STUDIES ON BIOLOGICAL ACTIVITY AND CONSTITUENTS OF ESSENTIAL OILS

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By

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CERTIFICATE

This is to certify that this thesis entitled "STUDIES ON BIOLOGICAL ACTIVITY AND CONSTITUENTS OF ESSENTIAL OILS" is an authentic record of the research work carried out by Rosamma M.K., 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,
12.08.2002.

Dr. P. Mohamed Shafi
(Supervising Teacher)

DECLARATION

I, Rosamma M.K., 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, 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.

C.U. Campus,
12.08..2002.

Rosamma M.K.

ROSAMMA M.K.

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CONTENTS

INTRODUCTION

Plants are an integral part of nature. They are designed with the unique purpose of life-sustaining force on earth. The natural essential oils and their aroma are perhaps the most remarkable products of plant metabolism and these products have influenced human thoughts and emotions from the beginning of our civilization. India is considered to be the ancient home of perfumes and aromatic plants, because it is blessed with a wide variety of soil and climatic conditions that support the enormous plant wealth.

Kerala is also well known for its spices and aromatics for many centuries. The major cultivated spices and aromatics had been a subject of thorough investigation in the past. But the lesser known plants are yet to be investigated. Therefore, the aim of this work is to investigate the constituents and properties of some essential oils made from the lesser known plants.

Essential oils have been so named because of their odour or essence. All the volatile oils are of vegetable origin. Plants chiefly in the tropics and subtropics produce essential oils in large quantity. According to the chemical theories of the ancient Greeks, matter is made up of four elements: earth, air, fire and water. The Pythogoreans and Aristotle added a fifth element, "ether", of which the heavenly bodies are supposedly made of. In their search of this fifth element (quinta essentia

or quintessence), alchemists made extensive investigations of the volatile oils of herbs and spices. Quinta essentia (quintessence) represents the efficient part of every drug. The very name essential oil recalls the paracelsian concept – the Quinta essentia. These oils are still referred to as essential oils in English language.[1] In ancient times perfumes were widely used for religious purposes as well as personal enhancement. The great world religions of Islam, Christianity, Buddhism and Hinduism employ fragrance in pursuance of their faith.

The essential oils are complex mixtures of hydrocarbons and their oxygenated derivatives. The main constituents of the essential oils are the terpenoids consisting of carbon atoms upto C_{15} (that is mono and sesquiterpenes) and their oxygenated derivatives such as alcohols, aldehydes, ketones etc. Applications of essential oils in many industries are well known. Due to their pleasant smell, they are commercially important specially in perfumery. The development of the modern perfumery industry started in France, the world centre of perfumery. The east is the birth place of most popular spices and flavourings. India, south east Asia and China have given us anise, basil, cardamom, cinnamon, clove, garlic, ginger, mustard, onions, pepper, tamarind and turmeric. Many of the spices popular today are indigenous to India. The biological properties such as antibacterial, antifungal, anthelmintic, insect repellant and insect attractant attributed to essential oils and their constituents have been investigated only in the recent decades.[2]

EXTRACTION OF ESSENTIAL OILS

Essential oils are not chemically pure substances but consist of several, often many compounds possessing different chemical and physical properties. The boiling points of the volatile oil components range in most cases from 150° to 300°C at 760 mm pressure.[3] The general methods used for the isolation of essential oils are expression, steam distillation, extraction using volatile solvents and adsorption in purified fats. The first authentic description of the distillation of real essential oils has been generally ascribed to the catalan physician, Arnold de Villanova.[4] Steam distillation is the widely used method for the extraction of essential oils. For that the plant material is macerated and steam distilled. When the essential oils goes into distillate it is extracted by the use of pure organic solvents like diethyl ether. Steam distillation may change or modify the components of essential oils, but liquid carbon dioxide extraction[5] can retain the true and natural characteristics of the essential oils.

Aromatic plants and their products particularly the essential oils are now becoming the most important export items from many developing countries in Asia. These valuable natural products sometimes comprise of more than 250 single compounds. Each of these constituents contribute to the beneficial effects of these essential oils.

IMPORTANCE OF ESSENTIAL OILS

Essential oil production and processing are important parts of the global flavour and fragrance industry. Mint and cinnamon are used in tooth paste, mouth wash or lozenges. Flavour of essential oils are used in baked goods, softdrinks, snack foods, liquors, sauces, gravies and other food products.[6] Some combinations of essential oils can be found in soaps, detergents, room freshners, paper, printing-ink, paint, candles, floor polishes, cosmetics etc. Along with aromatic chemicals, they constitute the creative building blocks for virtually all flavours and fragrances.[7] Essential oils are commercilly important as the source of natural perfumes and also of spices and flavourings in the food industry.[8]

BIOLOGICAL ACTIVITY AND APPLICATIONS OF ESSENTIAL OILS

The germicidal property of essential oils has been well known to mankind for a long time and these oils have been used for the purpose of preservation since very early times. Essential oils can either have 'static' effect, if they inhibit the growth of the micro organisms or 'cidal' effect, if they kill them.[9] In recent years essential oils have not only found extensive use in perfumery but also in the pharmaceutical industry due to their antiseptic, carminative, stimulative, expectorant, diuretic, counter irritant and rubefacient properties.[10] Because of their well known disinfectant properties they were considered to be excellent remedies against infections and epidemics. Since times immemorial, a number of essential

oil bearing plants are being used as folk medicine for a variety of ailments in various parts of the world. Sometimes the oils were believed to be a remedy for nearly all health problems. In remote ages physicians like Galen and Celsus recommended use of aromatic herbs as sovereign remedies for hysterical convulsions and as a calming and antispasmodic remedy for nervous tension and recommended mint, sage and myrtle for this purpose.[11] From ancient times to the most modern, essential oils are recognized as medicaments or parapharmaceuticals and are applied by advanced and most traditional doctors.

Even though many microbiological studies have been performed on essential oils, only little is known about the mechanism of action of essential oils. Their lipoid solubility and therefore their possibility to penetrate into the cells may influence the metabolism of the microorganisms and thus give an explanation of the effect.[12] This is in agreement with the observation of Malowan, who found that the antiseptic activity of many compounds – are dependent on their lipoid solubility.[13] 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[14] (phenol has the factor one).

THERAPEUTIC APPLICATIONS OF ESSENTIAL OILS

Therapeutic applications of essential oils and fragrance compounds have been known since ancient times. The fumigation is one of the most ancient

treatments meant to clean and refresh the air surrounding a sick person.[15,16] It is known that King Solomon and the Egyptian Queen Cleopatra used pillows filled with dried rose petals to facilitate sleep. In order to banish the bad, stale air, the Roman writer Plinius recommended fresh peppermint plants be hung in sick rooms. Also doctors tried to protect themselves against infectious air by sniffing essential oils via an artificial beak.

Essential oils are complex mixtures and as such act in different complicated ways. Therapeutic property of each essential oil is specific. Therefore individual chemicals isolated from essential oils are more often used than the oils themselves for the treatment. "Analgetica" the pain killers contain cinnomon, clove, eucalyptus, jasmine, mint, sage and pepper oils. Cold remedies "Antigrippe" include basil, cinnamon, pine, rosemary and thyme.

Vapo-Rub Ointments viz: Vicks and Tiger Balm are standard medicaments which contain essential oils. Vicks which contains eucalyptus, cedar wood, camphor, turpentine and menthol is widely used for the treatment of common cold and headache. Tiger-Balm containing peppermint, eucalyptus, clove and cinnamon oils, menthol and camphor is very useful for the relief of cold, headache, rheumatic and muscular pains.[6]

PSYCHOLOGICAL EFFECT OF ESSENTIAL OILS

Volatile components of essential oils can affect human body and mind positively and creatively. These are very powerful to create good feeling and also to calm and stimulate mind. Essential oils can be used for the treatment of mental and physical disorders. The accepted application of essential oils in aroma therapy are sniffing, inhalation, oral use and skin penetration.

In Europe, hop pillows were used in folk medicine as sedatives. Head space analysis of dried hops showed the presence of dimethylvinylcarbinol which is proved to have a distinct sedative-hypnotic effect. Rose oil is found to stimulate the central nervous system of psychotic patients. The oil increases the ability of concentration of healthy subjects, accelerates the working rate and improves the capacity to do work.

ESSENTIAL OILS AND INSECTS

The life of insects is influenced by volatile chemicals. Certain plants growing near other plants can save them from dangerous insects. Hemps (*Cannabis sativa*) were planted around vegetable gardens as repellents against a variety of worms and insects. Calamus oil can sterilise males of houseflies, so most probably an old custom of decorating houses in spring with calamous leaves is based on this property of the oil. Camphor, citronella, eucalyptus, clove and

cinnamon oils show insecticidal property. Oils of laurel, thyme and coriander attract insects. Rose oil in 1:1000 dilution will kill earthworms and leeches.

AGRICULTURAL USES OF ESSENTIAL OILS

Allelopathy deals with the chemical interactions of plants with each other. Some plants grow in perfect harmony with others, but some others perish without any apparent reason. There are many reasons for such effects including metabolites exchange in roots, organic and inorganic products washed away 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. Certain plants produce oils as toxins against their diseases. Allelopathy of oil bearing plants is still an under-investigated area with enormous potential.

In short essential oils have an important role in fields like medicine, agriculture, perfumery, flavour and food industry.

CINNAMOMUM ZEYLANICUM

CHAPTER 1

ANALYSIS OF *CINNAMOMUM ZEYLANICUM* LEAF ESSENTIAL OIL

SECTION I. INTRODUCTION TO ANALYTICAL METHODS

The characterisation of essential oils depends on the resolution power of the analytical tools. Odour or colour comparison was the early method used for the characterisation of essential oils. Later analytical techniques such as specific gravity, refractive index, distillation range, determination of iodine number and gas-liquid chromatography etc. have been employed for the determination of volatile components of essential oils.

GAS CHROMATOGRAPHY (GC)

Gas chromatography is the technique of choice for the separation of thermally stable and volatile organic and inorganic compounds. In gas chromatography, the sample is vapourised and injected onto the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase. The mobile phase does not interact with molecules of the analyte, its only function is to transport the analyte through the column. Two types of gas chromatography are encountered: Gas-Solid Chromatography (GSC) and Gas-Liquid Chromatography (GLC).

Gas-liquid chromatography accomplishes the separation by partitioning the components of a chemical mixture between a mobile gas phase and a stationary liquid phase held on a solid support. Gas-liquid chromatography finds widespread use in all fields of science, where its name is usually shortened as Gas Chromatography (GC). This powerful tool finds application in analyses of varied types – gases and pollutants, petroleum and petrochemicals, oils and fats, foods and flavours, alcohols and beverages, drugs and vitamins, steroids and alkaloids, blood and serum, proteins and lipids, pesticides and fungicides, radioactive isotopes, elemental organic analysis and a number of miscellaneous purposes.

Gas-solid chromatography uses a solid adsorbent as the stationary phase. Here the retention of analytes is the consequence of physical adsorption. Due to the nonlinear character of adsorption process, this technique has not found wide application except for the separation of certain low molecular weight gaseous species.[17]

The principal advantages of gas chromatography to an analyst are

(i) The technique has strong separation power and even quite complex mixtures can be resolved into constituents.

(ii) It is a micro method and only a few milligrams sample is enough for analysis and the sensitivity of the method is very high.

(iii) The speed of analysis is quite fast and gives good precision and accuracy

(iv) It involves relatively simple instrumentation; operation of a gas chromatograph and related calculations do not require highly skilled personnel and thus the technique is very suitable for routine analysis. The cost of equipment is relatively low and its life is generally long.

COMPONENTS OF A GAS CHROMATOGRAPHIC SET UP

Carrier Gas Supply

Carrier gas should be chemically inert; include helium, nitrogen and hydrogen. Choice of the gas depends on the detector used. The main purpose of this carrier gas is to transport sample components through the column. It should not interact with samples, stationary phase or contacted hardware. Its purity is very important since impurities may react with the sample components or the stationary phase and change the retention behaviour of the substrate. This may result in high background signal and reduction of detector sensitivity.

Column

Column is said to be the heart of a GC system. Remarkable separation takes place in this magic tube. Two basic types of columns which generally used are packed column and the open tubular or capillary column. Packed columns are constructed from tubing of stainless steel, nickel or glass. Inner diameters may

range from 1.6 to 9.5 mm. Length is often 3 m. These columns are packed with an inert support. For gas-solid chromatography the columns are packed with size graded adsorbents or porous polymers whereas for gas-liquid chromatography the packing is prepared by coating the liquid phase over a size-graded inert solid support. Unlike gas-solid chromatography, gas-liquid chromatography is applicable to high molecular weight compounds since gas to liquid mass transfer rates are fairly high and separations are achieved in less time. Further, due to linear gas-liquid partition isotherms, the elution bands are symmetrical showing no tailing; this helps in both qualitative and quantitative analyses and enhances the sensitivity of the technique. Also here the choice of liquid phases is wide open and the quality of liquids could be much more reproducible than that of adsorbents. Liquid phases are classified into the following groups:

a. Non polar: Such phases do not have any polar or polarisable groups.

eg: hexadecane, squalane etc.

b. Polar: These phases contain significant proportion of polar groups and retain selectively polar or polarisable solutes. Non polar solutes are eluted quickly in one group.

eg: dimethylsulpholane, versamid-900 etc.

c. Intermediate: This kind of liquid phases contain relatively low proportion of polar groups and dissolve both polar and non-polar solutes with slightly more affinity for polar ones.[18]

eg: didecylphthalate, benzylbiphenyl etc.

d. Hydrogen Bonding: In this group liquid phases involve in H-bonding type interactions with solutes.

eg: Diglycerol, tetrahydroxyethylethylenediamine (THEED) etc.

e. Specific: There are some stationary phases which interact with specific groups of solutes forming loose chemical complexes and are employed for specific separations only. eg: Tetracyanoethylpentaerithritol (TCEPE) is somewhat super selective for aromatics; on this phase benzene elutes out after C_{10}-C_{13} saturated hydrocarbons.

Capillary Columns

Capillary columns have an internal diameter of 1 mm or less. These are usually constructed of fused silica (a very high-purity glass) which has a much higher degree of cross linking within the silicon-oxygen matrix, than does ordinary glass. Capillary columns are of two types; wall-coated open tubular (WCOT) and support-coated open tubular (SCOT). WCOT columns are capillary tubes coated with a thin layer of the stationary phase. In SCOT columns the inner surface of the

capillary is lined with a thin film (≈ 30 μm) of a support material, such as diatomaceous earth. The efficiency of a SCOT column is less than that of a WCOT column but significantly greater than that of a packed column.[18]

Detectors

A detector located at the exit of the separation column, senses the presence of the individual components as they leave the column. The detector volume must be small to prevent the remixing of components separated on the column.

Thermal Conductivity Detector (TCD)

In TCD a heated filament is placed in the emerging gas stream. The amount of heat lost from the filament by conduction to the detector walls depends on the thermal conductivity of the gas phase.

Flame Ionisation Detector (FID)

The FID detector adds hydrogen to the column effluent. Subsequently the mixture is passed through a jet where it is mixed with external air and burned. This detector is the most widely used and generally applicable detector for gas chromatography. When ionisable material from the column effluent enters the flame and is burned, the current markedly increases. The current flowing through an external resistor is sensed as a voltage drop, amplified and finally sent to a

recorder. The FID responds proportionately to the number of $-CH_2-$ groups introduced into the flame.

Thermionic Emission Detector (TED), Sulphur-Chemiluminescence Detector (SCD), Electron Capture Detector (ECD), the Flame Photometric Detector (FPD), the Photo-Ionisation Detector (PID) are the other detectors which are used in gas chromatography.

Some Basic Parameters and Relationships in GC

Retention Time (t_R)

It is the time lapsed between sample introduction and appearance of peak maxima.

Gas Hold up Time (t_M)

It is the retention time of a solute (usually air) that has no affinity for the stationary phase.

Adjusted Retention Time (t'_R)

It is the difference between retention time and gas hold up time.

$$t'_R = (t_R - t_M)$$

Retention Volume (V_R)

Retention volume of a component is the volume of gas to carry a component maximum through the column

$$V_R = t_R . F_c$$

where F_c is the volume flow rate of the gas outlet corrected to the temperature of the column.

Adjusted Retention Volume (V'_R)

It is the difference between retention volume and hold up volume.

$$V'_R = V_R - V_M$$

$$V_M = t_M . F_c$$

$$V'_R = (t_R - t_M) F_c$$

Subscripts R and M refer to species that are retained and not retained on the column.

Gas chromatography 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 (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, low cost 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

$$I = 100 \left(\frac{\log t'_R (\text{subst}) - \log t'_R (n - Cz)}{\log t'_R (n - Cz+1) - \log t'_R (n\text{-}Cz)} + Z \right)$$

For n-alkanes the term $\log t'_R$ (subst) – $\log t'_R$ (n-Cz) reduces to zero and they have retention indices equal to the number of carbon atoms in the molecule multiplied by one hundred. A systematic method for expressing retention data used the Kovats retention indices RI.[19] The indices indicate where compounds will appear on a chromatogram with respect to straight chain alkanes injected with the sample. By definition the retention index for a normal paraffin is 100 times the number of carbon atoms in the compound regardless of the columns used or the chromatographic conditions. Thus the Retention index for pentane is 500, for hexane 600 and so on. Of course, the type of column and the operating conditions such as liquid loading and any pre-treatment must be specified.

Hyphenated Techniques

Gas chromatography is often coupled with the selective techniques of spectroscopy, thus giving so-called hyphenated methods that provide the chemist with powerful and pragmatic tools for identifying the components of complex mixtures. 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 these hyphenated techniques, identification of even trace components has 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. When we use GC-MS, the mass spectrometer is a universal detector for gas chromatographs since any compound that can pass through a gas chromatograph is converted into ions in the mass spectrometer. At the same time the highly specific nature of a mass spectrum makes the mass spectrometer a very specific gas chromatographic detector. Gas chromatography is an ideal separator whereas mass spectrometry is excellent for identification.

Gas chromatographic equipment can be directly interfaced with rapid scan mass-spectrometers of various types. As with GC-MS the interface between column and the detector is critical. In this instance a narrow light pipe having a length of 10 to 40 cm and an inside diameter of 1 to 3 mm is connected to the

column by means of a narrow tubing. The light pipe consists of a pyrex tube that is internally coated with gold. Often the light pipe is heated in order to avoid condensation of the sample components. Light pipes of this type are designed to maximise the path length for enhanced sensitivity while maximizing the dead volume to lessen band broadening.

The interface must provide the link between the two instruments. Almost all GC-MS interface systems contain an enrichment device. However, the high pumping speeds used in mass spectrometers may permit the total effluent from capillary GC column to be transported to the ion source of the mass spectrometer. When the chemical ionization reagent gas is used as the carrier gas, the effluent can be introduced directly into the mass spectrometer. Since the carrier gas molecules are usually much lighter than those of the sample, they can be removed by an effusion chamber.

The main advantages of a mass spectrometer as a detector for gas chromatography are its increased sensitivity and its specificity in identifying unknown or confirming the presence of suspected compounds.

GC-MS has been used for the identification of hundreds of components that are present in natural and biological systems. For example this procedure has permitted characterization of the odour and flavour components of foods, identification of water pollutants, medical diagnosis based on breath components and studies of drug metabolites.

SECTION II. ANALYSIS OF *CINNAMOMUM ZEYLANICUM* LEAF ESSENTIAL OIL

INTRODUCTION

The plant *Cinnamomum zeylanicum* belongs to the family Lauraceae. Lauraceae is a family of 45 genera and about 1100 species. Economically this family is important for the aromatic oils that are responsible for the fragrance of many of its members.[20] Cinnamon (*Cinnamomum verum* J.S. Presl, Syn. *Cinnamomum zeylanicum* Blume) and Cassia are two of the oldest and most famous spices known to man. Cinnamon, camphor and many fragrant woods are used in cabinet making. Species of about seven genera of the family are cultivated domestically as ornamental plants.

Cinnamon is an evergreen tree whose bark and leaves are found to be more aromatic. Size of the tree is moderate, 8 to 18 m in height and 50 cm in diameter, with reddish brown soft bark having numerous small warts; leaves are ovate or elliptic. Flowers are small in axillary or subterminal cymes or panicles.[21] Commercially two types of ethereal oils are extracted from cinnamon: leaf oil and bark oil. *Cinnamomum zeylanicum* is considered to be the source of true cinnamon. In addition to its culinary uses in Asian and European recipes, it has important applications in medicine too. Other varieties of cinnamon do not have as much fragrance as that of *Cinnamomum zeylanicum*. This tree is native to India and

Ceylon. For the extraction of leaf oil the leaves and tender twigs are harvested in May and November. After wilting of the leaves in shade for about 24 hours they are steam distilled for four to six hours. The essential oil content from leaves is 0.49 to 0.87%. Cinnamon oil is stomachic, carminative, emmenagogue and styptic. It is useful in anorexia, inflammations, stomachalgia, vitiated conditions of 'Vata' and tubercular ulcers.[21] The astringency is due to tannin. The odour causing essential oil contains Cinnamic aldehyde and Eugenol. This oil acts as a stimulant and is a powerful germicide.[22] It has been used since antiquity as a breath-sweetener, a tonic for the whole system, heart, stomach, liver, kidneys, gall and nerves. It was also considered a remedy for heart burn, nausea and diarrhoea and as a sedative for expectant mothers during child birth. In the flavouring industry, it is used as a modifier. The antiseptic nature is very effective and is very powerful to cure cold and any viral infection especially of the respiratory or intestinal system.

ECONOMIC AND MEDICINAL IMPORTANCE OF OTHER CINNAMON SPECIES

Cinnamomum camphora (L.) Nees & Eberm, *Cinnamomum burmannii* Blume (Malay Cinnamon), *Cinnamomum loureiri* Nees (Saigon Cinnamon), *Cinnamon tamala* Nees and Eberm are the other important species in the Lauraceae family.

Cinnamomum camphora is a source of camphor. Twigs and leaves are used to treat rheumatism, and muscular pain. It is very useful in bronchitis and pneumonia. In conjunction with menthol it relieves itching of skin.

Cinnamomum burmannii Blume is native to India, China and Malaysia. The bark contains essential oil and is used as a carminative and flavour. The bark oil of Cinnamomum loureiri which contains cinnamic aldehyde, phenols, pinene, phellandrene and caryophyllene is used to relieve nausea, flatulence and diarrhoea. *Cinnamomum tamala* leaf is used as a carminative and spice. It is also used for the treatment of colic pain.[23]

The bark of Ceylon Cinnamon exported as quills is used as a spice or condiment for flavouring cakes. Studies on its leaf oil is less compared to that of bark oil. Leaf oil is used in the manufacture of perfumes, used in soap, tooth pastes, hair-oil etc.

PREVIOUS WORK

The chemical studies on Cinnamomum concentrate mainly on the analysis of the volatile oils of bark and leaf of commercially important Cinnamomum species. *C. zeylanicum, C. cassia, C. camphora* are the species most extensively studied. As early as 1924 Glichiteh analysed the Cinnamon leaf oil and found that the oil contained about 50% eugenol. Pure linalool was identified from Indian Cinnamon leaf essential oil during its chromatographic analysis.[24] o-Methoxy eugenol,

caryophyllene, humulene, isocaryophyllene and benzyl benzoate in the non-phenolic portion and coniferaldehyde in the phenolic fraction. Angmor and coworkers carried out a compositional analysis of various plant parts of *Cinnamomum zeylanicum*. The major components in young and mature bark and leaf were cinnamic aldehyde and cinnamyl acetate. Herisset and coworkers used chromatographic analysis to differentiate *Cinnamomum zeylanicum* and *Cinnamomum cassia* oils. A lower cinnamaldehyde content combined with the presence of linalool and eugenol characterised *Cinnamomum zeylanicum* oil. In *Cinnamomum cassia*, cinnamaldehyde content was higher and linalool and eugenol were absent. The leaf essential oil of Ceylon Cinnamon was found to contain eugenol, methyl- and ethyl cinnamate by gas chromatographic analysis.[24]

On account of the industrial potential of Cinnamon leaf oil, Joy and coworkers carried out a study on the inter-relationship between the growth, yield and quality parameters in Cinnamon and identified elite types for aromatic leaf oil and eugenol yields.[25] Studies on the essential oil from the fruit rind of *Cinnamomum cecidodaphne* Meissn showed the presence of fairly good amount of methyl cinnamate, thymol, safrole, cineole and eugenol giving it a spicy flavour. Presence of linalool, linalyl acetate and nerol adds to its pleasantness. Possibility of application in perfumes and flavours is higher in this case. This oil showed considerable range of fungicidal activity.[26]

Pharmacological studies on the antiulcerogenic activity of Chinese Cinnamon has been reported. Chinese Cinnamon has been used in Chinese traditional medicine as a diaphoretic, an antipyretic and an analgesic.[27] The intraperitoneal administration of the aqueous extract of Chinese Cinnamon to rats markedly prevented ulcerogenesis induced by cold-stress.

The chemistry of *Cinnamomum zeylanicum* has received the most extensive attention compared to other species. Cinnamon leaf oil is yellow to brownish-yellow in colour and possesses a spicy but rather harsh odour.

The volatile compounds of *Cinnamomum glanduliferum* (Wal.) Nees leaves collected from Almora contained 1,8-cineole, linalool, camphor and α-terpineol as the significant components contributing to the scent. The oil found application for fragrant soaps and room sprays. The pharmaceutical application of the oil could be defined by the high concentration of 1,8 cineole and camphor.[28]

Investigation of the essential oil of Cinnamon leaf grown at Bangalore and Hyderabad was carried out by Mallavarapu and coworkers. In both oils eugenol was the major constituent. But they differed with respect to the relative amounts of linalool, cinnamaldehyde, cinnamyl acetate, β-caryophyllene and benzyl benzoate. The oil content of the Hyderabad leaf was found to be higher than that of Bangalore leaf.[29]

Investigation of essential oils of leaves of *Cinnamomum* Schaeffer members revealed a correlation between the leaf size and eugenol content among the variants of *Cinnamomum tamala*.[30] The variant possessing smaller leaves generally contained higher percentage of eugenol in its leaf oil. Thus smaller the leaf size higher the eugenol content. Another correlation noticed was between the refractive index and eugenol content of the oils. Higher the value of the refractive index, more will be the percentage of eugenol present.

Kiuchi and coworkers reported the nematocidal activity of *Cinnamomum zeylanicum* on screening of crude drugs used in Turkey for nematocidal activity on the larva of *Taxocara canis*. It was found that *Cinnamomum zeylanicum* was active even at a concentration as low as 0.1 to 0.2 mg/ml.[31] It has antioxidant property also.[32]

The leaf oil of *Cinnamomum zeylanicum* from various parts of India has been previously investigated.[33-36] Although eugenol is normally found as the main component (about 80%) of *Cinnamomum zeylanicum* leaf oil, benzyl benzoate has also been found as a major component in the leaf and bark oil of *Cinnamomum zeylanicum* from the Assam area of India[34] as well as in China.[35]

Cinnamomum zeylanicum is an interesting example of which leaves, stem bark and root bark yield oils differ in composition. In this species, in root bark oil, camphor is found as a characteristic constituent, leaf oil contains principally

eugenol, whereas in the stem bark oil, cinnamaldehyde predominates. The characteristic pungent, sweet and warm taste of cinnamon bark is due to the presence of an alcohol soluble pale yellow mobile essential oil which is very rich in cinnamaldehyde, reportedly upto eighty percent. It is used for the preparation of a number of ayurvedic and other pharmaceutical products. *Cinnamomum zeylanicum* leaves can be used as a substitute of 'Tejpat".[37]

PRESENT WORK

Plant Source and Extraction of Essential Oil

Fresh leaves of *Cinnamomum zeylanicum* were collected from neighbouring place of Calicut University, Kerala, South India. The species was identified by A.K. Pradeep, Department of Botany, Calicut University and a voucher specimen deposited (No. 29) in the specially maintained herbarium of Calicut University Chemistry Department.

Fresh leaves (250 g) were cut into small pieces and ground to a paste with 200 mL of distilled water using an electric mixer-grinder. It was then subjected to steam distillation for two hours. The distillate was extracted twice with 50 ml portions of diethyl ether and the combined extract dried using anhydrous sodium sulphate. Ether was evaporated to get light green essential oil. The yield was (2 g) 0.8% of the fresh weight.

EXPERIMENTAL

The oil composition was analysed by a combination of GC and GC-MS. GC analysis of the oil was carried out on a Shimadzu GC-14A (FID) and a Varian GC-3700 (FID) gas chromatographs fitted with a 30 m x 0.32 mm chemically bonded non polar FSOT-RSL-200 (film thickness: 0.25 μm: Biorad) and with a 30 m x 0.32 mm stabil wax (film thickness: 0.50 μm, Restek) fused silica column, respectively. The sample was injected by splitter. Hydrogen was used as carrier gas. The column temperature was programmed from 40°C (5 min) to 280°C (20 min) at 6° C/min. The compound identification was partly possible by injection of pure compounds and correlation with published retention index data.[36,38,39] GC/MS analysis was carried out on a Shimadzu GC-17A/QP 5000, on a HP-5890 GC/HP-5970 MSD and on a Finnigan MAT GCQ (Carrier gas: helium, EI mode, 70 eV, scan range: 40-450 amu and ion-source temperature 200°C each) equipped with Wiley/NBS and NIST libraries. For additional mass spectra correlations, published data were used.[39-42] By means of these combinations more than thirty constituents of the leaf oil of *Cinnamomum zeylanicum* could be identified.

The compounds identified are listed in order of elution from a non polar FSOT-RSL column and the percentage calculated by percentage peak area calculations of GC analysis are given below.

Table 1. Chemical composition of the leaf oil of *Cinnamomum zeylanicum*

Compound	Concentration (%)
(E)-2-hexenol	0.1
(Z)-3-hexenol	0.1
1-hexen-3-ol	0.1
hexanol	0.1
α-pinene	tr
(Z)-3-hexenyl acetate	0.1
(E)-2-hexenyl acetate	0.1
p-cymene	tr
β-phellandrene	tr
(E)-β-ocimene	tr
1,8-cineole	0.1
limonene	0.2
cis-linalool oxide (furanoid)	0.2
terpinolene	0.1
trans-linalool oxide (furanoid)	0.1
linalool	85.7
nonanol	0.3
borneol	0.1
terpinen-4-ol	0.3
α-terpineol	1.1
dihydrocarveol	tr
linalyl acetate	0.1
(E)-cinnamaldehyde	1.7
safrole	t
(E)-cinnamyl alcohol	0.1
eugenol	3.1
(E)-cinnamyl acetate	0.9

β-caryophyllene	2.4
α-humulene	0.2
eugenyl acetate	0.1
caryophyllene oxide I	0.1
spathulenol	0.2
benzyl benzoate	0.3

tr = trace (< 0.1%)

RESULTS AND DISCUSSION

The main compounds correlation higher than 1% calculated as percentage peak area of GC-FID analysis were linalool (85.7%), eugenol (3.1%), β-caryophyllene (2.4%), E-cinnamaldehyde (1.7%) and α-terpineol (1.1%).

This is the first time that linalool has been found to be present in such a concentration of more than 85% in any of the Cinnamomum species. The hitherto known highest concentration is 60% in cinnamomum sulphuratum.[30]

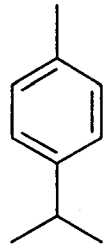
This oil sample has a very pleasant odour which can be attributed to the individual components present in it. The floral odour is due to the major component linalool and α-terpineol. Due to the presence of (E)-2-hexenol, (Z)-3-hexenol and their acetates along with 1-hexen-3-ol, the oil exhibits green-fresh note. The 'green' odour is the characteristic of hexenols and their derivatives and hexenals. The most prominent among them is cis-3-hexen-1-ol leaf alcohol. When the position of the double bond is shifted or the alcoholic group is shifted they became less 'green'.

The trans-3-hexen-1-ol is less odorous than the cis isomer.[43] Eugenol, β-caryophyllene, and (E)-cinnamyl acetate give a spicy touch while the fatty acids and their esters give fatty-moody note to this oil.

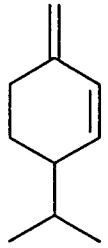
The high percentage of linalool can make this essential oil important in fine perfumery where a floral odour note is appreciated.

Structures of compounds identified in the leaf essential oil of *Cinnamomum zeylanicum* are given below.

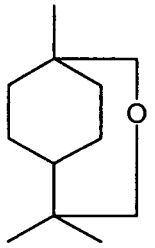
Structures of compounds identified in the leaf essential oil of *Cinnamomum zeylanicum*



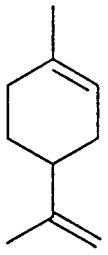
p-Cymene



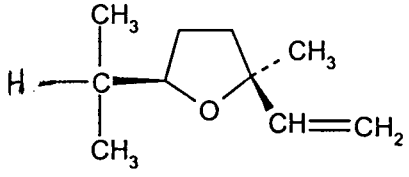
β-Phellandrene



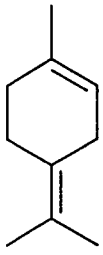
1,8-Cineol



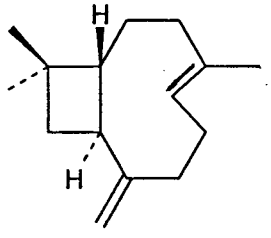
Limonene



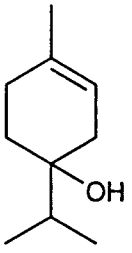
cis-Linalool oxide



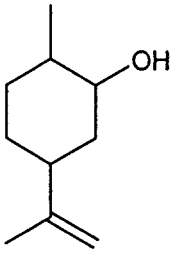
α-Terpinolene



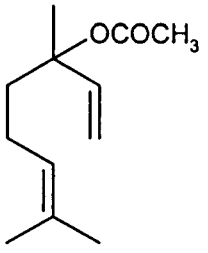
β-Caryophyllene



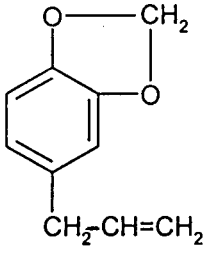
Terpinen-4-ol



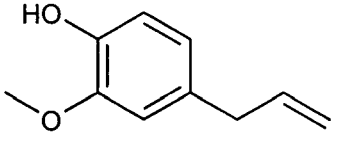
Dihydrocarveol



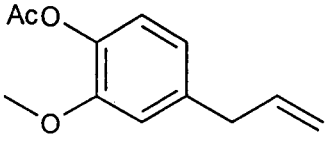
Linalyl acetate



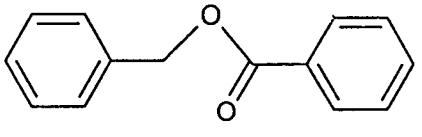
Safrole



Eugenol



Eugenyl acetate



Benzyl benzoate

SYZYGIUM TRAVANCORICUM

CHAPTER 2

SEASONAL CHANGE IN THE COMPOSITION AND OLFACTORIC PROPERTIES OF *SYZYGIUM TRAVANCORICUM* ESSENTIAL OIL

INTRODUCTION

Syzygium travancoricum is a member of the Myrtaceae family. It is a medium sized tree, a rare medicinal plant, very similar to *Syzygium cumini* but has larger leaves and smaller fruits[44-46]. Flowers are small and white. The species *S. cumini* and to a lesser extent *S. travancoricum* are known for their astringent, bactericidal, hypoglycemic and neuropsychopharmacological effects and for their significant odours. The water loving tendency of the plant is exemplified by its vernacular names Neernaval (Tamil) and Kollignaval (Malayalam). Traditionally this plant was used for curing diabetes and arthritis by the local people. *Syzygium travanrocium* is found solely in the Kerala region and no chemical studies have been performed on season dependent changes in the composition and aroma of the odorous essential leaf oil.

PREVIOUS WORK

No data was available on the essential oil of leaves of *Syzygium travancoricum*. Antifungal properties of crude leaf extracts (air dried leaves) of *S.*

travancoricum was studied by Radha and coworkers. The hexane extracts showed maximum activity against fungi.[46]

PRESENT WORK

The objective of this investigation was therefore to identify the components present and to investigate the character impact aroma compounds of the essential oils of *S. travancoricum* fresh leaves at different harvest times. Analysis was performed by gas chromatography combined with spectroscopy (GC-FID and GC-MS with columns of different polarity), gas chromatography combined with olfactory analysis (GC-O), and olfactory analysis alone (odour evaluation by professional perfumers). This combination is considered the most efficient for such analysis.[47,48]

EXPERIMENTAL

The leaves of *Syzygium travancoricum* were collected from the neighbouring village of Calicut University Campus in June 1999 (sample 1) and April 2000 (sample 2). It flowers in March. The plant material was identified by Dr. A.K. Pradeep, Department of Botany, Calicut University of Kerala. A voucher specimen (Voucher No.20) has been deposited in the specially maintained herbarium of the Department of Chemistry at Calicut University.

ESSENTIAL OIL EXTRACTION

The fresh leaves (500 g each) of the two samples of *Syzygium travancoricum* were cut into pieces and ground by means of an electric grinder, into a paste which was steam distilled for 3 hours. The distillate was extracted with diethyl ether (2 x 100 mL) and dried over anhydrous sodium sulphate. After removal of the solvent the yield of *S. travancoricum* essential leaf oil was (0.3 g) 0.06% of the fresh weight from sample 1 and (0.5 g) 0.1% of the fresh weight from sample 2.

OLFACTORY EVALUATION

The essential oils were diluted with dichloromethane, 10 µL of the solution was placed on a commercial odour strip (Dragoco), and the odour was characterised by professional perfumers.

GC ANALYSIS

GC analysis was performed with a Shimadzu GC-14A with FID and Shimadzu Chromatopac C-R6A integrator, and with a Varian GC-3700 with FID and Shimadzu Chromatopac C-RIB integrator. Compounds were separated on 30 m x 0.32 mm fused silica columns coated either with a 0.25 µm film of bonded non polar FSOT-RSL-200 (Bio-Rad-Munich, Germany) or with a 0.50 µm film of bonded polar stabilwax (Restek, Bellefonte, USA). The nonpolar column was maintained at 40°C for 5 min after injection, then programmed at 6°/min to 280°C which was maintained for 5 min. The polar column was maintained at 60°C for 5

min after injection then programmed at 10°/min to 280°C which was maintained for 5 min. Split injection was conducted with split ratio of 1:20 and 1:50 for the non polar and polar columns respectively, hydrogen was used as carrier gas at 2.5 and 3.5 kPa, respectively. For both columns the injector temperature was 250°C and the detector temperature 320°C. Quantification by percent peak area calculations was performed by use of the non-polar column. Some individual components could be identified by co-injection of pure compounds and comparison of their retention times (as Kovats indices) with published data.[36,38,39]

GC-O ANALYSIS

Gas chromatography-olfactory analysis ('Sniffing technique') was performed with a Fractovap 2101 GC equipped with a splitting system, a model 230 LT-Programmer, a model 160 electrometer (Carlo Erba, Milano, Italy) and a Kompensograph-III-Recorder (Siemens, Munich, Germany). Compounds were separated on a 30 m x 0.32 mm fused silica column coated with a 0.2 μm film of non polar FSOT-RSL-200 (Bio-Rad). The column was maintained at 40°C for 5 min after injection then programmed at 8°C/min to 230°C which was maintained for 20 min. Compounds were injected in splitless mode with hydrogen as carrier gas (pressure 1.8 kPa; column flow 2 mL/min). The detector (FID) temperature was 320°C, the injector temperature 250°C, and the sniffing capillary temperature 250°C. The column eluate sniffing split-ratio was 1:50, FID : nose. Peak to odour

impression correlations were performed by professional perfumers and fragrance chemists.

GC-MS ANALYSIS

GC-MS was performed with (i) a Shimadzu GC-17A chromatograph coupled with a Shimadzu QP5000 mass spectrometer and Compaq-ProLinea data system (class 5k-software) (ii) a Shimadzu GC-17A chromatograph coupled with a Shimadzu QP 5050 mass spectrometer and Pentium-II data system (Böhm, Horn, Austria; class 5k-software), (iii) a Hewlett Packard GC-HP 5890 chromatograph coupled with an HP-5970 MSD mass spectrometer and PC-Pentium data system (Comp Delphin, Vienna, Austria; Chemstation-software) and (iv) a Finnigan MAT GCQ with Gateway-2000 PS75 data system (Siemens, Munich, Germany; GCQ software). The columns and temperature programme used were as for GC analysis. Split injection was performed with helium as carrier gas. For the nonpolar column the split ratio was 1:50, the column head pressure 4.9 kPa, and the flow rate 0.5 mL min^{-1}, for the polar column the split ratio was 1:100, the column head pressure 105 kPa, and the flow rate 1.0 mL/min. Injector, interface and ion source temperatures were 250°, 300° and 200°C, respectively. The spectrometers were operated in electron-impact (EI) mode; the scan range was 41-450 amu. Compounds were identified by use of on-line Wiley, NBS and NIST-library spectra and literature MS data.[41,42,49-51]

RESULTS AND DISCUSSION

By GC and GC-MS analyses fifty and fortynine components could be identified in samples one and two respectively. GC-MS analysis were carried out using quadrupole MS-detectors, using polar and nonpolar columns. Another experiment used, ion trap MS-detector. This enabled the correct identification of the components by comparing with the published retention indices under these different conditions. The predominant components of these essential oils (concentration above 1%, calculated as the percentage peak area in GC-FID analysis with nonpolar column) were monoterpenes and sesquiterpenes. Sample 1 contained 44.7% trans-β-ocimene, 32.9% trans-β-caryophyllene, 6.7% α-humulene, 4.9% α-farnesene, and 3.6% alloaromadendrene. Sample 2 contained 21.2% trans-β-ocimene, 20.7% trans-β-caryophyllene, 8.1% nerolidol, 5.4% α-humulene, 4.2% cis-β-ocimene, 4.0% aromadendrene, 3.5% β-selinene, 2.8% α-farnesene, 2.7% α-selinene, 2% globulol, 1.8% ledene, 1.2% β-eudesmol and 1.1% spathulenol. Thirteen other compounds were present at concentrations between 0.1 and 0.6% in sample 1 and twenty eight compounds were present at concentrations between 0.1 and 0.9% in sample 2. The identified components of the essential leaf oils of *Syzygium travancoricum* is given in the following table. Compounds are given in order of decreasing concentration.

Table 2.1. Composition of the essential leaf oils of *Syzygium travancoricum*

Compound	Concentration (%)		Retention Indices (nonpolar/polar column)	Odour impression
	Sample 1	Sample 2		
trans-β-Ocimene	44.7	21.2	1257/1040	Herbal, woody
trans-β-Caryophyllene	32.9	20.7	1578/1421	Spicy
α-Humulene	6.7	5.4	1650/1493	Herbal, woody
α-Farnesene	4.9	2.8	1733/1492	Weak woody
Alloaromadendrene	3.6	0.9	1661/1477	Herbal
α-Calacorene	0.6	0.5	1919/1531	Spicy
γ-Gurjunene	0.4	0.4	1527/1407	Green-herbal
β-Selinene	0.3	3.5	1696/1479	Herbal-spicy
α-Copaene	0.3	0.6	1471/1374	Dark, herbal
Longipinene epoxide	0.2	0.9	1601/1399	Woody, pinene
Pinocarveol	0.2	0.8	1454/1132	Fresh, woody
Pinocarvyl acetate	0.2	0.7	1681/1277	Fresh, woody
cis-β-Ocimene	0.1	4.2	1234/1028	Herbal
Terpinolene	0.1	0.9	1282/1079	Terpinolene
α-Gurjunene	0.1	0.5	1592/1408	Herbal
β-Myrcene	0.1	0.9	1158/987	Spicy, herbal
Farnesol	0.1	0.7	2321/1699	Farnesol
2-Hexenal	tr	0.7	1201/823	Green
3-Hexenyl acetate	tr	0.3	1280/987	Green
Hexanol	tr	0.5	1329/849	Hexanol
1-Octen-3-ol	tr	0.5	1422/962	Mushroom
Carvone	tr	0.8	1717/1227	Herbal, dill
Geranyl acetone	tr	0.3	1820/1428	Fresh
Caryophyllene	tr	0.4	1550/1414	Herbal, spicy

Globulol	tr	2.0	2104/1571	Spicy
Elemol	tr	0.4	2076/1545	Spicy
α-Humulene epoxide	tr	0.3	1972/1221	Spicy
Nerolidol	tr	8.1	2002/1548	Nerolidol
trans-Carveol	tr	0.7	1791/1209	Caraway
δ-Cadinol	tr	0.8	2137/1638	Fresh, floral
β-Eudesmol	tr	1.2	2222/1677	Spicy
Spathulenol	tr	1.1	2035/1618	Spicy
α-Pinene	tr	0.7	1007/932	Pinene
α-Terpineol	tr	0.8	1650/1171	Fruity
Linalool	tr	0.4	1519/1082	Floral
Aromadendrene	tr	4.0	1649/1454	Spicy
α-Selinene	tr	2.7	1704/1509	Spicy
Ledene	tr	1.8	1671/1490	Spicy
Limonene	tr	0.6	1188/1020	Limonene
Terpinen-4-ol	tr	0.5	1609/1172	Terpinen-4-ol
α-Longipinene	tr	0.4	1541/1359	Woody, pinene
Fatty acids and their esters	2.7	3.3		
Higher hydrocarbons	0.9	0.7		
Unknown:	ca 0.5	0.4		

The fatty acids and their esters and the higher hydrocarbons identified in the samples are the following. The concentrations given in the brackets are sample 1 and 2 respectively.

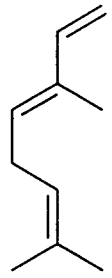
Tetradecanoic acid (0.3, 0.6), Hexadecanoic acid (0.9, 0.9), Hexadecanoic acid methyl ester (0.2, 0.3), Hexadecanoic acid ethyl ester (0.2, 0.1), octadecanoic acid (0.7, 0.9), octadecane (0.4, 0.2), Eicosane (0.4, 0.5). In addition Heptadecane (0.1) also was found to be present in sample 1.

Olfactoric evaluation of both the samples showed them as woody-herbal (hop-oil notes), spicy (clove-like), weakly aromatic-floral (tagetes like) and fruity and earthy-fatty in the background. The odour of Sample 2 was more intense and the oil content is higher. By gas chromatography-olfactometry, the odour elicited by all the compounds were studied and is given in the table above. The woody-herbal odour of the essential oil of the leaves of *S. travancoricum* can be attributed to trans-β-ocimene and humulene.[52,53] The term wood is used to describe an odour class which includes not only the odour of the essential oil derived from various trees such as cidarwood and sandalwood, but also some oils derived from Patchouli and Vetiver.[43] Ocimene and Caryophyllene derivatives were found to be responsible for the spicy notes and the aromatic-floral odour impression was because of the presence of ocimene derivatives and monoterpenes like linalool. The fruity-earthy tonality originated from the monoterpenes like α-terpineol and sesquiterpenes like nerolidol.[54] The more intense and stronger spicy-woody notes of sample 2 resulted from the higher concentration of aroma relevant compounds such as longipinene, pinocarveol derivatives, terpinolene, carvone, trans-carveol and α-pinene. Thus in relation to perfumery it can be stated that the essential leaf

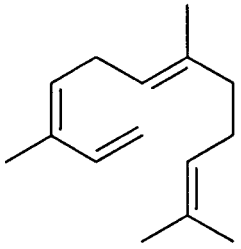
oil of *Syzygium travancoricum* could be useful in the perfume industry when herbal-spicy-woody odour notes are required (These odour notes are recommended in after-shaves, toilet-water etc.). If more intense spicy and woody odour notes are required, the leaves have to be harvested during April (Sample 2) and not in June.

Structures of the terpenoids present in this essential oil and not given in the previous chapter are given below.

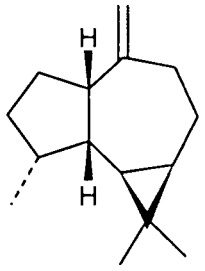
Composition of the essential oil of the leaves of *Syzygium travancoricum*



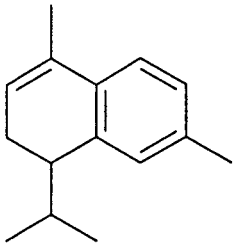
Trans-β-ocimene



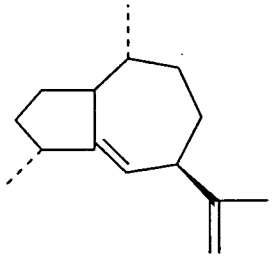
α-Farnesene



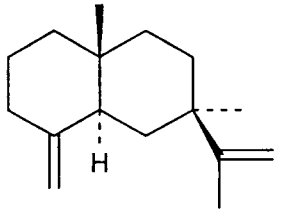
Alloaromadendrene



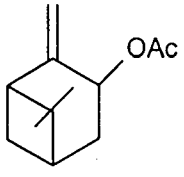
Calacorene



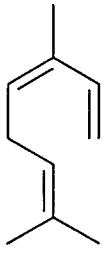
γ-Gurjunene



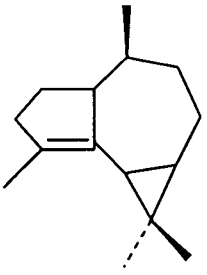
β-Selinene



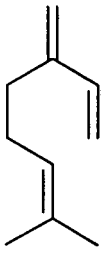
Pinocarvyl acetate



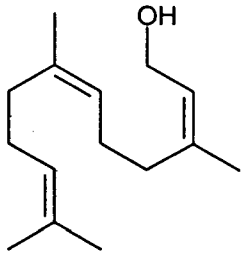
Cis-β-ocimene



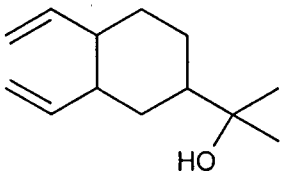
α-Gurjunene



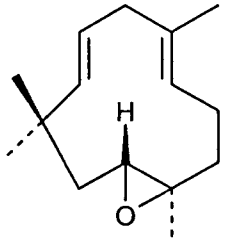
β-Myrcene



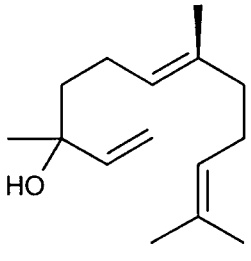
Farnesol



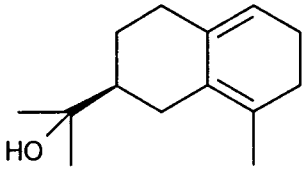
Elemol



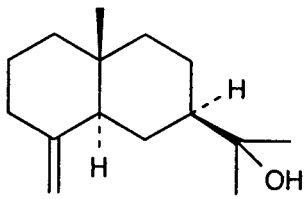
Humulene Epoxide I



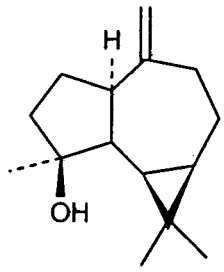
Nerolidol



Hedycaryol



β-Eudesmol



Spathulenol

ARISTOLOCHIA INDICA

CHAPTER 3

ANALYSIS OF *ARISTOLOCHIA INDICA* Linn. ESSENTIAL OIL

INTRODUCTION

Aristolochia indica Linn., commonly known as 'Indian birthwort' belongs to the family *Aristolochiaceae*. This family comprises of nearly eighteen genera and over six hundred species of mostly perennial climbing shrubs. The genus *Aristolochia* known to contain about five hundred species, is distributed mainly in subtropical and tropical regions. *Aristolochia indica* is a hardy climbing plant distributed in eastern and southern India. This plant requires good, ordinary and well drained soil and sunlight for cultivation. Of the many species of this genus, only five or six have been subjected to careful chemical analysis.[55-57] There is some information about the chemical composition of this plant, but studies of the volatiles from *Aristolochia indica* could not be found yet.

REPORTED PROPERTIES AND USES

The dried roots and occasionally the leaves constitute an important traditional Indian drug, known as 'Iswari' in Ayurveda, believed to have the power to destroy the toxic effects of all poisons especially snake poison.[58] It is also used as a gastric stimulant, bitter tonic and emmenagogue. The roots are used usually in the form of a tincture for the treatment of atonic types of dyspepsia, bowel troubles

in children and intermittent fevers. A decoction of the root is considered stimulant and febrifuge. In combination with black pepper and ginger it is used as a carminative for treating diarrhoea.[58] The dried powdered roots are given with honey to treat leucorrhoea. The leaf juice is used to treat diarrhoea, cholera and intermittent fevers. The seeds and leaves are useful for treating inflammations and dry cough. The powdered stem bark mixed in water with that of *Azadirachta indica* and *Cassia fistula* is administered orally as an antidote to snake bite by the Gonds of northern Andhra Pradesh. It is a purifier of blood and hence useful in skin diseases.[57] It is also used as a mild sedative and against intestinal worms. Eastern Madhya Pradesh the root pounded with black pepper is used to treat rheumatism.

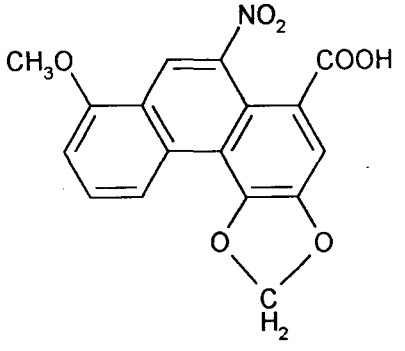
The objectives of the present work was the analysis of the essential oil of the aerial parts of *Aristolochia indica* by gas chromatographic-spectroscopic (GC-FID and GC-MS) and olfactoric methods and the identification of those compounds responsible for the characteristic odour and medicinal use.

PREVIOUS WORK

Rajashekharan and coworkers conducted the ethno-medico-botanical studies of *Aristolochia indica* (Cheriya Arayan) and *Aristolochia tagala* (Valiya Arayan) with the help of Kanitribes of Thiruvananthapuram District. As a panacea they are using these plants against snake poison and other ailments. *Aristolochia indica* is

prescribed in combination with other drugs for rat poison, snake poison, flatullence, intestinal colic and head ache. Paste form of root is a snake repellent. The roots of *Aristolochia indica* has been found to contain an alkaloid aristolochine ($C_{17}H_{19}O_3N$). The aroma of roots is due to an essential oil composed of sesquitrpenoid compounds with a trace of camphor.[59]

A rapid, sensitive and reproducible HPLC method based on photodiode array detection for quantitative determination of Aristolochic acid in *Aristolochia indica* has been described by Singh and coworkers.[60] The dried roots and rhizomes of *A. indica* constitute an important drug, used as a gastric stimulant and bitter tonic. The chief active principle of this drug is Aristolochic acid (8-methoxy-3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid) which possesses a wide range of activities including antifeedant, antitumor, immuno-modulating and antifertility activities. Clinically the compound is found effective against leukemia, tuberculosis, chronic bronchitis, bronchial asthma and pneumocardial diseases.

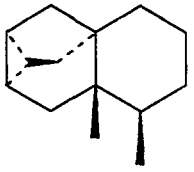


Aristolochic acid ($C_{17}H_{11}O_7N$)

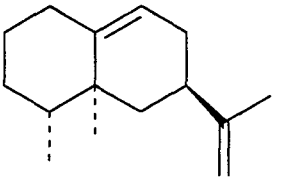
Biological evaluation of constituents of *A. indica* roots for fertility regulating activity revealed that an ethanol extract of *A. indica* roots decreased fertility in both rats and hamsters when administered postcoitally.[55]

A new type of sesquiterpene 12(S)-7,12-secoishwaran-12-ol from *Aristolochia indica* was isolated and identified by Pakrashi and coworkers.[61]

Govindachari and coworkers isolated two new sesquiterpene hydrocarbons namely ishwarane and aristolochene from *A. indica* and assigned the following structures:[62]



Ishwarane

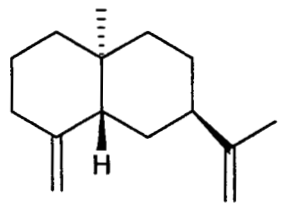


Aristolochene

The sesquiterpene alcohol ishwarol is also present in *A. indica*. Its structure was assigned on the basis of spectral data and correlation with the known tetracyclic sesquiterpene ketone ishwarone which is one of the chief constituents of the roots of *Aristolochia indica*.[63,64]

Ganguly and coworkers proposed proper evidence for the novel tetracyclic sesquiterpene ketone ishwarone which was isolated during 1935 by Rao and coworkers.[65,66]

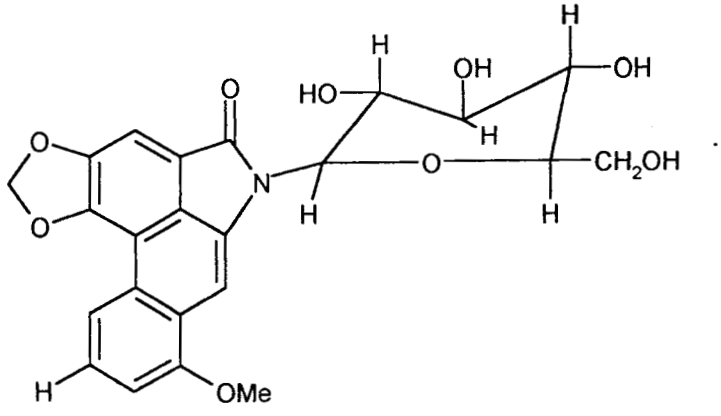
Another new sesquiterpene hydrocarbon belonging to the eudesmane group isolated from *A. indica* has been shown to be 5 βH, 7 β, 10α-selina-4(14),11-diene.[67]



5βH, 7β, 10α-Selina-4(14), 11-diene

Achari and coworkers reported the isolation of an N-glycoside and two rare naturally occurring steroids from *Aristolochia indica*.[68]

Pakrashi and coworkers isolated phenanthrene derivative aristololactam-β-D-glucoside and a sesquiterpene from the roots of *A. indica* which possesses interesting antifertility activity. Structure of aristololactam-β-D-glucoside is as shown below.[61,69,70]



Aristololactam -β-D-glucoside

Essential Oil Extraction

The aerial parts of *Aristolochia indica* (100 g) were cut into small pieces and ground into a paste using an electric grinder. The paste was steam distilled for 2.5 hours. The distillate was extracted with diethyl ether (2 x 50 mL) and dried over anhydrous sodium sulphate. After the removal of the solvent a yield of (.5 g) light green essential oil was obtained (i.e. 0.5% of the fresh weight of the sample).

Olfactoric evaluation

Olfactometric study enabled the identification of the compounds (Table 3.1, Identification 'O') responsible for different odours exhibited by it. 10 μL of a solution of the essential oil in dichloromethane was placed on a commercial odour strip (Dragoco Co.) and its odour was characterized by professional perfumers.

GAS CHROMATOGRAPHY

GC analysis was carried out using a Shimadzu GC-14A with FID and the integrator C-R6A-Chromatopac and a Varian GC-3700 with FID and the integrator C-RIB-Chromatopac (Shimadzu Co.). As columns, one 30 m x 0.32 mm bonded non polar FSOT-RSL-200 fused silica (film thickness 0.25 μm, Biorad Co.) and another 30 m x 0.32 mm bonded polar stabil wax (film thickness 0.50 μm; Restek Co.) were used. Carrier gas used was hydrogen. Injector temperature: 250°C, detector temperature: 320°C. Temperature programme was 40°C/5 min to

280°C/5 min with a heating rate of 6°C/min. Quantifications was done by Percent Peak area calculations (non polar column). Some single components could be identified by co-injection of pure compounds and correlation of their retention times (using Kovats indices) with published data.[36,38,39,74]

GAS CHROMATOGRAPHY – MASS SPECTROMETRY

The sample was analysed by the GC-MS system. Shimadzu GC-17A with QP 5000 and the data system Compaq-ProLinea (Class 5k-software), Shimadzu GC-17A with QP 5050 and data system Pentium-II (Böhm Co., Class 5k-software), Hewlett-Packard GC-HP5890 with HP5970 MSD and PC-Pentium (Böhm Co., Chem Station-software) and Finnigan MAP GCQ with data system Gateway-2000-PS75 (Siemens Co., GCQ-software) were used for analysis.

Carrier gas used: helium, injection temperature : 250°C, interface-heating: 300°C, ion-source heating: 200°C, EI mode, Scan range: 41-450 amu. For compound identifications Wiley, NBS and NIST library spectra as well as reference MS spectra data were used.[39-42, 48-50,75]

RESULTS AND DISCUSSION

The essential oil of the aerial parts of *Aristolochia indica* was olfactorically evaluated as smoky, leather like, woody-earthy with weak green Caryophyllene-terpinene and floral-fruity side notes.

By Gas Chromatographic-Spectroscopic system of analysis fifty-seven components were identified in the essential oil of the aerial parts of *Aristolochia indica*. The main compounds (concentration higher than 1%, calculated as percent peak area of GC-FID analyses) found were β-caryophyllene 58.4%, α-humulene 17.5%, ishwarone 2.8%, caryophyllene oxide I 1.4%, ishwarol 1.2%, linalool, 1.1% and α-terpinolene 1.0%.

The identified components of the essential oil of the aerial parts of *Aristolochia indica* are given in the following table 3.1.

Table 3.1: Composition of the essential oil of the aerial parts of *Aristolochia indica*

Compounds	Concentration (%)	Identification
β-caryophyllene	58.4	GC, GC-MS, O
α-humulene	17.5	GC, GC-MS, O
ishwarone	2.8	GC-MS
caryophyllene oxide I	1.4	GC, GC-MS
ishwarol	1.2	GC-MS
linalool	1.1	GC, GC-MS, O
α-terpinolene	1.0	GC, GC-MS O
ishwarane	0.8	GC-MS
aristolochene	0.7	GC-MS
cis-3-hexenol	0.5	GC, GC-MS, O
germacrene D	0.5	GC, GC-MS
octen-3-ol	0.4	GC, GC-MS, O

3-hexenyl acetate	0.4	GC, GC-MS, O
camphor	0.4	GC, GC-MS, O
nonanol	0.4	GC, GC-MS, O
humulene oxide	0.3	GC, GC-MS
nerolidol	0.3	GC, GC-MS, O
β-farnesene	0.3	GC, GC-MS, O
β-bisabolene	0.3	GC, GC-MS
pinocarveol	0.3	GC, GC-MS, O
δ-cadinol	0.3	GC, GC-MS, O
β-elemene	0.3	GC, GC-MS, O
α-terpineol	0.2	GC, GC-MS, O
β-farnesol	0.2	GC, GC-MS, O
octanol	0.2	GC, GC-MS, O
caryophyllene oxide II	0.2	GC, GC-MS
α-bisabolene	0.2	GC, GC-MS
phytol	0.2	GC, GC-MS
β-bisabolol	0.1	GC, GC-MS
germacrene A	0.1	GC, GC-MS
ledol	0.1	GC, GC-MS
2-octanol	0.1	GC, GC-MS
hexyl acetate	0.1	GC, GC-MS, O
thymol	0.1	GC, GC-MS, O
indole	0.1	GC, GC-MS, O
β-phellandrene	0.1	GC, GC-MS, O
tetradecanol	0.1	GC, GC-MS
5βH, 7β, 10α-selina4(14), 11-diene	0.1	GC, GC-MS

β-pinene	0.1	GC, GC-MS, O
borneol	tr	GC, GC-MS, O
terpinene-4-ol	tr	GC, GC-MS, O
β-selinene	tr	GC, GC-MS
hexanol	tr	GC, GC-MS, O
(12S)-7, 12-secoishwaran-12-ol	tr	GC, GC-MS
camphene	tr	GC, GC-MS, O
tricyclene	tr	GC, GC-MS
tetradecanoic acid	1.3	GC, GC-MS
hexadecanoic acid	1.0	GC, GC-MS
hexadecanoic acid methyl ester	0.5	GC, GC-MS, O
hexadecanoic acid ethyl ester	0.8	GC, GC-MS, O
octadecanoic acid	0.3	GC, GC-MS
octadecanoic acid methylester	0.3	GC, GC-MS
octadecane	0.3	GC, GC-MS
nonadecane	0.5	GC, GC-MS
eicosane	1.1	GC, GC-MS
docosane	0.4	GC, GC-MS

The gas chromatographic-spectroscopic data was correlated with olfactometric data and it was found that the identified main compounds β-caryophyllene, α-humulene, caryophyllene oxide I, linalool and α-terpinolene were responsible especially for the side notes (green-caryophyllene-terpinene as well as floral-fruity), while the characteristic smoky, leather-like and woody-earthy odour

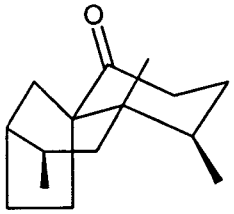
could be attributed to further sesquiterpenes such as humulene, octane-derivatives and N-heterocycles (in non-detectable concentration).

Some main or minor compounds of the investigated *A. indica* samples were ishwarane derivatives, with reported effects on fertility especially in animals. Therefore, these activities can be expected by the use of the essential oil of *Aristolochia indica* also.[55,62-66] This essential oil can be used as a powerful antimicrobial agent, since the identified main compounds β-caryophyllene, α-humulene, caryophyllene oxide I, linalool and α-terpinolene are well known natural products possessing antimicrobial effects.[76-78]

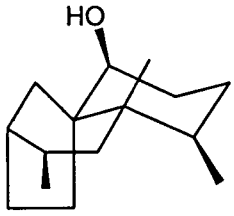
The combined concentration of β-caryophyllene and linalool is about sixty percent and evince sedative effects.[77] Therefore, *A. indica* essential oil can be used as a mild sedative. The characteristic smoky, leather-like, woody-earthy, green-caryophyllene-terpinene-like and floral-fruity odour of this essential oil is appreciable. Green aroma is attributed to leaf alcohol (cis-3-Hexen-1-ol).

The structures of the identified compounds of *Aristolochia indica* (structures given in earlier chapters are omitted) are given below.

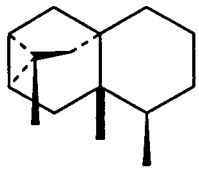
Structures of the compounds present in the essential oil of the aerial parts of *Aristolochia indica*



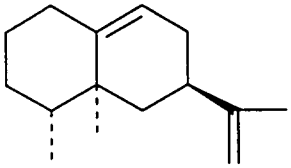
Ishwarone



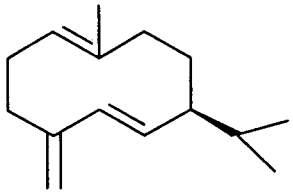
Ishwarol



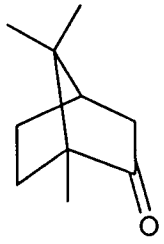
Ishwarane



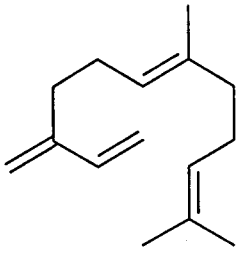
Aristolochene



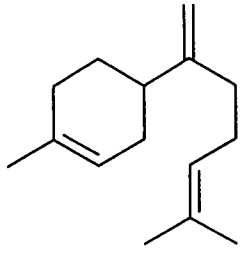
Germacrene D



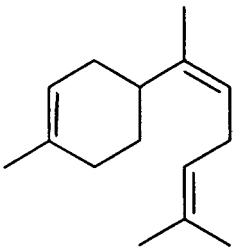
Camphor



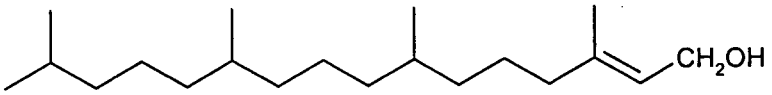
β-Farnesene



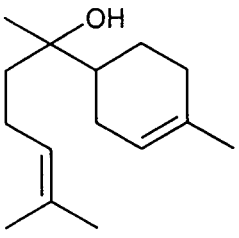
β-Bisabolene



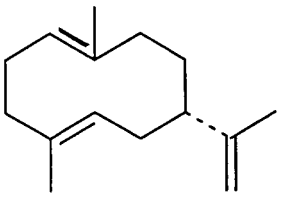
α-Bisabolene



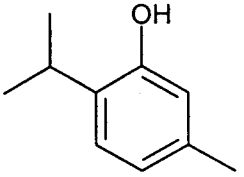
Phytol



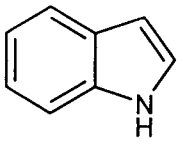
β-Bisabolol



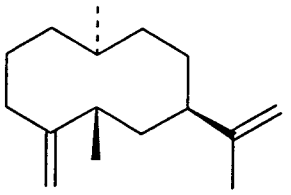
Germacrene A



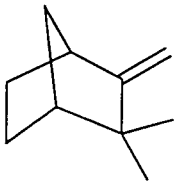
Thymol



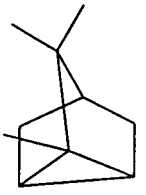
Indole



5βH,7β,10α-Selina4(14),11-diene



Camphene



Tricyclene

LEUCAS INDICA

CHAPTER 4

ANALYSIS OF THE ESSENTIAL OIL VOLATILES OF *LEUCAS INDICA* USING GC-FID, GC-MS AND OLFACTOMETRY

INTRODUCTION

Leucas indica R. Br. (Syn. *Leucas lavandulifolia*) belongs to Lamiaceae (Labiatae) family which consists of about 200 genera and 3200 species. Lamiaceae family is also known as aromatic or mint family. The generic name Leucas is derived from the Greek word Leukos meaning white, referring to the colour of the downy flowers.[79] Economically this family is important as a source of volatile aromatic essential oils and garden ornamentals. Some of them important for essential oils are salvia, lavender, rose mary and mint. In addition to many of the above, others serve as important culinary herbs valued for the flavour or aroma imparted to food.[20] This plant *Leucas indica* (L.) R. Br. ex Vatke (synonymous in Tamil language with Mosapullu) is wide spread in India, Himalayan region, Bangladesh, Malaysia and Mauritius.[80-82] *Leucas indica* is a hairs spreading herb growing upto a height of about 75 cm. Leaves are linear to lanceolate. This plant is found in a variety of habitats: on the way side, in waste lands, river banks (plains); shallow soil on exposed slopes by rocks (hills); and locally abundant in unweeded farmlands.

The species of *Leucas* R. Br. are rich in terpenoid contents and have great medicinal potential particularly as antihistaminic, antiseptic, carminative, febrifuge and wormifuge. The drug obtained commercially is known as "Dronapushpi" in trade which finds wide use in Ayurvedic drugs. *Leucas indica* is used in Indian folk medicine and for food preparations nearly in the same way as the better known species *Leucas aspera.* The whole plant is used medicinally. This is a reputed home remedy for worms, fever and intestinal catarrh in children.[83] It is also used in the treatment of migraine. Fresh juice of the plant is extensively used in jaundice and skin disease.[84]

The leaves are used as an expectorant, laxative, stomachic and tonic. They are useful in piles and sore eyes. The paste or decoction of fresh leaves is applied on old sores, wounds and dermatosis. The juice of fresh leaves is also applied against chronic head ache and cold. When the leaves are roasted and eaten with salt, it is considered to have febrifugal properties.[85]

Miris tribals of plains of Assam apply paste of young leaves to stop bleeding from the nose. In West Bengal the leaves are used in bronchitis, dyspepsia and leucoma. In Madhya Pradesh the leaves are bruised and a teaspoonful of juice is snuffed in the nostrils of the person suffering from snake bite.

Khonda, Kammaras and porjas tribals in Andhra Pradesh pour two or three drops of leaf or flower juice into the eyes to cure eye infection. Powder of dried

plant is used as a mosquito repellent. In Meghalaya particularly in Khasi and Jaintia hill region, the juice of the plant is mixed with the juice of *Rubiacordifolia* L. and *Nicotianatabacum* L. and applied on the bites of snakes and stings of poisonous insects as an antidote.[85]

No data about volatiles of any essential oils of *Leucas indica* has been published. Therefore the objective of this work was to identify the components of the essential oil of the fresh plant and also to find out the individual compounds responsible for the charactersitic pleasant odour using gas chromatographic, spectroscopic and olfactoric methods.

PREVIOUS WORK

The notable contributions reported in the case of other species of *Lamiaceae* family closely related to *Leucas indica* are the following.

Mahato and coworkers identified linifolioside, an isopimarane rhamnoglucoside from *Leucas linifolia*.[86] A triterpenoid lectone from *Leucas aspera* was reported by Pradhan and coworkers.[87] Saponin and Leucasin are triterpenes obtained from *Leucas nutans*.[88]

By cytological and biochemical analysis *Leucas vestita* it was found to be a potential source of monoterpenoid isobornyl acetate (37%). Other components are terpninen-4-ol (9%), piperitone oxide (7.7%) and piperitone (4.6%). The greenish

yellow coloured essential oil of *L. vestita* possesses a spicy odour. The chemical compounds were found to possess medicinal as well as aroamtic properties.[89]

Jelani and coworkers have made quantitative analysis of the presence of inorganic metals in the leaves of some species of Leucas R. Br. and recorded absence of cadmium in *Leucas cephalotes* Spreng. and absence of nickel in *Leucas stricta* Wall ex Benth and *Leucas zeylanica* (L.) R. Br. *Leucas cephalotes* maintains a considerable taxonomic distance from other species and has again found support from the chemosystematic work of Kumari and coworkers. They recorded that *Leucas aspera, Leucas lavandulifolia (Leucas indica)* and *Leucas decemdentata* show rather close chemical affinities.[90,91] Kamat and Singh confirmed the occurrence of terpenoids, steroids and alkaloids in the stem, root, leaves, inflorescence and seeds of the different *Leucas* species.[92] Essential oils of the following *Leucas* species has been studied previously.

(i) *Leucas cephalotes* (light yellowish green oil). The major components are terpinyl acetate (35.5%) and eugenyl acetate.

(ii) *Leucas linifolia* (pale yellow oil): Predominant compounds are methylcinnamate (24.5%) and piperitone (19.5%).

(iii) *Leucas stricta* (pale amber yellow coloured oil): Thujone (24.8%) and bornyl acetate are the majorcompounds.

(iv) *Leucas vestita*: (pale greenish yellow coloured oil) contains isoborynyl acetate (37%) and terpinen-4-ol (9%) as major compounds.

(v) *Leucas aspera* is very closely related to *Leucas indica*. From this about 0.2% light yellowish green oil is obtained. It is found that terpninyl acetate (28.1%) and isobornyl acetate (17.4%) are present as major components.[93]

PRESENT WORK

The aroma compounds of the essential oil of fresh plants of *Leucas indica* as well as the corresponding solid phase microextraction head space sample were analysed by GC-FID, GC-MS and olfactoric methods.

EXPERIMENTAL

Sample Preparation

The fresh plants of *Leucas indica* were collected in Kerala during the flowering season (month of July 2000) and the plant was identified by Dr. A.K. Pradeep, Department of Botany, Calicut University, Kerala. A voucher specimen (No. 33) has been deposited in the specially maintained herbarium of Calicut University, Department of Chemistry. One kg of fresh plants were crushed and ground in an electric mixer and was steam distilled for 3 hours and the distillate extracted with diethyl ether (2 x 100 mL). This extract was dried over anhydrous sodium sulphate. After the removal of the solvent, the yield was (1 g) 0.1 percent of

the fresh weight of the plant material. The essential oil was light yellowish brown in colour.

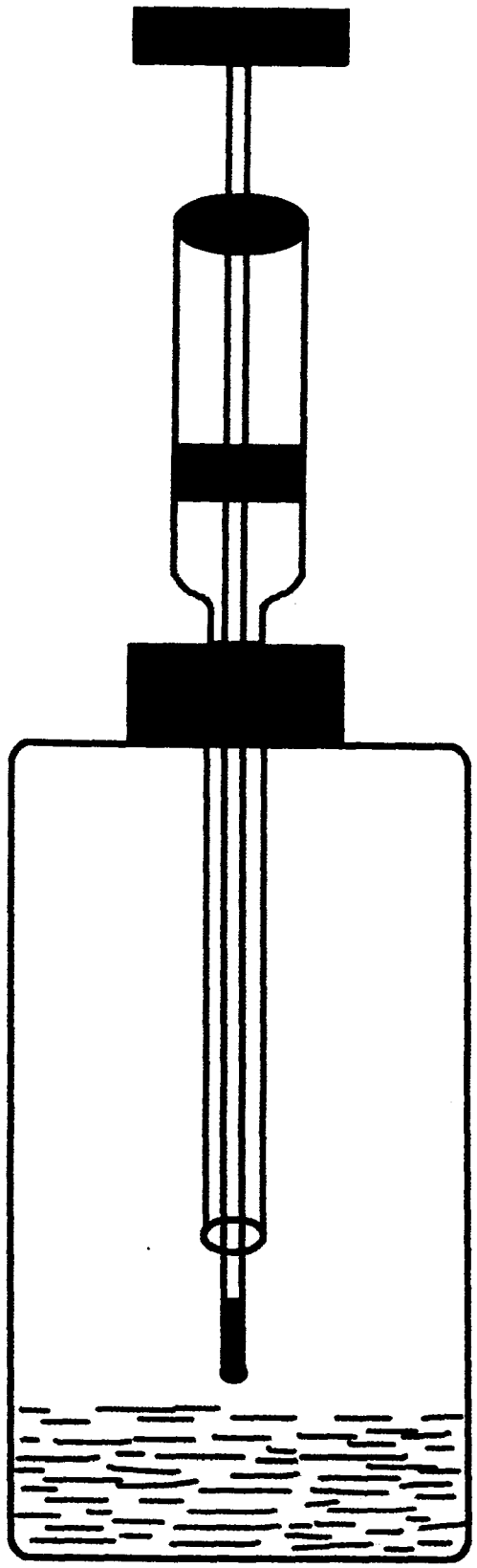
In the solid phase micro extraction of the essential oil of *Leucas indica*, the oil was deposited in a dark brown 5 mL flask, and extracted with a 2 cm – 50/30 μm Divinylbenzene Carboxen/Polydimethylsiloxane/Stable Flex coated glass fiber (Supelco, USA Cat-No. 5-7348) for 30 min at room temperature. Immediately afterwards the trapped volatiles on the fiber were directly analysed by GC-FID and GC-MS.

SOLID PHASE MICRO EXTRACTION (SPME)

Head Space Sampling

Beyond the conventional gas chromatoraphic analysis of gases and low viscosity liquids, some situations are more effectively handled by head space sampling. This is true when only the vapour above the sample is of interest as with perfumes or food products. In order to obtain the concentration of the volatile components solid phase micro extraction (SPME) is employed. More volatile components can be analysed by this method.

The SPME device consists of a fused silica fiber of about 1 cm length with a stationary phase coated on the outer surface and bonded to a stainless steel plunger and a holder that looks like a modified microliter syringe. The fused silica fiber



SPME Device

can be drawn into a hollow needle by using the plunger on the fiber holder. Organic analytes adsorb to the phase coating the fiber. Adsorption equilibrium is attained in two to thirty minutes. After sample adsorption, the fused silica fiber is drawn into the needle. The needle is withdrawn from the sample vial and introduced into the gas chromatograph injection, where the adsorbed analytes are thermally desorbed and delivered to a capillary GC column.

A thick phase coat is most effective for adsorbing/desorbing volatile analytes. With SPME we can achieve detection limits in the parts-per-trillion range for many volatiles and non volatile compounds. In short SPME is fast and easy and eliminates the cost and hazards associated with using organic solvents. It can be used for screening sample prior to a detailed analysis. Good precision under consistent sampling conditions also makes the technique viable in quantitative analysis. It reduces time and expense of sample concentration in analyses. Till now, the most successful applications of SPME are for analyses of volatile and semi-volatile organic compounds by coupling SPME with gas chromatography (GC). The use of SPME in combination with GC-MS offers a very sensitive and rapid method for the determination of odour active compounds in the microgram/litre range.

GC-FID

GC-FID analyses were carried out using a GC-14A with SPME sleeve adapted to injector, FID and C-RGA-Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID (Varian, Germany) and C-RIB-Chromatopac integrator (Shimadzu). The carrier gas was hydrogen; injector temperature, 250°C and detector temperature, 320°C. The temperature programme was: 40°C/5 min to 280°C/5 min, with a heating rate of 6°C/min. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused Silica, with a film thickness of 0.25 μm (Biorad, Germany) and 30 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 μm (Restek, USA). Quantification was achieved using peak area calculations and compound identification was partly carried out using correlations between retention times.[94-97]

GC-MS

For GC-MS measurements a GC-17A with QP 5000 (Shimadzu), SPME sleeve adapted to injector and Compaq-Pro Linea data system (Class 5K-software), a GC-HP 5890 with HP 5970-MSD (Hewlett-Packard, USA) and Chemstation Software on a Pentium PC (Böhm, Austria), a GCQ (Finnigan-Spectronex, Germany – USA) and Gateway-2000-PS75 data system (Siemens-Nixdorf, Germany, GCQ-Software) were used. The carrier gas was helium; injector temperature, 250°C; interface heating at 300°C, ion-source-heating at 200°C, E1-

mode was 70 eV, and the scan range was 41-450 amu. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 μm (Biorad, Germany) and 30 m x 0.32 mm bonded stabil wax, with a film thickness of 0.50 μm (Restek, USA). Mass spectra correlations were done using Wiley, NBS, NIST and other published data.[98-100]

RESULTS AND DISCUSSION

The essential oil was olfactorically analysed as follows. The essential oil was diluted with dichloromethane (1:10) and 10 μL of the sample was placed on a commercial odour strip (Dragoco Co., Germany) and was evaluated by professional perfumers as mushroom-like earthy, green, herbal (direction of dried tea-leaves) and sweet-fruity-lovage-like with smoky-burnt-tarry side-notes. Aroma impression is attributed to 1-Octen-3-ol (intense mushroom-like, forest-earthy, herbaceous), 3-octanone (herbaceous, spicy, buttery, mushroom-earthy) and trans-2-octen-3-ol (earthy, weak mushroom-like).

Using GC-FID and GC-MS (both with 2 columns of different polarity) ninety-three volatiles could be identified in this sample with the sesquiterpene β-caryophyllene (24.9%), the monoterpene linalool (24.4%), the octane derivatives 1-octen-3-ol (4.2%), 3-octanol (2.3%), trans-2-octen-3-ol (2.1%) and 3-octanone (2.0%) as well as hexane derivatives cis-hexen-3-ol (4.2%), hexanol (3.1%) and trans-2-hexen-1-ol (2.4%) as main compounds (concentrations higher than 2%,

calculated as % peak area using GC-FID with a non polar column). In addition more than 60 minor and 20 trace (concentration lower than 0.1%) components were found in the essential oil.

To get more information on the aroma compounds responsible for the characteristic odour of the essential oil, the head space of this *L. indica* sample was trapped using SPME and analysed by means of GC-FID and GC-MS. This is logical as an odour is elicited when the concerned compound reaches the olfactory buds in the nose. This is possible only when the compound is volatile. It was found that the composition of the SPME-head space sample is different only quantitatively but not qualitatively in accordance to the corresponding essential oil. Again β-caryophyllene (20.5%), linalool (19.8%), 1-Octen-3-ol (12.1%), cis-3-hexen-1-ol (11.6%), trans-2-hexen-1-ol (3.5%), hexanol (3.3%), 3-octanol (2.5%) and trans-2-octen-3-ol (2.3%) were identified as main compounds of this SPME-head space sample. Therefore, this analysis of the *Leucas indica* essential oil furnishes a more precise information about aroma-active constituents responsible for the characteristic aroma in qualitative and quantitative way. The green herbal notes observed for this oil were due to hexane derivatives (specially cis-3-hexen-1-ol), minor esters, mono and sesquiterpenes. Sweet fruity lovage-like odour can be attributed to some monoterpenes (eg: linalool and its derivatives) and sesquiterpenes (farnesene and β-bisabolene). Compounds of the essential oil of the fresh plant of *Leucas indica* and the corresponding solid phase micro extraction

head space sample (SPME) of the essential oil in the order of their Kovats indices (KI) using non polar column is given in the following table 4.1.

Table 4.1: Compounds of the essential oil of the fresh plants of *Leucas indica* and the corresponding SPME-headspace sample (SPME) of the essential oil in the order of their *Kovats indices* (KI, using a non-polar column) and concentrations in % (calculated as %-peak area of GC-FID analysis)

No.	Compounds	EO	SPME	KI
01	Isopropyl methyl ketone	0.2	nd	626
02	*cis*-1, 3, 5-Hexatriene	0.1	nd	640
03	*cis*-3-*Hexen*-I-ol	4.2	11.6	851
04	*trans*-3-Hexen-1-ol	1.4	0.8	856
05	*trans*-2-Hexen-1-ol	2.4	3.5	864
06	Hexanol	3.1	3.3	869
07	*cis*-3-Hexenyl formiate	0.3	tr	914
08	Isohexanol	0.1	nd	923
09	Camphene	0.7	0.5	952
10	1-Octen-3-ol	4.2	12.1	978
11	3-Octanone	2.0	0.6	982
12	β-Myrcene	0.4	0.5	984
13	*cis*-3-Hexenyl acetate	1.7	1.5	987
14	3-Octanol	2.3	2.5	990
15	*trans*-2-Octen-3-ol	2.1	2.3	999
16	*trans*-3-Hexenoic acid	0.4	0.1	1004
17	*cis*-4-Hexenoic acid	0.2	nd	1007
18	α-Terpinene	0.5	0.5	1016
19	*trans*-2-Hexenoic acid	0.3	tr	1023

20	Benzyl alcohol	1.4	1.8	1029
21	Acetophenone	1.2	1.8	1033
22	*trans*-2-Octen-1-ol	0.2	1.0	1041
23	γ-Terpinene	0.5	0.3	1056
24	Ocimenol	0.1	tr	1069
25	Guajacol	0.8	0.5	1071
26	Terpinolene	0.3	0.7	1074
27	*cis*-Linalool oxide	0.1	0.1	1081
28	*trans*-Linalool oxide	0.2	tr	1084
29	3-Methyl-1-hexanol	0.3	0.5	1089
30	Linalool	24.4	19.8	1094
31	2-Phenylethyl alcohol	1.6	1.8	1108
32	2-Methyl-1-octanol	0.1	nd	1119
33	*cis*-3-Hexenyl isobutyrate	tr	tr	1131
34	Camphor	0.8	0.3	1137
35	Benzyl acetate	0.6	0.5	1142
36	*trans*-2-Nonenal	0.2	tr	1147
37	Ethyl benzoate	0.5	nd	1153
38	Isoborneol	tr	tr	1157
39	*trans*-2-nonenol	0.5	0.1	1161
40	Borneol	0.2	0.1	1164
41	*cis*-3-Hexenyl butyrate	0.6	0.2	1169
42	Terpinen-4-ol	1.2	0.7	1173
43	*trans*-2-Hexyl butyrate	0.3	tr	1176
44	Ethyl octanoate	tr	nd	1181
45	α-Terpineol	1.1	1.1	1187

46	Octyl acetate	0.2	0.1	1190
47	Dodecane	tr	nd	1200
48	Methyl nonanoate	tr	tr	1207
49	Carveol	0.3	0.1	1216
50	Hexyl 2-methylbutyrate	tr	nd	1223
51	Pulegone	0.2	0.1	1228
52	2-Phenylethyl acetate	0.6	0.3	1236
53	Geraniol	0.3	0.4	1243
54	*trans*-2-Decenol	tr	nd	1252
55	Decanol	0.2	0.1	1263
56	Heptyl butyrate	tr	nd	1274
57	Propyl octanoate	tr	nd	1278
58	Bornyl acetate	0.1	tr	1280
59	Undecanol	0.1	tr	1285
60	Nonanoic acid	0.9	0.1	1288
61	Thymol	0.3	0.2	1291
62	Undecanal	tr	nd	1293
63	Nonyl acetate	tr	tr	1296
64	Tridecane	tr	nd	1300
65	Benzyl butyrate	0.2	0.1	1321
66	Octyl isobutyrate	tr	nd	1334
67	Eugenol	0.1	0.1	1351
68	Geranyl acetate	0.2	0.3	1362
69	*cis*-3-Hexenyl hexanoate	0.3	0.2	1367
70	Octyl butyrate	tr	nd	1371
71	Pentadecanol	0.2	0.1	1374

72	*trans*-2-Hexenyl hexanoate	tr	tr	1377
73	Decyl acetate	tr	nd	1393
74	α-Copaene	1.2	1.0	1398
75	β-Bourbonene	0.2	0.2	1412
76	Linalyl butyrate	0.8	0.5	1420
77	β-Caryophyllene	24.9	20.5	1426
78	α-Bergamotene	0.7	0.4	1435
79	*trans*-2, *cis*-6-Nonadienol	tr	tr	1442
80	α-Farnesene	0.2	tr	1459
81	α-Humulene	1.4	0.9	1466
82	β-Selinene	0.8	0.6	1477
83	Valencene	0.2	0.1	1483
84	α-Muurolene	0.3	0.2	1492
85	β-Bisabolene	0.3	0.1	1496
86	γ-Cadinene	1.3	1.2	1517
87	δ-Cadinene	0.4	0.3	1522
88	Nerolidol	0.9	0.8	1528
89	*cis*-3-Hexenyl benzoate	tr	nd	1551
90	Hexyl benzoate	tr	nd	1557
91	*cis*-3-Hexenyl octanoate	tr	tr	1563
92	Benzyl benzoate	0.4	0.1	1723
93	Palmitic acid	0.2	nd	1964

nd - not detected

For the sake of comparison the odour impression of the identified compounds of *Leucas* essential oil, taken from literature, also is given below[53,101-103] (Table 4.2)

Table 4.2: Compounds of the essential oil of the fresh plants of *Leucas indica* and their corresponding aroma impressions in accordance to published data

Compounds	Aroma impressions
Isopropyl methyl ketone	fruity
cis-1, 3, 5-Hexatriene	nad[1]
cis-3-Hexen-1-ol	green ("leaf alcohol"), fresh-grass-like
trans-3-Hexen-1-ol	intense green with bitter and fatty side-notes
trans-2-Hexen-1-ol	powerfull green-leafy with wine-like and fruity side-notes
Hexanol	alcoholic, ethereal
cis-3-Hexenyl formiate	green with vegetable-notes, slightly sweet
Isohexanol	alcoholic, medicinal-ethereal
Camphene	camphoraceous
1-Octen-3-ol	intense mushroom-like, forest-earthy, herbaceous
3-Octanone	herbaceous, spicy, buttery, mushroom-earthy
β-Myrcene	sweet-balsamic, plastic-side-note
cis-3-Hexenyl acetate	powerfull green, partly tea-leaf-like and fruity
3-Octanol	oily-nutty, herbaceous, melon and citrus side-notes
trans-2-Octen-3-ol	earthy, weak mushroom-like
trans-3-Hexenoic acid	diffuse cheese-like, mildly fruity
cis-4-Hexenoic acid	weak fruity, fatty
α-Terpinene	weak fresh-lemon-citrus-like
trans-2-Hexenoic acid	fatty, acrid musty odour, fruity, sweet on dilution

Benzyl alcohol	faint aromatic
Acetophenone	sweet, hawthorn-like, floral, almond-like, warm-aromatic
trans-2-Octen-1-ol	herbaceous, spicy, weak green-earthy
γ-Terpinene	herbaceous, citrus-notes
Ocimenol	diffuse and refreshing camphoraceous and lime-like, floral
Guajacol	burnt, smoky, medicinal, warm-woody
Terpinolene	sweet-piney, slightly sweet-anisic, plastic-like side-notes
cis-Linalool oxide	sweet-woody, floral-woody-earthy side-notes
trans-Linalool oxide	powerfull sweet-woody, floral
3-Methyl-1-hexanol	slightly fruity, alcoholic
Linalool	refreshing-clean-floral, citrus-lemon-orange notes
2-Phenylethyl alcohol	rose-like, honey-notes, floral
2-Methyl-1-octanol	fruity-green, slightly earthy
cis-3-Hexenyl isobutyrate	fruity-winey, sweet-green
Benzyl acetate	sweet, floral, fruity, fresh
trans-2-Nonenal	fatty-waxy
Ethyl benzoate	heavy, floral-fruity
Isoborneol	piney, camphoraceous
trans-2-nonenol	fruity-waxy
Borneol	camphoraceous, slightly sharp-earthy-peppery side-notes
cis-3-Hexenyl butyrate	wine-like, green, cognac- and brandy-like, slightly buttery
Terpinen-4-ol	spicy, nutmeg-like, woody-earthy, liliac-like
trans-2-Hexyl butyrate	green-fruity

Ethyl octanoate	floral, fruity, banana-like, pineapple-pear-like, brandy-notes
α-Terpineol	strong liliac odour, floral
Octyl acetate	fruity, floral, jasmin-notes, herbaceous
Dodecane	nad
Methyl nonanoate	wine-like, coconut-fatty-notes
Carveol	caraway and spearmint odour
Hexyl 2-methylbutyrate	sweet, fruity, green
Pulegone	diffuse herbaceous, mint-camphor-notes
2-Phenylethyl acetate	rose-like, sweet-honey side-notes
Geraniol	sweet-floral, rose-like, fruity
trans-2-Decenol	fatty-oily, waxy, rose-like side-notes
Decanol	floral-fruity, fatty-waxy
Heptyl butyrate	sweet, green, tea-like
Propyl octanoate	nad
Bornyl acetate	camphoraceous, pine-needle-like
Undecanol	citrus-lime-orange-lemon-mandarin-like, black currant, sweet
Nonanoic acid	cheese-like, waxy
Thymol	woody, burnt, smoky
Undecanal	fatty with orange and rose undertone
Nonyl acetate	fruity, soapy, gardenia-notes
Tridecane	nad
Benzyl butyrate	heavy, fruity, plum-like, floral
Octyl isobutyrate	fruity, citrus-notes, musty
Eugenol	strong spicy, cinnamon and clove-like
Geranyl acetate	rose and lavender-like, sweet-fruity

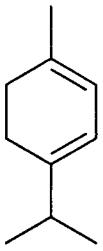
cis-3-Hexenyl hexanoate	diffuse green, fruity, pear-notes
Octyl butyrate	musty-fruity, weak citrus-notes
Pentadecanol	faint, bland-waxy, floral
trans-2-Hexenyl hexanoate	nad
Decyl acetate	orange and pineapple-like, rosy undertone
α-Copaene	weak woody
β-Bourbonene	vetiver-notes
Linalyl butyrate	bergamot-like, fruity, banana-like
β-Caryophyllene	terpene-odour, woody, spicy
α-Bergamotene	weak bergamot-like
trans-2, *cis*-6-Nonadienol	powerfull, green, vegetable-notes
α-Farnesene	mild, sweet, warm
α-Humulene	weak woody
β-Selinene	mild-warm-woody, herbaceous-peppery
Valencene	herbal-woody
α-Muurolene	nad
β-Bisabolene	pleasant-worm, sweet-spicy-balsamic
γ-Cadinene	mild, dry-woody, slightly tarry and medicinal
δ-Cadinene	woody, mild-medicinal
Nerolidol	rose and apple-like, green-citrus-like, woody-waxy
cis-3-Hexenyl benzoate	green, herbaceous, woody
Hexyl benzoate	woody, green, balsamic
cis-3-Hexenyl octanoate	nad
Benzyl benzoate	faint-sweet, balsamic, floral undertone
Palmitic acid	odour-less

nad - *no aroma* data available in the used literature.

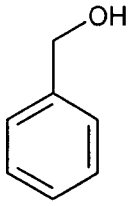
It can be concluded that hexane and octane derivatives, acetophenone, mono and sesquiterpenes, minor esters etc. are responsible for the charactersitic odour of the essential oil of fresh plants of *Leucas indica*. 1-octen-3-ol is the main aroma intense compound among octane derivatives.

Structures of the compounds identified but not given in other chapters are as follows:

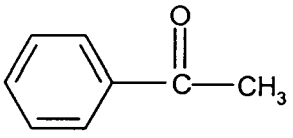
Composition of the essential oil of the fresh plants of *Leucas indica* from South India



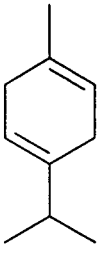
α-Terpinene



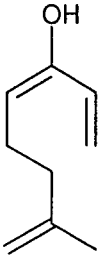
Benzyl alcohol



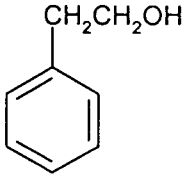
Acetophenone



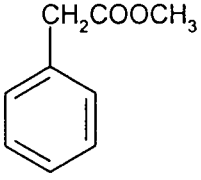
γ-Terpinene



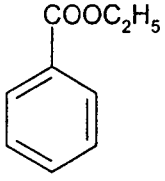
Ocimenol



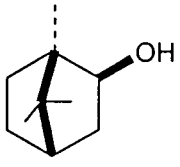
2-Phenylethyl alcohol



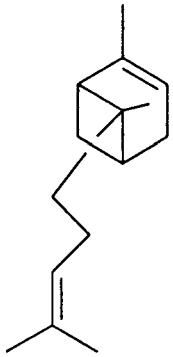
Benzyl acetate



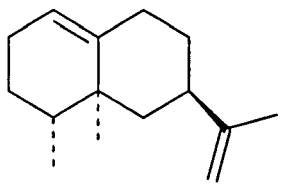
Ethyl benzoate



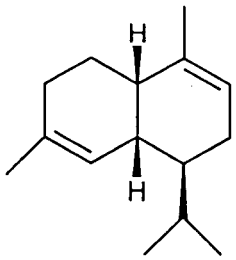
Isoborneol



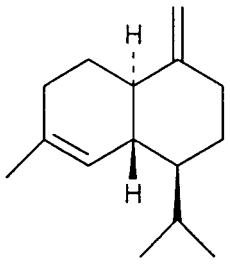
Bergamotene



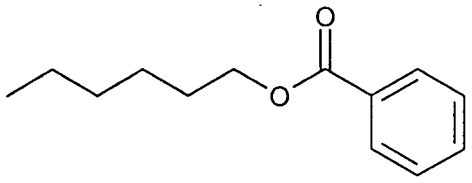
Valancene



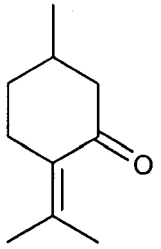
α-Muurolene



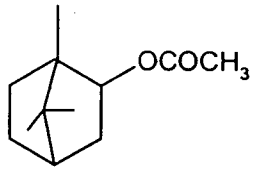
γ-Cadinene



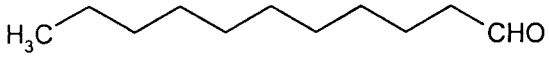
Hexyl benzoate



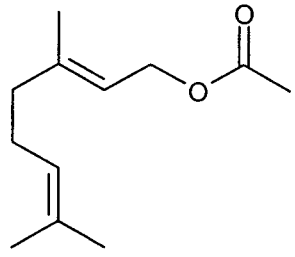
Pulegone



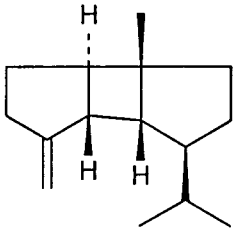
Bornyl acetate



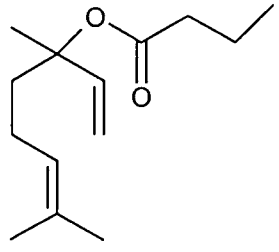
Undecanal



Geranyl acetate



β-Bourbonene



Linalyl butyrate

SYZYGIUM CUMINI

CHAPTER 5

ANALYSIS OF THE ESSENTIAL OIL OF THE FRESH LEAVES OF *SYZYGIUM CUMINI* (LINN.) SKEELS

Syzygium cumini (Syn. *Eugenia jambolana*) belongs to the *Myrtaceae* family which consists of about hundred genera and three thousand species. The main centre of its distribution is in tropical America and secondary centre is in Australia. The original home of *Syzygium cumini* is India or the East Indies. Myrtaceae family consists of giant trees and shrubs.[104] Throughout India this tree is found in moist localities of all forests upto 1,800 m usually along river banks and also cultivated as shade trees along road sides. *Syzygium cumini* is excluded from aquatic plants' lists because these are confined to the marine environment.[105]

Syzygium cumini is a smooth tree of four to fifteen metres in height. Sweet smelling leaves are leathery oblong-ovate to elliptic or obovate and six to twelve centimeter long, the tip being broad and shortly pointed. The panicles are borne mostly from the branchlets below the leaves, often being axillary or terminal and are four to six centimeter long. The flowers are numerous and scented. It is coloured pink or nearly white. The calyx is funnel shaped about 4 mm long and four toothed. The petals cohere and fall all together as a small disk. The stamens are very numerous and as long as the calyx. Fruit is oval to elliptic; 1.5 to 3.5 cm

long. The colour of the fruit is dark purple or nearly black. It is luscious, fleshy, edible and contains a single large seed.[106]

The members of this family are either astringent and tonic or aromatic and stimulant according as tannin or the essential oil predominates. Bark, leaves and fruits of *S. cumini* are found to be very useful due to their medicinal value. Kerala physicians recognise two varieties of *Syzygium* species: Locally called naval and nara which together is believed to constitute what is called Jambu dvaya (A pair of Jambu). Naval is usually equated with *S. cumini* and nara with *Syzygium caryophyllatum*. Rheede has illustrated these species under the vernacular Perin-njara and Njara respectively.[84]

PROPERTIES AND USES OF *S. CUMINI*

The bark is astringent, sweet, sour, acrid, refrigerant, carminative, diuretic, digestive, anthelmintic, stomachic and antibacterial. It is useful in diabetes, leucorrhoea, gastropathy and dermatopathy.[107] The fresh juice of the bark is given with goat's milk in the diarrhoea of children.[108] Decoction of dried bark is taken orally for venereal ulcers and also as a central nervous system stimulant.[109]

The leaves are antibacterial. The ash of the leaves of *Syzygium cumini* is used for strengthening the teeth and the gums. The tender leaves are used to arrest vomitting. The expressed juice of the leaves is used alone or in combination with other astringents in dysentery. Leaves are taken orally for leucorrhoea. The ash is

used externally to relieve the itching caused by centipede bite. Decoction of the root is taken as an antiemetic and to increase lactation in new mothers. Fruit is sour, acrid and sweet. It is a general tonic specially good for liver. It enriches the blood. It is a good lotion for ring-worm. The vinegar from the fruit is also a tonic, astringent, carminative and useful in diseases of the spleen.[110] Ayurvedic texts mention several types of drugs namely Mahajambuh and Rajajambuh prepared from bark and fruits of *Syzygyium cumini*.[84]

CUMINI AS TRADITIONAL MEDICINE

Dried leaves and dried bark are used to treat diabetes. In Brazil decoction of dried leaves is taken orally to treat diabetes.[111] In India decoction and fluid extract of dried bark is taken orally for diabetes.[112,113] Hot water extract of dried bark is taken orally for indigestion and as a blood purifier. Fruits are eaten to cure gastro-intestinal complaints. Hot water extract of dried fruits is used externally as an astringent and orally for stomach ulcers and to reduce acidity.[114]

Fluid extract of seeds is taken orally as an anti-inflammatory and hot water extract is an antipyretic. Fresh leaf juice is taken orally for blood pressure.[113,115-117]

Stembark juice is taken in for constipation and it is found useful to stop blood discharge in the feces when consumed mixing it with buttermilk. The experiment carried out shows that methanol extract of dried seeds, administered

intraperitoneally to mice at a dose of 25 mg/kg was active against acetic acid-induced writhing and was helpful in obtaining significant result.[118]

PREVIOUSLY REPORTED WORKS

Antidiabetic activity of the seed kernel of *Syzygium cumini* is well known. The hypoglycaemic activity of an aqueous suspension of the dried seed kernels of *S. cumini* was studied by Bhaskaran Nair and coworkers. Two standard hypoglycaemic drugs, tolbutamide and phenformin were used for comparitive study.[119] The results indicate that seed kernel of *S. cumini* exhibited maximum hypoglycaemic activity (4 g/kg dose).

The essential oils of the leaves, stem and fruits were chemically analysed. α-pinene, β-pinene limonene, cis and trans ocimene, bornyl acetate, β-caryophyllene, α-humulene are the major components found in leaf essential oil. Of the stem essential oil the predominant components are α-pinene, camphene, β-pinene, myrcene, limonene, cis and trans ocimene, bornyl acetate, α-copaene, α-humulene and δ-cadinene. The fruit essential oil was found to contain α-humulene and δ-cardinene. The fruit essential oil was found to contain α-pinene, camphene, β-pinene, myrcene, limonene, cis-trans ocimene, α-humulene as major components.[120]

Chemical examination of the essential oil from the leaves of *Syzygium cumini* was conducted by Khanna and he observed a sweet raw mango like aromatic odour comprising of fiftynine percent hydrocarbons and the rest were oxygenated derivatives. Major components were myrcene, β-pinene, γ-terpinene, terpinolene, β-phellandrene and bornylene. The oxygenated derivatives were methyl cinnamate, cuminaldehyde, α-terpineol, eugenol and borneol. α-Pinene, α-thujene, β-caryophyllene, nonyl alcohol, linalool, piperitone, safrole and a sesquiterpene hydrocarbon giving positive test for azulenes were also identified.[121]

A comparative study on extracts of *S. cumini* barks of different ages on dysentery and diarrhoea forming micro-organism showed the barks of young plants to have a better inhibitory effect on micro-organisms like *Salmonella, Viballerup, Shigella boydii* etc. Results showed that the water soluble fraction of the ethanolic extract of barks of the five year old plants had better inhibition effect on the above microorganisms.[122]

Antibacterial activity of Syzygium species were studied by Chattopadhyay and Sinha and found that ethanolic extracts of *S. andamanicum* and *S. cumini* stem bark inhibited the growth of all gram positive and most of the gram negative bacteria which were tested.[123]

S. cumini seeds may be acting as hypoglycaemic agents by increasing the insulin content through cathepsin B. The effects of oral administration of *S. cumini*

seeds and chloropropamide on blood glucose level and pancreatic cathepsin B in rat was studied and reported by Renu and coworkers.[124]

PRESENT WORK

The essential oil of the leaves of *S. cumini* (L.) Skeel was investigated by Gas chromatographic, Spectroscopic (GC-FID and GC-MS) and olfactoric methods to identify those compounds responsible for the characteristic odour and use of this plant in folk medicines.

EXPERIMENTAL

Plant Material

The leaves of *Syzygium cumini* was collected from the Calicut University Campus in May 1999 and the plant material was identified by Dr. A.K. Pradeep, Department of Botany, Calicut University of Kerala. A voucher specimen (No. 19) was deposited at the herbarium of the Department of Chemistry at Calicut University.

Essential Oil Extraction

The fresh leaves of *S. cumini* (400 g) were cut into small pieces and ground into a paste using an electric grinder. This paste was steam distilled for three hours. The distillate was extracted with diethyl ether (2 x 100 mL). It was then dried over

anhydrous sodium sulphate. After the removal of the solvent, pale yellow coloured essential oil (0.2 g) 0.05% of fresh weight of the leaves was obtained.

Olfactoric Evaluation

The essential oil was diluted with dichloromethane and evaluated olfactometrically 10 μL of the diluted essential oil was placed on a commercial odorostrip (Dragoco Co.) and its odour characaterised by professional perfumers.

Gas Chromatography

GC analysis were carried out using a Shimadzu GC-14A with FID and the integrator C-R6A-Chromatopac and a Varian GC-3700 with FID and the integrator C-RIB Chromatopac (Shimadzu Co). The columns of 30 m x 0.32 mm bonded unpolar FSOT-RSL-200 fused silica (film thickness 0.25 μm, Biorad Co.) and a 30 m x 0.32 mm bonded polar stabilwax (film thickness: 0.5 μm; Restek Co.) were used. Hydrogen was used as carrier gas. Injector-temperature 250°C, detector temperature 320°C, programme temperature 40°C/5min to 280°C/5 min., with a heating-rate of 6°C/min; quantifications by percent peak area-calculations (unpolar column). Some single components were identified by co-injection of pure compounds and correlation of their retention times (using Kovats indices) with published data.[36,38,39]

Gas-Chromatography – Mass Spectrometry

The *S. cumini* leaf essential oil was analysed by the GC-MS system Shimadzu GC-17A with QP 5000 and the data system Compaq-ProLinea class 5k-software) Shimadzu GC-17A with QP 5050 and data system Pentium II (Bohm Co, Class 5k-software) Hewlett Packard GC-HP 5890 with HP-5970 MSD and PC Pentium (Bohn Co, Chemstation – software) and Finigan MAT GCQ with data system Gateway 2000 – PS75 (Siemens Co, GCQ-software). Carrier gas used was helium, injector temperature 250°C, interface heating, 300°C, ion source heating 200°C EI mode, scan range 41-450 amu. For compound identification Wiley, NBS and NIST – Library Spectra (on line) as well as reference MS –Spectra data were used.[38,49]

RESULTS AND DISCUSSION

The essential oil was olfactorically evaluated as fresh-green-pinene like fruity-herbal-spicy (direction of Juniper berry oil) and in the background woody.

Using gas chromatographic – Spectroscopic system sixty four components could be identified in the essential oil of the leaves of *S. cumini*. The compounds identified and the corresponding concentration (%) are given in the following table.

Table 5.1: Composition of the essential oil of the leaves of *Syzygium cumini* from South-India

Compounds	Concentration (%)
Pinocarveol	15.1
α-Terpineol	8.9
Myrtenol	8.3
Eucarvone	6.6
Muurolol	6.4
Myrtenal	5.8
Geranyl acetone	5.6
α-Cadinol	4.6
Pinocarvone	4.4
trans-pinane	3.8
δ-Cadinol	3.5
para-Cymen-8-ol	2.7
cis-Carveol	2.2
Limonene oxide	1.8
Longipinene epoxide	1.6
Carvone	1.4
Bornyl acetate	1.2
Isopropyl formate	0.9
cis-3-Hexen-I-ol	0.9
cis-3-Hexenyl acetate	0.9
Dihydrocarvyl acetate	0.9
Perilla alcohol	0.8
α-Pinene	0.8
Fenchol	0.8
β-Terpineol	0.7
β-Pinene	0.7

Benzyl acetate	0.7
trans-β-Caryophyllene	0.6
Globulol	0.6
cis-2-Heptenal	0.5
Acetic acid	0.5
Verbenol	0.4
α-Pinene oxide	0.4
3-Penten-2-ol	0.3
2-Hexenal	0.2
Butyl acetate	0.2
Caryphyllene oxide	0.2
Nerolido epoxy acetate	0.2
Perilla aldehyde	0.1
Borneol	0.1
6-Methyl-5-Hepten-2-one	0.1
Citral	0.1
Linalool	0.1
Cumin aldehyde	0.1
Linalool oxide	0.1
β-Pinene oxide	0.1
9, 12-Octadecadienal	tr
α-Cadinene	tr
α-Terpinyl acetate	tr
Thujyl alcohol	tr
δ-Cadinene	tr
Hexanol	tr
α-Copaene	tr
β-Elemene	tr
Geraniol	tr

α-Humulene	tr
Ledol	tr
α-Bisabolol	tr
(more than 16C)	0.3
α-Bisabolol	tr
Tetradecanoic acid	0.7
Hexadecanoic acid	1.1
Hexadecanoic acid Methyl ester	0.3
Octadecanoic acid	0.1
Octadecane	0.1
Eicosane	0.2

The identified main compounds of the essential oil of the leaves of *S. cumini* are pinocarveol (15.1%), α-terpineol (8.9%), myrtenol (8.3%), eucarvone (6.6%), muurolol (6.4%), myrtenal (5.8%), geranyl acetone (5.6%), α-cadinol (4.6%) and pinocarvone (4.4%). Nearly forty side compounds especially mono and sesquiterpenes in a concentration range of 0.1% to 3.8% and twelve trace-components are additional constituents of this essential oil.

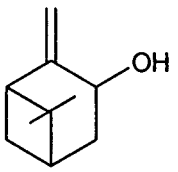
The fresh-green-pinene like odour impression has been attributed to the major components pinocarveol, eucarvone as well as hexane derivatives. The fruity herbal-spicy note is due to myrtenol, myrtenal, geranyl acetone and α-terpineol, α-cadinol together with pinene and caryophllene derivatives are responsible for the woody odour impression.

This essential oil of the leaves of *S. cumini* can play an important role in fine-perfumery where fresh-green-pinene odour notes are required. eg: in shower gels, deodourants, etc.

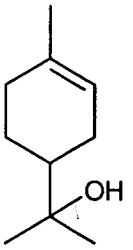
Pinocarveol, pinocarvone, eucarvone and other constituents of the leaf essential oil of *S. cumini* show positive effects in the treatment of coughs and colds and seem to be of interest for this reason. As mono and sesquiterpenes are known for their antimicrobial activity, many of the constituents identified show biological activities increasing the scope of various applications of this essential oil. The antibacterial and antifungal studies of this oil are included in one of the following chapters of this thesis.

Structures of the identified compounds and not included in earlier chapters are given below.

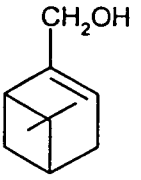
Composition of the essential oil of the leaves of *Syzygium cumini*



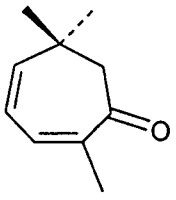
Pinocarveol



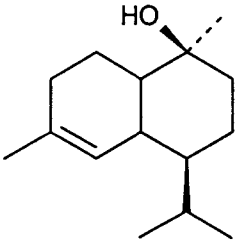
α-Terpineol



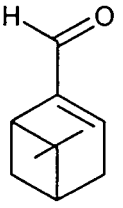
Myrtenol



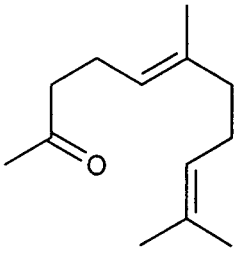
Eucarvone



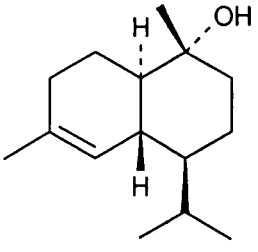
Muurolol



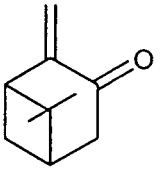
Myrtenal



Geranyl acetone



α-Cadinol



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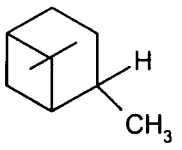

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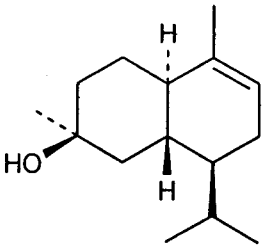

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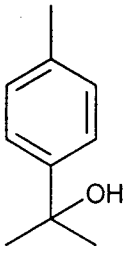

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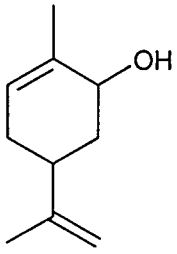

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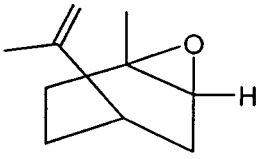

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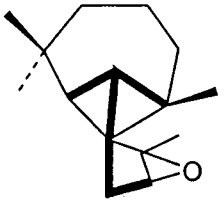

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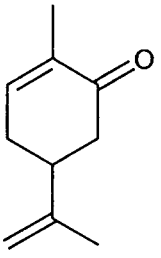

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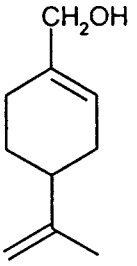

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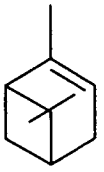

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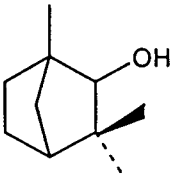

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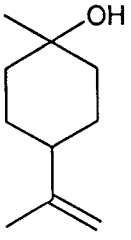

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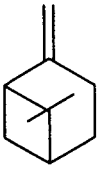

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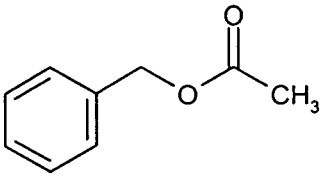

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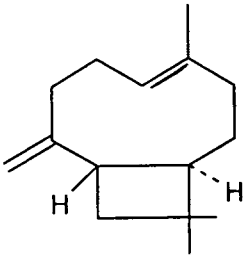

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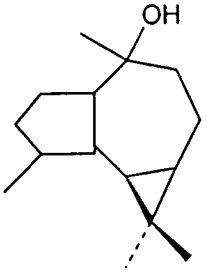

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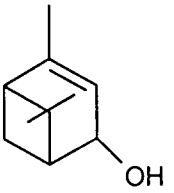

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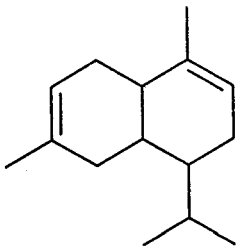

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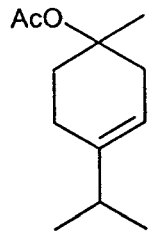

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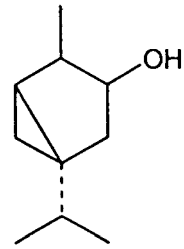

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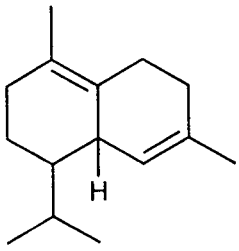

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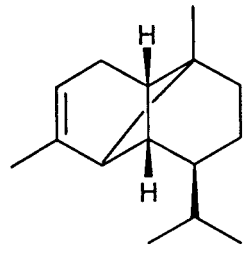

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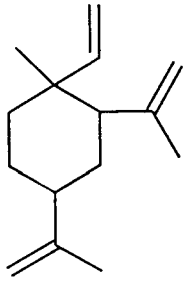

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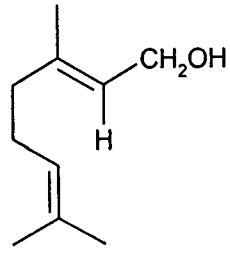

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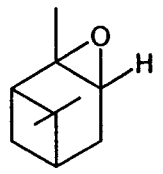

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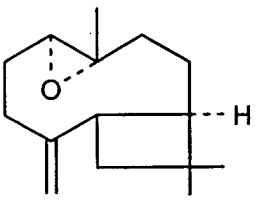

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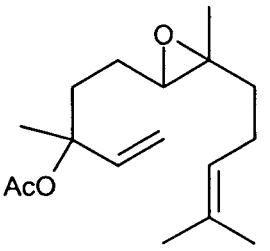

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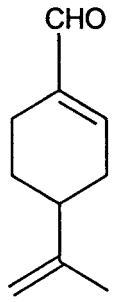

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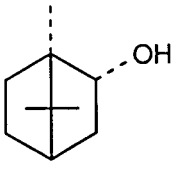

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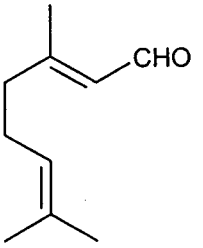

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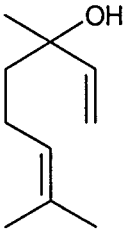

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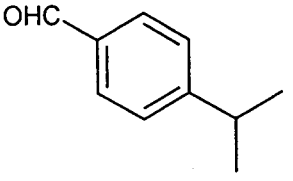

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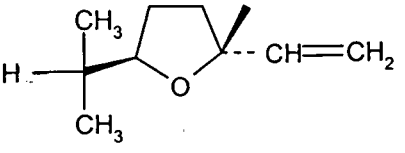

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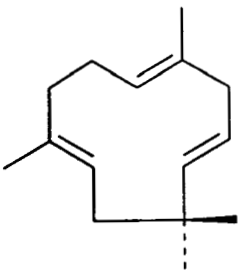
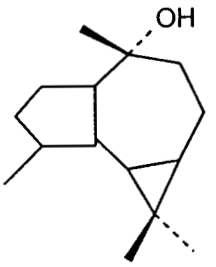


α-Humulene



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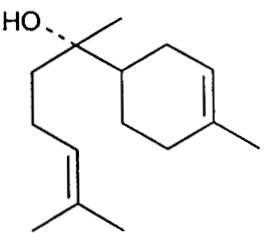
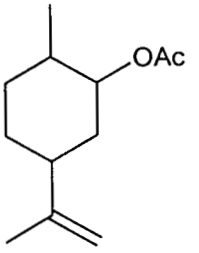


α-Bisabolol



Dihydrocarvyl acetate

CHAPTER 6

EVALUATION OF ANTIMICROBIAL PROPERTIES OF *SYZYGIUM CUMINI, SYZYGIUM TRAVANCORICUM* AND *ARISTOLOCHIA INDICA* ESSENTIAL OILS

SECTION I.

INTRODUCTION TO ANTIMICROBIAL STUDIES

Microorganisms are closely associated with the health and welfare of human beings: some of them are beneficial and others are detrimental. For example microorganisms are involved in the making of yogurt, cheese and wine; in the production of penicillin, interferon and alcohol; and in the processing of domestic and industrial waste. Microorganisms can cause disease, spoil food, and deteriorate materials like iron pipes, glass lenses and wood pilings. Microorganisms have a wider range of physiological and biochemical potentialities than all other organisms combined. For example, some bacteria are able to utilize atmospheric nitrogen for the synthesis of proteins and other complex organic nitrogenous compounds.

Each kind of microorganisms has specific growth requirements. Many microorganisms can be grown in or on a mixture of nutrients used in the laboratory to support growth and multiplication of microorganisms (culture medium). Some can grow in a medium containing only inorganic compounds, whereas others require a medium containing organic compounds (amino acids, vitamins or coenzymes). Some others require complex natural substances (peptone, blood

serum, etc.) and microorganisms like rickettsias cannot be grown in an artificial laboratory medium. On solid media microbes grow as colonies.

BACTERIA

The bacteria include organisms that possess rigid cell walls and when motile have flagella. One of the most important cytological features of bacteria is their reaction to a simple staining procedure called the gram stain. The procedure involves staining the cells with the dye crystal violet; all bacteria will be stained blue. The bacteria are next treated with an iodine solution and then decolourised with alcohol. Gram positive bacteria retain the crystal violet; Gram negative bacteria which lose the crystal violet, are counterstained by the safranin and hence appear red in colour.[125] The most plausible explanation for this difference in behaviour lies in the relative differences between the cell walls of the above two types of bacteria. Difference in the thickness of their cell walls is important. The cell walls of Gram negative bacteria are generally thinner (10 to 15 nm) than those of Gram positive bacteria (20 to 25 nm). Gram-negative bacteria contain a higher percentage of lipid than that of gram-positive bacteria. The cell wall of a bacterium is easily seen in the electron microscope. It usually appears as an envelope 50-100A° thick. The various structures of a bacterial cell differ from one another not only in their physical features but also in their chemical characteristics and in their functions. For example the gram-negative bacteria are more resistant to many antibiotics than Gram-positive bacteria.

Cell wall is very rigid structure that gives shape to the cell. Bacterial cell walls are usually essential for bacterial growth and division. Cells whose walls have been completely removed are incapable of normal growth and division. Bacterial cell walls are of considerable medical significance, because they are responsible for bacterial virulence. The shape determining part of the cell wall is largely peptidoglycan, an insoluble, porous, heteropolymer of alternating linked N-acetylglucosamine and N-acetyl muramic acid units.

As mentioned earlier the cell walls of gram positive bacteria is thick. The amount of peptidoglycan which is extensively cross linked is high and the lipid content is very less. The cell wall appears uniform and is devoid of outer membrane. It contains teichoic acid. The walls of many gram-positive bacteria can easily be destroyed by treating with lysozyme enzyme. It is also characterised by the simplicity of its amino acid composition.

The cell walls of Gram-negative bacteria are more complex, its appearance is not uniform and is composed of two layers. The outer membrane present, that surrounds a thin layer of peptidoglycan. This membrane serves as an impermeable barrier to prevent the escape of important enzymes, such as those involved in cell wall growth, from the space between the cytoplasmic membrane and the outer membrane. The outer membrane also serves as a barrier to various external chemicals and enzymes that could damage the cell. Percentage of peptidoglycan is low while the lipid content is high. Teichoic acid is absent. It involves low degree

of cross linkage. Lipopoly saccharides are the dominant surface feature of the outer membrane of gram-negative bacteria such as *E. coli* and *Salmonella typhimurium.*

Modes of Action of Antimicrobial Agents

Micro-organisms can be removed, inhibited or killed by various physical and chemical agents. Many antimicrobial agents affect more than one cellular target and many inflict both primary and secondary damages that eventually lead to cell death. The manner in which antimicrobial agents inhibit or kill can be attributed to the following kinds of actions.[126]

(i) Damage to the cell wall or inhibition of cell-wall synthesis

Several types of chemical agents damage the cell wall by blocking its synthesis, digesting it or breaking down its surface. Thus the structural integrity of bacterial and fungal cells get spoiled.

(ii) Alteration of the Permeability of the Cytoplastic membrane

When the cell membrane is disrupted a cell loses its selective permeability and can neither prevent the loss of vital molecules nor bar the entry of damaging chemicals.

(iii) Inhibition of enzyme action

Since microbial life depends upon an orderly and continuous supply of proteins to function as enzymes and structural molecules, inhibition of enzyme action will damage the microbial life too.

(iv) Alteration in protein function

Microbial cell functions properly only if proteins remain in a normal three dimensional configuration called native state. Some agents disrupt or denature proteins. Chemicals such as strong solvents (alcohols, acid and phenolics) also coagulate proteins. Other antimicrobial agents such as metallic ions attach to the active site of the protein and prevent it from interacting with its correct substrate. Such losses in normal protein function can arrest metabolism. In short a large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or to kill them.

Development of Resistance to Antibiotics

Drug resistance is one of nature's never-ending processes whereby organisms develop a tolerance for new environmental conditions. Drug resistance may be due to a pre-existing factor in the microorganism or it may be due to some acquired factors. For example, penicillin resistance may result from the production

of penicillinase by resistant organisms, which converts penicillin into inactive penicilloic acid.

Fungi

Fungi are widely distributed and are found wherever moisture is present. They are of great importance to human being in both beneficial and harmful ways. Fungi exist primarily as filamentous hyphae. A mass of hyphae is called a mycelium. Fungi are primarily terrestrial organisms, although a few are fresh water or marine. Many are pathogenic and infect plants and animals.[127]

The body or vegetative structure of a fungus is called a thallus. It varies in complexity and size, ranging from the single cell microscopic yeasts to multicellular molds, macroscopic puff balls and mushrooms. The fungal cell usually is encased in a cell wall of chitin. Chitin is a strong but flexible nitrogen-containing polysaccharide consisting of N-acetyl glucosamine residues.

Fungi grow best in dark, moist habitats, but they are found wherever organic material is available. Like many bacteria, fungi can secrete hydrolytic enzymes that digest external substrates. Then they absorb the soluble products. They are chemoorganoheterotrophs and use organic material as a source of carbon and energy.

Glycogen is the primary storage polysaccharide in fungi. Most fungi use carbohydrates (glucose or maltose) and nitrogeneous compounds to synthesize their own amino acids and proteins. Fungi usually are aerobic. Some yeasts however are faculatively anaerobic and can obtain energy by fermentation, such as in the production of ethylalcohol from glucose. Fungi are important decomposers that break down organic matter, they are used as research tools in the study of fundamental biological processes.

Fungi as Disease Agents

Fungi are known to cause infections and allergies. Fungi and their by-products such as (1-3)-β-D-glucan, mycotoxins, volatile organic copounds have also been implicated in other diseases and health effects. Mycoses are of two types: endemic and opportunistic. Former is related to the geographical distribution of certain fungal pathogens and latter are complications that occur in patients with weakened immune systems. Cryptococcosis is considered the most dangerous fungal disease in humans. It affects lungs and the meninges, the covering of the brain and spinal cord. Dermatomycosis is a general name for a fungal disease of the hair, skin and nails caused by a wide variety of fungi.[128]

Fungi have been associated with a number of allergic disorders in humans. The major allergic disorders caused by fungi are allergic asthma, rhinitis, sinusitis, bronchopulmonary mycoses and hypersensitivity pneumonitis. Sick building

syndrome is another major disease recognized recently in industrialized countries. The highly potent toxins produced by fungi are believed to be responsible for this devastating disease. About sixty species of fungi have been reported to be associated with human allergy and this number is rapidly growing as new fungi are added as allergens.[129] The fungi that cause superficial mycoses frequently spread from animals to humans.

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS

Minoterpenes and sesquiterpenes are the main constituents of essential oils. Many of the monoterpenes and their derivatives are important agents of insect toxicity. Recent research in the United States and the Netherlands has revealed an interesting twist on the role of volatile terpenes in plant protection. In corn, cotton and other species certain monoterpenes and sesquiterpenes are produced and emitted only after insect feeding has already begun. These substances attract natural enemies including predatory and parasitic insects that kill plant feeding insects and so help to minimize further damage. Thus volatile terpenes are not only defenses in their own right but provide a way for plants to enlist defensive help from other organisms. It is interesting to note that a mixture of basil and eucalyptus oil will kill hundred percent mosquito larvae at a concentration two to six times lower than individual oils.

Recently carvone, a monoterpene isolated from the essential oil of carumcarvi has shown its ability to inhibit the sprouting of potatoes during storage as well as fungicidal activity in protecting tubers from rooting without exhibiting mammalian toxicity.[130]

Essential oil of *Salvia officinalis* has also shown practical potency in enhancing the storage life of some vegetables by protecting them from fungal rootings.[131] There are several reports on the antifungal activity of essential oils. Essential oils from different plant species are known to exhibit various kinds of biological activities including antifungal, antimicrobial, cytostatic, insecticidal, allelopathic, antitoxidant and bioregulatory actions.[132]

From the essential oil yielding plants, those possessing bioactive potential against fungal and bacterial pathogens have been estimated to be about thirty eight and thirty four percent respectively. Out of the bioactive essential oil bearing plants, seventeen percent belong to the family Labiaceae followed by Compositeae, Umbelliferae, Myrtaceae and others. Because of their notable antimicrobial activity coupled with pleasing flavour, the essential oils can be used to treat microbial infections such as skin diseases.

The volatile oils of black pepper, clove, germanium oregano were assessed for antibacterial activity against twenty five different genera of bacteria. These included animal and plant pathogens and spoilage bacteria.[133] Essential oils

extracted from plants such as mentha, piperila L, *Lavandula officinalis* and from roots and flowers of radish are known to exhibit antimicrobial activity.[134] Essential oils on wood destroying fungi and phytopathogenic fungi has been studied in detail by Maruzzella and coworkers.[135]

SECTION II.

EVALUATION OF ANTIMICROBIAL PROPERTIES OF *SYZYGIUM CUMINI, SYZYGIUM TRAVANCORICUM* AND *ARISTOLOCHIA INDICA* ESSENTIAL OILS

Microbiological assays are used for the quantitative determination of antibiotics and inhibitory chemical agents and also the determination of the sensitivity of micro-organisms to these agents. A large number of plants belonging to Myrtaceae family are known to exhibit antimicrobial properties. Hence it was thought worthwhile to test the leaf essential oils of *Syzygium cumini* and *Syzygium travancoricum* for their antibacterial property against three gram positive bacteria – *Basillus sphaericus, Basillus subtilis, Staphylococcus aureus* and three gram negative bacteria – *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium*. The essential oil of *Aristolochia indica* of *Aristolochiaceae* family was also studied. Disk diffusion method was employed for this purpose.

The antimicrobial property exhibited by an essential oil is the combined effect of its constituents. The activity of individual components will invariably be different from one another, however isolation of each and every component from an essential oil, in order to study their individual properties is practically impossible. In this work the essential oil from *S. cumini* was separated into five different fractions by column chromatography and also by distillation. The composition of these five fractions were determined by GC and GC-MS. The

antibacterial property of all these five fractions also was investigated. This approach was helpful to access the relative efficacy of constituents to control the growth of bacteria. The antifungal properties of *S. cumini* essential oil also was studied.

WORK REPORTED

Syzygium cumini leaf extract is known to produce inhibitory effect against *Aerobactor aerogenes, Basillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*.[46,136,137] From the antibacterial point of view *S. cumini* was found to be more potent than *S. andamanicum* and *S. samarangense*. Ethanolic extracts of *S. andamanicum* and *S. cumini* stem bark inhibited the growth of all gram positive and most of the gram negative bacteria tested.[135]

Essential oils on wood destroying fungi and phytopathogenic fungi has been studied in detail and is found that the oils from onion and garlic exhibit high activity.[138,139] Essential oils and their volatile substances such as thymol and menthol have been used as antibacterial agents for a long time. Several essential oils possessing substances which may be bactericidal or bacteriostatic in nature have shown very encouraging results in laboratory experiments.[140-143] The study of the antibacterial and antifungal properties of essential oils from different species of the same family shows that 1:1 combination of the oils were more effective than the individual oils.[144,145]

PRESENT WORK

Plant Material

S. cumini, S. travancoricum and *Aristolochia indica* were collected from the Calicut University Campus and surroundings as given in previous chapters. These three plants are known to exhibit medicinal properties. The essential oils to be tested were extracted from fresh leaves by hydrodistillation method as explained earlier.

EXPERIMENTAL

(i) ANTIBACTERIAL ACTIVITY

The nutrient agar culture medium was prepared by dissolving readymade nutrient agar in distilled water (23 mL/lit.) by heating till it boiled and then sterilising by autoclaving at 15 lbs pressure (121°C for 20 min.). The medium was poured into sterile Petridishes and allowed to solidify and dry. The inoculum was inoculated uniformly over the medium. Within 15 minutes after the plates were inoculated small paper disks impregnated with known amount (5 μL/disk) of essential oil was applied to the surface of the inoculated plates with sterile forceps. Disks were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface. The spatial arrangement of the disks were not closer than 15 mm to the edges of the plate and far enough apart to prevent overlapping of zones of inhibition. Within 15 minutes after the disks were applied, the plates were

inverted and placed in an incubator at 35°C, until bacterial growth was observed. After 16 to 18 hours of incubation, the plates were examined and the diameter of the zone of complete inhibition was measured to the nearest whole millimeter by sliding calipers.

(ii) ANTIFUNGAL ACTIVITY

The readymade Potato Dextrose Agar (PDA) medium (Himedia 39 g) was dissolved in distilled water (1000 mL) and heated to boiling until it dissolved completely. The medium and Petridishes were autoclaved at pressure of 15 lb/inc^2 for 20 min. The medium was poured into sterile Petridishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (one week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the essential oil in DMSO (Dimethyl Sulphoxide) and different concentrations were made (5, 10, 20 µg). After inoculation, cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentrations of test solutions (5, 10, 20 µg) were added. Controls were maintained with DMSO and Nystatin (20 µg). The treated and the controls were kept in an incubator at room temperature for 24 h to 96 h. Inhibition zones were measured and diameter was calculated in millimeter. Three to four replicates were maintained for each treatment.

The volatile fraction of *S. cumini* and *S. travancoricum* were tested for their antibacterial activities. The micro-organisms (gram positive and gram negative bacteria) were cultured at IICT, Hyderabad. The chemical analysis of the essential oils and the identification of various compounds present in them help us to find out the cause of microbial activity of these oils. The concentration of essential oil used in each evaluation was 5 μL of essential oil or 5 μL of 30 mg/mL solution of benzyl penicillin sodium (PEN-G). The result obtained during the antibacterial evaluation of the two essential oils are tabulated below (Table 6.1).

Table 6.1. Antibacterial Activity of *S. cumini* and *S. travancoricum* Essential Oils

Bacteria	Zone of inhibition (mm)		PEN-G
	S. cumini	*S. travancoricum*	
Basillus sphaericus	16	11	20
Basillus subtilis	13	10	19
Staphylococcus aureus	14	12	18
Escherichia coli	12	11	9
Pseudomonas aeruginosa	17	11	8
Salmonella typhimurium	20	12	10

Under the same conditions the antibacterial activity of *Aristolochia indica* essential oil was also evaluated (Table 6.2).

Table 6.2. Antibacterial activity of *Aristolochia indica* essential oils

Bacteria	Zone of inhibition *Aristolochia indica*	PEN-G
Basillus sphaericus	7	20
Basillus subtilis	9	19
Staphylococcus aureus	8	18
Escherichia coli	8	9
Pseudomonas aeruginosa	10	8
Salmonella typhimurium	7	10

S. cumini essential oil was found to be not only antibacterial but also antifungal. It was tested for their antifungal activity against five fungi – *Aspergillus niger, Aspergillus terreus, Chrysosporium tropium, Cladosporium cladosporiodes* and *Rhizopus oryzae*. Concentration of the essential oil used was 10 µL/disc. Negative control used was diethylether (concentration 10 µL/disc). The concentration of positive control Nystatin was 20 µg. Zone of inhibition was along with 6 mm disc diameter. The result obtained is given below.

Table 6.3. Antifungal activity of *S. cumini* leaf essential oil

Compounds	Zone of Inhibition (mm)				
	Aspergillus niger	*Aspergillus terreus*	*Chrysoporium tropicum*	*Cladosporium cladosporioides*	*Rhizopus oryzae*
S. cumini oil	24	16	13	17	11
Negative control (diethyl ether)	--	--	--	--	--
Positive control (Nystatin)	28	34	18	22	19

Results and Discussion

Plant extracts of *Syzygium* species are well known antibacterials.[123,137,144] The antibacterial activity of *S. cumini* essential oil was found to be good while that of *S. travancoricum* was moderate. Essential oils of plants belonging to the family Myrtaceae are known for their biological activities which is attributed to the presence of 1,8-cineole.[146] Though *S. cumini* and *S. travancoricum* essential oils tested did not contain 1,8-cineole, they showed considerable antibacterial activity especially against *Salmonella typhimurium*. Therefore, the activity can be attributed to other compounds present in the oils. The mono and sesquiterpenes present in this oil having bactericidal properties are linalool, camphor, geraniol, α-terpineol, β-caryophyllene, nerolidol and cadinene derivatives. *S. cumini* oil showed good antifungal activity specially against *Aspergillus niger*.[147-149]

The essential oil of *Aristolochia indica* was moderately active against the six organisms studied. This can be attributed to β-caryophyllene, α-humulene, caryophyllene oxide I and linalool as they are well known antimicrobials.[78,147-149]

S. cumini essential oil which showed good antibacterial activity was further studied by fractionation. The components present in the various fractions were analysed by GC-MS and the antimicrobial activity was studied separately. The results are given in section III.

SECTION III

SEPARATION OF *SYZYGIUM CUMINI* ESSENTIAL OIL INTO DIFFERENT FRACTIONS

(i) By column chromatography

(ii) By distillation

(i) 2 g of *S. cumini* essential oil was column chromatographed using silica gel (80 g, Qualigens 60-120 mesh). The column was successively eluted with n-pentane (200 ml) and diethyl ether (200 mL). Three fractions of 200 mL, 100 mL and 100 mL were collected and the solvent evaporated to get fractions 1, 2 and 3.

(ii) Distillation of essential oil

The *S. cumini* essential oil was taken in a micro distillation set and kept in paraffin oil at 250°C. Fractions 4 and 5 were obtained by distilling 3 ml of the essential oil at 250°C for 15 minutes. The distillate is fraction 4 and the residue remained in the flask is fraction 5.

All the five fractions were analysed by GC and GC-MS.

EXPERIMENTAL

GC and GC-MS Analysis of Five *Syzygium* Essential Oil Samples

GC Conditions

Gas chromatographic analyses were performed with a Shimadzu GC-14A with FID and Shimadzu Chromatopac C-R6A integrator, and with a Varian GC-3700 with FID and Shimadzu Chromatopac C-RIB integrator (all Shimadzu, Koyoto, Japan). Compounds were separated on 30 m x 0.32 mm (i.d.) fused silica columns coated either with a 0.25 μm film bonded non-polar FSOT-RSL-200 (Bio-Rad, Munich, Germany), HP-5MS (Hewlett-Packard, Palo Alto, USA) or with a 0.50 μm fil of bonded polar Stabilwax (Restek, Bellefonte, USA). The non-polar column was maintained at 40°C for 5 min after injection, and then programmed at 6°C/min to 260°C which was maintained for 5 min. Split injection was conducted with split ratio of 1:20 and 1:50 for the non-polar and polar columns, respectively; hydrogen was used as the carrier gas at 2.5,2.5 and 3.5 kPa respectively. For all columns the injector temperature was 250°C and the detector temperature 320°C. Quantification by percent-peak-area-calculations was performed by use of the non-polar FSOT-RSL-200 column. Some individual components could be identified by co-injection of pure compounds and comparison of their retention times (as Kovats indices) with published data.[36,38-40,150,151]

GC-MS Conditions

GC-MS condition was exactly as given in chapter five.

The compounds identified in each of the essential oil fraction is listed in Table 6.4.

TABLE 6.4

Identified compounds of *Syzyium* essential oil fractions 1-5 (concentration calculated as %-peak-area of GC-FID using a polar carbowax column)

No.	Compound	EOS1	EOS2	EOS3	EOS4	EOS5
01	α-pinene	1.21	0.12	0.17	12.23	0.40
02	camphene	0.22	0.05	0.09	0.34	0.05
03	isopropanol	0.02	0.01	0.04	0.02	0.01
04	propanol	0.01	0.02	0.03	0.02	0.02
05	β-pinene	5.14	0.09	0.02	26.31	1.15
06	β-myrcene	0.72	0.37	0.13	0.91	0.08
07	pent-1-en-3-ol	nd	0.04	nd	tr	nd
08	α-phellandrene	0.18	0.07	0.01	0.59	0.08
09	nopinene	0.13	0.03	0.02	0.14	0.01
10	α-terpinene	0.29	0.05	0.03	0.33	0.03
11	3-methyl butanol	0.18	0.02	0.01	0.13	nd
12	limonene	1.52	0.59	0.18	4.77	0.93
13	sabinene	0.34	0.15	0.07	1.10	0.07
14	1,8-cineole	0.12	0.35	0.06	0.05	tr
15	2-hexenal	0.14	0.21	0.09	0.41	0.02

16	*trans*-β-ocimene	0.13	0.04	0.08	0.11	0.03
17	γ-terpinene	0.39	0.19	0.08	0.52	tr
18	*para*-cymene	0.16	0.22	0.05	0.73	0.07
19	β-phellandrene	0.05	tr	0.02	0.14	nd
20	α-terpinolene	0.96	0.77	0.02	1.44	0.57
21	3-hexenyl acetate	0.07	0.32	0.49	1.01	0.24
22	artemiseole	tr	0.64	0.23	0.73	0.18
23	hexanol	0.05	0.42	0.97	1.79	tr
24	3-hexenol	0.09	1.28	5.49	10.37	0.52
25	2-hexenol	0.45	0.33	0.77	1.32	0.04
26	2-phenylethyl alcohol	0.16	0.51	0.12	0.36	tr
27	octen-3-ol	0.44	0.39	0.16	0.39	0.07
28	linalool oxide I	0.12	0.28	0.18	0.93	tr
29	α-cubebene	0.21	0.14	0.13	0.23	nd
30	linalool oxide II	0.14	0.33	0.28	0.45	tr
31	α-ylangene	0.42	0.18	0.13	0.15	0.07
32	α-campholene aldehyde	0.57	0.56	0.24	0.46	0.51
33	α-copaene	1.48	0.17	0.06	0.24	0.62
34	linalool	0.10	2.35	0.42	1.27	0.83
35	octanol	0.08	0.39	0.26	0.40	0.07
36	β-bourbonene	0.72	0.32	0.05	0.04	0.24
37	pinocamphol	0.11	0.48	0.20	0.93	0.41
38	α-gurjunene	1.07	tr	nd	0.06	0.29

39	pinene hydrate	0.08	nd	0.16	0.07	tr
40	*para*-menth-3-enol	0.03	tr	0.11	tr	nd
41	pinocamphone	0.06	0.30	0.12	0.69	0.14
42	fenchol	0.15	2.58	0.43	1.19	0.99
43	isomenthol	0.05	0.24	0.17	0.07	0.11
44	pinocarvone	0.07	3.61	0.69	1.27	0.88
45	β-elemene	0.14	tr	nd	nd	tr
46	terpinen-c-ol	0.19	2.39	0.98	1.23	1.79
47	nopinone	nd	1.77	1.04	1.13	1.47
48	germacrene D	0.71	tr	nd	0.05	tr
49	dihydro-α-terpineol	nd	tr	0.26	0.14	nd
50	caryphyllene	3.17	0.50	0.19	0.26	1.16
51	sabinene hydrate	0.45	0.18	0.19	0.33	tr
52	myrtenal	0.68	5.36	1.73	1.07	3.12
53	β-cadinene	0.65	tr	nd	nd	tr
54	pinocarveol	0.27	9.40	6.03	3.74	6.11
55	allo-aromadendrene	10.74	tr	tr	0.43	2.07
56	T-cadinene	tr	0.14	nd	0.22	0.62
57	*cis*-verbenol	nd	0.37	0.56	0.19	tr
58	caryophyllene oxide I	0.07	0.16	0.04	0.09	nd
59	α-terpineol	0.12	13.29	21.78	5.29	16.86
60	α-humulene	2.48	tr	nd	nd	tr
61	α-amorphene	1.43	tr	nd	nd	tr

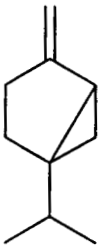
62	borneol	tr	1.01	0.54	0.60	tr
63	ledene	0.77	0.12	0.03	nd	nd
64	*para*-menth-1,5-dien-8-ol	tr	1.90	2.25	0.76	1.93
65	γ-muurolene	1.58	0.63	0.21	0.13	0.63
66	junipene	0.16	0.24	tr	nd	3.50
67	α-muurolene	9.48	tr	tr	tr	tr
68	verbenone	tr	1.76	2.51	0.94	0.05
69	β-cedrene	0.27	nd	0.17	0.07	tr
70	β-selinene	0.29	tr	tr	nd	tr
71	carvone	0.31	0.95	0.25	0.18	0.61
72	δ-cadinene	31.05	0.56	0.10	0.63	6.88
73	γ-cadinene	7.94	0.80	tr	0.21	2.20
74	*para*-mentha-1(7),2-dien-8-ol	tr	nd	0.48	0.16	nd
75	nopol	nd	nd	0.16	nd	tr
76	myrtenol	0.13	5.26	2.95	1.37	3.25
77	methyl salicylate	1.16	0.66	0.21	0.26	0.64
78	α-cadinene	1.82	0.67	0.28	tr	1.06
79	*trans*-carveol	0.39	1.25	0.71	0.45	0.95
80	*para*-cymenol	0.26	0.92	0.73	0.55	0.75
81	calamenene	0.20	0.11	0.18	0.26	0.43
82	*cis*-carveol	0.11	1.08	0.78	1.40	2.01
83	benzyl alcohol	0.15	0.35	1.65	tr	1.08
84	α-calacorene	0.34	0.28	0.22	0.17	0.28

85	pinene epoxide	0.15	0.50	0.69	0.18	0.22
86	valencene	0.27	0.38	0.48	tr	0.73
87	perilla alcohol	0.25	0.69	1.19	nd	1.02
88	caryophyllene oxide II	0.07	0.93	0.27	tr	0.47
89	ledol	0.31	1.15	1.82	0.16	0.83
90	cubenol	0.53	1.44	0.38	0.10	2.28
91	α-cedrol	0.14	1.72	0.61	0.02	0.55
92	viridiflorol	0.08	0.14	0.34	tr	0.22
93	aromadendrene oxide	0.06	0.22	0.39	0.04	0.02
94	globulol	0.05	0.20	0.51	tr	0.11
95	T-cadinol	0.30	5.49	2.40	0.19	3.84
96	T-muurolol	0.25	5.62	3.53	0.08	4.00
97	δ-cadinol	0.10	1.23	3.84	0.17	1.97
98	γ-cadinol	0.14	0.57	0.28	0.19	0.12
99	α-bisabolol	0.09	0.54	0.40	0.12	0.41
100	α-cadinol	0.23	7.01	21.17	0.35	11.06
101	torreyol	nd	0.13	0.14	0.23	0.19
102	α-bergamotol	0.02	0.27	0.26	0.08	0.07

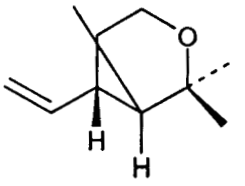
Total number of compounds identified in all the fractions are one hundred and two. In fraction **1**, the major components are δ-cadinene (34.05%), allo-aromadendrene (10.74%), alpha-muurolene (9.48%), δ-muurolene (7.94%), β-pinene (5.14%) while in fraction **2**, α-terpineol (13.29%), pinocarveol (9.4%), α-cadinol (7.01%), t-muurolol (5.62%), t-cardinol (5.49%) and myrtenal (5.36%) are major ones. In fraction **3**, α-terpineol (23.78%), α-cadinol (22.17%), pinocarveol (6.03%), 3-hexen-1-ol (5.49%), myrtenol (3.95%) are more abundant components. β-Pinene (28.31%), α-pinene (14.23%), 3-hexen-1-ol (12.37%), α-terpineol (6.29%) and α-terpineol (16.86%), α-cadinol (11.06%), δ-cadinene (6.88%), α-cadinene (6.38%) are the main compounds present in fractions **4** and **5** respectively.

The structures of the identified compounds in the fractionated *Syzygium cumini* leaf essential oil are given below.

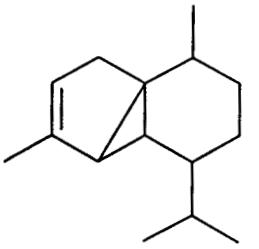
Structures of the identified compounds in the fractionated samples of *Syzygium cumini* leaf essential oil



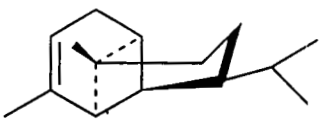
Sabinene



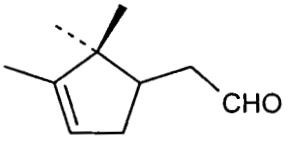
Artemiseole



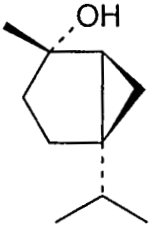
α-Cubebene



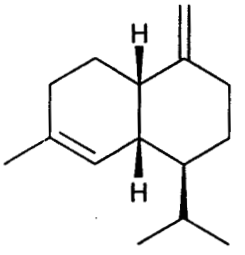
Ylangene



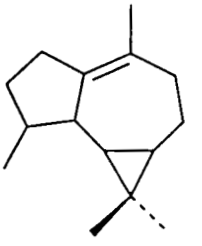
α-Campholene aldehyde



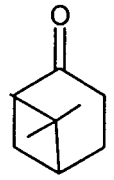
Sabinene hydrate



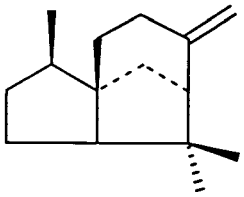
γ–Amorphene



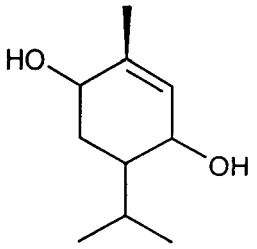
Ledene



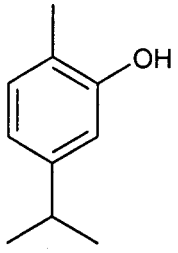
Nopinone



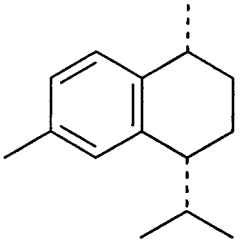
β- Cedrene



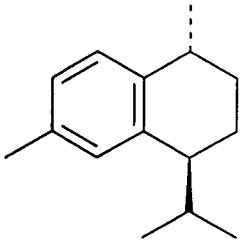
P-Menth-1(7)-ene-2,8-diol



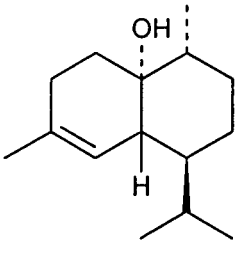
p-Cymenol



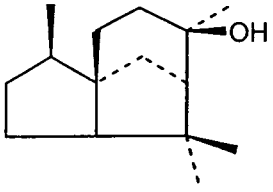
Cis-Calamenene



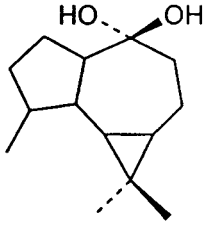
Trans-Calamenene



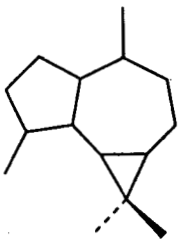
Cubenol



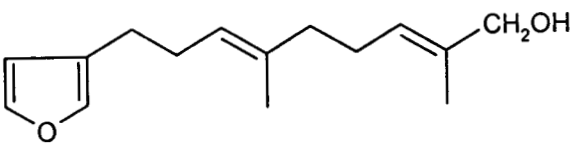
α-Cedrol



Viridiflorol



Aromadendrine



Torreyol

The fractionated samples were studied for their antibacterial activity using disc diffusion method (as given previously).

Essential oil fractions were tested for their antibacterial property against three gram positive bacteria – *Basillus sphaericus, Basillus subtiles, Staphylococcus areus* and three gram negative bacteria *E. coli, Pseudomonas aeruginosa* and *Klebsiella aerogenes.* Concentration of the essential oil is 5µL/disc. The negative control diethyl ether concentration is 5µL/disc and that of positive control PEN-G is 30mg/mL. Zone of inhibition includes disc diameter (6 mm).

The result obtained is as shown in tables 6.5 & 6.6.

TABLE 6.5

Antibacterial Activity of Essential Oil Fractions From *Syzygium cumini* leaves

Fractions	*Bacillus sphaericius (mm)*	*Bacillus subtilis (mm)*	*Staphylococcus areus (mm)*
1	7	10	9
2	17	25	14
3	15	28	17
4	10	18	13
5	11	20	13
Negative control (Diethyl ether)	Nil	Nil	Nil
Positive control (PEN-G)	20	19	18

TABLE 6.6

Antibacterial Activity of Essential Oil Fractions From *Syzygium cumini* Leaves

Fractions	*E coli* *(mm)*	*Pseudomonas auruginosa* *(mm)*	*Klebsiella aerogenes* *(mm)*
1	7 (7)	Nil (Nil)	11 (16)
2	6 (7)	Nil (Nil)	8 (10)
3	9 (9)	Nil (Nil)	10 (12)
4	7 (8)	Nil (Nil)	7 (9)
5	6 (8)	Nil (Nil)	8 (14)
Negative control (Diethyl ether)	Nil	Nil	Nil
Positive control(Streptomycin)	28	29	30

The result obtained when the antibacterial (gram negative) activity of essential oil fractions from *S. cumini* was tested at a higher concentration of 10μL/disc is given in brackets (Table 6.6).

Results and Discussion

Activity of *Syzygium cumini* essential oil fractions against gram positive bacteria was found to be more pronounced compared to gram negative bacteria.

There was no remarkable change observed in the activity against gram negative bacteria when this experiment was conducted with increased concentration (10 μL/disc instead of 5 μL/disc) of the essential oil fractions. These fractions showed no activity against *Pseudomonas aeruginosa*.

S. cumini whole essential oil which was isolated during the month of May showed remarkable activity against gram positive as well as gram negative bacteria. Essential oil fractions tested for antibacterial activity was isolated during the month of November and showed less activity against gram negative bacteria. This difference in activity may be due to seasonal variations in composition.

Fraction **3** with higher percentage of oxygenated terpenoids showed good activity against gram positive bacteria. The activity of fraction **2** also was found to be comparable.

Fraction **1** which contained mainly non oxygenated compounds was the least active.

In general the fractions containing more of the oxygenated terpenoids were found to be more active against bacteria while the fractions containing non oxygenated terpenoids in larger portions were less active.

CONCLUSION

The chemical investigation of plants has been a favourite area of research around the globe as well as in India for several decades. Kerala is blessed with a wide variety of plants whose biological activity are still unknown. Studies conducted on local plant wealth are very limited, but there is ample scope in this field so as to expose biological activity of plant products and their applications in different fields such as medicine, agriculture, etc. Therefore, the aim of this research work was to identify and separate, medicinally and commercially useful essential oil constituents of different plants by GC, GC-MS, GC-MS-O techniques.

The work presented in this thesis comprises of the analyses of essential oils from five plants. Among the five essential oils extracted studies were conducted upon the antibacterial properties of three of the essential oils. One of the essential oils was fractionated by column chromatography and by distillation. All the five fractions thus obtained were investigated for their constituents and antibacterial property. Antifungal property of one of the essential oils was also researched upon.

More than two hundred known compounds were identified in these various essential oils and their fractions. Findings of olfactory evaluation of the essential oils gave valuable contributions to perfumery. Some of the odour notes can be of use in after shave lotions and personal health products.

Analysis of the essential oil of leaves of *Cinnamomum zeylanicum* is given in Chapter 1. Gas Chromatographic Analysis and the combined Gas Chromatography Mass Spectrometry (GC, GC-MS) analysis led to the identification of thirty nine compounds and a high percentage (85.7) of linalool was obtained. Based on this work a paper entitled "Analysis of *Cinnamomum zeylanicum* Blume Leaf Oil from south India" has been published in the Journal of Essential Oil Research (J. essent. Oil Res., 13, 2001, 442).

Chapter 2 exposes the seasonal changes in the composition and olfactoric properties of *Syzygium travancoricum* essential oil. The essential oil from the leaves collected during the month of June was analysed by GC, GC-MS and forty five compounds were identified. By noting the major components present in the essential oils, various medical and commercial applications are also identified. This work has been published as "Analysis of the Essential Oils of the Fresh Leaves of *Syzygium cumini* and *Syzygium travancoricum* from South India" in the Journal of Essential Oil Bearing Plants (J.E.O.P., 2, 1999, 68). *Syzygium travancoricum* is a rare plant and little studies were conducted on this plant earlier. Essential leaf oil from *Syzygium travancoricum* plants collected during the month of April was analysed by GC-FID, GC-MS and GC-O methods. The essential leaf oil content obtained from this species was higher than that of the species collected in the month of June. The aroma was also a little intense in the latter. The difference in essential oil content may be due to seasonal variations. This work has been

published under the title "Analysis of the Composition and Aroma of the Essential Leaf Oil of *Syzygium travancoricum* from South India by GC-FID, GC-MS and Olfactometry: Seasonal Changes of Composition" in the Journal Chromatographia (Chromatographia 53, 2001, S-372).

Chapter 3 consists of analysis of the essential oil of the aerial parts of *Aristolochia indica*. GC-FID, GC-MS and Olfactoric methods were used to identify fifty six compounds. This work has been published as a paper entitled "Analysis of the Essential Oil of the Aerial Parts of the Medicinal Plant *Aristolochia indica* Linn. (Aristolochiaceae) from South India" in the Journal Scientia Pharmazeutica (Sci. Pharm.) 68, 2000, 309.

Chapter 4 presents the analysis of the essential oil volatiles of *Leucas indica* using GC-MS and olfactometry resulting in the identification of ninety three compounds. The head space of this *Leucas indica* was trapped using Solid Phase Micro Extraction (SPME) and analysed by means of GC-FID. The head space analysis threw more light upon the aroma compounds responsible for the characteristic odour of the essential oil. Correlation of compounds and corresponding aroma impression in accordance with published data also gave valuable information.

This work has been published as a book chapter entitled "Analysis of the Essential Oil Volatiles of *Leucas indica* from South India" (Recent Res. Devel.

Agricultural & Food Chem., S.G. Pandalai (Ed.), Research Sign Post, Thiruvananthapuram, India).

Analysis of the essential oil of the fresh leaves of *Syzygium cumini* and the findings there of are discussed in Chapter 5. By GC and GC-MS techniques, sixty four compounds were identified. A paper has been published on this work in the Journal of Essential Oil Bearing Plants and the title is "Analysis of the Essential Oils of the Fresh Leaves of *Syzygium cumini* and *Syzygium travancoricum* from South India" (J.E.O.P., 2, 1999, 68).

Chapter 6 deals with the antimicrobial studies of various essential oils extracted. It also contains the study of separated fractions of one of the essential oils.

Antibacterial activity of *Syzygium cumini* and *Syzygium travancoricum* leaf essential oils was studied against three gram positive and three gram negative bacteria. It was found that *Syzygium cumini* essential oil was more antibacterial than *Syzygium travancoricum*. A short report on "Antibacterial activity of *Syzygium cumini* and *Syzygium travancoricum* Leaf Essential Oils" has been accepted for publication in the Journal Fitoterapia. Antibacterial activity of *Aristolochia indica* essential oil also showed moderate activity against the same type of bacteria under similar conditions. A short report on "Antibacterial Activity of the Essential Oil

from *Aristolochia indica*" has been accepted for publication in the journal Fitoterapia.

Since *Syzygium cumini* essential oil was found to be more antibacterial, it was subjected to further studies. The antifungal activity of the same was evaluated and found to be active against the five fungi tested. *Syzygium cumini* essential oil was separated into five fractions by column chromatography and by distillation and their antibacterial activity was also investigated. These five fractions were analysed using GC, GC-MS techniques and identified one hundred and two compounds.

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