GC,MS	1377
3. β-Elemene	0.2	0.7	GC,GC-MS	1398
4. trans-β-Faranasene	0.8	0.9	GC,GC-MS	1412
5. trans-β-Bergamotene	0.6	0.7	GC,GC-MS	1423
6. β-Caryophyllene	2.3	4.6	GC,GC-MS	1427
7. α- Bergamotene	0.3	0.8	GC,GC-MS	1431
8. Germacrene	0.2	0.4	GC,GC-MS	1453
9. α- Muurolene	tr	0.5	GC,GC-MS	1478
10. cis- α- Bisabolene	0.2	0.6	GC,GC-MS	1494
11. δ-Cadinene	0.4	0.5	GC,GC-MS	1500
12. cis-Nerolidol	0.8	0.9	GC,GC-MS	1522
13. Elemol	0.1	0.4	GC,GC-MS	1527
14. tran-Nerolidol	0.2	0.2	GC,GC-MS	1545
15. Caryophyllene oxide	0.7	0.9	GC,GC-MS	1563
16. β-Eudesmol	4.8	6.9	GC,GC-MS	1634
17. β-Bisabolol	0.4	0.9	GC,GC-MS	1659
Aromatic compounds				
1. Benzyl alcohol	0.3	tr	GC,GC-MS	1033
2. Benzoic acid	nd$^{\$}$	0.7	GC,GC-MS	1077
3. Benzyl acetate	0.3	tr	GC,GC-MS	1141
4. Hexyl salicylate	0.2	0.8	GC,GC-MS	1664
5. Benzyl benzoate	0.2	0.7	GC,GC-MS	1739
6. Phenylethyl benzoate	nd	0.4	GC,GC-MS	1840

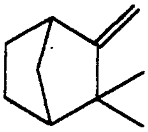
Compound	[A]	[B]	Identification	RI^{+}
Aliphatic compounds				
1. 1-Hexen-3-ol	0.7	tr	GC,GC-MS	768
2. Hexanal	tr	nd	GC,GC-MS	777
3. Hexan-2-ol	0.2	tr	GC,GC-MS	784
4. trans-Hex-2-enal	0.1	tr	GC	830
5. Cis-3-Hexenol	0.4	nd	GC,GC-MS	845
6. trans-2-Hexenol	0.1	nd	GC	851
7. Hexanol	0.3	0.1	GC,GC-MS	855
8. Cis-3-Hexenyl acetate	0.2	nd	GC,GC-MS	990
9. trans-2- Hexenyl acetate	0.1	nd	GC,GC-MS	999
10. Hexyl acetate	0.1	tr	GC,GC-MS	1010
11. Cis-3-Hexenyl decanoate	0.3	tr	GC,GC-MS	1760
12. 1-Octen-3-ol	tr	0.9	GC,GC-MS	964
13. Octanal	nd	0.1	GC	983
14. Octan-2-ol	tr	0.1	GC	993
15. trans-3-Octenol	tr	0.1	GC	1035
16. Cis-3-Octenol	tr	0.2	GC,GC-MS	1040
17. Octanol	0.1	0.1	GC	1055
18. 1-Nonan-3-ol	tr	0.4	GC,GC-MS	1064
19. Nonanal	nd	0.1	GC,GC-MS	1086
20. Decanal	0.2	0.1	GC,GC-MS	1179
21. trans-2-Decenol	tr	0.2	GC	1249
22. Decenol	0.2	0.4	GC	1255
23. Nonanoic acid	0.7	0.9	GC,GC-MS	1282
24. Eicosane	2.2	1.2	GC,GC-MS	2000

$^{+}$Retention indices from the unpolar column

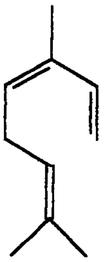
*trace compound with concentrations less than 0.1%

$^{\$}$ not detected

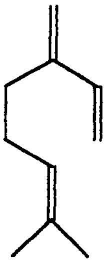
Structures of compounds identified in *Clerodendrum* essential oils (Structures given in other chapters are omitted)



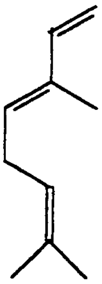
Camphene



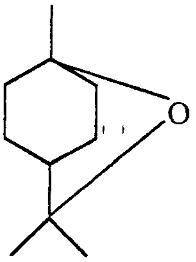
Ocimene



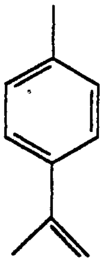
Myrcene



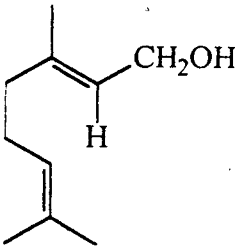
(E) β-Ocimene



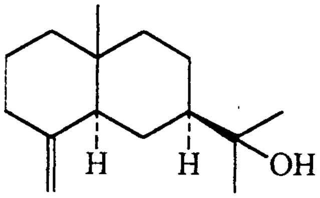
1,8-Cineole



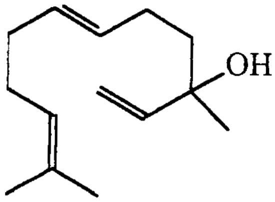
p-Cymenene



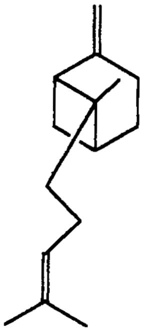
Geraniol



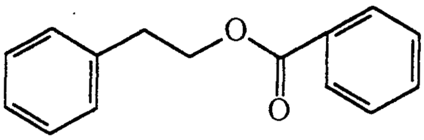
β-Eudesmol



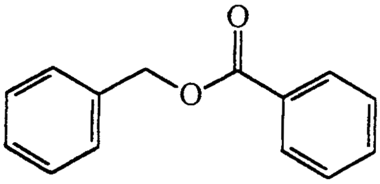
Cis-Nerolidol



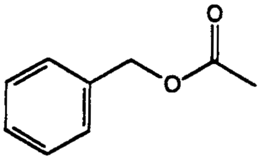
β-Bergamotene



Phenyl ethyl benzoate



Benzyl benzoate



Benzyl acetate

III. 7.b Olfactoric studies

The olfactoric characteristics of the leaf oil and root bark oil of *C.infortunatum* evaluated by professional perfumers were described as follows. The leaf oil is dominated by a fresh green (leaf)- pinene tope-note with green woody-fruity side notes and a smokey-fatty background note. The root-bark oil is dominated by pinene-woody and weak fruity top notes with earthy-moody and woody-root like side notes as well as smokey-fatty-gum-like background notes. The observed odour characteristics are correlated with identified chemical compounds as follows.

The fatty acids and their esters and partly some nonane-derivatives are responsible for the fatty back ground and side-notes and in combination with the detected higher hydrocarbons also for the gum-like notes. Additionally these compounds may play a fixative propertiy of both essential oils. The green notes from the essential oil of the leaves of *C.infortunatum* are attributed to hexane derivatives and the fresh notes to camphene and 1,8 cineole. The pinene impressions of both oils are caused by α- and β-pinenes as well as sabinene. The fruity odour is caused by limonene, benzyl acetate, α-terpineol, decanal, decanol and geraniol. The woody impressions of the oils are caused by β-eudesmol[144], caryophyllene derivatives and other sesquiterpenes. The earthy moody characteristics of the root oil is caused by octane derivatives.

In conclusion, the essential oils of the leaves and root bark of *Clerodendrum infortunatum* have interesting compositions with identified compounds partly responsible for reported medicinal applications especially as a laxative, as a remedy against certain skin diseases or tumours, as a drug with insecticidal, cercaricidal and fungicidal as well as spasmolytic activities. The high amount of fatty acids and their esters in the oils is remarkable, which is known from fatty oils, but not common in essential oils.

ANALYSES OF THE ESSENTIAL OIL OF THE LEAVES OF
THE MEDICINAL PLANT COLEUS ZEIZANICUS

Saidutty A “Chemical investigation of some indigenous plants” Thesis.
Department of Chemistry, University of Calicut, 1999

CHAPTER IV

ANALYSES OF THE ESSENTIAL OIL OF THE LEAVES OF THE MEDICINAL PLANT *COLEUS ZEYLANICUS*

IV.1 Introduction

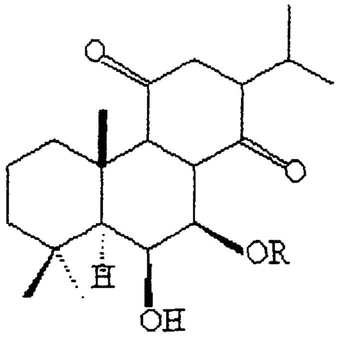
Coleus Zeylanicus (Benth) *Cramer* syn. *Plectranthus zeylanicus* Benth belongs to the family, *Lamiaceae (Labiatae). Lamiaceae* is a large family comprising ca. 180 genera and 3500 species, which are cosmopolitan in distribution with their greatest development in the mediterranean. The members grow under various habitat conditions at different altitudes and show a high degree of adaptability with their occurrence ranging from the Arctic to the Himalayas. The family is represented in India by ca. 64 genera and 340 species of which 60 species and 5 varieties are strictly endemic to Peninsular India.[145]

Coleus zeylanicus is an annual herb with stems rooting at lower nodes. The stems are four angled and hirsute. Leaves are broadly ovate to suborbicular, rounded to truncate at base, coarsely crenate-serrate, sparsely hirtellous on both surfaces and thick with amber coloured oil globules and terminal panicles. The plant is endemic to Ceylon where it is cultivated as a medicinal herb. This plant is also cultivated in Kerala in many places for its aromatic roots and for medicinal purpose. It is interesting to note that this species is also known as *Iriweriya* in Sinhala language[146.147]. *C.zeylanicus* is used in *Ayurvedic* medicine as a drug "Hribera" or "Balam" against pyrexia (fever depression), jaundice and gout. Further folk medicinal applications for this *Coleus* species in India are reported for the cooling, carminative, tonic, dyspepsia curing, antiemetic, antidermatitic, antiulcerogenic, bleeding disorders regulating and anti-diarrhoic properties. Further *C.zeylanicus* in composition with other drugs is mentioned as *Ayurvedic* medicament against indigestion and dysentery[146,147].

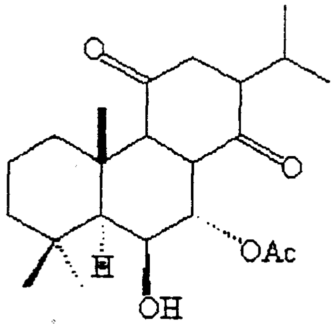
Leaves of *Coleus amboinicus* are used as a flavouring agent. They are also used in the treatment of urinary diseases. Decoction of the leaves is given for chronic cough and asthma. A decoction of the leaves of *C. blumei* is used in dyspepsia. The roots of *Coleus forskohlii* are edible. They are considered to be the wild ancestor of the tuber varieties known as Kaffir potatoes. Tubers of *C.parviflorus* (Koorkan Kizhangu) are edible and used as a substitute for potatoes where as the fresh fragrant roots of *Coleus vettiveroides* are used for decoration of temple images and for dressing hair.

IV.2. **Work so far reported.**

Mehrotra and co-workers[148] carried out phytochemical studies on *Coleus zeylanicus*. The genus *Coleus* has been investigated as a rich source of highly oxidized abietane and labdane diterpenoids[149]. The isolation of a unique labdane diterpenoid forskolin (coleonol) from the plant *Coleus forskohlii*[150,151] which is a experimental drug[152] for glaucoma, congestive cardiomyopathy and asthma has generated further interest in the diterpenoids of *Coleus* and related genera. The phyto chemical studies on the ethanolic extract of the plant *Coleus zeylanicus* *by* Mehrothra and co-workers[148] led to the isolation of two new abietane type diterpenoids which are characterised as 7β-acetoxy-6β-hydroxy royleanone(1) and 7β, 6 β- dihydroxyroyleanone(2). The known stereo isomer, 7α- acetoxy–6 β– hydroxy royleanone (3) has also been isolated form the plant, *Coleus zeylanicus*.



1 R=Ac
2 R=H



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A preliminary biological screening of root extract of *Coleus forskohlii* Briq.(syn. *C.barbatus*) indicated hypotensive and spasmolytic activities[153,154]. The benzene extract of roots afforded labdane diterpenoids including coleonol (forskolin), which as mentioned earlier is a potential drug for treatment of glaucoma, congestive cardiomyopathy and asthma because of its unique adenylate cyclase stimulant activity[155]. Further examination of the ethanolic extract of the roots of *C. forskohlii* by Ahmad and Viswakarma[156] yielded caffeic acid and a new monoterpene glycoside, coleoside, characterised as cuminyl-O-β-D- glucopyranosyl (1→2)- β-D- galactopyranoside. Studies of Ammon and Muller[157] have shown that forskolin is a hypotensive agent with spasmolytic, cardiotonic and platelet aggregation inhibitory activity. It has been shown to be a powerful activator of enzyme adenylate cyclase in various tissues. Forskolin is thought to act on the catalytic sub-unit and also on the coupling mechanism of guanine regulatory sites with the catalytic sub unit. It is now clear that forskolin elicits psychological responses, which have been shown to be AMP-dependent.

Phytochemical studies on *Coleus forskohlii* verified the fact that the genus *Colues*, is a rich source of highly oxidized abietane and labdane diterpenoids. Various diterpenoids have been isolated from different parts of *C.forskohlii*. Coleon E, coleon F, barbatusin, cyclobutatusin have been isolated from the leaves of the plant. Diterpenoids coleonol, coleonol B, coleonol C, coleonol D, coleonole E,coleonol F, deoxycoleonol, coleosol ,coleol, coleonone, 3 β-hydroxy-3-deoxybarbatusin are found to be present in the roots of *C. forskohlii*. The new labdane diterpenoids I, II and III are also present in the roots along with naphthopyrone and crocetin dialdelyde. The other diterpenoids isolated form *Coleus forskohlii* are barbatusol, 6β-hydroxycarnosol and plectrin [158,159].

Leaves of *Coleus amboinicus* Lour. Syn. *C. aromaticus* contain oleanolic, 2α, 3α - dihydroxyolean-12- en-28-oic, crategolic, pomolic, euscaphic, tormentic, ursolic and 2 α, 3 α, 19 α, 23- tetrahydroxyurs-12-en-28-oic acids. Leaves also contain cirsimaritin, β-sitosterol-β- D-glucoside,salvigenin,6-methoxygenkwanin,qercetin,chrysoeriol, uteolin, apigenin, eriodictyol and taxifolin. Two new pigments coleon S and coleon T are isolated from leaves and stems of *Coleus caninus*. The aerial parts of the same plant contain α-amyrin, tormentic acid, kumatakenin, 3,7- dimethylquercetin and β-sitosterol[158,159].

Essential oil analysis of the plants of the genus *Coleus* Lour has not been reported much in literature. Essential oil of *C.froskohlii* of is reported to show antimicrobial activity. Chemical analysis of *Coleus amboinicus* leaf oil shows the presence of thymol (41.30%), carvacrol (13.25%), 1,8- cineole (5.45%), eugenol (4.40%) and β-caryophyllene (4.20%) as the major components[159].

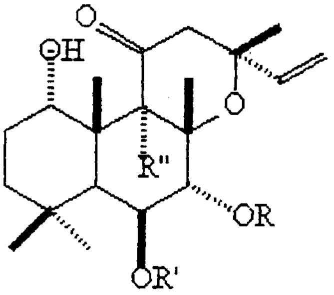
Biological Activity

The various compounds isolated from different *Coleus species* are found to show biological and pharmacological activities. Colenol showed hypotensive and spasmolytic activities. Coleonol lowered blood pressure of anaesthetised cats and rats and of spontaneously hypertensive rat; in small doses it had positive inotropic effect on isolated rabbit heart and on cat heart *invivo*, where as large doses had depressant action on central nervous system. Coleonol also exhibited nonspecific spasmolytic activity on smooth muscle of gastrointestinal tract.

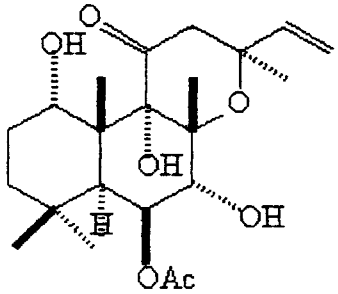
Forskolin, isolated form *Coleus forskohlii* stimulated renin release form isolated perfused rat kidney two-fold at 0.1 μM or three-fold at 1. μM; stimulation was blocked by angiotensin but not by the β-adrenoceptor antagonist, atenolol. Topical ocular application of forskolin lowered intraocular pressure (IOP) in rabbits, monkeys and human volunteers. A topical suspension of forskolin (1.0%) significantly lowered IOP (70.0%) in one hour but remained significant for atleast 5 hrs. Forskolin increased force of contraction and pacemaker activity of isolated guinea pig atrium. It potentiated Ca^{2+}-dependent contraction in atrium. Forskolin lowered Na^+ - K^+ - ATP ase and increased adenylate cyclase activities in rat heart slices. Forskolin and cardiac glycosides had additive positive inotropic effects on guinea pig atrium. It inhibited norepinephrine-induced contractions in isolated guinea pig artery and angiotensin II-induced contractions in guinea pig colon and aorta. Forskolin (1.0 μm) inhibited arachidonate and ADP – induced serotonin release in human platelets by 100.0% and 60.0% respectively. It enhanced or inhibited phosphorylation of human platelet proteins, depending on the molecular weight of protein involved.

Barbatusin showed inhibitory activity at 200 and 400 mg/kg against Lewis lung carcin and lymphocytic leukaemia P388 respectively in mice. Barbatusol (3mg/kg, i.v.) in rats, induced potent lowering blood pressure associated with discrete bradycardia. The labdane diterpenoids I, II and III showed blood pressure lowering and cardioactive properties. Finally, naphthopyrone (0.5 mg/kg,i.v.) decreased blood pressure in cats from 110 mm to 30 mm Hg in 2 minutes lasting 45 minutes; it had LD_{50} 150 mg in cat.

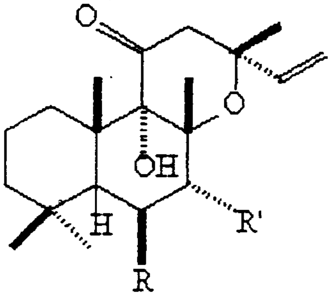
New compounds reported from *Coleus* species



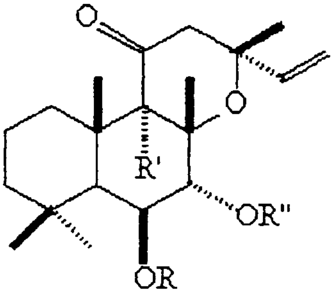
Coleonol
R = Ac , R' = H , R" = OH
Coleonol B
R = H , R' = Ac , R" = OH
Deoxycoleonol
R = Ac , R', R" = H



Coleonol C



Coleonol D
R = OH , R' = OAc
Coleol
R , R' = H

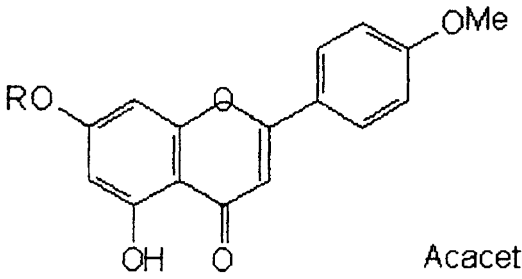


Coleonol E
R , R' = H , R" = Ac
Coleonol F
R = Ac , R' = OH , R" = H

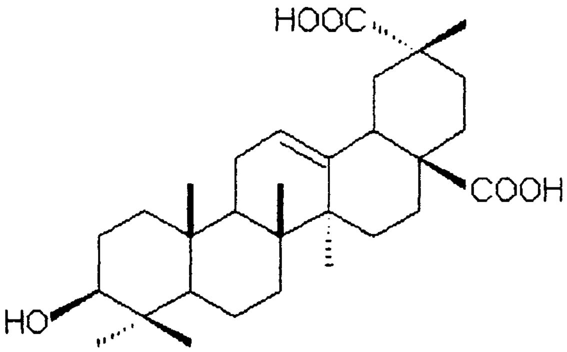
NB4358

546

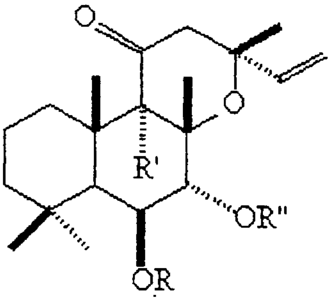
TH
SA1/C



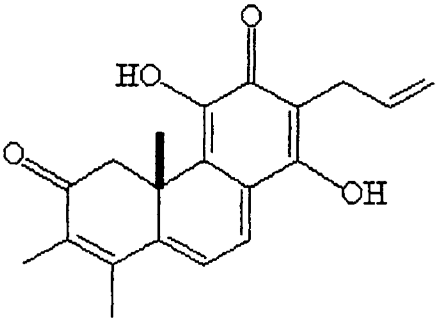
Acacetin 7-O-methylglucuronate
R = Gluc.acid(6-OMe)



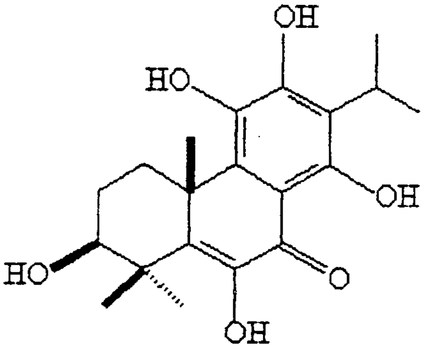
Serratagenic acid



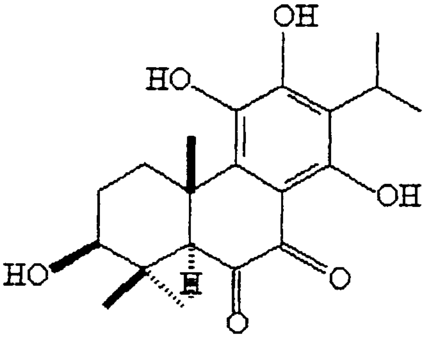
Coleonol E
R , R' = H , R" = Ac
Coleonol F
R = Ac , R' = OH , R" = H



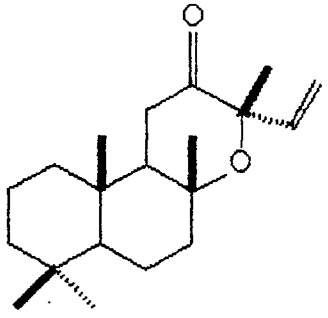
Coleon F



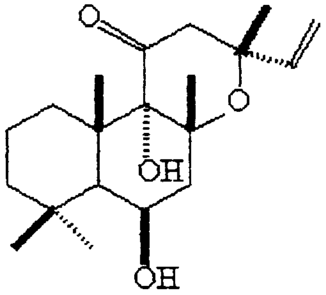
Coleon S



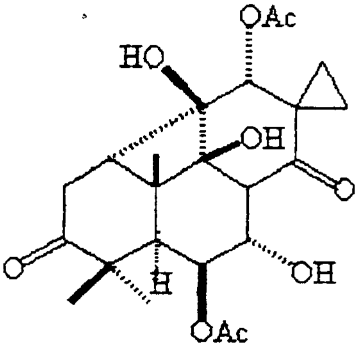
Coleon T



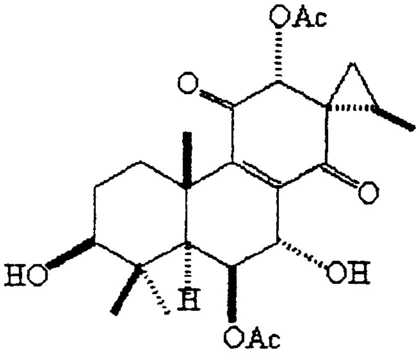
Coleonone



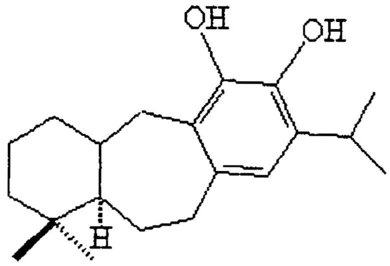
Coleosol



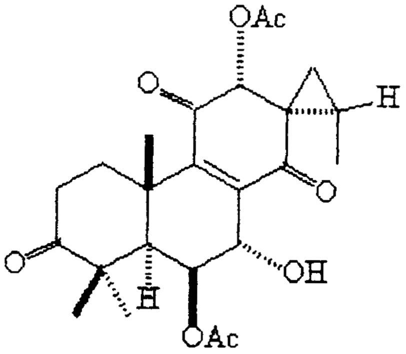
Cyclobutatusin



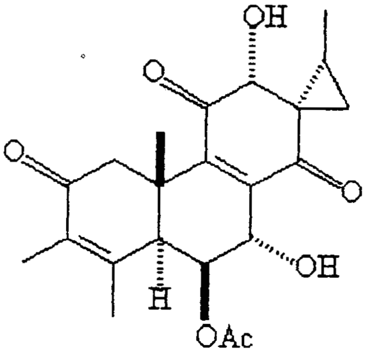
3-b-Hydroxy-3-deoxybarbatusin



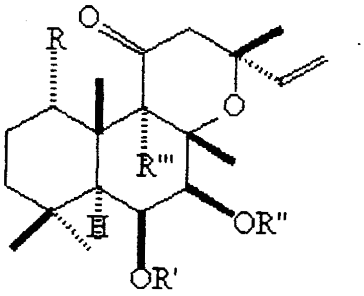
Barbatusol



Barbatusin

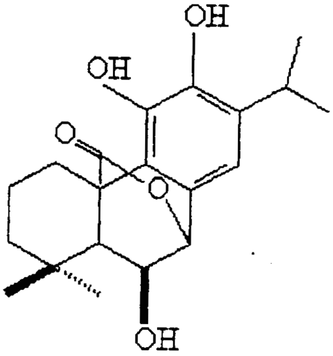


Plectrin



Labdane Diterpenoids

I R ,R' , R''' = H , R'' = Ac
II R , R''' = OH , R' = H , R'' = Ac
III R , R''' = OH ,R' = Ac , R'' = H



6-b-Hydroxycarnosol

IV.3 Present Work

Coleus zeylanicus (Benth) *Cramer* syn. *Plectranthus zeylanicus* Benth. is much used in traditional Ayurvedic medicine but less studied phytochemically about its chemical constituents. Only less volatile compounds like diterpeniods[148] were reported to be present in this plant. No information is available about the components of essential oils of this species where as the essential oils of much better known species like *C.forskohlii*, *C.aromaticus* and *C.laciniatus* were studied by various groups of scientists[160-163]. Therefore the present work was aimed to identify those compounds of the essential oil of the leaves, responsible for the characteristic odour as well as for the reported folk medicinal applications[145,146].

IV.4. Plant Material and isolation of essential oil

The leaves of the plant, *Coleus zeylanicus* were collected during December 1997 form Calicut University Campus. The plant was identified by Dr. A.K. Pradeep, Department of Botany, Calicut University. Voucher specimens are kept in the Herbarium, Department of Chemistry, Calicut University. One Kg of fresh leaves of the plant were cut into small pieces and ground into a paste with one litre of water using a mixer-grinder. This material was subjected to steam distillation for 3 hours. The distillate was extracted with diethyl ether (2 x 100 mL), dried with anhydrous sodium sulphate and the ether distilled off. The weight of the essential oil thus obtained was 0.25 g (yield 0.025% of wet weight).

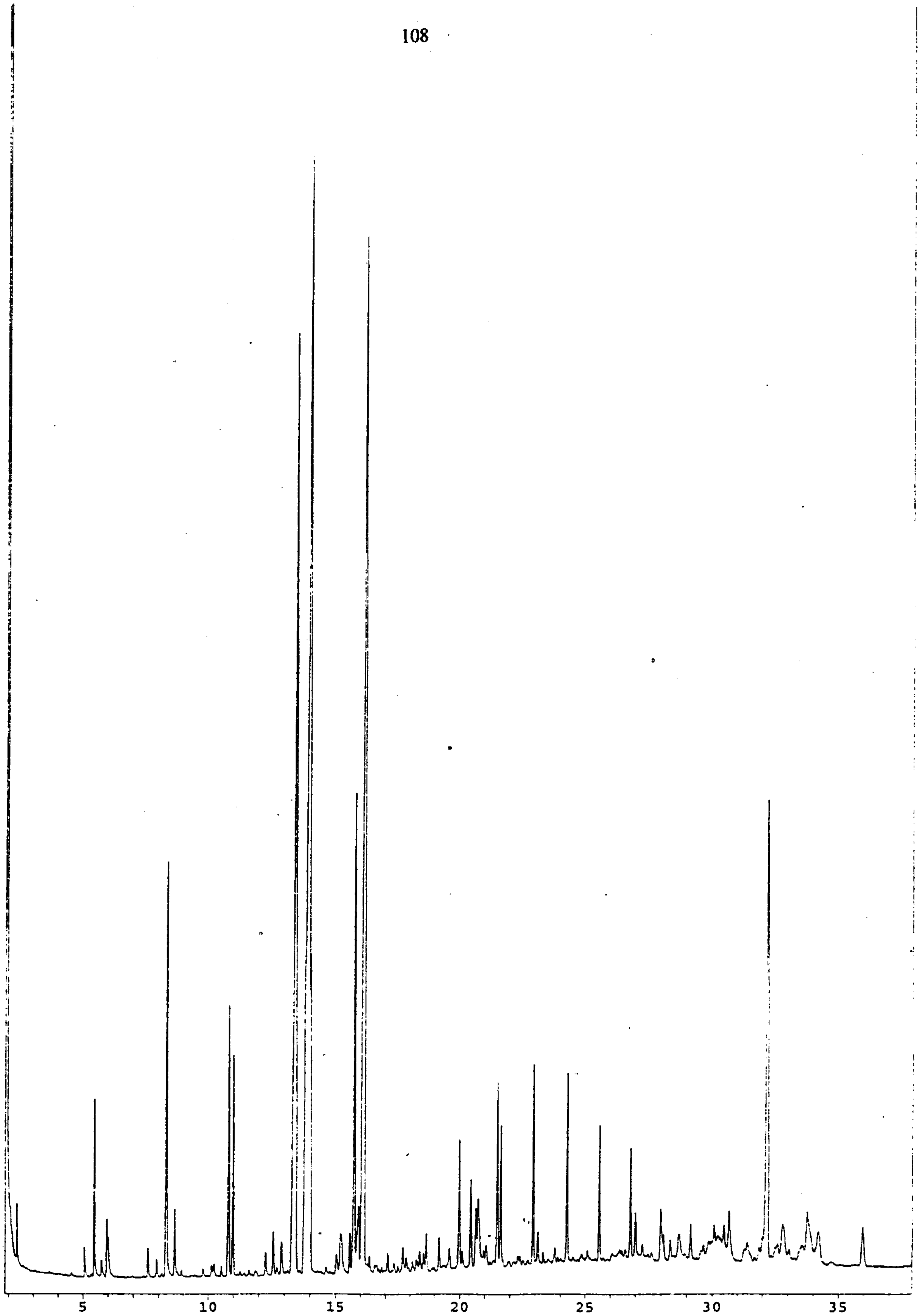
IV.5 Instrumentation

The volatiles of the leaf essential oil of *Coleus zeylanicus* was analysed by a Shimdzu 14 A-GC and Varian 3700-GC fitted with FID and integrator systems using hydrogen as carrier gas. Gas chromatographic–spectorscopic (GC-MS) studies were done using a Shimadzu GC-17 A with QP-5000 and Hewlett-Packard GC-HP5890 with HP 5970-MSD, using helium as carrier gas. Mass Spectral Studies were carried out in the 41-450 amu scan range (Ei mode 70 eV). Columns used for both analyses were 30 mx 0.32 mm bonded FSOT-RSL-200 fused silica (film thickness 0.25 micron, Biorad Co.) The percentage composition were calculated on the basis of relative percentage peak area. Compound identifications were done by co-injection of pure compound and by retention time correlations[164-166].

IV.6. Results and Discussions

a) Identification of components

The essential oil of leaves of *Coleus zeylanicus* was analysed by chromatographic- spectroscopic and olfactoric methods. More than eighty compounds were detected in the oil. Of these sixty four compounds were identified by the above techniques. The various compounds identified along with their percentages and methods of identification are listed in the table IV.1



Gas Chromatogram of *Coleus zeylanicus* leaf oil

Table IV.1

Constituents of the essential oil of *Coleus zeylanicus* leaves.

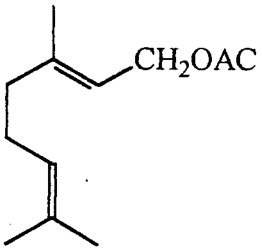
Compound	%	**Identification**
Monoterpenes		
1. Geraniol	36.1	GC,GC-MS,O
2. Geranyl acetate	20.5	GC,GC-MS,O
3. Nerol	16.9	GC,GC-MS,O
4. Neryl acetae	4.8	GC,GC-MS,O
5. Linalool	1.9	GC,GC-MS,O
6. Geranic acid	0.4	GC,GC-MS,R
7. Nerolic acid	0.4	GC,GC-MS,R
8. Linalyl propionate	0.3	GC,GC-MS,R
9. trans-Linalool oxide (furanoid)	0.1	GC,GC-MS,R
10. Linalyl acetate	0.1	GC,GC-MS,O
11. Geranial	t	GC,GC-MS,O
12. Limonene	t	GC,GC-MS,O
13. Myrcene	t	GC,GC-MS,R
14. Neral	t	GC,GC-MS-O
15. α-Pinene	t	GC,GC-MS,R
16. β-Pinene	t	GC,GC-MS,R
17. Sabinene	t	GC,GC-MS,R
18. γ-Terpinene	t	GC,GC-MS,R
19. Terpinen-4-ol	t	GC,GC-MS,O
20. α-Terpineol	t	GC,GC-MS,O
Sesquiterpenes		
1. δ-Cadinol	0.3	GC,GC-MS,O
2. δ-Cadinene	t	GC,GC-MS,R

Compound	%	**Identification**
3. β-Caryophyllene	t	GC,GC-MS,R
4. α-Cubebene	t	GC,GC-MS,R
5. β-Elemene	t	GC,GC-MS,R
6. Elemol	t	GC,GC-MS,R
7. Eudesmol	t	GC,GC-MS,R
8. α-Franesene	t	GC,GC-MS,R
9. Farnesol	t	GC,GC-MS,R
10. Germacrene D	t	GC,GC-MS,R
11. Gurajunene	t	GC,GC-MS,R
12. α-Humulene	t	GC,GC-MS,R
13. α-Muurolene	t	GC,GC-MS,R
14. Nerolidol	t	GC,GC-MS,O
15. Valencene	t	GC,GC-MS,O
Diterpenes		
1. 8-β-13-β-Kaur-16-ene	0.1	GC,GC-MS,R
2. Kauran alcohol *	0.1	GC,GC-MS,R
3. Kauran alcohol *	t	GC,GC-MS,R
Higher Hydrocarbons (>16 C)		
1. Octadecane	1.3	GC,GC-MS,R
2. Nonadecane	0.6	GC,GC-MS,R
3. Eicosane	1.4	GC,GC-MS,R
Fatty acids and their esters		
1. Myristic acid	0.5	GC,GC-MS,R
2. Myristic acid methyl ester	0.2	GC,GC-MS,R
3. Myristic acid ethyl ester	0.4	GC,GC-MS,R
4. Palmitic acid	1.7	GC,GC-MS,R
5. Palmitic acid methyl ester	0.4	GC,GC-MS,R

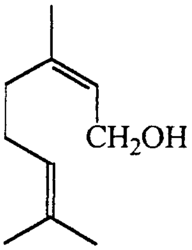
Compound	%	**Identification**
6. Stearic acid	0.6	GC,GC-MS,R
7. Stearic acid ethyl ester	0.5	GC,GC-MS,R
Hexane derivatives		
1. (E)-2- Hexenal	1.7	GC,GC-MS,R
2. (E)-2- Hexen-1-ol	0.7	GC,GC-MS,R
3. 1-Hexanol	0.6	GC,GC-MS,R
4. (Z)-1-Hexen-3-ol	0.5	GC,GC-MS,O
5. Hexylacetate	t	GC,GC-MS,R
6. Hexanal	0.2	GC,GC-MS,R
Others		
1. 1-Octen-3-ol	2.1	GC,GC-MS,O
2. 1-Octen-3-yl acetate	2.0	GC,GC-MS,R
3. 3-Octanol	0.9	GC,GC-MS,R
4. Octyl acetate	0.6	GC,GC-MS,R
5. Benzyl acetate	0.2	GC,GC-MS,O
6. Acetic acid	0.1	GC,GC-MS,O
7. 2-Methyl-6-hepten-3-ol	0.1	GC,GC-MS,R
8. Decanol	t	GC,GC-MS,R
9. Dodecane	t	GC,GC-MS,R

O-olfactoric detection
R- retention time correlation according to ref. 12-14
t- trace compound (<0.1%)
*-position of –OH group not characterised

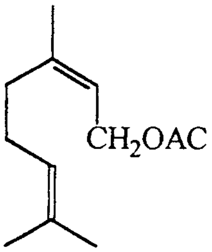
Structures of terpenes identified in *Coleus* leaf oil
(structures given in previous chapters are omitted)



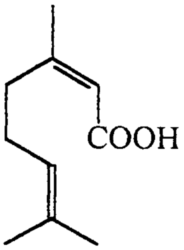
geranyl acetate



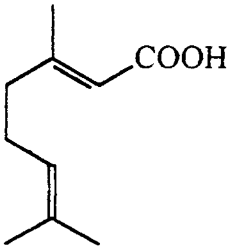
nerol



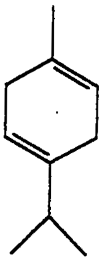
neryl acetate



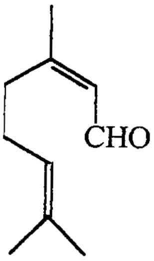
Nerolic acid



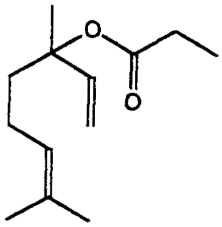
Geranic acid



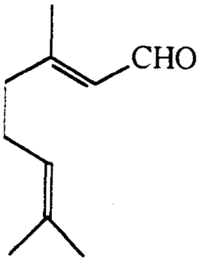
γ-Terpinene



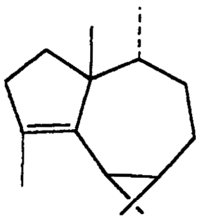
Neral



Linalyl propionate



Geranial



α-Gurjunene

Monoterpenes constituted the major class of compounds (81.5%) in the leaf essential oil of *Coleus zeylanicus*; the other major classes of compounds were sesquiterpenes (0.3%), fatty acids an their esters (4.3%), higher hydrocarbons(3.3%) hexane derivatives (3.7%) and octane derivatives (5.6%). Geraniol (36.1%), geranyl acetate (20.5%), nerol (16.9%), neryl acetate (4.8%), 1-octen-3-ol (2.1%), 1-octen-3-yl acetate (2.0%), linalool (1.9%) and (E)-2- hexenol (1.7%) were the major components in the oil.

Coleus zeylanicus is known to have antimicrobial, antifungal, disinfectant and antiseptic activities. In Ayurveda, the various parts of the plant are employed for different therapeutic uses. The chromatographic-spectroscopic analysis of the volatiles of the leaf oil allows the correlation of the constituents and various applications of the plant described in literature. The chemical components in the leaf essential oil responsible for various functions of the plant are given in the table IV.2 in accordance with the findings in the literature[167-179].

Table IV.2.

Biological activities of identified constituents of *Coleus zeylanicus*

1. **Bactericidal (antimicrobial), Disinfectant and antiseptic activity.**
   Geraniol, geranyl acetate, nerol, neryl actetate, linalool, delta-cadinene, β-caryophyllene, β-ionone, nerolidol, α-pinene, γ-terpinene, α-terpineol.
2. **Fungicidal activity**
   Geraniol, geranyl acetate, linalool, limonene, α-pinene, γ-terpinene.
3. **Against gastric disorders**
   Geraniol, geranyl acetate, linalool, limonene, α-pinene, γ-terpinene.
4. **Mild sedative properties**
   Geraniol, geranyl acetate, nerol, neryl acetate, linalool, limonene, β-caryophyllene, α-pinene, germacrene-D.
5. **Against stomachic disorder**
   Geraniol, linalool, α-pinene
6. **Spasmolytic effects**
   Geraniol, geranyl acetate, nerol, linalool, β-caryophyllene, germarene D.
7. **Use as aromaticum**
   Geraniol, nerol, linalool, limonene. α-pinene.
8. **Use as carminativum**
   Gerniol, geranyl acetate, linalool, limonene, pinene, γ-terpinene
9. **Diuretic effects**
   Geraniol, linalool, α-pinene, β-pinene, cadinene derivatives, α-terpineol, terpinen-4-ol.
10. **Expectroic activity**
    Pinene-derivatives, limonene
11. **Anthelmintic activity**
    Geraniol, nerol, nerolidol, α-terpineol
12. **Analgetic and antirheumatic effects**
    Pinene-and terpinene-derivatives.

IV.6.b. Olfactoric evaluation

Two hundred microlitres of the essential oil which was diluted with dichloromethane was placed on a commercial odour strip (Dragoco Co.) and the odour evaluated after 10 seconds by professional perfumers. The odour of the leaf essential oil of *Coleus zeylanicus* was described as green-fresh and violet – geraniol (top notes), weak sour in the direction of rhubarb stewed fruit and smelling of cod-liver oil (basic notes) as well as fatty in the back ground. The identification of components in the essential oil provided odour–component correlations.

Green odour denotes an odour reminiscent of green foliage. (E)-2-Hexenal which is known as leaf aldehyde; (Z)-3-hexenol which is called leaf alcohol, the other hexenals and hexenols and hexane derivatives in general are responsible for the green odour[179]. Hatanaka[217] found that as the double bond in the hexenols and hexenals moves form 2 to 5, the odour shifts form fruity, fresh and sweet through leafy and grassy green, and then insect green and vegetables-like green, to oily-fatty and herbal. The leaf aldehyde, (E)-2-hexenal (1.7%) was found to be present along with other hexane derivatives in the leaf essential oil of *C.zeylanicus* explains the green-fresh notes of the oil. Limonene and sabinene were also found to contribute to the green odour.

The violet-geraniol odour notes are due to geraniol, geranyl acetate, nerol, neryl acetate, linalool derivatives and also due to beta-ionone. The sour odour impressions can be attributed to the acids present in the oil namely acetic acid, geranic acid and nerolic acid. The cod-liver oil and fatty odour notes stem from nonane and fatty acid derivatives.

In conclusion, the many biological and pharmacological applications of the plant, *Coleus zeylanicus* may be partly attributed to the compounds identified in the essential oil along with the less volatile or non-volatile secondary metabolites isolated from the plant. Also the leaf essential oil of *Coleus zeylanicus* may be utilised as a natural source of geraniol like Palmarosa oil[180], which can be isolated either for use as it is or for the preparation of other fragrance and flavour molecules. *Coleus zeylanicus* leaf oil may be used as a subtitute for Palmarosa oil (East Indian geranium oil) in the preparation of perfumes, particularly soap fragrances where its greater tenacity is a highly desirable quality. Like palmarosa oil geraniol, geranyl acetate and nerol constitute the major portion (ca. 75%) of the *Coleus zeylanicus* leaf oil.

PHYTOCHEMICAL STUDIES ON COMMIPHORA CAUDATA

Saidutty A “Chemical investigation of some indigenous plants” Thesis.
Department of Chemistry, University of Calicut, 1999

CHAPTER V

PHYTOCHEMICAL STUDIES ON *COMMIPHORA CAUDATA*

V.1 Introduction

Commiphora caudata Engl.Syn. *Protium caudatum* W.A. belongs to the myrrh family of flowering plants, *Burseraceae* in the rue order of Rutales. The family *Burseraceae* comprises about 20 genera and more than 600 species. They are native primarily to tropical America but few species occur in Africa and Asia. The plants are trees or shrubs with scattered compound glanddotted leaves and small flowers, balsms and resins are contained in the passages formed by separation of cell-layers or destruction of cells. The small flowers are usually unisexual, with pentamerous or tetramerous clayx. The fruit is a two- to five-seeded drupe or capsule. The family consists of plants which are valuable in many respects. The gumbo-limbo or incense tree (*Brusera simaruba*), has the light reddish brown wood that is used for fishing floats, its fragrant resin is used in incense. The oleogum resin from several species of the genus, *Boswellia* from Somaliland and Arabia, called frankincense was used in biblical times in incense, in medicine and for embalming. Myrrh is the resin from plants of the genus *Commiphora*, from the same countries and from Northern Abyssinia. Elemi resins are obtained from other genera of the family, and species such as *Aucomea klaineana* produce useful timber[181,182].

The plant *Commiphora caudata* is distributed in dry forests of Deccan and Carnatic in Bellary, Cuddapah, Mysore, South Arcot and rarely in Kerala and Tamil nadu. It is a handsome deciduous tree with papery bark and greyish soft wood. Its leaves are alternate, imparipinnate, leaflets opposite. The flowers are small in fascicles, fruits ovoid or sub-globose, fleshy drupes about the size of a pea. Roots of *Commiphora caudata* are astringent, sweet, cooling, aphrodisiac and diuretic, and are useful in diabetes, strangury, fever and vitiated conditions of vata and pitta. The leaves are anodyne and are useful in rheumatalgia. The plant is called Hill-mango in English, Ilkatah in Sanskrit, Kondamamidi in Telugu, Kondamavu in Kannada, Kiluvai in Tamil and Idinjil in Malayalam [183,184,185].

V.2 Work so far reported on *Commiphora* species.

A study of ten members of the *Burseraceae* family including *Commiphiora caudata* reveals that the family is morphologically and chemically homogeneous [186]. It contains quercetin and its methoxylated derivatives, myricetin and gallic acid. The genus *Commiphora* is the most advanced taxon of the family in containing flavones and absence of pyrogallol systems, proanthocyanidins and tannins. The placement of *Protium caudatum* W.& A. in *Protium* rather than in *Commiphora* (*Commiphora caudata* Engl.) is supported. Chemically the family is closely related to the *Anacardiaceae*. The presence of primitive flavonoids such as biflavones, myricetin, proanthocyanidins and tannins keep this as one of the primitive families of the *Rutales*.

Commiphora mukul syn. *C.wightii* is one of the most phytochemically analysed species of the genus, *Commiphora*. Satyavati[187] reports on the development of gum guggulu, a potent hypolipidemic agent from *C.Mukul*. *Commiphora mukul* [188] is used as a herbal drug in the Unani system of Medicine for cardiovascular diseases. The herb was analysed by flame AAS and ICP-AES to determine if its therapeutic action can be associated with the elements present in it. Cadmium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, nickel, phosphorous, lead and zinc were some of the elements which play various roles in cardiovascular affections. An effort was made to correlate the role of these elements in cardiac diseases. The leaf extracts of *Commiphora wightii*[189] shows nematicidal properties against root-knot-nematode, *Meloidogyne incognita*. Juvenile hatching of *M.incognita* eggs is greatly inhibited by the extract. The hypolipidemic potential of guggul (*C.mukul*) is effectively coupled with the antianginal efficacy of pushkarmool (*Inula racemosa*) to yield a combination capable of providing relief of chest pain as well as improved functional status in 200 patients having ischemic heart disease. Improvements in grades assigned at the pretreatment level for precordial pain and dyspnea, restoration of normal ECG patterns, and significant reductions in cholesterol, triglycerides and total lipid level indicate a beneficial effect of treatment with combined drug. The underlying mechanisms for the drug action have also been considered which include reduced risk of cardiac events associated with antiischemic therapy through the cardioprotective action of guggul and post-exercise ECG restoration and PGE-2-like enhancing potential of pushkarmool[190].

The effects of the administration of 50mg of guggulipid[191] or placebo capsules twice daily for 24 weeks are compared as adjuncts to a fruit and vegetable- enriched prudent diet in the management of 61 patients with hypercholesterolemia in a randomized fashion. Guggulipid decreased the total cholesterol level by 11.7%, the LDL by 12.5%, triglycerides by 12.0% and total cholesterol/HDL cholesterol ratio by 11.1% from the post diet levels, where as the levels were unchanged in the placebo group. The HDL cholesterol level showed no changes in both groups. The lipid peroxides, indicating oxidative stress, declined 33.3% in the guggulipid group without any decrease in the placebo group. The combined effect of diet and guggulipid for 36 weeks was as great as the lipid-lowering effect of modern drugs. Side effects of guggulipid were head-ache, mild nausea, eructation and hiccup in a few patients.

The medicinal value of *Commiphora wightii*[192] has been believed by tribals to be mainly due to its yield of guggulipid, which has been significantly shown to have hypocholesteromic, antiseptic, antipathogenic and antiparasitic properties. It is also used for nonspecific diarrhoea and dysentery. Amoebic dysentery is a common disorder of a large number of people in tropics. The crude extracts of oleogum-resin obtained from *C.wightii* is found to be effective against *E.histolytica* and is considered to be comparable with quassinoids, ailanthinone and bruceantin. The aqueous extract of the resin of the plant, containing a steroid significantly inhibited the maximal edema response and the total edema response during six hours of Carrageenan-induced rat paw edema [194]. Further a crystalline steroidal fraction when given intraperitoneally at 1 or 5 mg/100g inhibited development of primary inflammation[193] induced in rats by Freund's adjuvant and reduced severity of secondary inflammation; its effect against primary inflammation was less than that of hydrocortisone but it was more effective in reducing severity of secondary lesions. Oleoresin (2 and 20 mg/100g, peritoneally or orally) decreased weights of uterus, ovaries and cervix in female rats where as glycogen and sialic acid levels in these organs increased. It was useful as an antifertility agent. Purified guggulu steroid mixture at 0.2mm completely inhibited ADP-adrenaline-or serotonin-induced platelet aggregation [194].

The important secondary metabolites isolated from *C.mukul* syn. *C.wightii* are as follows. Monocyclic diterpenes, α-camphorene and cembrene isolated from resin; allyl cembrol isolated from the plant is characterised as 2-hydroxy-4,8,12-trimethyl-1-isopropyl-3,7,11-cyclodecatriene. Cholesterol, 4, 17 (20)-trans-pregnadien-3,16-dione, 4,17(20)-cis-pregnadien-3,16-dione and three new sterols, guggulsterols I, II and III are isolated from gum resin. Gum resin is also found to contain cembrene A, mukulol (allyl cembrol), octadecan-1,2,3,4-tetrol and eicosan-1,2,3,4-tetrol[193]. Quercetin, quercetin-3-O-α-L-arabinoside, quercetin-3-O-β-D-galactoside,quercetin-3-O-α-L-rhamnoside,quercetin

3-O-β-D-glucuronide, ellagic acid and pelargonidin –3,5-di-O-glucoside are isolated from flowers. Guggulsterol-VI and Z-guggulsterol are isolated from gum resin along with 20α-hydroxy-4-pregnen-3-one and 20β-hydroxy-4-pregnen-3-one. Also, linoleic, oleic, stearic and palmitic acids, sitosterol, stigmasterol, cholesterol, campesterol and α-spinasterol are identified in the seed oil [194].

The stem bark of *Commiphora kua*[195] has yielded three unstable C-22 compounds which rapidly degraded to give artefacts identified as mansumbin-13 (17)-en-3,16-dione, 3-beta-hydroxymansumbin-13 (17)-en-16-one and 16-oxo-3,4-seco-mansumbin-4(28), 13(17)-dien-3-oic acid. Spectroscopic evidence is advanced suggesting that the unstable parent compounds are the corresponding 16-hydroperoxides. Gum exudate[196] of *C.kua* contains a new bisabolene, 2-methyl-5-(5′-hydroxy-1′,5′-dimethyl-3′-hexenyl) phenol together with known bisabolene xanthorrhizol and furanosesquiterpenoid, 2-O-methyl-8,12-epoxy germaora-1(10),4,7,11-tetraene.

The resin obtained from *Commiphora incisa*[197] contains 2 novel triterpenes which are identified as 1α-acetoxy-9,19-cyclolanost-24-en-3β-ol and 29-norlanost-8,24-dien-1α,2α,3β-triol. The aqueous extract of the resins of *C.incisa*[193] significantly inhibited both the maximal edema response and the total edema response during 6 hours of Carrageenan-induced rat paw edema. The octanordammarane triterpenes, mansumbinone and mansumbinoic acid isolated from the resin of *C.incisa* are separated and tested. Administered prophylactically, mansumbinone proved to be more than 20 times less potent than indomethacin and prednisolone in inhibiting Carrageenan-induced rat paw edema. However, molar potency of mansumbinoic acid is within an order of magnitude of those of indomethacin and prednisolone.

Ethyl acetate extract of the scented myrrh[198] (*Commiphora guidottii* Chiov.) gives the sesquiterpene T-Cadinol. It is shown to have a concentration dependent smooth muscle relaxing effect on the isolated guinea pig ileum and a dose-dependent inhibitory effect on choleratoxin-induced intestinal hypersecretion in mice. Three known and one novel furanogermacranes have been isolated from the resinous exudate of *Commiphora holtziana* Engl[199]. Structure of the novel compound was determined as 1,2-epoxyfurano-10(15)-germacran-6-one.

The volatile resin exuded from the stem bark of *Commiphora rostrata*[200] is examined and 22 oxygenated alkane components are identified by GC-MS. The potential value of this material in the defense of the plant against predators and fungal pathogens is discussed. The major alkanone constituents of the resin of *C.rostrata*, 2-decanone and 2-undecanone and a series of structural analogues are bio-assayed for thier repellency against maize weevil, *Sitophilus zeamis* in olfactometric assays. All the aliphatic ketones and aldehydes showed comparable or greater activity than the synthetic commercial insect repellent, N,N′-diethyltoluamide (DET). In the alkanone series the C-8 and C-9 compounds demonstrated significantly higher activity than their shorter or longer chained congeners. The results show that the resin constituents may play allomonal role in the ecosystem where the plant thrives. On-line coupling of supercritical fluid chromatography to atmospheric pressure chemical ionisation mass spectrometry was used to analyse a complex mixture of di- and tri- acylglycerols extracted from the tree *Commiphora guillaumini* Per.[202] Components including the ant attractant 1,2- dioleoyl glycerol are identified by mass spectrometry using Skimmer fragmentation in both positive and negative mode.

A survey on the food procurement practices with special focus on the dietary use of wild plants in the two villages of South Niger revealed that *Commiphora africana*[203] is among the wild plants used in the diet, both in the drought and rainy season. The species contained high concentrations of calcium, iron, copper and / or zinc. The root of *Commiphora merker*[204] is found to contain a new pentacyclic triterpene, 2α, 3β, 23- trihydroxyolean–12-ene with anti-inflammatory and analgesic activity.

Sample of resins collected from *Commiphora myrrha*[205] and *C. holtziana* have yielded wide range of sesquiterpenes, notably furanosesquiterpenes based on eudesmane, elemane and germacrane. Also the oil form *C.myrrh* gum resins contains isofuranogermacrane, lindestrene, furanoeudesma-1,3-diene and furanodiene[206]. Gas chromatographic- Mass spectrometric[207] analysis of the essential oil of

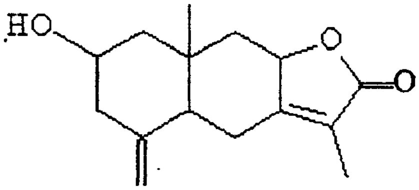
C.myrrha shows the presence of 15 compounds in which furanoeudesma-1, 3- diene is the main constituent.

In vivo and *in vitro* experimental studies on *C.myrrha* shows that it is an indigenous fertility regulating agent[208]. Further, myrrh, a resin extracted from North African shrubs of the genus *Commiphora,* is used since antiquity as a perfume and as remedy for human and animal diseases. Following the Gospel reporting, the offer of myrrhed wine before crucifixion, Dolaro and co-workers[209] isolated three sesquiterpenoids namely curzarene, furanoeudesma-1, 3-diene and furanodiene from hexane extract of *C. myrrha*. Furanoeudesma –1, 3-diene displaced concentration dependent specific binding of [3H] diprenorphine to brain membranes and showed structural similarities with peptide PLO17 (morphiceptin) and DPDPE (m and d opioid agonists respectively). These results explain the analgesic effect of myrrh and indicate one of the reasons why myrrh had such a widespread use as a medicinal remedy in the past. Isolinalyl acetate, 3- epilupenyl acetate, lupeone, 3-epi-α-amyrin, α-amyrone, β-eudesmol acetate isolated from the Arabian Plant. Also a sesquiterpene alcohol, commiferin with m.p 170°C is isolated from *C.myrrha*[193] Also, β -sitosterol, cedrelone and siderin which is a characterised as 6,7-dimethoxy-4-methyl coumairn are isolated from the roots of *Commiphora Stocksiana* [193]

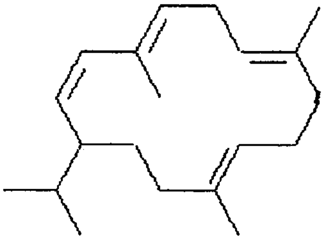
The genotoxic, cytotoxic and anti-tumour properties *Commiphora molmol*[210] are studied in normal and Ehrlich ascites carcinoma cell-bearing mice. In normal mice, the genotoxic and cytotoxic activity were evaluated on the bases of the frequency of micronuclei and the ratio of polychromatic to normochromatic cells in bone marrow, which was substantiated by the biochemical changes in hepatic cells. The anti-tumour activity of *C. molmol* was evaluated from the total count and viability of Ehrlich ascites carcinoma cells and their nucleic acid, protein, malondialdehyde, and elemental concentrations in addition to observations on survival and the trend of changes in body weight. The tumours at the site of injection were evaluated for histopathological changes. Treatment with *C.molmol (*125-500 mg/kg) showed no clastogenicity but was found to be highly cytotoxic in normal mice. The results obtained in the Ehrilich ascites carcinoma-cell bearing mice revealed the cytotoxic and antitumour activity of *C.molmol* which was found to be equivalent to those of the standard cytotoxic drug cyclophosphamide. On the basis of non-mutagenic, antioxidative and cytotoxic potential of *C.molmol* as observed in this study, its use in cancer therapy seems to be appropriate. The anticlastogenic and biochemical potentials[211] of *C.molmol* are studied in Swiss albino mice treated with cyclophosphamide. The *C.molmol* treatment (125-500 mg/kg) showed no mutagenicity. This caused a highly significant and dose-dependent mito-depressant effect in the femoral cells and reduction of RNA levels in hepatic cells as compared with the control. Cyclophosphamide treatment showed significant increase in the frequency of micronuclei, cytotoxity and reduction in the contents of nucleic acids and proteins. Pretreatment with *C.molmol* could neither alter the biochemical and cytological effect of cyclophosphamide nor show any additive effect on both treatments.

The aqueous suspension of *C.molmol* (oleogum resin) has been screened for its potential to protect gastric mucosa against the ulcers caused by 80% ethanol, 25% NaCl, 0.2M NaOH, indomethacin and combined indomethacin-ethanol treatment.[212] *C.molmol* pre-treatment at doses of 250, 500 and 1000 mg/kg provided dose-dependent protection against the ulcerogenic effects of different necrotizing agents used. Treatment of rats with 1 mL of 80% ethanol was found to cause depletion of stomach wall mucus, reduction in the concentration of protein, nucleic acids and NP-SH groups in the stomach wall. Ethanol treatment also caused histopathological lesions including necrosis, erosion, congestion and haemorrhage of the stomach wall. Pre-treatment with *C.molmol* provided a dose-dependent protection against all these effects. *C.molmol* also offered protection against mucosal damage caused by indomethacin and its combination with ethanol. The protective effect of *C.molmol* observed is attributed to its effect on mucus production, increase in nucleic acid and non-protein sulfhydryl concentration, which appears to be mediated through its free radical scavenging, thyroid stimulating and prostaglandin inducing properties.

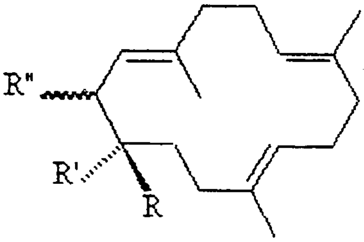
New compounds reported from *Commiphora* species



Commiferin

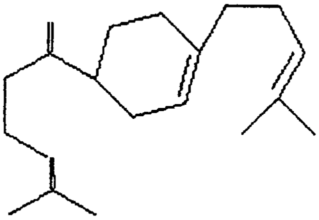


Cembrene

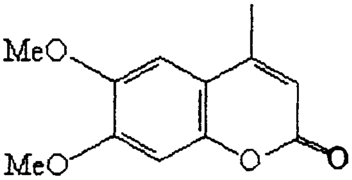


Cembrene A $R = CMe{=}CH_2$, $R', R'' = H$

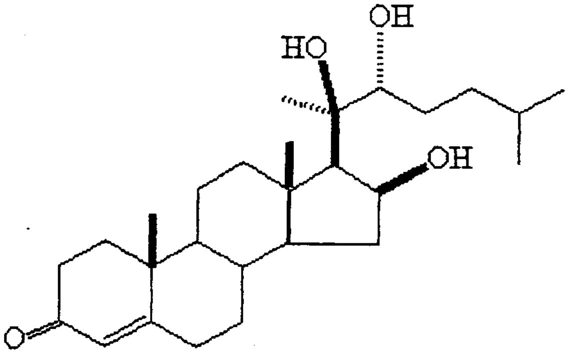
Mukulol $R = H$, $R' = CHMe_2$, $R'' = OH$



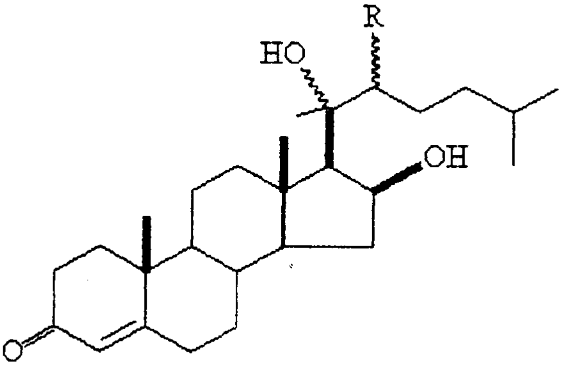
α-Camphorene



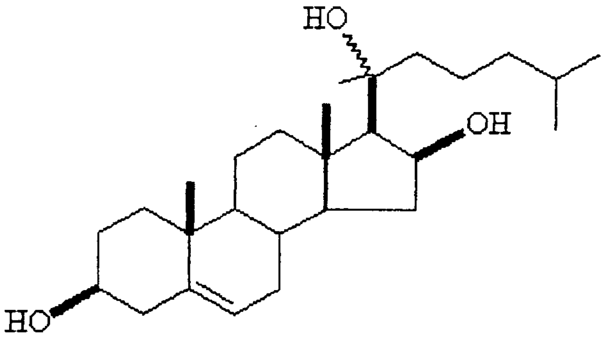
Siderin



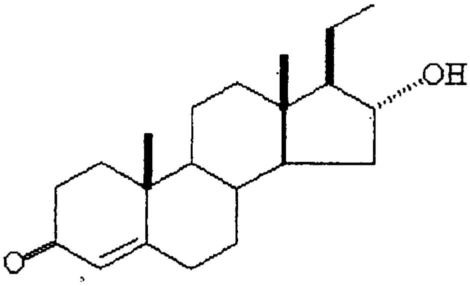
Guggulsterol I



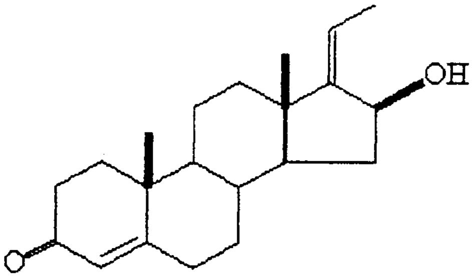
Guggulsterol I R = OH
Guggulsterol III R = H



Guggulsterol II



Guggulsterol VI



Z-Guggulsterol

Present Work

Commiphora caudata is one of the least phytochemically studied species of the genus, *Commiphora.* Only one group[187] of scientists worked on this species. Not much is known about the secondary metabolites of the plant. So the present work is aimed to isolate the secondary metabolites from the stem bark of the plant and to analyse and identify them by chromatographic as well as spectroscopic methods.

V. 4 Materials and methods

Plant material

The stem bark of the plant was collected from Punnayurkulam village of Thrissur District, Kerala and was authenticated by Dr. A K Pradeep of Botany Department, Calicut University.

Melting point determination

All the melting points of crystalline isolates were determined using Toshniwal Cappillary Melting point apparatus and are uncorrected.

Infrared absorption spectroscopy (IR)

The IR spectra of the compounds were recorded using a Shimadzu FTIR-8101-A spectrometer and the spectra were determined as KBr pellets.

Nuclear Magnetic Resonance spectroscopy (NMR)

The ^{1}HNMR spectra are recorded on a Varian UNITYplus-300 spectrometer using $CDCl_3$ as solvent with tetramethylsilane (TMS) as internal standard. The chemical shifts are reported in ppm(s).

Electron impact mass spectra (EIMS)

The electron impact mass spectra were recorded on a Finnigan TSQ 70 mass spectrometer.

Gas Chromatography-Mass spectroscopy (GC-MS)

The gas chromatographic-mass spectroscopic studies of S_1 was carried out using a Varian 3400 GC, using helium as carrier gas. The sample was kept at 40^0C and heated to 330^0C at a rate of 7^0C per minute.

Column Chromatography

Column chromatographic separation of the crude and semipurified extract were carried out using silica gel (Acme, 60-120 mesh).

Thin Layer Chromatographic Analysis (TLC)

Thin layer chromatographic plates were prepared using TLC grade silicagel-G (Acme), layer thickness 0.2mm (prepared using Stahl apparatus).

Reagents

Liebermann-Burchard Reagent:- The reagent was prepared as follows.

Chemicals required

Acetic anhydride	5 mL
Con. Sulphuric acid	5 mL
Ethanol	50 mL

Acetic anhydride and 97% sulphuric acids were mixed carefully with cooling and ethanol was added to the mixture with cooling.

Treatment of the plates: Heated to 110°C until maximal visualisation of the spot.

V.5 Extraction and Fractionation of the petroleum ether extract of *Commiphora caudata*

The stem bark of *Commiphora caudata* collected was dried in shade. The dry powdered material (1.5Kg) was extracted thrice (3x2.5L) with petroleum ether. The combined extract was concentrated under reduced pressure to yield 10g of viscous dark brown liquid. It was adsorbed in silica gel (150g) and chromatographed over silica gel (60-120 mesh) using a column of length one metre and diametre two centimetre. The column was eluted with petroleum ether-benzene mixture, benzene and petroleum ether-benzene-ethyl acetate mixture. Several 25 mL portions were collected and each of them checked by thin layer chromatography. Fractions were pooled together according to their homogeneity judged from the tlc analysis. Table V.1 summerises the fractions, eluant compositions and volume of different fractions.

Table V.1 chromatographic fractions of petroleum ether extract of the stem bark of *C. caudata*.

Fraction	Eluant composition	Volume	Components
1	Petroleum ether-benzene	500 mL	S_1
2	Petroelum ether-benzene- ethyl acetate (1:1:1)	500 mL	S_2

V.6 Isolation of compounds from different chromatographic fractions of petroleum ether extract.

Fraction 1 obtained on elution with petroleum ether-benzene-mixture, on evaporation gave colourless waxy compound. It was dissolved in warm ethyl acetate and adsorbed in silica gel (100g, 60-120 mesh). The material was again chromatographed over silica gel in a column of 50 cm length and 1 cm diametre. The column was eluted with petroleum ether-benzene mixture of varying compositions. Several 25 mL portions were collected and portions of homogeneity checked by tlc, were pooled together. The fraction obtained on eluting with 4:1 petroleum ether-benzene mixture (180mL) on evaporation gave a colourless compound. The compound was washed with cyclohexane three times and re-crystallised from petroleum ether to yield 200 mg of a colourless compound, S_1.

Fraction 2 obtained by elution with petroleum ether-benzene-ethylacetate mixture was deep green in colour, which on evaporation gave a green mass. The examination of it gave a deep pink spot on spraying with Liebermann–Burchard reagent. It was dissolved in ethylacetate and adsorbed on silica gel (100g, 60-120 mesh). After drying it was chromatogfaphed over silica gel and eluted with benzene,

benzene-ethyl acetate mixture (4:1). The latter portion on evaporation and recrystallisation from benzene gave shining crystals of S_2 (250 mg).

V.7 Results and discussion

a. Characterisation of S_1

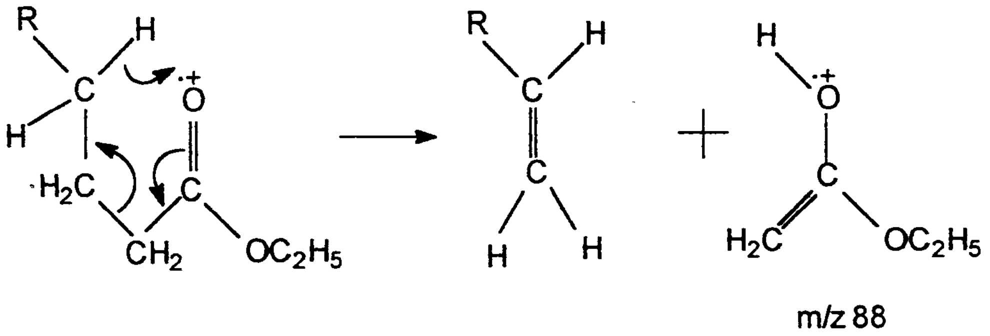
The compound S_1 isolated as above, was a colourless compound with m.p. 58°C. This compound did not answer Liebermann-Burchard colour reaction, indicating that it was not a triterpenoid or sterol. It gave a dark colour on spraying with con.H_2SO_4 and strong heating. It gave a single spot on tlc in petroleum ether-benzene (3:1) solvent system. I R spectrum of the compound showed absorption at 2926.6, 2851.1, 1736.1, 1464.1, 1373.3, 1178.6, 729.2 and 719.5 cm^{-1}. The absorption at 2926.6, 2851.1, 1736.1, 1178.6, 729.2 and 719.5 cm^{-1} suggested the presence of ester of higher saturated acid. But the mass spectrum of the compound with peaks at m/z values 412, 410, 396, 257 etc could not be interpreted for a single ester molecule. The ^{1}HNMR spectrum of the compound had a triplet at δ 4.05, multiplets at δ 4.6 and 5.4 along with other peaks in the high field region. These observations also could not be interpreted for a single compound. It was therefore presumed that S_1 was not a single pure compound but a mixture of compounds. As its m.p. was only 58°C, a GC-MS analysis was attempted. By this approach twenty five compounds could be identified in the sample. The identified compounds and their concentrations are given in the table V.2

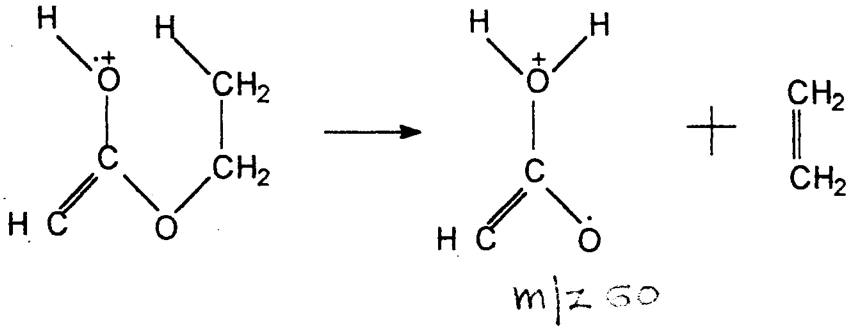
Table V.2

Compounds and their percentages identified in the GC-MS analysis of S_1 from *Commiphora caudata*

Compound	Common name	%
1. Ethyl hexoanoate	Ethyl caproate	0.01
2. Nonanal		1.2
3. Ethyl octanoate	Ethyl caprylate	0.41
4. Decanal		0.52
5. Ethyl nonanoate		0.25
6. Ethyl-9-oxo-nonanoate		0.55
7. Ethyl decanoate	Ethyl laurate	0.31
8. Ethyl tetradecanoate	Ethyl myristate	0.77
9. 6,10-dimethylundecan-2-one		1.09
10.Ethyl pentadecanoate		0.44
11.Ethyl hexadecanoate	Ethyl palmitate	5.16
12.Ethyl hepatadecanoate		0.44
13.Ethyl octadec-9-enoate	Ethyl oleate	9.58
14.Ethyl octadecanoate	Ethyl stearate	1.94
15.Ethyl nonadecanoate		0.22
16.Ethyl eicosanoate	Ethyl arachidate	1.21
17.Ethyl decosanoate	Ethyl behenate	3.91
18.Ethyl tricosanoate		1.14
19.Ethyl tetracosanoate	Ethyl lignocerate	20.60
20.Ethyl pentacosanoate		2.51
21.Ethyl hexacosanoate	Ethyl cerotate	12.74
22.Ethyl heptacosanoate		0.82
23.Ethyl octacosanoate	Ethyl montanate	2.1
24.Ethyl nonacosanoate		0.46
25.Ethyl tricontanoate		1.96

Results of GC-MS analysis showed that ethylesters of higher saturated acids form the major constituents as indicated by the IR spectrum. A typical feature of the mass spectra of ethyl esters of fatty acids is the presence of a prominent peak at m/z 88 corresponding to the McLafferty ion, $CH_2=C(OC_2H_5)-^{+\cdot}OH$. This peak forms the base peak in many of the higher ethyl esters. The peak at m/z 60 corresponding to double McLaffety ion of ethyl esters, is also prominent in these esters. The McLafferty rearrangements of ethyl esters leading to ions with m/z 88 and 60 are shown below.





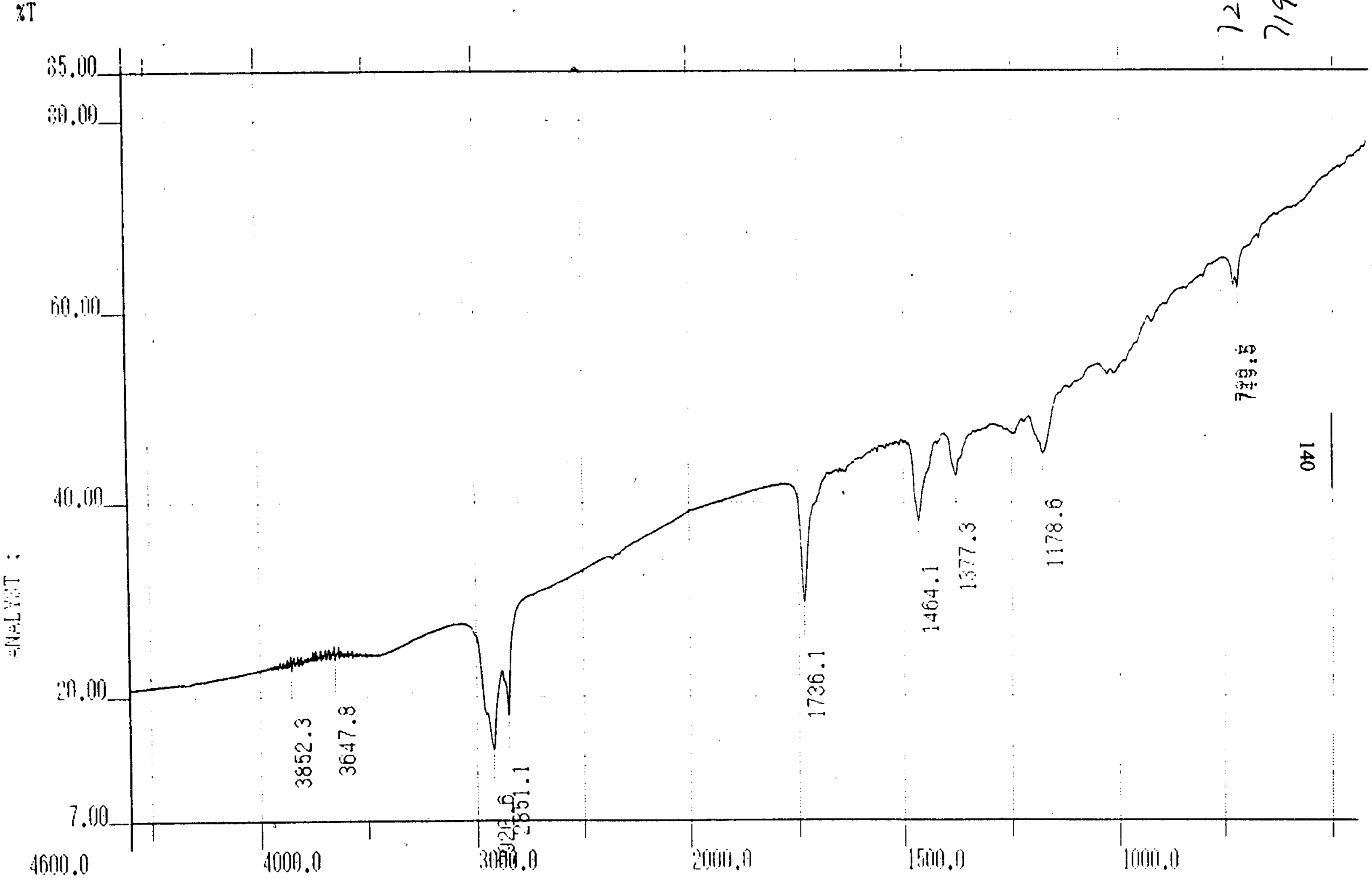
A few spectra showing this feature are provided (page number 144). Further, the (M – 88) ion which is formed along with m/z 88 ion, undergoes fragmentation to give C_nH_{2n-1} ions which give prominent peaks at m/z 41, 55, 69, 83, 97 etc.

The mass spectrum of ehtyl-9-oxo-nonanoate is worth mentioning. It is one of the compounds identified along with nonanal, decanal and 6,10-dimethylundecanone in addition to the esters. The spectrum shows peaks at m/z 88, 60, 41, 55, 69, 83, 111 etc. The peaks at m/z 88 and 60 corresponds to the McLafferty ion and double McLafferty ion respectively. The (M – 88) neutral molecule containing an olefinic bond and an aldehyde functional group undergoes fragmentations typical of aldehyde and alkene to give peaks at m/z values 111, 41, 55, 69, 83 etc. The fragmentation pattern of the compound is given in page No 145.

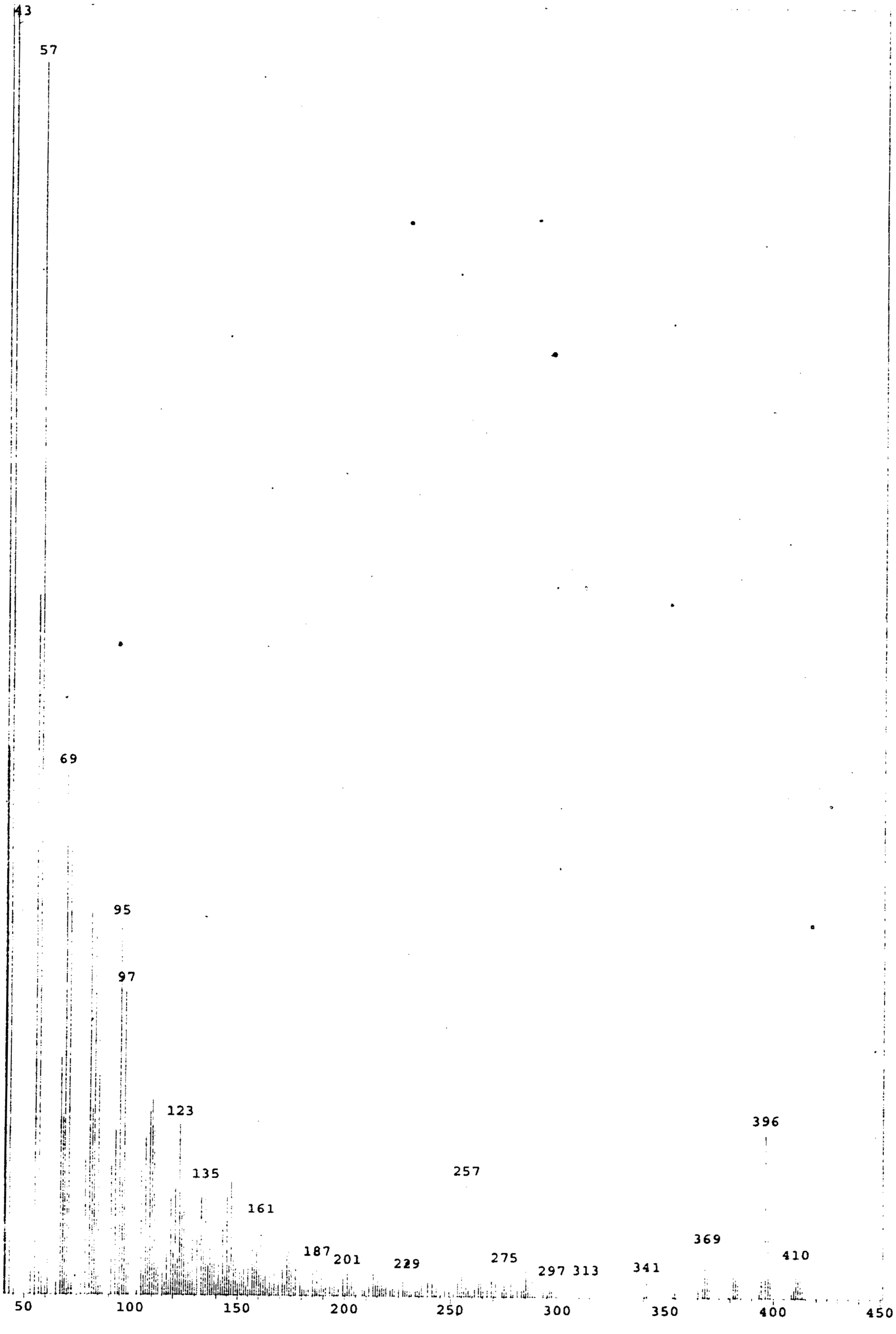
GC-MS analysis of S_1 showed that the content of esters of fatty acids with even number of carbon atoms exceeds far more than those with odd number of carbon atoms. This is in accordance with the fact that most naturally occurring fatty acids are even numbered due to their mode of biosynthesis. Fatty acids are biosynthesised by successive condensation of two carbon units[214]. The esters present in the stem bark of *Commiphora caudata* have characteristic structural functions. It is found that esters and oils accumulated in the bundle sheath surrounding the vascular bundles of leaves and stems have significance in the defence mechanisms.

```
------------ PARAMETERS OF SPECTRUM --
MEASURING MODE : %T
    RESOLUTION : 4.0 cm-1
   NO. OF SCAN : 40
          GAIN : AUTO
      DETECTOR : DETECTOR 1 (2.0 mm/sec)
   APODIZATION : HAPP-GENZEL
       REMARKS : S1
       ANALYST :
```

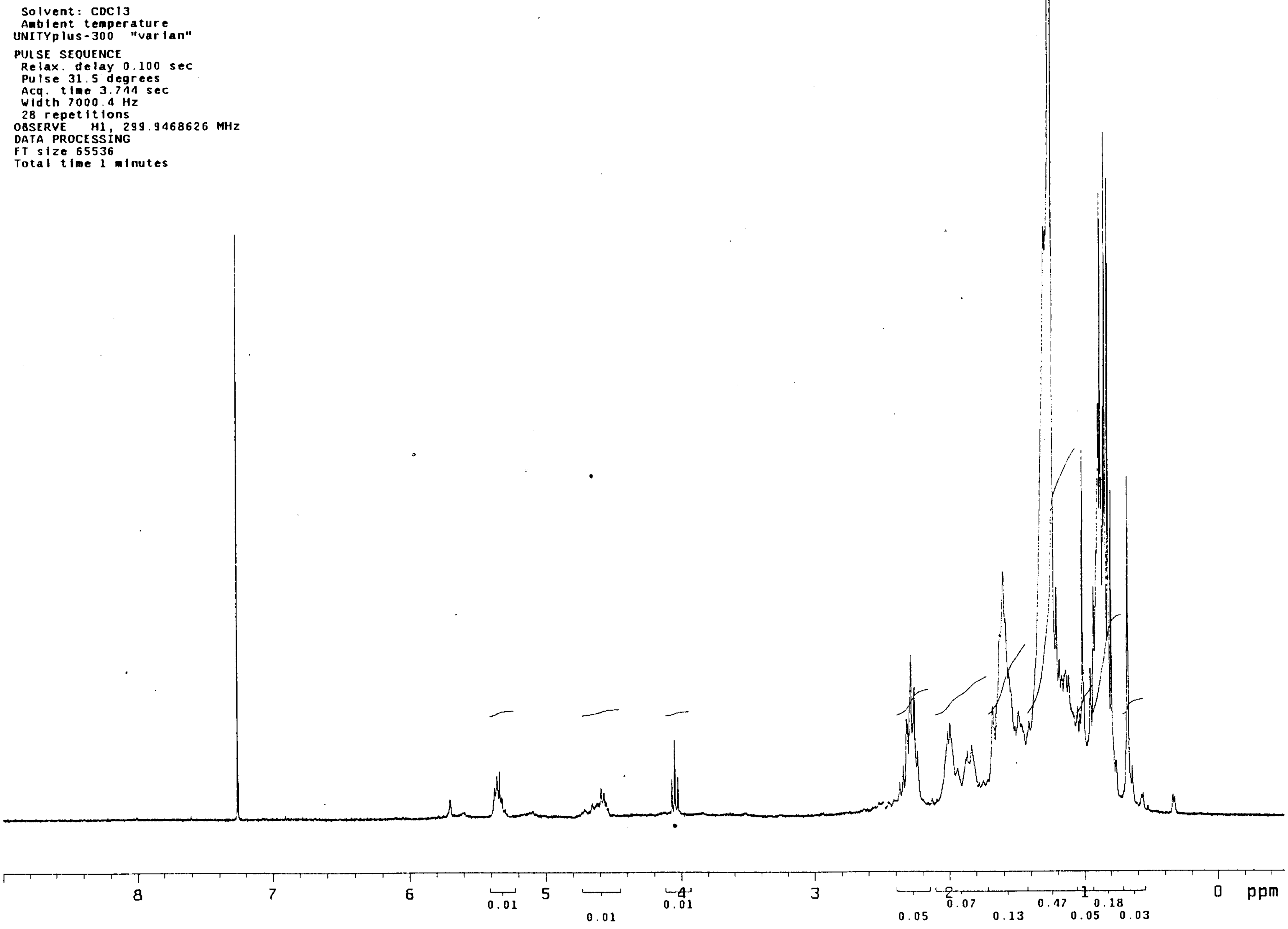
729.2
719.5



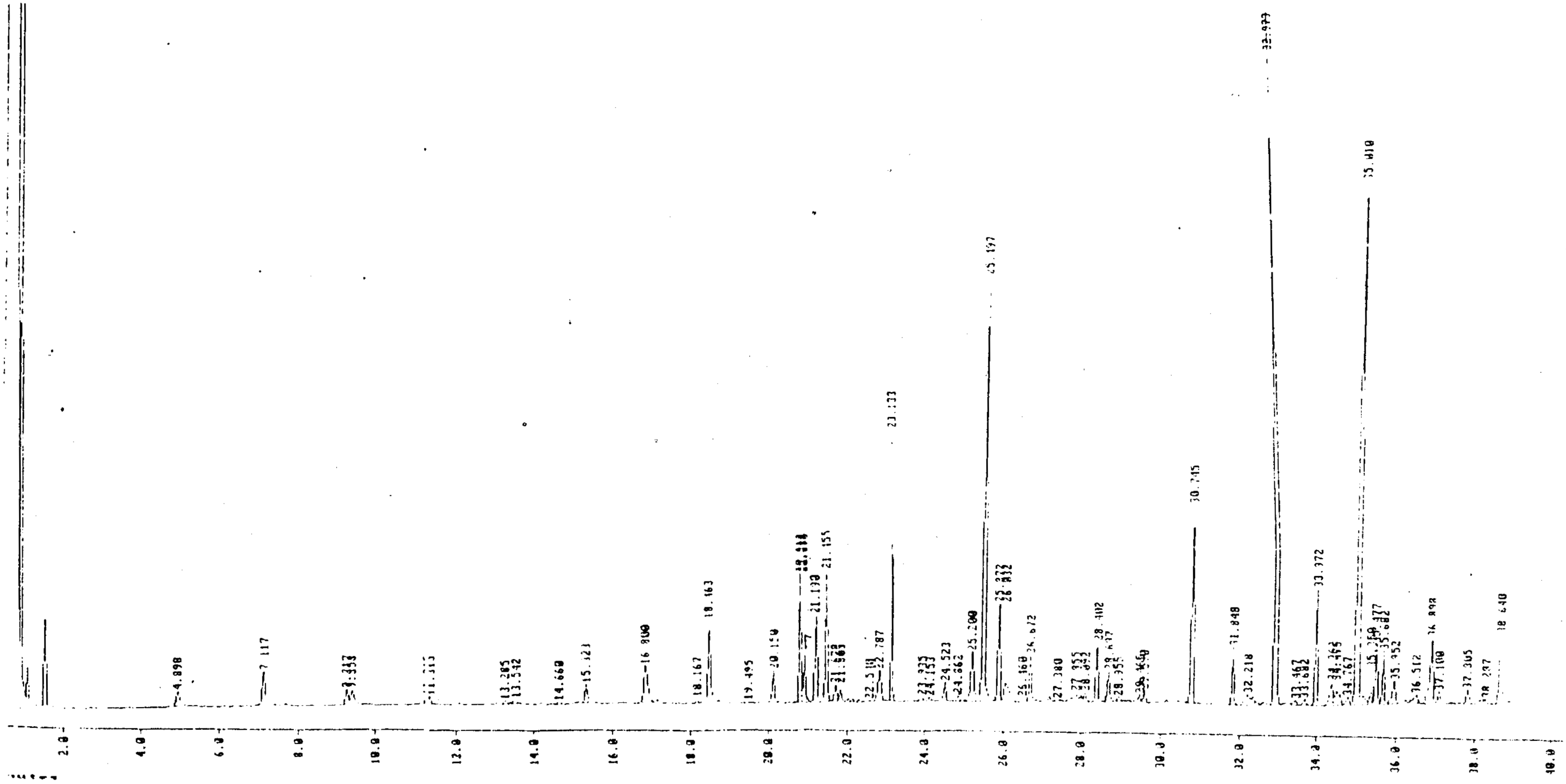
IR Spectrum of S_1 (mixture of compounds)



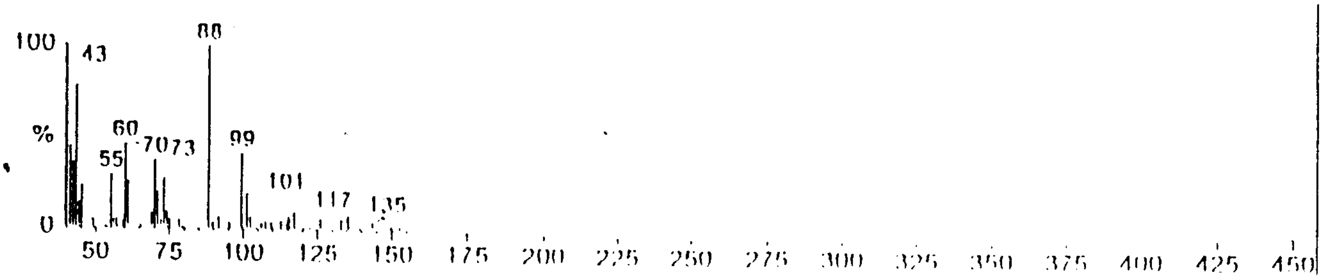
EI Mass Spectrum of S_1 (mixture of compounds)



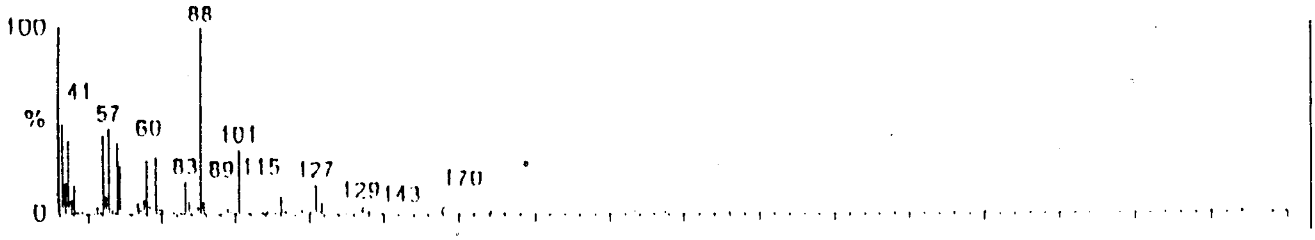
^{1}H NMR Spectrum of S_1 (mixture of compounds)



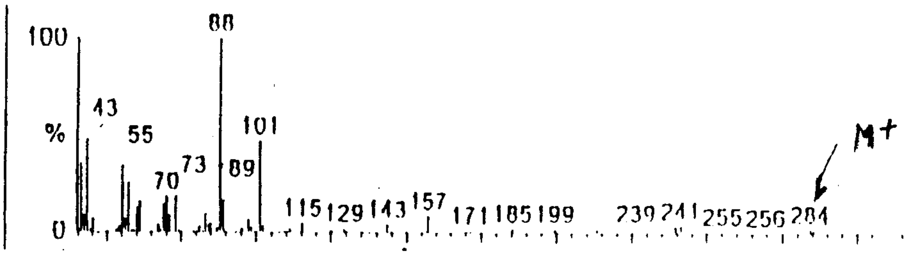
Gas Chromatogram of S_1(mixture of compounds)



EI Mass Spectrum of ethyl hexanoate

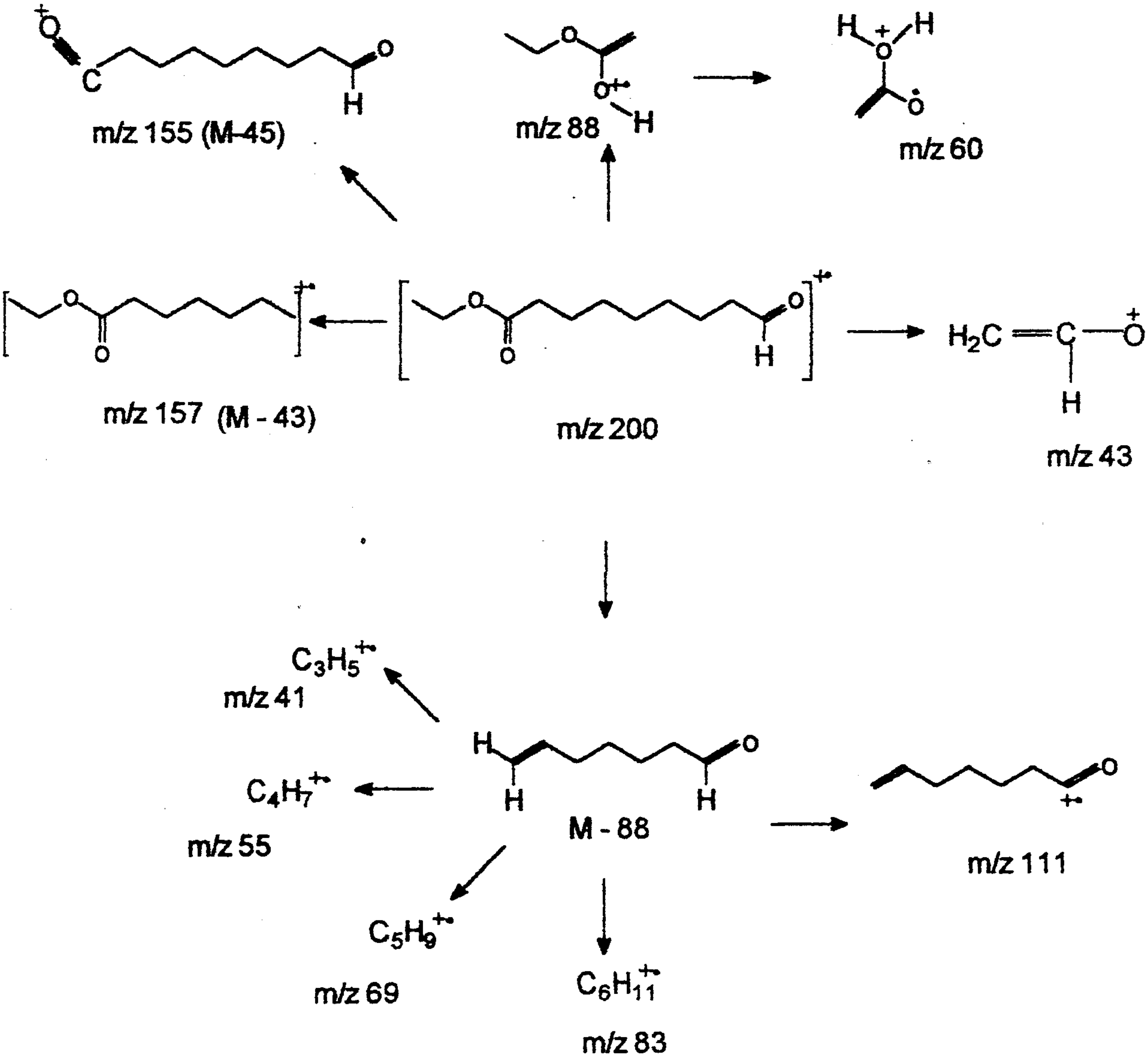


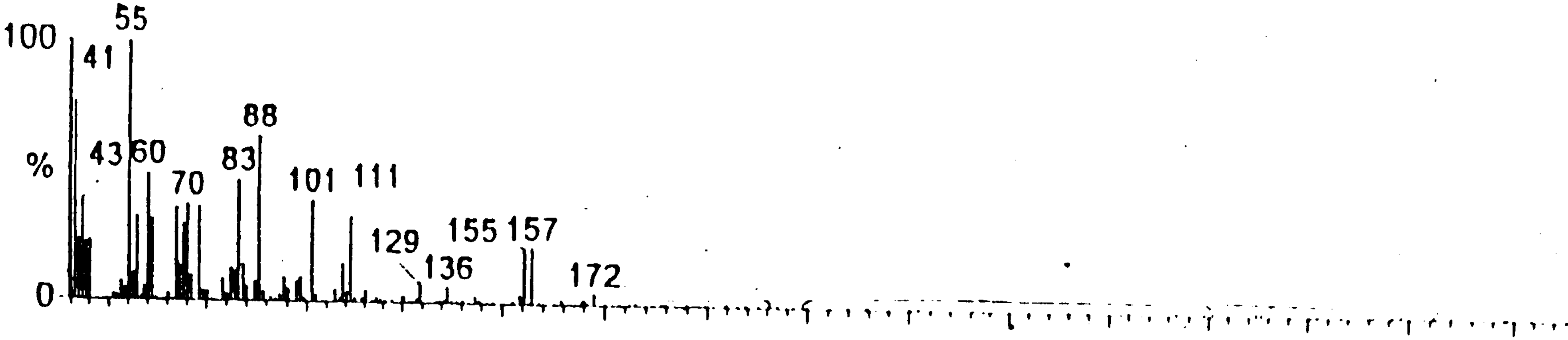
EI Mass Spectrum of ethyl octanoate



EI Mass Spectrum of ethyl hexadecanoate

Fragmentation pattern of Ethyl-9-oxo-nonanoate





EI Mass Spectrum of ethyl –9-oxo-nonanoate

V. 7.b Characterisation of S_2

S_2 was isolated from the petroleum ether extract on elution with a 1:1:1 mixture of petroleum ether-benzene-ethyl acetate as a white powdery substance. On recrystallisation from methanol it gave colourless needles with m.p 139°C. Leibermann-Burchard reagent gave a play of colours indicating that it was a sterol. EI Mass spectrum of this compound showed M^+ at m/z 414 and base peak at m/z 55. IR absorption spectrum showed the presence of hydroxyl group (broad absorption band at $3441.4 cm^{-1}$), bands due to gem-dimethyl group (doublet at 1383.1 and $1385.1 cm^{-1}$), -C-H stretching and bending bands at (2981, 2816, 1464, $1470 cm^{-1}$) were also observed in the spectrum. ^{1}HNMR spectrum was quite comparable with that of β-sitosterol. A direct comparison of R_f value with that of an authentic sample established its identity. The derivatives, acetate (m.p.125^0C) and benzoate (m.p.127^0C) of the compound were prepared and was found to be identical with β-sitosterol acetate (m.p.127^0C) and β-sitosterol benzoate (m.p.145^0C)[216]. Mixed m.p. with an authentic sample was undepressed.

V.8 Experimental

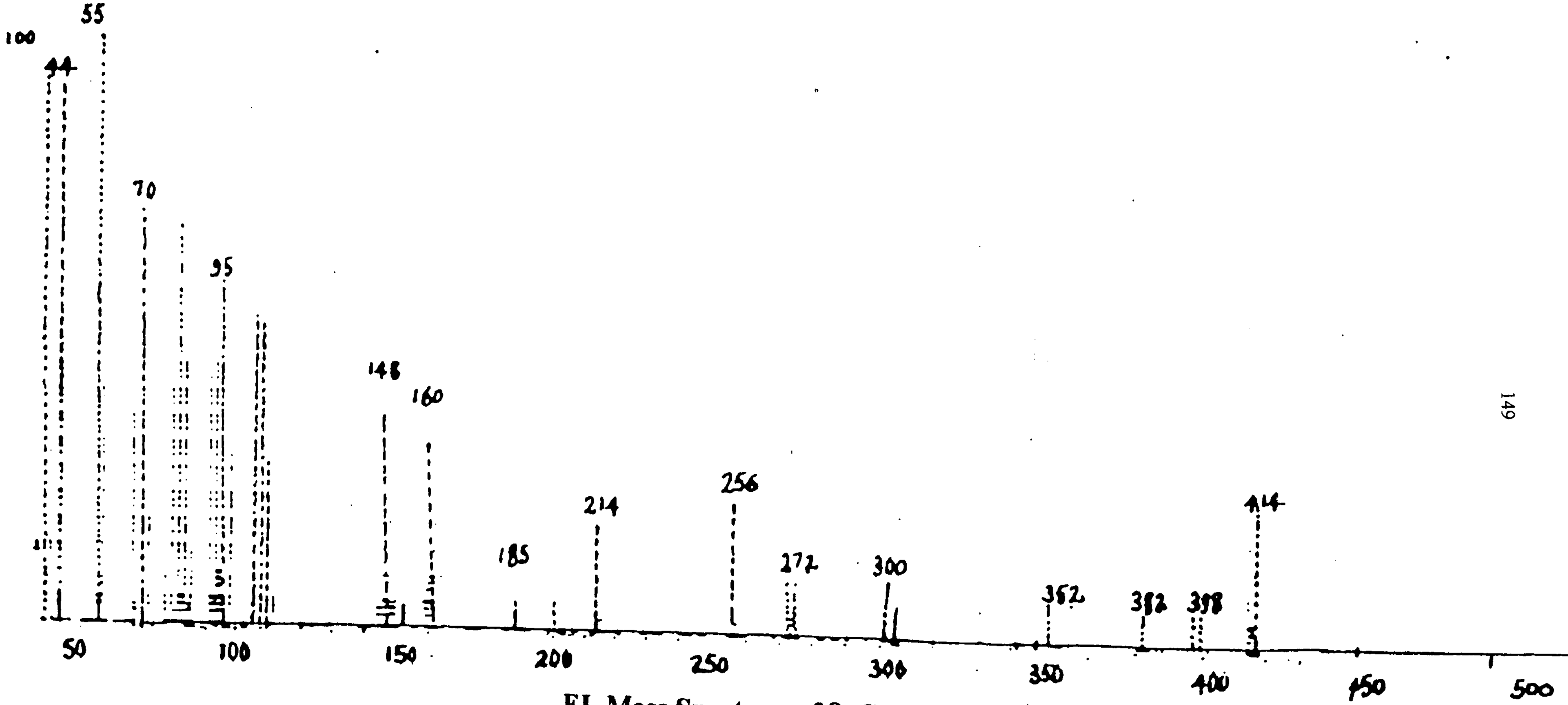
1. Acetylation of S_2 (β-sitosterol)

100 mg of S_2 was refluxed with 3 mL pyridine and 1 mL acetic anhydride for 2 hours. Poured the reaction mixture into cold water,

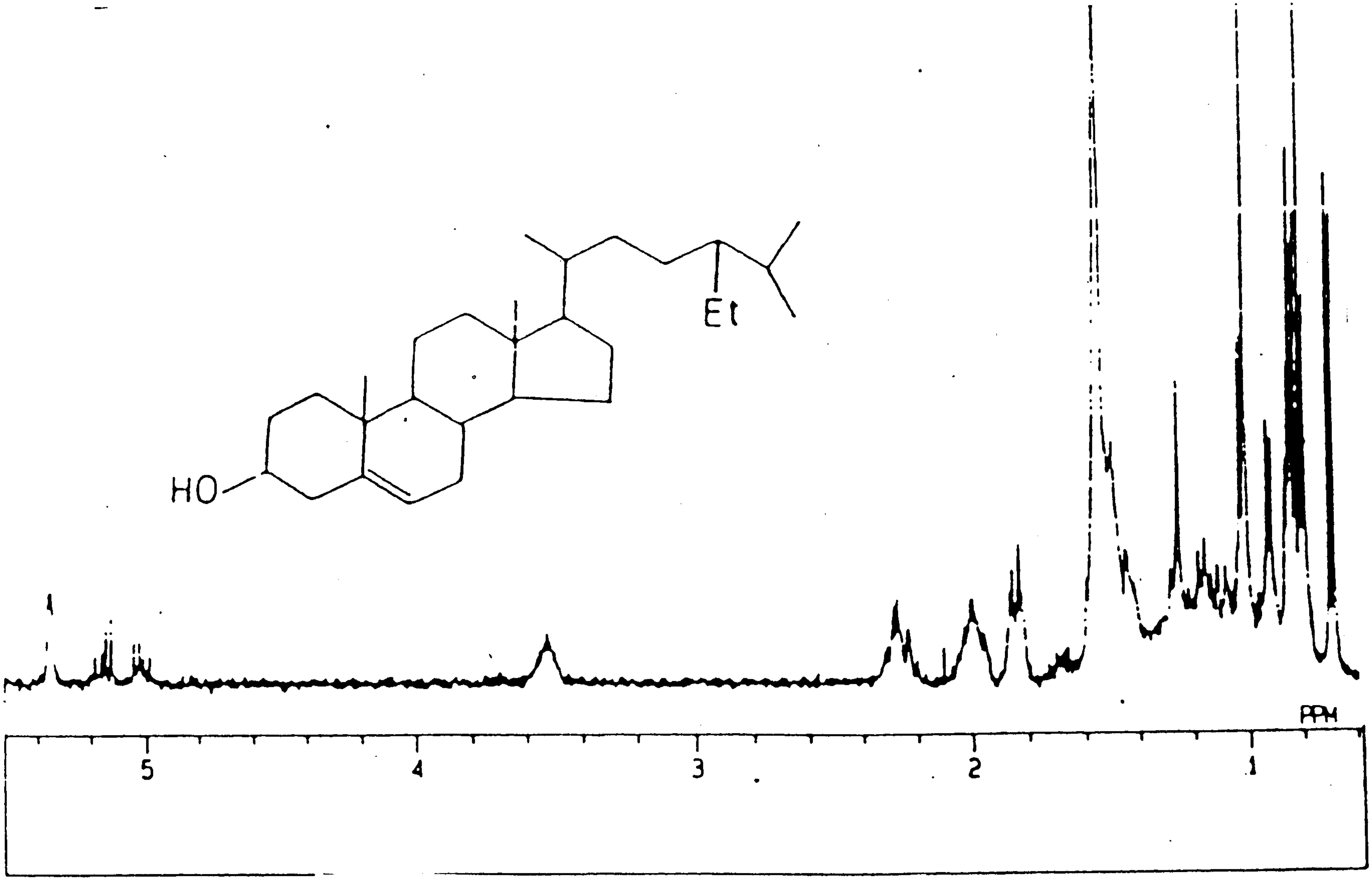
filtered, washed with water and then re-crystallised from petroleum spirit. The melting point of the compound was 133^0C.

2. Benzoylation of S_2 (β-sitosterol)

100 mg S_2 was refluxed with 2 mL freshly distilled benzoylchloride and 5 mL pyridine on a sand bath for 2 hours. The reaction mixture was then poured into cold water, filtered, washed with water and recrystallised from benzene. The compound obtained melted at 144^0C



EI Mass Spectrum of β- Sitosterol



^{1}H NMR Spectrum of β- Sitosterol

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