CHAPTER I

INTRODUCTION
"God who gives the wound gives the salve!"

Rightly has this been said as disease, decay and death have always coexisted with life and the study of disease and their treatment must have been contemporaneous with the dawn of human intellect. It is good for a man perplexed and lost amongst many thoughts to come into closer intercourse with nature and to learn her ways and catch her spirit. It is no fancy to believe that if the children of this generation are taught a great deal more than it used to be taught of nature and the ways of God in nature, they will be provided with the material far-far healthier, happier, less perplexed and anxious lives than most of us now living.

The progress in the study of medicinal plants is closely connected with the essential needs of life and with the advance of civilization. The study of medicinal plants is one of the mightiest suggestive agents active in the present day life, it affects both the individual and the groups who are constantly facing new threats and menaces, by offering new promises, new openings and unexpected horizons for the future.

"Nothing more difficult than a beginning" wrote Byron, of Poetry, and his remark may aptly be applied to the history of
medicine, for it is no easy task to describe the means by which our primitive ancestors discovered and developed the art of healing. The first written document treating medicaments and medication indicates that fragment plants have always been considered to possess prophylactic power, especially against contagious diseases. A study of the history of materia-médica reveals that essential oils are some of the most important constituents of medicinal plants. As a matter of fact, the great potency of many of the well-known Indian medicinal plants has been shown on careful investigation in recent years to be entirely due to the presence of essential oils. It is due to this that the use of medicinal plants and their extracts on open wounds was a general practice from times immemorial. The spices and aromatics have from the very beginning been administered to the needs and welfare of man and hence very much appreciated by him.  

Natural perfume is one of the most remarkable phenomena of plant metabolism and no wonder, the history of aromatics is perhaps the most romantic story of any vegetable product. Through the 'corridors' of time and throughout the mist of ages, the fragrance impulse has always influenced human beings. The primitive man must have been struck, not only by the elegance and vivid colouring of the flowers, but also by the
fragrance of the vegetation around him. It is also almost certain that this must have aroused his curiosity as much as did the utilisation of plants for his food and clothing.\(^4\)

The universal appreciation of the eastern spices and aromatics constituted the first foundation of international trade and were among the earliest articles of barter and commerce. India was the first to discover musk, a rich and alluring perfume, treasured through the ages. But as in many other fields of human endeavour, it was in the occident, however, that their first attempts reached full development.

One of the earliest reference to medicinal use of plants is found in Ebers Papyrus (which was discovered in \(1873\)) this dates approximately the 16th century B.C. It described more than 700 herbal remedies including many which are familiar today. The list mentions poppy, castor oil, squills, aloes and caraway. The scholar emperor Shen Nung (2735 B.C.) compiled a book of herbs and is credited with observing the antifebrile effects of 'Chang-Shan' which has now been shown to contain antimalarial alkaloids.\(^5\) He also noticed the diaphoretic and stimulatory effect of the drug 'Ma-Huang' from which almost 5000 years later Na Cai isolated the active alkaloid ephedrine. The antileprotic action of 'Chaulmogra' fruit was known to
ancient Indians. 'Ipecacuacha' root was used in Brazil and the far east for the treatment of dysentery and diarrhea. Its alkaloid emetine still continues to be an important drug for amoebiasis.

One great herbal drug of the 17th century which has survived the test of time, is the 'Cinchona' bark, from which later quinine was extracted. Similarly the roots of an indigenous plant 'Rauwoofia Serpentina' has been widely used in India and Malaya as an antidote to insect and snake bites. Vakil has investigated its use in hypertension and found it to have a marked hypotensive action. In the field of the treatment of tuberculosis indigenous plants are proving of great value. Japanese workers have isolated from vine named 'Stephania Cepharantha' and from 'Stephania Sanskil' the alkaloid 'Cepharanthine' which is being used for treatment and prophylaxis of tuberculosis. Recent studies on various species of Podophyllum have indicated the presence of anticancer constituents in the plant. A few examples thus cited above clearly bring out how researches in the field of medicinal herbs have ameliorated human sufferings.

Composition of the drugs were progressively investigated and many new components were identified and manufactured commercially. The industry of synthetic and isolated aromatics
which had its origin mainly in the work of the great explorers, illustrious names among which are those of \( G. \) Aschan, E. Gildemeister, H. Walbaum, S. Bertram, A. Hesse, G. Kleber, F. Kremer, P. Barbier, L. Bouvetault, E. Charabot, L. Puzicka and J. L. Simonsen whose classical investigations are still being carried on with ever greater enthusiasm by Barton in London, S. N. Thattacharya and Sukh Dev in India, not to mention, other diligent and skilled workers in the United States, British Commonwealth, France, Germany and the Far East.

**CHEMISTRY OF PLANT PRODUCTS:**

Plant products may often provide materials of biological activity. The chemical composition of a plant is completely ascertained only when the nature and quantity of all the chemical individuals of which it is composed are known. Seasonal changes, differences in habitat and chemical nature of the soil and artificial interference by man in cultivation can produce far reaching changes in the qualitative as well as quantitative composition of the plant.

The efficacy of the plant products has most often been shown to be due to the active principles contained therein. These active principles fall under the following group of compounds:

(1) Essential oils, (ii) Alkaloids, (iii) Glycosides,
Essential oils:

Paracelsus Von Hohenheim (1493-1541) considered essential oils as "the last possible and most sublime extractive, the 'quinta essentia' which represents the efficient part of every drug." Though the truth of this theory has not been fully corroborated the original term 'Essentials' from the 'Quinta Essentia' is still retained.

Centuries ago it was discovered that the active principles responsible for the odours of plants could be separated from them by gentle heating with water. In course of time a number of methods developed for the isolation of the essential oils from various plant materials are (i) steam and water distillation, (ii) expression, (iii) enflourage, (iv) maceration and (v) solvent extraction method. Details of these methods are described in standard works on essential oils. The investigations on the chemistry of essential oils were started by Dumas followed by others. Berthelot (1827-1907), studied mainly the hydrocarbons with the formula C_{10}H_{16} contained in these oils and Kekule (1829-1896) mentioned the name 'terpene' for these hydrocarbons in 1866. Tilden (1842-1926) introduced nitrosyl chloride as a reagent for terpenes in 1895. Wallach (1847-1931) perfected the reaction and Semmler (1860-1931) followed him and isolated many terpenes and terpenic derivatives from essential oils.
The constituents present in essential oils have been classified into four groups which are characteristic of the majority of the essential oils.\(^{15}\)

1. Terpenes, related to the isoprene or isopentane.
2. Straight chain compounds, devoid of any side branches.
4. Miscellaneous compounds.

Each of these groups have been discussed in details by various workers in the field.\(^{16-22}\)

**INDIA'S HERITAGE**

The history of aromatics in India can be traced from the remote past. The earliest mention of the medicinal use of plants is to be found in Rigveda written between 4500 and 1600 B.C. In Atharvaveda, which is a later production, the use of drugs is more varied in many instances of charms etc. It is in the Ayurveda, which is considered as an Upaveda that definite properties of drugs and their uses have been given. Ayurveda which was written by Charaka and Sushruta deals with medicine and there is a remarkable description of the materia-medica as it was known to ancient Hindus. It also indicates that Indians were well acquainted with the primitive apparatus of distillation. Some books of Pali, Sanskrit and Islamic literature also deal with the Indian perfumery industry in the past. Huen-tsang and Fanien,
the two famous Chinese travellers have described India as the land of aromatic grasses, flowers, fruits, woods, rhizomes, resins and gums.  

Sandalwood oil, aromatic spices and various other oils of Indian origin have been favourites of the West for centuries and because of enormous aromatic wealth of the country, it was but natural, that there developed with them an extensive trade in essential oils, perfumes and other raw materials. This was responsible in the country for the development of a number of centres of high class perfumes viz., Kannauj, Jaunpur, Bangalore, Poona etc., and in view of the growing trade in the world market, the government of India has started a number of research centres in the country. India thus became known to the entire civilized world as an important place for the essential oil industry.

PRESENT POSITION:

India once a forerunner, has, however, lagged behind the West in keeping pace with the modern developments of the art of perfumery. Because of lack of scientific knowledge and growing competition, the essential oil industry in India has suffered a set back and lost the hold in the world market. But with the dawn of independance and recognition of this industry as a scheduled industry by the popular government, there developed
a number of research centres and industrial concerns under the
guidance of C.S.I.R. to explore new sources of income from the
nursery of forest resources. A research committee\textsuperscript{24} was also
constituted which surveyed successfully Vetiver, Jasmine, Roses,
Citrus plants and high class Patchouli plants for their essential
oils; some of the other items available in large quantities in
the country are oils of lemongrass, sandalwood, turpentine,
palmarosa, ginger, eucalyptus, cardamum etc. With the gradual
impetus to the essential oil industry many new terpenic compounds
like Jasnone\textsuperscript{25}, Jatamansone\textsuperscript{26}, Longifolene\textsuperscript{27}, Constunolide\textsuperscript{28} and
Azulenes\textsuperscript{29} have been successfully synthesised at important centres
of research like the National Chemical Laboratory, Poona etc.

Some of the spices and essential oil bearing raw
materials having a monopoly status in the world such Ajowan seeds,
Costus roots, Pepper, Indian Pimmsed etc., are exported as such
to foreign countries. A number of essential oils such as nutmeg,
caraway, patchouli, geranium, citronella etc., are imported in
large quantities. A glance at table 1 reveals the average import
of essential oils in India during the years 1965-67 amounting to
Rs. 91,88,938 and including perfumes and flavour materials
amounting to Rs. 98,33,616 where as the average export\textsuperscript{31} for this
period amounts to Rs. 2,91,24,106 and including perfumes and
flavour materials to Rs. 2,96,22,396. These figures even though
encouraging warrant further development of this industry. In this
connection it is gratifying to note that the government has established a number of Regional Research Laboratories such as at Hyderabad, Jammu (Kashmir), Bangalore, Kanpur, and a number of universities are also busy in this project. The Central Drug Research Institute, Lucknow, and Forest Research Institute, Dehradun are also exploring new resources of income from the unexploited aromatic wealth of the country lying hidden in the treasure of forest resources, to earn a good foreign exchange and to put the national economy on a sound footing. It is hoped that by the joint efforts of the scientists, industrialists and the government, the industry will develop and capture the world market and once again regain the past glory of the country.
### TABLE I

Essential oils, perfumes and flavour materials (Imports)\(^{30}\)

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Twelve month ended</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April'64 to March'65.</td>
<td>April'65 to March'66.</td>
</tr>
<tr>
<td>Bergamot oil</td>
<td>(Rs.)</td>
<td>(Rs.)</td>
</tr>
<tr>
<td>Citronella oil</td>
<td>392871</td>
<td>396118</td>
</tr>
<tr>
<td>Clove oil</td>
<td>1127202</td>
<td>623072</td>
</tr>
<tr>
<td>Geranium oil</td>
<td>275100</td>
<td>263628</td>
</tr>
<tr>
<td>Patchouli oil</td>
<td>10812</td>
<td>1259713</td>
</tr>
<tr>
<td>Peppermint oil</td>
<td>245241</td>
<td>446749</td>
</tr>
<tr>
<td>Ylang Ylang oil</td>
<td>2017134</td>
<td>1800566</td>
</tr>
<tr>
<td>Natural essential oils NES</td>
<td>258437</td>
<td>185120</td>
</tr>
<tr>
<td>Resinoids</td>
<td>1964852</td>
<td>1575974</td>
</tr>
<tr>
<td>Other items</td>
<td>384161</td>
<td>341404</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td>8619725</td>
<td>8786768</td>
</tr>
<tr>
<td>Synthetic perfumes and flavour material.</td>
<td>909870</td>
<td>100406</td>
</tr>
<tr>
<td>Mixtures, Aromatic chemicals, synthetic essential oils.</td>
<td>617544</td>
<td>-</td>
</tr>
</tbody>
</table>

Total group 551. | 9529595 | 9504718 | 10436534
## TABLE I

Essential oils, perfumes and flavour materials (Export)\textsuperscript{31}

<table>
<thead>
<tr>
<th>Essential oils.</th>
<th>Twelve months ended</th>
<th>June \textsuperscript{66} to March \textsuperscript{67}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April \textsuperscript{64} to March \textsuperscript{65}</td>
<td>April \textsuperscript{65} to March \textsuperscript{66}</td>
</tr>
<tr>
<td><strong>Citronella oil</strong></td>
<td>2200</td>
<td>28333</td>
</tr>
<tr>
<td><strong>Keara oil</strong></td>
<td>60875</td>
<td>51406</td>
</tr>
<tr>
<td><strong>Lemongrass oil</strong></td>
<td>14021214</td>
<td>5970325</td>
</tr>
<tr>
<td><strong>Palmarosa oil</strong></td>
<td>1990368</td>
<td>1491025</td>
</tr>
<tr>
<td><strong>Sandalwood oil</strong></td>
<td>14582088</td>
<td>13466152</td>
</tr>
<tr>
<td><strong>Vetiver oil</strong></td>
<td>243246</td>
<td>668294</td>
</tr>
<tr>
<td><strong>Other items</strong></td>
<td>1288733</td>
<td>1076442</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>32188724</td>
<td>22751977</td>
</tr>
<tr>
<td><strong>Synthetic perfumes and flavour material etc.</strong></td>
<td>660146</td>
<td>541693</td>
</tr>
</tbody>
</table>

Total group 551. 32848870 23298670 32724648
BIOSYNTHESIS

The first attempts to propound a theory for the biosynthesis of terpenoids were stimulated by the fact that the vast majority of the simpler members of this class had structures which could be built up in principle, of two or three isoprene units. The simple rule that the terpenoids are theoretically divisible into isoprene units holds good to a very large extent. Thus the monoterpene, have the molecular formula \((\text{C}_5\text{H}_8)_2\) and the sesquiterpenes \((\text{C}_5\text{H}_8)_3\) and both are built up of isoprene units as proposed by Wallach. The nature of the union of these units - head to tail - has, however, been discovered later, and is governed by the isoprene rule. This rule has been elaborated and reviewed by Ruzicka.\(^{32}\)

The problem of biosynthesis therefore resolves itself as follows - firstly, there is the question of the creation of five carbon units; secondly, the manner in which the combination of these units takes place to give the postulated precursors of the various terpenoid families, and finally there is the mechanism of cyclisation and rearrangement of these precursors or their equivalents to give the individual terpenoids.

During the past 20 years, evidence has been accumulated indicating that the isoprene biogenetic hypothesis is probably an extension of a more fundamental rule, in which the repeating unit is
a simple molecule - acetic acid (as active acetate). It has been envisaged that the isoprene unit may be derived from three molecules of acetic acid with the loss of a carboxyl group; the intermediate product senecioic acid(I) being found in nature (in certain species of senecio), its semi-dimethyl groups are reactive, being activated by the carboxyl group through the double bond. It may be observed that the five carbon atoms are derived alternately from the methyl and the carboxyl groups of the two original acetic acid molecules.

\[
\begin{align*}
\text{CH}_3\text{COCH} + \text{CH}_3\text{COOH} & \xrightarrow{\text{H}_2\text{C}} \text{CH}_3\text{COCCH}_3 \\
\text{acetic acid} & \xrightarrow{\text{H}_2\text{C}} \text{CH}_3\text{COCCH}_3 \text{(}-\text{H}_2\text{O}) \\
\text{HOCCH}_2\text{C} & \xrightarrow{\text{H}_2\text{C}} \text{CH}_3 \xrightarrow{\text{H}_2\text{C}} \text{C}=\text{CHCOOH}
\end{align*}
\]

\text{Senecioic acid (I)}

This hypothesis has been supported by the experiments of Bonner and his collaborators on the formation of rubber in vitro by seedlings and isolated stem sections of the Guayule plant. By using isotopically labelled acetate, it has been shown that acetate is incorporated into the rubber formed by the plant, showing that the added acetic acid participates in rubber
biosynthesis. Bloch and his associates have established the utility of acetic acid as the primary building unit for the synthesis of cholesterol in the animal tissues, and have thus revived the hypothesis of Channon and Robinson that squalene, an acyclic terpene is an intermediate in the biosynthesis. This view has further been substantiated by Langdon and Bloch with the isolation of radioactive squalene from the livers of rats fed with squalene and C\textsuperscript{14}-acetate; the radioactive squalene being partly converted into radioactive cholesterol. Bloch has suggested that squalene is formed from the isoprenoid units like

\[
\begin{array}{c}
\text{C - m = C} \\
\text{m - C - m = C}
\end{array}
\]

where m, and C are respectively carbon atoms derived from methyl and carboxyl fragments of the acetic acid molecule. Evidence has also been provided for the formation of Lanosterol as an intermediate between squalene and cholesterol, and a probable route outlined as below.

\[
\text{Acetate} \rightarrow \text{isoprene unit} \rightarrow \text{squalene} \rightarrow \text{lanosterol} \rightarrow \text{cholesterol.}
\]

The cyclisation of squalene(II) to lanosterol(III) has been represented by Ruzicka as follows:
The formation of other triterpenes can also be envisaged by other modes of cyclization. It has, however, been suggested that the mevalonic lactone (β-hydroxy-β-methyl-γ-valerolactone) (IV) is a much better precursor than senecioic acid. \(^{16}\) Investigations by Lynen established that the sequence of events follow as shown in the chart.

\[
\begin{align*}
\text{CH}_3 - C - & \text{SCoA} + H_2\text{CH}_2 & \text{C} = \text{SCoA} & \iff \text{HSCO} + \\
0 & & 0 & & \text{CH}_2 & \text{C} = \text{CH}_2 & \text{C} = \text{SCoA} & 0 & 0
\end{align*}
\]

acetyl coenzyme A  \hspace{1cm} \text{acetoacetyl coenzyme A}
Acetyl coenzyme A + Acetoacetyl coenzyme A → \( \text{P-hydroxy P-methyl glutaryl CoA} \)

\[ \begin{align*}
2\text{TPNH} & \quad \text{ATP} \\
& \quad \text{ADP} \\
\text{mevalonic acid} & \quad \text{mevalonic acid 5-phosphate} \\
& \quad \text{Mevalonic acid pyrophosphate} \\
& \quad \text{isopentenyl pyrophosphate} \\
& \quad \text{B} \quad \text{B-dimethyl allyl pyrophosphate}
\end{align*} \]
A scheme for the formation of acyclic monoterpenes from seneconic acid can easily be planned as given in standard works (Tavormina, Gibbse et al.) and then the monocyclic monoterpenes can be formulated by the well known cyclisations of open chain precursors, for example, geraniol may give rise to \( \alpha \)-terpineol, and thence to limonene; citral may afford menthol which can be oxidised to menthene; cineole and carvone may originate from \( \alpha \)-terpineol. Bicyclic monoterpenes may also arise from acyclic precursors, for example, possible synthesis of pinene from ocimene envisaged by Ruzicka. In sesquiterpenes also farnesol and related acyclic compounds follow routes analogous to geraniol - three possible modes of cyclisation giving rise to three main types of cyclic
sesquiterpenes, thus developing a farnesol rule in sesquiterpenes corresponding to isoprene rule. The azulenes and other large ring sesquiterpenes for example, guaiol and caryophyllene can also be derived from farnesol. Thus the well known sesquiterpene exception to the isoprene rule, eremophilone can certainly be envisaged as derived from a normal hypothetical precursor in which migration of a methyl group causes a rearrangement. Similarly the diterpenes can be derived from a regular isoprene tetrramer - the phytol rule. Biochemical type synthesis are in progress at the present day by laboratory cyclisation of acyclic polymers and provide useful and interesting models for the enzymatic processes involved. These have been reviewed by MacMillan.

**FIXED OIL**

Fats and oils not only are an essential part of the diet of men and animals, but they play an important role in many industries. Consequently, the life and progress of a nation depends in no small measure on its supply of these products. The demand for fats and oils increases with the population and through the discovery of new uses for these substances.

While the quantity of oils and fats from land and marine sources may be limited, supplies of these from vegetable origin can be unlimited for the reason that vegetation is in
greater abundance than the animal wealth particularly in the
tropical and subtropical regions of the world. For a country
like India, which has already abundance of oil seeds—both
cultivated and from forest sources, their proper and full utiliza-
tion for the various industries is of prime importance. In this
connection, it became necessary to have as thorough and intimate a
knowledge as possible about the composition and chemistry of every
individual oil and fat.

The fatty acids, as the name signifies, comprise
the building units of all natural fats, and in addition, of oils
and waxes. They occur in nature in various combinations, the
most important being glycerides (V) as in fats, phosphatides (VI),
as in phospholipids like lecithin, and esters (VII) of monohydric
alcohols, as in waxes like spermaceti (consisting of esters of
octyl alcohol, \(C_{16}H_{33}OH\), with lauric, myristic and palmitic acids).

\[
\begin{align*}
CH_2\cdot O \cdot CO \cdot R & \quad CH_2\cdot O \cdot CO \cdot R \\
| & | \\
CH \cdot O \cdot CO \cdot R' & CH\cdot O \cdot CO \cdot R' \\
| & | \\
CH_2\cdot O \cdot CO \cdot R'' & CH\cdot O \cdot P \cdot (OH) \cdot OR
\end{align*}
\]

(V) \hspace{2cm} (VI) \hspace{2cm} (VII)

\(R, R', \text{ and } R'' = \text{ alkyl radicals alike or different;}
\)
\(B = \text{ nitrogen base like choline.}\)
The fatty acids were thought to consist of a straight chain of carbon atoms with a methyl group at one end, and a carbonyl group at the other. Investigations in recent years have, however, established the existence in nature of an appreciable number of fatty acids having branched aliphatic chains, cyclic substituents, triple bonds, keto groups, hydroxy groups, and various systems of double bonds. The naturally occurring fatty acids nevertheless can be simply divided into saturated and unsaturated acids. Both the classes have been exhaustively dealt with in standard works. 47-49

Certain fatty oils of plant origin possess the ability, when exposed on a surface to air, to form dry, tough and durable films. These vegetable oils are, therefore, classified, depending on the ability into non-drying (iodine value below 100), semidrying (iodine value between 100 and 130), and drying (iodine value above 130), oils. This property of forming a hard film depends on the proportion of highly unsaturated acids present in the oils and finds an important application in the paint industry. Thus the semidrying oils contain substantial percentages of monoethenoid acids (like oleic) together with lesser amounts of more highly unsaturated acids (like linoleic and linolenic), whereas the drying oils contain large percentages of the diethenoid and triethenoid acids.
Distribution, formation and utilization by plants:

Fats and oils are widely distributed both in the animal and vegetable kingdoms. In plants, they are found chiefly in the spores, seeds, and fruits, but they occur also in the leaves, roots and other vegetative organs. Their function in the leaves is not yet clear, but those in the spores, seeds and some tubers constitute a food reserve to be drawn upon during germination and the early life of the plant. In the early stages of germination the total fat present undergoes little diminution, but following this period it rapidly diminishes. During the later stages, the fat contains a considerable portion of free fatty acids. Rhine\textsuperscript{50} states that during germination the fat is not transported as such but is first converted into carbohydrates before being transported into the plant.

According to the generally accepted view, the fat is formed from carbohydrates. It is noteworthy that the fatty acids found in fats contain an even number of carbon atoms. The fact that C\textsubscript{18} acids preponderate would suggest the hypothesis that fats are derived from polysaccharides or perhaps, from three molecules of glucose.

Formation of lower acids may take place from simple sugars instead of complex polysaccharides. There is considerable evidence that the fatty acids are produced first and then at a
later stage these are combined through the agency of lipase with glycerol to form the triglycerides.\textsuperscript{51-53} Also it is believed that glycerol is formed from carbohydrates.

In general there appears to be some relationship between the composition of fats and the ecological conditions. The fats, with a few exceptions, from tropical plants are characterised by containing notable percentages of saturated acids, whereas those from plants growing under colder climatic conditions contain large proportion of unsaturated acids. Consequently, drying and semidrying oils are of more frequent occurrence in the plants of temperate climates than in those found in the tropics. On the other hand those of the nondrying class predominate in tropical regions.\textsuperscript{54}

With the application of modern methods for the investigation of fats, including those developed by Hilditch\textsuperscript{55} and co-workers, it has been possible to determine the component glycerides of fat and a more intimate insight has been obtained not only as to their character and quality but also the way in which these are combined to form glycerides. Hilditch\textsuperscript{55} has found a striking similarity in the mixtures of glycerides composing the seed fats from closely related plants.

\textbf{Unsaponifiable matter :}

In addition to glycerides, which constitute by far
the major portion of the fats, the fixed oils contain small percentages of unsaponifiable matter, which includes all those substances which are not saponified by alkali and are soluble in ether and light petroleum. This consists of sterols and small quantities of other alcohols (e.g., tocopherols) and hydrocarbons, such as squalene (VIII), \( (C_{30}H_{50}) \), tricosane \( (C_{30}H_{62}) \) and pentatriocnate \( (C_{35}H_{72}) \). These may be separated from sterols and other alcohols by well known methods. 58

\[
\begin{align*}
\text{CH}_3 & & \text{CH}_3 & & \text{CH}_3 \\
(CH_3)_2C=CH_2(CH_2)_2C=CH_2 & & (CH_2)_2C=CH_2(CH_2)_2C=CH_3 & & (CH_2)_2C=CH(CH_3)_2
\end{align*}
\]

(VIII)

**Sterols**:

The sterols are crystalline polycyclic, secondary alcohols containing an aliphatic side chain. These are widely distributed in nature, occurring both as free and as esters of higher aliphatic acids, and may be classified on the basis of their occurrence as zoosterols (in animals), phytosterols (in plants) and mycosterols (in kryptogams, particularly in fungi). In plants sterols have no known function, although these have profound importance in the animal metabolism as hormones, co-enzymes, bileacids and provitamin D. The steroidal alkaloid,
tomatine(IX), may be somehow related to flowering since its concentration may be photoperiodically controlled in the same way as the flowering response.\textsuperscript{59} Pregnane type of sterols have recently been found in plants.\textsuperscript{60} A thorough survey of plant sterols is given by Börjesson.\textsuperscript{61}

![Chemical structure](image)

(IX)

Both the saturated and the unsaturated sterols occur in nature, but the latter for the most part, are present in much larger quantities. All the sterols show optical activity, those which are unsaturated being laevoratory.

The following table gives a few phytosterols commonly met with.\textsuperscript{62}
<table>
<thead>
<tr>
<th>NAME</th>
<th>FORMULA</th>
<th>m.p.(_c)</th>
<th>(\alpha)_D</th>
<th>Position of double bond</th>
<th>SOURCE(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stigmasterol</td>
<td>C(<em>{29})H(</em>{48})(\delta)</td>
<td>170</td>
<td>-49</td>
<td>5:6</td>
<td>Soyabean, Calabarbean.</td>
</tr>
<tr>
<td>Ergosterol</td>
<td>C(<em>{28})H(</em>{44})(\delta)</td>
<td>165</td>
<td>-130</td>
<td>5:6 5:8</td>
<td>Ergot, Yeast.</td>
</tr>
<tr>
<td>Brassicasterol</td>
<td>C(<em>{28})H(</em>{46})(\delta)</td>
<td>145</td>
<td>-64</td>
<td>5:6</td>
<td>Rapeseed oil.</td>
</tr>
<tr>
<td>-spinasterol</td>
<td>C(<em>{29})H(</em>{48})(\delta)</td>
<td>168</td>
<td>-27</td>
<td>7:8</td>
<td>Spinach, Sengoroot.</td>
</tr>
<tr>
<td>-sitosterol</td>
<td>C(<em>{29})H(</em>{50})(\delta)</td>
<td>140</td>
<td>-36</td>
<td>5:6</td>
<td>Cottonseed, Talland, Wheat germ oils.</td>
</tr>
<tr>
<td>-sitosterol</td>
<td>C(<em>{29})H(</em>{50})(\delta)</td>
<td>148</td>
<td>-43</td>
<td>5:6</td>
<td>Soyabean and wheat germ oils.</td>
</tr>
</tbody>
</table>
MODERN METHODS OF ANALYSIS

The enormous development in the chemistry of plant products is the outcome of the application of physical and biochemical methods to their studies in addition to the usual chemical methods of reductive and oxidative degradations and specific colour reactions, which have led to the discovery of a large number of compounds and ultimately to the determination of their structure and synthesis.

The following techniques are of immense help in phytochemical investigations: Chromatography, colorimetry, magnetic resonance spectroscopy, mass spectrometry, X-ray crystallography, Raman spectra and others. These are briefly reviewed below:

Chromatography:

It has become a prominent method of isolation, purification and characterisation of constituents that one comes across during the phytochemical investigations e.g., Rothe\textsuperscript{64} has utilised it to separate oxygenated compounds and hydrocarbons from essential oils. Nigam and Kumari\textsuperscript{65} have used it for the analysis of the essential oils of citrus decumana, carrot and lemon oil, and isolated gratissimol, a new sesquiterpene alcohol from the oil of ocimum gratissimum. Several reviews\textsuperscript{66-68} to illustrate the
general utility of the technique are available and its various forms given below:

1. Partition chromatography.
2. Adsorption chromatography.
3. Thin layer chromatography.
4. Vapour phase chromatography.
5. Ion exchange chromatography.

It would, however, be worthwhile to give a brief resume of some of the important ones used in the present investigations.

**Thin layer chromatography (TLC):**

TLC is a very valuable supplement to column and paper chromatography and has become of great importance in analytical separation of natural products. It not only combines the advantages of paper and column chromatography but in certain respects, it is superior to either of them. Kirchner, Miller and Keller have published a number of papers on the separation of terpenes and essential oils on chromatostrips, or on sheets for two dimensional separations and described an apparatus for preparing chromatoplates. Peitsma extended the technique by preparing large plates (5"x7") and used them to separate the constituents of essential oils. The wider applications of TLC were, however, not visualised until 1956 when Stahl extended the application of the technique to separate the components other
than terpenes. Several papers notably by Demole, Stahl and Wollison et al. and recent books by Benderath, Truter, Bobbitt, Stahl, Marini and Calvin give theoretical and practical review of the technique.

**Gas liquid chromatography :**

It has also been referred to as vapour phase chromatography and is a new technique. Gas liquid chromatography has generally been employed for the analysis of gases and liquids with boiling points up to 400°C. The separation in the column is obtained mainly by partition. The stationary phase, in the separation tube consists of a carrier which can be loaded with liquids of various polarities. The gas or vapour mixtures to be analysed is led through the column at a definite rate by means of an inert carrier gas (mobile phase), the length of the column is adjusted to the amount of substances under analysis. The gases used are nitrogen, hydrogen or helium. In the analysis of essential oils the stationary phases that are used, are polyethylene glycol and silicone oil. By programming the temperature of the capillary columns, the polar stationary phases can be used without rapid deterioration of the life of the column. Bevitt, and Cheshire have discussed conditions which give maximum performance in GLC analysis of essential oils. Recently the interpretation and value of GLC data for essential oils has been reviewed by Kingston and the characterisation of
linalool and linalyl acetate\textsuperscript{37} and isomeric citral-\textsubscript{L} and -B has successfully been accomplished.\textsuperscript{38}

The application has also been extended to unsaponifiable matter, aliphatic alcohols, tocopherols, and triterpenoid alcohols.\textsuperscript{39}

**Absorption spectroscopy:**

Now a days, it has become an important tool for an organic chemist. The adsorption spectroscopy using ultra-violet and visible light, was the earliest physical method employed for the examination of molecular structure. Usually the data obtained from ultra-violet spectra is used in conjunction with other spectral data for this purpose.

**Ultra-violet absorption spectroscopy:**

The advantage of this technique is mainly in terpenoids in the detection of conjugation. Empirical rules proposed by Woodward and modified by Fiese\textsuperscript{40} are available for predicting the positions of the maxima of the high intensity absorption bands (ca 7-20,000) of such systems as conjugated dienes, trienes and conjugated ketones etc. The position of the maximum is dependent on the basic chromophore and the degree of substitution while exception may be met with, these rules
nevertheless afford a valuable guide. If a solvent other than alcohol is used a correlation of the values may be needed.

An example of the use of this method may be cited in the work of Mequillin\(^{91}\) who, by a comparison of the ultra-violet absorption spectra of their derivatives like the oximes and 2:4 dinitrophenyl hydrazones, concluded that \(\alpha(XI)\) and \(\beta(XI)\) eryperones were structural isomers and not stereo-isomers.

\[
\begin{align*}
(X) & \quad \quad \quad \quad (XI)
\end{align*}
\]

Visible and ultra-violet spectra are of great assistance in locating the positions of nuclear alkyl groups in the azulenic series\(^{92}\).

**Infra-red spectroscopy**

Infra-red technique has been widely employed in the structural diagnosis, particularly in the field of natural products, such as alkaloids, sesquiterpenes, steroids and steroidal sapogenins, where the compounds isolated in the pure
form are many a times available in small quantities. The absorption is measured in the 650 cm⁻¹ - 400 cm⁻¹ region of the spectrum yielding a complex absorption curve containing many bands or peaks some of which are characteristics of groups in the terpene molecule. Bellamy⁹³ and Pace⁹⁴ have given good reviews for the empirical interpretation of the infra-red absorption of organic compounds. The technique has also been used for the quantitative estimation of terpenes like camphor in sassafras oil.

**Nuclear magnetic resonance spectroscopy:**

N.M.R. technique is at present a very powerful tool in the hands of organic chemist. It is gradually being applied more and more in the study of the structure of various terpenoid compounds. NMR studies on essential oil include the detection and identification of double bonds, the determination of number of rings and the analysis of mixture of isomeric terpenes. Its main advantages are the speed and certainty with which it can identify certain functional groups under wide variety of conditions, without the necessity of precise calibration.

**Mass spectrometry:**

Mass spectrometry has proved useful for finding out the accurate molecular weights. Thus, it has been successfully utilised to distinguish readily between C₃₀ and C₃₁ substances
in triterpenoids and long chain fatty acids. By this method even the substances containing isotopic oxygen such as $^{16}\text{O}$ and $^{18}\text{O}$ or isotopic chlorine $^{35}\text{Cl}$ and $^{37}\text{Cl}$ can be separated and identified according to their different mass. Applications of the method to essential oils are also innumerable. The importance of the technique can well be understood by taking the example of ylangene and copaene. The I.R. spectra demonstrate similarly, showing absorption at 790 and 780 cm$^{-1}$ respectively indicating a trisubstituted olefin. U.V. spectra of the two are strikingly similar, showing the presence of one vinyl proton, one vinyl methyl, one unsplit methyl and two doublet methyls. Both have got the same molecular weight, 204. These were shown to be steric-isomers by Hunter and Brogden by observing the different mass spectral cracking pattern of the two isomers. The details of the use of mass spectrometry for the characterisation of plant products has been given by Mathieson, Paech and Tracy and Pakrashi.

Raman spectra:

The Raman spectra of 15 essential oils has been discussed by Mohan who found the data to be adequate for the identification of the major constituents of the oils. Michel studied the carbonyl Raman bands of a large number of esters, ketones and aldehydes. Priner studied the Raman and
infra-red spectra for the derivatives of eugenol, anethol and estragole.

X-Ray:

The X-ray diffraction method is the most direct of the physical methods of structure determination. Structures deduced from chemical and other physico-chemical evidence have been confirmed by the plot of electron density maps. A remarkable application of this method has been in the elucidation of the structure of the sesquiterpene longifolene(XII) at a time when little was known about the chemistry of the compound. The structure assigned by X-ray diffraction studies has been confirmed by chemical methods.

( XII)
PROBLEM TAKEN AND WORK DONE

Phytochemistry is a dynamic science and much of the fascination of the subject is due to the ever increasing utility of its advancements. Each time some new facts are discovered for an old idea amended, having a profound impact on the human life. This gives a great impetus to the new researcher for working with zeal and stemmimg with confidence on new lines of investigation.

Systematic study, harnessing and utilization of the natural resources of any country are among the most important of the activities which should be undertaken by the community for its proper growth and development. Among the vegetable resources a prominent place must be assigned to the medicinal plants.

India, with its wide variability of soil and climatic conditions, is one of the richest sources of fixed and essential oils. Essential oils are some of the most important constituents of Indian medicinal plants. As a matter of fact, the great potency of many of the well known Indian medicinal plants has been shown on careful investigation in recent years to be entirely due to the presence of essential oils. Thus the physiological action of Mentha�iperita is due to
menthol, of ocimum sanctum to eugenol, of pychotisajwan to thymol and so on.

The advent of some of the physical techniques like gas liquid chromatography and nuclear magnetic resonance, recently, in addition to the already known and much used methods like colorimetry, polarography, thin layer and column chromatography and ultra-violet and infra-red spectroscopy have added stimulus to the phytochemist and have made his task rather simpler and more accurate.

Being inspired by the enormous wealth of the plant products of the country and their utility as a source of national wealth and for the amelioration of human sufferings, and with the availability of improved physical methods of analysis in addition to the well established chemical methods, it was thought desirable to work up some fixed and essential oils which have remained hitherto unworked or have been investigated at a time when the above facilities were not available. In addition to this the present author has studied the antimicrobial efficiency of some essential oils against certain pathogenic and non-pathogenic organisms.

With the above object in view, the present investigations were undertaken and the findings divided into three parts:
Part I: Consisting of studies on the essential oils from the seeds of:
(i) Majorna hortensis.
(ii) Anethum sowa.

Part II: Deals with the chemical investigations of the fixed oils from the seeds of:
(i) Cleome viscosa.
(ii) Diospyros melanoxylon.

Part III: Studies on the antimicrobial activity of essential oils.

Each one is briefly summarised below:

PART I: Studies on the essential oils from the seeds of:

(i) Majorna hortensis (Family - Labiate):

The essential oil, from the seeds of hitherto unworked plant Majorna hortensis, has been obtained in an yield of 0.8%, as a pale yellow mobile liquid with a characteristic smell. It has been found to contain methyl chavicol (26.6%), α-terpinol (14.7%), ocimene (6.4%), cadinene (4.3%), Δ3-carene (6.2%), β-caryophyllene (4.6%), d-linalool (9.5%), geranyl acetate (7.8%), citral (5.4%), eugenol (11.2%) and a new hydrocarbon (0.8%).
(ii) *Anethum sowa* (Family - Umbelliferae):

The essential oil of *Anethum sowa* obtained from the seeds in an yield of 1.9% has been completely analysed and found to contain carvone (47.8%), d-limonene (36.1%) dihydrocarvone (2.1%), dillapiol (5.2%) and Δ-pherllandrene (2.7%). Though the presence of these constituents had been recorded by the previous workers, their percentage composition had not been mentioned. In addition to the above constituents, the presence of Δ-pinene (2.4%) and Δ-carphene (1.2%) has also been confirmed by the present author. Thus the essential oil, has been completely analysed and its percentage composition put on a firm footing.

**PART II** Chemical examination of fixed oil from the seeds of:

(i) *Cleome viscosa* Linn (Family - Caparadaceae):

The constituent groups of the seeds of *Cleome viscosa* have been found to be as follows:

- Moisture (8.02%), ash content (5.49%), proteins (31.28%),
- fixed oil (9.00%), total sugars (6.34%), residue (fibre+ nitrogen free extract etc.) (39.99%).

**ASH** The ash has been found to contain water insoluble ash (78.64%), acid insoluble ash (0.83%), alkalinity of total ash (3.68 ml. of 0.1 N acid/gram of the sample) and ash analysed
which contained carbonate, phosphate, sodium, potassium and calcium.

**Proteins:** Total protein presence was estimated by semi micro Kjeldahl's method and found to contain 31.25%.

**Sugars:** Qualitative and quantitative estimations of sugar were made and paper chromatography technique was used for the identification of the constituents. Presence of glucose, fructose and sucrose was established in the seeds.

- Total reducing sugar = 3.025 %
- Total sugar = 6.345 %

**Fixed oil:** A greenish yellow fixed oil obtained in an yield of (9.00%) from the seeds, was found to consist of:

- Saponifiable matter = 85%.
- Saturated acids: 65.8% (palmitic acid, 30.6% and stearic acid 45.2%).
- Unsaturated acids: 34.2% (oleic acid, 19.4% and linoleic acid 14.8%).
- Unsaponifiable matter: \( \beta \)-sitosterol and \( \beta \)-amyrin: 0.6613 %.

(ii) *Dyospyros melanoxylon* (Family - Ebenaceae):

The seeds of *Dyospyros melanoxylon* have been found to contain the following percentage composition:

- Moisture (9.67%), ash content (6.02%), proteins (Kjeldahl's method) (29.4%), fixed oil (3.5%), total sugars (3.94%) and
residue (fibre + nitrogen free extract etc.) (47.45%).

**Ash:** Found to contain - water insoluble ash (69.81%), acid insoluble ash (0.741%), alkalinity of total ash (2.90 ml. of 0.1 N acid/gram of the sample) and showed the presence of chloride, carbonate, phosphate, sodium, potassium, iron and magnesium.

**Proteins:** The total protein percentage was estimated by semi micro Kjeldahl's method to be 29.4.

**Sugars:** Qualitative and quantitative determinations were made and paper chromatography technique was used for identification of sugars. Dextrose, fructose, mannose and galactose were found to be present in the seeds.

- Total sugar = 3.96%
- Total reducing sugar = 1.969 %

**Fixed oil:** A yellow fixed oil was obtained in an yield of 3.5% from the seeds which was revealed to contain:

- Saponifiable matter = 79%
- Saturated acids = 78.37%
  - Myristic, 7.67%; Palmitic, 22.43%; & stearic acid 48.27%.
- Unsaturated acids = 21.63%
  - Oleic acid, 11.69%; linoleic acid, 6.64% and palmitolic acid 3.30%.
- Unsaponifiable matter = 1.02%
  - Lupeol, B-sitosterol and stigmasterol.
PART III - Studies on the antimicrobial activity of essential oils:

Essential oils (total eight) of Zanthoxylum alatum, Kaempferia galanga, Pavonia odorata, Xanthium strumarium, Ophiorrhiza mungos, Andropogon ivavancusa, Justicia procumbans and Anethum sowa have been studied for their antimicrobial activity against certain pathogenic and non-pathogenic organisms.

The antibacterial organisms included for the studies were B.anthrax, S.aeruginosa, E.coli, H.pertuisis, S.aureus and S.leutea. The antifungal organisms included for the studies were A.niger, A.terreus, M.canis, C.tropicalis, T.mitata agrpttea and C.albicans.

The zones of inhibition were measured for their activity and the minimum concentrations of the essential oils required to inhibit the growth of the organisms were also studied. Comparative studies were made with the above mentioned essential oils in both the cases, of antibacterial and antifungal activities. The concentrations of the essential oils used were in 1 : 100, 1 : 250, 1 : 500 and 1 : 1000 ratio. It is interesting to note that even in very low concentrations (1 : 500, 1 : 1000) the essential oils of Kaempferia galanga, Ophiorrhiza mungos, Justicia procumbans and Anethum sowa showed promising results.
Potent antimicrobial agents have a great medicinal
value because most of the antifungal agents are synthetic in
nature and none of them are free from some side effects. The
antifungal results obtained by the essential oils of Justicia
procumbans and Anethum sowa are very stimulating for their
application in the skin diseases. Though all the essential
oils responded satisfactorily for their antimicrobial activities,
the oil of Justicia procumbans in particular gave very promising
results as such it can be placed as a most potent antimicrobial
agent among the other oils tested. The next important oil is
that of Anethum sowa which also gave significant results.

These antibacterial and antifungal studies of the
essential oils mark a new approach to cure the human sufferings,
specially the common skin diseases and infections which are caused
by pathogenic bacteria and fungi.

It is now being realised that even though all such
studies may not lead to discovery of potent remedies from plant
sources, the background information regarding the chemical
structure may open the door for a creative synthesis. We are
yet at the threshold of making use of plants for the suffering
humanity, and nature only knows what is in store for us.


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