CHAPTER IV
PHYTOCHEMICAL ANALYSIS

4.1. INTRODUCTION

Plants have been used as medicine for millennia. A survey conducted by World Health Organisation (WHO) revealed that about 20,000 plant species were being used as medicine all over the world out of the around 2,50,000 existing plant species on earth. It has been estimated that over 6,000 plants were used in traditional folk and herbal medicine, respectively about 75% of the medicinal needs of the third world, 95% in Africa (Shivaa and Neelakantan, 2001). The traditional insight embrined in indigenous system of medicine need scientific validation especially in its chemical parameters.

Natural product chemistry is an ancient science. The preparation of food stuffs, colouring matters, fibers, toxins, medicinals and stimulants are examples of activities as old as mankind. The plant chemicals are often classified as either primary or secondary metabolites. The primary metabolites such as carbohydrates, lipids, proteins etc. are common to all plants and are involved in the primary metabolic process of building and maintaining plant cell (Kawfman et al., 1999). The beneficial medicinal effects of plant materials typically result from the combinations of secondary derived from primary metabolites are more limited in distribution in the plant kingdom, being restricted to a particular taxonomic group.

Phytochemicals are major source of dyes, flavors, sweetener, aromas, perfumes, insecticides, ant parasitic drugs and many other substances. Further research on plant will provide apart from drugs, additional sources of these industrial raw materials. All this potential justifies the broadest and most exhaustive phytochemical research.

Many plants contain mixture of volatile monoterpenes and sesquiterpenes called essential oils in the glandular hairs that lend a characteristic odour to their foliage. They are highly concentrated, aromatic oily liquids obtained from a variety of aromatic plant materials including flowers, buds, seeds, rhizomes, leaves, bark, wood, fruits and roots. These aromatic compounds formed by plant as by products or as final
metabolic products are stored in certain organs of the plant a spectrum of essential oils present in the members of Zingiberaceae (Ibrahim and Zakaria, 1987).

In India about 20% of the 400 tones of chemicals estimated to be used annually in perfumes and flavors are obtained from essential oils, which represent an important class of indigenously developed starting material for perfumery and flavor industry. The term aromatherapy, in brief, fragrance for health is the remedial treatment for mental and physical disorders by the application of essential oils. Despite, the continuing inventions of synthetic aromatics, essential oils still remain as the most important part of fragrances (Ranade, 1993). The indigenous use of essential oil and their components offer enormous scope in developing plant derived biosafe products of direct utility to humans (Khanuja, 2000).

The individual chemicals isolated from essential oils are more often used than oils and the intimate knowledge of essential oil composition helps to evaluate the quality of oils that allows a better and specially directed application of it (Buchbauer, 2000). Chemists in the late eighteenth century took a jump from the world of myths into modern science. They began to separate, purity and finally analyse the compounds produced in living cells. Natural product chemistry has brought great stimulus towards the development of refined separation techniques such as column chromatography, gas chromatography, high performance thin layer chromatography, paper chromatography, electrophoresis etc. These methods have made it possible to isolate compounds present in extremely small quantities in biological system.

4.1.1. Extraction

In phytochemical analysis, selection of the plant is an important step. The plant material is collected, dried and powdered for further analysis. Extraction is the next stage and there are two main types of extraction processes viz cold extraction and hot extraction. In cold extraction, the finally powdered plant material is kept covered with the solvent at room temperature with occasional stirring. The plant material may be extracted repeatedly till all the desired compounds are extracted. Cold extraction is recommended for thermo labile metabolites like alkaloids, proteins etc. Hot extraction is more efficient and soxhlet extractor is the usual choice for hot extraction. Here, hot solvent is percolated through the powdered plant material and the extract is boiled to
get back hot solvent vapour, which is condensed and recycled. Thus extract keeps getting concentrated with time.

4.1.2. Chromatographic techniques

In phytochemical analysis, chromatographic techniques are widely used for detection and separation of individual compounds from extract. The Russian botanist, Mikhail S. Tswett, first introduced chromatography in the beginning of the 20th century. It is a separation technique where the separation is effected by different migration of solute molecules between the stationary and mobile phases.

4.1.3. Gas chromatography – Mass spectrometry

In recent years, the combined GC-MS apparatus has emerged as one of the most important of all techniques in natural product research. Gas chromatography provides an effective resolution of the individual components in a mixture. Identification of these separated components can frequently be accomplished through their characteristic molecular fingerprints, mass spectra (Berezkin, 1983; Grob, 1997).

Gas chromatography is an ideal separator, whereas mass spectrometry is excellent for identification. Any compound that can pass through a gas chromatograph is converted to ions in the mass spectrometer. At the same time, the specific nature of a mass spectrum makes the mass spectrometer a very specific gas chromatographic detector. One incompatibility problem here is the difference in pressure required for the operation of a gas chromatograph and mass spectrometer. The gas chromatograph operates at high pressure and the mass spectrometer operates under high vacuum. Another problem is the pressure of high volumes of carrier gas and relatively little sample in the effluent from the gas chromatograph. The interface unit of GC-MS helps to operate both gas chromatograph and a mass spectrometer without degrading the performance of either unit.

Two types of mass spectrometers were used for GC-MS works: magnetic sector mass spectrometers and quadrupole mass filters. Three main characteristics that affect the compatibility of GC-MS units are scanning speed, sensitivity and useful dynamic range. The scan repetition rate for magnetic sector instruments is 3-4 Hz. In quadrupole mass filters, the scan repetition rate is 4-8 Hz. The linear mass scale of the
quadrupole mass filter is ideally suited for digital control that is important for routine analysis with high sample throughout.

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950’s by Roland Gohlke and Fred MCLafferty. These sensitive devices were bulky, fragile and originally limited to laboratory settings. The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. (http://en.wikipedia.org/wiki)

The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules take different amount of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio.

Source [http://en.wikipedia.org/wiki/]
These two components, used together, allow a much finer degree of substage identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (eg. flame Ionization Detector) detects multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.

Samples are introduced to the column via an inlet. This inlet is typically an injection through a septum. Once in the inlet, the heated chamber acts to volatilize (vaporise) the sample. In a split system, a constant flow of carrier gas moves through the inlet. A portion of the carrier gas flow acts to transport the sample into the column. Another portion of the carrier gas flow gets directed to purge the inlet of any sample following injection (spectrum purge). Yet another portion of the flow is directed through the split vent in a set ratio known as the split ratio. In a splitless system, the advantage is that a larger amount of sample is introduced to the column. However, a split system is preferred when the detector is sensitive to trace amounts of analyte and there is concern about overloading the column.

Mass spectrometer can be used as real time detectors for gas chromatography. The total ion current is measured and recorded as a function of time. It is a measure of the total number of ions formed from the material in the effluent. Gas chromatography provides an effective resolution of their individual components in a mixture. GC-MS provides the characteristic molecular ion peak and fragment ion peaks from a separated compound in an essential oil. Individual spectra are compared with known spectra in a suitable database such as Wiley, Nist or Flavor and Fragrance Database and then further of the constituent in Relative Retention Indices (RRI) of essential oil. Constituents calculated using n-alkanes as standards, also helps in identification of
constituents. The main advantages of a mass spectrometer as a detector for gas chromatography are its increased sensitivity and its specificity in identifying unknowns or confirming the presence of suspected compounds.

4.1.4. Purge and Trap GC-MS

For the analysis of volatile compounds a Purge and Trap (P&T) concentrator system may be used to introduce samples. The target analytes are extracted and mixed with water and introduced into an air tight chamber. An inert gas such as Nitrogen (N\textsubscript{2}) is bubbled through the water, this is known as purging. The volatile compounds move into the head space above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber. The volatile compounds are drawn along a beated line onto a ‘trap’. The trap is a column of absorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then beated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system. P&T GC-MS is particularly suited to Volatile Organic Compounds (VOCS) and Aromatic Compounds (BTEX) associated with petroleum [http://en. Wikipedia. org/wiki].

Applications of GC-MS include drug detection, fire investigation, environmental analysis explosives investigations and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

The present study is undertaken to identify the various highly valuable chemicals present in the plant Kaempferia galanga. Volatile oil of dried rhizome of K. galanga obtained by water distillation was determined by Supinya et al. (2005), and the major chemical constituents were identified as ethyl-p-methoxycinnamate (31.77%), methyl cinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadacane (6.41%) respectively. Haxane rhizome extracts of K. galanga were investigated by Presanakumary et al. (1994) and they reported that the oil quality remains the same irrespective of the geographical type. Cyclohexane oxide derivatives
(Pancharoen et al., 1989) diterpenes (Prawat et al., 1993) from Kaempferia species were reported.

As the phytochemical investigations on K. galanga are rather less known, the present study is under taken with GC-MS analysis of ethanol extract of Kaempferia galanga.

4.2. Review of Literature

The composition of the volatile oil from the roots of Peucedanum praeruptorum were analysed by GC-MS technique. Thirty-eight chemical constituents, comprising 77.97% of the total content were identified. The main components were beta -phellandrene (9.52%), alpha -bisabolol (8.44%) and beta -pinene (5.22%) (Chen-BingHua et al., 2002). The chemical constituents of the essential oil from Pelargonium graveolens leaves were analysed by Rana et al. (2002). Thirty compounds accounting for 99.1% of the oil were identified and the main components identified were citronellol (33.6%), geraniol (26.8%), linalool (10.5%), citronellyl formate (9.7%) and p-menthone (6.0%).

The essential oils of Zingiber ottensii and Z. zerumbet fresh rhizomes were analysed by a combination of capillary GC and GC-MS (Sri-Nurestri et al., 2005). Twenty-eight and eighteen components were identified from the rhizomes of Z. ottensii and Z. zerumbet, respectively. The components were determined by comparing their retention indices with those reported in the literature and their mass spectral data with those from the mass spectral database. The most abundant component of Z. ottensii and Z. zerumbet rhizome oil was zerumbone, representing 37% and 73% of the total oil, respectively. Other major components of Z. ottensii were terpinen-4-ol (16.8%), alpha -humulene (10.9%) and sabinene (7.2%), whilst that of Z. zerumbet were alpha -humulene (5.9%), camphene (2.8%) and caryophyllene oxide (2.7%).

The essential oil of Curcuma zedoaria was obtained from fresh rhizomes and analysed by a combination of capillary GC and GC-MS. Twelve components (79.41% of the total) were detected, and identified by comparing with Kovats Indices and their mass spectral data. The essential oil was made up of monoterpenoids and sesquiterpenoids, with furanogermentone (45.23%) as the major component (Malek et
The rhizomes and leaves of *Alpinia galanga*, each gave 2.2% of oils, which were analysed by GC and GC-MS (Raina *et al.*, 2002). The rhizome oil contained 84 constituents, comprising 100% of the oil, of which the major ones were 1, 8-cineole [eucalyptol] (11.2%), alpha-turmerone (11.1%), beta-caryophyllene (9.8%), ar-turmerone (7.3%) and beta-sesquiphellandrene (7.1%). The leaf oil contained 83 components, comprising 97.4% of the total oil, of which the main constituents were terpipolene (26.4%), 1, 8-cineole (9.5%), alpha-phellandrene (8%) and terpinen-4-ol (7.4%).

The chemical composition of the essential oils of the rhizomes of the common ginger (*Zingiber officinale*) and three ginger-lilies (*Hedychium coccineum, H. flavescens* and *H. coronarium*) grown in Mauritius was investigated by GC and GC/MS (Gurib-Fakim *et al.*, 2002). *Zingiber officinale* oil was characterized by the presence of geranial (16.3%), neral (10.3%), zingiberene (9.5%), beta-sesquiphellandrene (6.3%) and ar-curcumene (5.1%). The oils of the ginger lilies were characterized as follows: *H. coccineum*: (E)-nerolidol (44.4%), trans-sesquisabinene hydrate (24.2%); *H. flavescens*: linalool (35.0%), 1, 8-cineole [eucalyptol] (15.3%), beta-pinene (14.7%), alpha-terpineol (14.5%) and alpha-pinene (5.3%); *H. coronarium*: alpha-muurolol (16.8%), alpha-terpineol (15.9%), 1, 8-cineole (11.2%), an unknown sesquiterpene alcohol (7.0%), alpha-fenchyl acetate (5.6%), citronellal (5.5%) and (E)-methyl cinnamate (5.1%).

The volatile constituents of *Helietta longifoliata* leaves were analysed by gas chromatography -mass spectrometry. Twenty-five constituents were identified representing *ca.* of 96% of the oil, and limonene (17.50%), germacrene D (16.60%), elemol (11.81%), bicyclo-germacrene (11.67%), guaiol (11.53), and epi-alpha-bisabolol (7.24%) were the most abundant components (Moura *et al.*, 2002).

Aromatic profiles and key aroma components of banana and yellow passion fruit essences were studied using gas chromatography-mass spectrometry and Gas Chromatography-Olfactometry (GCO) systems (Jordan *et al.*, 2001). A total of 43 components were quantified in banana essence. Among them 26 components contribute to the aromatic profile in banana essence but isoamyl acetate, 2-pentanol acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-methyl-butanol, acetal, isobutyl
acetate, hexanal, ethyl butyrate, 2-heptanol, and butyl butyrate contribute to and define the aroma in this fruit since they were detected by all GCO panellists in all replications. A total of 62 compounds were quantified in yellow passion fruit essence. Analysis by GCO revealed that a total of 19 components contribute to the overall flavour in this essence, where 3-hydroxy-2-butane, ethyl butyrate, 2-heptanol, ethyl hexanoate, linalool, isoamyl acetate, (Z)-3-hexenyl hexanoate, and hexyl hexanoate were recognized by the three panellists in all replications. Mailhebiau and Suvarnalatha (2002) identified sixty seven components from leaf oil of Cupressus arizonica by GC/MS, the major constituents were delta -3-carene (21%), alpha -pinene (14%) and sabinene (11%).

The volatile components of fresh leaves and roots from Anthriscus sylvestris (L.) Hoffm. were analysed by GC and GC-MS. The monoterpene fraction (69-70%) dominated, while beta-phellandrene (39-45%) was the main component in both the leaf and the root oil. Other components in the leaf oil were beta-myrcene (17%), sabinene (6.2%), Z-beta -ocimene (5.4%) and benzene acetaldehyde (4.1%). In the roots Z-beta -ocimene (16.9%) and alpha-pinene (4.6%) were the major components (Bos et al., 2002).

The chemical composition of the essential oils of Calea clematidea leaves and flowers was analysed by GC and GC/MS (Flach et al., 2002). The essential oil of the leaves showed a high content of a new natural epoxy terpenoid, named clemateol (ca. 70%), with minor amounts of o-vanillin (6.5%), spathulenol (4.2%), alpha -terpinene (4.0%), germacrene B (2.9%), yomogi alcohol (1.8%), (E)-caryophylene (1.7%), 0 -cymenene (1.6%), and alpha-gurjunene (1.5%), while the essential oil of the flowers was characterized by a higher content of thymol methyl ether (ca. 80%), with minor amounts of clemateol (4.8%) and o-cymene (4.7%).

Sadaquat-Ali et al. (2002) analysed the essential oils of the leaves, flowers and rhizomes of Alpinia zerumbet, A. purpurata, Hedychium coronarium and H. gardnerianum growing wild in Fiji using GC/MS. The rhizome and leaf oils of A. zerumbet were found to be rich in beta-pinene (4.0%, 10.0%), 1, 8-cineole [eucalyptol] (28.1%, 13.2%) and terpinen-4-ol (41.4%, 40.9%), respectively. The rhizome oils of A. purpurata contained alpha-pinene (24.9-36.1%) and beta-pinene
(65.8-71.3%), while the leaf and flower oils of the same species contained alpha -pinene (79.6-81.0%), beta -pinene (29.4-43.0%) and beta -caryophyllene (0-24.2%), respectively. The rhizome and leaf oils of H. coronarium were rich in alpha -pinene (10.6%, 20.9%), beta -pinene (31.4%, 53.6%), 1, 8-cineole (55.9%, 11.9%) and beta -caryophyllene (0%, 17.7%), respectively. The rhizome oils of H. gardnerianum were rich in alpha -pinene (10.4-11.0%), beta -pinene (31.0-32.1%), 1, 8-cineole (27-29.5%) and linalool (22.6-28.1%). The leaf and flower oils contained alpha -pinene (18.4%, 6.6%), camphene (23.7%, 17.4%), beta -pinene (22.1%, 17.0%), linalool (13.1%, 4.6%) and beta -caryophyllene (7.7%, 17.4%)

The essential oils of the rhizomes and the leaves of Alpinia galanga were analysed by capillary GC and GC/MS. The oils of the rhizomes and the leaves from the two places were found to contain similar constituents (Mallavarapu et al., 2002). The rhizome oils contained limonene (3.7% and 3.5%, respectively), 1, 8-cineole[eucalyptol] (33.0% and 30.2%, respectively), camphor (5.0% and 14.0%, respectively), alpha -terpineol (9.3% and 2.3%, respectively), alpha -fenchyl acetate (12.7% and 1.1%, respectively) and (E)-methyl cinnamate (5.3% and 2.6%, respectively), as the major constituents. The major constituents of the leaf oils from the same locations were: alpha -pinene (6.6% and 6.3%, respectively), camphene (5.0% and 5.1%, respectively), beta -pinene (21.5% and 23.5%, respectively), 1, 8-cineole (34.4% and 30.7%, respectively) and camphor (7.8% and 12.8%, respectively).

Ahmad and Jantan (2003) analysed the rhizome and leaf oils of Boesenbergia stenophylla R. M. Sm. by gas chromatography on two columns of different polarity, retention indices and GC-MS. The oils were found to possess compositional similarities but quantitative differences in the concentration of each component. The oils are natural sources of methyl (E)-cinnamate, constituting 49.9-53.4% of the oils. They are also rich in sesquiterpenoids (39.8 and 40.3%, respectively) with delta -elemene, beta -elemene, alpha -santalene, alpha -humulene, gamma -muurolene, spathulenol, caryophyllene alcohol and kaur-16-ene as the main representatives.

The essential oil yield from the aerial parts of Artemisia campestris was analysed using GC and GC/MS and 38 compounds were identified (Chalchat et al.,
The principal components were sesquiterpene alcohols: spathulenol (9.2%) and 4-hydroxy-9-epi- beta -caryophyllene (3.0%); and monoterpenes hydrocarbons: beta -pinene (9.1%), alpha -pinene (3.4%), limonene (2.5%) and germacrene D (3.3%).

The chemical composition of the essential oil from Artemisia iwayomogi was analysed by means of GC and GC-MS. Eighty-five constituents were identified representing 96.23% of the total oil. Camphor (19.31%), 1, 8-cineole [eucalyptol] (19.25%), borneol (18.96%), camphene (4.64%) and beta-caryophyllene (3.46%) were the major components (Yu-HyeonHee et al., 2003).

The content and composition of the essential oil of Eupatorium cannabinum were studied by Judzentiene (2003). Oils were prepared by hydrodistillation and analysed by GC and GC-MS. In the eight inflorescence and leaf oils, germacrene D was the principal component constituting upto 11.7-25.6% of the oils. The other constituents in appreciable amount were neryl acetate (tr - 8.9%), beta -bisabolene (0-8.0%), and beta -ylangene (0.6-6.2%). Sixty eight identified components formed up (53.8-76.9%) of total oil content. Monoterpenes and oxygenated monoterpenes made up to 8.9-16.7%, while sesquiterpenoids made up to 38.9-69.2% of the oils.

The essential oils from bark and leaves of Cedrelopsis grevei an aromatic and medicinal plant from Madagascar, have been examined separately by means of GC-MS. The oil constituents were identified according to their mass spectra and their relative retention indices determined on both polar and non-polar stationary phase capillary columns. A total of 55 compounds have been identified constituting 76.7% (bark) and 91.6% (leaves) of the volatile constituents. Both oils were found to have a similar composition; however the relative percentages of some compounds notably differed. The bark essential oil contained beta -pinene (17.1%), cis-sesquisabinene hydrate (12.8%) and caryophyllene oxide (7.0%) as the main components whereas the leaf essential oil was largely dominated by trans- beta -farnesene (35.6%); beta -pinene (12.8%), cis-sesquisabinene hydrate (9.8%) and ar-curcumene (8.6%) were also present as major components (Gauvin et al., 2004).

The constituents of the volatile oil obtained from the fruits of Aristolochia contorta by steam distillation were analysed by GC-MS (Zhang et al., 2004). Fifty-eight components were separated and identified. The volatile oil had high levels of
monoterpenes and sesquiterpenes (67.66%). The major sesquiterpenes were caryophyllene (30.59%) and caryophyllene oxide (15.30%).

Ibrahim *et al*. (2004) examined the Galangal oils distilled from the rhizomes and seeds of *Alpinia galanga* by capillary GC and GC/MS. The rhizome oil was rich in 1, 8-cineole (40.5%). Other compounds that were found in appreciable amounts in the oil were the sesquiterpenoids, beta -bisabolene (8.4%), (Z,E)-farnesol (3.8%), beta -caryophyllene (3.6%) and (E)- beta -farnesene (3.2%). The seed oil was characterized by its richness in sesquiterpenoids with the major ones being beta -bisabolene (37.6%), (E)- beta -farnesene (22.7%), (E,E)-farnesyl acetate (7.9%), (Z,E)-farnesol (3.9%) and beta -caryophyllene (3.0%).

Hymete and Rohloff (2003) analysed volatile fractions from leaves (0.21%) and flowers (0.48%) of *Ocimum urticifolium* by GC and GC-MS. Twenty-seven compounds comprising 89.0% of the leaves oil and fifty-five compounds representing 63.9% of the flower oil have been characterised. The main sesquiterpenes found in the leaf oil were delta -cadinene (17.2%) followed by beta -caryophyllene (14.5%) and gamma -muurolene (10.5%), while the monoterpane (Z)- beta -ocimene (22.7%) and the phenylpropanoid elemicine (8.8%) were detected as other major oil components. In flower oil, beta -bisabolene (19.2%) and beta -caryophyllene (10.5%) were the main sesquiterpenes while linalool (2.3%), (Z)- beta -ocimene (2.4%) and camphor (1.5%) were major monoterpeneic hydrocarbons beside elemicine (6.4%). The presence of elemicine, a biologically active phenylpropanoid compound in these oils is significant.

GC-MS is used to extract and analyse the essential oil from the aerial parts of *Microsorium fortunei*. More than 23 peaks are separated and 21 compounds representing 98.39% of the oil are identified. The main constituents of *M. fortunei* essential oils are 1-hexanol, hexanoic acid and glutamic acid (Sun and Cheng, 2004).

Steam distilled oil of the fresh leaves of lemon grass, *Cymbopogon citratus*, was analysed by GLC and GC-MS (Mohd-Ali, 2004). Twenty-two compounds comprising ~99.7% of the volatile oil were characterized. The oil contained high amount of monoterpenes (95.9%), with geranial (52.3%), cis-pinocarveol (20.2%), neral (9.8%) and limonene-1, 2-epoxide (3.6%) being the major constituents. GC and
GC-MS analysis of volatile oil obtained from *Piper nigrum* L. resulted in the identification of 49 components accounting for 99.39% of the total amount, and the major components were beta-caryophyllene (24.24%), limonene (16.88%), sabinene (13.01%), beta-bisabolene (7.69%) and alpha-copaene (6.3%). The acetone extract of pepper showed the presence of 18 components accounting for 75.59% of the total amount. Piperine (33.53%), piperolein B (13.73%), pipera- mide (3.43%) and guineensine (3.23%) were the major components (Gurdip-Singh et al., 2004).

The composition of flower and leaf oils of *Achillea eriophora* was analysed by Jaimand and Rezaee (2004). The percentages of flower and leaf oils, calculated on the basis of dry weight and analysed by GC and GC/MS, was 1 and 0.9% and 1.2 and 0.9%, respectively. The major constituents, determined by steam distillation method, in flower were 1, 8-cineole [eucalyptol] (45.0%), beta-pinene (16.6%) and (E)-nerolidol (7.6%), and in leaf were 1, 8-cineole (41.5%), (E)-nerolidol (10%) and beta-pinene (9.8%). The major constituents, determined by hydro-distillation method in flower were 1, 8-cineole (41.3%), beta-pinene (12.4%) and alpha-thujene (6.5%), and in leaf were 1, 8-cineole (41.0%), beta-pinene (13.8%) and terpinen-4-ol (9.1%).

The volatile oil of *Lippia javanica* was prepared by hydrodistillation of leaves, flowers and stems, and characterized by GC-MS. The major component was 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one (Manenzhe, 2004). The GC-MS analysis of the essential oil, obtained from steam distillation of the oleoresin of *Pistacia atlantica* var. *mutica*, has led to the identification and quantification of eleven terpenoids, alpha-pinene (70%), beta-pinene (1.94%), 3-carene (0.2%), carveol (2.18%), epoxypinene (2.15%), limonene oxide (9%), myrtenol (5.31%), limonene (0.62%), citral (5.72%), alpha-phellandrene (0.2%), and beta-myrcene (0.3%) (Delazar et al., 2004).

The volatile compounds obtained by hydrodistillation of the aerial parts of *Rosmarinus officinalis* were analysed by GC/MS. Thirty compounds were characterized representing 98.2% of the essential oil with 1,8-cineole (29.5%), 2-ethyl-4,5-dimethylphenol (12.0%) and camphor (11.5%) as the major components (Touafek et al., 2004). The chemical constituents of leaf oils of *Elephantopus scaber* L. from 12 locations in Southern China were investigated using GC/MS. A total of 24
compounds were detected, of which 20 were identified by their mass spectra fragmentation patterns. The major compounds include hexadecanoic acid (8.19-39.22%), octadecadienoic acid (trace-29.22%), five alkane homologues, i.e., n-tetradecane (1.19-5.26%), n-pentadecane (3.22-12.05%), n-hexadecane (2.38-16.26%), n-heptadecane (2.48-15.32%), and n-octadecane (1.39-9.59%), as well as tetramethylhexadecanol (2.06-4.31%) (Wang-Li et al., 2005).

Twenty-four constituents were identified from leaves of *Podocarpus fleuryi* by GC-MS analyses (He-DaoHang, 2005). The identified constituents represent 97.29% of the peak area of the oil. Terpenes and alcohols are major chemical constituents in the oil. The main compounds were alpha-pinene (8.67%) (-)-beta-elemene (2.68%), beta-caryophyllene (3.97%), and (+)-ledene (2.11%), germacrene D (21.56%).

The petroleum ether fractions of ethanol extract from the leaves of *Cunninghamia lanceolata* was analysed by the capillary GC-MS method (Gao et al., 2006). Thirty-eight components were isolated, and thirty-six of them were identified. The identified composed 98.06% of the ether fraction. 2-hydroxy-1, 3-propanediyl octadecanoic acid ester (16.929%), heptacosane (15.178%), 2-(octadecyloxy) ethyl hexadecanoic acid ester (11.038%), 1-heptatriacotanol (6.877%) and oleic acid (6.531%) accounted for most of the ether. The main components were saturated fatty acid esters (33.834%) and hydrocarbon (22.031%).

The chemical compositions of volatile oils of the leaves, stem, rhizomes and roots of the medicinal plant Galangal (*Alpinia galanga*) from South Kerala, were analysed by capillary GC and GC-MS. Monoterpene and sesquiterpene compounds along with methyl cinnamate were identified in all the six oils. The major constituents of leaf oils were fenchyl acetate (20.7% leaf oil-I) and beta-caryophyllene (40.5% leaf oil-II) along with beta-farnesene, caryophyllene oxide, and 1,8-cineole. The main constituent of the stem oil (I) was cubenol (28.4%) followed by humulene (5.1%), germacrene (4.9%) and cadinene (2.5%). Galangal rhizome oil (I) had carotol (26.7%), 1,8-cineole (10.8%), fenchyl acetate (4.8%), beta-caryophyllene (5.8%), methyl cinnamate (2.7%) and rhizome oil (II) contained 1,8-cineole (30.3%), beta-pinene (6.5%), camphor (5%), fenchyl acetate (7.2%), methyl cinnamate (2.5%) along with limonene, camphor, alpha-terpineol, and cubenol.
The root oil (I) was found to have fenchyl acetate (30.5%) along with 1, 8 cineole and limonene (Menon, 2006).

GC-MS analysis of the essential oil of the mature fruits of *Lythraea molleoides* detected the presence of limonene (89.89%), alpha-pinene (3.48%), beta-pinene (2.63%), alpha -terpineol (1.27%), myrcene (0.64%), sabinene hydrate (0.54%), 4-terpineol (0.28%), camphene (0.22%) and delta -3-carene (0.13%) (Shimizu et al., 2006).

The essential oil from fresh and dried rhizomes of *Hedychium coronarium* on GC-MS analysis resulted in the identification of 44 and 38 constituents representing 93.91% and 95.41%, respectively. The major components of the essential oil from fresh and dried *H. coronarium* rhizome were 1, 8-cineole (41.42%, 37.44%), beta -pinene (10.39%, 17.4%) and alpha -terpineol (8.8%, 6.7%) (Beena et al., 2006).

GC/MS of the volatile components of the aerial part of *Macfadyena unguis-cati* revealed 74 compounds, 52 of them (representing 75.97%) were identified by Aboutabl et al. (2006). The major compound is n-decane (12.21%) followed by phytol (12.19%). The saponifiable fraction of the petroleum ether extract showed 21 fatty acid identified as methyl esters. 37 compounds were identified in the unsaponifiable fraction; representing 93.26%, beta -amyrin, squalene, beta -sitosterol and 3 alpha, 5-cyclo-ergosta-7, 22-dien-6-one were identified in the USM. Determination of LD<sub>50</sub> of different extracts showed that total ethanol extract is the safest (4.9 g/kg) followed by petroleum ether extract (4.5 g/kg) and ethyl acetate extract having the least LD<sub>50</sub> (3.1 g/kg).

The chemical composition of the volatile oil *Artemisia scoparia* was studied by GC-MS. Nineteen components (99.51% of the total composition) were identified. Beta -Pinene (19.01%), capillin (17.45%), limonene (15.11%), myrcene (10.95) were found to be the major constituents of the oil (Negahban et al., 2006).

Oils from *Mentha rotundifolia* were analysed by GC-FID and GC/MS (Brada et al., 2007). The results revealed the presence of two chemotypes. One chemotype contained high proportions of piperitone oxide (19.7-31.4%) and piperitenone oxide (27.8-29.4%). The other chemotype had piperitenone (54.9%) and piperitenone oxide (17.6%) as its major constituents.
Essential oils obtained by hydrodistillation from the rhizomes and leaves of *Amomum pterocarpum* (Zingiberaceae) were analysed by Sabulal *et al.* (2007). Thirty-six constituents each were identified from the rhizome (95.7%) and leaf oils (92.6%). Beta-pinene was the major constituent in both the rhizome oil (65.5%) and the leaf oil (41.7%). Phytol (26.5%) was the other major constituent in the leaf oil.

The composition of the essential oil from the aerial parts of *Levisticum officinale* was analysed by GC and GC-MS. The main components of the oil were beta-pinelandrene (42.5%), alpha-terpineol (27.9%), cis-ocimene (7.5%) and dehydro-1, 8-cineol (6.8%) (Reza and Abbas, 2007).

Maccioni *et al.* (2007) analysed the essential oil of the flowering aerial parts of *Teucrium scorodonia* L. by GC and GC-MS. All the identified compounds were sesquiterpene hydrocarbons. The main ones were germacrene B (26.2%) and beta-caryophyllene (25.2%).

Sghaier *et al.* (2007) analysed the phytochemical composition of the essential oil of *Teucrium ramosissimum* (aerial parts). A total of 68 compounds, accounting for 99.44% of the essential oil, were identified by GC and GC/MS. The major compounds were beta-eudesmol (61; 44.52%), caryophyllene oxide (56; 9.35%), -thujene (1; 5.51%), sabinene (4; 4.71%), and T-cadinol (59; 3.9%).

The essential oil from the aerial parts of *Salvia leucantha* Cav. (Lamiaceae) was analysed by GC, GC/MS and NMR spectroscopy (Anuradha *et al.*, 2007). The oil was found rich in sesquiterpene hydrocarbons; beta-caryophyllene (13.9%), alpha-guaiene (12.6%), cis-muurola-3,5-diene (10.8%), germacrene D (13.8%) and bicyclogermacrene (8.7%). Bornyl acetate constituted 23.9% of the oil.

The chemical composition of the essential oil of *Juniperus oxycedrus* from Algeria was studied by Dob *et al.* (2006). The oil was obtained from the leaves of the plant by hydrodistillation and analysed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) techniques. The yield of essential oil is 0.1%, based on dry weight. Eighty-nine components representing 82.3% of the total oil were identified. The results show that oxygenated monoterpene (41.0%) composes the major part of oil and the main components are trans-pinocarveol (7.0%), cis-verbenol (6.3%), and manoyl oxide (6.0%).
The chemical components of *Paulownia tomentosa* were separated and identified by GC-MS. Out of the sixty-nine compounds (89.33% ) identified, the most abundant were benzyl alcohol (13.276%), 1, 2, 4-trimethoxybenzene (8.342%), phenol, 2-methoxy-3-(2-propenyl) (6.141%), phenol, 3, 4-dimethoxy-(3-986%), tricosane (3.682%) and pentacosane (3.238%) (Wang-Xiao, 2006).

Sarkhail *et al.* (2006) reported that the main compounds *Phlomis olivieri* Benth. (Lamiaceae), were germacrene D (66.1%), beta -selinene (5.1%), beta -caryophyllene (4.2%) and alpha -pinene (4.2%). A comparison of the composition of this oil with other oils of *P. olivieri* from different regions showed that germacrene D and beta-caryophyllene are main compounds of all oils.

The essential oil of *Dracocephalum moldavica* L. (Labiatae) were analysed by means of GC and GC/MS and identified ninety components. The major constituents of the essential oil were limonene (19.8%), alpha -pinene (14.4%), methyl geranate (8.5%), geranyl acetate (7.9%), carvacrol (7.8%) and geranial (5.4%). The essential oil of *D. moldavica* is rich in monoterpenoids (Morteza-Semnani *et al.*, 2007).

Liu-Ling *et al.* (2007) analysed the essential oils of *Piper nigrum* L. and *Piper longum* L. Thirty compounds were separated and identified from *P. nigrum*. The main components were beta -caryophyllene (23.49%), 3-carene (22.20%), D-limonene (18.68%), beta -pinene (8.92%) and alpha -pinene (4.03%). Forty-five compounds were separated from *P. longum* and identified. The main components were beta -caryophyllene (33.44%), 3-carene (7.58%), eugenol (7.39%), D-limonene (6.70%), zingiberene (6.68%) and cubenol (3.64%).

The chemical composition of the essential oil from *Tanacetum polycephalum* was analysed by GC and GC/MS and 39 compounds constituting 94.02% of the oil were identified, the major components being borneol (28.30%), beta -pinene (10.10%), alpha -pinene (6.5%), camphene (6%), alpha -terpineol (5.16%) and 1, 8-cineol (5.10%) (Amiri, 2007).

Farsam *et al.* (2007) studied the chemical composition of the essential oil of *Chimonanthus fragrans*, an aromatic plant by GC and GC-MS. Forty-nine components were identified corresponding to ~98.12% of the total components of the essential oil with 0.12% yield. The major components were elemol (20.06%), beta -
caryophyllene (9.51%), beta-elemene (8.65%), bicyclo-germacrene (8.15%), gamma-elemene (7.2%), germacrene-D (5.65%), trans-beta-ocimene (5.5%), sabinene (3.65%), linalool (2.6%), caryophyllene oxide (2.3%), and delta-cadinene (1.95%).

The essential oil obtained from the stem bark of *Croton urucurana* was analysed by GC and GC-MS. 83 compounds were identified and borneol (14.7%), bornyl acetate (5.2%), 1-isopropyl-7-methyl-4-methylene-1,3,4,5,6,8-hexahydro-2H-naphthalen-4a-ol (14.7%), sesquicineole (10.5%) and gamma-gurjunene epoxide (5.4%) were the main components (Simionatto et al., 2007).

The chemical compositions of the leaf essential oils of *Beilschmiedia brenesii*, *Cinnamomum paratriplinerve*, *Cinnamomum tonduzii*, *Nectandra smithii*, *Ocotea endresiana* and *Ocotea praetermissa* (Lauraceae), determined by GC-MS (Agius et al., 2007). *B. brenesii* leaf oil was dominated by (E)-caryophyllene (43.4%), alpha-humulene (26.5%), and (Z)-beta-ocimene (19.3%). Pinenes dominated the leaf oils of *C. paratriplinerve*, *N. smithii*, *O. endresiana* and *O. praetermissa*. Germacrene D (16.0%) was also abundant in *N. smithii* oil while alpha-humulene (14.3%) was abundant in *O. endresiana* oil. The leaf oil of *C. tonduzii* was composed largely of alpha-pinene (17.4%), germacrene D (16.8%) and (E)-caryophyllene (14.1%).

The composition of the essential oil of *Zosima absinthifolia* (Umbelliferae) was studied by GC and GC/MS (Shafaghat, 2007). Among the 21 identified components constituting 96% of the oil, octyl acetate (24.69%), beta-caryophyllene (22.24%) and (Z)-beta-ocimene (8.9%) were the major constituents. In the essential oil of the plant, sesquiterpenes predominated over monoterpenes.

*Hypericum perforatum* volatile oils were extracted by supercritical-CO$_2$ fluid extraction and the compounds were identified using gas chromatography mass spectrometry (GC-MS). In total 47 compounds were identified; caryophyllene oxide, spathulenol, cyclododecane and dodecanoic acid were the main components (Lv-Ying Gang et al., 2007). Sixteen volatile compounds were identified from the n-hexane extract of the buds of *Syzygium aromaticum* by using gas chromatography-mass spectroscopy (GC-MS). The major components were eugenol (71.56%) and eugenol acetate (8.99%). The dichloromethane extract of the buds yielded limonin and ferulic
aldehyde, along with eugenol. The flavonoids tamarixetin 3-O-beta-D-glucopyranoside, ombuin 3-O- beta -D-glucopyranoside and quercetin were isolated from the ethanol extract (Nassar et al., 2007).

The essential oils from the green branchlets of Cupressus dupreziana A.Camus were analysed by GC and GC/MS (Ramdani et al., 2007). The main constituents of the essential oils were alpha -pinene (27.2%-44.2%), germacrene D (16.2%-27.2%) and Delta 3-carene (14.2%-26.7%). Dongmo et al. (2007) analysed the essential oil of fresh leaves of Cinnamomum zeylanicum by GC and GC/MS. The oil contains 11 components among which eugenol (89.1%), linalool (4.3%), benzoate benzyl (3.1%) and cinnamaldehyde (1.5%) were the main components.

The essential oils of Cassia sophera Linn. was analysed by GC-MS and a total of 42 compounds have been identified. The major constituents are palmitic acid (22.82%), linoleic acid (8.32%), elaidic acid (19.16%), stearic acid (9.86%), 5-isopropyl-6-methyl-3-heptyne-2,5-diol (6.44%), undecyl lauric acid (6.61%), oleic acid (2.1%), arachidic acid (3.57%) and 3 alpha , 7 beta -dihyodxy-5 beta , 6 beta -epoxycholestane (5.9%) (Mostafa, 2007).

GC and GC/MS analysis of the essential oil of aerial part of Lamium maculatum showed the presence of thirty compounds which account for 99.9% of the total composition (El-Sayed, 2008). Twenty-four components representing (93.56%) of the oil were identified. The major components were beta -caryophyllene (14.80%), caryophyllene oxide (13.84%), Z, E- alpha -franesene (10.11%), dihydroedulan I (9.13%), alpha -humulene (6.06%), bornyl formate (6.03%) and alpha -bisabolene (5.34%).

Analysis of the leaf essential oil of Callistemon pinifolius and C. salignus using GC and GC-MS resulted in the identification of fifty five and fifty one compounds, respectively. 1, 8-Cineole (39.1% and 40.8%), alpha -pinene (26.7% and 24.2%), and (E)- beta -terpineol (9.6% and 13.5%), were the major constituents (Saxena et al., 2008).

The essential oils of Acanthospermum hispidum DC. aerial parts, and leaves of Tithonia diversifolia have been characterised by combined gas GC and GC-MS analyses by Gbolade et al. (2008). Twenty-nine components have been fully identified.
and grouped into monoterpane hydrocarbons (22.2%), oxygenated monoterpenes (4.6%), sesquiterpenes hydrocarbons (58.2%) and oxygenated sesquiterpenes (10.8%) in *A. hispidum*. The main constituents of the oil were beta-caryophyllene (28.0%), alpha-pinene (15.9%) and bicyclogermacrene (11.0%) among the hydrocarbon compounds, and bisabolol (8.9%) and carvacrol methyl ether (4.1%) among the oxygenated components. *T. diversifolia* essential oil comprised of seventeen components and was characterised by a predominant content of monoterpane hydrocarbons (87.9%), cis-beta-octimene (43.7%), alpha-pinene (28.6%) and limonene (12.0%) being the main compounds.

The essential oil and oleoresins (ethanol, methanol, CCl4 and isoctane) of *Zingiber officinale* were extracted respectively by hydrodistillation and Soxhlet methods and subjected to GC-MS analysis. Geranial (25.9%) was the major component in essential oil; eugenol (49.8%) in ethanol oleoresin, while in the other three oleoresins, zingerone was the major component (33.6%, 33.3% and 30.5% for methanol, CCl4 and isoctane oleoresins, respectively) (Gurdip et al., 2008).

Volatile constituents of the dried leaves and stems of *Nasturtium officinale* R. Br. were analysed by GC and GC/MS. The major volatile constituents of the leaves were 2-phenylethyl isothiocyanate (72.9%), pulegone (8.0%), heptyl isothiocyanate (4.9%) and 4-phenyl butyl isothiocyanate (3.2%), while the main volatile constituents of the stems were 2-phenylethyl isothiocyanate (83.5%), 4-phenylbutyl isothiocyanate (6.9%), pulegone (2.2%) and sec-butyl isothiocyanate (1.9%) (Afsharypuor and Salehi, 2008).

The chemical composition of essential oils extracted from *Xylopia frutescens* Aubl. (Annonaceae) fruit was examined by Sena-Filho *et al.* (2008). GC/MS detected 23 terpenoid compounds, in which the principal components were germacrene D (24.2%), linalool (12.1%), beta-pinene (8.0%), cis-sabinene hydrate (7.9%), trans-pinocarveol (7.8%), alpha-copaene (7.0%) and limonene (5.6%). Other constituents found were sabinene, m-cymene, 1, 8-cineole, beta-octimene, perillene, cis-sabinol, alpha-campholenal, isopinocamphone, terpinen-4-ol, verbenone, trans-carveol, carvone, perillyl alcohol, alpha-cubebene, delta-cadinene and elemol.
Essential oils of rhizomes and aerial parts of *Kyllinga brevifolia* were analysed by GC-FID and GC/MS (Guilhon *et al.*, 2008). The oils reveal a high content of the diterpenoids belonging to the labdane group, mostly manoyl oxide (6.8%-31.1%), 13-epi-manoyl oxide (5.7%-26.1%), 11 alpha-hydroxymanoyl oxide (5.9%-16.2%) and 1 beta-hydroxymanoyl oxide (4.6%-22.1%). Hexane extract obtained of the rhizomes collected in Santarem Novo was rich in manoyl oxide (30.4%), 11 alpha-hydroxymanoyl oxide (26.7%) and 1 beta-hydroxymanoyl oxide (14.7%).

Jjunor *et al.* (2008) were analysed the essential oils from the leaves and bark of *Bursera hollickii* by GC and GC/MS. Sixty-two (62) components were identified from the leaf oils which constituted ca. 99% of the oils, while sixty-three (63) components were identified from the bark oils which constituted ca. 97% of the oils. Monoterpene represented the major components of the oils with alpha-pinene (49.8% and 34.8%), beta-pinene (11.0% and 10.6%), terpinolene (0.7% and 13.4%) and alpha-terpineol (5.7% and 8.9%) being the major component. Of the sesquiterpenes, the predominant components were beta-caryophyllene (4.8% and trace) and alpha-humulene (3.4% and 0.7%).

The essential oil from the fruits of *Ferulago longistylis* Boiss. (Apiaceae) was analysed by GC and GC/MS resulted in the identification of 59 compounds. The major constituents found were 2, 3, 6-trimethylbenzaldehyde (29%), alpha-pinene (17%), (Z)-beta-ocimene (16%), sabinene (6%), myrcene (6%) and bornyl acetate (4%) (Ozkan, 2008).

The chemical composition of the essential oils obtained from the fruits of *Pimpinella affinis* Ledeb. (Apiaceae) was analysed by GC and GC-MS techniques. Twenty-four components were identified in the essential oil of *P. affinis*, whose major constituents were geijerene (17.68%), limonene (12.86%), pregeijerene (9.92%), germacrene D (8.54%) and trans-beta-ocimene (4.94%) (Mohammadreza, 2008).

The volatile oil of *Cymbopogon proximus* was analysed by GC/MS (El-Tahir *et al.*, 2008). The chromatogram showed 8 peaks corresponding to 8 components, with piperitene representing 72.44% of the oil’s composition. Vaverkova *et al.* (2008) analysed essential oil of leaves and flowers of various *Tanacetum* spp. by GC/MS.
A total of 19-34 compounds, representing 70-93% of the oils in flower of *Tanacetum parthenium* and in various aerial parts of *T. balsamita* were revealed. The major constituents were borneol, bornyl acetate, camphor, camphene and 1, 8-cineole [eucalyptol].

Chemical analysis of volatile constituents from native *Schizandra chinensis* was conducted by GC and GC/MS spectrometric analyses (Lim *et al.*, 2008). The major constituents were alpha -pinene (7.46%), beta -pinene (30.66%), 1,8-cineole (4.81%), and 4-methyl-1-(1-methylethyl)-3-cyclohexene-1-ol (7.90%) from the stems, and camphene (10.05%), beta -pinene (17.67%), sabinene (4.02%), 1-limonene (4.04%), 1-methyl-4-(1-methylethyl)benzene (4.97%), linalyl acetate (7.86%), and linalool oxide (4.84%) from the roots.

GC-MS analysis of the essential oil from *Foeniculum vulgare* seed showed the presence of 31 components accounting for 95.2% of the total amount (Muhammad *et al.*, 2008). The major component was trans-anethole (70.1%). The analysis of ethanolic and methanolic seed extracts showed the presence of 9 components, including linoleic acid (56%), palmitic acid (5.6%) and oleic acid (5.2%).

Verdian and Hadjiakhoondi (2008) identified 39 compounds from the essential oil of *Laurus nobilis* L. (Lauraceae) by GC and GC-MS. The main compounds were 1, 8-cineole, trans-sabinene hydrate, alpha -terpinyl acetate, methyl eugenol, sabinene, eugenol and alpha -pinene. The essential oil from leaves of *Coriandrum sativum* L. (Apiaceae), obtained by hydro-distillation was analysed by gas chromatography-mass spectrometry (Matasyoh *et al.*, 2009). Out of 27 peaks, 24 components, which constitute 92.7%, were identified in the oil. The oil was dominated by aldehydes and alcohols which accounted for 56.1% and 46.3% of the oil, respectively. The major constituents were 2E-decenal (15.9%), decanal (14.3%), 2E-decen-1-ol (14.2%) and n-decanol (13.6%). Other constituents present in fairly good amounts are 2E-tridecenc-1-al (6.75%), 2E-dodecenal (6.23%), dodecanal (4.36%), undecanol (3.37%), and undecanal (3.23%).

The essential oils from the dried leaves, pseudostems and rhizomes of *Alpinia conchigera* Griff. were analysed by capillary GC and GC-MS. Forty one compounds were identified and the most abundant components in the leaf oil included beta -
bisabolene (15.3%), beta-pinene (8.2%), beta-sesquiphellandrene (7.6%), chavicol (7.5%) and beta-elemene (6.0%), while beta-bisabolene (19.9%), beta-sesquiphellandrene (11.3%), beta-caryophyllene (8.8%) and beta-elemene (4.7%) were the main components in the pseudostem. In the rhizome, 1,8-cineole (17.9%), beta-bisabolene (13.9%), beta-sesquiphellandrene (6.8%) and beta-elemene (4.0%) were the major components (Halijah-Ibrahim et al., 2009).

Liang et al. (2009) reported that the essential oils of *Salvia miltiorrhiza* are beta-caryophyllene (12.2-31.7%), beta-caryophyllene oxide (1.4-11.6%), alpha-caryophyllene (4.8-10.6%), cadinadiene (7.4-29.3%), and hexadecanoic acid (3.9-18.8%).

The chemical composition of the *Lippia rugosa* essential oil was determined by GC-MS. Geraniol (51.5%), nerol (18.6%) and geranial (10.4%) were the main components of Lippia oil (Tatsadjieu et al., 2009). The essential oils obtained from leaves of two *Myrtus communis* varieties (baetica and italic) were investigated by GC and GC-MS at their different phenological stages by Wannes et al. (2009). The highest essential oil yield was observed at the flowering stage with 0.6% (w/w) for italic and 0.4% (w/w) for baetica and 49 compounds were identified. The main essential oil leaf compounds of both myrtle varieties, belonging to the monoterpene class, were alpha-pinene, 1,8-cineole, limonene and linalool and their percentages showed significant changes during the phenological stages.

Volatile constituents of *Ocimum sanctum* and *O. basilicum* were extracted using various solvents and their chemical constituents were identified and quantified by using GC-MS in optimized conditions (Vani et al., 2009). The profiles of extract from both species were compared in an effort to investigate effects of seasonal variation on their chemical compositions. The predominant volatile constituents in *O. sanctum* and *O. basilicum* were found to be methyl eugenol and methyl chavicol, respectively, during different months of analysis.

GC and GC-MS analyses of the essential oils of leaves, stems and inflorescences of *Piper marginatum* revealed the presence of 40 components accounting, respectively, for 99.6%, 99.7% and 99.1% of the leaf, stem and
inflorescence oil, the most abundant being (Z)- or (E)-asarone and patchouli alcohol (Autran et al., 2009).

Bajpai et al. (2009) examined the chemical composition of the essential oil isolated from the floral parts of Nandina domestica by hydrodistillation. The GC-MS analysis determined 79 compounds (which represented 87.06% of total oil), were present in the oil containing mainly 1-indolizino carbazole (19.65%), 2-pentanone (16.4%), mono phenol (12.1%), aziridine (9.01%), methylcarbinol (4.6%), ethanone (3.3%), furfural (2.96%), 3,5-dimethylpyrazole (1.29%) and 2(5H)-furanone (1.32%).

The steam volatile components of Chile pepper (Capsicum annuum L. var. glabriusculum) at two ripening stages (green and red) were analysed using GC and GC-MS; 140 constituents were identified, of which hexyl isopentanoate, hexyl 2-methylbutanoate, limonene, hexyl isohexanoate, (E)-2-hexenal, isopentyl isopentanoate and (Z)-3-hexenyl isopentanoate were found to be the major constituents (Forero et al., 2009).

The constituents of the essential oils of leaf, petiole, shoot and terminal shoot of Cinnamomum malabaricum were determined by Leela et al. (2009). Thirty-nine compounds, constituting 95% of the oil, were identified in the leaves. Major constituents of the leaf oil were (E)-caryophyllene (28.6%), (E)-cinnamyl acetate (15.1%), bicyclogermacrene (14.4%) and benzyl benzoate (8.5%). Twenty-eight compounds, representing 98% and 97% of the oil, were identified in the petioles and shoots, respectively, whereas in the oil of the terminal shoots 34 compounds, accounting for 97%, were identified. The essential oils of the petioles, shoots and terminal shoots were dominated by linalool (77.8-79.4%).

Chutia et al. (2009) analysed the essential oil fully matured ripen fruits of Citrus by GC and GC-MS. Thirty seven different components were identified constituting approximately >=99% of the oil. The major components were limonene (46.7%), geranial (19.0%), neral (14.5%), geranyl acetate (3.9%), geraniol (3.5%), β-caryophyllene (2.6%), nerol (2.3%), neryl acetate (1.1%) etc.
4.3. Materials and Methods

4.3.1. Plant collection

*Kaempferia galanga* were collected from Kerala Agricultural University, Vellanikara Trissur Dt., Kerala, India and identified at Rapinat Herbarium (RHT), St. Joseph’s College (*Autonomous*), Tiruchirappalli, Tamil Nadu. A voucher specimen was prepared and deposited in the Rapinat Herbarium.

4.3.2. Extraction

Collected plants were shade dried. Rhizomes, roots and leaves were powdered separately. The dried and powdered materials were extracted by hot extraction process using a soxhlet extraction device with solvent ethanol for 72 hours at a temperature not exceeding the boiling point of the solvent. The filtrates were concentrated in vacuum rotary evaporator at 60°C in order to reduce the volume. The paste like extracts were stored in labeled screw capped bottles and kept in refrigerator at 4°C. The extracts were subjected to GC-MS.

4.3.2.1. GC-MS analysis

Gas Chromatography-Mass Spectrometry analysis was performed with a Fisons GC-MS instrument. A splitless mode was chosen with helium as carrier gas. The column was DBS MS of 30 m in length, 0.25 mm in diameter and 0.25 mm film thickness and 1μl (1 mg/ml) the active fractions (substances) dissolved in ethanol was injected in the following conditions, injector temperature, 280°C carrier gas, helium, pressure 150 Kpa, lionization mode E⁺ solvent delay (min) 2.00, temperature gradient, 20°C per minute from 100 to 315°C. The analysis was carried out at the Food Testing Laboratory of Indian Institute of Crop Processing Technology (IICPT), Thanjavur, TamilNadu, India.

4.4. Result and Discussion

The chromatograms of phytochemical components of rhizome, leaves and roots of *K. galanga* are shown in Fig 4.1, 4.2, 4.3 respectively. Mass spectra were used to identify and structurise the components comparing with those in NIST (National Institute of Standards and Technology) Library.

The plant extracts appeared to have 72 compounds as shown in Table 4.1. Seventeen compounds in rhizome alone, fourteen in root and thirty in leaf. Two
compounds were common for all extract *ie.* n-Hexadecanoic acid and 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester. Eucalyptol 2-Propenoic acid, 3-phenyl-, ethyl ester, Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1â, 4âa, 8âa)-. 2-Propenoic acid, 3-phenyl-, ethyl ester (Synonym: Cinnamic acid, ethyl ester), Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1â, 4âa, 8âa)- (Synonym: ç-Muurolene) are present in rhizome and leaf. Pentadecane, 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol are present in leaf and root. Octadecanoic acid is present in rhizome and root.

Activity of ethanolic extract of *K. galanga* rhizome, leaf and root are shown in Table 4.2, 4.3, 4.4 respectively. P-menth-1-en-8-ol, 7-Octen-2-ol, 2-methyl-6-methylene, 1-Deoxy-d-mannitol, Phytol, ç-Tocopherol are showing anti cancer activity (Table 4.5). Oleic Acid, á-Pinene, Isopinocarveol, Santolina triene, 9-Octadecenoic acid (Z)-, methyl ester, 9, 12-Octadecadienoic acid, methyl ester are having cancer preventive action (Table 4.6). Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro- 7-methyl-4-methylene-1- (1-methylethyl)-, (1â, 4âa, 8âa)-, (+)- Cycloisosativene, Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis (1-methylethenyl)-, [1S-(1â, 2â, 4â)]- (Synonym: (-)-á-Elemene), Caryophyllene, ç-Tocopherol, 1H-Cycloprop[e]azulene, 1a, 2, 3, 4, 4a, 5, 6, 7b-octahydro-1, 1, 4, 7-tetramethyl-, [1aR-(1âa, 4â, 4âa, 7bâ)]- [Synonyms: à-Gurjunene], are having anti tumor activity (Table 4.7).

The other important activities of *K. galanga* are antioxidant (Table 4.8), hypocholesterolemic (Table 4.9), anti-inflammatory (Table 4.10), anti-achene (Table 4.11), antiseptic (Table 4.12), anti-androgenic (Table 4.13), sedative (Table 4.16), anesthetic (Table 4.17), and analgesic (Table 4.18). This plant also shows antimicrobial (Table 4.15) especially antibacterial activity (Table 4.14). Some phytocomponents are known to posses herbicidal, nematocidal, fungicidal, vermicidal, pesticidal, insecticidal and termiticidal action (Table 4.19).

Eucalyptol, á-Pinene, Isopinocarveol, Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-(Synonym: (-)-Borneol, and Santolina triene are having herbicidal activity. Eucalyptol, á-Pinene, Isopinocarveol, Bicyclo [2.2.1] heptan-2-ol,
1,7,7-trimethyl-, (1S-endo)-(Synonym: (-)-Borneol, Santolina triene, 9,12-Octadecadienoic acid, methyl ester, p-menth-1-en-8-ol, 7-Octen-2-ol, 2-methyl-6-methylene- and n-Hexadecanoic acid are showing nematicidal properties.

Eucalyptol, á-Pinene, Isopinocarveol, Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-(Synonym: (-)-Borneol, (+)-Cycloisosativene, Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro- 7-methyl-4-methylene-1- (1-methylethyl)-,(1ä,4ä,8ä)-(Synonym: ç-Muurolene), 1H-Cycloprop[e]azulene, 1a, 2, 3, 4, 4a, 5, 6, 7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aè, 4â, 4aä, 7bè)]-[Synonyms: à-Gurjunene, Santolina triene, 3H-3a,7-Methanoazulene, 2, 4, 5, 6, 7, 8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3äà,4ä,7ä and Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1ä, 4aä, 8aä)- are having the activity of fungicide.

Eucalyptol, á-Pinene, Isopinocarveol, Bicyclo[2.2.1]heptan-2-ol, 1, 7, 7-trimethyl-, (1S-endo)-(Synonym: (-)-Borneol, Oleic Acid, Santolina triene, 9,12-Octadecadienoic acid, methyl ester, Eucalyptol, p-menth-1-en-8-ol and 7-Octen-2-ol, 2-methyl-6-methylene- are act as insecticide. There are some phytochemical compounds in K. galanga where no activity so far reported (Table 4.20). The structures of new compounds were established by spectral analysis.

The constituents of the rhizome of K. galanga include cineol, borneol, 3-carene, camphene, kaempferol kaempferide cinnamaldehyde, p- methoxy cinnamic acid and ethyl cinnamate (Anonymous, 1959). Wong et al. (1992) investigated the essential oil of K. galanga rhizome and identified fifty four compound. The major constituents were ethyl trans -p-methoxycinnamate (51.6%), ethyl cinnamate (16.5%) pentadecane (9.0%), 1.8-cineole (5.7%), delta-car-3-ene (3.3%) and borneol (2.7%). Terpenoid constituents amounted to 16.4%. According to Seidemann (1992) the main constituents of rhizome of K. galanga were cinnamic acid ethyl ester and p-methoxycinnamic acid ethyl ester. The main ingredient of K. galanga volatilization oil was verified as ethyl 3-(4-methoxyphenyl)-(E)-acrylate (Hong-Li, 2008). Puthan et al. (1926) reported that the oil of K. galanga from India possessed ethylcinnamates and ethyl p-methoxycinnamates as the main compound together with paraffin hydrocarbon. Another report indicated that the main components of K. galanga oil
found to be β-phyllandrene, α-terpineol, ethylcinnamate and dihydroβ-sesquiphellandrene (Sudibyo, 2000).

The present study also coincides with the results obtained from other Zingiberaceae members. The essential oil from fresh and dried rhizomes of *Hedychium coronarium* on GC-MS analysis resulted in the identification of 44 and 38 constituents representing 93.9% and 95.41% respectively. The major components of the essential oil from fresh and dried *H. coronarium* rhizome were 1, 8-cineole, beta-pinene and alpha terpineol. The aromatic oil has antifungal as well as antibacterial effects (Beena, 2007). Arambewela *et al.* (2007) analysed the volatile oil of rhizome of *Alpinia galanga* (Zingiberaceae) by GC and GC-MS. Sixteen compounds were identified and reported to be used as emmenagogue, aphrodisiac, anti-inflammatory and in the treatment of of bronchitis, heart disease, chronic enteritis, kidney stone, diabetes and rheumatism etc.

The essential oils of *Zingiber ottensii* and *Z. zerumbet* fresh rhizomes were analysed by a combination of capillary GC and GC-MS (Sri-Nurestri *et al.*, 2005). The most abundant component of *Z. ottensii* and *Z. zerumbet* rhizome oil was zerumbone. Other major components of *Z. ottensii* were terpinen-4-ol, alpha-humulene and sabinene whilst that of *Z. zerumbet* were alpha-humulene, camphene and caryophyllene oxide.

The essential oil of fresh rhizomes of *Curcuma zedoaria* was made up of monoterpenoids and sesquiterpenoids, with furanogeremenone as the major component (Malek *et al.*, 2004). The rhizome oil of *Alpinia galanga* contained 84 constituents of which the major ones were 1, 8-cineole [eucalyptol], alpha-turmerone, beta-caryophyllene, ar-turmerone and beta-sesquiphellandrene. The leaf oil contained 83 components, of which the main constituents were terpipolene, 1, 8-cineole, alpha-phellandrene and terpinen-4-ol (Raina *et al.*, 2002). The rhizome oils of *A. galanga* contained limonene, 1,8-cineole [eucalyptol], camphor, alpha–terpineol, alpha-fenchyl acetate and (E)-methyl cinnamate, as the major constituents. The major constituents of the leaf oils from the same locations were: alpha-pinene, camphene, beta-pinene, 1,8-cineole and camphor (Mallavarapu *et al.*, 2002). According to Ibrahim *et al.* (2004) the rhizome oil *A. galanga* was rich in 1, 8-cineole.
Gurib et al. (2002) reported that *Zingiber officinale* oil was characterized by the presence of geranial, neral, zingiberene, beta-sesquiphellandrene and ar-curcumene. The oils of *H. coccineum* were characterized as (E)-nerolidol, trans-sesquisabinene hydrate; *H. flavescentes*: linalool, 1,8-cineole [eucalyptol], beta-pinene, alpha-terpineol and alpha-pinene; *H. coronarium*: alpha–muurolol, alpha-terpineol, 1,8-cineole, an unknown sesquiterpene alcohol, alpha-fenchyl acetate, citronellal and (E)-methyl cinnamate.

The rhizome and leaf oils of *Alpinia zerumbet* were found to be rich in beta-pinene, 1,8-cineole [eucalyptol] and terpinen-4-ol, respectively. The rhizome oils of *A. purpurata* contained alpha-pinene and beta-pinene, The rhizome and leaf oils of *H. coronarium* were rich in alpha-pinene, beta-pinene, 1, 8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively. The rhizome oils of *H. gardnerianum* were rich in alpha-pinene, beta-pinene, 1,8-cineole and beta-caryophyllene, respectively.

Essential oils of rhizomes and leaves of *Amomum pterocarpum* (Zingiberaceae) were analysed by Sabulal et al. (2007). Thirty-six constituents each were identified from the rhizome and leaf oils and beta-pinene was the major constituent in both the rhizome oil and the leaf oil.

The most abundant components identified in the leaf oil of *Alpinia conchigera* included beta-bisabolene, beta-pinene, beta-sesquiphellandrene, chavicol and beta–elemene. Beta-bisabolene, beta-sesquiphellandrene, beta-caryophyllene and beta–elemene were the main components in the pseudostem and In the rhizome, 1,8-cineole, beta-bisabolene, beta-sesquiphellandrene and beta–elemene were the major components (Halijah-Ibrahim et al., 2009).

Antioxidant and antimicrobial activity of 13 Zingiberaceae species were reported by Habash et al. (2000). Antioxidant compound from the rhizomes of *Kaempferia rotunda* was reported by Lotulung et al., (2008). Antioxidant compound from the rhizomes of *K. galanga* was reported (Mohanty et al., 2008; Vankar et al., 2006). Huang et al. (2008) published the sedative activity of hexane extract of *K. galanga* and its active compounds. Kim et al. (2008) reported that ethyl cinnamate
and ethyl p-methoxycinnamate identified from *K. galanga* have larvicidal activity. According to Insun *et al.* (1999) the ethanolic fraction of *K. galanga* has a specific site of action on anal gills of the *Culex quinquefasciatus* larvae, by destruction of the irregular ridge–like reticulam on surface of gills which function as ionic regulators. Larvicidal activity of *K. galanga* also reported by Pitasawat *et al.* (1998). Insecticidal constituents isolated from *K. galanga* by Pandji *et al.* (1993) showed pronounced toxicity against neonate larvae of *Spodoptera littoralis*.

Anti-inflammatory activity of aqueous extract of *K. galanga* was reported by Sulaiman *et al.* (2008). Choochote *et al.* (2007) published the repellent activity of *K. galanga*. Xiano *et al.* (2006) studied the effect of rhizome oil of *K. galanga* on tumor growth and human gastric cancer. Ethyl-p-methoxycinnamate has been reported to show many biological activities, such as anticancer (Zheng *et al*., 1993) and anti-monoamine oxidase activities (Noro *et al*., 1983). Anti-allergic activity of *K. galanga* was described by Tewtrakul and Subhadhirasakul (2007). Ethyl cinnamate (EC) isolated from rhizomes of *K. galanga* was used for treatment for hypertension (Rozana *et al*., 2002). Vasorelaxant effects of the chloroform extract of *K. galanga* on smooth muscles of the rat aorta was reported by Mustafa *et al.* (1996). Mackeen *et al.* (1997) reported that ethanol extract of *K. galanga* displayed cytotoxicity against HeLa (human cervical carcinoma) cells (CD50 values of 10-30 micro g/ml). According to Choi *et al.* (2006) two cinnamates, ethyl trans-cinnamate and ethyl p-methoxycinnamate (100% at 60 micro g ml⁻¹) from *K. galanga* were responsible for nematicidial activity. Chandra *et al.* (2003) reported that ethanol extract of *K. galanga* showed strong inhibitory effect on the growth of cancer cells.

In conclusion this study has justified the traditional use of *Kaempferia galanga* for treating various diseases and for many ayurvedic drug preparations.