CHAPTER I

INTRODUCTION
1. HISTORICAL IMPORTANCE

From the dawn of civilization man, gifted with the sense of smell, has been attracted by the aromatic plants for their odour appeal. He has always tried to increasingly utilise odoriferous plants for his pleasure and well-being. From ancient times spices derived from aromatic plants have been used as flavouring agents for foods and drinks. Their use as offering to deities, as incense, in medicine, for aesthetic purposes, as the principal agents for embalming the dead has been in vogue from times immemorial.

The knowledge of perfumery materials as drugs, flavouring agents and fragrance has thus been growing steadily from the advent of human race, and the vast store of this accumulated knowledge has helped a great deal in the growth of modern perfumes and medicines.

Perfumes are one of the most remarkable phenomena of plant metabolism. It has been known for many centuries that the fragrance possessed by many plants is associated with volatile liquids which can be extracted from the leaves, fruits, flowers and from other parts of the plants. These have come to be known as the essential or volatile oils. Such oils were isolated originally by pressure or by gentle heating and later by steam distillation.

The systematic identification of the chemical constituents of essential oils and aromatic chemicals began
in the 19th century and this led to the isolation of acyclic, alicyclic and aromatic compounds which were named terpenes because of their association with the oil of turpentine. The number of chemical compounds of proved or potential usefulness, as the active perfumery materials from plants, has since been growing. Many useful perfumery compounds occur in nature and to list a few, e.g., ionones, geraniol, rose-furan, cinnamyl alcohol etc. present a broad and representative range of different odours. A number of oxygen-containing derivatives of terpenes were discovered, many of them crystalline, which were known as "camphors". This term was later on dropped and the name terpenes was used to describe such compounds related to terpenes.

The role that perfumes play in day to day living verges on the fantastic. Beginning with the morning cup of tea and ending with the moment we finally rest our weary head on a pillow, we are using perfumes without being consciously aware of the fact. Soaps, toiletries, cosmetics, foods and beverages, soft drinks, chewing tobacco, cigarettes, tea, agarbatti, pharmaceuticals and even the industrial products like leather, plastics and textiles, all of these are perfumed. Perfumes have pervaded life to such an extent that they now need to be treated as indispensable.
India has a long record of achievements in the field of essential oils, aromatic chemicals and drugs. With her vast expanse of territory and varied climatic conditions, physical features and the fertility of soil, India has been a rich nursery of medicinal and aromatic plants throughout the ages.

The value of Indian perfumery materials, spices and drugs was recognised far and wide in the ancient world. For centuries India was one of the leading perfume manufacturing countries of the world. Although due to mechanization some western countries have outpaced India in recent years but due to growing awareness among the young Indian scientists and technologists, for the last two decades, the time is not far off when India will retrieve its age-old supremacy in the field of perfumes and drugs.

It has now been established that India has all the requisites for greater diversification of essential oil products - variety of soils, climate and altitude. There is a growing feeling among experts and manufacturers that if India is to regain her position as a leading perfume producing country, the producers should have easy access to a wide range of aromatic chemicals. At present in Europe and America, a modern perfume can contain as many as 200 ingredients of which ninety percent are synthetic ones - mainly byproducts of coal-tar, petrochemicals and other similar bases, though natural ingredients do form an
essential part of the compound.

Kashmir is famed for its scenic beauty and luxuriant flora which include many economic plants used by men to alleviate diseases. The most important essential oil bearing plants which grow there are, Juniperus, Cedrus, Skimmia, Mentha, Artemisia and many others. The essential oils from these plants are utilized by various industrialists for the manufacture of industrial products and medicines.

2. ESSENTIAL OILS

The history of essential oils dates back to the use of perfumes in early stages of human culture. The investigations on the chemistry of essential oils were started by Dumas\(^1\). Essential oils find an amazingly wide and varied application in many industries for scenting and flavouring of all kinds of consumer products. The action of heat, atmospheric oxygen and enzymes may often produce changes in the plant products. Seasonal changes, differences in habitat and the chemical nature of the soil also produce changes in the qualitative as well as in the quantitative composition of the oils.

Various methods utilised for the extraction of essential oils are:-- 1. Steam and Water distillation methods  2. expression  3. enfleurage  4. maceration and 5. solvent extraction method. Details of these are described in standard books on essential oils.\(^2\-4\)
The simpler mono- and sesquiterpenes are the chief constituents of essential oils. Goodwin\(^5\) has suggested that the number of different terpenes present in the plants may be greater than that of any other group of natural products.

3. RECENT DEVELOPMENTS

Modern advance in science has considerably added to our knowledge of the aromatic odoriferous substances. The separation of these principles in pure form from plant material led to their synthesis and the production of artificial scents and perfumes. Our ancestors had a limited number of aromatic substances for their use, and the advances in this field have provided a great variety of synthetic perfumes. Besides this many more aromatic plants with pleasant fragrant smells have been discovered and are being gradually brought into use. Perfumed preparations such as hair oil, soap, skin-creams, lotions, smelling salts etc. are increasingly in use. The demand for the aromatic substances has thus increased enormously during recent years.

With the advent of the year 1970 there has been a huge influx of new compounds in the chemistry of terpenoids and up to this time some one thousand sesquiterpenes have been discovered\(^6\) and more and more are likely to be added in the subsequent years. The new sesquiterpenes are coming to light at a quick rate because of the fact that the original suggestion
of Wallach\textsuperscript{7} that sesquiterpenes are formed by head to tail condensation has been found valid only in certain cases and a number of exceptions to this rule have been found to exist. Wallach suggested the isoprene nature of this class of compounds and the first sesquiterpenoid (farnesol) was isolated in 1913.

The variety of the structures among these sesquiterpenoids and the wide natural occurrence of these compounds in essential oils has increased the interest of many chemists searching for flavour and fragrant substances, and has led to the discovery of many new compounds. An obstacle to the use of sesquiterpenoid compounds in flavours and fragrances is in most cases the difficult synthesis of the complicated structures. Until now the derivatives of easily accessible sesquiterpenoids have been used. Continuously new sesquiterpenoids are being isolated and their structure determined.

4. MODERN TECHNIQUES USED FOR THE STUDY OF ESSENTIAL OILS

It can be said that essential oils have achieved a great importance if one takes into account their perfumery value. These are the most complex mixtures synthesised by nature. For studying these, chromatographic and spectroscopic techniques are very commonly used now-a-days. Column chromatographic technique\textsuperscript{8} separates mixture of various terpenic compounds, and thin layer chromatography\textsuperscript{9} offers the cheapest and the quickest method for their resolution.
Use of silver nitrate impregnated silicagel G plates is very common now-a-days for the separation of ethylenic hydrocarbons which form complexes with the Ag+ ions.

Gas liquid chromatography picks up even those compounds present in very low percentages and along with mass spectrometry has almost revolutionized the field. GLC has been mainly used in the present thesis to find out the composition of different terpenic compounds. Perkin Elmer 881 equipped with Reoplex 400 and SE-30 columns and AIMIL-NCL dual column gas chromatographs have been used in the present investigation. Lot of theoretical background regarding GLC has been given in text book reviews, journal reviews and in various articles. SE-30 is a silicone nonpolar column and the advantage with this is that the temperature can be raised even upto 300°. Reoplex 400 is a polyester column and an intermediate between polar and nonpolar but in this the temperature can be raised only upto 200°.

Among the spectroscopic techniques use of IR, NMR and UV is very common for determining the structures of new compounds. Mass spectrometry gives the molecular weight of a compound. Details of these methods are described in standard books. Recently C-13 NMR spectroscopy has been introduced which helps in determining the structures of new compounds. The increase in literature in essential oils has made way for new books and review articles. In India
review articles have been published by Sukhdev, Bhattacharya and Mehta. The progress in the field of terpenes has been given by Simonsen, Guenther, Guha, Barton and Mayo and Tsutsui and Tsutsui.

5. PROBLEM TAKEN AND WORK DONE

The present study deals with the chemical examination of the essential oils extracted from the leaves of Artemisia absinthium, Artemisia vestita, Artemisia vulgaris and Artemisia scoparia. Besides the essential oils of Artemisia species, ten other essential oils have been screened for antimicrobial activity. In order to see if fixed oils could also compare (even though in a small measure) with the antimicrobial activity of essential oils, two new fixed oils were isolated, chemically examined and their activity measured. Thus the fixed oils of Conium maculatum and Brassica oleracea var. acephala have been isolated and their activity measured. The oils, however, were found to be active only against one organism of Staphylococcus aureus, showing thereby that fixed oils, unlike essential oils have low power of antimicrobial activity. The essential oils of Artemisia absinthium, Artemisia vestita and Artemisia vulgaris have also been investigated for insecticidal properties.

Genus Artemisia:

Genus Artemisia (family Compositae) has attracted the attention of various workers for a long time.
It is a large genus and comprises of 280 species of which 34 are found in Jammu & Kashmir state. It is commonly called "Wormwood" in India and "Sagebrush" in America. Artemisia species have been grouped into two classes on the basis of the production of an anthelmintic drug, santonin. The two classes are santonin bearing and non-santonin bearing.

Artemisia plants have been extensively used in medicine as antispasmodics. In Kashmir the plant has been known from ages and in the ancient times the aqueous extract of leaves was used by people for the eradication of round and thread worms. Recently the essential oil of Artemisia annua has been reported to possess antimycotic activity even up to the dilution of 1:2000. Polyphenols from the fruit of Artemisia transiliensis have been reported to show antioxidant properties for fat preservation. Srebocan and Stern have reported that the essential oil of Artemisia caerulescens caused epileptic seizures in mice which assumed dog like postures. The oil of Artemisia is also reported to cause cramps in animals, and higher amount results in convulsions and death. Certain species like Artemisia capillaris have the property of choleretic effects. From the juice of Artemisia absinthium certain antiseptic substances have been prepared. A gelatin capsule filled with 10% emulsion of the oil of Artemisia tridentata was found effective in the treatment of human ulcers.
A number of sesquiterpene lactone compounds have been reported from this genus by Asplund, \textsuperscript{47} Geissman, \textsuperscript{48-53} Shafizadeh, \textsuperscript{56-58} Abbasov \textsuperscript{59} and Gonalez. \textsuperscript{60} The structures of some of these are given below.
Certain preparations from *Artemisia monosperma* have found application as antispasmodic in the treatment of colic and in conditions associated with arterial hypertension. Keeping in view the medicinal importance of the genus *Artemisia*, the author was encouraged to undertake the chemical examination of the leaves of *Artemisia absinthium*, *Artemisia vestita*, *Artemisia vulgaris* and *Artemisia scoparia*.

1. *Artemisia absinthium* Linn.

   It is an aromatic and bitter herb found in Kashmir at 5000-7000 ft. It is distributed over north Asia and Afghanistan. Its flowers are used as vermifuge and as tonic in intermittent fevers. It exercises a powerful influence over the nervous system and its tendency to produce headache and other nervous disorders is well known to travellers in Kashmir and Ladakh.

2. *Artemisia vestita* Wall.

   It grows abundantly in Srinagar at 5000 ft. and is a shrub with fern like leaves. When crushed the leaves emit a fine odour.

3. *Artemisia vulgaris* Linn.

   It grows throughout the hilly districts of Kashmir. The plant is considered to be a valuable stomachic
and antispasmodic. Externally it is used in fomentations given in skin diseases. The leaves and the tops are administered in nervous and spasmodic affections connected with debility, in asthma and diseases of the brain. 64

4. **Artemisia scoparia** Waldst & Kit.

It is found in western Himalayas from Kashmir to Lahul at 5000-7000 ft, in Sind, Punjab, upper Gangetic plain and western Tibet at 7000-12000 ft.

6. **RESULTS OBTAINED ON INVESTIGATION**

The essential oil of **Artemisia absinthium** has been chemically analysed by column, thin layer and gas liquid chromatography. The various terpenic compounds identified are: - $\alpha$-thujene (0.96%), $\alpha$-pinene (6.88%), camphene (0.31%), p-cymene (0.23%), 1,8 cineole (0.97%), methylheptenone (0.09%), $\beta$-phellandrene (10.54%), caryophyllene oxide (1.39%), $\alpha$-terpineol (2.31%), thujyl alcohol (20.23%), geraniol (1.58%), thujyl acetate (1.79%), caryophyllene (5.41%), $\alpha$-himachalene (6.94%), $\alpha$-cadinene (0.36%) and elemol (1.59%). These compounds have been identified by GLC on SE-30 column. GLC on Reoplex column identified methylheptenol (2.23%), $\alpha$-thujone (9.2%) and anisaldehyde (2.57%). S-guaiaazulene has been identified by column chromatography. Three unknown sesquiterpenic compounds were obtained from the column.
chromatography. These have been studied by spectroscopic techniques. The structure of one of these (AA-2) has been established as sesquiterpene acetate related to himachalenes.

The essential oil of *Artemisia vestita* has been analysed by TLC, GLC and column chromatography. GLC on SE-30 column identified the following compounds: \(\alpha\)-thujene (0.03\%), \(\alpha\)-pinene (0.30\%), \(\Delta^3\)-carene (0.22\%), myrcene (0.53\%), methylheptenone (0.53\%), \(\beta\)-phellandrene (0.70\%), 1:8 cineole (0.58\%), \(\alpha\)-terpinene (1.53\%), thujyl alcohol (5.29\%), \(\alpha\)-terpineol (3.26\%), citral 'b' (2.40\%), citral 'a' (2.61\%), nerol (1.88\%), geraniol (1.70\%), \(\alpha\)-thujone (4.46\%), \(\beta\)-caryophyllene oxide (1.37\%), \(\beta\)-caryophyllene (0.87\%), elemol (2.59\%), \(\beta\)-eudesmol (13.10\%), 7(11)-selinene-4-ol (4.80\%), \(\alpha\)-himachalene (7.6\%) and nerolidol (3.36\%). Thujyl acetate (5.64\%) and anisaldehyde (0.53\%) have been identified on Reoplex column.

The essential oil of *Artemisia vulgaris* has been found to contain: \(\alpha\)-thujene (traces), \(\alpha\)-pinene (traces), p-cymene (0.11\%), camphene (traces), sabinene (0.14\%), limonene (2.01\%), methylheptenone (2.92\%), 1:8 cineole (4.98\%), thujyl acetate (1.61\%), \(\alpha\)-terpineol (2.26\%), thujone (9.61\%), thujyl alcohol (3.35\%), geraniol (6.45\%), borneol (3.55\%), linalool (25.42\%), \(\beta\)-caryophyllene (6.39\%), elemol (6.50\%) and \(\alpha\)-cadinene (traces).

The essential oil of *Artemisia scoparia* has been
analysed by column and thin layer chromatography. The various compounds identified are: α-pinene (0.4%), limonene (4.0%), camphene (0.5%), α-cadinene (5.0%), 1,8 cineole (3.2%), methylheptenone (9.1%), eugenol (10.0%), carvone (20.9%), dihydrocarvone (1.9%), α-thujone (35.0%) and geranyl acetate (9.0%).

Besides Artemisia species, ten other essential oils have been screened for antimicrobial activity. It has been found that all the essential oils are having fairly good activity against various bacteria and fungi. Among the three species of Artemisia screened for insecticidal properties, Artemisia vesitita has shown higher toxicity (a 20% solution showing 93.3% of mortality).
REFERENCES

1 Dumas., Leibig's Annaleu der pharmacie, 6, 245,(1833).


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