CHAPTER I

INTRODUCTION
Right from the appearance of human being on the planet, diseases and decay which co-existed with him, have been the most vital problems threatening the survival of mankind.

It was therefore natural for the human being to make search for substances which could ward off diseases are cure them. In his search for such substances and due to easy availability of plants around him, he made an elaborate study of plants and was able to recognise the curative values of various plants for various ailments. His studies when compiled and developed into the monographs known as pharmacopeias.

In our country, such a study is of great importance because of its ancient Ayurvedic system of medicine and the mediaeval Greco-Arab system which found in every home in this country under the popular name "Unani-Tib". Infact despite of the advent of the Allopathic system of medicine, the indigenous system of medicine is still the main source of relief for a large number of ailments for the majority of people of the Indian sub continent. The scourge of diseases is causing micro-organisms is increasing day by day not only to plants and animals but also by human beings. During the last decade a lot of research activity in the evolution of new antimicrobes has been carried out. About one third of all pharmaceuticals are derived from plants and over 60% of the pharmaceutical preparation are plant based\(^1\). Side effects of drugs derived from plants are less than those of synthetic ones. Vane\(^2\) has rightly—pointed out that out of
every 3000 chemical synthesised, only one is likely to be successful in its clinical trial.

The antimicrobial properties of volatile constituents have been well known to mankind, these have been used as preservatives, fungicides and germicides. It is seen that some essential oils, if applied pure, are fairly active germicides. The majority of the essential oils however, are more valued for their antiseptic than for their disinfectant action.

Since the volatile oils contain a number of compounds, it is not possible to generalise their activity on any class of micro-organisms. In 1887, Chamberland found that Anthrexbacilli contained in the blood were killed by the oils of Vespetro in 18 hours and by the oils of Angelia in 42 hours against salmonella typhi.

Starting from the nineteenth century up to the beginning of the twentieth century, the antimicrobial properties of essential oils have been investigated by Khurana, Smith, Hathmacher, Dyche-Teague, Lord-Husa, Bryant. Many constituents of the essential oil have also been studied thoroughly against many types of micro-organism, showed the useful utilization of saffrol for the disease of mouth, and foot.

The aromatic plants like ocimum are grown in each house and because of its great therapeutic value, it is still worshiped by Hindus. The practice of offering generally aromatic
flowers to God in India is very old and common. In addition to this aromatic plants were used as important ingredients of Yajnas in religious rituals. In our country our ancestors used to grow pleasant smelling plants like jasmin, rose, marigold etc., in their houses. The pleasant smelling compounds isolated from these aromatic plants is known as essential oils and is defined as odoriferous bodies obtained generally from plant sources. Essential oil is liquid at room temperature and volatalises without decomposition. It occurs in various parts of plants but is restricted only to some of their special portions such as leaves, bark, roots, flowers and fruits. The Himalayan pine is the type in which the oil is present only in the flowers. Cinnomum oil is confined to the bark, leaves, and the roots. The essential oil are composed of a number of chemical compounds such as hydrocarbons, alcohols, esters, aldehydes, lactones, oxides and ketones etc.

Volatile constituents find varied applications in many industries including syrups, drugs, pharmaceuticals, flavouring of foods, scenting, paints, insecticides, beverages, detergents, cosmetics, soaps etc. Many substances derived from essential oils are also applied to the skin and hair of human body for cosmetic and hygienic purposes. Such substances help for the up-keep of human health. In many cases the ornamental value and the aesthetic value of the products have been increased by the addition of various essential oils.
The antimicrobial activity of essential oils have been recognised since long time. Essential oils and their constituents have been reported to be associated with curative on diseases like CARUNOMA, INFLAMATION, HYPERTENSION and CATARRHS OF BRONCHI and LUNGS and also cure diseases of RESPIRATORY TRACT. The pepperment odour stimulates secretion of gastces and thus enhances digestion and due to these perfumery industry has been in existence in this country. Indian perfumers (Attars) have earned reputations for their aroma and flavours through out the world. In early day the Indian essential oils, aromatic spices and various other oils had a monopoly and were much demand in abroad. The important essential oils produced these day are of polmarosa, Cedar wood, Sandal wood, Mint, Vetiver and Terpentine.

Even their inspite of having a glorious, the perfumery industry in this country lagging behind the western countries in keeping pace with the modern developments in the art of perfumery with the result that essential oil industry was not in a healthy condition.

As such there is an imperative and argent need to make vigorous attempts to revitelise this industry by utilising modern sophisticated technology and the author has attributed in this direction with a view to analyse the indigenous volatile constituents by modern techniques and study their antimicrobial properties in order to discover, cheaper thrapeutic agents of more
potential values to ameliorating human sufferings.

A deep sweep in the available literature of preceding decade has indicated that the analysis of essential oils has paved an important way. Some recent applications of HPLC are described below:

(1) Monoterpene glucosides from \textit{Schizonepeta tenuifolia}\textsuperscript{11},
(2) Sesquiterpene lactone from \textit{Veronica multa}\textsuperscript{12},
(3) Isopimarane diterpene from \textit{Amaracus akhdarensis}\textsuperscript{13},
(4) Diterpene from genus \textit{Amaracus}\textsuperscript{14}, and
(5) Limonoid from \textit{Trichila roka}\textsuperscript{15}.

In addition to HPLC, GC/MS and GC/FTIR has also proved to have very important role in the field of essential oils. Few significant applications of GC/MS and FTIR analysis are described below:

(6) "GC/MS and GC/FTIR Analysis of essential oil of celery seed" by \textit{Cu Jian-Qin et al.}\textsuperscript{16},
(7) "The composition of lemon petitigrain oil, citrus Lemon", by \textit{Ekunduyo and Bakare et al.}\textsuperscript{17},
(8) "The chemical composition of Lovage Head space and Essential oils produced by Solvent Extraction with Various Solvents", by \textit{Jian-Qin Cu and Antoine Gaset et al.}\textsuperscript{18},
(9) "Major constituents of the Essential oil from leaves" by \textit{Neth and Bordoloi}\textsuperscript{19}.
(10) "Sweet orange leaf oil of Aromatic plants" by Zhenia Fleisher and Alexander Fleisher

(11) "A Paradox Database for GC/MS Data on composition of Essential oils and other Volatiles" by Lenord M. Libbey

(12) "The essential oil of cordia cylindrostachya Roem and Schult grown on Aruba" by Fun and Baerheim Svendsen

(13) "The Essential oil of Lippia Adoensis leaves and flower" by Stella, Elakovitch and Babajide, Oguntimein

(14) The essential oil of Greater Galarage from Malaysia" by Herman, L. DE Pooter, et al. and

(15) "Volatile aroma constituents of Shri Lankan Ginger", by Alexander J. et al.

Spectroscopic aids (UV, IR, NMR, 13-C NMR and Mass) have also displayed a very significant importance in the analysis of essential oils e.g.

(16) "Chemical examination of the aerial parts of ROTHIA TRIFOLIATA", by Sastry and Bhagavan et al.

(17) "Volatile compounds from Triticum Aestivum", by Thomos R. Hamilton-Kemp and Roger A. Andersen

(18) "Volatile oil, constituents of SAGEBRUSH", by William, Epstein and Larry, Gaudioso, and

(19) "Composition of Essential oil from of EUCALYPTUS DELEG'TENSIS", by Roderick J. Weston.
The applied utility of essential oil can be gauged by the large number of publications appearing in literature submitting reports about the (Antibacterial/Anifungal) activities of the essential oil which may potentially be used as antimicrobial agents. Some of the recent antimicrobial activity (Antibacterial/Anifungal) of the naturally occurring essential oil are described below:

(20) "Fungitoxic activity of cumaldehyde, main constituents of the Cuminum cyminum oil", by Singh and Upadhyay.

(21) "Chemistry and antimicrobial activity of the constituents of the leaves of Ardisia solonacea", of Khan and Ashraf et al.

(22) "A potential bactericidal agent", by Saxena and Jain.

(23) "Fungitoxicity of some essential oils against Macrophomia phaseoline", by Dwivedi and Dwivedi et al.

(24) "Antimicrobial activity of essential oil of Brazilian plants", by Craveiro and Clark.

(25) "Antibacterial activity of phenolic and nonphenolic fraction of some Indian Medicinal Plants", by Patel and Gandhi et al.

(26) "Antimicrobial activity of some plant essential oil", by Subbarao and Satyanarayana, and

(27) "In Vitro Antimicrobial Studies of the Essential Oil of Ixora Coccinea Linn.", by Yadava.
In view of the volatile oils and in order to extend our present knowledge about the plant which may be used more potentially against the diseases caused by micro-organisms, it was thought worthwhile to carry out investigations on the following plants.

(i) *Anisomeles indica* (Linn.)$^{38,39}$

It belongs to family Labiatae. It is found in throughout India ascending to 6000 ft. in the Himalayas. It is useful as an astringent and carminative. The whole plant has a strong scent resembling camphor.$^{40}$

(ii) *Majorana hortensis* (Moench)$^{41,42}$

The plant *M.hortensis* belongs to natural order Labiatae and is commonly known as "Murwa" in Hindi and "Bantulsi" in Kumaon. It is cultivated in Indian gardens and grows in any well drained fertile garden loam. The Ayurvedic system of medicine has described it to be useful in acute diarrhoea, paralytic limbs and toothache, in addition to this oil is employed to a small extent in high grade flavour preparations and perfumes and in soap and liqueur industries.

(iii) *Eupatorium triplinerve* (Vahl.Syn.)$^{43-44}$

The plant *E.triplinerve* belongs to natural order "Compositae" and is known as "Ayapana" in Hindi and Bangali. It is distributed chiefly in tropical America and has migrated
to India. Now it grows in Indian garden also. It is used in
treatment of stimulant and a hot infusion is emetic and
diaphoretic, in addition to this its fresh leaves is used as a
haemostatic agent.45
**PROBLEM TAKEN AND WORK DONE**

Plant chemistry has developed much of the fascination because of its enormous advancements and due to its increasing utility. In recent years huge number of new plant constituents have been isolated, which have established their reputation as useful starting materials for the preparation of even life saving drugs.

As such plants are the richest source of drugs and therefore science of medicine from the early times centered around the plants having curative values.

Uncontrolled technological advancements and changing living habits of human beings has created a scenario resulting and in an undesirable environment for human being. Therefore there appears to be an urgent need for the isolation of new compounds related specifically from medicinal plants.

In addition to above knowledge of relationship of biological activity to structure pertaining to the volatile constituents is desirable not only for the discovery of novel therapeutic more potential and cheaper drugs but also for unravelling the secret of the therapeutic reputation of the plants.

It therefore clearly transpries that their still exists enormous degree of fascination in the isolation and structural elucidation of plant constituents and establishing their antimicrobial properties. Therefore the author took up this challenging
task in case of *Anisomeles indica*, *Majorana hortensis* and *Eupatorium triplinerve* and his findings are summarised below:

(1) **CHROMATOGRAPHIC EXAMINATIONS OF ESSENTIAL OILS OF**

**ANISOMELES INDICA, EUPATORIUM TRIPLINERVE AND MAJORANA HORTENSIS LEAVES**

The essential oils obtained from the leaves of *Anisomeles indica*, *Eupatorium triplinerve* and *Majorana hortensis* by steam distillation were subjected to thin layer, column and gas liquid chromatographic analysis. The essential oil of *A.indica* was found to consist of $\alpha$-pinene (7.0%), $\beta$-pinene (3.0%), d-limonene (3.0%), methyl chavicol (3.2%), $\alpha$-thujene (3.5%), citral (9.5%), borneol (2.13%), 1,8-cineole (11.9%), nerol (2.2%), $\alpha$-terpineol (2.10%), eugenol (24.5%), azulene (6.0%) and caryophyllene (5.2%) where as the essential oil of leaves of *E.triplinerve* consist of myrcene (2.54%), methyl chavicol (1.14%), nerol (7.89%), bornyl acetate (1.60%), $\alpha$-phellandrene (2.50%), geranenol (6.99%), $\beta$-pinene (2.04%), azulene (1.0%), $\alpha$-thujene (9.37%), 1,8-cineole (7.92%), caryophyllene (1.20%), limenlyl acetate (1.91%), $\alpha$-terpinolene (5.90%), elemol acetate (12.0%), chavicol (4.34%), limonene (1.2%), and borneol (18.9%).

The essential oil of *M.hortensis* leaves was found to be mixture of $\alpha$-pinene (6.32%), $\beta$-pinene (2.41%), d-limonene (2.94%), camphene (1.92%), bornyl acetate (3.51%), $\beta$-ocimene (2.13%), $\alpha$-thujene (1.12%), 1,8-cineole (7.32%), myrcene (2.84%), p-cymene (4.0%), d-$\alpha$-terpineol (4.8%), chavicol (3.25%),
carvacrol (6.7%), geraniol (1.94%), eugenol (26.0%), azulene (1.92%) and caryophyllene (1.60%).

(2) SPECTROSCOPIC (UV,IR) STUDIES OF ESSENTIAL OILS OF A.indica, E.triplinerve and M.hortensis LEAVES

The essential oils from the leaves of Anisomeles indica, Eupatorium triplinerve and Majorana hortensis have also been studied by U.V. and I.R. spectral analysis.

Quantitative estimations of the components of these essential oils by spectroscopy gave the results which are tabulated below:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>E.Oil of plant</th>
<th>Percentage of constituents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methylchavicol</td>
</tr>
<tr>
<td>1.</td>
<td>A.indica</td>
<td>9.76</td>
</tr>
<tr>
<td>2.</td>
<td>M.hortensis</td>
<td>3.60</td>
</tr>
<tr>
<td>3.</td>
<td>E.triplinerve</td>
<td>4.30</td>
</tr>
</tbody>
</table>

The various constituents in the above essential oils were also confirmed by I.R. spectral studies.

(3) COLOURIMETRIC STUDIES OF ESSENTIAL OILS OF M.hortensis, A.indica and E.triplinerve LEAVES

The quantitative estimations of the constituents of the above essential oils was also done colourimetrically. The results are tabulated below:
<table>
<thead>
<tr>
<th>S. No.</th>
<th>E.Oil of No. plant</th>
<th>Percentage of constituents (%)</th>
<th>α-terpineol</th>
<th>Eugenol</th>
<th>1,8-cineole</th>
<th>Limonene</th>
<th>Citral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Majorana hortensis</td>
<td></td>
<td>4.90</td>
<td>25.50</td>
<td>7.50</td>
<td>2.40</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Anisomeles indica</td>
<td></td>
<td>3.00</td>
<td>25.00</td>
<td>12.00</td>
<td>2.80</td>
<td>10.00</td>
</tr>
<tr>
<td>3.</td>
<td>Eupatorium trilinerve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.00</td>
<td>-</td>
</tr>
</tbody>
</table>

(4) STUDIES ON ANTIMICROBIAL ACTIVITIES ON ESSENTIAL OILS OF Eupatorium trilinerve, Majorana hortensis and Anisomeles indica LEAVES

The essential oils obtained from the leaves of E.trilinerve, M.hortensis and A.indica were studied for their antimicrobial activities. The essential oil of E.trilinerve was found to be highly active against Escherichia coli, Proteus vulgaris, Aspergillus flavus and Penillium digitatum, where as the essential oil of M.hortensis was found to be highly active against Bacillus anthracis, Proteus vulgaris, Salmonella stanely, Streptococcus agalacties, Aspergillus fumigatus and Aspergillus nigher. The essential oil of A.indica was having high antimicrobial activity against Bacillus anthracis, Proteus vulgaris, Salmonella stanely, Aspergillus fumigatus and Aspergillus niger.
REFERENCES


