CHAPTER I

INTRODUCTION
Man right from the dawn of civilization has been trying to find the curative value of plant products to combat the diseases and alleviate human sufferings. Plants are also indispensable for human beings for nourishment, protection and defence.

India with its wide variability of soil and climatic conditions induces a luxuriant growth of innumerable medicinal and aromatic herbs and plants. The oldest documents to show the use of medicinal plants in India is found in Rigveda¹. In India the last 50 years have seen considerable progress in the field of plant products. The recent isolation of plant constituents which possess cancer², asthma³ and tumor inhibition⁴ properties and are useful in high blood pressure and to cure mentally handicapped⁵ has encouraged the modern phyto-chemist to isolate compounds of varied applications.

Distribution, characterization and medicinal uses of plants of Indian origin have been described from time to time in standard volumes by many workers like Ainslie⁶, Bentley and Trimer⁷, Chopra et al.⁸ Chopra and Handa⁹, Dastur¹⁰, Haridas¹¹, Kirtikar and Basu¹², Moos¹³, Mukhopadhyaya¹⁴, Nadkarni¹⁵, Zimmer¹⁶ and in Wealth of India¹⁷.
CHEMISTRY OF PLANT PRODUCTS

Though countless types of compounds exist in the plant kingdom, the active principle contained therein has been observed to fall under the following groups: (1) alkaloids (2) carbohydrates (3) colouring matter (4) essential oils (5) fixed oils (6) glycosides (7) proteins (8) steroids (9) terpenoids (10) coumarins (11) enzymes and (12) tannins. The details regarding the chemistry, importance etc. of these types of compounds have been reviewed in standard works and through research communications from time to time. Some of the types of compounds, investigated by the present author have been reviewed briefly below:

**Essential Oils:**

Occurrence, isolation, significance, importance and the chemistry have been dealt with in standard works\textsuperscript{19-24}.

**Biosynthesis:**

Though many theories\textsuperscript{25-27} have been put forward from time to time, it was Ruzicka\textsuperscript{28,29} who first enunciated the famous 'isoprene rule'.

Biosynthesis of terpenes as shown in the chart can be achieved in three parts: (1) Synthesis of C\textsubscript{5} unit in the living organism (2) Arrangement of these to form cyclic terpenoids and (3) Cyclization of acyclic terpenes to cyclic terpenoids.
\[
\begin{align*}
\text{CO}_2 & \quad \text{3-methyl crotonyl-CoA} \quad \leftrightarrow \quad \text{isovaleryl-CoA} \quad \leftrightarrow \quad \text{leucine} \\
\text{ATP} & \quad \text{ADP} + \text{P} \\
& \quad \text{B-methyl glutaconyl-CoA} \\
& \quad \text{H}_2\text{O} \\
& \quad \text{B-hydroxy-B-methyl-glutaryl-CoA} \quad \xrightarrow{2\text{TPN}} \quad \text{mevalonic acid} \\
& \quad \text{Acetoacetyl CoA} \\
& \quad \text{Acetyl CoA} \\
& \quad \text{Carbohydrates,Fats} \\
& \quad \text{mevalonic acid-5-P} \quad \xrightarrow{\text{ATP}} \\
& \quad \text{ADP} \quad \xrightarrow{\text{ATP}} \\
& \quad \text{mevalonic acid-5-\text{PP}} \quad \xrightarrow{\text{ATP}} \\
& \quad \text{ADP} + \text{CO}_2 \quad \xrightarrow{\text{ATP}} \\
& \quad \text{isopentenyl-PP} \\
& \quad \text{Dimethylallyl-PP} \\
& \quad \text{Monoterpenoids} \quad \xrightarrow{\text{Geranyl-PP}} \\
& \quad \text{Sesquiterpenoids} \quad \xrightarrow{\text{Farnesyl-PP}} \\
& \quad \text{Cyclic triterpenes} \quad \xrightarrow{\text{3-squalene}} \\
& \quad \text{Steroids} \quad \xrightarrow{\text{Steroidal hormones}} \\
& \quad \text{Bile acids.}
\end{align*}
\]
Discovery of isopentenyl pyrophosphate(I) by Lymen\textsuperscript{30} and Bloch\textsuperscript{31} as an active intermediate and its conversion to farnesyl pyrophosphate(II) the precursor for sesquiterpenoids (like cadinene, nerolidol, bisabolene, eudesmol etc.) triterpenoids (like squalene) and tetraterpenoids (carotenoids) has solved many a problem in the biosynthesis of terpenoids which are the main constituents of the essential oils. Geranyl pyrophosphate(III) formed by the combination of isopentenyl phosphate and its isomer B-8 dimethyl allylpyrophosphate acts as a key substance for the synthesis of monoterprenoids (like citral, menthol, puligol, limonene, \(\alpha\)-terpeniol, carvone etc.), bicyclic monoterpenoids (like camphor, \(\alpha\)-thujene, \(\alpha\)-pinene, fenchol etc.) through an intermediate carbonium ion.

\[ \text{H}_2\text{C} = \text{C} - \text{CH}_3 \]
\[ \downarrow \]
\[ \text{CH}_2 \]
\[ \text{CH}_2\text{OPP} \]

Isopentenyl pyrophosphate

\[ \text{II} \]

Farnesyl pyrophosphate

\[ \text{III} \]

Geranyl pyrophosphate

\textit{Glycosides}:

A great variety of glycosides occur in low concentrations in nearly all the plants (in seeds, leaves, bark and roots). On hydrolysis with inorganic acids, glycosides\textsuperscript{32} give a sugar portion and a hydroxylic compound the aglycon, which may be an
alcohol, phenol\textsuperscript{33,34}, sterol\textsuperscript{35,36}, flavanol\textsuperscript{37}, anthraquinone\textsuperscript{38}, triterpenoid\textsuperscript{39,40} etc. Depending upon the nature of glycosidic linkage, glycosides are classified as O-glycosides, N-glycosides\textsuperscript{41}, C-glycosides\textsuperscript{42,43}, S-glycosides\textsuperscript{44} etc.

The function of glycosides has not been clearly understood. There are various views about their functions, e.g. glycosides may serve as sugar reserve, as waste products of plant metabolism, as means of detoxication by the removal of harmful substances such as phenols, or as defence mechanism. Cardiac glycosides have marked physiological activity and are largely used in medicine.

**Fixed Oils:**

The distribution of oils and fats of vegetable origin is unlimited as compared to land and marine sources. Michel Chevreal\textsuperscript{45,46} was the first to show that the fixed oils and fats comprise of triglycerides (i.e., fatty acid esters with glycerol) as the major constituents. They have been classified by many authors into non-drying, semi-drying and drying depending upon the increasing degree of unsaturation measured in terms of iodine absorption\textsuperscript{47-49} (iodine value). Though fats and oils are important for their calorific value as food (energy supplied by fats is 9 calories per gram), they are also put to industrial use in the manufacture of detergents, emulsifying agents\textsuperscript{50}, soaps, candles, paints, varnishes, lubricants, illuminants, cosmetics and pharmaceuticals.
Fats and oils contain small quantities of unsaponifiable matter consisting of hydrocarbons, sterols\textsuperscript{51,52}, vitamins and a variety of compounds. The importance of unsaponifiable matter can be exhibited from the fact that sitosterols from soyabean oil form the starting material for the commercial production of testosterone\textsuperscript{53} and vitamin E is obtained in appreciable quantities from wheat germ and cotton seed oil\textsuperscript{54}.

Chemical nature and structure of glycerides have been extensively dealt with in standard works\textsuperscript{55-63}.

\textbf{Proteins:}

Proteins which are polymeric amides derived from the monomers (\(\alpha\)-amino acids), occupy an important position among chemical substances because they are the very substances of life. Proteins perform diverse biological functions in the living system. Proteins like keratin serve as structural materials, proteic hormones like insulin regulate metabolic processes, protein like haemoglobin (in blood) transport oxygen within the body and enzymes which are also proteic catalyse biological reactions.

The significance, synthesis\textsuperscript{64}, importance and chemistry of proteins and amino acids\textsuperscript{65} obtainable on their hydrolysis have been reviewed by many authors\textsuperscript{66-72}. 
Carbohydrates:

Carbohydrates which include familiar substances like glucose, sucrose, starch, cellulose etc., provide us with the basic needs of life like food in the form of starch containing vegetation, clothing in the form of cellulose fibres (cotton, rayon etc.) and shelter (wood used to make houses and furniture is almost cellulose). Sucrose (common sugar) is one of the most important commercial commodities produced in the world.

The chief function of the carbohydrates is to supply energy. They are the main raw materials, necessary for the synthesis of fatty acids and some amino-acids in biological systems. Apart from their uses as calorie supplying agents, they are indispensable in the production of paper, fibres, plastics, drugs, vitamins, lacquers, photographic films etc.

MODERN METHODS OF ANALYSIS

To determine the structure of a compound, it has been customary to subject the compound to a detailed examination of its physical properties (melting point, boiling point, refractive index, optical rotation, solubility etc.), functional group analysis by a series of chemical tests and degradative (reduction and oxidation) methods. Enormous development in the field of phyto-chemistry has been possible in the past two decades with the introduction of modern techniques like chromatography (paper, thin layer, column and gas-liquid), colorimetry, spectroscopy (ultra-violet, infra-red, nuclear magnetic resonance and mass). Some of these methods have been used by
the present author in the present investigations and have therefore, been reviewed briefly below:

**Chromatographic Methods:**

Chromatographic techniques (paper, TLC, column and GLC) are indispensable in phytochemical analysis for isolation, purification and identification of different plant constituents. These techniques have been successfully applied for studying saturated and unsaturated fatty acids\(^74-76\), methyl esters of fatty acids\(^77\), unsaponifiable matter\(^78-79\), proteins\(^80\), gums\(^81-85\), sugars\(^86,87\), terpenes and essential oils\(^88-93\), terpenoid alcohols\(^94\) etc. by many research workers to reveal their composition both qualitatively and quantitatively.

The theory and different methods of practising these techniques have been reviewed in standard works\(^95-107\).

**Colorimetry:**

Qualitative and quantitative analysis of many natural products\(^108\) such as proteins, amino-acids etc. colorimetrically is being largely used these days. Kokita *et al.*\(^109\) Tillerd\(^110\) and Ilarionov\(^111\) have successfully applied the technique for studying various fats and oils. Detection and determination of many constituents of essential oils like geraniol\(^112\), citral\(^113\), cineol\(^114\), α-phelladrene\(^115\), thymol\(^116\), azulene\(^117\) and coumarin\(^118\) by colorimetric methods have been studied by many workers. During the last 25 years several methods\(^119,120\) have
been proposed and modified for the colorimetric estimation of amino-acids.

**Infra-red Spectroscopy :**

Infra-red spectroscopy has been of great service to the phytochemist in the elucidation of the structure of plant products, particularly for the characterization functional groups. The supremacy of the technique has been exhibited in the identification of new plant products belonging to the groups of alkaloids $^{121}$, steroidal sapogenins $^{122}$, steroids $^{123}$, sesqui-terpenes $^{124-126}$, terpenoids and fats and their derivatives $^{127-129}$ by many research workers. The details regarding the interpretation and applications of the method have been reviewed in standard works $^{130-137}$.

**Nuclear Magnetic Resonance Spectroscopy :**

NMR spectroscopy, introduced by Block $^{138}$ and Purcell $^{139}$ has been used by phytochemists to study biochemical problems $^{140}$, macromolecular $^{141,142}$ systems, pharmaceutical preparations $^{143-144}$ and to decide the correct one among many possible structures like Daucol and Carotol $^{145-147}$, Nerumbone and Humulene $^{148}$. Interpretation and importance of the technique have been reviewed by many authors $^{149-154}$.

**Mass Spectrometry :**

Mass spectrometry is increasingly being used in structure determination of natural products. Nevertheless, much
structural information regarding molecular weight, deduction of molecular formula and to decide between alternative structures can be obtained eventhough the complete structure can not be elucidated by mass spectrometry alone. The details of the use of mass spectrometry for the characterisation of plant products has been given by many authors^{155-157}.

PROBLEM TAKEN AND WORK DONE

Phytochemistry, a dynamic science plays an important role in medicine. Plants produce many products which may be used in medicine, cosmetics, dying and as food materials etc. Isolation of active principles which possess anti-cancer, anti-inflammation, antileprosy, antifertility and a wide range of properties from Indian aromatic and medicinal plants and their successful utilization to alleviate human sufferings has encouraged the research workers to continue investigations on new drugs from the plant kingdom. with an everincreasing zeal.

In this country, in general, a large number of vegetable drugs are used as household remedies by the common people, out of which some are described in 'Ayurvedic' and 'Unani' books and some still remains unpublished. Many of the plants have also still remained unworked phyto-chemically and pharmacologically or incompletely worked, of which re-examination is necessary with the advent of the modern scientific technology which has been revolutionised during the last fifty years with the
introduction of more sophisticated instruments carrying out more precise work.

The last few years have also seen the isolation of interesting types of compounds from the Indian essential oils which have become very important due to their antimicrobial activity. A time has come for the scientists of various disciplines to find out substances from plants to replace the usual important sources of food materials and imported materials. With these ends in view and to make an humble contribution to a distant goal, the author has studied the chemical nature of some plant products like essential oils, fixed oils, proteins, carbohydrates, glycosides, pectins and the antimicrobial activity of some Indian essential oils against human and plant pathogens. The findings are briefly reviewed below:

1. Chemical Examination of the Essential Oil from *Limnophila heterophylla*.

A pale yellow essential oil has been isolated in an yield of 0.11% by the steam and water distillation of the plant of *Limnophila heterophylla*. On chemical investigation the oil has been found to have the percentage composition: p-cymene (2.93%), α-pinene (3.92%), d-limonene (38.84%), d-cadinene (6.19%) and α-eudesmol (46.65%).

The essential oil of the plant can serve as a good source for α-eudesmol and d-limonene.

The brownish-yellow and brown oils extracted in yields of 21% and 4.8% respectively from the seeds of Kigelia pinnata DC and Cyamopsis tetragonoloba Taub have been found to be the glycerides of:

**Kigelia pinnata** DC: palmitic (21.30%), stearic (47-60%), oleic (22.29%) and linoleic (8.81%) acids. The unsaponifiable matter has been found to contain β-sitosterol, lupeol and an unidentified fraction (probably a hydrocarbon).

**Cyamopsis tetragonoloba** Taub: palmitic (14.29%), stearic (42.54%), oleic (38.21%), linoleic (2.99%) and linolenic (1.97%) acids. Unsaponifiable matter contains β-amyrin, β-sitosterol and stigmasterol.

The oil of Kigelia pinnata DC can be tried for domestic purposes after examining its toxicity.


The hydrolysates of the proteins and the carbohydrate portions extracted from the defatted material has revealed the presence of amino-acids and free sugars respectively, studied by different chromatographic techniques. The percentage composition of amino-acids present in the protein of K. pinnata
has been estimated colorimetrically and the results are presented below:

**Kigelia Pinnata** DC: The protein hydrolysate has been found to contain cystine (13%), lysine (9%), glycine (10%), aspartic acid (8%), glutamic acid (16%), alanine (6%), proline (11%), valine (8%) and leucine (19%). The carbohydrate portion of the seeds has been found to contain arabinose, glucose, inositol, lactose and raffinose.

**Cyamopsis tetragonoloba** Taub: This protein consisted of cystine, histidine, aspartic acid, threonine, proline, alanine, methionine and leucine. The carbohydrate portion contained arabinose, galactose, glucose, mannose, ribose and sucrose.

**Diospyros periquina** Gurke: Hydrolysis of the protein shows the presence of amino-acids: histidine, lysine, glycine, aspartic acid, glutamic acid, alanine, proline, methionine, leucine and tryptophan (tryptophan has been identified from the alkali hydrolysate of the protein) whereas fructose, glucose, galactose, lactose, mannose and sucrose are present in the carbohydrate portion.

The protein isolated from *Cyamopsis tetragonoloba* containing some essential amino-acids can be tried as an admixture to protein deficient diet.
4. Chemical Examination of the Fruits of Diospyros perigrina Gurke.

Brownish-yellow fixed oil has been found to be a glyceride of myristic (4.3%), palmitic (19.79%), stearic (29.68%), oleic (28.36%), linoleic (11.57%) and palmitoleic (6.28%). The unsaponifiable matter consists of β-sitosterol.

The alcoholic extract has been found to contain β-sitosterol D-glucoside.

Preliminary studies of the pectin precipitated from the water extract of the fruits on acid hydrolysis reveal the presence of arabinose, galactose and galacturonic acid in it.

5. Antimicrobial Screening of Some Indian Essential Oils.

Five essential oils from the leaves of Skimmia laureola, Cymbopogon flexuosus, Cinnamomum zeylanicum, Geranium and Eucalyptus citriodora have been isolated by water and steam distillation method and tested for their activity at six different dilutions against Vibrio cholerae, Salmonella paratyphi (human pathogens), Bacillus anthracis (pathogen of domesticated animals), Xanthomonas malvacearum (plant pathogen), Keratinophyton terreum (keratinophilic fungi), Trichophyton mentagrophytes (strong dermatophyte) and Malbranchea pulchella var. sulphurea by agar-filter paper disc diffusion technique.

In the second part of the same chapter sixteen essential
oils, isolated from various Indian medicinal plants have been tested in different combinations for their activity against *Salmonella typhi* (human pathogen) and found to compare favourably with chloramphenicol used as the reference antibiotic.

Some of the essential oils tested can be used as anti-microbial agents against some diseases, infections and destruction caused by the micro-organisms saved. The results obtained in the investigation of the activity of essential oils in combinations against *Salmonella typhi* could be of use medicinally for combating typhoid after examining the toxicity of the mixtures.
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