THE CHEMISTRY OF THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

A THESIS SUBMITTED TO THE UNIVERSITY OF THE PUNJAB IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY

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These studies were undertaken to investigate the chemistry of the essential oils of the Pakistani species of the family Umbelliferae. Pakistan, being mostly an agricultural land, possesses an enormous wealth of natural products because of its geographical conditions. It is stated that 174 species belonging to 56 genera of the family Umbelliferae, occur in different parts of the country. Among these only a few are cultivated as crops while the rest of the species grow wild in the hilly tracts and arid zones annually and go waste without being exploited. Whether cultivated or wild, most of the species are believed to have the potentiality of becoming pharmaceutically and/or commercially important and the local people particularly living in the hilly areas where returns from the other crops are poor, are eagerly looking forward to find if these or other economic species can be commercialized because of their constituents such as essential oils and medicinal products.

These studies are in fact, a part of the work carried out to evaluate the potential sources of essential oils in the country and help millions of farmers to get more money from their small land holdings by resorting to cultivation of much valued essential oil bearing plants.

The essential oils of the different species obtained by steam distillation were studied for their physico-chemical constants and chemical compositions. The small amounts of pure compounds, isolated from the complex mixtures in essential oils, by adsorption chromatographic methods, were studied for their structural elucidation and identification by the spectroscopic techniques, which are faster, require small amount of the pure compounds and help in achieving the results which are difficult otherwise.
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SUMMARY

The percentage essential oil yield, physico chemical constants and chemical composition of the essential oils of different Pakistani species of the plant family Umbelliferae are presented here.

Scaligeria aitchisonii was studied for the first time and found to contain essential oil (0.12%) having specific gravity 0.86, refractive index 1.4741, acid number 0.56, ester number 20.15 and ester number after acetylation 57.5. The chemical composition of the essential oil revealed the presence of $\alpha$-thujene (0.30), $\alpha$-pinene (0.14), sabinene (0.10), $\alpha$-phellandrene (2.39), $\beta$-pinene (0.66), $\Delta^3$ carene (0.07), $\alpha$-terpinene (0.26), p-cymene (29.56), $\gamma$-terpinene (35.72), terpinolene (0.06), linalyl acetate (4.84), ethyl cinnamate (1.23), cinnamaldehyde (2.37), linalool (12.73), and eugenol (3.74%).

Foeniculum vulgare, wild variety, was studied to yield essential oil (1.35%) with specific gravity 0.95, refractive index 1.5239, acid number 1.25, ester number 3.25 and ester number after acetylation 12.27. The chemical composition of the essential oil revealed, $\alpha$-pinene (2.96), camphene (0.54), sabinene (0.21), $\alpha$-phellandrene (0.51), $\beta$-pinene (0.24), $\beta$-phellandrene (0.31), $\delta$-limonene (10.45), $\gamma$-terpinene (0.26), trans-anethole (67.28), fenchone (8.29), fenchyl alcohol (0.84), p-anisaldehyde (2.23) and $\alpha$-terpineol (1.24%).

Pleurospermum stylosum was studied for the first time and recorded to contain essential oil (0.08%) having specific gravity 0.86, refractive index 1.4614, acid number 0.46, ester number 0.75 and ester number after acetylation 55.07. The essential oil was found to contain $\alpha$-thujene (0.25), $\alpha$-pinene (0.78), sabinene
(0.87), α - phellandrene (20.10), β - pinene (1.23), β - phellandrene (0.56), α -
terpinene (0.18), p-cymene (2.63), d-limonene (0.74), γ - terpinene (34.93), methyl n-
amyl ketone (10.34), geranial (6.34), neral (3.41), borneol (2.85), nerol (7.73),
linalool (1.67) and angelicin (0.16%).

*Psammogeton stocksii* was studied for the first time and found to yield
essential oil (0.069%), with specific gravity 0.87, refractive index 1.4639, acid
number 0.72, ester number 31.25, and ester number after acetylation 60.59. The
chemical composition indicated α - pinene (14.50), camphene (0.86), sabinene (0.74),
α - phellandrene (2.13), β - pinene (2.20), p-cymene (5.93), d-limonene (5.14), γ -
terpinene (31.68), terpinolene (1.41), geranyl acetate (7.50), camphor (4.04), α -
terpineol (15.00), geraniol (2.09), and bergaptene (1.01).
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INTRODUCTION

The name essential oil is derived from the fact that this is an admixture of substances found in the essences\(^1\) or volatile compounds which give many plants their characteristic odour. The essential oils also known as ethereal oils, are defined as, the oils obtained by the steam distillation of plants\(^2\). Some of the low boiling components of the essential oils are lost during steam distillation process, which led to the development of modified methods for the extraction of essential oils. In the light of these, the essential oils are defined as volatile odoriferous mixture of compounds obtained from the plants\(^3\). These mixtures contain the odorific constituents of plants and are used in medicines, flavourings, and perfumes. A somewhat more detailed definition was put forward by Parry\(^4\) which states that for all practical purposes, the essential oil may be defined as odoriferous bodies of an oily nature, obtained almost exclusively from the vegetable source, generally liquid at ordinary temperature and volatile without decomposition. The volatility and plant origin are the characteristic properties of the essential oils.

The essential oils are important agro-based industrial products. They find application in various types of industries, manufacturing food products, beverages, pharmaceuticals, cosmetics and paints.
1.1 HISTORY

In the recorded history of mankind, perfumes were used by the Egyptians; as an offering to their deities, for aesthetic purposes during their lives and as principal agents for embalming their dead. It was customary for the priests to burn incense before the gods in the temples, and this incense probably consisted of aromatic gums, resins and oleoresins mixed with perfumed woods. The Greeks and Romans learnt the secret art of perfumery from Egyptians. Dioscoride (23-79 B.C.), the author of "De Materia Medica" mentioned oil of turpentine and gave partial information about methods of producing it.

An extensive trade in odoriferous oil and ointment was carried on in the ancient countries of orient, Greece and Rome. These were produced by placing flowers and roots, etc., in best quality fatty oils contained in glass bottles and subjecting to a warming influence of the sun and finally separating the odoriferous oil from the residual solid constituents. Sometimes the flowers, etc., were macerated with wine before fatty oil was added, and the product obtained by digestion was filtered and then boiled to honey consistency.

In fact the Arabs were the first who mastered the technique of obtaining essential oil from the naturally occurring organic materials. The Arabs contributed a lot and earned their names by developing and introducing the distillation techniques. In the tenth century, an Arab physician, Avicenna, made efforts to extract the essential oil from the flowers by distillation. He was fortunate enough, however, to isolate from the rose flowers some of its perfume in the form of an oil or otto (Attar) and produced rose water. Therefore, the first description of rose water had been reported by an Arab historian, Ibn-e-Khuludune. The distillates of plants under the
name of the Araqiyyat had been in use in the muslim materia medica. Musk, Jasmine were extracted and tinctures were also prepared by the Arabs. It was the muslim queen Noor Jehan\(^5\) who was the first lady who extracted the "Attar" or oil of roses in the subcontinent of Indo-Pakistan. It would not be an exaggeration to say that the art of essential oils production reached its zenith under the patronage of muslim rulers. Their hygienic ways of life, social customs and beliefs, all contributed towards the development of this art. Incenses were burnt in their homes to purify air, delicate scents were extensively used on garments and bodies to impart them sociability. The religion of Islam enjoins upon its followers to make judicious use of perfumes and essential oils. The essences were also employed to add taste and acceptability to their beverages and sherbets. As they did not drink wine, their non alcoholic drinks developed in the direction of utility, taste and flavour derived from the herbs. These factors were mainly responsible to put the art of production of essential oil on commercial footing.

The knowledge of essential oil production was spread over Europe by the crusaders during the 13th century\(^7\). Arnold de Villanova prepared the distilled waters by the distillation of different plants and praised the remedial qualities of distilled waters which became speciality of medieval and post-medieval European pharmacies. Distillation was considered a means of separating the essential from the crude and non essential\(^6\) with the help of a fire. A great Swiss medical reformer, Bombastus Paracelsus Von Hohenheim (1493-1541) gave essential oil a special meaning. His theory was that it is the last possible and most sublime extract, the Quinta essentia which represents the efficient part of every drug and that the isolation of this extract should be the goal of pharmacy. It was the "Krauterbuch" of Adam Lonicer (1528-1586), the first Edition of which appeared at Frankfort in 1551, which may be
regarded as a significant turning point in understanding the nature and importance of essential oils. Lonicer stressed the medicinal value of many marvelous and efficient oils of spices and seeds. Giovanni (1537-1615) in his "De Destillatione Libri IX" written about 1563, not only differentiated distinctly between expressed fatty and essential oils but described their preparation, the ways of separating the volatile oils from water and the apparatus used for this purpose. In the seventeenth and eighteenth centuries, it was chiefly the pharmacists who improved methods of distillation and made valuable investigations into the nature of essential oils.

The revolution in the science of chemistry, which began at the end of 18th century with the work of Lavoisier (1743-1794), resulted in a new and illuminating approach to the investigations of the nature of essential oils\(^4\). Submitting the turpentine oil to elementary analysis, Houton de La Billardiere\(^6\) found the ratio of carbon to hydrogen to be five to eight, the same ratio that was later established for all terpenes, sesquiterpenes and polyterpenes. The systematic study may be said to have begun with the analysis of a number of steroptenes by the great French Chemist, Dumas\(^8\) (1800-1884). His investigations were of considerable importance in the further development of chemistry of volatile oils. He reported the empirical formulae of camphor, anethole, borneol and others and at the same time studied the formation of addition products of some terpenes with the purpose of characterising them. Bertheiot (1827-1907) devoted his work primarily to the hydrocarbons contained in the oils. In 1866, the name of terpenes was introduced in a text book written by Kekule (1829-1896), who apparently coined this term. Tilden (1842-1926) one of the greatest English chemist introduced nitrosyl chloride as a reagent for terpenes\(^6\). This reaction was perfected and was used with excellent results by the German chemist, Wallach (1847-1931). Tiemann and Semmler\(^8\) (1895) established the true structures
of terpineol. The structure of α-pinene was established in the preceding year by Wagner. Before the end of the nineteenth century a number of essential oil constituents were obtained and involved either careful fractional distillation or a simple chemical treatment for the separation of these compounds in relatively pure state. Menthol, santalol, borneol, citronellal and cinnamic alcohol were separated and were sold commercially in substantial quantities. This very active and for reaching research was the result as well as the cause of the wide expansion in the use of essential oils during the latter half of the nineteenth century. Gradually the use of essential oils in the medicinal drugs became quite subordinate to their employment in the production of perfumes, beverages, food stuffs, etc. The work of Wallach and his pupils and of Semmler (1860-1931) and collaborators on terpenes and terpene derivatives introduced what might probably be called the Elizabethan age of essential oil industry. Discovery followed discovery; one essential oil after the other was thoroughly investigated and its composition elucidated. Newly identified constituents were synthesized and many of them manufactured. It should be emphasized in this connection that Wallach's work also laid the foundation for another important chapter in the chemistry of essential oils, viz., the analysis and assay of products which because of high price, were prone to fraudulent manipulation and adulteration by unscrupulous producers or dealers.

The increased demand of essential oils by European societies which all of a sudden became sophisticated through acquisition of wealth, colonies and industries opened up ways for the enhanced production of essential oils based on principles of plant breeding, mechanized agriculture, engineering and mass production. Prior to the world war first, essential oil factories in and around New York city, London, Leipzig and Grasse used to distil essential oils from the plant materials mainly cultivated in
the vicinity or from dried plants which could be shipped from abroad at low cost. The oils produced in these factories were much superior in quality to those produced by the natives using primitive methods in the other parts of the world. The operating expenses of these factories including high standards of living and consequently high wages and salaries involved, amortization of invested capital, taxes and other general over head expenses increased the cost of production of essential oils. The problem of shipping space for bulky raw materials which arose during the war, forced local growers in various countries abroad to install their own distillation units and to process their own plant materials for oil. As a result after world war first, the high cost of transporting raw materials and increased cost of production prevented the manufacturers in Europe and the United States from competing with native producers abroad. Thus, production of essential oils in many instances reverted from a highly developed centralized units to a scattered and primitive ones. Today only a few essential oils are produced by modern methods, among these are the natural flower oils in Grasse region of Southern France and the citrus oils in California and Florida. However, most of the essential oils are still imported from the developing countries like, Indonesia, Singapore, Sri Lanka, Tanzania, India and Pakistan. The aromatic plants are cultivated in the world over; the greatest centre is perhaps Grasse district of France, where the vast gardens of roses, violets, orange blossoms, Jasmine and other fragrant flowers stretch all around the area for miles. England is unsurpassed for its lavender and Bulgaria is famous for its roses. Brazil, Paraguay, China, Japan, Taiwan and India have a monopoly over mint oil and menthol, while many countries in the South East Asia still form a major source of spices, oil of citronella, cinnamon, clove, pepper, cardamom and ginger. Since the beginning of the century the European nations have become highly industrialized, with the result the labour has become very
expensive, this factor alone has greatly affected the cultivation of aromatic plants. The amount of essential oil in plants is so small that in order to produce one pound of rose oil, 4000 pounds of rose flowers are required to be distilled. The continuous consumption of scents and essential oils has attained such proportion that the supply from the natural sources cannot meet the demand. Presently the world wide sale of perfumes and related products is estimated to be around 6 billion US dollars, of which the essential oils from the natural products contribute around 16%. The high cost of cultivation and processing of aromatic plants has been increasing exponentially over the years. This urged the chemists to develop the synthetic substitutes. In the 2nd half of 19th century a few synthetic aroma chemicals were synthesized. Vanillin was prepared from the glucoside coniferin in 1876; it was also obtained by the oxidation of isoeugenol in 1896. Similarly coumarins and heliotropin were produced in 1878 followed by terpineol in 1890, isoeugenol 1890, linalool and linalyl acetate in 1891 and ionones in 1893. In 1960, α-pinene, a major constituent of turpentine oil was subjected to intensive research to synthesis a number of conventional and non-conventional fragrant compounds. In the early 1920's Ruzicka synthesized nerolidol and farnesol. The synthetic production of aromatic chemicals have now fortified the demand of natural essential oils and perfumes. The synthetic menthol, next to vanillin, has the highest volume of consumption and is now being produced over 2000 tons per year, whereas, natural menthol production is around 36000 tons per year. Research and development in the last 30 years with the help of modern instrumental techniques, especially gas chromatography, u.v., i.r., n.m.r. and mass spectography have revealed the secrets and complexities of natural oils and have resulted in their being duplicated synthetically. It, however, must be admitted that the uniqueness in nature has not still been fully revealed or captured. This fact alone mentions nature's
supermacy over synthetics in odour and when formulating the more expensive compounds.

The essential oil industry in its present state is not limited to the production, distribution of essential oils and improvement of methods, nor to the establishment and maintenance of standards of quality alone, but it has come more and more to be concerned with the isolation and synthesis of fragrant compounds, which occur in the threshold concentrations in the essential oils and find their way into so many products of advanced civilization. Botany, agriculture, pharmacy and chemistry, engineering and knowledge of world market, commercial ingenuity and responsibility have all contributed to the development of modern essential oil industry. It is the maintenance of this combination which will keep up the high standard and the general usefulness of this industry.

1.2 STATE OF ESSENTIAL OIL INDUSTRY IN PAKISTAN

In the beginning of 20th century, not only the supplies of different articles of necessity were meagre in under-developed countries, but the wants of man also were not large in number. With the spread of education and concomitant march towards the progress of civilization, ideas of mirth, merriment, refinement and luxury began to seize the minds of men more and more. The people ignored the salient features of their culture and civilization and were deceived into a blind worship of the false glamour of western culture and civilization. With the passage of time, social and cultural values have altogether changed as the people changed their own and adopted the life style of western nations. They were now very particular about their make up. Perfumes, scents and cosmetics are extensively used for grooming of both men and women. Their eating and drinking options have also changed. Beverages and ice
creams have become food items, which are now relished frequently. The market of such products is ever expanding. More than 200 factories manufacturing personnel products, cosmetics, beverages and ice creams have been established all over the country. These factories are consuming raw materials especially, essential oils, perfumes, beverage concentrates and the ice cream mixes imported from the western countries. The most modern class of the society does not appear to be satisfied with the indigenous products of the country. They have an intense desire for the articles produced by the advanced nations and countries. They make resort to ready made articles. The western products based on perfumes and flavours cater conveniently to the needs of the modern men and women. Multinationals are engaged in producing these products and flooding the markets of under developed countries, allured by advertisement through press and electronic media. It is true, that the products have found their way even to our village life as well, but their impact on the rural life has so far remained limited. With the low level of income and dominating influence of the nature in the lives of the village folks, it is unlikely that the imported products can become a dominant part of rural life.

In order to meet the requirements of the local industries, government has to import the essential oils and the products based on these in the form of beverage concentrates, ice cream mixes, perfumes, scents and flavours. Surprisingly enough, a review of import statistics report for the past two years i.e. 1990-91 and 1991-92, (table-1) highlights that an amount over Rs. 260 million is being spent per year for the import of the essential oils and their isolates.
Table-I
Statistical Import Data
(Rs in thousands)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>April - June</td>
<td>Cumulative from July 1991</td>
</tr>
<tr>
<td>1.</td>
<td>Essential oil, perfume, flavouring materials</td>
<td>81.043</td>
<td>249.207</td>
</tr>
<tr>
<td>2.</td>
<td>Menthol</td>
<td>1.540</td>
<td>11.245</td>
</tr>
<tr>
<td>3.</td>
<td>Thymol</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4.</td>
<td>Turpentine oil</td>
<td>--</td>
<td>0.229</td>
</tr>
</tbody>
</table>

Thus inflicting heavy foreign exchange drain on the national exchequer and consequently creating a continuous, uncertain and adverse balance of payment position. For a country like Pakistan with limited resources at its disposal, it is not possible and justified to spare such a huge amount of foreign exchange for the import of essential oils and other luxury items. Consequently, due to lack of sufficient funds, the important national building projects could not be materialized and the country could not make any tangible headway towards economic and industrial development. The nations which have not fully exploited the God given resources, mineral or agricultural through pains-taking research and development activities have to face the similar type of economic, cultural and social problem as we are confronting with.

Pakistan is basically an agricultural country. The soil is fertile and is irrigated by one of the best and largest systems of canals in the world. The physical and climatic conditions in Pakistan are extremely favourable for growing different kinds of essential oil bearing plants. In fact, a number of aromatic plants and herbs grow wild in hilly areas and arid zones annually and go waste without being exploited. The
scientists of PCSIR Laboratories Complex, Lahore have done a commendable job pertaining to natural resources of essential oil bearing plants within the country. The studies were carried out in order to obtain a basic information for the potential sources of such oils and consists of work on different aspects such as survey, collection of the species, extraction of the oil, physico-chemical characterization and percentage composition of various essential oils. It is satisfying to note that more than 70 species of the plant family Umbelliferae alone were collected and preserved in herbarium. With limited facilities at disposal in terms of sophisticated instruments and relying upon pains-taking chemical analysis, more than 60 species of the different plant families were analyzed. A great deal of work has been published and is available in the form of reports, papers and publications. These investigations have exposed the potential resources and revealed the chemical composition of these oils and thus are a great asset and can serve as a base and prove a great help to those who want to study these oils with respect to their industrial applications thus exploiting the large natural resources or to develop new agricultural sources of such oils. Some of the existing potential essential oil sources: i) citrus fruits, Pakistan ranks third in the production of a variety of citrus fruits in the world. It is estimated that 250 tonnes of essential oil can be distilled from the fruit peels, a waste product of the fruit processing industry. Ironically 15 tonnes of the oil is imported every year to meet the requirement of the local industry. However 60 tonnes of orange flower water is distilled and used in the preparation of syrup, Rooh-e-Afza by M/s. Hamdard Foundation Ltd, Pakistan each year. ii) Juniper berries, the world’s second largest plantation of Juniper macropoda, spreading over an area of 250,000 acres exists in Baluchistan. It can yield 400 tonnes of berries which contain 1.5-3.0% of the essential oil. iii) Cymbopogon Jawaranussa is wildly growing in the arid zones
and is plentifully available in Mianwali, Muzaffargarh, Jhang, Campbellpur, Jehlum and Bahawalpur districts. It contains 2% essential oil\(^2\). The major constituent piperitone 90% of the oil is used as a basic material for the synthesis of menthol and thymol.\(^iv\) Skimmia laureola, is an evergreen wildly growing shrub. The leaves contain 1.5% essential oil\(^2\) with linalool and linalyl acetate as the major constituents. The oil can be used or exported as perfume. \(v\) Pine trees, 1000 kilo litres\(^1\) of the oil is distilled from the oleoresin from pinus roxburghi. Most of the oil is used in paint industry, while pines are the starting materials for the synthesis of many aroma chemicals. \(vi\) Pine needles, another source of essential oil, so far unexploited. Pine needle oil has a fragrant and refreshing odour and can be easily obtained from the trees where felling is taking place. \(vii\) Eucalyptus trees, the essential oil obtained from the Eucalyptus camaldulensis contains more than 70% cineole\(^1\). It is extensively used in Pharmaceuticals. The quality of the oil obtained from the E. citriodora is also good. It contains more than 85% citronella\(^1\). Unfortunately, eucalyptus species are not being processed. \(viii\) Sweet basil, this crop is cultivated on large scale in the Province of Sind near Hyderabad. The essential oil is distilled for export purposes. The oil contains methyl chavicol as the major constituent. \(ix\) Mentha arvensis, is cultivated as a cash crop. The oil of this variety is rich in menthol (80-90%) content and is a commercial source of natural menthol. There is good market for this oil within the country as well as abroad. \(x\) Rose, Rosa damascena grows abundantly in Pakistan. It contains a small quantity of the oil and not distilled. The flowers find market, fetching good price. \(xi\) Umbellifers, a brief description of potential sources of essential oil of the plant family Umbelliferae of Pakistan are given in a table form. (table-2).
Table-2

POTENTIAL SOURCES OF ESSENTIAL OILS OF THE PLANT FAMILY UMBELLIFERAE OF PAKISTAN

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Source (Plant Name)</th>
<th>Common Name</th>
<th>Area of Collection</th>
<th>Availability</th>
<th>Essential oil (%)</th>
<th>Major Components</th>
<th>Possible Commercial Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anethum graveolens (seeds)</td>
<td>Dil, Sowa</td>
<td>Lahore, Gujranwala</td>
<td>Cultivated</td>
<td>2.5</td>
<td>α-pinene, hexyl methyl phthalate and bergapten.</td>
<td>Flavouring pickles and foods.</td>
</tr>
<tr>
<td>3.</td>
<td>Apium graveolens (seeds)</td>
<td>Celery, Karfas, Ajrwan,</td>
<td>Lahore</td>
<td>Cultivated</td>
<td>2.5</td>
<td>Limonene, Butyl phthalide and sedanonic anhydride.</td>
<td>Improvement of taste and aroma of prepared foods.</td>
</tr>
<tr>
<td>5.</td>
<td>Carum carvi (seeds)</td>
<td>Caraway Zera siah</td>
<td>Lahzar Naran</td>
<td>Wild (Abundant)</td>
<td>3.5-6.7</td>
<td>Limonene, carvone and carvendiol</td>
<td>Flavouring foods and drinks.</td>
</tr>
<tr>
<td>6.</td>
<td>Carum roxburghianum (seeds)</td>
<td>Halapwan</td>
<td>Donekan village, Emmabari</td>
<td>Cultivated</td>
<td>3.8</td>
<td>Limonene, cadinene, and β-cyclaclevendulic acid.</td>
<td>Used as condiment.</td>
</tr>
<tr>
<td>7.</td>
<td>Coriandrum sativum (seeds)</td>
<td>Castander Dhania</td>
<td>Lahore, Gujranwala</td>
<td>Cultivated</td>
<td>0.5</td>
<td>Limonene, γ-terpine, linalyl acetate, geranyl acetate, linalool and geranial</td>
<td>Employed in per junery.</td>
</tr>
<tr>
<td>8.</td>
<td>Cuminum cyminum (seeds)</td>
<td>Cumin Safed zera</td>
<td>Quetta, Mastung</td>
<td>Cultivated</td>
<td>3.0</td>
<td>β-pinene, γ-terpine, cuminaldehyde, 1,3-p-menthadiene-7-ol and 1,4-p-menthadiene-7-ol</td>
<td>Seeds added to many food preparations as condiments, local drinks &amp; curry powders.</td>
</tr>
<tr>
<td>No.</td>
<td>Plant Name</td>
<td>Common Name</td>
<td>Origin</td>
<td>Cultivation</td>
<td>Essential Oil Components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
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<td>--------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Dorema ammoniacum (gum)</td>
<td>Quetta</td>
<td>Wild (Abundant)</td>
<td>0.48</td>
<td>α-pinene, citronellyl acetate and dammol alcohol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Ferula assafoetida (gum)</td>
<td>Maslahk, Spinkaraz Quetta</td>
<td>Wild (Abundant)</td>
<td>20.74</td>
<td>α-pinene secondary butyl propanoyl disulphide and α-terpinenol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Ferula costata (seeds)</td>
<td>Quetta, Urak</td>
<td>Wild (Abundant)</td>
<td>2.5</td>
<td>β-caryophyllene, cadinen and farnesol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Ferula narthex (seeds)</td>
<td>Chitral</td>
<td>Wild</td>
<td>1.1</td>
<td>Δ^1-carene, geranyl acetate and α-terpinenol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Ferula cepoda (seeds)</td>
<td>Hazarganj</td>
<td>Wild (Abundant)</td>
<td>3.7</td>
<td>Limonene, myristicin and terpinenol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Foeniculum vulgare (seeds)</td>
<td>Throughout the country</td>
<td>Cultivated</td>
<td>2.5</td>
<td>Asafoetida, fenchone, anisaldehyde.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Psammomene canescens (seeds)</td>
<td>Ziarat, Mastlah, Maks</td>
<td>Wild (Abundant)</td>
<td>5.8</td>
<td>Limonene, myristicin and methyl eugenol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Pycnochyca aucheriana (seeds)</td>
<td>Machh</td>
<td>Wild</td>
<td>0.35</td>
<td>β-pinene, limonol &amp; myristicin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The situation with respect to the production of essential oils in Pakistan is not very encouraging. There is a need to patronize this industry. Experience of the last so many years has revealed that the farmer's cooperation is readily forthcoming if he is convinced that the cultivation of the aromatic plants is a profitable proposition, which it really is. He has to be provided assistance at every stage from the nursery to the sale of the distillate. The essential oil industry in Pakistan has not so far been established on the sound footings. As for as the growers are concerned, there are no assured buyers of their produce. Conversely, an industry can not be established unless there is an assured supply of the raw materials. Essential oil consumers and buyers should come forward and assure the purchase of the essential oils at reasonable prices. It will only be then that this industry will take its foot hold.

The field of oil production is labour intensive proposition at the level of growing of aromatic plants. It will not only help millions of farmers to get more money from their small land holdings by resorting to cultivation of essential oil bearing plants but will create more job opportunities in the rural areas and thus will help to ameliorate the condition of rural population by raising their income. The Government of Pakistan is committed to eradicate the cultivation of narcotic plants from the face of this land. It is therefore, stressed upon the growers to switch over the cultivation of the quality valuable aromatic plants and help in ridding the country and world of the drug culture. Our agriculturists should now come forward and take a lead in cultivating the exotic as well as local plants rich in essential oils that fetch high price in the world market. It will contribute in the achievement of the objective of improving the lot of the farmer. This will not only provide essential oils to the relevant industries but will open a new and profitable field for the manufacturers. It will also contribute to the employment of innumerable workers and their families.
often primitive people in developing, manufacturing, controlling, advertising, marketing and selling of these oils.

1.3 CHEMISTRY OF ESSENTIAL OILS

Chemically the essential oils are composed of organic compounds, usually hydrocarbon, alcohol, aldehyde and ketone in nature. It is with the advancement of science that a better knowledge of the constituents of the oils is gained. It is found that the oils contain chiefly liquid and more or less volatile compounds of many classes of organic substances. Some of these are aromatic compounds, still others are aliphatic substances which belong largely to the ester or aldehyde classes. In general, however, most of the compounds belong to the general class of substances known as terpenes. The name terpene is derived from the English word "Turpentine". The terpenes are generally colourless liquids which are lighter than water and boil in the 140-190°C temperature range. They are insoluble in water, highly refractive and optically active. The terpenes are the unsaturated hydrocarbons which have a distinct architectural and chemical relation to the simple isoprene molecule \( \text{C}_5\text{H}_{10} \). They have the molecular formula \( \text{C}_{10}\text{H}_{16} \), thus are constituted by two isoprene units combined by head to tail union.

The terpenes may be open chain or cyclic substances containing two isoprene units. Related to these are sesquiterpenes (three isoprene units), diterpenes (four isoprene units), tri and polyterpenes. Terpene derivatives are made up of isoprene units to which have been added the elements of water (H and OH) or simply an oxygen atom to give \( \text{C}_{10}\text{H}_{18}\text{O} \) and \( \text{C}_{10}\text{H}_{16}\text{O} \). The functional groups are more frequently alcohols, aldehydes, ketones and esters.
It is evident that terpenes molecules contain six hydrogen atoms fewer than
the molecule of the corresponding saturated hydrocarbon C_{10}H_{22}. Since every
double bond or ring closure reduces the amount of hydrogen by two atoms, the
terpenes may be divided into four groups^{15}.

i. Acyclic mono terpenes

ii. Monocyclic mono terpenes

iii. Bicyclic mono terpenes

iv. Tricyclic mono terpenes

i. Acyclic mono terpenes: The molecules of this class of terpenes
contain an open chain of carbon atoms and three double bonds. Among
the acyclic terpenes, the hydrocarbons: myrcene and ocimene, are
relatively less important but the corresponding alcohols: geraniol,
erol, linalool and aldehydes: citral, geranial,neral, are of some
interest. For example, geraniol occurs in the oil of geranium, whereas
its partially hydrogenated derivative citronelhol occurs in rose oil. The
aldehydes corresponding to these alcohols are citral and citronellal.
These substances occur in lemon oils and essential oil of citronella^{16}.

ii. Monocyclic mono terpenes: This class of terpenes contains a single
ring of carbon atoms and two double bonds in the molecule. A
saturated monoterpene has the formula C_{10}H_{16} and is called menthane.
If the compounds have the empirical formula C_{10}H_{16}, there must be
two double bonds since the ring occurs in the place of one of the three
double bonds present in acyclic terpenes. These hydrocarbons are
generally called menthadienes. Many representatives of this class of
menthadienes are found among the terpene fractions of essential oils,
for example, dipentene formed by the polymerization of isoprene
molecules under the influence of acids, is one such a compound.

\[
\text{Dipentene}
\]

By arranging the double bonds in different ways it is possible to
make a total of 32 isomers of this class of compounds. These include possible
optical antipodes and their racemic mixtures and cis-trans forms. Some of
these terpenes with their derivatives occur in nature. For example limonene,
terpinolene, terpinenes, phellandrenes and p-cymene. Limonene is perhaps,
the most widely distributed in nature. The optically active forms both (+) and
(-) are found in the oils of citrus fruits, pine leaves, peppermint and many
other essential oils in both the racemic and optically active forms. Oxygen
containing derivatives (alcohols and carbonyl compounds) of the hydrocarbons
also belong to the monocyclic terpene group and include α, β, γ-terpineols,
menthol, piperitol, isopulegol, carvomenthol as alcohols; pellitolaldehyde,
phellandral as aldehydes; menthone, pulegone, isopulegona, pipritone,
carvomethone, carvone as ketones and 1,8-cineole, ascaridole as oxides many
of these substances can be converted into each other by relatively simple
chemical reactions. Carvone is an important ketone which occurs in several essential oils. d-Carvone is the main constituent of caraway seed (50-60\%) and dill seed oil, l-carvone is the principal constituent of spearmint oil (upto 70\%) and dl-carvone occurs in ginger grass oil.

iii. Bicyclic mono terpenes: The third group of monoterpenes has two rings leaving room for only one double bond for a formula C\textsubscript{10}H\textsubscript{16}. This type of terpenes include carene, pinene, camphene, bornylene, isobornylene and fenchene. Through arrangement of these different structures other ring systems can be derived. Molecular rearrangements, shifting of double bonds to other places in the molecule, oxidation or dehydrogenation and hydrogenation occur more or less readily, whereas, treatment with acid may open these rings. Pinene is perhaps the most abundant hydrocarbon in nature. It is the chief constituent of turpentine oil distilled from the oleo-resin of several genera and species belonging to the family Pinaceae. Oxygen containing derivatives include thujyl alcohol, sabinol, myrtenol, borneol, fenchyl alcohol as alcohols; myrtenal as aldehydes; thujone, umbellulone, carone, verbenone, camphor and fenchone as ketones.

iv. Tricyclic mono terpenes: The fourth group of monoterpenes, whose molecules contain no double bond while the carbon atoms form three rings. The compounds belonging to this group of terpenes are not important as they are not present in essential oils. Teresantalic acid\textsuperscript{6} is the only naturally occurring representative of this group.

The most important are the monocyclic and bicyclic terpenes. These
terpenes along with derivatives are usually present in the essential oils.

The essential oils in addition to the terpenes $C_{10}H_{16}$ often contain more complicated hydrocarbons of the same composition but of higher molecular weight. Their composition can be expressed by the general formula $(C_5H_8)_n$. For monoterpenes $n=2$; for polyterpenes $n$ is greater than 2. The polyterpenes can be divided into sesquiterpenes $C_{15}H_{24}$ and diterpenes $C_{20}H_{32}$ etc. The monoterpenes and sesquiterpene are termed as terpenes and are steam volatile. While di-$C_{20}H_{32}$ tri- and polyterpenes are not steam volatile and are not found to be present in the essential oils. Sesquiterpenes have molecular formula $C_{15}H_{24}$ and are thus constituted by three isoprene units. These are unsaturated compounds containing 8 hydrogen atoms less than the corresponding saturated hydrocarbons and may be divided like monoterpenes into:

i. Acyclic sesquiterpene,
ii. Monocyclic sesquiterpene,
iii. Bicyclic sesquiterpenes,
iv. Tricyclic sesquiterpenes.

1. Acyclic sesquiterpenes: The molecules of this class of terpenes contain an open chain of carbon atoms with four double bonds and include some of the terpenes and their derivatives. Sesquicitirolene is present in the sesquiterpene fraction of citronella oil. $\beta$-Farnesene is present in the essential oil of hops. Farnesol is widely distributed in flower oils of rose jasmine, acacia and cyclamen. Nerolidol, an isomer of farnesol is found in neroli essential oil.
ii. Monocyclic sesquiterpenes: The compounds of this class of terpenes contain one ring and three double bonds in the molecules. Among the monocyclic sesquiterpenes, hydrocarbons, bisabolene and zingiberene are relatively more important. Bisabolone is widely distributed in nature and is found in the essential oils of bergamot and myrrh; zingiberene is the main constituent of the essential oil of ginger root.

iii. Bicyclic sesquiterpenes: The compounds of this class of terpenes contain two rings and two double bonds in the molecule and include some of the terpenes and their derivatives. Hydrocarbons, cadinene, selinene and caryophyllene are relatively more common in nature. Cadinene having a reduced naphthalene skeleton is reported to be present in the essential oils of cubebs. Selinene another hydrocarbon with reduced naphthalene skeleton is found in celery seed essential oil. Caryophyllene may contain six membered carbon ring or condensed with four carbon ring, is reported to be present in the clove bud, clove stem, cinnamon and sandal-wood oils.

iv. Tricyclic sesquiterpenes: The compounds of this class of terpenes contain three rings and one double bond in the molecule. This class of terpenes includes santalene, cedrene, gurjunene, longifolene and vetivene. The cedrene which constitutes 60-70% American red cedar wood oil is used for scenting of soaps. Longifolene is reported in the turpentine oil and has no industrial application. Santalene is present in sandal wood oil. Vetivene is isolated from the vetiver root oil. These terpenes are not used as such in industries.
1.3.1 Formation Of Essential Oils In Plants

There is no common history for such a long variety of components usually found in the essential oils. Due to similarity and certain chemical reactions between a number of components, they were classified, i.e., straight chain hydrocarbons, benzene derivatives, terpenes and miscellaneous compounds. These components were formed from major plant constituents (carbohydrates, lipids and proteins) under genetic control as direct products of a metabolic pathway or as a result of interaction between pathways or end products.

The straight chain hydrocarbons, on the basis of structural similarity were connected with fatty acid metabolism and might be formed from the lipids via several different pathways. These included β-oxidation, hydroxyacid cleavage and oxidations via lipoxygenase enzymes. The primary products of these pathways were aldehydes and ketones, various oxidations, reductions and esterifications also yielded substantial quantities of acids, alcohols, lactones and esters.

The formation of benzene derivatives was connected with amino acid metabolism. The aromatic amino acids like tryosine and phenylalanine might serve as important precursors to this class of essential oil components. It was suggested that cinnamic acids served as intermediates for many of such components. Simple esterification of cinnamic acid to methyl and ethyl cinnamate had been usually reported in essential oils. Removal of acetate group from the cinnamic acid yielded benzoic acids. The benzoic acid might be further transformed by esterification to yield benzyl esters and by reduction to yield various benzaldehydes and benzyl alcohols. Decarboxylation would yield phenols.
Due to the specific structural configuration, the formation of terpenes in plants had been the interest of many investigators and gave rise to most of the speculations. Francesconi et al.\textsuperscript{19} suggested that there was a generic reason for the prevalence of compounds with 5, 10 & 15 C atoms and that the transition from C\textsubscript{5} to C\textsubscript{10} occurred through condensation of two molecules of isovaleraldehyde (Me\textsubscript{2}CHCH\textsubscript{2}CHO) which gave an isomer of citronellal. From which in turn citronellal and rhodinal were derived. Citronellal might give rise to geraniol by a reversible reaction. This made it easy to conceive of changes to cyclic compounds, carbohydrates, terpenes, ketones etc. All transformations thus involved slow reactions such as hydration, dehydration, oxidation, reduction etc. which were compatible with plant metabolism. "Ring\textsuperscript{20} stated that as most of the constituents of essential oil contained 5, 10, 15 C atoms, the higher compounds were derived from isovaleraldehyde by the condensation of the latter. The formation of isovaleraldehyde was due to the oxidation of amyl alcohol, which was considered to be the compound from which essential oils were formed. Isovaleraldehyde condensed into isocitronellal, which isomerized into rhodinal, citronellal and geraniol, which in turn were converted into rhodinol, citronellol, linalool, menthone, Isopulegol and citral. The presence of three basic compounds, i.e., valeric acid, valeraldehyde and amyl alcohol was proved to be present in citrus oil. Simpson\textsuperscript{21} also made an attempt to indicate in order the probable natural process involved in the formation of essential oils.

From the established facts regarding the chemistry of terpenes it may be concluded that C unit was not the actual structure undergoing condensation and that more complex compounds were involved, which split off certain groups after condensation had taken place. This would include precursors as described by Hall, viz., phosphoric acid ester, as the sugar precursors and their degradation products.
and protein complexes carrying the condensing structures which released the terpene compounds when formed. The regular head to tail union might be predetermined in the compound from which the terpene was formed. The terpenes already formed readily underwent secondary changes, such as reduction, oxidation, esterification and cyclization and this fact might explain the large variety of derivatives of the same pattern. These families of terpenes might have their origin in independently formed key terpenes such as geraniol, citral, pinene, etc. Higher terpenes might have been formed through a condensation of lower terpenes of the same or different chain lengths, whereby quite often derivatives from the regular and symmetrical architecture could be observed. No indications were available that would justify connecting the terpenes directly with other essential oil components, such as, straight chain hydrocarbons or propyl benzene derivatives. Although the majority opinion favoured a connection through carbohydrate metabolism and there was no reason to assume that these products were formed in the same phase of these processes. Other essential oil components showed structural features strongly suggesting connection with fat and nitrogen metabolism. From the chemical evidence, the conclusion might be drawn that the complexity of the oil composition was caused by excretion or secretion of products formed in metabolic processes taking place in the plants and the origin of essential oils was intimately connected with the vital processes in the plants.

According to Potty et al. the metabolic pathways leading to terpene formation in citrus fruit essential oil were only partially known, while it was postulated that terpene formation involved mevalonic acid. The mechanism of mevalonic synthesis was unknown. Most plants synthesized mevalonic acid from acetate. However, key enzymes leading from acetate to mevalonic acid had not been found in citrus fruit. The incubation of mevalonic acid with orange extracts and (ATP) resulted in the
formation of mevalonate 5-phosphate (MASP) and mevalonate 5-pyrophosphate (MASPP). The activation step preceded decarboxylation and dehydration of the mevalonate to form the intermediates isopentyl pyrophosphate (IPP) and dimethyl allyl pyrophosphate (DMAPP). The next step in terpene synthesis involved a head to tail condensation of DMAPP and IPP to form linaloyl pyrophosphate and other acyclic and cyclic monoterpenes. Linalool was the major monoterpenone formed from incubation of C\textsuperscript{13}-labelled mevalonate with orange juice vesicles while neither nerol nor geraniol were detected by Potty and Braemmer. George Nascimento and Cori detected significant quantities of these terpenoids when mevalonic acid was incubated with cell free extracts of orange. The interconversion of terpenes could occur via several pathways. The conversion of neryl pyrophosphate and linaloyl pyrophosphate to terpinolene, \( \alpha \)-terpineol and limonene was determined by means of stabilization of the carbonium ion intermediate. The interconversions of geraniol, geranial, citronellol and citronellal was caused by enzyme capable of oxidation/reduction reactions.

1.4 LOCATION IN PLANTS

The essential oils secreted as a result of various metabolic processes, had been observed to be accumulated in the form of oily droplets in some cells or spaces in the plant tissues. The oils had been found in different cell groups distinguished as internal and external gland cells. The external glands were epidermal cells or modification of these, such as excretion hairs. These glands contain oil usually accumulated outside the cell between cuticle and the rest of the cell wall. The cuticle being thin skin, was liable to break with a slight touch. Thus, on touching the plant, the observer immediately feels its well known flavour. The internal glands were located throughout
the plant, they were formed by the deposition of the oil between the walls of the cells. The intercellular glands often had grown to form long canals, coated on the inside with a layer of thin walled cells. This coating was stated to have a double function, the separation of the other tissues from the oil and formation of the oil. The oil formed in the epithelial cells or in the membrane was stated to pass through the cell wall into the interior of the gland. These oil glands were unequally distributed throughout the plant. According to Strauss\textsuperscript{22}, the volatile oils secreted as a result of various metabolic processes in plants, accumulated in the specific cells or vessels of a variety of organs, leaves, flowers, fruits, stem or roots of the plant in a manner characteristic of the family of the individual plant. According to Pogorelova\textsuperscript{23}, the different organs and tissues, such as epidermal fellogen and cambial cells, conducting elements of central cylinders, leaves, flowers and fruits of the specie Valeriana officinalis did not contain essential oil while the stolon, rhizome, bark and hypodermal tissues of the roots contained essential oil during different stages of growth.

The mentha species showed that the lower surface contained 10-25 glands per sq.mm., the upper surface 1-6 per sq. mm., and the dimensions and number of glands increased near the large vein. According to Welch\textsuperscript{24}, there existed relationship between the oil glands and oil yield. No doubt, the theoretical values for certain species with small yield agreed fairly well with the actual yields, but in the majority of species there was such a wide variation that it was impossible for such determination to predict accurately the approximate yield of the oil. Guilliermond\textsuperscript{25} disputed the exact place of formation of essential oils in plants by detecting the droplet of essential oil in the cytoplasm by staining with indophenol blue. However, from these studies it was speculated that the essential oils were formed in the regions of photosynthetic activity, where carbon dioxide was reduced and synthesized to carbohydrates. This
idea would be supported by experiments which attempt to establish correlations between oil secretion and known metabolic processes in the plants. Such observations were found in studies on the effect of climate and growth conditions on essential oil content.

1.4.1 Functions In Plants

The functions of essential oils in plants were not thoroughly understood in the past. They were considered as the waste products of the plants. The observations based on recent research revealed that they have specific functions in the plants.

The essential oils being volatile compounds give many plants their characteristic odour and smell, which is constantly diffused from the plants into the air, thus dispersing the odour, characteristic to the plant in the surrounding atmosphere. The insects were reported to be very sensitive to the essences. It was observed that oil bearing plants were attractive to certain insects while others acted as repellent. Some useful insects were probably attracted to visit the flowers, thus contributing towards more effective cross pollination, influencing ultimately the bearing quality of the plants. The volatile components of Aristolochia debilis, Heterotropa spp. and Crataeva religiosa plants and their essential oils were found to attract Byasa alcinous, Luebdorflia japonica and Hbomoia glaucippe insects[26].

The essential oils due to their penetrating and irritating odour were observed to act as repellents for certain insects and animals. The presence of essential oil in roots, woods, leaves, flowers and fruits might act as protection in a number of plants against the plant parasites and against the depredation of animals[27]. The basic cause of formation of essential oil seemed to protect the plants from environmental germs.
Although plants were reported to produce many types of defence chemicals but maximum antibiotic activity was associated with the essential oils which possessed all the qualities of man made chemical warfare agents: vapourizing and spreading into the vast area, penetrating, paralysing and destructive to harmful agents like insects, bacteria, fungus, germs and animals. The essential oils of Foeniculum vulgare, Pimpinella anisum and anethole were reported to possess the insect repellent properties. The essential oil of Ducusia ismaelis Asch exhibited significant antimicrobial activity against Staphylococcus aureus, Bacillus subtilis and Candida albicans. Linalool was found an effective insecticide and acaricide. It was active against adults, larvae and eggs and could be used against animal parasites and house flies, thus when flies were placed on a surface treated with 0.7% linalool, 100% mortality was observed. Turpentine, lavender oils and resene terpenes were reported most effective insecticides against Colorado beetle, white American butterfly and pear bug in green houses. The response of Colorado potato beetles to volatile components of tansy was studied and recorded that the potato beetles exhibited avoidance behaviour. While α-pinene, the component of tansy oil was found to attract beetles. The essential oils obtained from Japanese cypress or cedar showed termite repellency. It was found that α-citral (Geraniol), β-Citral (Neral), components of Cymbopogon citratus essential oil showed antibacterial action against gram -ve and gram +ve organisms. The essential oil from Ocimum sanctum plant showed considerable antifungal and antibacterial activity when tested against 6 fungal and four bacterial strains. Thujone essential showed rodent repellent property and was used as an important constituent in rodent repellent powders.

The essential oils were reported to act as central nervous system depressants. The presence of these oils in different parts of the plants might be acting to dissuade
the animals and insects by inducing sedative and narcotic effects\(^{37}\) and thus defending the plants from the harmful insects and animals.

The essential oil components were considered as moderator in intracellular oxidation\(^{38}\) to protect the plant cells against the action of atmospheric agents. The essential oils of anise, peppermint, bitter almonds, sassafras, spearmint and wintergreen were reported to be more or less resistant to oxidation\(^{39}\). These oils were studied to have constituents which could be hydrogen donors in oxido-reduction reactions and determined the antioxidant or hydrogen donor action of the oil constituents. Phenols were found to be excellent donors. Secondary and tertiary alcohols and aldehydes also showed strong activity\(^{38}\). While hydrocarbons were inactive in the dark, but became active in the light. On the other hand primary alcohols, terpene oxides, ketones were found inactive. Gyame\(^{40}\) found lemon grass oil as the most effective antioxidant in the preservation of Shea butter. The preservative activity of the oil might be attributed to the citral, a major component in the essential oil.

Some observers maintained that the essential oils functioned as a reserved food, as a means of sealing wounds or as a varnish to prevent excessive evaporation of water but these opinions did not appear to be supported by experiments. It was also reported that the plants which contained a considerable amount of essential oils were prevented from becoming too warm since heat was absorbed in the vapourization of oils. But results from experiments showed that effect was negligible.
1.5 DIFFERENT METHODS FOR THE EXTRACTION OF ESSENTIAL OILS

Thousands of essential oils have been investigated of which, according to Langenous, some 150-200 species have been exploited for commercial purposes. The essential oils are obtained from the various parts of plants. They come from the seeds and from almost every other part of plant; cinnamon oil for example is obtained from the bark and the leaves.

The yield and the quality of the oil largely depend on the handling and storage conditions of aromatic plant materials. As soon as the plant material is harvested, certain changes start taking place and these include: loss of water content of plant material; loss of volatile oil; changes in physicochemical values of the oil and deterioration of the oil present in the plant material. Therefore, it is best to start extracting the essential oil from the material as soon as it is harvested but it is not always possible. The bulk of the crop comes all at one time and therefore, storage of the plant material has to be done. Necessary precautions are taken during the storage and these include keeping the crop in dry atmosphere at possible low temperature and under shade. Some plant materials, for example bitter almonds, patchouli, leaf and orris roots contain the major components of their essential oils in the bound form, some times as glycosides. The plant materials do not release their essential oils immediately after harvesting. These types of materials are kept under specific storage conditions during which the bound oils are released by the action of the enzymes present in the plant material.

The essential oils are enclosed in oil glands or oil sacs present in the cellular structure of the plant materials. The extraction of the essential oil depends entirely on
the rate of diffusion of the oil through the plant tissues to an exposed surface from where the oil can be removed by a number of processes. In order to hasten the process of extraction the plant material must be broken down to some extent. This process of disintegration is commonly known as comminution. The extent of comminution required varies with the nature of plant material. Flowers, leaves and other thin and non-fibrous parts can be distilled without comminution.

There are different methods, depending upon the stability of the oil, for the extraction of the oil from the plant materials. If the stability of essential oil is high, the extraction may be accomplished by water or steam distillation. This process subjects the oil to the harsh action of heat and water, thus resulting in an oil which may not be that the nature has produced originally. So the delicate oils like flower oils or floral extraits and essences, which are often referred to the trade as absolutes, have been termed natural perfume materials. They are extracted chiefly by means of volatile solvents and enfleurage and maceration.

Steam Distillation

Steam distillation may be defined as "the separation of components of a mixture of two or more liquids by virtue of the difference in their vapour pressure". The principle of extraction by this method is that the oil from oil glands travels to the cell walls under the influence of water and heat to the exposed portions from where steam carries it along with to the receiver. The essential oil from different seeds and other plant materials on large scale can be recovered by steam distillation. In steam distillation, live steam, usually of a pressure, higher than atmosphere, is generated in a separate steam boiler, and injected into the plant charge within the retort. This type of distillation is referred to as direct steam distillation. Many aromatic plants are
distilled today with direct live steam distillation at atmospheric pressure. No water is kept in the bottom of the still, live steam, saturated or super saturated and frequently at pressure higher than atmosphere is introduced through open or perforated steam coils below the charge and proceeds upward through the charge above a supporting grid, but in laboratory or on small scale production of essential oils, the steam is produced in a steam vessel and introduced in the glass flask or steel vessel which contains the plant material. The essential oil with water vapours is collected through water cooled condenser into a receiver. A mixture of n-hexane and diethyl ether is added to the distillate and shaken thoroughly in a separating funnel. It is allowed to stand until two distinct organic and aqueous layers are formed in the separating funnel. The aqueous layer is discarded and the organic layer is dried over anhydrous sodium sulphate. The yellow coloured organic layer is filtered into a dry flask and distilled under reduced pressure. The last traces of the solvent are removed leaving a yellow coloured oil. The oil is collected and stored in a dry, air tight dark coloured glass bottles in the atmosphere of nitrogen.

Expression^6, 41

This method is used for the extraction of essential oils from the citrus fruits. The essential oil is contained in numerous oval shaped sacks, distributed irregularly in the outer coloured portion of the peel. The essential oils of lemon, orange, and grapefruit are very sensitive to heat. They deteriorate rapidly under the influence of heat and water, so cannot be extracted by the steam distillation. These oils are best obtained by cold expression. The method consists in pressing the peels of these fruits by various techniques in order to rupture the walls of oil sacs, which release the oil in the form of emulsion with water and cell debris. The essential oil is separated by
decantation, centrifugation and filtration from the water and cell debris.

**Enfleurage**

This method is restricted to those flowers (jasmine, tuberose and violet) which after picking, continue their plant physiological activities in forming and emitting perfume. The essential oils of these flowers are too delicate to withstand relatively high temperature, action of boiling water or steam as they undergo hydrolysis, polymerization, resinification under adverse effects. This method is carried out only in France. In order to recover these flower oils with natural odour and fragrance, enfleurage method is used. This method is based on the principle that the fat possesses high power of absorption of fragrance when the fragrant flowers are brought in contact with fat layers in closed chamber. Enfleurage chamber is equipped with large number of chassis. A chassis consists of a rectangular wooden frame 2 inch high 20 inch long and 16 inch wide. The frame holds a glass plate upon both sides of which, a suitable blend of semi solid fat of good keeping quality is applied. When piled one above the other the chassis form air tight compartments with layers of fat on the upper and lower side of each glass plate. Every morning during the harvest the freshly picked flowers are strewn by hand on top of the fat layer of each glass plate. The chassis are then piled up and left in the cellars for 24 hours or longer depending upon the type of flowers. After 24 hours, when the flowers have emitted most of their essential oil and started to wither, they are removed by skilled and experienced workers. This is known as defleurance. Immediately after the defleurance, the chassis are recharged with fresh flowers. In the case of jasmine flowers the enfleurance process lasts for 70 days. The fat is saturated with flower oil with its typical fragrance. It is called pomade, and is used as such or extracted with high proof alcohol. The alcohol
washings are called extract. It gives natural flower fragrance as emitted by living flowers.

Maceration\textsuperscript{6,41}

This method is used for the flowers, which cease their plant physiological activities in forming and emitting their perfume immediately just after picking. In such cases it is required to shorten the long enfleurage process by immersion of the flower petals in the molten fat at temperature 45-60 degree centigrade for 1-2 hours. The same batch of hot fat is treated with several batches of fresh flower petals, until the fat becomes quite saturated with flower essential oil. The exhausted flowers are removed and the fragrant fat is known as pomade. This pomade can be extracted with high proof alcohol and concentrated to obtain the absolutes of flower oils.

Extraction\textsuperscript{6,41}

The essential oil is extracted with volatile solvents which are selected according to the plant material to be treated. Benzene with or without admixture of acetone or petroleum ether, in the cold or at boiling point is used for the extraction of essential oils from flowers in specially designed extractors. The solvent penetrates the cell walls of the plant material and not only dissolves the essential oil but also waxes, albuminous matter and colouring matter. The solvent is removed by distillation at low temperature leaving behind the semi solid "concretes". The latter is extracted with high proof alcohol, the wax free alcoholic solution is distilled under reduced pressure to remove the alcohol and finely the essential oil, the so called "absolute" free from wax and alcohol is obtained.
Liquid Carbon dioxide Extraction

A wide range of aromatic raw materials starting from fruits, vegetables, spices, herbs, seeds, flowers and other parts of the plants can be extracted for their essential oils by this method. The plant material is reduced in size and packed in a closed extraction vessel fitted with storage tank and compressor via heat exchanger. The compressor pumps the extractant carbon dioxide at a constant temperature from its storage tank via heat exchanger to the extraction vessel. Liquid carbon dioxide has low viscosity, it can penetrate readily into plant material to be extracted. After extraction has been accomplished, the solvent phase is evaporated leaving behind the flavouring oil. Since carbon dioxide is a gas, the removal of it can be achieved by slightly raising the temperature or by decreasing the pressure.

The chief advantages of this process are:

The essential oil is obtained in a highly concentrated and pure form, free from water, alcohol, sugars, acids and other residues. The essential oil retains the aroma of fresh material from which it is extracted. The extraction procedure does not involve elevated temperatures. Therefore, there are less chances of the deterioration of the essential oil. The solvent is non-inflammable and non-explosive and can be removed at ordinary temperature. The solvent is odourless, so it does not interfere with or alter the natural aroma of the extract. The solvent is non-toxic, so its use with food products is safe.

1.6 USES OF ESSENTIAL OILS

Essential oils find an amazingly wide and varied application in many industries for the scenting and flavouring of all kinds of consumer's finished
products, some of these are luxuries, most of them necessities in our advanced civilization. Many of these products contribute directly to our health, happiness and general well being.

Since the origin of civilization, even in the most ancient ones, essential oils and their isolates had been used by the man for their beneficial and physiological effects. Essential oils possess nice, pleasant and acceptable odour, perceived through sense of smell and recorded in the sense of memory. Moreover, in the recent years it has been found that certain odours (pheromone) act as sex attractants\(^\text{42}\). These oils also possess the antiseptic, healing, soothing, curing and beautifying qualities. So they are extensively used in perfumes, cosmetics and toilet products not only to provide with nice, pleasant and commercially acceptable odour, but also to enhance the cleanliness of a person and his environments; to ward off skin troubles; impart healing, soothing and curing properties; cover up imperfections and to beautify and mask bad odours of the body. Today, a large number of perfumes and personal products, toilet and dental preparations, containing essential oils as important constituents are available in the market. These products are used by the people in their every day life, both at work and in social and family circumstances.

Spices with their essential oil constituents have been used since time immemorial as the important ingredient of our food. The spices increase appetite, help in food digestion and are carminative in action\(^\text{44}\). They are indispensable to man in order to bring about proper digestion of food. The digestive juices containing digestive enzymes such as pepsin, trypsin, lipase, amylase, etc., are secreted into the stomach and intestines only when stimulated by the smell and taste of pleasantly flavoured food\(^\text{43}\). The individuals digest more food with pleasant taste, a fact equally
true in the reverse. Spices such as caraway, coriander, black pepper, cloves, cardamom, cinnamon, fennel seeds, mustards, table sauces, chilli and other aromatic plant materials are widely used as condiments in the preparation of our foods both in kitchen and in baked goods industries, manufacturing biscuits, cakes, crackers, doughnuts, sandwich fillings and puddings etc.

The essential oils are generally effective antioxidants\textsuperscript{39,40}. They act as food preservatives and hence increase the shelf life of food items. They also exhibit the antimicrobial activities and kill pathogenic and putrefactive organisms\textsuperscript{45} like standard antibiotics and hence are used in food industry not as flavouring and palatable constituents but also as preservatives in food and pickle industry for improving the shelf life of the vegetable oils and fats, dehydrated soups, meats and vegetables, prepared cake mixes, fruit butters, jam, jellies, sour and sweet pickles, etc.

The essential oils have penetrating and irritating odour. The presence of these oils in roots, woods, leaves and fruits of the plant act as protection against plant parasites and against depredation of animals. The man has exploited and made use of these oils in the various formulations to ward off trouble creating insects and animals. Various exterminators and insecticides\textsuperscript{46}, i.e., Bedbug sprays, cattle sprays, cockroach powders\textsuperscript{47}, fly sprays, mosquito repellents\textsuperscript{47} plant sprays, shampoos for the control of lice, ticks and flees\textsuperscript{48} and naphthalene balls are manufactured from these oils. The essential oils obtained from Japanese cypress or cedar were found to exhibit termite repellency\textsuperscript{49}. The wood work in the buildings and furnitures, when treated with wax containing 0.1-0.3% of the essential oil, was recorded to be saved from termite attack upto 7 - 8 years\textsuperscript{49}. 
The essential oils being volatile, are constantly diffused from the flowers into the air. The insects, which are sensitive to these oils are attracted to visit these flowers. These insects not only collect the nectar and produce a marketable crop of high cash value, but also pollinate many valuable plant species. These insects contribute towards most effective cross pollination, influencing, ultimately the fruit bearing quality of the plants and trees. This characteristic of the essential oil has been exploited in the preparation of insect and animal attractants. Japanese beetle attractants and rat baits containing essential oils as active constituents, are available in the market.

Increasingly volatile oils and their aromatic isolates serve also for the covering of somewhat objectionable odour as for instance, in case of artificial leather, acceptable and useful articles can be made from raw materials that were formerly discarded or overlooked because of disagreeable odour. In most instances the incorporation of aromatics into products, such as synthetic rubber, baby pants, natural and synthetic latices, surgical supplies, rubber toys and water proofing compounds, has opened a new and a profitable field for the manufacturers.

The volatile oils of chenopodium, wormseed and tansy are well known for their anthelmintic activities. They are used for the deworming of tape, round and hook worms in animals, while the essential oils of anethum soya, eucalyptus, citridora and cymbopogon were reported to exhibit marked nematocidal activity against larvae of Red-knot and M. Incognita.

The essential oils or their constituents are generally used in remedies for cold, coughs, bronchitis and asthma. It is possible that the excreted oil irritates the mucosa of the respiratory tract. Even a small concentration of the essential oil of
anise, eucalyptus, fennel, peppermint, storax and thyme stimulates the activity of ciliated epithelium of the respiratory tract bringing about increased expectoration. The essential oils from eucalyptus, pine, violet, basil, thyme have therapeutic value in the diseases of respiratory organs are used in the cough syrups, inhalants, cough drops, elixirs and other medicinal preparations.

The activity of the cardiovascular system was reported to be influenced by some essential oils. The essential oils extracted from onion and garlic had been recorded to reduce the level of cholesterol and triglycerides in the blood serum. The essential oil constituents, such as citral, citronellol, geraniol, nerol, α-terpineol and limonene were studied to show antithrombotic activity.

Most of the essential oils exert an irritating action on the skin and mucosa. If the oil is allowed to remain in contact with the skin for long time, redness and inflammation results and finally blisters are formed. Apparently this action accounts for the use of these oils against skin parasites. The linalool had been reported as an insecticide and acaricide. It was active against adults, larvae and eggs and could be used as a shampoo against animal ecto-parasites. Some oils when applied on the skin in suitable concentrations, increase the local circulation (nutritive and healing irritation) and produce redness showing thereby their healing action. The healing action of terpeneless oil of mint on wounds and affections of skin had been reported by Alfonso. The effect of essential oils against lichens and eczemas had been studied and a preparation containing bergamot oil, against skin diseases had been reported. Several oils which did not produce irritation or redness effects on the skin, were used as counter irritant in arthritis, rheumatism, etc. The essential oil from Curcuma rhizoma had been recorded to show anti-inflammatory activity in mice.
and rats and inhibited gastric ulcer formation in rats. The essential oils of birch, lavender, mint, camphor, sassafras, savin, bay and rosemary had been studied to be effective for the treatment of affection of the scalp \(^{59}\), owing to various phenols and higher alcohols, which they contain.

The essential oils inhibit the growth and kill pathogenic bacteria like standard antibiotics. Sandal wood oil\(^{45}\) had been reported feebly active against B. coli and putrificactive organisms but showed specific activity against staphylococcus in urine as medium for the growth of these micro organisms. The essential oils of thyme, citron, Juniper and mint showed bactericidal action\(^{60}\) on Meningococci, Staphylococci, diphtheria bacilli and earth's bacillus.

The fungus diseases are very difficult to cure. Ring worm is a skin disease caused by the growth of the fungus on the skin. The investigations of the previous workers revealed that the essential oils exhibited fungistatic and fungicidal activities\(^{61}\). The essential oil of Ocimum sanctum was reported to show antimicrobial activity \(^{62}\), when tested against 6 fungal strains.

The essential oils were reported to have a remarkable effect on the functioning of different parts of the brain. The oils have depressant action on the central nervous system, causing sedation\(^{63,64}\), relaxation and tranquilization. Some oils depress the higher centres of the brain producing sedation and sleep. The oils have depressant action on the motor cortex and thus act as anticonvulsant.

The essential oils showed animal and bird repellent activities. This property of the oil had been utilized to prevent dogs and cats\(^{65,66}\) from urination in treated areas, e.g., outside the walls of the basement and also to protect the field crops against deer.
and rodent damages. The spearmint oil and 1-carvone had been recorded to show bird repellent activity when tested by coating the rice seed with these oils. This can be utilized for protecting the seeds from the birds in the fields at the time of sowing.

1.7 UMBELLIFERAE

Umbelliferae is a natural and rather large family in the plant kingdom. The number of species of the family, cultivated as well as grown wild in the world, has been recorded to be about 3000. They grow mainly in the north temperate and subtropical regions and are absent from the tropical regions except in the mountains. Most of the plants of the family umbelliferae grow wild in various parts of the world.

Plants are annual, biennial or perennial. The stems of the plants are hollow, erect, decumbent or prostrate, mostly herbaceous, pubescent or spiny. The branches are alternate or dichotomous. The roots are adventitious or taproot; fibrous, short or elongated, tuberous, fusiform or globose. The leaves are simple or compound, petiolate or sessile; leaf bases are usually sheathing; leaf lamina is variously shaped. The inflorescence is polygamous or bisexual; the umbels is a compound or sometimes simple. Involucral bracts 1 to many or lacking. The rays 1 to many. Involucel of 1 to many bractlets or lacking. The Calyx teeth are evident or obsolete. The petals 5, usually 3 lobed, central lobe inflexed, sometime outer petals larger. The stamens 5, carpels 2, attached to a carpophore; carpophore sometime lacking. The ovary is inferior, 2-celled with 1 anatropous ovule in each cell; styles are 2, often swollen at the base forming a stylopodium. The fruit is schizocarpic, consisting of 2 mericarps terete or compressed; mericarps usually with 5 primary ridges with furrows.
The family can be easily recognized by its inflorescence which is an umbel. The name is also derived from the typical umbel type of inflorescence. Pakistan being an agricultural country and because of its geographical conditions possesses an enormous wealth of natural plants. About 167 species belonging to 56 genera of the family Umbelliferae have been reported to occur in different parts of the country. Among these species *Anethum graveolens* (Dill), *Coriandrum sativum* (Coriander), *Cuminum cyminum* (Cumin), *Daucus carota* (carrot), *Foeniculum vulgare* (Fennel), *Trachyspernum ammi* (Ajwain) are cultivated as crops. The rest of the species grow wild, frequently in mountainous terrains at different heights. A few of the wild species also grow in plains in different parts of the country. The growth of these plants mainly depends upon the climatic conditions of the various regions of the country. It has been reported that some of the wild species can be cultivated under suitable conditions and these can therefore be adopted as useful commercial crops. Whether cultivated or wild, most of the species have the potentiality of becoming pharmaceutically and/or commercial important.

In the Indo-Pakistan subcontinent, a large number of vegetable plants has been utilized locally in both the old and new materia medica. The plants of the family were extensively used for various ailments and their remarkable medicinal value was fully recognized by the ancient people of sub-continent. The different parts of plants such as roots and seeds or their products such as oils and aqueous distillates known as "Arqiyat" have been employed as medicines since time immemorial. Essential oils recovered from the seeds of umbelliferae species have been used against cholera, pneumonia, bronchitis, asthma, hysteria, cough and intestinal disorders.
The seeds and the essential oils of the Umbelliferae species have been frequently employed in baked foods, pickles, condiments, pharmaceutical preparations, confectioneries and in insect and animal repellent preparations. Pakistan is quite rich with respect to the cultivated growth of umbelliferae plants, while the different regions of the country possess a variety of the wild growing species of the family. The plants of the family are reported to be a good source of both volatile and fixed oils.

_Scaligeria aitchisonii_

The plant grows wild in Pakistan and is found in Swat, Hazara, Chitral and Kashmir from 2-3000 m. It is up to 50 cm tall. The stem is 1 or more from the base. Branches are glabrous, reddish brown when dry. The root is globose, 1 cm in diameter. The leaves are deciduous, 2-3 pinnate; lower leaves are 10 cm long; upper are shorter pinnatisect; segments are shorter, less than 5 mm long and 0.5-1 mm broad; no leaves are found at the fruiting stage. The rays are 6-12.

The pedicles 10-15 and are 2-3 times longer than the fruits. The fruits are avoid, 1.5 mm long, and smooth. The base is cordate. The inner seed face sulcate.

_Foeniculum vulgare (Mill)_

Fennel is a common cultivated plant in the plains and has a wide distribution. It grows wild in chitral, Kaghan and Swat valleys. The leaves are used as vegetable and the fruits as a spice and as a carminative.

The plants are up to 2 m tall, glabrous, glaucous, the stem is striate. The leaves are 3-4 pinnate; segments filiform, up to 4 cm long; leaf base sheathing. Rays 5-30.
1-6 cm long. The carpophore is divided to the base. The fruit is oblong to ovoid, 3-5 mm long, glabrous.

**Pleurosporum stylosum (Clarke)**

It is a wild plant and found in mountains at high altitudes, growing among rocks. It is found in Gilgit, Kaghan Valley, Babusar village, Chitral, Swat, Bishigram. Plants are 3-150 cm tall. Tap root is thick. Stem base is fibrous. The leaves are 2-pinnate; pinnae lanceolate to oval; segments serrate to pinnatifid. The umbels are lateral and terminal. The rays are 10-30, 3-8 cm long, scabrous. Calyx teeth is obsolete. Petals are white. The fruit is broadly ovate, 4-5 mm long.

**Psammogeton stocksii (Boiss)**

It is a wildly growing plant distributed in Baluchistan at Makran, Pasni, Mund, Turbat etc.

Plants are 15-40 cm tall, and branched. The stem is glabrous to puberulous. Basal and lower leaves are petiolate, upper leaves are sessile to subsessile, reduced. Peduncles are terminal and lateral, upto 10 cm long. Rays are 5-12, upto 2 cm long, glabrous to puberulous. Petals are white to pinkish. Stylopodium is conical; styles are 1.5 mm long, divergent, light brown to purple. The fruit is oval-ovoid, 2-2.5 mm long; pubescent; hairs T shaped; inner seed face is plane.
1.8 AIMS AND OBJECTIVES

The studies on the chemistry of essential oils of the Pakistani species of the family Umbelliferae were undertaken to evaluate and obtain basic information for use in developing and exploiting Allah's given agricultural resources mainly to replace the poppy crops in the tribal areas of Pakistan with equally valued essential oil bearing crops in order to check and eradicate the culture of heroin which is menace to the life and welfare of the masses. The dissemination of information and know-how on all relevant aspects including, extraction, processing, chemical composition and application of essential oils, will help to diversify agricultural production and create rural employment opportunities by introducing high valued essential oil bearing crops and will check the flow of population towards cities. Thus the number of aromatic plants and herbs of plant family Umbelliferae, which grow wild in the hilly tracts and zones annually and go waste without being exploited will be utilized for the production of essential oils. This will serve as an import substitute and also cut down foreign exchange expenditure, a great drain on essential oil imports. This will help to patronize the essential oil industry on scientific and modern techniques and ultimately establish the industry on sound footings. This will also help millions of farmers to get more money from their small land holdings by resorting to the cultivation of essential oil bearing crops and will improve the lot of the rural population and solve their economic, cultural and social problems.
GENERAL METHODS

The recent development of various techniques has no doubt made possible the accurate analysis of the essential oils, but some of the classical methods for determining their purity and value, based on the measurement of physical characteristics and qualitative estimation of certain components, such as alcohols, esters, acids and aldehydes are still in vogue. The methods employed include the determination of specific gravity, refractive index, optical rotation, amount of alkali required for the free acid neutralization, the amount of alkali used for the saponification of esters (ester number) and the amount of alkali required for the saponification of esters formed after the esterification of the essential oil.

Specific Gravity of volatile oils may be determined by the Westphal balance or pycnometer, the later being official method and the more accurate of the two. It is one of the most important physical property and is specific for each pure oil and its constituents. Therefore, the degree of variation from its true value gives an indication of the relative purity. By definition, the specific gravity of an essential oil is defined as "the ratio of the mass of the essential oil to the mass of an equal volume of water at 40°C or other specified temperature". The specific gravity of essential oils vary between 0.84 and 1.2. The oils lighter than water, such as orange, coriander, turpentine and rosemary oils are usually rich in hydrocarbons, alcohols, esters and ketones. Oils with specific gravities that approach or exceed 1.0, e.g., anise, cinnamon, clove and sassafras oils usually contain chiefly aldehydes, phenols, phenolic derivatives or certain esters. The specific gravity of any essential oil is not absolutely constant, since it is influenced by such factors as the maturity of the plant from which the oil is obtained and the age of the oil, as well as by the methods of
preparation and purification. The determination of specific gravity of essential oil is made at a constant temperature, as a slight variation in temperature brings about considerable change in its volume due to its large coefficient of expansion. The specific gravity of essential oils is usually determined at 25°C.

**Refractive Index** is a valuable physical constant for the identification and detection of essential oils and is a criterion of their purity. It is defined as "the degree of deflection of a beam of light that occurs, when it is passed from one transparent medium to another". In mathematical terms, it is the ratio of the sine of the angle of incidence to the sine of the angle of refraction. Abbe's refractometer with temperature control is usually used for the determination of this physical constant. It gives value up to the 4th decimal place. The measurement is usually made at 25°C. The temperature must be controlled and recorded because with the rise of temperature, the value of refractive index decreases. The refractive index is denoted by \( n \ D \) where \( n \) is the refractive index at 25°C taken with sodium light (D-line). The refractive index does not vary greatly with different essential oils being between about 1.46 and 1.61 at 20°C. In some cases, however, the determination may serve for the detection of extraneous matter.

**Acid number** is an important chemical constant related to the quality of the essential oil. Most essential oils contain only very small amounts of the free acids, consequently the acid content is usually reported as an acid number rather than as percentage calculated as a specific acid. But the acid number of an oil is often increased as the oil ages, especially if the oil is improperly stored. Processes such as oxidation of aldehyde and hydrolysis of esters increase the acid number. Oils which have been thoroughly dried and which are protected from air and light show little
change in the amount of the acid. The acid number of a volatile oil is determined by titrating the free acids present in one gram of the oil, with standard potassium hydroxide solution using phenolphthalein as indicator. The acid number of an essential oil is defined as the number of milligrams of potassium hydroxide required to neutralize the free acids present in one gram of the oil. In determining the acid number of an essential oil, dilute alkali solution must be used, since many of esters, e.g., formates, normally present in essential oil are capable of saponification even in the cold in the presence of strong alkalies. Moreover, phenols will react with the alkali hydroxides making it necessary to use special indicators (such as phenol red) for oils containing large amount of phenolic bodies.

**Ester number.** Determination is of great importance in the evaluation of many essential oils. The esters which occur as normal constituents of essential oils are esters of monobasic acids and are mostly the acetates of alcohols, e.g., borneol, geraniol, terpineol. When these esters are refluxed with alcoholic potassium hydroxide, they are saponified and yield the free alcohol and potassium salt corresponding to the acid component of the ester. The process of saponification may be represented by the reaction $R_{1}COOR_{2} + KOH \rightarrow R_{1}COOK + ROH$. The number of milligrams of potassium hydroxide required to saponify one gram of the essential oil represents the ester value or ester number of the essential oil. The determination of the total esters of the essential oil serves to detect the adulteration and to establish the quality of those oils, valued for their ester content. The ester values of the oils that contain appreciable amounts of aldehydes or phenols cannot be estimated accurately by saponification with an alkali, since variable amounts of the later are consumed by reaction with aldehydes and phenols or their decomposition products.
Alcohol Number, the determination of the total alcohols occurring free and combined as esters, is sufficient to establish the purity and value of an oil with respect to its content of alcoholic constituents. The alcohols in essential oils are present both in free and combined forms as esters. The total alcohols present in the oil are determined by transforming the free alcohols into the corresponding acetates by boiling oil with acetic anhydride in an acetylation flask and then determining the saponification value of the acetylated product. When the alcohol occurs partly free and partly combined in the form of an ester, a correction factor must be employed. In some cases ordinary methods of esterification by acetylation fail and it is necessary to resort to special procedure. For most oils containing primary and secondary alcohols, the standard method of esterification with acetic anhydride using fused sodium acetate as a catalyst, is satisfactory and gives results of an accuracy of 0.5 percent.

Resolution of Essential Oils into Different Constituents. The oils are resolved into their different constituents by chromatographic techniques. Chromatography may be defined as a separation of molecular mixtures by distribution between two or more phases, one phase being essentially two dimensional (a surface) and the remaining phase or phases, being a bulk phase brought in contact in a countercurrent fashion with the two dimensional phase, various types of chromatography are possible depending upon the physical states of the phases involved. The use of the solid as the stationary phase with a liquid as a mobile phase is generally referred to as adsorption chromatography. The separation of components of the essential oils by adsorption chromatography depends on adsorption-desorption equilibrium between compounds adsorbed on the surface of the stationary phase and in the moving liquid phase. The extent of adsorption of a compound depends upon the polarity of the molecule, the activity of adsorbent and the polarity of the mobile liquid.
phase. The actual separation of the compounds in a mixture is dependent on the relative values of the adsorption desorption equilibrium constants for each of the components in the mixture. In general the more polar a functional group in the compound is, the more strongly it would be adsorbed on the solid phase. Organic compounds in the order of increasing polarity are stated as: hydrocarbons, olefins, ethers, halogen compounds, aromatics, ketones, aldehydes, esters, alcohols, amines, mercaptans, acids and strong bases.

The activity of adsorbent depends upon the type of material and the mode of its preparation. The commonly used adsorbents in the order of increasing activity are reported to be as: cellulose, starch, sugars, magnesium silicate, calcium sulphate, silicic acid, Florisil, magnesium oxide, aluminium oxide, activated charcoal. The choice of the proper adsorbent will depend on the type of the compounds to be chromatographed. Silicic acid is relatively mild adsorbent useful for the separation of various constituents present in the essential oils. The solvents generally used as eluent are stated in the order of their increasing polarity as: petroleum ether, cyclohexane, carbon tetrachloride, benzene, methylene chloride, chloroform, diethyl ether, ethyl acetate, pyridine, acetone, n-propanol, ethanol, methanol, water and acetic acid. Since the entire separation is dependent on the establishment and maintenance of equilibrium conditions, the polarity of the solvent system is generally increased by the slow increase of concentration of more polar solvents. The rate of change of solvent polarity will depend upon the similarity or dissimilarity of components in the mixture to be separated. If closely related compounds are to be separated, for example isomeric olefins or alcohols, the change in the solvent system may be limited to a few percent every 100 ml. The separation of compounds having distinctly different functional groups can often be accomplished by a much more rapid change in the
solvent composition, for example, 25 percent every 100 ml.

The application of chromatographic separation procedures is more an art than a science. When faced with separation problem involving a totally unknown composition of the oil, it is usually best to carry out a crude and rapid trial chromatographic separation with a small portion of a mixture, using the information and experience gained in the trial run to carry out a more efficient separation on the remainder of the material. Such a procedure usually results in saving of time and material. Thin layer chromatography can often serve as an excellent guide to the conditions for column chromatography.

**Thin Layer Chromatography** is used as a model and guide for column chromatography. It is a special application of adsorption chromatography in which thin layer of adsorbent supported on a flat surface is utilized instead of column adsorbent. The most commonly used adsorbents used in TLC in order of importance are silica gel, alumina, kieselguhr and cellulose. The first two are more important for general use. The choice of the best eluent for TLC will depend on the type of adsorbent and the components in the mixture to be separated. The same general rules for column adsorption chromatography also apply to TLC. However, since only one solvent system is generally used in TLC, trial chromatographic separations with a variety of solvents should be carried to determine which solvent leads to the best separation. A series of small individual plates are prepared, spotted and developed in different solvents.

The commonly used plates are 10 by 20 cm or 20 by 20 cm. An aqueous slurry is prepared from commercial TLC adsorbent according to the directions on the container. The preparation of the plates is best accomplished by means of an
applicator. The plates are air dried and placed in an oven at 105°C for one hour or more to activate them for use in adsorption chromatography.

Dilute solution of the oil is made in a volatile solvent and applied in the form of small spots with the help of fine glass capillary about 1 cm from the edge of the plate. If more than one application is necessary allow the spot to dry before re-applying more sample. Allow the spotting solvent to evaporate before placing the plate in the developing chamber. Solvents are usually chosen by trial and error using solvents of different polarities. The spotted plate is placed in the glass chamber fitted with a cover. The level of the solvent in chamber should be below the level of the spot of the plate. Developments such that when the solvent runs about three-fourth of the way-up the plate, the plates are removed from the development chamber and allowed to dry in the air. The spots are visualized by the iodine vapours, the use of ultraviolet light on fluorescent dye containing layers and charring with sulphuric acid. The most commonly used indicator is iodine vapour. The developed and dried plate is placed in a close container containing a few crystals of iodine. The iodine vapour is adsorbed into the areas of the plate containing organic compounds; brown spots due to iodine charge transfer complexes appear on the white plates. The method is usually non-destructive. The spot may be marked and the plate gently warmed to allow the iodine to sublime out of the layer, leaving the unchanged compound.

TLC can be used for the relative small preparative separations employing thicker layers of adsorbents (2000 u) and applying the sample as a band instead of spots. The plates are developed in a solvent system which ensures fine separation of the various components present in the mixture in the form of separate bands. These bands are visualized by the use of indicators. The bands are then scraped from the
plate and the material is leached from the adsorbent with diethyl ether. TLC may be used as a tentative means of identification.

Ultraviolet Spectroscopy

Ultraviolet spectra arise from electron transitions among the energy levels of the molecule.

The theory of ultraviolet spectroscopy is that when a molecule is radiated with a visible or ultraviolet light, valence shell electrons of the molecule are excited from the ground states to higher energy states. A molecule will require certain amount of energy for these excitations. This amount of energy is provided by visible and ultraviolet light. Maximum absorption occurs at points where the energy of the light of certain wave-length becomes equal to that required for excitation. These points of maximum absorption are recorded by a spectrophotometer on a graph as a function of wavelength.

The upper limit of the ultraviolet portion of the electromagnetic spectrum is 400μm. The lower limit is the lowest practical wavelength 200 μm. For ultraviolet region the source is a hydrogen discharge lamp producing a continuous spectrum from 200 to 350μm. The dispersion unit, a prism or a combination of prism and grating while the detector is usually some form of photo-multiplier.

Ultraviolet spectra of compounds are usually determined in the vapour phase or in solution. In the preparation of a solution, a sample is accurately weighed and its dilute solution is made to a desired concentration. One centimeter square quartz cells are rinsed several times with solvent, dried and filled with the solution. The solvent
most commonly used are cyclohexane, 95% ethanol and 1, 4- dioxane. Solvent which is inert to the solute is used.

Ultraviolet spectroscopy has been much used in terpenoid chemistry. Its main application being the detection of conjugation. In simple acyclic dienes, \( \lambda \) max is 217-228. If the diene is heteroannular \( \lambda \) max is 230 - 240 nm. For homoannular diene, \( \lambda \) max is 256 - 265. If an \( \alpha, \beta \)-unsaturated carbonyl system is present, the \( \lambda \) max is 220 - 250 nm, and there is also a weak band at \( \lambda \) max 315 - 330 nm. The absorption maximum of a diene system is affected by substituents. The position of absorption maximum depends on their number and type and for calculating \( \lambda \) max from the molecular structure of the compound Woodward has developed a set of empirical rules.

Infrared Spectroscopy

Infrared Spectroscopy arise from the vibration of the molecule itself. A bond in an organic molecule may stretch or it may bend relative to other bonds. For occurrence of each stretching or bending vibration in a molecule a definite quantum of energy is required. This quantum of energy is provided by infrared part of the electromagnetic radiation. Out of many possible stretching and bending vibrations only those vibrations are capable of absorbing infrared radiations, which cause a change in the dipole moment. Also bending vibrations occur at longer wavelength than stretching vibrations. Infrared radiation is produced by electrically heating the source of radiation which is an incandescent filament of rare earth oxides. The radiation from the source is dispersed to obtain bands of narrow wavelength with the help of a dispersion system, consisting either of sodium chloride or in some case a prism-grating combination. The detector is usually a thermocouple or bolometer,
which measures the radiant energy by means of its heating effect. The heat is converted to an electric current, which is amplified. The amount of attenuation required is a direct measure of the absorption by the sample or compound. The movements of the attenuator is recorded by the chart pen. Infrared spectra may be obtained for gases, liquids or solids. Liquids may be examined neat or in a solution. Neat liquids are examined between plates with or without spaces. Volatile liquids can be examined neat in sealed cells. The amount of material needed is between 1 mg and 10 mg. Infrared absorption spectra are usually obtained by placing the sample in one beam of a double beam infrared spectrometer and measuring the relative intensity of transmitted (and therefore absorbed) light energy versus wavelength (or wave number).

Different kinds of atoms have different masses and different kinds of bonds have different strength. Therefore, when a molecule absorbs infrared radiations, its bonds vibrate at different frequencies (wave numbers). From the data available about the positions of absorption of various bonds in various molecules, it is possible to correlate the bond stretching frequency and the type of bond and thus to determine the functional groups present in the molecule.

Infrared spectroscopy is very useful in terpenoid chemistry and is valuable for detecting the presence of a hydroxyl group (3400 cm\(^{-1}\)) or a carbonyl group (saturated: 1750 - 1700 cm\(^{-1}\); \(\alpha, \beta\)-unsaturated 1700 - 1600 cm\(^{-1}\)). It is particularly also useful for detecting the presence of isoprenyl or isopropylidene groups and may often distinguish between cis and trans--isomers.)
Nuclear Magnetic Resonance

The title of this branch of spectroscopy makes it clear that this is concerned with the property of the nucleus. The n.m.r. spectroscopy is useful for the detection of the alkyl part of the molecule and indicates the proton environment. The instrument can be described in terms of following components.

1) A strong magnet whose homogeneous field can be varied continuously and precisely over a relatively narrow range. This is accomplished by means of the sweep generator.

2) Radio frequency Oscillator.

3) A radio frequency receiver.

4) A recorder calibrator and integrator.

5) A sample holder which positions the sample relative to the main magnetic field, the transmitter coil and the receiver coil. The sample holder also spins the sample to increase the apparent homogeneity of the magnetic field.

A sample, a liquid or a solution in a suitable solvent is contained in a 5 mm glass tube. Ordinarily, about 0.4 ml of a liquid or some where between 10 to 50 mg of a solid dissolved in 0.4 ml solvent is used with the radio frequency oscillator at 60 mega cycles per second, the sweep generator periodically sweeps the main magnetic field in the immediate vicinity of 14092 gauss. For protons, the range of the sweeps is of the order of 1000 cycles per second or so. Calibration of chemical shifts is in dimensionless units, i.e., parts per million (ppm) or tau (τ) values, from a reference marker. Peak areas are measured by the integrator which, on the A-60 spectrum.
superimposes a series of steps on the absorption peaks; the step heights are proportional to the number of proton under the respective peaks.

N.M.R. spectroscopy has been used to detect and identify double bonds, to determine the nature of end groups and also the number of rings present and to ascertain the orientation of methyl groups in the molecule. In certain cases, definite structures have been assigned on the basis of nmr spectra.

Mass spectroscopy

Mass spectroscopy is a very versatile technique and can help in finding precisely and efficiently the molecular weight, molecular formula and the complete structure of an organic compound. In fact, UV, IR, NMR and MS give information about the structure of a compound only when combined together.

In mass spectrometer, a substance in a gas phase at very low pressure (10 mm), is bombarded by high energy electron beam usually of the order of 70 ev. As a result the molecule is broken into small fragments or positive ions which are then recorded in the increasing or decreasing order of their masses. The result of interaction of high energy electron with the molecule is that the molecule is excited and then it ejects an electron to become itself a positive ion. The mass of the ion is equal to the molecular weight of the substance and ion is called molecular ion. The positive ion then undergoes fragmentation or rearrangement can take place to produce only those ions which are relatively stable.

A mass spectrometer consists of three units i.e., ionization chambers the analyzer and recorder. The ionization chamber is maintained at 10 -10 mm pressure and the sample is heated to a temperature at which it volatilizes at that pressure.
filament of Rhenium is heated to emit a beam of electrons of an energy of about 70 ev. The electron bombardment converts the molecule into ions which are accelerated out of the source by an electric field as soon as they are formed. The neutral molecules are pumped out of the ionization chamber as they are not accelerated. The positive ions are then accelerated to the analyzer where they are separated into groups according to their mass to charge ratio, m/e. Since most of the ions are singly charged, the separation is according to mass m. The separation is possible between masses which differ by 0.01 mass units.

A very small quantity of a pure sample is injected in the sample probe from where it is sucked into the reservoir which is maintained at 10 mm pressure. The vapours of the sample enter the ionization chamber where they are bombarded by high energy electrons (70 ev) which cause them to ionize. Un-used electrons, neutral fragments and the material other than the positive ions are pumped out of this chamber whereas the positive ions are accelerated by a series of accelerating voltages. These positive ions are deflected and then recorded as their m/e versus relative abundance. Since charge on the ion is usually one m/e is essentially the mass m of the ion. The recording is done on a photographic ultraviolet recording paper by five separate galvanometer mirrors simultaneously. Two others forms (digital or bar) of the spectrum could be obtained with the help of a computer, along with the recorded film.

In a mass spectra the molecular ion peak (Mpeak) is the one which represents the molecular weight of the compound. It is obtained in the higher mass region other peaks in this area of higher mass are M+1 and M+2 peaks obtained due to the presence of heavier isotopes. Base peak is the one which corresponds to the most stable ion and is assigned 100% intensity. It is the highest peak in the spectrum.
Mass spectroscopy is increasingly used as a means of elucidating the structure of terpenoids. Thus, it is possible to determine molecular weight, molecular formula, the nature of various functional groups, and the complicated fragmentation patterns of double bonds. Since even simple terpenoids give complicated fragmentation patterns, structural identifications of an unknown terpenoid by means of mass spectroscopy must be carried out, with some caution. It is possible, however, to identify a terpenoid by comparison of its mass spectrum with the reference spectrum of an authentic specimen.
LITERATURE SURVEY

Sage\textsuperscript{73} studied the essential oil from the entire plant of the sweet fennel of Spanish origin and recorded that the oil did not contain a notable quantity of anethole and was entirely different from the oil distilled from the fruit of Foeniculum vulgare. Two samples of the oils were examined to possess specific gravity 0.9203, 0.9340; refractive index 1.495, 1.4980; optical rotation, +46, +24; solubility in 90\% alcohol 1 in 1. It was recommended that this oil should be called "plant" oil in distinction to "Fruit" oil usually prepared.

Frerichs\textsuperscript{74} studied the constants of the fennel oil in order to improve the inadequate specifications of the Ger. Pharm., so as to check possible adulteration with fenchone or substitution by a mixture of stenanis oil and fenchone.

Vinogradova and Novotel\textsuperscript{75} studied that methyl chavicol fraction of fennel oil could be isomerized into anethole by reacting 1 part of the fennel oil with 2 parts of the solid KOH at 1\textdegree\textsuperscript{C} for two hours.

Shchetinin\textsuperscript{76} separated anethole which was approximately 60\% of the fennel oil and reported that only half of this amount could be brought to pharmacopeol product by gradual rectification. However, residual anethole was used for the synthesis of anisaldehyde with 50\% yield.

Miller\textsuperscript{77} made a comparative study of fennel oil of flowering plants grown during two successive years and observed that the oils were quite similar to those, distilled from stalks, fruits and leaves of the wild fennel but were not USP quality. The use of maleic anhydride adducts for the identification of terpene hydrocarbon was successfully applied to the fennel oil of the flowering plants. Fenchone was
found to be the principal ketone in the oil. However, difference in the soil composition was observed to affect the ketone content in the oil.

Garcia and Pulido\textsuperscript{78} analyzed a number of samples of essential oils of Spanish Anethum foeniculum obtained from common source, or prepared by steam distillation and recorded that their specific gravity varied from 0.813 - 0.962, refractive index 1.4960-1.5153 and optical rotation was found irregular, some oils were laevorotatory others dextrorotatory or inactive. Several oils were resolved into their different fractions. These fractions were also studied for their physical constants.

Akacic and Rogina\textsuperscript{79} studied that Foeniculum macdonicum and fructus foeniculi contained 2.6% volatile oil, which was found to possess specific gravity 0.9755 - 0.9774, refractive index 1.5467-1.5478, optical rotation + 6.6 to 6.8. The volatile oil on fractionation gave 60\% pure anethole.

Gleisberg and Hatratti\textsuperscript{80} recorded that the essential oil content in the unripe fruits of foeniculum vulgare was higher than that in the ripe fruits, there was no increase in the oil content of the ripe fruit after harvesting but rather a small decrease was noted.

Aubrey Carson\textsuperscript{81} determined the percentage amount of volatile oil and standard deviations with the modified method and reported that fennel contained 2.25 ± 0.65 of the volatile oil.

Yves and Jovan\textsuperscript{82} studied eighteen samples of essential oils of both bitter and sweet fennel (Foeniculum vulgare) and reported; 50-85\% trans anethole, < 0.3\% cis anethole, 3.20 \% estragole and 0.7-2.2\% fenchone.
Yves\textsuperscript{83} reported that the toxicity of cis-anethole was markedly high than that of trans-anethole. Cis-anethole was determined by IR spectrometry as it gave a distinct absorption band at 731 cm\textsuperscript{-1} and 691 cm\textsuperscript{-1}. However, cis-anethole, trans-anethole and estragole were determined in a mixture with G.C. as they gave three distinct peaks.

Fernandes et al\textsuperscript{84} determined the essential oil of the fruits of fennel plants, growing wild in fertile plains at the mouth of Mata river and reported that the average amount of volatile oil by using a technique based on distillation was 3.5%. It was somewhat lower than the yield obtained in extraction for analysis.

Fernandes et al\textsuperscript{85} investigated the average yields of essential oil of wildly growing Portuguese fennel plants, at the end of flowering and from the ripe fruits were 0.5 and 3.55% respectively. The chemical composition of the oil revealed that it contained \(\alpha\)-phellandrene, fenchone, estragole, anethole, anisaldehyde and anisic acid. Fenchyl alcohol, however, was not found.

Michiichi and Motoo\textsuperscript{86} distinguished 4 kinds of crude drugs containing anethole, sweet fennel, anise and star anise from their essential oils by using I.R. absorption spectra. The anethole was detected by the characteristic absorption of a propenyl group at 961 cm\textsuperscript{-1}. In sweet fennel, characteristic absorption of fenchone at 1743 cm\textsuperscript{-1} as considered. For anise and star anise oils the absorption at 1371 cm\textsuperscript{-1} which was stronger in the star anise oil was used. This absorption was considered to be due to concerted effect of iso-propyl group in cineole, terpineol and phellandrene.

Hevieta\textsuperscript{87} studied that the anethole separated from the Romanian foeniculum oil showed specific gravity 0.9672; refractive index 1.5290; optical rotation + 17.5:
F.p. +1; solubility in 90% alcohol 0.5 vol/vol. Anethole which was separated by freezing the raw at -28°C and pressing the frozen product upto 300 atmosphere, showed specific gravity 0.9868, refractive index 1.560; F.P. 20.2, m.p. 22.5, solubility in alcohol (90%) 0.5 vol/vol. After 3 months storage at 22-25 in brown bottles the constants were; 0.9876, 1.560, 19, 21.5, 1/1 respectively.

Walther and Richard recorded maximum yield of essential oil from the seeds of fennel collected before the plants were fully ripe and also observed that the time of maximum yield could be varied with different strains of fennel as well as conditions of cultivation.

Nasir et al. reported that fennel fruits of Pakistani variety, contained 1.3% of the essential oil.

Mogens wellendorf conducted gas and thin layer chromatographic studies of essential oil from the fennel seeds using polyglycol -chromosorb column at 180 and He as the carrier gas. Thin layer chromatography was studied using silica gel as an adsorbent and developments were, however, made with saturated solutions of SbCl₃ in CHCl₃.

Wahid and Ikram reported that the fruits of Foeniculum vulgare gave 2.03-2.17% oil having specific gravity 0.973, optical rotation +13.9, refractive index 1.544, solubility in 80% ethanol 1.7 and solidification point 11.6.

Kuechler et al. studied the composition of essential oil extracted from the fruits of Foeniculum vulgare plant by gas chromatography and reported the amounts of anethole, fenchone and relative retentions of the unknown constituents of the oil.
Topalov et al.\textsuperscript{93} studied that the essential and fatty oils of fennel fruit occurred in the shell and seed and reported that better quality of essential oil was obtained if the ground fruits were extracted with diethyl ether followed by distillation.

Topalov et al.\textsuperscript{94} investigated that besides the location of the oil cells in the fruit shell of \textit{Foeniculum vulgare}, 25\% of the essential oil was found in the endosperm of the seed absorbed in the fixed oil.

Flueck and Kast\textsuperscript{95} studied the effect of different kinds of milling of fennel fruit on the essential oil content and reported that the loss was greatest with the beating cross mill showing 37-67\% loss, pestle method was intermediate showing 4.6-12\% loss and the least loss was recorded in the ball mill method, i.e., 0-2.5\% of the essential oil. The loss occurred was due to low boiling components as shown by gas and thin layer chromatographic studies, with retention of relatively larger amounts of anethole. There was no oxidation of anethole to anisaldehyde and anisic acid.

Solotowitsch and Chiketi\textsuperscript{96} investigated fennel ethereal oil by gas chromatography and identified anethole, fenchone, phellandrene and methyl chavicol in the oil.

Regina and co-worker\textsuperscript{97} studied and identified the components of essential oil of Polish \textit{Foeniculum vulgare} by thin layer chromatography. The oil was diluted 1:20 with 95\% ethanol then applied on the glass plate covered with silicic acid, starch and water. The ascending method used was benzene and it required 1-2 hours. Developers used were 0.25\% \textit{KMnO}_4, 10\% \textit{SbCl}_3 in \textit{CHCl}_3, 25\% \textit{CCl}_4 \textit{CO}_2 \textit{H} and 2,4-dinitrophenyl hydrazine.
El-Hamidi and Ahmed\textsuperscript{98} studied that the essential oil content in plant of Foeniculum vulgare increased up to a certain point then decreased. In fruit, however, the oil increased up to the time of maturation. The active principles of the essential oil, which appeared in all stages of growth of the plant and fruit were found to be anethole, fenchone and phellandrene.

Osisiogu\textsuperscript{99} investigated that the fruits of Foeniculum vulgare of Indian origin, cultivated in Nigeria, yielded 2.0-2.4\% of essential oil. It was found to contain 86\% anethole and no fenchone by TLC using silica gel G.

Toth\textsuperscript{100} differentiated by gas chromatography the essential oils from fennel fruits from Germany, Poland, Hungary and other European areas, India, China, Japan and Argentina and reported that the newly identified components of the oils were $\beta$-pinene, $\beta$-myrcene, p-cymol and 8 unidentified components. Whereas, oils from the herb and fruit were found to be qualitatively similar but there was a quantitative difference to permit distinction of the two. The fruit of Foeniculum vulgare var. vulgare (bitter fennel) showed high fenchone 12.22\%, relatively high $\alpha$ - pинene (1.8-4.7\%) and low limonene (1.5-2.5\%) content, while Foeniculum vulgare var. dulce (sweet fennel) had a low fenchone (1\%) and $\alpha$-pинene (0.4-0.8\%) and high limonene (4.7-5.4\%) content, however, strong variations were found in the composition of materials from the various geographical areas. The fennel root oil was found radically different from the fruit with dill apinale as the chief component (> 90\%): The oil composition was relatively constant for the various varieties and geographical origins. The following compounds reported by others as components of fennel oil could not be found in either fruit or herb oil: anisic acid, valeric acid, p-methoxy acetophenone, anise ketone, camphor, p,p′dimethoxy stilbene, foeniculin, fenchyl
alcohol, ethyl fenchyl alcohol, thiofenchone, thymohydroquinone, 2,3- dimethyl butadiene, ocimene and α - terpinene. The results of the other authors varied partly because they used strongly autoxidised oil.

Koichi et al\textsuperscript{101}, separated 14 components from Chinese star anise oil by precision distillation and gas chromatography and identified each component by TLC and IR spectral analysis. For comparison the same procedure was applied to fennel oil from Europe and reported that 3 of the above 14 components were not detected.

Jakub et al\textsuperscript{102}, studied the chemical composition of several samples of essential oils of Oleum foeniculi and Oleum anisi, by thin layer chromatography (TLC) and gas liquid chromatography (GLC). A 10\% solution of the essential oil of Oleum foeniculi in 70\% aqueous ethanol was applied on silica plates using benzene as the developing solvents. The development zone was made visible with iodine vapour followed by spraying with 2,4- dinitrophenyl hydrazine. The best GLC separation was achieved with 20\% PEGA column on chromosorb W, using a stainless steel column 2m. long oven temperature 180°C and nitrogen gas flow 80 ml per minute. All the samples of the essential oil showed anethole, fenchone, anisaldehyde and terpene. TLC of the essential oil of Oleum anisi was performed on silica gel plates with benzene as the developing solvent. The developed zones were made visible by spraying with Vanillin in concentrated sulphuric acid. GLC data of the essential oil using the same conditions as for previous oil, showed the presence of α-pinene, limonene, anethole, anisaldehyde and an unidentified substance.

Paukov et al\textsuperscript{103}, investigated the essential oil of fennel by means of gas liquid chromatography with capillary column.
Jerzy\textsuperscript{104} investigated anethole in substantia and in fennel oil by determining methoxy group with III. method. The determination of the methoxy group with pyridine - HCl, however was reported to be troublesome, time consuming and results were highly scattered. The determination of unsaturation by the addition of iodine monochloride and by Hg (OAC) method gave high results especially in the case of fennel oil.

Bukala \textit{et al}\textsuperscript{105} studied the chemical composition of essential oil of Foeniculum vulgare by dividing the oil into hydrocarbon and oxygenated fractions by fractional distillation. The hydrocarbon and oxygenated fractions were subjected to gas chromatography using 170 cm columns of 20% carbowax 20M or polyethylene glycol adipate on chromosorb W, flame-ionization detector and column temperature 110°C for hydrocarbons and 180°C for oxygenated fractions. The hydrocarbon fraction was found to contain, α - pinene 4.33, camphene 0.27, β-pinene 0.37, myrcene 0.83, α - phellandrene 0.34, limonene 2.16, β-phellandrene 0.59, γ-terpinene 0.96 and p-cymene 0.45%. The oxygenated fraction was found to be composed of fenchone 21.68, camphor 0.39, α - terpineol 3.02, methyl chavicol 0.43, anethole 63.25 and anisaldehyde 0.95%.

Schantz and Juvonen\textsuperscript{106} prepared aqua Foeniculi and aqua Anisi by dissolving 0.5 gm of essential oils in 1000 gm of water according to Norwegian Pharmacopoeia and investigated the constituents of essential oils and of the solutions, and found by gas chromatography that the composition changed in solutions during storage for one year. The distribution of oils between water and filter paper or talc (dispersing additives) was determined gravimetrically. The loss of essential oil was 8-20% higher when talc was used than with filter paper in the preparation of aqua
solution. Aqua Foeniculi lost 50 and aqua Anisi 70% essential oils when stored at 10-15°C for a period of one year. The percentage of anethole, the main constituent of the oil was reduced from 64.5 and 89.4% to 14 and 64.8% on dissolution and to 4.36 and 11.2% during storage for one year in aqua Foeniculi and aqua Anisi respectively. Anethole was autoxidised to its β-glycol, the amount of which was found to be increased initially during storage but decreased there after on further autoxidation via anisaldehyde to polymers.

Karlsen et al.\textsuperscript{107} studied analysis of the essential oils and morphological measurements of the fruits of Foeniculum vulgare var. dulce (sweet fennel) and Foeniculum vulgare var. vulgare (bitter fennel) and their hybrids, using plants cultivated in Kazanlik. Monoterpene hydrocarbons identified were α- and β-pinenes, α-thujene, α-fenchene, camphene, Δ^1-carene, sabinene, α - phellandrene, cis-octimene, terpinolene, trans-octimene and p-cymene. There were differences in the quantities of these hydrocarbons but the amount of estragole compared with anethol was relatively constant in the essential oils of the first two plants. Direct gas chromatography of green fruits showed relatively more, i.e. 3-7 time more fenchone than the brown ones. Hybrid was found to inherit a lower content of fenchone of the female plant.

Trenkle\textsuperscript{108} investigated the essential oil obtained by the steam distillation of Foeniculum vulgare roots and identified by gas chromatography α - and γ- terpinene, terpinolene, α-pinene, β-pinene, β-myrcene, α - phellandrene, p-cymene, limonene, myristicin and petersilienapioi in addition to dillapiol.

Shah et al.\textsuperscript{109} reported that the fruit from a strain of a fennel grown in Ooty, India, contained 8.5% essential oil, i.e., more than twice that of best oil yielding
Russian fennel, although differing in morphology and sensory characters, this fennel was identified by kew authorities as Foeniculum vulgare (fennel). The oil was totally devoid of anethole and the main constituent being estragole.

Rothbaecher and Kraus\textsuperscript{110} studied the physico chemical constituents of essential oil obtained by the steam distillation of crushed seeds of Foeniculum vulgare and recorded: specific gravity 0.9684; refractive index 1.5355; optical rotation 14.2\textdegree; acid number 0.502 and ester number 5.624. The oil was fractionated by column chromatography into various fractions which were studied and identified by thin layer chromatography and IR spectral analysis. It was found to contain limonene, α -phellandrene, camphene, α - pinene and anethole.

Svendsen and Karlsen\textsuperscript{111} reported that the presence of cis-anethole in the essential oils of Foeniculum vulgare, Pimpinella anisum, and Foeniculum dulce (anethole containing plants), was not a distillation artifact as it was also detected in the whole fruits of the species by the gas chromatography.

Fertman and Lesnov\textsuperscript{112} determined and identified the composition of essential oils in plant raw materials by the use of thin layer chromatography (TLC) on alumina and reported it as a quick qualitative method developed for the determination of certain essential oils of plant origin.

Trenkle\textsuperscript{113} obtained by steam distillation, the essential oils of the roots, root barks, stems, leaves, unripe and ripe fruits of Romanian (Rum 66) Foeniculum vulgare plants, cultivated at Hamburg. The essential oils were separated into their components by column, thin layer and gas chromatography procedures. The root oil was found to contain α- and β- pinenes, camphene, myrcene, α- and β-
phellandrene, α- and β-terpinene, cis and trans- allo- ocimene, Limonene, p-cymol, terpinolene, trans-anethole myristicin and apiol. The oils of the two varieties were found qualitatively the same but different quantitatively in component percent. The oil from fruit and herb were observed qualitatively similar. In the unripe fruit the fenchone content was found much higher but trans-anethole was only 0.5 that of the ripe fruit. The unripe fruits were noted to have relatively high content of α-pinene, myrcene, α- phellandrene, limonene and γ-terpinene.

Ballarian, C. and Ballarian, J \(^{114}\), differentiated fennel oil and aniseed essential oils by thin layer chromatography on the basis of fenchone content which occurred in good amounts in the fennel oil but not in anise oil. Both oils contained large amounts of anethole. Fenchone was very unreactive by the addition of iodine and spraying with starch. A colour spot was formed by using KMnO\(_4\) and vanillin- H\(_2\)SO\(_4\); fenchone turned gray blue while anethole and anisaldehyde turned brown. Fenchone could be coloured by many H\(_2\)SO\(_4\) containing reagents if heated after spraying.

Kravet \(^{115}\) stated that the amount of anethole could be determined in the freshly processed anise seed and fennel oils by the measurement of refractive index of the oils, but recommended gas liquid chromatography and bromide bromate methods for the detection of anethole in oils which were stored for a long time.

Thielemann\(^{116}\) identified fennel oil and oil of Pimpinella anisum by the presence and absence of fenchone. Both oils contained anethole in large amounts but only fennel oil contained an appreciable amount of fenchone. The anethole and fenchone were separated and determined by thin layer chromatography on silica gel G plates, activated for 2 hours at 110°C using a 95:5 benzene-ethylacetate mixture. The plates when sprayed with concentrated sulphuric acid, anethole gave a reddish -yellow
spot in the cold while fenchone became only visible after heating at 100°C. The Rf factors of anethole and fenchone were recorded to be 44 and 62 respectively. The spots were made visible by spraying with a solution of 1 gram 2,4- dinitrophenyl hydrazine in 1000 ml of ethanol containing 36% HCl. It was followed by spraying with 0.2% K₄Fe(CN)₆ in 2N HCl that the hydrazones of ketones were coloured blue immediately, those of aldehydes became olive green slowly and the spot of the unsaturated compounds changed their colour only after a long time.

Brasil and Gilberto determined with the help of gas chromatography and IR spectra, fenchone 2.53, fenchyl alcohol 2.07 and anethole 90.21% in the essential oil of Foeniculum vulgare collected in Rio Grande do Sul.

Muhammad Y.A. et al detected anethole in anise and fennel oil by differential spectrophotometric method in which anethole absorption was measured against a blank of brominated anethole at 258 nm. The results were stated to be more precise and had equivalent or greater accuracy than those obtained by conventional spectrophotometric methods.

Emborg et al reported 1.22 and 1.50% oil yields from the Foeniculum vulgare herb and fruit respectively. The quality of the oil as reflected by its major and minor components, which were determined by combined thin layer and gas chromatography and by mass spectral and IR analysis. 19 constituents were identified in the herb oil and 14 in the fruit oil, corresponding to 96 and 98 % of the total oil contents. However, trans - anethole, fenchone, estragole and limonene represented 79.2% of the herb and 93.1% of the fruit oil.
Muhammad Ashraf and Muhammad Khurshid Bhatti\textsuperscript{120} reported that fresh seeds of \textit{Foeniculum vulgare} of Pakistan variety yielded 2.0-2.5\% of the essential oil. The chemical composition as determined by gas chromatography, indicated $\alpha$-pinene 3.0, camphene 0.65, $\alpha$-phellandrene 0.44, limonene 4.56, fenchone 10.20, methyl chavicol 3.5, anethole 74.85, anisaldehyde 1.80 and p-anisic acid 1.00\%.

Fournier et al\textsuperscript{121}, separated trans-anethole from fennel oil and purified by the THN 102 preparative gas chromatography. Also isolated 0.55 gm, 99.8\% pure anethole from 0.8gm oil in 8 minutes time using low pressure 1205 mm and temp. 120 °C.

Conan\textsuperscript{122} defined the standards for the essential oil of fennel produced in Reunion Island and reported identification of the constituents of fennel essential oil by gas chromatography and IR spectrometry.

Duquenois et al\textsuperscript{123}, stated that the quantitative differences between anethole, estragole and fenchone contents in the essential oils must be taken into consideration in order to recommend specification for the standardization of pharmaceutical fennel.

Hethelyi and Tetenyi\textsuperscript{124} determined the essential oil content and composition of the \textit{Foeniculum vulgare} oil by subjecting the plant material to pyrolysis at 1000°C prior to gas chromatography. The pyrolysis products were introduced into the column by the N carrier gas. The column was packed with diethylene glycol succinate on chromosorb W, flame ionization detector was used. The contents of anethole, estragole and fenchone were determined in several samples.

Frazao et al\textsuperscript{125}, reported the physico chemical characteristics of fenchone and anethole, the major components of \textit{Foeniculum vulgare} essential oil.
Fehr\textsuperscript{126} investigated the change of content of essential oil in relation with the storage time in 9 samples of anise and 7 samples of fennel fruits and recorded that anise samples showed an average 1\% decrease of essential oil per month, while the decrease of essential oil content in fennel samples ranged 0.01 and 0.153 ml per month.

Kraus et al\textsuperscript{127} determined and compared the contents of Romanian fennel oil with the previously known components of the essential oil, and observed the presence of many oxidation products and other artifacts, e.g., anisaldehyde, anisic acid and acetaldehyde, however, the presence of anisaldehyde and acetaldehyde and increase of the contents of p-cymol and camphor was recorded in the aged oil.

Meckes\textsuperscript{128} published a review article covering the fennel fruit anatomy, characteristics of fennel drug powder and impurities, adulterants and components of fennel.

Rozas et al\textsuperscript{129} investigated the essential oils of seeds and flowers of Foeniculum vulgare and identified \(\alpha\)-pinene, \(\beta\)-pinene, myrcene, limonene, p-cymene, anisaldehyde as the minor components and fenchone, estragole and anethole as the major components of the essential oil by gas chromatography - mass spectroscopy.

Formacek and Kubeczka\textsuperscript{130} reported that by elimination of signals of nonprotonated nuclei and calculation of average signal intensity\(^1\)C atom as a measurement characteristic, it was possible to quantitatively analyze the essential oils by \(^{13}\)C NMR. The relative errors in the analysis of a mixture of limonene, pinene and linalool was found less than 5\%. Fennel oil was analyzed for 12 components by 2
variants and the results compared with each other and with the results of quantitative
capillary gas chromatography.

Stahl\textsuperscript{131} studied the chemical composition of essential oils of Foeniculum
vulgare from bulb, fruit and roots by gas chromatography and reported trans-anethole
and limonene as the major components of the bulb and fruit oils, while limonene and
parsley -apiole were recorded as the major components in the root oil. The oil
composition of various bulb layers was also studied and observed that trans anethole
was prevalent in all layers, whereas the oil from the outer layer was recorded to be
rich in monoterpenic hydrocarbons and thus stated to be inferior in quality.

Chernyshova et al\textsuperscript{132} studied the essential oil from whole fennel plant and
investigated the possibilities for its processing.

Fouraste and Stanislas\textsuperscript{133} determined the essential oil contents in drugs
stored in kraft paper , polyethylene and coloured and tinted glass containers and
reported that kraft paper was the best container for storing the extracts and
polyethylene was the least suitable container for the storage of essential oil containing
drugs. The vernaine oil stored in polyethylene containers lost its oil by 80% in three
years, however, the loss of eucalyptus oil content was found to be much lower.

Kubeczka and Formacek\textsuperscript{134} studied the application of direct carbon-13 NMR
spectroscopy in the analysis of volatile oils and reported the results of \textsuperscript{13}C NMR
analysis of fennel oil in comparison with the gas chromatography.

Lawrence\textsuperscript{135} in a review article entitled "Progress in essential oils" covering
the composition of various essential oils with many references, has also reported the
composition of fennel oil.
Akgul\textsuperscript{136} investigated the essential oil of Turkish fennel seeds collected from 8 different provinces and out of 31 constituents, quantitatively isolated, the major were reported to be trans-anethole, 75.68-86.52%; limonene 4.2-9.15; estragole 3.25-5.21; fenchone 1.05-2.80; \( \alpha \)-terpinene 0.86-1.57 and \( \gamma \)-pinene 0.47-1.14%. The monoterpane hydrocarbon fraction was found to contain 12 compounds showing qualitative and quantitative difference in the hydrocarbon contents of different oils.

Akgul\textsuperscript{137} identified by gas chromatography 31 constituents of the essential oils from Turkish fennel seeds collected from eight different provinces during the two respective seasons, 1983 and 1984 and reported that the main characteristic of the oil was the highest content of trans anethole (75.68-86.52%), the other important components were limonene 4.25-9.15, estragole 3.25-5.21, fenchone 1.05-2.80, \( \gamma \)-terpinene 0.86-1.56 and \( \alpha \)-pinene 0.47-1.14%. Anisaldehyde, anis ketone and cis-anethole reported as the autoxidation products of trans-anethole, were found generally in the samples. Carvone, citral, octanal, citronellal and \( \alpha \) -terpineol that occurred rarely in fennel oils were also identified in some samples.

Peiffer et al\textsuperscript{138}, studied the effect of the shape and materials of stills on the quality of essential oils. The stills were reported to be prototype of alembics, retorts and Rosenbut stills containing glass, ceramic, copper and tin. The hydrolysis of trans-anethole in Foeniculum vulgare essential oil was predominantly affected by the size and shape of the stills, while the material of construction of stills, the length, cross section and collection channel inside the stills affected the degradation reaction occurring during the distillation process.

Akgul and Bayrak\textsuperscript{139} studied by gas chromatography the volatile oil composition of various parts of bitter fennel (Foeniculum vulgare var.vulgare)
growing as a wild Turkish plant and reported that the major component of all the oil samples was trans-anethole (29.70, 37.07, 54.22, 61.08 and 64.71% in leaf, stem, flowering umbel, flowers and fruits respectively) while the other main components were: α-pinene in leaves, stem, flowering umbel and flowers; α-phellandrene in leaves, stem and flowering umbel fenchone in the fruit oil. The volatile oils of flowering umbel, flower and fruit were recorded to contain high amount of oxygenated compounds in gradually increasing percentage.

Sur et al\textsuperscript{140} developed gas chromatographic techniques for the determination of biological active monoterpenoids in fennel and water infusions and reported that analysis required 1-2 grams of the plant material and 100-200 grams of infusion and took not more than 1.5 hour including distillation and gas chromatography. The techniques allowed the analysis of the herbs and infusion without the determination of essential oil content and weighing of isolated oils from chromatography.

Gurdipet et al\textsuperscript{141} investigated essential oil from the seeds of Foeniculum vulgare by GC-MS and reported the presence of 20 components, out of which 18 comprising 96.04% of the total oil had been identified.

Zhao et al\textsuperscript{142} determined 27 constituents of essential oils from leaves, flowers and fruits of Foeniculum vulgare by GC-MS spectrometry and reported: limonene content was 57.8% of the oil from leaves, 34.2% from the flowers and 13.1% from fruits; the trans-anethole contents 21.8% in the oil from leaves, 41.2% from flowers and 63.4% from fruits.
4.1: MATERIALS, INSTRUMENTS AND REAGENTS

4.1.1: MATERIALS

1. Scaligeria aitchisonii (Nasir), above ground parts of the plant at flowering stage, collected from Shogran (Kaghan Valley) NWFP, Pakistan.

2. Foeniculum vulgare wild variety, matured seeds collected from plants wildly growing at shogran (Kaghan Valley) NWFP, Pakistan.

3. Pleurosporum stylosum (Clarke) above ground parts of the whole plant at flowering stage, collected from Babusar (Kaghan Valley) NWFP, Pakistan.

4. Psammogeton Stocksii (Boiss), above ground parts of the plant, collected from Turbat, Baluchistan, Pakistan.

4.1.2: INSTRUMENTS

1. Infrared spectrophotometer (Hitachi 270-30).


3. Mass spectrometer JEOL Model JMS-A X 505 H Mass Spectrometer combined with Hewlett 5890 Packard Gas Chromatograph and Data Acquisition and reprocessing performed by JEOL JMA-DA 5500 system.
4. Gas Liquid Chromatographic Analyzer, Pye-Unicam 104
   Gas Chromatograph instrument equipped with flame ionization detector.

5. Ultra-Violet Spectrophotometer, Beckman Model 36

6. Melting point of the solid compounds, determined on Fisher Johns Melting point apparatus.

7. Refractive Indices of the oils, determined on Abbe's Refractometer.

8. Compounds fractionated on PLC were visualized under UV lamp giving UV light of 366 nm.

4.1.3: SOLVENTS

1. n-Hexane, solvent used in essential oil extraction and eluent in column and thin layer chromatography, was distilled to remove traces of higher alkanes and xylenes.

2. Diethyl ether (Merck), solvent and eluent in column and thin layer chromatography.

3. Ethanol, eluent in column and thin layer chromatography and solvent in crystallization and UV absorption spectra determination was obtained by refluxing Ethanol (2 litre) with quick lime (250 g) on water bath for 6
hours. Allowed to stand overnight then distilled on steam bath.


5. n-Propyl alcohol, as eluent in preparative TLC.

6. Ethyl acetate (BDH), eluent in preparative TLC.
   Methylenediacetamide, (BDH), eluent in preparative TLC.

4.1.4: CHEMICAL/REAGENT

1. 0.1 N Oxalic acid, prepared from accurately weighed (6.3 g) oxalic acid and distilled water to make the volume 1 litre.

2. 0.1 N Sodium hydroxide, dissolved sodium hydroxide (4.2 g) in distilled water to make volume 1 litre and standardized against Oxalic acid (0.1 N) using Phenolphthalein as indicator.

3. 0.5 N HCl, added HCl (50 ml) in distilled water to make 1 litre and standardized against standard sodium hydroxide using phenolphthalein as indicator.

4. 0.5 N Alcoholic Potassium hydroxide, dissolved KOH (28.4 g) in minimum amount of distilled water added ethanol (95%) to make the volume 1 litre and standardized against standard HCl using phenolphthalein indicator.
5. Neutral Ethanol, ethanol neutralized immediately before the use, by titration with Sodium hydroxide (0.1 N) using phenolphthalein indicator.

6. 1 % phenolphthalein, dissolved phenolphthalein (5 g) in 500 ml ethanol (95 %).

7. Anhydrous sodium sulphate (BDH), heated anhydrous sodium sulphate in oven at 102°C to remove the last traces of moisture then cooled in desiccator before use as drying agent.

8. 0.2 % 2,7-dichlorofluorescein in methanol was prepared to use as fluorescent dye in TLC.


10. Acetic anhydride (Riedel).

11. Keisel gel G (E.MERCK), as adsorbent in thin layer chromatography.

12. Silica gel 60 (E.MERCK), heated at 104°C in oven for 1 hour to activate and use as an adsorbent in column chromatography.
4.1.5: ADSORBENT COLUMN AND THIN LAYER

CHROMATOGRAPHY PLATES \(^{144,145}\)

Adsorbent column: A plug of wool or cotton was placed in the bottom of a glass column provided with means of regulating the flow of eluent through the column. The plug was pressed and leveled with glass rod, in order to provide a square base for the adsorbent column. The column was rinsed with least polar solvent and fixed with stand in vertical position to produce a uniform packing. The adsorbent was slurried with the solvent of least polarity and added rapidly to the empty column. A loose plug of cotton was inserted to protect the top of the column from being disturbed by the solvent addition during the elution process.

0.25 mm/0.5 mm thin layer chromatography plates: Silica gel (60 g) was added to distilled water (120 mL) and shaked vigorously in a flask to make a homogenous slurry. Applied the slurry with the help of an applicator to make 0.5 mm thickness silica gel coat on 5 glass plates (20 cm X 20 cm). Similarly for 0.25 mm thickness plates, silica gel (30 gm), distilled water (60 mL) was used. The plates were dried in the open and activated at 105\(^{\circ}\)C in an oven for 1 hour.

0.5 mm Silver nitrate (10 %) impregnated silica gel coated plates: Silica gel (60 g) was added to silver nitrate solution (120 mL) containing (6 g) silver nitrate in distilled water and shaked vigorously to make a homogenous slurry. Applied the slurry with the help of an applicator to make 0.5 mm thickness silica gel coat on 5 glass plates (20 cm X 20 cm). The
plates were dried in the open but in darkness and activated at 105°C in an oven for 1 hour.

4.2: EXTRACTION OF ESSENTIAL OILS

A weighed amount of each plant material was crushed in pestle and mortar, in order to rupture the maximum cell walls of oil glands and then immediately charged to a flask fitted with water cooled condenser. The steam was produced in a separate steam generator and passed through the material employing a glass tube with holes made on it, to ensure uniform circulation of steam through the material. The steam carried along with volatile oil from the plant materials, got condensed in the water cooled glass condenser. The condensate was collected in a glass receiver, kept in the ice-cooled water in order to prevent evaporation of low boiling constituents of essential oils. The distillation was continued until no further droplets were noticed in the distillate. The distillate was kept in the receiver for a few minutes, so as to allow maximum amount of essential oil to come at the top in the form of an oily layer. The essential oil was separated from the aqueous layer with the help of a separatory funnel. The aqueous layer was saturated with sodium chloride and further extracted with diethyl ether in order to recover partially miscible oxygenated constituents of the oil. The ethereal extracts were dried over anhydrous sodium sulphate. The solvent was carefully removed under the reduced pressure using rotary vacuum evaporator. The yields of the essential oils were recorded after combining these two fractions in each oil. The oils were
packed in amber coloured glass sample bottles for further investigations. The percent yields of the essential oils are given in Table-3.

TABLE - 3

TIME OF DISTILLATION AND PERCENTAGE YIELDS OF THE ESSENTIAL OILS FROM PLANT SPECIES UNDER INVESTIGATION.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>TIME OF DISTILLATION (hr.)</th>
<th>% AGE YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaligeria aitchisonii</td>
<td>8</td>
<td>0.12</td>
</tr>
<tr>
<td>Foeniculum vulgare</td>
<td>12</td>
<td>1.35</td>
</tr>
<tr>
<td>Pleurospermum stylosum</td>
<td>7</td>
<td>0.08</td>
</tr>
<tr>
<td>Psammogeton stocksii</td>
<td>9</td>
<td>0.069</td>
</tr>
</tbody>
</table>

4.3: DETERMINATION OF PHYSICO-CHEMICAL CONSTANTS

4.3.1: SPECIFIC GRAVITY

The specific gravities of the essential oils were determined with specific gravity bottle.

The specific gravity bottle (.5 mL) was first cleaned, dried and weighed along with the stopper. The bottle was filled with distilled water, recorded the temperature of the water. The stopper of the bottle was carefully inserted, so that no air
bubble was enclosed. The excess amount of the water which forced out through capillary tube was wiped with tissue paper, so that no water remained sticking to it and the bottle filled with water was weighed. The bottle was then emptied, again dried and filled with the essential oil. Recorded the temperature of the essential oil. The stopper was then inserted carefully. The oil which forced out through capillary tube was wiped and finally weighed. The specific gravities of essential oils were then calculated as:

\[
\text{Room temperature} = T
\]

\[
\text{Weight of empty specific gravity bottle} = A
\]

\[
\text{Weight of empty specific gravity bottle and water} = B
\]

\[
\text{Weight of the water} = B - A = X
\]

\[
\text{Weight of the specific gravity bottle + essential oil} = C
\]

\[
\text{Weight of the essential oil} = C - A = Y
\]

Specific gravity of the essential oil \[= \frac{Y}{X}\]

The specific gravities of the essential oils are given in Table-4.

4.3.2: REFRACTIVE INDEX

Abbe's Refractometer was used for the determination of refractive indices of the essential oils. The refractometer was standardized with distilled water which has a refractive index 1.3330 at 20°. A drop of the essential oil of each specie under investigation was placed between the prisms of the refractometer.
The telescope was rotated to bring the border line of the total refraction to the junction of cross wires in the telescope. The refractive indices of the essential oils were observed up to the 4th decimal place from the scale appeared on the instrument and recorded the room temperature. The refractive indices of the essential oils are given in Table-4.

4.3.3: ACID NUMBER

Weighed out the essential oil (1 gram) with accuracy in a titration flask and dissolved it in neutral ethanol (25 mL). Titrated the solution with standard potassium hydroxide solution (0.1 N) using phenolphthalein as indicator. The titration was continued until faint pink colour persisted. The difference in the initial and final readings of potassium hydroxide in the burette gave the volume of potassium hydroxide (0.1 N) required for the neutralization of free acids present in 1 gram of the essential oil. The acid values were calculated as:

\[
\text{Weight of empty titration flask} = A
\]
\[
\text{Weight of empty titration flask + essential oil} = B
\]
\[
\text{Weight of the essential oil} = B - A = X
\]
\[
\text{Amount of neutral ethanol added} = 25 \text{ mL}
\]
\[
\text{Normality of potassium hydroxide} = 0.1 \text{ N}
\]
\[
\text{Volume of potassium hydroxide used} = Y
\]

\[
\text{Acid number} = \frac{\text{Eq.Wt. of KOH} \times \text{Normality of KOH} \times \text{Vol.of KOH used}}{\text{Wt. of the essential oil in grams}}
\]

Acid number of the essential oils are given in Table-4.
Weighed out the essential oil (1 gram) with accuracy in alkali resistant saponification flask (100 mL). Added 95% pure neutral alcohol (5 mL) and 3 drops of phenolphthalein indicator (1%) and neutralized the free acids with standard aqueous potassium hydroxide solution (0.1 N). Then added alcoholic potassium hydroxide solution (0.5 N; 10 mL) measured accurately from the burette. An equal amount of alcoholic potassium hydroxide (0.5 N) was taken into an empty alkali resistant saponification flask (100 mL) for blank experiment. These flasks were fitted with water cooled condensers. Reﬂuxed the contents of the flasks on water bath and as soon as the ethanol started boiling. Occasionally shaken the ﬂasks with swirling motion. Boiled the solution for 30 minutes. After the oil was completely dissolved. Removed and permitted to cool at room temperature for 15 minutes. Titrated the excess alkali with standard hydrochloric acid (0.5 N) using phenolphthalein as indicator, disappearance of pink colour indicated the end point.

\[
\begin{align*}
\text{Wt of the oil} & = A \\
\text{Volume of alcoholic KOH (0.5N) used in the test} & = 10 \text{ mL} \\
\text{Volume of alcoholic KOH (0.5N) used in the blank} & = 10 \text{ mL} \\
\text{Normality of HCl} & = 0.5 \\
\text{Volume of HCl used in the blank} & = 10 \text{ mL} \\
\text{Volume of HCl used in the test} & = X \text{ mL} \\
\text{Volume of alcoholic KOH (0.5 N) used in the saponification} & = 10-X \\
\text{Ester number} & = \frac{\text{Eq.Wt. of KOH X Normality of KOH X volume of KOH used}}{\text{Wt. of the Essential oil in grams}} \\
\end{align*}
\]

The ester numbers of the essential oils are given in table-4.
4.3.5: ESTER NUMBER AFTER ACETYLATION\textsuperscript{146,147}

Introduced the under investigated essential oil (4 mL) into acetylation flask (100 mL), added acetic anhydride (4 mL) and anhydrous sodium acetate (0.8 g). Boiled the mixture gently for exactly 1 hour, cooled, disconnected the flask from the condenser, transferred the mixture to a small separatory funnel, rinsed the acetylation flask with three successive portion of warm water (5 mL) and added the rinsings to separatory funnel. When the liquids had completely separated, rejected the aqueous layer and washed the remaining oil with successive portions of sodium carbonate saturated solution until the washings were alkaline to litmus. The washed oil was then dried over anhydrous sodium sulphate and filtered.

Transferred the dry acetylated oil (3 mL) to a tared saponification flask (100 mL), noted the exact weight, added alcoholic potassium hydroxide (0.5 N; 25 mL), connected the flask with reflux condenser and boiled the mixture on a water bath for exactly 1 hour.

Allowed the mixture to cool, added 10 drops phenolphthalein and titrated the excess alkali with \(H_2SO_4\textsuperscript{4/8M}(0.5 N)\). Performed a blank determination with the same quantities of the same reagents and in the same manner to calculate the ester number after acetylation.

\[
\text{Weight of acetylated oil in the saponification flask} = X \text{ g}
\]

\[
\text{Vol. of alcoholic KOH (0.5 N) added in the test} = 25 \text{ mL}
\]

\[
\text{Vol. of alcoholic KOH (0.5 N) added in the blank} = 25 \text{ mL}
\]
Normality of HCl accurately standardized = 0.5
Volume of HCl used in the blank = A
Volume of HCl used in the test = B
Volume of KOH (0.5N) used for the saponification of acetylated essential oil = A-B

\[ \text{Eq. Wt. of KOH (A-B)} \times \frac{1}{2} \]

Ester number after acetylation = \-----------------------\[ \frac{\text{Wt. of the acetylated E. oil in grams}}{\text{Wt. of the acetylated E. oil in grams}} \]

Ester numbers after acetylation of the essential oils of the species are given in table-4:

**TABLE-4**

**PHYSICO CHEMICAL CONSTANTS OF THE ESSENTIAL OILS OF THE SPECIES UNDER INVESTIGATION**

<table>
<thead>
<tr>
<th>CONSTANT</th>
<th>SCALIGERIA AITCHISONII</th>
<th>FOeniculum vulgare</th>
<th>PLEUROSPERMUM STYLOSUM</th>
<th>PSammoGETON STOCKSII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>26* 0.86</td>
<td>25* 0.95</td>
<td>30* 0.86</td>
<td>31* 0.87</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>25* 1.4741</td>
<td>24* 1.5239</td>
<td>30* 1.4614</td>
<td>31* 1.4639</td>
</tr>
<tr>
<td>Acid number</td>
<td>0.56</td>
<td>1.25</td>
<td>0.46</td>
<td>0.72</td>
</tr>
<tr>
<td>Ester number</td>
<td>20.15</td>
<td>3.25</td>
<td>0.75</td>
<td>31.25</td>
</tr>
<tr>
<td>Ester number after acetylation</td>
<td>57.5</td>
<td>12.27</td>
<td>55.07</td>
<td>60.59</td>
</tr>
</tbody>
</table>

* temperature
4.4: RESOLUTION OF THE ESSENTIAL OILS BY COLUMN AND THIN LAYER CHROMATOGRAPHY

The essential oils of the species were resolved into hydrocarbons and oxygenated fractions by column chromatography. The essential oil (10 g) was dissolved in normal hexane (100 ml) and carefully added to the top of the column (130 cm x 4.2 cm i.d.), uniformly packed with activated silica gel (350 g) slurried in n-hexane according to PAST0144. The column was eluted with n-hexane and a mixture of n-hexane with increasing proportions of diethyl ether as eluent. The eluted mixture was checked from time to time and elution with one solvent system was continued until decrease in the amount of eluted compound was noted with the help of a watch glass. The hydrocarbon fractions of the oils were eluted with n-hexane, while the oxygenated fractions with 1-25% diethyl ether in n-hexane. The last fractions of the oils were, however, eluted with a mixture of 5% ethanol in diethyl ether. Eighty to eighty five fractions, 60-70 ml each were collected for the essential oils of the under investigated species.

For the essential oil of Scaligeria aitchisonii, 80 fractions were collected in the following order:

Fractions: 1-51 (C₆H₁₄ 100%), 52-57 (C₆H₁₄--Et₂O, 98+2), 58-59 (C₆H₁₄--Et₂O, 95+5), 60(C₆H₁₄--Et₂O, 92+8), 61-63 (C₆H₁₄--Et₂O, 85+15) 64-75 (C₆H₁₄--Et₂O, 80+20) and fractions 76-80 (C₆H₁₄--Et₂O, 75+25),
For the essential oil of *Foeniculum vulgare* 84 fractions were collected in the order given as:

Fractions: 1-14 \((C_6H_{14} 100 \%)\), 15-68 \((C_6H_{14}--Et_2O, 98+2)\), 69-75 \((C_6H_{14}--Et_2O, 97+3)\), 76-77 \((C_6H_{14}--Et_2O, 96+4)\), 78 \((C_6H_{14}--Et_2O, 95+5)\), 79-80 \((C_6H_{14}--Et_2O, 92+8)\), 81 \((C_6H_{14}--Et_2O, 90+10)\), 82-83 \((C_6H_{14}--Et_2O, 75+25)\) and fraction 84 \((C_2H_5OH--Et_2O, 5+95)\).

For the essential oil of *Pleurospermum stylosum* 84 fractions were collected in the order as:

Fractions: 1-50 \((C_6H_{14} 100 \%)\), 51-60 \((C_6H_{14}--Et_2O, 98+2)\), 61-62 \((C_6H_{14}--Et_2O 97+3)\), 63-71 \((C_6H_{14}--Et_2O, 95+5)\), 72-82 \((C_6H_{14}--Et_2O, 80+20)\), and fractions 83-84 \((C_2H_5OH--Et_2O, 5+95)\).

For the essential oil of *Psammogeton stocksii* 83 fractions were collected in the following order:

Fractions: 1-52 \((C_6H_{14}, 100 \%)\), 53-62 \((C_6H_{14}--Et_2O, 97+3)\), 63 \((C_6H_{14}--Et_2O, 95+5)\), 64-65 \((C_6H_{14}--Et_2O, 90+10)\), 66-80 \((C_6H_{14}--Et_2O, 80+20)\), 81-83 \((C_2H_5OH--Et_2O, 5+95)\).

The fractions were checked on 0.25 mm thickness silica gel coated activated TLC plates prepared as instructed by Pasto\textsuperscript{144} and Stahl\textsuperscript{145}. The fractions were spotted with the help of a fine glass capillary tube on the activated plates. The plates were developed in eluent mixture contained in the tank. The developed plates were dried and visualised by either iodine vapours, or charring with sulphuric acid or by the use of ultraviolet light on fluorescein containing silica gel layer. The successive fractions showing identical spots on TLC plates were combined, thus giving 6 to 7 fractions of the essential oils of the under investigated species.
The fractions showing more than one spot on TLC plates were rechromatographed and purified on 0.5 mm thickness preparative layer chromatographic plates (PLC). The fractions were weighed and applied in the form of bands on the activated plates, with the help of a fine glass capillary tube. Thus 40 mg of the substance could be loaded on one plate. The plates were developed in a suitable eluent mixture of n-hexane and diethyl ether. But fractions number 4 of the essential oils of Pleurospermum stylosum and psammogetion stocksii were re-chromatographed and purified on silver nitrate (10%) impregnated silica gel coated, activated plates. The plates were developed in an eluent system; methylene dichloride-chloroform-ethyl acetate-n-propanol (45 + 45 + 4.5 + 4.5)\(^ {145} \). The developed plates were dried, sprayed with 2,7-dichlorofluorescein solution (0.2%) used as locating reagent to make the bands visible under uv light of 366 nm. The similar bands after scraping, were transferred to small conical flasks and extracted with diethyl ether, which after filtration and distillation under reduced pressure with rotary vacuum evaporator, gave the weight of the components. The components purified from successive fractions having identical Rf values were combined and weighed which gave the weight of the resultant final purified fractions of the essential oils.

**SCALIGERIA AITCHISONII**

From the column chromatography of the essential oil of Scaligeria aitchisonii:

Fractions: 1-51, which showed a single spot on TLC, were com-
bined. The removal of the solvent by distillation under reduced pressure gave the first fraction (6.548 g). It was found to be a mixture of terpene hydrocarbons by GLC.

Fractions 52-57 were found a mixture of two compounds by TLC and were combined. The removal of the solvent by distillation under reduced pressure gave the compound mixture (0.783 g) which was eventually separated into individual components by preparative layer chromatography (PLC) using eluent system, n-hexane-diethyl ether (90:10). The major component (0.405 g) was the 2nd fraction of the essential oil. The minor fraction (0.378 g) having Rf value identical to 1st fraction, was added to the 1st fraction of the essential oil.

Fractions 58-59, a mixture of two components by TLC were combined. After the removal of the solvent by distillation gave compound mixture (0.123 g). It was separated by PLC using solvent system, n-hexane-diethyl ether (85:15). The major component, (0.079 g) was added to the 2nd fraction due to identical Rf value. The minor component (0.043 g) was the 3rd fraction of the essential oil.

Fraction 60, which showed a single spot on TLC, yielded a compound (0.080 g), after the removal of the solvent by distillation. It was added to 3rd fraction of the oil because of identical Rf value.

Fractions 61-63, showing single spot on TLC were com-
bined. After the removal of the solvent by distillation gave the fourth fraction (0.147 g) of the essential oil.

Fractions 64-73, being a mixture of two components on TLC were combined and gave compound mixture (1.337 g) after the removal of the solvent by distillation. It was separated on PLC using eluent system n-hexane-diethyl ether (80+20) into individual components. The major component (1.246 g) was the 5th fraction of the essential oil while the minor component (0.090 g) having identical Rf value was added to the 4th fraction.

Fractions 74-75, showing single spot on TLC were combined. The removal of the solvent by distillation under reduced pressure gave a single compound (0.027 g). It was added to the 5th fraction due to identical Rf value.

Fractions 76-80 which showed a single spot on TLC were combined. The removal of the solvent by distillation gave the sixth fraction (0.374 g) of the essential oil.

FOeniculum vulgare

From the column chromatography of the essential oil of Foeniculum vulgare:
Fractions 1-14, which showed a single spot on TLC, were combined. The removal of the solvent by distillation under reduced pressure, using rotary vacuum evaporator gave the first fraction (1.406 g). GLC revealed it to be a mixture of hydrocarbons.
Fractions 15-68, a mixture of two compounds by TLC, were combined. The removal of the solvent by distillation under reduced pressure gave compound mixture (6.87 g). It was resolved into individual components by PLC, using eluent system, n-hexane-diethyl ether (90+10). The major component (6.728 g) was the 2nd fraction of the essential oil while the minor component (0.142 g) was added to the 1st fraction due to its identical Rf value.

Fractions 69-74, showing a single spot on TLC were combined. The distillation of the solvent under reduced pressure gave the third fraction (0.829 g) of the essential oil.

Fractions 75-77, showing a single spot on TLC, were combined and distillation of the solvent under reduced pressure gave the fourth fraction (0.069 g) of the oil.

Fractions 78-80, mixture of two components on TLC, were combined and distillation of the solvent under reduced pressure obtained a compound mixture (0.213 g). It was resolved by PLC using solvent system, n-hexane-diethyl ether (82+18) into individual components. The major component (0.198 g) was the 5th fraction of the oil while the minor component (0.015 g) was added to the 4th fraction of the oil due to its identical Rf value.

Fraction 81, a mixture of two compounds on TLC was distilled under reduced pressure to remove the solvent and obtained a compound mixture (0.040 g). It was resolved into individual components on PLC using eluent system, n-hexane-diethyl
ether (75+25). The major component, (0.025 g) was added to 5th fraction of the essential oil due to its identical Rf value while the minor component (0.014 g) was the 6th fraction of the essential oil.

Fractions 82-83, showing a single spot on TLC were combined. The distillation of the solvent under reduced pressure yielded a compound (0.110 g). It was added to 6th fraction of the essential oil of Foeniculum vulgare due to its identical Rf value.

PLEUROSPERMUM STYLOSUM

From the column chromatography of the essential oil of Pleurospermum stylosum:
Fractions 1-50, showed a single spot on TLC. These were combined and distilled under reduced pressure to remove the solvent and obtained the first fraction (6.087 g) of the essential oil. GLC revealed it to be a mixture of hydrocarbons.

Fractions 51-60, a mixture of two components on TLC, were combined. The distillation of the solvent under reduced pressure yielded a compound mixture (1.069 g). It was eventually separated into individual components by PLC using solvent system n-hexane-diethyl ether (90+10). The major compound (1.00 g) was the 2nd fraction of the essential oil, while the minor component (0.068 g) was added to 1st fraction as it had Rf value identical to the 1st fraction of the oil.
Fractions 61-62, showed three spots on TLC. They were combined and distilled under reduced pressure to remove the solvent and obtained compound mixture (0.257 g). It was separated by PLC using eluent system n-hexane-diethyl ether (90+10) into three components. The major component (0.153 g) was the 3rd fraction, the minor component (0.034 g) was added to the 2nd fraction, while the intermediate component (0.069 g) due to its identical Rf value was added to the 1st fraction of the oil.

Fractions 63-71, showing a single spot on TLC, were combined. The distillation of the solvent under reduced pressure gave a single compound (0.821 g). It was added to the 3rd fraction of the essential oil on the basis of identical Rf value.

Fractions 72-82, showing a single spot on TLC were combined and distilled under reduced pressure to remove the solvent and obtained compound (1.225 g). GLC revealed it to be a mixture of three components. These were separated into individual components on AgNO₃ (10 %) impregnated silica gel coated activated plates, using eluent system methylene dichloride-chloroform-methyl acetate-n-propanol (45 + 45 + 4.5 + 4.5)¹⁴⁵. A good separation was accomplished with a run of 15 cm. But it was improved further by following up the double development using methylene dichloride-chloroform (40 + 60). The developed plates were dried, sprayed with 2,7-dichlorofluorescein solution (0.2 %) to make the bands visible under uv light of 266 nm. The similar bands after scraping, were transferred to small conical flasks and extracted with diethyl ether. Which after filtration and distillation under
reduced pressure gave three components. The first component (0.285 g) gave fine crystals and formed the fourth fraction. The second component was the fifth fraction (0.772 g), while the third component was the sixth fraction (0.167 g) of the essential oil.

Fractions 83-84, showing single spot on TLC, were combined. The distillation of the solvent under reduced pressure gave rise to crystalline material and formed the seventh fraction (0.016 g) of the essential oil of the pleurosperrnum stylosum. It was fluorescent under uv light 366 nm.

PSAMMOGETON STOCKSII

From the column chromatography of the essential oil of psammogeton stocksii:
Fractions 1-52 showing a single spot on TLC were combined. The distillation under reduced pressure removed the solvent and gave the first fraction (6.162 g) of the essential oil. It was found to be a mixture of hydrocarbons from GLC of the fraction.

Fractions 53-62, mixture of two components on TLC, were combined, distilled the solvent under reduced pressure and obtained a compound mixture (1.029 g). It was separated into individual components by PLC using eluent system, n-hexane-diethyl ether (90+10). The major component (0.730 g), was the 2nd fraction of the essential oil while the minor component (0.297 g), was added to 1st fraction because of its identical Rf value.
Fraction 63, showing a single spot on TLC was distilled to remove the solvent under reduced pressure and obtained a compound (0.020 g). It was added to the second fraction of the oil because of its identical Rf value.

Fractions 64-65, showing a single spot on TLC, were combined. The distillation of the solvent under reduced pressure gave rise to crystalline compound and formed the third fraction (0.404 g) of the essential oil.

Fractions 66-80, showing single spot on TLC, were combined, distilled under reduced pressure to remove the solvent and gave a compound (1.710 g), but GLC of the fraction revealed it to be a mixture of two compounds, the compounds were separated on AgNO₃ (10 %) impregnated silica gel coated activated plates using eluent system, methylene dichloride-chloroform-ethyl acetate-n-propanol (45 + 45 + 4.5 + 4.5)₁⁴⁵, the developed plates were sprayed with 2,7-dichlorofluorescein solution (0.2 %) to make the bands visible under uv light of 266 nm. The similar bands after scraping were transferred to small conical flasks and extracted with diethyl ether. Which after filtration and distillation under reduced pressure gave two components. The major component formed the fourth fraction (1.50 g) of the essential oil, while the minor component formed the fifth fraction (0.209g) of the oil.

Fractions 81-83, showing a single spot on TLC, fluorescent under uv light of 366 nm, were combined. The distillation
of the solvent under reduced pressure gave rise to a fine crystalline compound forming sixth fraction (0.103 g) of the essential oil.
4.5: RESOLUTION BY GLC AND GC/MASS SPECTRAL ANALYSIS OF THE FIRST FRACTION COMPONENTS OF THE ESSENTIAL OILS

The 1st fractions were subjected to GLC and GC/Mass spectral analysis.

Gas chromatographic analysis of 1st fractions in n-hexane were carried out on a pye-unicam-104 instrument equipped with a flame ionization detector, using a 25 m X 0.22 mm. I.d, SE-30 coated WCOT fused silica column. Hydrogen was used as a carrier gas with a flow velocity of 26 cms/sec. and split ratio 1:100. The column temperature programmed at 60° for 0 min. with 4°/min. rise to 210°, while detector and injector temperatures of 300° and 250° respectively were used. Various components were identified by their retention times. Percentage composition of individual components was calculated on the basis of peak area using SP-4100 (Spectra Physics) computing integrator.

GC/mass spectra of 1st fractions in n-hexane were determined on JEOL model JMS-A x 505 H Mass spectrometer combined with Hewlett 5890 Packard Gas Chromatograph. Samples were injected on a WCOT fused silica column, coated with SE-30 and Helium as a carrier gas, split ratio 1:100, EI positive mode, electric energy 70 ev, ionization current 300 uA, ionization source temperature 250°, interface temperature 230° column temperature programmed at 60° for 4 min. with a 6°/min. rise to 230°. Data acquisition and reprocessing were performed by JEOL JMA-DA 5500 system with MS-48 TK library search system.
The 1st fractions of the essential oils were resolved into peaks. The retention time and fragmentation pattern of mass spectrum of each peak of the chromatogramme in the order of increasing intensity are given in Table-5.

**TABLE-5**

RESOLUTION AND SPECTRAL ANALYSIS OF THE 1ST FRACTION COMPONENTS OF ESSENTIAL OILS

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<th>GC/MS</th>
<th>m/e (%)</th>
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**FOeniculum Vulgare**

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     121(3.21).

49  10.614  0.31  228  93(100.00), 136(21.78), 77(20.5),
     44(13.75), 65(6.85), 119(5.85),

54  11.93  10.45  251  68(100.00), 93(65.95), 136(35.98),
     121(26.25), 79(24.98), 107(21.75),
     53(17.5), 41(15.85).

58  13.307  0.26  282  93(100.00), 136(45.75), 121(38.5),
     77(30.25), 65(12.75), 106(9.5),
     53(8.98).

PLEUROSPERMUM STYLOSUM

12  7.006  0.243  167  93(100.00), 32(59.32), 77(29.77),
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14  7.44  0.772  174  93(100.00), 32(32.30), 77(22.40),
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18  9.146  0.862  202  93(100.00), 77(27.67), 136(19.45),
     41(15.25), 121(8.37), 69(8.97),
     53(5.28), 105(3.02).

19  9.483  20.089  208  93(100.00), 69(66.30), 41(55.81),
     79(43.57), 136(40.47), 53(14.73),
     121(35.27), 107(14.16).

21  9.918  1.228  211  93(100.00), 69(71.84), 41(63.22),
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**PSAMMOGETON STOCKSII**

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93(100.00), 69(65.5), 41(51.5), 79(15.1), 121(7.8), 53(7.7), 136(6.5), 32(42.5).
119(100.00), 134(26.25), 91(10.98), 77(6.01), 65(6.00), 41(4.89), 103(3.25).
68(100.00), 93(56.5), 136(31.7), 79(23.5), 121(22.7), 107(22.5), 53(11.5), 39(10.29).
93(100.00), 136(44.5), 121(33.55), 77(28.15), 43(14.95), 105(10.10), 65(6.15), 51(5.05).
121(100.00), 136(76.5), 93(75.95), 79(38.5), 39(24.18), 103(16.25), 53(10.25) 68(8.15).
4.5.1: SPECTRAL ANALYSIS OF THE OXYGENATED FRACTIONS OF THE ESSENTIAL OILS OF THE SPECIES

The oxygenated fractions were subjected to infrared (ir), ultraviolet (uv), nuclear magnetic resonance (¹H-nmr), gas chromatograph/mass spectral analysis.

Infrared spectra of the oxygenated fractions of the essential oils were recorded as neat thin films between rock salt plates or mulls in nujol on i.r.spectrometer (Hitachi 270-30).

Nuclear magnetic resonance (nmr) spectra of the oxygenated fractions of the essential oils were determined in deuterated chloroform (CDCl₃) with tetramethyasilane or chloroform as the internal standard on 90 & 100 MHz NMR spectrometer (JEOL-JNM-EX 90).

GC/Mass spectra of the oxygenated fractions in diethyl ether were determined on JEOL Model JMS-AX-505H Mass Spectrometer combined with Hewlett 5890 Packard Gas Chromatograph. Samples were injected on WCOT fused silica column, coated with SE-30 and Helium as carrier gas, split ratio 1:100, EI positive mode, electric energy 70 ev, ionization current 300 uA, ionization source temperature 250°, interface temperature 230° column temperature programmed at 60° for 4 min. with a 6°/min. rise to 230°. Data acquisition and reprocessing were performed by JEOL JMA-DA 5500 system with ms-49 TK library search system.
Ultraviolet spectra of the oxygenated fractions in ethanol were recorded on Beckman Model 36 uv spectrophotometer, chart rate 50 nm/min.

Abbreviations used in the dissertation stand for:

I.R.: All absorptions are given in cm$^{-1}$

- OOP: Out of plane bend vibrations,
- $b =$ broad, $w =$ weak, $m =$ medium,
- $s =$ strong

U.V.: All the absorptions are given in nanometres (nm) under the $\lambda_{\text{max}}$

NMR: All absorptions are given in parts per million (ppm) on $\delta$ scale

- $s =$ singlet, $d =$ doublet, $t =$ triplet, $m =$ multiplet, $q =$ quartet, Hz = Hertz.

SPECTRAL ANALYSIS OF OXYGENATED FRACTIONS OF THE ESSENTIAL OILS OF SPECIES

**TABLE - 6**

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<th>Fraction</th>
<th>% age</th>
<th>Characterization</th>
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<td>I.R.: (neat) (cm$^{-1}$) 3110-3020 w, 2985, 2935, 2875 s, 1740 s, 1650 w, 1460, 1370 m, 1260 s, 1035 m, 940 845 w, 838 w,</td>
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</tbody>
</table>
$^1$H-NMR: (CDCl$_3$) $\delta$ (ppm), 1.5 (s, 3H), 1.6 (s, 3H), 1.7 (s, 3H), 1.85 (m, 4H), 1.95 (s, 3H), 5.0 (complex, 1H, $J_1 = \sim 7$ Hz, $J_{allylic} = \sim 0$-1 Hz), 5.1 (dd, 1H, $J_{PC} = \sim 10$ Hz, $J_{ab} = \sim 1$-2 Hz), 5.2 (dd, 1H, $J_{AC} = \sim 16$ Hz, $J_{CB} = \sim 10$ Hz).

M.S.: m/e (%) 196 (1.21), 154 (2.72), 136 (33.62), 121 (54.33), 107 (16.47), 93 (100.00), 80 (57.88), 69 (51.20), 55 (21.05), 43 (57.92), 32 (2.28).

U.V.: (EtOH) (nm) $\lambda_{max} = 209$

I.R.: (neat) (cm$^{-1}$) 3090-3025 m, 2990-2910 m, 1980, 1775 overtones, 1730 s, 1655-1455 s, m, 1470, 1370 m, 1180 s, 1050 s, 980 s, 780, 690 s.

$^1$H-NMR: (CDCl$_3$) $\delta$ (ppm), 1.3 (t, 3H, $J = \sim 7$ Hz), 4.25 (q, 2H, $J = \sim 7$ Hz), 6.4 (d, 1H, $J = \sim 14$ Hz), 7.75 (d, 1H, $J = \sim 14$ Hz), 7.3-7.6 (complex, 5H, $J_1 = \sim 6$ Hz, $J_2 = \sim 1$-3 Hz, $J_3 = \sim 0$-1 Hz).

M.S.: m/e (%) 177 (53.44), 176 (99.88), 158 (24.66), 148 (100.48), 131 (99.87), 103 (99.95), 91 (20.24), 77 (99.9), 63 (10.91), 51 (55.64), 39 (6.35).

U.V.: (EtOH) (nm) $\lambda_{max} = 275, 215, 203$

I.R.: (neat) (cm$^{-1}$) 3090-3010 m, 2820, 2750 m, 1980, 1755 overtones, 1690 s, 1630 s, 1610-1455 s, m, 1090 m, 970 s, 755, 690 s.

$^1$H-NMR (CDCl$_3$) $\delta$ (ppm), 6.5 (dd, 1H, $J_1 = \sim 16$ Hz, $J_2 = \sim 7$ Hz), 7.35 (d, 1H, $J_1 = \sim 16$ Hz, $J_2 = \sim 0$-1 Hz), 7.25 (complex m, 5H), 9.66 (d, 1H).

M.S.: m/e (%) 133 (8.67), 132 (82.33), 131 (100.00), 103 (52.46), 91 (2.6), 77 (34.78), 51 (23.05), 39 (3.80).

U.V.: (EtOH) (nm) $\lambda_{max} = 287, 220, 205$. 

3
I.R.: (neat) (cm⁻¹) 3440 b, 3100-3000 w, 2980-2860 s, 1640 w, 1460 s, 1380 m, 1125 m, 1000-700 m, 840 w.

¹H-NMR: (CDCl₃) δ (ppm) 1.25(s, 3H), 1.5(s, 3H), 1.64(s, 3H), 1.48(dt, 2H, J₁ = ~ 7 Hz, J₂ = ~ 1-2 Hz, 1.72(s, 1H), 2.0(q, 2H, J = ~ 7 Hz), 5.0 (complex, 1H, J₁ = ~ 7 Hz) J₂ = ~ 0-1 Hz), 5.08 (d, 1H, J₁ = ~ 10 Hz, J₂ = ~ 0-1 Hz), 5.18(dd, 1H, J₁ = ~ 16 Hz, J₂ = ~ 1-2 Hz). 5.88(dd, 1H, J₁ = ~ 16 Hz, J₂ = ~ 10 Hz).

M.S.: m/e (%) 155(0.30), 154(2.70), 136(47.85), 121(66.62), 107(23.14), 93(100.00), 80(76.98), 71(92.51), 55(90.24), 41(88.07).

U.V.: (EtOH) (nm) λ_max 209.

63.57

I.R.: (neat) (cm⁻¹) 3500 b, 3095-3000 w, 2980-2850 m, 1870-1740 overtones, 1620-1430 s, 1470, 1360 m, 1270 s, 1240 s, 1020 s, 845-800 m, 960-700 w.

¹H-NMR (CDCl₃) δ (ppm) 1.95(d, 2H, J = ~ 7 Hz), 3.80(s, 3H), 5.72-6.5 (m, 3H) 6.8-6.94(m, 3H).

M.S.: m/e (%) 165(21.2), 164(100.00), 149(75.30), 131(55.58), 121(48.73), 103(69.19), 91(52.22), 77(65.90), 65(25.17), 55(41.22), 39(24.88).

**FOeniculum VULGARE**

67.28

I.R.: (neat) (cm⁻¹) 3100-3000 m, 2990-2850 m, 1890, 1770 overtones, 1670 w, 1620-1470 s, w, 1460, 1380 w, 1250 s, 975 s, 840 s.

¹H-NMR: (CDCl₃) δ (ppm) 1.8 (d, 3H, J = ~ 7Hz) 3.75(s, 3H), 6.09(dq, 1H, J₁ = ~ 16 Hz, J₂ = ~ 7 Hz), 6.2(bd, 1H, J₁ = ~ 16 Hz, J₂ = ~ 0.1 Hz), 6.84(dd, 2H, J₁ = ~ 8 Hz, J₂ = ~ 1 Hz), 7.28(dd, 2H, J₁ = ~ 8 Hz, J₂ = ~ 1 Hz).

M.S.: m/e (%) 149(11.00), 148(100.00), 133(22.3), 117(23.4), 105(16.2), 91(10.05), 77(12.5), 63(4.80), 51(5.95), 39(3.50).
U.V.: (EtOH) (nm) $\lambda_{max}$ 260, 209

8.29

I.R.: (neat) (cm$^{-1}$) 2995-2895 s, 1755 s, 1470, 1385 m,

$^1$H-NMR: (CDCl$_3$) $\delta$ (ppm) 0.7-1 (complex 1H),
1.01 (s, 6H), 1.1 (s, 3H), 1.35-1.88 (complex, m, 5H),
2.2 (bs, 1H).

M.S.: m/e (%) 153(2.8), 152(24.64), 137(3.14),
109(8.17), 81(100.00), 69(46.64), 53(4.74),
41(19.95).

U.V.: (EtOH) (nm) $\lambda_{max}$ 260 w, 210

0.84

I.R.: (neat) (cm$^{-1}$) 3430 bs, 2995-2900 s,
1470, 1380 w, 1095 m,

$^1$H-NMR: (CDCl$_3$) $\delta$ (ppm) 0.85 (s, 3H), 1.00 (s, 3H),
1.1 (s, 3H), 1.25-1.72 (m, 8 H), 3.32 (b, 1H).

M.S.: m/e (%) 155(1.98), 154(17.64), 139(18.41),
136(18.40), 121(56.61), 111(66.59), 93(61.92),
81(100.00), 69(86.16), 55(52.25), 43(66.65).

U.V.: (EtOH) (nm) $\lambda_{max}$ 209

2.23

I.R.: (neat) (cm$^{-1}$) 3090-3010 w, 2980-2950 m,
2850-2740 m, 2010, 1910 overtones, 1695 s,
1600-1480 sm, 1440, 1390 w, 1255 s, 1190 s, 1020 s,
1155, 1390 sw, 830 s.

$^1$H-NMR (CDCl$_3$) $\delta$ (ppm) 3.82 (s, 3H), 6.84 (d, 2H,
$J_1 = \sim 8$ Hz, $J_2 = \sim 1$ Hz), 7.84 (d, 2H, $J_1 = \sim 8$ Hz,
$J_2 = \sim 0-1$ Hz), 9.8 (s, 1H).

M.S.: m/e (%) 137(6.52), 136(70.17), 135(100.00),
107(17.42), 92(14.51), 77(28.21), 64(9.28), 51(5.89),
39(6.67).

U.V.: (EtOH) (nm) $\lambda_{max}$ 280, 225, 208
I.R.: (neat) (cm$^{-1}$) 3440 b, 3100-3000 w, 2990-2870 s, 1665 w, 1470,1375 s, 1390-1375 m, 1190 s, 1000-700 m,

$^1$H-NMR: (CDCl$_3$) $\delta$(ppm), 1.19 (s, 6H), 1.35 (m, 4H), 1.62 (s, 3H), 1.98 (complex 3H), 2.2 (b, 1H), 5.3 (b, complex 1H).

M.S.: m/e (%) 136(69.99), 121(73.90), 107(11.06), 93(85.43), 81(44.69), 68(25.04), 59(100.00), 43(30.00).

U.V. (EtOH) (nm) $\lambda_{max}$ 209

PLEUROSPERMUM STYLOSUM

I.R. (neat) (cm$^{-1}$) 2990-2855 s, 1725 s, 1470, 1365 m, 1172 m,

$^1$H-NMR (CDCl$_3$) $\delta$(ppm), 0.85 (t, 3H, J = ~7 Hz.), 1.3 (complex, 4H), 1.55 (q, 2H, J = ~7 Hz), 2.15 (s, 3H), 2.4 (t, 2H, J = ~7 Hz).

M.S.: m/e (%) 115(6.5), 114(79.47), 99(28.57), 85(19.92), 71(92.04), 58(100.00), 43(99.61).

U.V.: (EtOH) (nm) $\lambda_{max}$ 270-202

I.R. (neat) (cm$^{-1}$) 3190-3010 w, 2990-2855 s, 2780, 2710 m, 1680 s, 1620 m, 1450, 1380 s, 1155 m, 980, 850 m, 845 s,

$^1$H-NMR: (CDCl$_3$) $\delta$(ppm) 1.6 (s, 3H), 1.7 (s, 3H), 2.16 (s, 3H), 2.26 (complex, 4H), 5.17 (b, 1H), 5.85 (d, 1H), 9.96 (s, 1H).

1.98 (s, 3H), 2.58 (t, 2H)

M.S.: m/e (%) 153(1.74), 152(14.90), 137(12.49), 123(11.70), 119(5.12), 109(13.27), 94(24.20), 84(37.56), 69(100.00), 54(9.84), 41(73.27),

M.S m/e (%) 153(0.50), 152(4.63), 137(9.17), 134(9.78), 119(16.41), 109(29.71), 94(38.86), 84(34.97), 69(100.00), 59(17.60), 41(83.17).
U.V.: (EtOH) (nm) \( \lambda_{\text{max}} \) 240.

I.R.: (nujol) (cm\(^{-1}\)) 3325 b, 2980-2890 s, 1470, 1380 s, m, 1120 w.

\(^1\)H-NMR: (CDCl\(_3\)) \( \delta \) (ppm), 0.85 (s, 9H), 1.1-1.4 (m, 6H), 1.7 (m, 2H), 4.0 (d, 1H),

M.S.: m/e (%) 155 (0.14), 154 (1.29), 139 (15.12), 121 (7.51), 110 (22.15), 95 (100.00), 81 (11.91), 67 (10.25), 55 (7.79), 41 (24.85), 32 (7.51).

U.V.: (EtOH) (nm) \( \lambda_{\text{max}} \) 210.

I.R.: (neat) (cm\(^{-1}\)) 3360 b, 3080-3010 w, 2990-2880 s, 1675 w, 1450, 1380 m, 1020 s, 838 w, 820 w,

\(^1\)H-NMR: (CDCl\(_3\)) \( \delta \) (ppm) 1.6 (s, 3H), 1.65 (s, 3H), 1.72 (s, 3H), 2.05 (s, 2H), 2.1 (s, 3H), 4.07 (d, 2H),
J \( \approx \) 7 Hz, 5.08 (b, 1H, J \( \approx \) 0.1 Hz), 5.4 (t, 1H, J \( \approx \) 7 Hz)

M.S.: m/e (%) 155 (5.57), 154 (46.60), 136 (67.82), 121 (80.27), 111 (44.48), 93 (99.69), 84 (89.94), 69 (99.51), 68 (100.00), 55 (41.25), 41 (99.73), 31 (6.54).

U.V.: (EtOH) (nm) \( \lambda_{\text{max}} \) 220.

I.R.: (neat) (cm\(^{-1}\)) 3440 b, 3090-3000 w, 2980-2960 s, 1638 w, 1460, 1370 m, 1125 m, 840 w, 1000-700 m,

\(^1\)H-NMR: (CDCl\(_3\)) \( \delta \) (ppm) 1.2 (s, 3H), 1.48 (d, t, 2H),
J \( \approx \) 7 Hz, J \( \approx \) 1-2 Hz, 1.5 (s, 3H), 1.6 (s, 3H), 1.72 (s, 1H), 2.0 (q, 2H, J \( \approx \) 7 Hz), 5.0 (t, 1H)
J \( \approx \) 7 Hz, J \( \approx \) 0-1 Hz), 5.08 (d, 1H)
J \( \approx \) 10 Hz, J \( \approx \) 0-1 Hz), 5.18 (dd, 1H)
J \( \approx \) 16 Hz, J \( \approx \) 1-2 Hz), 5.88 (dd, 1H)
J \( \approx \) 16 Hz, J \( \approx \) 10 Hz).

M.S.: m/e (%) 155 (0.28), 154 (2.70), 136 (45.27), 121 (63.24), 107 (20.19), 93 (100.00), 80 (73.16),
71 (89.28), 55 (87.92), 41 (85.07).
U.V.: (EtOH) (nm) $\lambda_{\text{max}}$ 209.

7. U.V.: (EtOH) (nm) $\lambda_{\text{max}}$ 298,250.

PSAMMOGETON STOCKSII:

2  7.50  I.R.: (neat) (cm$^{-1}$) 3090-3000 w, 2990-2860 s,
1740 s, 1680 w, 1460,1370 m, 1390,1375 bifurcate,
1240 s, 1025 m,960,840 w, 838 w.

$^1$H-NMR (CDCl$_3$) $\delta$(ppm) 1.57(s, 3H),
1.65(s, 6H), 2.04(s, 7H), 4.5(d, 2H, J = ~ 7 Hz),
5.01 (b,s, 1H, J$_1$ = ~ 7 Hz., J$_2$ = ~ 0-1 Hz).
5.3(t, 1H, J = ~ 7Hz.)

M.S.: m/e (%) 196(0.44), 136(27.23),121(26.06),
107(7.74), 93(51.99), 80(17.95), 69(100.00),
53(8.14), 41(46.18),32(3.10).

U.V. (EtOH) (nm) $\lambda_{\text{max}}$ 212

3  4.04  I.R.: (nujol) (cm$^{-1}$) 2980-2870 s, 1755 s, 1465,1380 w

M.S.: m/e (%) 153(4.98),152(44.95),137(6.22),
108(51.93), 95(100.00), 81(70.35), 69(35.59),
55(30.52),41(37.58),

4  15.00  I.R.: (neat) (cm$^{-1}$) 3455 b, 3100-3010 w, 2990-2870 s,
1660 w, 1460, 1380 s, 1390 m, 1180 s,1000-700 m.

$^1$H-NMR (CDCl$_3$) $\delta$(ppm) 1.16(s, 6H) 1.23(t, 4H,
J = ~ 7 Hz), 1.62(s, 3H), 1.95(complex 3H), 2.19(b,
1H)5.35(b, 1H).

M.S.: m/e (%) 136(64.23), 121(68.72),
107(8.34), 93(79.78), 81(39.98), 68(20.98),
59(100.00), 43(27.67).

U.V. (EtOH) (nm) $\lambda_{\text{max}}$ 209

5  2.09  I.R. (neat) (cm$^{-1}$) 3360 b, 3080-3010 w, 2990-2870 s,
1678 w, 1460,1385 m, 1010 s, 840 w.
\[^1\text{H}-\text{NMR} \text{ (CDCl}_3)\ \delta \text{(ppm)} 1.58 (s, 3H), 1.65 (s, 7H), 2.02 (s, 4H), 4.1 (d, 2H), 5.1 (b, 1H), 5.38 (t, 1H).\]

\text{M.S.: m/e} \% \ 155 (3.15), 154 (27.06), 136 (35.48), 121 (70.83), 111 (43.25), 93 (85.67), 84 (49.05), 69 (100.00), 55 (31.10), 41 (99.86), 31 (5.68).

\text{U.V. (EtOH) (nm)} \lambda_{\text{max}} 233, 213

\begin{array}{c}
6 & 1.03 \\
\end{array}

\text{M.S.: m/e} \% \ 217 (13.65), 216 (100.00), 201 (37.25), 188 (13.12), 173 (52.0), 157 (2.05), 145 (20.97), 131 (2.69), 108 (5.34), 89 (8.64), 63 (5.98), 51 (7.33), 40 (50.7)

\text{U.V. (EtOH) (nm)} \lambda_{\text{max}} 311, 267, 258, 241

\text{m.pt.: 186}^\circ
RESULTS AND DISCUSSIONS

5.1: IDENTIFICATION OF THE COMPOUNDS OF THE 1ST FRACTIONS OF ESSENTIAL OILS OF THE SPECIES

Identification was affected by comparison of retention times and fragmentation pattern of each peak of the chromatogramme.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>7.017</td>
<td>167</td>
<td>α-thujene</td>
<td>149</td>
<td>S. aitchisonii</td>
</tr>
<tr>
<td>12</td>
<td>7.006</td>
<td>167</td>
<td>-do-</td>
<td></td>
<td>P. stylosum</td>
</tr>
</tbody>
</table>

α-thujene

The base peak is formed by the loss of isopropyl group, attached to the qua-ternary C and weak bond is broken to give a fragment at m/e 93.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>7.346</td>
<td>174</td>
<td>α-pinene</td>
<td>150,151</td>
<td>S. aitchisonii</td>
</tr>
<tr>
<td>37</td>
<td>7.371</td>
<td>173</td>
<td>-do-</td>
<td></td>
<td>F. vulgare</td>
</tr>
<tr>
<td>14</td>
<td>7.344</td>
<td>174</td>
<td>-do-</td>
<td></td>
<td>P. stylosum</td>
</tr>
<tr>
<td>26</td>
<td>7.415</td>
<td>173</td>
<td>-do-</td>
<td></td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>
The relatively low abundance of molecular ion peak is consistent with the view that the molecular structure of the compound is rather strained or crowded; the base peak m/e = 93 corresponds to the loss of 43 mass units and relative abundance of the ion m/e = 41 is less than one quarter of the base peak. The failure to detect the isopropyl ion strengthens that the loss of 43 mass units is not an entity. Therefore, the groups elided may be obtained by the breaking of two tertiary bonds with the removal of or concomitant hydrogen migration. The occurrence of gem dimethyl group as a part of ring system is common feature of many terpenes.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>8.028</td>
<td>185</td>
<td>Camphene</td>
<td>151,152</td>
<td>F. Vulgare</td>
</tr>
<tr>
<td>30</td>
<td>8.046</td>
<td>186</td>
<td>-do-</td>
<td></td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>

The facile elimination of a methyl radical is consistent with the presence of gem dimethyl, in which the methyl-carbon
bonds are also allylic to the double bond; the removal of 29 mass units to yield m/e = 107 may arise from the fission at the tertiary centres and one of which is also allylic, with hydrogen migration in either direction; the base peak m/e = 93 may be accounted for the elision of dimethyl with hydrogen loss from the various ions; the possible fragmentation as it does at two tertiary centres with or without hydrogen migration, yields either of these ions,

The ions m/e 77 & 79 or not so readily explained. It is possible to arise from a process involving a random rearrangement but convincing evidence on this point is lacking. The phenomenon of high abundance of the ion m/e = 93, as examined and reported by Friedman and Wolf\textsuperscript{148} by making isotopic labelling of certain carbon atoms of camphene indicates that the ion is a cyclic structure either of the form I or II.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND IDENTIFIED</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>9.128</td>
<td>202</td>
<td>Sabinene</td>
<td>151,153</td>
<td>S.aitchisonii</td>
</tr>
<tr>
<td>44</td>
<td>9.121</td>
<td>201</td>
<td>-do-</td>
<td></td>
<td>F.vulgare</td>
</tr>
<tr>
<td>18</td>
<td>9.146</td>
<td>202</td>
<td>-do-</td>
<td></td>
<td>P.stylosum</td>
</tr>
<tr>
<td>34</td>
<td>9.146</td>
<td>202</td>
<td>-do-</td>
<td></td>
<td>P.stocksii</td>
</tr>
</tbody>
</table>
The molecular ion peak is relatively of low abundance due to rather strained molecular structure of the compound. The base peak m/e = 93 = M - 43 corresponding to the loss of 43 mass units can be formed in one step decomposition directing from the molecule at qua-ternery carbon atom, because of the complexity of the different structures involved, it is not advisable to make any detailed postulates as to how the various kinds of molecules decompose.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>9.353</td>
<td>206</td>
<td>α-phellandrene</td>
<td>151</td>
<td>S. aitchisonii</td>
</tr>
<tr>
<td>45</td>
<td>9.325</td>
<td>206</td>
<td>-do-</td>
<td></td>
<td>F. vulgare</td>
</tr>
<tr>
<td>19</td>
<td>9.483</td>
<td>208</td>
<td>-do-</td>
<td></td>
<td>P. stylosum</td>
</tr>
<tr>
<td>35</td>
<td>9.35</td>
<td>207</td>
<td>-do-</td>
<td></td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>

The spectrum is dominated by m/e = 93 = M - 43. A comparison with other peaks in the spectrum and with other spectra of this group indicates that this fragment is more easily
formed than from any other monocyclic hydrocarbon investigated. This can be explained by the presence of two conjugated double bonds in the ring and binding of the isopropyl group in the allylic position to one of the double bonds.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>9.902</td>
<td>211</td>
<td>β-pinene</td>
<td>151,154, 155</td>
<td>S. aitchisonii</td>
</tr>
<tr>
<td>46</td>
<td>9.889</td>
<td>211</td>
<td>-do-</td>
<td></td>
<td>F. vulgare</td>
</tr>
<tr>
<td>21</td>
<td>9.918</td>
<td>211</td>
<td>-do-</td>
<td></td>
<td>P. stylosum</td>
</tr>
<tr>
<td>37</td>
<td>9.92</td>
<td>211</td>
<td>-do-</td>
<td></td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>

β-pinene

The relatively low intensity of the molecular ion peak due to rather strained or crowded molecular structure of the compounds; the absence of isopropyl ion and formation of base peak m/e = 93 = M - 43 corresponding to the loss of 43 units, indicate that the loss of 43 mass units is not an entity and the groups elided may be obtained by the breaking of the two tertiary bonds with the removal or migration of hydrogen. A point of distinction between the two pinene isomers arises from the abundance of the ion m/e = 41 which for β-pinene is more than half of the base peak, while for α-pinene it is less than one quarter.
The occurrence of gem dimethyl group as a part of the ring is a common feature of many mono terpenes.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>10.614</td>
<td>228</td>
<td>β-phellandrene 151,155,156</td>
<td>F.vulgare</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>10.62</td>
<td>229</td>
<td>-do-</td>
<td></td>
<td>P.stylosum</td>
</tr>
</tbody>
</table>

\[ \beta - \text{phellandrene} \]

The spectra is dominated by m/e = 93 = M - 43. This fragment is more easily formed from the phellandrene than any other mono cyclic hydrocarbon. This can be explained by the presence of two conjugated double bonds and the binding isopropyl group in the allylic position to one of the double bonds in the molecule. The difference between the spectra of α & β-phellandrene is only due to the presence of diffused peaks in β-phellandrene arising from the decompositions 136\(^+\)→94\(^+\) + 42, 94→79\(^+\) + 15 but not for α-phellandrene.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10.942</td>
<td>235</td>
<td>(\Delta^3)-Carene 151,155,157</td>
<td>S.aitchisonii</td>
<td></td>
</tr>
</tbody>
</table>
$\Delta^3$-carene

The relatively low abundance of molecular ion peak is due to strained molecule. The base peak corresponds to the loss of 43 mass units. The loss of 43 due to breaking of two tertiary bonds with the hydrogen migration.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>11.245</td>
<td>240</td>
<td>$\alpha$-terpinene 151,158</td>
<td>S.aitchisonii</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>11.218</td>
<td>239</td>
<td>-do-</td>
<td>P.stylosum</td>
<td></td>
</tr>
</tbody>
</table>

$\alpha$-terpinene

The parent molecule ion is of a reasonable abundance. The ring system is also strengthened by the distribution of the double bond. A methyl of the gem-dimethyl group may be readily fragmented as the carbon-methyl bonds are allylic to the 3,4 double bonds in the ring. A possible reaction is the ring opening process arising from the fission of the doubly allylic carbon carbon bonds. Thus for $\alpha$-terpinene open ion structures of m/e = 121 and 93 may be obtained by fission at allylic bonds or at qua-ternary centres.
<table>
<thead>
<tr>
<th>GC PEAK</th>
<th>RETENTION</th>
<th>GC/MASS</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO.</td>
<td>TIME</td>
<td>SCAN NO.</td>
<td>IDENTIFIED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>11.85</td>
<td>250</td>
<td>P-cymene</td>
<td>151,155,159</td>
<td>S. aitchisonii</td>
</tr>
<tr>
<td>26</td>
<td>11.64</td>
<td>247</td>
<td>-do-</td>
<td></td>
<td>P. stylorum</td>
</tr>
<tr>
<td>42</td>
<td>11.65</td>
<td>247</td>
<td>-do-</td>
<td></td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>

\[
\text{P-cymene}
\]

Being an aromatic hydrocarbon, gives large peaks at 
\(m/e = 134 = M\), \(m/e = 119 = M - 15\) (base peak) and \(m/e = 91 = M - 43\). But the peak \(m/e = 80\) is missing in the spectrum of P-cymene.

<table>
<thead>
<tr>
<th>GC PEAK</th>
<th>RETENTION</th>
<th>GC/MASS</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO.</td>
<td>TIME</td>
<td>SCAN NO.</td>
<td>IDENTIFIED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>11.93</td>
<td>251</td>
<td>d-limonene</td>
<td>151,155,160</td>
<td>F. vulgare</td>
</tr>
<tr>
<td>27</td>
<td>11.847</td>
<td>251</td>
<td>-do-</td>
<td></td>
<td>P. stylorum</td>
</tr>
<tr>
<td>43</td>
<td>11.874</td>
<td>252</td>
<td>-do-</td>
<td></td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>

\[
d\text{-limonene}
\]
The spectrum has peaks at m/e = 136 = M, m/e = 121 = M - 15, m/e = 107 = M - 29 & m/e = 93 = M - 43. But the base peak for limonene is m/e = 68 = 1/2 M corresponding to one isoprene unit. It is possibly due to retero Diel's Alder reaction.

<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND IDENTIFIED</th>
<th>REFERENCE SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>13.611</td>
<td>287</td>
<td>γ-terpinene 151,155,161</td>
<td>S. aitchisonii</td>
</tr>
<tr>
<td>58</td>
<td>13.307</td>
<td>282</td>
<td>-do-</td>
<td>F. vulgare</td>
</tr>
<tr>
<td>30</td>
<td>13.601</td>
<td>285</td>
<td>-do-</td>
<td>P. stylosum</td>
</tr>
<tr>
<td>48</td>
<td>13.468</td>
<td>283</td>
<td>-do-</td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>

γ-terpinene

It has a strong peaks at m/e = 136 = M, m/e = 121 = M - 15, m/e = 93 = M - 43 base peak. γ-terpinene has the same strong peaks but of greater intensities. The base peak m/e = 93 is formed not only directly from the molecule ion but also from m/e ≤ M - 15 = 121. m/e = 121 is formed due to the fragmentation not of allylic methyl group. The intensity of m/e = 121 is as great as that of α-terpinene due to non conjugated double bonds in the molecule.
<table>
<thead>
<tr>
<th>GC PEAK NO.</th>
<th>RETENTION TIME</th>
<th>GC/MASS SCAN NO.</th>
<th>COMPOUND</th>
<th>REFERENCE</th>
<th>SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>14.795</td>
<td>319</td>
<td>terpinolene</td>
<td>155,162</td>
<td>S. aitchisonii</td>
</tr>
<tr>
<td>49</td>
<td>14.795</td>
<td>319</td>
<td>-do-</td>
<td></td>
<td>P. stocksii</td>
</tr>
</tbody>
</table>

The parent molecular ion peak is most abundant because the double bonds are so distributed as to strengthen the otherwise weak bond system associated with the gem di-methyl group attached to the cyclo-hexane ring. There are no doubly allylic bonds, and most probable form of dissociation in this instance is by rupture of a bond at quaternary centre.
5.2: IDENTIFICATION OF OXYGENATED FRACTIONS OF THE ESSENTIAL OILS OF THE SPECIES

Identifications were made from spectral investigations of the oxygenated fractions isolated from the essential oils.

SCALIGERIA AITCHISONII

FRACTION NO. 2:

IR: (neat) (cm⁻¹)

3110, 3020 W olefinic C-H stretch

2985, 2935, 2875 S aliphatic C-H stretch

1740 S ester C=O stretch

1650 W olefinic C=C stretching vibrations

1460, 1370 M aliphatic C-H in plane bend

1260 S ester C-O asymmetric stretch

1035 M ester C-O symmetric stretch

940, 845 W olefinic C-H (OOP)bending vibrations

838 W Olefinic C-H (OOP)bend corresponding to isopropylidene group

¹H-NMR (CDCl₃) δ (PPM)

1.5 S 3H, \( \text{CH}_3 \) \( \text{C}-\text{O}-\text{C}-\text{CH}_3 \) down field shift is due to electron withdrawing effect of C-O-C-CH₃ group.

1.6 S 3H, \( \text{H}_3\text{C}>\text{C}-\text{C}-\text{C}-\text{H}_3 \) the peaks due to two methyl groups attached to the same C atom indicate that the two groups experience different anisotropic effect of -C=C-
1.85 m 4H, \( \text{C}=\text{CH-CH}_2-\text{CH}_2-\text{C-0-C-CH}_3 \)

1.95 s, 3H, \(-\text{O-C-CH}_3\) it is slightly overlapped with the adjacent peak.

5.0 complex 1H, \( \begin{array}{c} \text{H}_3\text{C} \\ \text{H}_3\text{C} \end{array} \text{C}=\text{CH-CH}_2 \)

\( J = \sim 7 \text{ Hz}, \quad J_\text{allylic} = \sim 0-1 \text{ Hz.} \)

5.1 dd 1H, \( \begin{array}{c} \text{R}\text{C=C}< \text{H}_a \\ \text{H}_c \text{C}\text{=C}< \text{H}_b \end{array} \) due to \( H_b \) proton.

\( J_{bc} = \sim 10 \text{ Hz.} \)
\( J_{ab} = \sim 1-2 \text{ Hz.} \)

5.2 dd 1H, \( \begin{array}{c} \text{R}\text{C=C}< \text{H}_a \\ \text{H}_c \text{C}\text{=C}< \text{H}_b \end{array} \) due to \( H_a \) proton.

\( J_{ac} = \sim 16 \text{ Hz.} \)
\( J_{ab} = \sim 1-2 \text{ Hz.} \)

5.9 dd 1H, \( \begin{array}{c} \text{R}\text{C=C}< \text{H}_a \\ \text{H}_c \text{C}\text{=C}< \text{H}_b \end{array} \) due to \( H_c \) proton.

\( J_{ac} = \sim 16 \text{ Hz.} \)
\( J_{cb} = \sim 10 \text{ Hz.} \)

**MS:**

<table>
<thead>
<tr>
<th>M/e</th>
<th>%</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>196</td>
<td>1.21</td>
<td>molecular ion peak, the even m/e indicates no or an even number of N atoms. The peak is very small.</td>
</tr>
<tr>
<td>154</td>
<td>2.72</td>
<td>formation of 154(^+) is due to some rearrangement.</td>
</tr>
<tr>
<td>136</td>
<td>33.62</td>
<td>loss of CH(_3)COOH formed due to the transfer of H from ( \gamma )-carbon to carbonyl group by ( E_1 ) rearrangement and formation of alkene RCH=CH(_2).</td>
</tr>
<tr>
<td>121</td>
<td>54.33</td>
<td>loss of branched CH(_3) group from the m/e 136.</td>
</tr>
<tr>
<td>107</td>
<td>16.47</td>
<td>loss of CH(_3)-CH(_2) from the m/e 136.</td>
</tr>
<tr>
<td>93</td>
<td>100.00</td>
<td>loss of ( \begin{array}{c} \text{H}_3\text{C} \ \text{H}_3\text{C} \end{array} \text{CH} ) ion from the m/e 136.</td>
</tr>
</tbody>
</table>
formation of $80^+$ is due to some rearrangement.

formation of $\text{H}_3\text{C} \overset{\text{H}_3\text{C} > \text{C}=\text{CH} - \text{CH}_2}{\text{H}_3\text{C}}$, $69^+$ due to allylic fragmentation.

formation of $\text{H}_3\text{C} \overset{\text{H}_3\text{C} > \text{C}=\text{C}^+\text{H}}{\text{H}_3\text{C}}$ due to vinyl cleavage.

formation of $\text{H}_3\text{C} \overset{\text{H}_3\text{C} > \text{C}^+\text{H}}{\text{H}_3\text{C}}$ or $\text{CH}_3\text{C}=\text{C}$ ion.

formation of $32^+$

UV(EtOH) (nm)

$\lambda_{\text{max}} = 209$

No $\lambda_{\text{max}}$ above 220, the intense absorption at 209 nm is due to ethanol used as solvent.

The molecular ion peak of the mass spectrum indicates that there are probably no hetero atoms in the molecule other than O and that the compound has a molecular weight of 196 amu. The nmr spectrum shows the presence of 20 H atoms.

The i.r. indicates the presence of aliphatic, olefinic, ester and isopropylidene groups in the molecule. U.V. does not show any absorption above 220 nm while the chemical shifts, integration and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum determine that the fraction of the essential oil is 3,7-dimethyl-1,6-octadien-3-yl acetate, commonly known as linalyl acetate. Which is confirmed on the basis of reported ir164, nmr165 and mass166 spectral data.

\[ \text{H}_3\text{C} \overset{\text{O} - \text{C} - \text{CH}_3}{\text{H}_3\text{C}} \]

Linalyl Acetate
FRACTION NO. 3:

IR (neat) (cm⁻¹)
3090, 3025, M aromatic and olefinic C-H stretching vibrations over lapped.
3000
2990-2910 M aliphatic C-H stretch
1980-1755 overtones the pattern indicates mono substituted aromatic ring.

1730 S ester C=O stretch.
1655, 1580, S, M aromatic ring C=C skeletal in plane stretching vibrations. The presence of these bands indicates the aromatic ring.
1500, 1455.

1470, 1370 M aliphatic C-H in plane bend.
1180 S ester C-O asymmetric stretching vibrations.
1050 S ester C-O symmetric stretching vibrations.
980 S trans olefinic C-H (OOP) bend.
780, 690 S aromatic C-H/C-C (OOP) bending vibrations indicating mono substituted aromatic ring.

¹H-NMR: (CDCl₃) δ (ppm)

1.3 t, 3H , CH₃-CH₂-O-C=O protons on methyl carbon at a β-position from hetero atom typically absorb at δ 1.3 J = ~ 7 Hz due to free rotation.

4.25 q, 2H CH₃-CH₂-O-C=O protons of methylene group adjacent to O typically absorb at δ 4.25. The up field triplet and down field quartet indicate the presence of ethyl group in the molecule.

6.4 d, 1H, J= ~ 14 Hz due to Hₐ proton
7.75 d, 1H, J = ~ 14 Hz due to Hb proton

7.3-7.6 complex, 5H, due to 5 aromatic protons.
J₀ = ~ 8 Hz Protons on aromatic ring with

Jₜ = ~ 1-2 Hz 0 CH=CH-C=O-C₂H₅ substituent.
Jₚ = ~ 0-1 Hz

MS: m/e (%) source

177 53.44 M + 1 isotope peak 53% of M indicating a maximum of 48 C atoms. This shows too many carbon atoms when the mass of the M at m/e 176 is taken into consideration. Abnormally large. M+1 peak can be caused by ion-molecule reactions resulting in proton transfer to hetero atom, oxygen₁⁶⁹.

176 99.88 M molecular ion peak, the even m/e indicates no or an even number of N atoms. The intensity of the peak is high.

158 24.66 M-18 formation of 158⁺

148 100.48 M-28 formation of C₆H₅CH=CHC=OCH, 148⁺ due to McLafferty rearrangement and loss of H₂C=CH₂

131 99.87 M-45 loss of O-CH₂-CH₃ group from the molecular ion.

103 99.95 M-73 loss of -C=O-CH₂-CH₃ from the molecular ion.

91 20.24 M-85 formation of tropylium ion C₇H₇ the intensity of the peak is small due to complexity of the path leading to the formation of tropylium ion.

77 99.9 M-99 formation of phenyl group C₆H₅

63 10.91 M-113 formation of 63⁺
a common ion $^{+}{C_{4}H_{3}}$, found for non-alkyl substituted phenyl after the elimination of $HC\equiv CH$.

loss of C atom from the m/e 51 ion. a common ion found for aromatic ring and formation of $C_{3}H_{3}$.

UV (EtOH) (nm)

$\lambda_{\text{max}} = 275,215,203$

conjugated aromatic absorption showed $\lambda - \lambda^*$ indicating benzoid derivative.

The molecular ion peak of the mass spectrum indicates that there are probably no hetero atom in the molecule other than possibly O and that the compound has a molecular weight of 176 amu. The n.m.r. spectrum shows the presence of 12 H atoms.

The i.r. indicates the presence of alkyl, olefinic, ester groups and mono substituted aromatic ring in the molecule. U.V. shows absorptions typical of an extended conjugated aromatic system. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation of various peaks in mass spectra confirm that the fraction is ethyl cinnamate. Which is further confirmed on the basis of reported uv$^{167}$, and mass$^{168}$ spectral data.

\[
\begin{align*}
&\text{O} \\
&\| \\
&\text{CH} = \text{CH} - \text{C} - \text{OC}_{2}\text{H}_{5} \\
&\text{ethyl cinnamate}
\end{align*}
\]

FRACTION NO. 4

IR: (NEAT) (cm$^{-1}$)

3090-3010 M aromatic ring and olefinic C-H stretching vibrations overlapped.
2820,2750 M aldehydic C-H stretch

1980-1755 overtones the pattern indicates mono substituted aromatic ring.

1690 S aldehydic C=O stretch.
Aldehydes normally absorb at 1725 cm⁻¹ but conjugation decreased the aldehydic C=O frequency to 1690 cm⁻¹.

1630 S olefinic C=C stretch, normally it absorbs at 1647 cm⁻¹ but conjugation has decreased the frequency to 1630 cm⁻¹.

1610,1580 S,M aromatic ring C=C skeletal in plane stretch vibrations.
1500,1455

1390 M aldehydic C-H bend

1090 S trans olefinic C-H (OOP) bend

755,690 S aromatic C-H/C-C (OOP) bend, indicating mono substituted aromatic ring.

¹H-NMR (CDCl₃) δ (PPM)

6.5 dd, 1H \[ \text{J}_{\text{trans}} = \sim 16 \text{ Hz.} \quad \text{due to } H_a \text{ proton} \]
\[ \text{J}_{\text{ab}} = \sim 7 \text{ Hz.} \]

7.35 d, 1H \[ \text{J}_{\text{trans}} = \sim 16 \text{ Hz.} \quad \text{due to } H_c \text{ proton} \]
\[ \text{J}_{\text{allylic}} = \sim 0-1 \text{ Hz.} \]

7.25 complex 5H, \quad \text{due to aromatic ring protons.}

9.66 d, 1H, \[ \text{H-C}=O \text{, J = \sim 6 Hz. aldehydic protons.} \]

MS: m/e (%) source

133 8.67 M + 1 isotope peak 10% of the M indicating a maximum of 9 C atoms.
132 82.33 M molecular ion peak, the even m/e indicates no or an even number of N atoms. The intensity of the peak is high. The aromatic aldehydes are characterised by a large molecular ion peak.

131 100.00 M-1 the loss of proton and the formation of M-1 peak larger than the molecular ion peak.

103 52.46 M-29 probably due to loss of HC≡O

91 2.6 M-40 formation of tropylum ion, C\textsubscript{7}H\textsubscript{7}\textsuperscript{+}, the intensity of the peak is low due to complexity of the path leading to the formation of tropylum ion.

77 34.78 M-55 formation of phenyl ion, C\textsubscript{6}H\textsubscript{5}, common ion found for compounds containing benzene ring.

51 23.05 M-81 a common ion found for non-alkyl substituted phenyls after elimination of HC≡CH from the phenyl ion.

39 3.80 M-93 loss of C atom from the m/e 51 ion, a common ion found for phenyl ion.

UV(\text{EtOH}) (nm)
\lambda_{max} 287, 220, 205 conjugated aromatic absorptions, * benzoid derivatives showing \( \pi \rightarrow \pi^* \) and \( n \rightarrow \pi^* \)

The molecular ion and isotope peaks of the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O, the maximum number of C atoms is 9 and that the compound has a molecular weight of 132 amu. The nmr spectrum shows the presence of 8 H atoms. The number of unsaturation sites and cyclic rings as calculated by the formula\textsuperscript{144} is 6.

The i.r. indicates the presence of trans olefin, aldehyde group and mono substituted aromatic ring in the molecule. U.V.
spectrum also shows an aldehyde group and absorption peaks typical of conjugated aromatic system. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectra determine that the fraction is cinnamaldehyde. Which is confirmed on the basis of reported uv\(^{167}\), ir\(^{169}\), nmr\(^{169}\) and mass\(^{155,169}\) spectral data.

![Cinnamaldehyde](image)

Cinnamaldehyde

FRACTION NO. 5:

I.R. (neat) (cm\(^{-1}\))

3440 Broad alcoholic O-H stretch. The broadness of the absorption peak is due to intermolecular hydrogen bonding.

3100-3000 W olefinic C-H stretch

2980-2860 S aliphatic C-H stretch.

1640 W olefinic C=C stretch

1460, 1380 M aliphatic C-H in plane bend

1125 M alcoholic C-O stretching vibrations corresponding to tertiary alcohol.

1000-700 M C-H (OOP) bending in terminal olefins.

840 W C-H (OOP) bend, corresponding to isopropylidene group
$^1$H-NMR (CDCl$_3$) & (PPM)

1.25 S, 3H, \( \frac{\text{HO-C-CH}_3}{\text{CH}_3} \) downfield shift is due to electron withdrawing effect of oxygen of OH group attached to adjacent C.

1.5 S, 3H, \( \frac{\text{H}_3\text{C}}{\text{C}=\text{CH}} \) \( \text{H}_3\text{C} \) downfield shift is due to anisotropic effect of C=C attached to the adjacent C atom.

1.6 S, 3H, \( \frac{\text{H}_3\text{C}}{\text{C}=\text{CH}} \) \( \text{H}_3\text{C} \) slight downfield shift as compared to aforementioned methyl group attached to the same carbon atom is due to different anisotropic effect of C=C.

1.48 dt, 2H \( \text{-CH}_2\text{-CH}_2\text{-C-CH}_3 \) J = $\sim$ 7 Hz and J = $\sim$ 1-2 Hz

1.72 S, 1H, due to O-H group

2.0 q, 2H, due to \( \text{CH-CH}_2\text{-CH}_2 \) J = $\sim$ 7 Hz

5.0 complex, 1H, \( \text{H}_3\text{C} \) \( \text{C}=\text{CH-CH}_2 \) due to CH, \( \text{J} = \sim 7 \text{ Hz} \), \( J_{\text{allylic}} = \sim 0-1 \text{ Hz} \) It forms triplet with the adjacent methylene protons, but allylic coupling with the protons on methyl groups made it complex.

5.08 d, 1H, \( \frac{\text{H}_3\text{C}}{\text{C}=\text{C}<\text{H}_a} \) \( \text{H}_3\text{C} \) due to H$_b$ proton, forms doublet with H$_c$ proton which is not further resolved.

\( J_{bc} = \sim 10 \text{ Hz} \).
\( J_{ab} = \sim 0-1 \text{ Hz} \).

5.18 dd, 1H, \( \frac{\text{R}}{\text{C}=\text{C}<\text{H}_a} \) \( \text{R} \) due to H$_a$ proton

\( J_{ac} = \sim 16 \text{ Hz} \)
\( J_{ab} = \sim 1-2 \text{ Hz} \).
5.88 dd, 1H, \[ R > C = C < H_a \]
\[ H_b \] due to \( H_c \) proton

\[ J_{ac} = \sim 16 \text{ Hz} \]
\[ J_{cb} = \sim 10 \text{ Hz} \]

**MS:** m/e (% Source

155 0.30 M+1 isotope peak 11 % of the M indicating a maximum of 10 C atoms.

154 2.70 \( M \) molecular ion peak, the even m/e indicates no or an even number of N atoms. The peak is very small indicating tertiary alcohol.

136 47.85 M-18 a prominent peak at m/e 136 usually found in the spectra of alcohols. It is due to the loss of OH and H from the \( pC \). The loss is also exaggerated by the molecular decomposition of higher alcohols on hot inlet surface.

121 66.62 M-33 terpene alcohols show a fairly strong peak at m/e 121 due to the loss of \( H_2O \) together with the loss of branched \( CH_3 \) group.

107 23.14 M-47 loss of -CH\(_2\)-CH\(_3\) from the fragment m/e 136.

93 100.00 M-61 loss of \( H_3^+ CH_- \) from the peak at m/e 136.

80 76.98 M-74 the loss of the fragment 74 and formation of the peak at m/e 80\(^+\) is due to some rearrangements.

71 92.51 M-83 fragmentation is probably at C-C next to the hetero atom with the \( OH \) formation of ion + \( CH_3-C-CH=CH_2 \). It is also due to allylic fragmentation, the ion is stable due to the
presence and delocalization of double bond. The presence of this peak in the spectrum confirms that the compound is tertiary alcohol.

55 90.24 M-99

the higher R.A indicates the formation of more stable ion \( \text{H}_3\text{C}^+ \text{C} = \text{CH} \) due to vinylic fragmentation.

41 88.07 M-113

the formation of \( \text{H}_2\text{C}^+ \text{C} \) or \( \text{C}_3\text{H}_5^+ \). The higher R.A value supports the formation of this very stable ion due to the presence and delocalization of double bond.

UV(EtOH) (nm)

\( \lambda_{\text{max}} = 209 \)

No \( \lambda_{\text{max}} \) above 220, the intense absorption at 209 nm is due to ethanol used as solvent.

The molecular ion and isotope peaks of the mass spectrum indicate that there are probably no hetero atoms in the molecule other than O, the maximum number of C atoms is 10 and that the compound has a molecular weight of 154 amu. The nmr spectrum of the compound shows the presence of 18 H atoms. The number of sites of unsaturation and cyclic rings as determined by formula is 2.

The ir indicates the presence of alkyl, olefin, tertiary alcohol, isopropylidene groups in the molecule. UV spectrum does not show any absorption above 220. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum determine that the fraction of the oil is 3,7-dimethyl-1,6-octadien-3-ol, commonly known as linalool. Which is confirmed on the basis of reported ir\(^{170}\), nmr\(^{171}\) and mass\(^{172,173}\) spectral data.

![Linalool](image-url)
FRACTION NO. 6:

IR: (neat) (cm⁻¹)

3500     Broad   inter-molecular H bonded alcoholic O-H stretch
3095,3000  W   aromatic C-H stretch.
2980-2850 M    aliphatic C-H stretch

1870-1740, overtones due to 1,2,4 tri-substituted aromatic ring.

1620,1510, S   aromatic ring C==C skeletal in plane stretching
1450,1430 vibrations, the presence of these bands indicates the aromatic ring.

1470,1360 M    aliphatic C-H in plane bend.

1270     S   C-O asymmetric stretch of alkyl aryl ether.
1240     S   C-O stretch of C-OH.

1020     S   C-O symmetric stretch of alkyl aryl ether.

845-800 M   aromatic C-H out of plane bend, characteristic absorption band of 1,2,4 tri-substituted aromatic ring.

960-700 W   olefinic C-H (OOP) bending vibration due to terminal ≡ CH₂ group and Cis and trans olefinic
>C=C<

¹H-NMR (CDCl₃) δ (PPM)

1.95 d, 2H, Ar-CH₂-CH=CH₂ the down field chemical shift
of protons of methylene group
J = ~ 7 Hz. is due to electron withdrawing environment.
3.80 s, 3H, Ar-OCH₃  an isolated downfield singlet is  an evidence of methoxy group  attached to benzene ring.

5.5-6.5 m 4H  i  CH₂-CH=CH₂  due to methine, methylene and  ii  OH  hydroxyl group protons.

6.8  m, 3H,  aromatic ring protons

MS:  m/e (%) source

165  21.20 M+1  isotope peak 21-20 % of the M indicating ~19 C atoms in the molecular ion. It is too many C atoms when the mass of the M at m/e 164 is taken into consideration. Abnormally large M+1 peak can be caused by ion molecule reactions resulting in proton transfer to hetero atom oxygen.

164  100.00 M  molecular ion peak, the even m/e indicates no or an even number of N atoms. The intensity of the peak is high.

149  75.30 M-15  loss of CH₃ group from β position of benzene ring.

131  55.58 M-33  loss of 2H and OCH₃ group from the molecular ion.

121  48.73 M-43  loss of H, CH₃ and -CH=CH₂ groups due to cleavage of the benzene derivatives.

103  69.19 M-61  formation of 103⁺
The molecular ion peak in mass spectrum indicates that there are no hetero atoms probably other than O and that the molecular weight of the compound is 164 amu. The nmr spectrum shows the presence of 12 H atoms.

The ir indicates the presence of alkyl, olefin, ether, phenol groups and 1,2,4-trisubstituted aromatic ring in the molecule. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectra determine that the fraction is 1-Hydroxy-2-methoxy-4-allyl benzene, commonly known as eugenol. Which is further confirmed on the basis of reported ir174, nmr175,176 and mass177 spectral data.

Eugenol
<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-thujene</td>
<td>0.30</td>
</tr>
<tr>
<td>α-pinene</td>
<td>0.14</td>
</tr>
<tr>
<td>sabinene</td>
<td>0.10</td>
</tr>
<tr>
<td>α-phellandrene</td>
<td>2.39</td>
</tr>
<tr>
<td>β-pinene</td>
<td>0.66</td>
</tr>
<tr>
<td>Δ3-carene</td>
<td>0.07</td>
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<tr>
<td>α-terpinene</td>
<td>0.26</td>
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<tr>
<td>p-cymene</td>
<td>29.56</td>
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<tr>
<td>γ-terpinene</td>
<td>35.72</td>
</tr>
<tr>
<td>terpinolene</td>
<td>0.06</td>
</tr>
<tr>
<td>linalyl acetate</td>
<td>4.84</td>
</tr>
<tr>
<td>ethyl cinnamate</td>
<td>1.23</td>
</tr>
<tr>
<td>cinnamaldehyde</td>
<td>2.37</td>
</tr>
<tr>
<td>linalool</td>
<td>12.73</td>
</tr>
<tr>
<td>eugenol</td>
<td>3.74</td>
</tr>
</tbody>
</table>

Material unrecovered from the column. 5.83
UV (EtOH) (nm)

$\lambda_{\text{max}}$ 260, 209 benzoid derivative showing intensive absorption due to $\lambda \rightarrow \lambda^*$ transition.

The molecular ion peak and the isotope peak of the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly oxygen, the maximum number of C atoms is 10 and the compound has a molecular weight of 148 amu. The nmr spectrum shows the presence of 12 H atoms. The number of sites of double bonds and cyclic rings as determined by the formula is 5.

The ir indicates the presence of aromatic, aliphatic, trans olefinic, ether groups and 1,4 disubstituted benzene ring in the molecule. Trans olefinic band at 975 cm$^{-1}$ is quite pronounced. The uv spectrum indicates the extended conjugation of aromatic double bonds. The chemical shifts, integration and splitting pattern of different peaks in nmr and fragmentation of various peaks in the mass spectrum determine that the compound is p-methoxy-propenyl-benzene, commonly known as trans anethole. Which is further confirmed on the basis of reported ir$^{178}$ and mass$^{179}$ spectral data.

![Chemical Structure](image)

Trans Anethole

FRACTION NO. 3

IR: (neat) (cm$^{-1}$)

2995-2895 s, aliphatic/alicyclic C-H stretch

1755 s, ketone C=O stretch, normally the frequency is 1715 cm$^{-1}$ but the penta cyclic ring strain raised the frequency to 1755 cm$^{-1}$.

1470, 1385m, The C-H in plane bend

$^{1}$H-NMR (CDCl$_{3}$) $\delta$ (PPM)

0.7-1 complex 1H, $H_b$ upfield shifting of one proton of
the methylene group is due to diamagnetic effect of the C=O group.

1.01 s, 6H,
the protons of two methyl groups are deshielded due to anisotropic effect of C=O.

1.1 s, 3H,
the CH₃ group shifts downfield due to anisotropic effect of the C=O.

1.35-1.88 complex m, 5H,
due to 5 protons of two methylene and one methine groups present in the molecule.

2.2 broad,s, 1H,
downfield shift of the 2nd proton of the methylene group, as it experiences a different anisotropic effect of C=O group.

**MS:** m/e (%) source

<table>
<thead>
<tr>
<th>m/e</th>
<th>%</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>153</td>
<td>2.8</td>
<td>M + 1 isotope peak 11% of the M indicating maximum 10 C atoms.</td>
</tr>
<tr>
<td>152</td>
<td>24.64</td>
<td>M molecular ion peak, the even m/e indicates no or even number of N atoms, the peak is of reasonable size.</td>
</tr>
<tr>
<td>137</td>
<td>3.14</td>
<td>M - 15 loss of CH₃ group.</td>
</tr>
<tr>
<td>109</td>
<td>8.17</td>
<td>M - 43 loss of C=O from m/e 137⁺ peak.</td>
</tr>
<tr>
<td>81</td>
<td>100.00</td>
<td>M - 71 loss of H,C=O &amp; H₃C⁺C groups from the molecular ion.</td>
</tr>
<tr>
<td>69</td>
<td>46.64</td>
<td>M - 83 probably formation of H₃C⁺C=CH=CH₂ ion</td>
</tr>
<tr>
<td>53</td>
<td>4.74</td>
<td>M - 99 formation of C₄H₅</td>
</tr>
<tr>
<td>41</td>
<td>19.95</td>
<td>M - 111 formation of C₃H₅</td>
</tr>
</tbody>
</table>

U.V. (EtOH) (nm)

λ_max = 260,210 weak n→π⁺ transition at 260 nm due to non conjugated ketone.
The molecular ion peak and isotope peak (M+1) of the mass spectrum indicate that there are probably no heteroatoms in the molecule other than possibly 0, the maximum number of C atoms is 10 and that the compound has a molecular weight of 152 amu. The nmr spectrum shows the presence of 16 H atoms. The number of sites of unsaturation or the cyclic ring as determined by the formula is 3.

The ir indicates the presence of alkyl and carbonyl groups in the molecule but the absorption of C=O group at higher frequency (1755 cm⁻¹) is an evidence to determine that the compound has a penta cyclic ketone group in the molecule. The uv shows a weak absorption peak at 260 nm, due to C=O. While the chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in the mass spectra determine that the fraction is 1,3,3-trimethyl bicyclo[2,2,1] heptan-2-one commonly known as fenchone. Which is further confirmed on the basis of reported ir and mass¹⁸O spectral data.

![Fenchone](image)

FRACTION NO. 4

IR: (neat) (cm⁻¹)

3430 broad, s alcoholic O-H stretching vibrations, broadness due to inter molecular hydrogen bonding.

2995-2900 s, aliphatic C-H stretch

1470, 1380, w aliphatic C-H in plane bend

1095 m alcoholic C-O stretch corresponding to secondary alcohol.

¹H-NMR (CDCl₃) δ (ppm)

0.85 s, 3H, due to methyl group. The methyl group protons normally absorb at 0.85.

1.00 s, 3H, due to another methyl group
present in the molecule, the downfield shift of the proton is due to the field effect exerted by the OH group.

1.1 s, 3H,

the downfield shift of the methyl group is due to its close proximity to -OH group through spatial interaction.

1.25-1.72 m, 8H,

due to methylene/methine protons on C₄,C₅,C₆ & C₇ & the OH proton.

3.32 broad 1H
due to the proton attached to the C atom to which the hydroxyl group is also attached. Downfield shift of the protons is due to the electron withdrawing effect of the adjacent OH group.

MS: m/e (%) source

155 1.98 M + 1 isotope peak, 11 % of the M peak indicating 10 C atoms in the compound.

154 17.64 M molecular ion peak, the even m/e indicates no or even number of N atoms. Molecular ion peak is small.

139 18.41 M - 15 loss of CH₃ group.

136 18.40 M - 18 loss of H₂O, common in alcohols.

121 56.61 M - 33 terpenic alcohols show a fairly strong peak formed after the loss of both H₂O and CH₃ (due to the presence of branched methyl groups).

111 66.59 M - 43 loss of C=O from m/e 139, common in alcohols.

93 61.92 M - 61 formation of 93⁺

81 100.00 M - 73 formation of C₆H₅⁺, after elimination of C₄H₉O from the molecular ion.

69 86.16 M - 85 formation of C₅H₉, probably

\[ \text{H}_2\text{C=CH-CH} < \text{CH}_3 \]
formation of $\mathrm{H\equiv C\left<_{\mathrm{CH}_3}^+\right>}$ after the elimination $\mathrm{CH}_2$ from m/e 69.

formation of $\frac{\mathrm{H}_2\mathrm{C}}{\mathrm{H}_3\mathrm{C}}\mathrm{C\equiv C}$

formation of $\frac{\mathrm{H}_2\mathrm{C}}{\mathrm{H}_3\mathrm{C}}\mathrm{C\equiv C}$

U.V. (EtOH) (nm)

$\lambda_{\text{max}} = 209$

No $\lambda_{\text{max}}$ above 220 therefore has no diagnostic value.

The molecular ion peak and the isotope peak (M+1) of the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O, the maximum number of C atoms is 10 and the compound has a molecular weight of 154 amu. The nmr spectrum shows the presence of 18 H atoms. The number of sites of unsaturation or cyclic rings, as determined from the formula is 2.

The ir indicates the presence of secondary alcohol and alkyl groups in the molecule. UV does not give any absorption peak above 220, while the chemical shifts integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum determine that the compound is $1,3,3$-trimethyl-bicyclo$[3.3.0]$ heptan-2-ol, commonly known as fenchyl alcohol. Which is further confirmed on the basis of reported ir$^{181}$, nmr$^{182}$ and mass$^{172,183}$ spectral data.

Fenchyl Alcohol

FRACTION NO. 5

IR: (neat) (cm$^{-1}$)

3090, 3010 w. aromatic C-H stretch
2980,2950 m, aliphatic C-H stretch.

2830, m, characteristic peak of methoxy group.

2850,2740 m, aldehydic C-H stretch

2010,1910 overtones the pattern of these low intensity bands indicates a para-substituted aromatic compound.

1695 s, aldehydic C=O stretch in conjugated aromatic compound, normally it is at 1735 cm\(^{-1}\) but conjugation has decreased the frequency of C=O by 40 cm\(^{-1}\).

1600,1580, s,m aromatic ring C==C skeletal stretching bands.

1500,1480

1440,1390 w, aliphatic C-H in plane bend.

1255 s, C-O asymmetric stretch of alkyl aryl ether.

1190 s, due to methoxy group

1020 s, C-O symmetric stretch of alkyl aryl ether.

1155,1390 s,w C-H in plane bending vibrations of aldehydic C-H group.

830 s, the C-H (ODP) bending of aromatic ring, the characteristic absorption band in 1,4-disubstituted benzene ring.

The medium absorption at 2740 cm\(^{-1}\) accompanied by strong C=O absorption band at 1695 cm\(^{-1}\) is a good evidence for the presence of HC=O group, conjugated to the aromatic ring.

\(^1\text{H-NMR} \text{ (CDCl}_3\text{)} \delta \text{ (ppm)}

3.82 s, 3H,Ar-O-CH\(_3\) an isolated singlet in the downfield at 3.82 ppm is an evidence of methoxy group attached to benzene group.

6.84 d, 2H, aromatic \(H_a, H_a^\prime\), atoms adjacent to OCH\(_3\) group, AA' XX' system.

\(J_{ab} = \sim 0-1\text{ Hz.} \quad J_{ab'} = \sim 8\text{ Hz.}\)
aromatic \( H^1, H^5 \) protons adjacent to \( -CHO \) group, \( \text{AA}^{\text{-XX}} \) system.

9.8 s, H, CHO

Due to \( \text{CHO} \) group proton.

MS: m/e (% source

137 6.52 M + 1

isotope peak 9% of the M indicates a maximum of 8 C atoms.

136 70.17 M

molecular ion peak, the even m/e indicates no or even number of N atoms. The molecular ion peak is large.

135 100.00 M - 1

loss of proton. The peak is larger than the molecular ion peak, common in aromatic aldehydes due to the formation of more stable ion, \( \text{Ar-C}^\equiv\text{O} \)

107 17.42 M - 29

probably due to loss of \( \text{HC}^\equiv\text{O} \).

92 14.51 M - 44

probably due to loss of \( \text{CH}_2 \) group β to benzene, the formation of \( \text{C}_6\text{H}_4\text{O} \) indicates that 0 is directly attached with the benzene ring.

77 28.21 M - 59

formation of phenyl group \( \text{C}_6\text{H}_5 \).

64 9.28 M - 72

loss of \( \text{C}^\equiv\text{O} \) from m/e 92 ion with the formation of \( \text{C}_5\text{H}_4 \).

51 5.89 M - 85

loss of \( \text{HC}^\equiv\text{CH} \) from m/e 77 ion with the formation of \( \text{C}_4\text{H}_3 \).

39 6.67 M - 97

loss of C atom from the m/e 51, with the formation of \( \text{C}_3\text{H}_3 \).

U.V. (EtOH) (nm)

\( \lambda_{\text{max}} \) 280, 225, 208

conjugated aromatic aldehyde showing intense \( n \rightarrow \pi^* \)

The molecular ion peak and the isotope peak of the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O. The maximum number of C atoms is 8 and that the compound has a molecular weight of 136 amu. The
nmr spectrum of the compound shows the presence of 8 H atoms. The number of sites of unsaturation and cyclic rings as determined by the formula is 5.

The ir shows the presence of aromatic, aliphatic, olefinic methoxy and aldehydic groups in the molecule. UV shows the presence of aldehydic group and absorptions typical of an extended conjugated aromatic system. The chemical shifts, integrations and splitting patterns of different peaks in nmr and fragmentation pattern of various peaks in mass spectra determine that the fraction of the oil is p-methoxy benz-aldehyde, commonly known as p-anisaldehyde. Which is further confirmed on the basis of reported uv\textsuperscript{167}, ir\textsuperscript{184,185} and mass\textsuperscript{186} spectral data.

\[
\text{OCH}_3
\]

\[
\text{C} = \text{O}
\]

FRACTION NO.6 p-Anisaldehyde

IR: (neat) (cm\textsuperscript{-1})

3440 broad alcoholic O-H stretch, broadness is due to intermolecular H bonding.

3100-3000 w, the absorption between 3100-3000 cm\textsuperscript{-1} is due to olefinic C-H stretch.

2990-2870 s, C-H stretch in aliphatic/alicyclic compounds.

1665 w, olefinic C=C stretch.

1470,1375 s, aliphatic C-H in plane bend.

1390,1375 m, aliphatic C-H in plane bend indicating geminal dimethyl group present in the molecule.

1190 s, alcoholic C-O stretch corresponding to tertiary alcohol.

1000-700 m, C-H (OOP) bending vibrations in olefin.
$^1$H-NMR (CDCl$_3$) $\delta$ (ppm)

1.19 s, 6H, due to two methyl groups present in the molecule. Downfield shift is due to OH group present on the adjacent C.

1.35 m, 4H, due to two CH$_2$ groups protons in the molecule.

1.62 s, 3H, due to methyl group attached to unprotonated C atom, downfield shift is due to the anisotropic effect of the C=C.

1.98 complex, 3H, due to the methylene and methine protons. The downfield shifts are due to the presence of double bond and OH group respectively attached to the adjacent C atom.

2.2 broad, 1H due to OH group proton.

5.3 broad 1H complex methine proton gives a complex (very broad peak), due to its coupling with adjacent CH$_2$ and allylic coupling with protons of methylene and methyl groups.

**MS:** m/e (%) source

154 Nil M In tertiary alcohols molecular ion peak is almost absent.

136 69.99 M - H$_2$O a distinct and some times prominent peak usually found in spectra of alcohols. The path is consistent with the loss of OH and -Hatom.

121 73.90 M - 33 loss of CH$_3$ group from the m/e 136 fragment.

107 11.06 M - 47 formation of $^{107}+$. 

93 85.43 M - 61 loss of $^3$H$_2$CH from the m/e 136 and probably formation of $^93^+$. 
loss of CH$_3$ and H$_3$C-OH due to cleavage of weak bonds at qua-ter-nary and tertiary C atoms.

loss of H$_3$C$_3$OH and forma-

H$_3$C CH$_3$$^+$

$\text{CH}_2$=C-CH=CH$_2$ due to homolytic réterô-D-iels-Alder reaction.

formation of H$_3$C$_3$O-H, 59$^+$ due to the fragmentation of weak bond at tertiary C atom. The formation of 59$^+$ due to the cleavage of C-C bond next to the oxygen atom with elimination of largest group confirms that the compound is tertiary alco-

hol.

formation of H$_3$C=CH, 43$^+$.

U.V. (EtOH) (nm)

$\lambda_{\text{max}}$ 209 intense absorption is due to ethanol used as solvent.

The i.r. indicates the presence of aliphatic, olefinic, tertiary alcoholic and geminal dimethyl groups in the molecule. U.V.spectrum does not show any absorption above 220 nm. The nmr shows the presence of 18 H atoms, while the chemical shifts, integrations and splitting pattern of different peaks in nmr, fragmentation pattern of various peaks and the presence of the base peak at m/e = 59 due to formation of H$_3$C$_3$O-H, confirm that the under investigated fraction of the oil is 1-methyl-4-isopropyl-1-cyclohexen-8-ol, commonly known as $\alpha$-terpineol which is further confirmed on the basis of reported ir$^{187},$ nmr$^{188}$ and mass$^{172,189}$ spectral data.

\[ \text{\alpha-terpineol} \]
TABLE 8

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF THE FOENICULUM VULGARE SEEDS (Wild variety)

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
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<td>α-pinene</td>
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<td>camphene</td>
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<td>sabinene</td>
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<tr>
<td>β-phellandrene</td>
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<td>trans-anethole</td>
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<tr>
<td>fenchone</td>
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<tr>
<td>fenchyl alcohol</td>
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<tr>
<td>p-anisaldehyde</td>
<td>2.23</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Material unrecovered from the column. 4.64
PLEUROSPERMUM STYLOSUM

FRACTION NO. 2

IR: (neat) (cm⁻¹)

2990-2855 s, aliphatic C-H stretch.

1725 s, ketonic C=O stretch.

1470,1365 m, aliphatic C-H in plane bending vibrations.

1172 m, C-\(\text{CO-C}\) stretching and bending vibrations.

\(^1\)H-NMR (CDCl₃) \(\delta\) (ppm)

0.85 t, 3H, CH₂-CH₂ the chemical shift of methyl proton is normally at 0.85 ppm.

1.3 complex, 4H, CH₃-CH₂-CH₂-CH₂-CH₂-C=O the chemical shift of the methylene protons is normally at 1.2 ppm in alkanes.

1.55 quintet, 2H, CH₂-CH₂-CH₂-C=O methylene protons give quintet because of coupling with two neighbouring CH₂ groups.

2.15 s, 3H, H₃C-C=O the chemical shift of protons on methyl group adjacent to C=O normally falls at 2.1.

2.4 t, 2H, CH₂-CH₂-C=O the triplet is due to its coupling with the adjacent CH₂, and downfield shift is due to anisotropic effect of adjacent C=O.

MS: m/e (%) source

115 6.5 M + 1 isotope peak 8 % of the M indicating a maximum of 7 C atoms.

114 79.47 M molecular ion peak, the even m/e indicates no or an even number of N atoms. The molecular ion peak, is conspicuous. Molecular ion peak in ketones is usually quite pronounced.

99 28.57 M-15 loss of CH₃ group from the molecular ion due to the cleavage of C-C adjacent to the 0 atom.
85 19.92 M - 29  loss of CH₂-CH₃ group from the molecular ion.

71 92.04 M - 43  loss of CH₃CO group and formation of

CH₃-CH₂-CH₂-CH₂-CH₂-H

OH

58 100.00 M - 56  formation of CH₃-C-CH₂ with the elimination of CH₂=CH-CH₂-CH₃. One alkyl group attached to C=O is greater than C₃ atoms. Cleavage of C-C bond,β to Oxygen occurs with H migration from ¹C to give a major peak (McLafferty rearrangement).

43 99.61 M - 71  loss of larger alkyl group attached to C=O from the molecular ion and formation of CH₃-C=O.

U.V. (EtOH),(nm)

λ<sub>max</sub> 270,202 weak absorption at 270 nm is due to n→π* transition of ketone carboxyl group.

The molecular ion peak and isotope peak in mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O. The maximum number of C atoms is 7 and that the compound has a molecular weight of 114 amu. The nmr spectrum shows the presence of 14 H atoms. The number of unsaturated sites and cyclic rings as calculated by the formula is 1.

The ir indicates the presence of alkyl and ketone groups uv shows weak absorption at λ<sub>max</sub> 270 nm. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum confirm that the fraction is methyl n-amyl ketone.

\[
\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}-\text{CH}_3
\]

Methyl n-Amyl Ketone
FRACTION NO.3

IR: (neat) (cm$^{-1}$)

3190-3010 w, olefinic C-H stretch.
2990-2855 s, aliphatic C-H stretch.
2780,2710 m, aldehydic C-H stretch
1680 s, aldehydic C=O stretch. Normally it is 1735. But conjugation has decreased the frequency of C=O.
1620 m, olefinic C=C stretching vibrations. Normally the C=C stretch is at 1647 but the decrease is due to conjugation with the C=O group as the conjugation decreases the frequency of C=C by 25 cm$^{-1}$.
1450,1380 s, aliphatic C-H in plane bend.
1155 m, aldehydic C-H bend.
980,850 m, olefinic C-H OOP bending vibrations.
845 s, olefinic C-H (OOP) bend corresponding to isopropylidene group \( \text{H}_3\text{C} > \text{C}=\text{CH} - \text{H}_3\text{C} \)

$^1$H-NMR (CDCl$_3$) $\delta$ (ppm)

1.6 s, 3H, \( \text{H}_3\text{C} > \text{C}=\text{CH} - \text{H}_3\text{C} \) downfield shift of the methyl protons is due to anisotropic effect of adjacent C=C group.

1.7 s, 3H, \( \text{H}_3\text{C} > \text{C}=\text{CH} - \text{H}_3\text{C} \) the methyl group is attached to the same C atom but the slight downfield shift than the aforementioned methyl group is due to the fact that the protons of the methyl group experience different anisotropic effect.

2.16 s, 3H, $\text{CH}_3$-C=C=CH-C=O the downfield shift is due to the anisotropic effect of the C=C group and conjugated C=O group.

2.26 complex, 4H, $\text{CH} \cdots \text{CH}_2\cdots \text{CH}_2\cdots \text{C}=\text{CH}_3$ due to overlapping of the peaks formed by the coupling of the two methylene groups with their neighbouring protons. The complex peak looks as a broad based singlet.
5.17 broad 1H, $^{H_3C}C=CH-CH_2$\underline{H_3C}$ forms a triplet by coupling with the adjacent methylene protons but due to allylic coupling with the protons of methyl groups, it appears as a broad complex.

5.85 d, 1H, $C=CH-CHO$ the chemical shift and doublet formation indicate that CH is attached with CHO group on one side and with unprotonated C through double bond on the other side, with the result doublet is formed only due to coupling with CHO proton.

9.96 s, 1H, $=CH-CHO$ due to aldehyde proton.

The spectrum also shows the presence of another isomer as indicated by the extra peaks at:

1.98 s, 3H, due to methyl group protons, the downfield shift is caused by the anisotropic effect of the neighbouring C=C and HC=O.

2.58 t, 2H, due to methylene protons and the downfield shift is caused by the anisotropic effect of the neighbouring C=C and HC=O.

The formation of singlet at 1.98 ppm and triplet at 2.58 ppm is due to the cis-isomer of the compound. The chemical shifts of $CH_3$ and $CH_2$ protons in trans and cis-isomers are different due to different magnetic shielding effect of the carbonyl double bond.$^{163}$

**MS:**

<table>
<thead>
<tr>
<th>m/e</th>
<th>(%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>153</td>
<td>1.74</td>
<td>M + 1</td>
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<tr>
<td>152</td>
<td>14.90</td>
<td>M</td>
</tr>
<tr>
<td>137</td>
<td>12.49</td>
<td>M - 15</td>
</tr>
<tr>
<td>123</td>
<td>11.70</td>
<td>M - 29</td>
</tr>
</tbody>
</table>

isotope peak 11 % of the M indicating a maximum of 10 C atoms.
molecular ion peak, the even m/e indicates no or even number of N atoms. The peak is small.
loss of branched $CH_3$ group present in the molecule.
the loss of CHO group from the molecular ion, common in aldehydes and is due to the cleavage of the C-C bond adjacent to the hetero atom.
119 5.12 M - 33 formation of 119⁺
109 13.27 M - 43 loss of HC=CHOH
94 24.20 M - 58 the formation of 94⁺ is due to some rearrangement.
84 37.56 M - 68 the formation of 84⁺ is due to some rearrangement.
69 100.00 M - 83 loss of $\text{H}_3\text{C}^+\text{C}^\text{CH}=\text{CHO}$ ion and formation of $\text{H}_3\text{C}^+\text{C}^\text{CH}^\text{CH}_2$ due to allylic $\text{H}_3\text{C}^+$ fragmentation.
54 9.84 M - 98 formation of $\text{C}_4\text{H}_6^+$
41 73.27 M - 111 formation of $\text{C}_3\text{H}_5^+$

**MASS SPECTRA OF THE SECOND ISOMER:**

153 0.50 M + 1 isotope peak 11% of the M indicating a maximum of 10 C atoms.

152 4.63 M⁺ molecular ion peak, the even m/e indicates no or even number of N atoms. The peak is small.

137 9.17 M - 15 loss of $\text{CH}_3^-$ group present in the molecular ion.

134 9.78 M - 18 loss of $\text{H}_2\text{O}$ from the molecular ion.

119 16.41 M - 33 probably due to loss of $\text{H}_2\text{O}$ and $\text{CH}_3^-$ group from the molecular ion


94 38.86 M - 58 formation of 94⁺ due to some rearrangement.

84 34.97 M - 68 formation of 84⁺ is due to some rearrangement.

69 100.00 M - 83 formation of $\text{H}_3\text{C}^+\text{C}=\text{CH}^\text{CH}_2$ and loss of $\text{CH}_3^-$; $\text{H}_3\text{C}^+\text{C}=\text{CH}-\text{CHO}$ group due to allylic fragmentation.

59 17.60 M - 93 formation of 59⁺
The molecular peak and the isotope peak in mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O, the maximum number of C atoms is 10 and compound has a molecular weight of 152 amu. The nmr spectrum shows the presence of 16 H atoms. The number of sites of unsaturation and cyclic rings as calculated from the formula is 3.

The IR indicates the presence of aliphatic, olefinic aldehyde and isopropyldiene groups in the molecule. UV shows absorption peak at \( \lambda_{\text{max}} \) 240 nm due to \( \alpha \)-unsaturated carbonyl group. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum determine that the under investigated fraction is 3,7-dimethyl-2,6-octadien-1-al, commonly known as citral.

But nmr spectrum also shows the presence of another isomer as indicated by the extra peaks, i.e., singlet representing three protons due to methyl group and triplet representing two protons due to methylene group. These extra peaks indicate the presence of cis-isomer of the compound, called neral in the fraction. The GLC and GC/MS also shows the presence of another peak constituting 35% of the fraction. The fragmentation pattern of various peaks in the mass spectrum further confirms the presence of cis-isomer. Therefore it is inferred that the citral fraction is a mixture of two isomers; trans isomers called geranial constituting 65% while the cis-isomer called neral 35% of the fraction.
FRACTION NO. 4

IR: (nujol) (cm⁻¹)

3325 broad alcoholic O-H group
2980-2890 s, aliphatic C-H stretch
1470, 1380 s, m, aliphatic C-H bend
1120 w, alcoholic C-O stretch corresponding to secondary alcohol

¹H-NMR (CDCl₃) δ (ppm)

0.85 s, 9H, due to 9 protons of 3 methyl groups. Methyl group protons normally absorb at 0.85.
1.1-1.4 m, 6H, due to 6 protons of 3 methylene groups.
1.7 m, 2H, one proton due to methine group the other due to OH group.
4.0 d, 1H, H-C-OH due to one proton attached to C to which OH group is attached.

MS: m/e (%) source

155 0.14 M + 1 isotope peak 11% of the molecular ion peak indicating 10 C atoms.
154 1.29 M⁺ molecular ion peak, the even m/e indicates no or even number of N atoms, the intensity of the peak indicates that it is either primary or secondary alcohol as the size of the peak in tertiary alcohol is very small and even undetectable.
139 15.12 M - 15 loss of CH₃ group due to cleavage of weak bond at quaternary C atom.
121 7.51 M - 33 loss of CH₃ and H₂O groups from the molecular ion peak.
110 22.15 M - 44 formation of 110⁺ is due to some rearrangement.
95 100 M - 59 loss of CH$_3$ and CH$_2$-CHOH due to cleavage of weak bonds at quaternary and tertiary C atoms and formation of C$_7$H$_{11}$.

81 11.91 M - 73 formation of C$_6$H$_9$

67 10.25 M - 87 formation of C$_5$H$_7$

55 7.79 M - 99 formation of C$_4$H$_7$

41 24.85 M - 113 formation of H$_2$C=C-CH$_3$, 41$^+$

32 7.51 M - 122 formation of 32$^+$

U.V. (EtOH) (nm)

$\lambda_{\text{max}}$ 210 has no diagnostic value.

MELTING POINT: 205°C

The molecular ion peak and the isotope peak in the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O, the maximum number of C atoms is 10 and compound has a molecular weight 154 amu. The nmr spectrum shows the presence of 18 H atoms. The number of sites of unsaturation and cyclic rings as calculated from the formula is 2.

The ir indicates the presence of aliphatic/alicyclic, alcoholic groups in the compound, uv does not show any absorption peak above 220 nm, the compound is solid crystalline and melts at 205°C. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum determine that the compound is 2-hydroxy-camphane commonly known as borneol which is further confirmed on the basis of reported mt.pt.$^{190}$, ir$^{191}$, nmr$^{192}$ and mass$^{172,193}$ spectral data.

![Borneol](image)
FRACTION NO.5

IR: (neat) (cm\(^{-1}\))

3360 broad s  alcoholic O-H stretch
3080-3010 w,  olefinic C-H stretch.
2990-2880 s,  aliphatic C-H stretch.
1675    w,  olefinic C=C stretch.
1450,1380 m,  aliphatic C-H in plane bend.
1020    s,  C-O stretching vibrations corresponding to primary alcohols.
838     w,  olefinic C-H (OOP) bending vibrations corresponding to isopropylidene \(\ce{H3C>\ce{C=C-H}}\) group.
820     w,  olefinic C-H (OOP) bending vibrations.

\(^1\)H-NMR (CDCl\(_3\) ) \(\delta\) (ppm)

1.6  s,  \(3\)H, \(\ce{\overset{\wedge}{\text{H}_3\text{C}>\text{C=CH-}}}\) the downfield shift of methyl protons is due to the proximity of double bond attached to the adjacent C atom.

1.65 s,  \(3\)H. \(\ce{\overset{\wedge}{\text{H}_3\text{C}}>\text{C=CH-}}\) the slight downfield shift than the aforementioned methyl protons is due to the fact that it experiences a different anisotropic effect.

1.72 s,  \(3\)H, HC=CH-C\(_2\) downfield shift is due to the anisotropic effect of the double bond attached to the adjacent C atom.

2.05 s,  \(2\)H, HC-CH\(_2\)-CH\(_2\) due to protons of methylene group but it appears as a singlet.

2.1 s,  \(3\)H, CH\(_2\)-CH\(_2\)-C\(_2\) due to two protons of methylene group and one proton of OH group. It also appears as a singlet.

4.07 d,  \(2\)H, \(\ce{\overset{\wedge}{\text{H}}\text{C=CH}_2\text{OH}}\) the chemical shift of the methylene protons indicates bonding to OH group on one side and \(\ce{\overset{\wedge}{\text{H}}\text{C}}\) group on the other side, the split is due to methine proton resulting in doublet.
the downfield shift of the methine proton is due to presence of adjacent double bond. It forms triplet with the adjacent methylene protons but due to allylic coupling with the protons of methyl groups it appears as a broad singlet due to the complex nature of the multiplet.

the chemical shift and triplet formation indicates that methine group is attached with -CH₂OH on one side and double bond with non protonated C on the other, resulting in a triplet formation with the CH₂OH protons.

isotope peak 11% of M indicating a maximum of 10 C atoms.

the molecular ion peak, the even m/e indicates no or an even number of N atom. The intensity of peak indicates that the compound is either primary or secondary alcohol as the size of the peak in tertiary alcohol is very small and even undetectable.

due to the loss of OH group and H atom. This loss is also exaggerated by the thermal decomposition of higher alcohols on the hot inlet surface. The peak is most noticeable in the spectra of primary alcohols.

the terpene alcohol due to the presence of branched CH₃ group show a fairly strong peak at m/e 121, owing to the loss of H₂O and CH₃ groups.

loss of H₃C >CH isopropyl group from the molecular ion.

the strong peak at m/e 93 is due to
the loss of \( \text{H}_3\text{C} \text{CH}_2 \) group from the \( \text{H}_3\text{C} \) fragment m/e 136.

84  89.94 M-70  the loss of fragment 70 and formation of peak at m/e 84 is due to some rearrangement.

69  99.51 M-85  probably due to the allylic fragmentation of the molecular ion, the loss of \( \text{CH}_2=\text{C}-\text{CH}-\text{CH}_2\text{OH} \) and formation of \( \text{H}_3\text{C}>\text{C}=\text{CH}-\text{CH}_2 \) m/e 69 with higher value of R.A. indicating the stability of fragment induced by the delocalization of double bond present in the molecule.

68  100.00 M-86

55  41.25 M-99  the formation of \( \text{H}_3\text{C}>\text{C}=\text{CH} \) due to vinylic cleavage.

41  99.73 M-113  the formation of \( \text{H}_2\text{C}>\text{C}\) fragment

31  6.54 M-123  formation of \( \text{CH}_2\text{OH} \) is due to the cleavage of \( \text{C}-\text{C} \) bond adjacent to hetero atom. The presence of this peak in the spectrum of the compound confirms it to be a primary alcohol.

U.V. (EtOH) (nm)

\[ \lambda_{\text{max}} = 212 \]

No \( \lambda_{\text{max}} \) above 220.

The molecular ion peak and the isotope peak in the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O, the maximum number of C atoms is 10 and that the compound has a molecular weight of 154 amu. The nmr shows the presence of 18 H atoms. The number of unsaturated sites and cyclic rings as indicated by the formula is 2.

The ir indicates the presence of aliphatic, olefinic, primary alcoholic and isopropylidene groups in the molecule. UV does not show any absorption above 220 nm, while the chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum confirm that the fraction of the oil is 3,7-dimethyl-2,6-octadien-1-ol, commonly known as nerol. Which is further confirmed by the reported mass 172,194 spectral data in literature.
FRACTION NO. 6

IR: (neat) (cm⁻¹)

3440 broad s, alcoholic O-H stretch
3090-3000 w, olefinic C-H stretch
2980-2960 s, aliphatic C-H stretch
1638 w, olefinic C=C stretch
1460,1370 m, aliphatic C-H in plane bending vibrations.
1370 m,w C-H in plane bending does not bifurcate.
1125 m, alcoholic C-O stretching vibrations corresponding to tertiary alcohols.
840 w, olefinic C-H (OOP) bend corresponding to isopropylidene group. \( \text{H}_3\text{C} \) \( >\text{C} = \text{CH} \)
840 w, olefinic C-H (OOP) bend corresponding to isopropylidene group.
1000-700 m, C-H (OOP) bending vibration in olefins.

\( ^1\text{H-NMR (CDCl}_3\text{)} \) δ (ppm)

1.2 s, 3H,HO-C-CH₃ downfield shift of methyl protons is due to OH group on adjacent C atom.

1.48 d,t 2H, -CH₂-CH₂-C- downfield shift of methylene proton is due to electron withdrawing effect of OH group attached to the adjacent C atom.

J = ~ 7 Hz.
J = ~ 1-2 Hz.
1.5 s, $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
downfield shift of methyl protons is due to the presence of double bond on the adjacent C atoms.

1.6 s, $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
slight downfield shift as compared to the previous methyl group attached to the same C atom is due to different anisotropic effect of C=C.

1.72 s, $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
due to alcoholic OH group.

2.0 q, $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
downfield shift is due to the presence of double bond on the adjacent C atom.

5.0 t, $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
forms triplet with the adjacent methylene protons but due to allylic coupling with protons of methyl groups, the triplet is not further resolved.

5.08 doublet, $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
doublet due to $\text{H}_b$ proton, $\text{H}_b$
forms doublet due to coupling with cis-proton and as the vicinal coupling constant is small, the doublet is not further resolved.

5.18 d,d $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
$\text{H}_a$ proton forms doublet of doublet due to coupling with trans $\text{H}_c$ and vicinal $\text{H}_b$ protons.

5.88 d,d $\text{H}_3\text{C} \text{H}_ \text{C}$

$\text{H}_3\text{C} > \text{C}=\text{CH}^-$
$\text{H}_c$ proton forms doublet of doublet due to coupling with trans $\text{H}_a$ and cis-$\text{H}_b$ protons.

MS: m/e  (%) source

155 0.28  M + 1 isotope peak 11% of the M indicating a maximum of 10 C atoms.

154 2.70  M molecular ion peak, the even m/e indicates no or an even number of N atoms. The peak is very small indicating tertiary alcohol.

136 45.27  M - 18 a prominent peak at m/e 136 usually found in the spectra of alcohols. It is due to the loss of OH and H atom.
terpene alcohols due to the presence of branched methyl group show a fairly strong peak at m/e 121. Loss of H$_2$O together with the loss of CH$_3$ group.

loss of -CH$_2$CH$_3$ from the fragment at m/e 136.

loss of -CH<CH$_3$ from the peak at m/e 136.

loss of fragment 74 and formation of peak at m/e 80 is due to some rearrangements.

fragmentation is probably at C-C next to the hetero atom with the formation of $^>C^+CH=CH_2$ it is due to allylic fragmentation. The ion is stable as supported by higher R.A. value. The presence of this peak in the spectrum indicates that the compound is tertiary alcohol.

the higher R.A. indicates the formation of more stable ion $^>C=CH$.

probably the formation of $^>C^+CH_2$.

The higher R.A. value supports the formation of this very stable ion due to the presence and delocalization of double bond in the fragment.

U.V (EtOH) (nm)

$\lambda_{max} = 209$ No $\lambda_{max}$ above 220. Intense absorption in the 200-220 nm is due to ethanol used as solvent.

The molecular ion peak and the isotope peak in the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O, the maximum number of C atoms is 10, and that the compound has a molecular weight of 154 amu. The nmr shows the presence of 18 H atoms. The number of unsaturated
sites and cyclic rings as indicated by the formula is 2.

The ir indicates the presence of aliphatic, olefinic, tertiary alcoholic and isopropylidene groups in the molecule. UV does not show any absorption above 220 nm, while the chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum determine that the fraction of the oil is 3,7-dimethyl-1,6-octadien-3-ol commonly known as linalool. Which is further confirmed on the basis of reported ir170,nmr171 and mass172,173 spectral data.

![Linalool Structure]

FRACTION NO.7

Melting point 137°C

UV(EtOH) (nm) \( \lambda_{\text{max}} \) 298,250

It was identified as angelicin on the basis of melting point 137°C (lit. 138-139.5°C)\textsuperscript{190} and u.v analysis \( \lambda_{\text{max}} \) 298,250 nm reported (295,248 nm)\textsuperscript{202}. 
<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-thujene</td>
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<td>sabinene</td>
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<td>α-phellandrene</td>
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<tr>
<td>β-phellandrene</td>
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<td>α-terpinene</td>
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<td>angelicin</td>
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<td><strong>Material unrecovered from the column.</strong></td>
<td><strong>5.23</strong></td>
</tr>
</tbody>
</table>
PSAMMOGETOM STOCKSII

FRACTION NO. 2

I.R. (neat) (cm⁻¹)

3090-3000 w, olefinic C-H stretch.
2990-2860 s, aliphatic C-H stretch.
1740 s, C=O stretch in esters.
1680 w, olefinic C=C stretch.
1460,1370 m, aliphatic C-H in plane bend.
1390,1375 m, bifurcation of the peak at 1370 cm⁻¹ indicates the presence of geminal dimethyl group.
1240 s, C-O asymmetric stretch in esters.
1025 m, ester C-O symmetric stretching vibrations.
960,838 w, olefinic C-H (OOP) bending vibrations.
838 w, olefinic C-H (OOP) bend corresponding to isopropylidene group present in the molecule.

¹H-NMR (CDCl₃) δ (ppm)

1.57 s, 3H, \( \text{H}_3\text{C} \)\( \text{H}_3\text{C} \text{C} \) the downfield shift of methyl protons is due to the anisotropic effect of the adjacent C=C.
1.65 s, 6H, \( \text{H}_3\text{C} \)\( \text{H}_3\text{C} \text{C} \)\( \text{H}_3\text{C} \text{C} \)\( \text{H}_3\text{C} \text{C} \) a singlet of methyl protons formed slightly downfield due to different anisotropic effect of double bond attached to the adjacent C atom, is overlapped by the singlet due to another methyl group proton having double bond attached to the adjacent C atom. Thus a singlet representing 6 protons appears at 1.6 ppm.
2.04 s, 7H, \( \text{H}_3\text{C} \text{-C}=O, \)\( \text{H}_3\text{C} \text{-C}=O, \) protons of CH₃ group bonded to C=O typically absorb near 2.0 ppm but the peak formed by overlapping of peaks due to coupling of protons of two methylene groups with the neighbouring protons also absorb at 2.04
ppm. Thus a singlet representing 7 protons is exhibited at 2.04 ppm.

4.5 d, 2H, C=HC-CH₂-O-C-CH₃
J = 7 Hz

The doublet formation and the chemical shift of methylene protons indicate bonding to O-C=OR on one side and -CH=C- on the other side. The split is due to methine protons resulting in doublet formation.

5.01 broad singlet 1H, H₃C=CH-CH₂
J = 7 Hz
J_{allylic} = 0-1 Hz

Forms triplet with the adjacent methylene protons but due to allylic coupling with the protons of two methyl groups it appears as a broad singlet due to complex nature of the multiplet.

5.3 t, 1H, C=CH-CH₂-O-C-CH₃
J = 7 Hz.

Formation of a triplet and its downfield shift indicate that CH is attached through a double bond to non protonated C atom on one side and CH₂-O-C-CH₃ on the other.

MS: m/e (%) Source
196 0.44 M
The molecular ion peak is very small, terpen acetates usually do not show detectable molecular ion peak.

136 27.23 M-60
Loss of CH₃COOH formed due to the transfer of H from the carbon \( \gamma \) to carbonyl group by E1 rearrangement.

121 26.06 M-75
Loss of branched CH₃ group from m/e 136.

107 7.74 M-89
Loss of CH₂-CH₃ group from the m/e 136.

93 51.99 M-103
Loss of \( \text{H}_3\text{C-CH} \) ion from m/e 136.

80 17.95 M-116
Formation of 80⁺ due to some rearrangement.
formation of $\text{C}_2\text{H}_5^+$ due to doubly allylic fragmentation and loss of $\text{CH}_2\text{C}=\text{CH}\text{CH}_2\text{O}-\text{C}-\text{CH}_3$

formation of $\text{C}_4\text{H}_5^+$

formation of $\text{C}_2\text{H}_5^+$

formation of $\text{C}_2\text{H}_5^+$

The molecular ion peak indicates that there are probably no hetero atoms in the molecule other than oxygen and the molecular weight of the compound is 196 amu. The nmr shows the presence of 20 H atoms.

The ir shows the presence of aliphatic, olefinic, ester, geminal dimethyl and isopropylidene groups in the molecule. UV does not give any diagnostic peak. While the chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in the mass spectrum determine that the fraction of the oil is trans-3,7-dimethyl-2,6-octadien-1-yl-acetate, commonly known as geranyl acetate. Which is confirmed on the basis of reported ir$^{195}$ and mass$^{196}$ spectral data in literature.

FRACTION NO. 3

IR: (nujol) (cm$^{-1}$)

2980-2870 s, aliphatic or alicyclic C-H stretch

1755 s, ketonic C=O stretch. Normally the frequency of C=O in ketone is 1715 cm$^{-1}$ but the rise of fre
frequency of C=O group to 1755 cm\(^{-1}\) is due to strain caused by the pentacyclic ring.

1465, 1380 w, aliphatic C-H in plane bend.

**MS:**

<table>
<thead>
<tr>
<th>m/e</th>
<th>(%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>153</td>
<td>4.98</td>
<td>isotope peak 11% of M, indicating maximum 10 C atoms.</td>
</tr>
<tr>
<td>152</td>
<td>44.95</td>
<td>molecular ion peak, the even m/e indicates no or an even number of N atoms. The peak is of considerable size.</td>
</tr>
<tr>
<td>137</td>
<td>6.22</td>
<td>loss of CH(_3) from the molecular ion.</td>
</tr>
<tr>
<td>108</td>
<td>51.93</td>
<td>formation of 108(^+) due to some rearrangement.</td>
</tr>
<tr>
<td>95</td>
<td>100.00</td>
<td>formation of C(<em>7)(</em>{11}) due to the fragmentation of the weak bond at tertiary and qua-ternary C atoms.</td>
</tr>
<tr>
<td>81</td>
<td>70.35</td>
<td>formation of C(_6)H(_9)</td>
</tr>
<tr>
<td>69</td>
<td>35.59</td>
<td>formation of C(_5)H(_9)</td>
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<td>55</td>
<td>30.52</td>
<td>formation of C(_4)H(_7)</td>
</tr>
<tr>
<td>41</td>
<td>37.58</td>
<td>formation of 41(^+)</td>
</tr>
</tbody>
</table>

**MELTING POINT:** 175\(^0\)C

The molecular ion peak and the isotope peak of the mass spectrum indicate that there are probably no hetero atoms in the molecule other than O, the maximum number of C atoms is 10 and that the compound has molecular weight 152 amu.

The ir indicates the presence of aliphatic/alicyclic and pentacyclic ketone in the molecule. The compound is crystalline having melting point 175\(^0\)C. The fragmentation pattern of various peaks in the mass spectrum determines that the compound is 2-carbonyl camphane, commonly known as camphor. Which is confirmed on the basis of reported m.pt.\(^\text{190}\) ir\(^\text{197}\) and mass\(^\text{198}\) spectral data.

![Camphor](Camphor.png)
FRACTION NO.4

IR: (neat) (cm\(^{-1}\))

3455 broad alcoholic O-H stretch, broadness due to intermolecular H bonding.

3100-3010 w, olefinic C-H stretch

2990-2870 s, aliphatic/ Alicyclic C-H stretch

1660 w, olefinic C=C stretch.

1460,1380 s, aliphatic C-H in plane bend

1390, m, broad and is not clearly bisected. However, the broadness may be due to geminal dimethyl group present in the molecule.

1180 s, alcoholic C-O stretch corresponding to tertiary alcohol.

1000-700 m, C-H (OOP) bending vibrations in olefin.

\(^1\)H-NMR (CDCl\(_3\)) \(\delta\) (ppm)

1.16 s, 6H, due to two methyl groups present in the molecule, the downfield shift is due to O-H group on the adjacent C atom.

1.23 t, 4H, \(J = 7\) Hz. due to two methylene groups protons in the molecule.

1.62 s, 3H, due to CH\(_3\) group, downfield shift is due to double bond on the adjacent C atom.

1.95 complex, 3H, due to methylene and methine protons.

2.19 broad, 1H, due to OH group proton.

5.35 broad, 1H, methine proton gives a broad peak due to coupling with the adjacent CH\(_2\) and allylic coupling with methylene and methyl protons.
MS: m/e (%) source

154 Nil M in tertiary alcohols molecular ion peak is almost absent.

136 64.23 M-H₂O a distinct and some times prominent peak usually found in the mass spectra of alcohols. It is due to the loss of OH and H from the β carbon.

121 68.72 M-33 loss of CH₃ group from the fragment ion m/e 136.

107 8.34 M-47 Formation of 107⁺

93 79.78 M-61 loss of H₃C>CH from the fragment ion m/e 136.

81 39.98 M-73 loss of CH₃ and H₃C>C=O

68 20.98 M-86 loss of H₃C>C-CH=CH₂ and formation of CH₃-C-CH-CH₂, 68⁺ due to retero-Diels-Alder reaction.

59 100.00 M - 95 formation of >COH, 59⁺ due to the fragmentation of weak bond at tertiary C atom. The formation of 59⁺ due to cleavage of C-C bond next to hetero atom with the elimination of largest group of the molecule confirms that the compound is tertiary alcohol.

43 27.67 M-111 formation of H₃C>CH, 43⁺

U.V. (EtOH) (nm)

λₘₐₓ 209 intense absorption is due to ethanol (solvent), has no diagnostic value.

The i.r. indicates the presence of aliphatic, olefinic, tertiary alcoholic and geminal dimethyl groups in the molecule.
U.V. spectrum does not show any absorption above 220 nm. The nmr shows the presence of 18 H atoms, while the chemical shifts, integrations and splitting pattern of different peaks in nmr, fragmentation pattern of various peaks and the presence of the base peak at m/e = 59 due to formation of \( \text{H}_3\text{C} - \text{C}=\text{CH} - \text{OH} \), confirm that the fraction of the oil is 1-methyl-4-isopropyl-1-cyclohexen-8-ol, commonly known as \( \alpha \)-terpineol. Which is further confirmed on the basis of reported ir\(^{187}\), nmr\(^{188}\) and mass\(^{172,189}\) spectral data.

\[ \alpha \text{-Terpineol} \]

**FRACTION NO. 5**

**I.R. (neat) (cm\(^{-1}\))**

- 3360 broad, alcoholic O-H stretch.
- 3080-3010 w, olefinic C-H stretch.
- 2990, 2940 s, 2870, aliphatic C-H stretch.
- 1678 w, olefinic C=C stretch.
- 1460, 1385 m, aliphatic C-H in plane bending vibrations.
- 1010 s, alcoholic C-O stretching vibrations indicating primary alcohol.
- 840 w, olefinic C-H (OOP) bend corresponding to isopropylidene, \( \text{H}_3\text{C} - \text{C}=\text{CH} \) group.
- \( ^1\text{H-NMR (CDCl}_3\text{)} \delta \text{ (ppm)} \)

\( 1.58 \text{ s, } 3\text{H, } \frac{\text{H}_3\text{C}}{\text{H}_3\text{C}} - \text{C}=\text{CH} \) due to protons on methyl group, downfield shift due to double bond on the adjacent C atom.
1.65 s, 7H, $\text{H}_3\text{C} > \text{C}=\text{CH}$
due to six protons of two methyl
groups and one proton of hydroxyl
group.

2.02 s, 4H, =\text{CH}-\text{CH}_2-\text{CH}_2-C=
the protons of two methylene
groups appear to form singlet at
2.02 ppm.

4.1 d, 2H, C=\text{CH}-\text{CH}_2\text{OH}
downfield shift is due to elec
tron withdrawing effect of -OH
group.

5.1 broad, 1H, $\text{H}_3\text{C} > \text{C}=\text{CH}-\text{CH}_2$
forms triplet with the adjacent
methylen protons but due to
coupling with protons of methyl
groups at allylic position it
appears as a broad complex.

5.3B t, 1H, C=\text{CH}-\text{CH}_2\text{OH}
downfield shift is due to the
double bond attached directly and
also due to electron withdrawing
environment. The triplet is due
to adjacent methylene protons.

MS:

<table>
<thead>
<tr>
<th>m/e</th>
<th>(%)</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>3.15</td>
<td>M+1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>isotope peak 11% of the molecular ion peak indicating 10 C atoms.</td>
</tr>
<tr>
<td>154</td>
<td>27.06</td>
<td>M</td>
</tr>
</tbody>
</table>
molecular ion peak, the even m/e indicates no or even number of N atoms. The intensity of peak indicates that it is either primary or secondary alcohol, as the size of the peak in tertiary alcohol is very small and even undetectable.

136 35.48 M-18
this peak is most noticeable in the spectra of primary alcohols and is due to loss of OH group and H atom . This loss is also exaggerated by the thermal decomposition of higher alcohols on hot plate surface.

121 70.83 M-33
the terpene alcohols due to the presence of branched CH$_3$ group show a fairly strong peak at m/e 121, formation of C$_9$H$_{13}^+$. 

111 43.25 M-43
loss of $\text{H}_3\text{C} > \text{CH}$, isopropyl group from $\text{H}_3\text{C}$
the molecular ion and formation of C$_{7}$H$_{11}$O.
The strong peak at m/e 93 is due to formation of \( C_7H_2 \) and loss of \( H_3C\overset{2}{\sim}CH \) group from the m/e 136. The loss of fragment 70 and formation of peak at m/e 84 is due to some rearrangement.

Due to doubly allylic fragmentation of molecular ion with the formation of \( H_3C\overset{2}{\sim}CH-CH_2 \) and loss of \( H_2C-\overset{2}{\sim}CH_2OH \). The 69 with higher intensity indicates the stability of the fragment, induced by the delocalization of double bond present in the molecule.

The formation of \( H_2C-\overset{2}{\sim}CH_2OH \) due to the vinylic cleavage.

The formation of \( H_2C-\overset{2}{\sim}CH_2 \) due to the vinylic cleavage of \( C-C \) bond adjacent to hetero atom. The presence of this peak in the spectrum of the compound confirms it to be a primary alcohol.

UV (EtOH) (nm)

\[ \lambda_{\text{max}} 233,213 \quad \text{A shoulder at 233 nm is not diagnostic, no } \lambda_{\text{max}} \text{ above 220.} \]

The molecular ion peak and isotope peak (M+1) of the mass spectrum indicate that there are probably no hetero atoms in the molecule other than possibly O, the maximum number of C atoms is 10 and that the compound has molecular weight of 154 amu. The nmr shows the presence of 18 H atoms. The number sites of unsaturation and cyclic rings as calculated by the formula is 2.

The ir indicates the presence of aliphatic, olefinic, primary alcoholic and isopropylidene groups in the molecule. UV does not give any diagnostic peak above 220 nm. The chemical shifts, integrations and splitting pattern of different peaks in nmr and fragmentation pattern of various peaks in mass spectrum determine that the compound is 3,7-dimethyl-2,6-octadien-1-ol, commonly known as geraniol. Which is further confirmed on the
basis of reported ir\textsuperscript{199}, nm\textsubscript{r}\textsuperscript{200} and mass\textsuperscript{201} spectral data in literature.

\begin{center}
\begin{tikzpicture}
\draw (0,0) -- (1,0) -- (1,1) -- (0,1) -- cycle;
\draw (0,1) -- (1,2);
\draw (1,1) -- (1.5,1.5);
\draw (1.5,1.5) -- (2,1.5);
\draw (2,1.5) -- (2,2);
\draw (2,2) -- (1.5,3);
\draw (1.5,3) -- (1,3);
\draw (1,3) -- (0,2);
\draw (0,2) -- (0,1);
\draw (0,1) -- (0,0);
\node at (0.5,0.5) {CH\textsubscript{2}OH};
\node at (1,1.5) {Geraniol};
\end{tikzpicture}
\end{center}

FRACTION NO. 6

Crystalline solid m.pt. 187°

<table>
<thead>
<tr>
<th>m/e</th>
<th>(%)</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>217</td>
<td>13.65</td>
<td>m + 1, isotope peak 13.65% of the molecular ion peak indicates 12 C atoms present in the molecule.</td>
</tr>
<tr>
<td>216</td>
<td>100.00</td>
<td>m, molecular ion peak, the even m/e indicates no or an even number of N atoms. Molecular ion peak is a base peak.</td>
</tr>
<tr>
<td>201</td>
<td>37.24</td>
<td>M - 15, loss of CH\textsubscript{3} from the molecular ion peak.</td>
</tr>
<tr>
<td>188</td>
<td>13.30</td>
<td>M - 28</td>
</tr>
<tr>
<td>173</td>
<td>52.08</td>
<td>M - 43, loss of 43 mass units from the molecular ion peak.</td>
</tr>
<tr>
<td>157</td>
<td>2.06</td>
<td>M - 59</td>
</tr>
<tr>
<td>145</td>
<td>21.00</td>
<td>M - 71</td>
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<tr>
<td>131</td>
<td>2.59</td>
<td>M - 85</td>
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<tr>
<td>108</td>
<td>5.34</td>
<td>M - 108</td>
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<tr>
<td>89</td>
<td>8.66</td>
<td>M - 127</td>
</tr>
<tr>
<td>63</td>
<td>5.98</td>
<td>M - 153</td>
</tr>
<tr>
<td>51</td>
<td>7.34</td>
<td>M - 165</td>
</tr>
<tr>
<td>40</td>
<td>49.74</td>
<td>M - 176</td>
</tr>
</tbody>
</table>
U.V.: (EtOH) (nm)

\[ \lambda_{\text{max}} = 312,268,250 \text{ nm}. \]

The molecular ion peak and isotope peak of the mass spectrum indicate that there are probably no hetero atom in the molecule other than possibly oxygen, the number of C atoms is 10 and compound has a molecular weight 216 amu.

It was identified, bergaptene by comparing melting point 187°C (lit. 188-190°C)\(^{190}\) u.v. analysis 312,268,250 (lit.311 nm)\(^{202}\) and reported mass spectral data\(^{203}\).
<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>PERCENTAGE</th>
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</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>14.50</td>
</tr>
<tr>
<td>camphene</td>
<td>0.86</td>
</tr>
<tr>
<td>sabinene</td>
<td>0.74</td>
</tr>
<tr>
<td>α-phellandrene</td>
<td>2.13</td>
</tr>
<tr>
<td>β-pinene</td>
<td>2.20</td>
</tr>
<tr>
<td>p-cymene</td>
<td>5.93</td>
</tr>
<tr>
<td>d-limonene</td>
<td>5.14</td>
</tr>
<tr>
<td>γ-terpinene</td>
<td>31.68</td>
</tr>
<tr>
<td>terpinolene</td>
<td>1.41</td>
</tr>
<tr>
<td>geranyl acetate</td>
<td>7.50</td>
</tr>
<tr>
<td>camphor</td>
<td>4.04</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>15.00</td>
</tr>
<tr>
<td>geraniol</td>
<td>2.07</td>
</tr>
<tr>
<td>bergaptene</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Material unrecovered from the column. 5.75
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