CHAPTER 5

GENERAL INTRODUCTION
Herbalism has been one of the main branches of medicine for centuries in all parts of the world. There are ample proof for the application of the crude plant extracts and decoction in therapy world over as well as in India. But the rationalisation of the science of therapeutics as we know is comparatively of recent origin. Thus chemical investigation along with pharmacological examination of chemical constituents from plants of medicinal value has gained much importance in the therapeutic world. This idea encouraged me to take up the phytochemical investigation incorporated in the present thesis. The chemical investigation in this field gained pace with the rapid development of new techniques like chromatography, spectrometry and various other physical methods.

Ayurveda is one of the ancient systems of medicine in India. Susruta and Charak (1000 B.C.) wrote comprehensive treatises on the subjects. Charaka has elaborately described the classification, diagnosis and treatment of various diseases. Susruta Samhita was, on the other hand, the main source of knowledge about surgery in ancient India. Ayurveda literally means the "science of life". It defines life (Ayurveda) as the union of body, sense, mind and soul. The present Ayurvedic materia medica is stupendous and contains as many as 8000 published recipes. In addition there exist a large number of unpublished recipes held as "family secret" by traditional
practitioners. Some 1200 drugs are in frequent use either in the form of single drugs or as compounded formulations. 'Sidha' system which is even older than Ayurvedic system is greatly practised in South India and consists of treatment of diseases by the use of various minerals.

Since the dawn of human creation, man had to suffer from various ailments and diseases. His worries to be relieved of diseases led to search for remedies that could provide cures for them. His attention was naturally diverted towards the plants since they were easily and abundantly available. It was, therefore, natural that in its early stages, medicinal science developed around such plants as were observed to have some therapeutic properties. The search for medicinal plants have continued through centuries. It goes to the credit of the people of India that they were acquainted with a far larger number of medicinal plants than the natives of any other country on the face of the earth.

India has a vast store of folkloric medicine practised mainly among the tribal and village people. The practitioners of magicoreligious medicine confirmed himself to tribal and village population, each fitting well into the cultural and social life of his own setting. It has been estimated that about more than 2000 herbs are being used by these practitioners.

The plants and plant products are the greatest gift of nature for the service of man-kind. The use of the plants to prevent and cure diseases goes for back in the history of man. A transition
from the primitive to rational medicine is evident from the documents, many of which are of great antiquity and reveal that a large number of herbal and aromatic plants have been used in India. Thus, the plants have been used for defence, protection and nourishment by human beings with the dawn of civilization. The primitive man used the raw material and raw extracts of the plants to help those in sorrow need and sickness without the knowledge of their chemical composition. With the growth of civilization, the multifarious use of plant products began to be appreciated and in the course of time, their uses in medicine, flavours, perfumes, dyeing, cosmetics, edible fats etc. developed. More than in any other branch of science, empiricism has been developed in medicinal chemical research. As such, the plants have been thoroughly investigated throughout the ages and their importance understood either for beauty or utility.

The Greco-Arabic medicine was introduced in India by the Muslims rulers and come to be known as 'Unani-Tibb'. Some valuable contributions of this period are 'Dymock's Pharmacographica Indica', 'Watt's Dictionary of Economic Raw materials' and 'Hooker's Flora of British India'. The pioneering work of Sir R.N. Chopra is praiseworthy in this direction.

Chemistry has a very vital role to play in improving the material life of the people at large. Phytochemistry, a dynamic science, play an important role in medicine. It helps in determining the chemical constituents and structure of active principles and opens the door for newer synthetic analogue. The isolation of active
principles which possess the physiological and pharmacological activity from the medicinal plants and their successful utilization to alleviate human sufferings have encouraged researchers to continue the investigation of new drugs from natural sources.

There are numerous reasons why drug chemists investigate plants for their constituents. Firstly, one can consider that purely scientific curiosity has motivated some investigations which may possibly has been undertaken because the plant had not previously been investigated. Another important reasons for the chemical investigation of plants is to establish the therapeutic claims of the traditional system to withdraw the toxic drugs from use and to standardise the medicine so that each patient receives effective dose in reproducible dose regimens.

Human beings, right from the dawn of civilization, have been trying to find out the curative value of natural products to combat the diseases and alleviate human sufferings. The history of indigenous drugs in India can be traced from the 'Rigveda', the oldest repository of human knowledge particularly in 'Ayurveda'.

More commonly plants have been investigated for specific classes of constituents such as terpenoids, steroids, alkaloids, flavonoids and related compounds or they may have been examined as part of a chemotaxonomic study in which relationship between plants are assessed on the basis of chemical constituents.

The medicinal properties of the plants depend upon the presence of one or more physiological active compounds. Therefore,
it becomes necessary to isolate the physiological active principles from plants in pure form, to study their exact composition and finally to establish their structure by means of physico-chemical examinations and then subject them to physiological tests. If proved effective, attempts are made to devise the cheaper methods of synthesis. Further, by slightly modifying the composition of natural drugs, it may be possible to increase the physiological activity of the drugs.

Various plant products possess significant physiological and pharmacological activities for example cytotoxic active compound, isolated from Ipomopsis aggregata, showed anticancerous activity against the KB cell culture. The bark of Diospyros montana Roxb., has been investigated to establish the antitumor activity. Oil of Hydnocarpus wightiana is used as remedy for the treatment of leprosy. The methanolic extract of the stem of Ochrosia acuminata (Au-Koe-Lo-Moo in Chinese) showed potent antileukemic activity in vivo against P-388 lymphocytic leukemia growth at T/C $>180\%$ (50 mg/kg). The methanolic extract of the roots of Morus indica found to possess CNS depressant, analgesic and significant local anaesthetic actions. Hypnea musciformis wulfen showed antispasmodic activity and anti-inflammatory activity against rats hind paw oedema induced by commercial carrageenan. Pterocarpus marsupium Roxb. (Leguminosae) is advocated for the treatment of diabetes mellitus in Indian medicine and hypoglycemic activity in normal and alloxanised albino rats. The flavonoid present in Pterocarpus marsupium showed a consistent effect on normal blood sugar levels but it effectively reversed the alloxan induced changes in blood sugar level and beta-cell population in the
pancreas. It also showed a protective effect when it was given prior to alloxan administration. The novel action of drug on the pancreatic beta-cells and absence of acute toxicity may offer a new hope to the diabetics in future.

The great diversity and complexity of the chemical nature of the natural products and their presence some times in very poor amounts in plants, provide a major obstacle in the progress of the phytochemistry. However, the availability of the modern physico-chemical techniques such as TLC, GLC, UV, IR, NMR, MASS, ESR spectroscopy etc. has helped the chemists the structure of the constituents from the plants. It has now become possible for an organic chemists to investigate successfully the various biological compounds even if they occur in very small amounts. This has given a significant impetus to the development of drugs from plant sources.

Unfortunately a single test can not be applied for the determination of the medicinal value of the plants. A test for antibacterial activity for example will not reveal the presence of substance possessing narcotic effect or having action on the heart. There are certain constituents like "hasish" and "opium" which are not distributed in the plant tissues uniformly. Further, it is also possible that substance may not be present in the plant at all the stages of its growth. Climatic conditions also effect the chemical constituents in the plants.

The important field of research in phytochemistry lies in the investigation of the modification of drugs and the relationship
between chemical structure and physiological action. When the mechanism of drug action is known, it become possible to synthesise the relative new drugs possessing greater therapeutic value and less toxicity. The development of 'Aspirin' is a classical example of this fact. 'Aspirin' is a modified drug of the natural drug 'Salicin' and is used in the treatment of rheumatic and neuralgic pains. As a further example the knowledge of the mechanism of the action of 'Connine' led chemists to synthesise a wide range of new drugs. 'Procaine' and 'Novacaine' which possess local anaesthetic properties.

The enormous development in the chemistry of natural products has been made possible on account of the development of the physico-chemical techniques. By the application of these techniques, the work of the chemists with regards to the identification of organic constituents has become easier. The introduction of chemical and biological assaying methods, the potency of the drugs have made their use much safer.

The references⁹⁻¹⁵ relating to the isolation and study of natural products by the use of different chromatographies will indicate the importance of this particular technique.

Paper electrophoresis is now being increasingly used in the separation of the alkaloids and various other plant products. The references¹⁶⁻¹⁸ of the work in which this technique has been employed to reveal its importance.

Ultra-violet and infra-red spectroscopy are modern tools for
the phytochemists. Their importance are evident from the
references 19-21.

Nuclear electron resonance and nuclear proton magnetic
resonance spectroscopy, which are the modern techniques, have also
been used in the analysis of the natural products. Their importance
is evident from the references 22-25.

X-rays analysis plays an important role in establishing the
structure of higher molecules such as alkaloids, sterols, mucilages,
tannins, waxes and essential oils 26,27.

Phytochemistry has not only enriched modern medicine but has
also provided valuable leads for drugs designing. It has also
contributed greatly to the development of 'organic chemistry',
especially the newer techniques of isolation, purification, structural
elucidation and reaction mechanisms of the compounds.

A systematic examination of the plants for getting the new
drug is obviously an arduous task for the plant biochemists.
Nevertheless, many notable successes already recorded to give
encouragement for the continuation of the work. Plants and other
natural sources can provide thousands of substances of which a few can
reasonably be considered useful as drugs which will not be provided by
the chemists for years to come. Thus the science of medicine has
developed on the basis of curative and preventive properties on
diseases of different plant products. Thus this was main reason for
investigation of all types of plants for achieving success in the
utilisation of natural products for benefit of mankind.
The most common biologically active constituents of plants fall under the followings groups of compounds.

(1) Flavonoids;  (2) Saponins and Sapogenins;  (3) Terpenoids;
(4) Tannins;  (5) Alkaloids;  (6) Steroids;  (7) Enzymes;  (8) Proteins
and amino acids;  (9) Resins;  (10) Aliphatic ketones and alcohols;
(11) Lactones and coumarins;  (12) Fatty acids;  (13) Essential oils;
(14) Anthraquinones;  (15) Carotenoids and so on.

The description of all the groups of organic substances mentioned above is beyond the scope of this thesis. However, a brief description of the anthraquinones, sterols and terpenes has been given here as the author has been engaged mainly in the study of compounds belonging to these chemical groups.

**ANTHRAQUINONES**

The quinone constitute an important class of naturally occurring pigments, about fifty percent of which are known to occur in higher plants, distributed amongst various families. The pigments are mainly present in leaves, barks, roots and to certain extents in other part of plants. Over 1200 quinones have been isolated from micro-organisms, particularly lower fungi, a few have been found to be present in lichens and some are elaborated by certain insects and marine animals. Numerous quinonic colouring matters are known as the metabolic products of the micro-organisms. The pigments of lichens so far isolated, except usnic acid, are the pulvinic acid derivatives or the quinonic compounds, e.g. anthraquinones and terphenylquinone. They usually occur in the form of glycosides and occasionally in the
free state. Structurally, quinones are related to benzoquinones, naphthoquinones or anthraquinones. In some cases miscellaneous complex structure are present e.g. alphin pigments\textsuperscript{28} elaborated by insects. Among those the pigment related to anthraquinone constitutes the largest group\textsuperscript{29}. About half of the total numbers occur in the plants of 'Rubiaceae family' which were studied intensively by Perkin et al\textsuperscript{30} and the remaining are mostly the fungal product studied mainly by Raistrick\textsuperscript{31} and Shibata\textsuperscript{32}.

The quinones are coloured dioxo derivatives of dihydro aromatic systems, the oxygen atom occupying positions which are either ortho or para (or their equivalent polycyclic compounds) to each other. The quinonic pigments are distinguished from other group of pigment by their oxido-reductive nature and the distinct colouration given by the addition of caustic alkali or conc. $\text{H}_2\text{SO}_4$. Magnesium acetate exhibits beautiful colouration with $\alpha$-hydroxy quinone in alcoholic solution. This colour reaction suggests the relative positions of substituted hydroxyls in the anthraquinone nucleus e.g. 1,4-dihydroxyanthraquinone (orange) and 1,2-dihydroxyanthraquinone (blue)\textsuperscript{29}.

Anthraquinones occur as complex mixture which can be separated either by the use of different solvents or by the application of chromatographic method employing magnesium carbonate, magnesol, magnesium oxide, silicic acid and silica gel as adsorbents. Paper and thin layer chromatographies have also been occasionally employed for the separation and characterization of these compounds.
Ultra-violet and infra-red spectral investigations on anthraquinones have been performed by various workers\textsuperscript{33-35}.

The study of bianthraquinones were initiated by Raistrick laboratory in London and continued by Shibata\textsuperscript{32} and other in Tokyo (Japan). On reductive cleavage by heating with alkaline sodium dithionite, bianthraquinone afforded two molecules of anthraquinones. Some of the recently investigated anthraquinones are: anthraquinonoid pigments of Japanese *Rubia cordifolia*\textsuperscript{36}; new bianthraquinones from *Cassia sciamea* Lam\textsuperscript{37}; averythin, an anthraquinone from *Aspergillus resicolour*\textsuperscript{38}; bianthraquinone from *Cassia occidentalis*\textsuperscript{39}; chrysophanol -8-O-β-glucoside from the flowers of *Woodfordia fruticosa*\textsuperscript{40}; new anthraquinone pigments from *Acacia leucopholea*\textsuperscript{41}; new anthraquinone pigments from the stem bark of *Melia azedarach* Linn\textsuperscript{42}; new anthraquinone glycosides from the stem bark of *Aphanamixis polystachya*\textsuperscript{43} and anthraquinones from the stem bark of *Maesopsis eminii*\textsuperscript{44}.

**PHYSIOLOGICAL ACTIVITY OF THE QUINONES**

Many natural quinones and quinols have antibiotic and this is probably important in some cases to protect the surrounding tissues against invading micro-organisms. The mode of action appears to be complex\textsuperscript{45}.

Anthraquinone glucosides are important cathartic compounds. They are used as purgative and are widely employed in geriatric and pediatric medicine for their unique pharmacological effects\textsuperscript{46}. 
The production of anthraquinones in tissue culture of *Cassia secca* L., *Cassia angustifolia*, *Cassia tora* and *Digitalis purpurea* have been reported. Sennosides - A and B isolated from the leaves of *Cassia acutifolia* and *Cassia angustifolia* are widely used as cathartics. Recently Tsuji has established the structure of an antiviral-antibiotic compound named as julimycin B-II from the metabolites of *Streptomyces shiodaensis*.

**STEROIDS**

Sterols are polycyclic, hydroaromatic secondary alcohols and may occur in free state, glycosides or as esters. Invariably they occur whenever life exists and are found associated with fats and oils of both vegetables and animals origin.

The sterols have been mainly divided into three groups according to their occurrence: (1) Zoo-sterols, obtained from animals, (2) Phyto-sterols, obtained from plant kingdom and (3) Myco-sterols, obtained from micro-organisms including fungi.

Sterols have been divided into various groups according to their structural pattern in their skeleton as given below:

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<th>SERIES</th>
<th>RING A/B TRANSFUSED</th>
<th>RING-A,B AND C FUSED</th>
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<tbody>
<tr>
<td>C 19</td>
<td>Androstane</td>
<td>Ia</td>
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<tr>
<td>C 21</td>
<td>Allopregnane</td>
<td>Ib</td>
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<tr>
<td>C 24</td>
<td>Allocholane</td>
<td>Ic</td>
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<td>C 27</td>
<td>Cholastane</td>
<td>Id</td>
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<tr>
<td>C 28</td>
<td>Ergostane</td>
<td>Ie</td>
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<td>C 29</td>
<td>Stigmasterone (Sitostane)</td>
<td>If</td>
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<tr>
<td></td>
<td>Iso-Androstane</td>
<td>IIa</td>
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<td>Pregnane</td>
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<td>Cholane</td>
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<td>Co-prostane</td>
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<td>Co-prositostane</td>
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(a) $R = \text{H}$
(b) $R = \text{-CH}_2\text{-CH}_3$
(c) $R = \text{-CH-CH}_2\text{-CH}_2\text{-CH}_3$
(d) $R = \text{-CH-CH}_2\text{-CH}_2\text{-CH}<\text{CH}_3$
(e) $R = \text{-CH-CH}_2\text{-CH}_2\text{-CH}<\text{CH}_3$
(f) $R = \text{-CH-CH}_2\text{-CH}_2\text{-CH}<\text{CH}_3$

**PHYSIOLOGICAL ACTIVITY OF THE STEROIDS**

Sterols seem to have an important function in animal metabolism as hormones, co-enzymes, bile acids and vitamin-D. β-sitosterol shows significant antipyretic and anti-inflammatory activities.
TERPENOIDS

The terpenoids represent a compound which has a carbon skeleton (i) either constructed directly from isoprenoid units or (ii) has at some stage in its biogenesis a carbon skeleton so constructed. Terpenoidal chemistry has an attraction for organic chemists because of its use in the preparation of perfumes, flavours, protective coatings, pharmaceuticals, insecticides and codensation catalysts etc.

Terpenes are classified as: monoterpenes \((C_5H_8)_2\); sesquiterpenes \((C_5H_8)_3\); diterpenes \((C_5H_8)_4\); triterpenes \((C_5H_8)_6\); tetraterpenes \((C_5H_8)_8\) and polyterpenes \((C_5H_8)_n\). Most of the known triterpenoids have been found to have either tetracyclic or pentacyclic structure.

Pentacyclic triterpenes occur in the plant as a glycoside or as a free form. This class can be divided into the following series-(i) oleanane (e.g. \(\beta\)-amyrin); (ii) lupane (e.g. lupeol) and (iii) ursane (e.g. \(\alpha\)-amyrin). These are the most widespread pentacyclic triterpenes found in nature.

PHYSIOLOGICAL ACTIVITY OF THE TERPENES

Triterpenoids have been observed to have very interesting physiological activities. Helvolic acid, cephalosporin and fusidin have been studied as antibiotics in detail. Fusidin with penicillin shows a remarkably high anti-staphylococcal activity\(^53\). The cucurbitacin-E shows tumour inhibiting and anti-cancerous properties\(^53\).