HERBAL DRUGS & RESEARCH METHODOLOGY

Three decades ago, only few had any appreciation of the number of remedies that had their origin from herbal medicine, and most had vague knowledge of herbal medicine, traditional medicine or other forms of complementary and alternative medical practices. For a variety of reasons, more individuals nowadays prefer to take personal control over their health with the use of herbal medicines, not only to prevent diseases but also to treat them. This is particularly true for a wide variety of illnesses readily treated at home (common cold, etc.). Herbal products are also commonly used by patients with certain chronic medical conditions, including breast cancer (12%), liver disease (21%), human immunodeficiency virus (22%), asthma (24%) and rheumatologic disorders (26%). WHO estimates that about three-quarters of the world's population currently use herbs and other forms of traditional medicines to treat their diseases.

Even as we entered into the new century with its exciting prospect of gene therapy, herbal medicines remain one of the common forms of therapy available to the world population. The acceptance and recognition of herbal medicine has been in part due to the acknowledgement of the value of traditional and indigenous pharmacopoeias, the incorporation of some medicines derived from these sources into pharmaceuticals, the need to make health care affordable for all and the perception that pharmaceutical drugs are increasingly over prescribed, expensive and even dangerous. Another important perception fomenting this interest is that natural remedies are somehow safer and more efficacious than remedies that are pharmaceutically derived.

Traditional medicine using herbal drugs exists in every part of the world. The major areas are Chinese, Indian and European traditions. The philosophies of these traditional medicines have some resemblance to each other but differ widely from modern Western medicine. In view of the progress of Western medicine not only new synthetic drugs but also herbal drugs have to fulfill the international requirements on quality, safety and efficacy. Herbal drugs have the advantage of being available for patients in the geographical area of the special traditional medicine. The development procedure of herbal drugs for world-wide use has to be different from that of synthetic drugs.

Practically every country develops its own medical system, which includes the ancient civilization of China, Egypt and India. Thus, the Indian Medical System-Ayurveda came into existence. The raw materials for Ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture

of products. Also, Siddha, Unani and Tibb are traditional health care systems have been flourishing for many centuries. Apart from these systems there has been a rich heritage of ethnobotanical usage of herbs by various colorful tribal communities in the country.

Vast ethnobotanical knowledge exists in India from ancient time. Our work over four decades, both in the field and literary studies, has resulted in a dictionary of Indian folk-medicine and ethnobotany that includes 2532 plants. India has about 45,000 plant species; medicinal properties have been assigned to several thousand. About 2000 figure frequently in the literature; indigenous systems commonly employ 500. Despite early (4500-1500 BC) origins and a long history of usage, in the last two centuries Ayurveda has received little official support and hence less attention from good medical practitioners and researchers. Much work is now being done on the botany, pharmacognosy, chemistry, pharmacology and biotechnology of herbal drugs. The value of ethnomedicine has been realized; work is being done on psychoactive plants, household remedies and plants sold by street drug vendors. Statistical methods are being used to assess the credibility of claims. Some recent work in drug development relates to species of *Commiphora* (used as a hypolipidaemic agent), *Picrorhiza* (which is hepatoprotective), Bacopa (used as a brain tonic), *Curcuma* (anti-inflammatory) and *Asclepias* (cardiotonic). A scrutiny of folk claims found 203 plants for evaluation. Less well known ethnomedicines have been identified that are used to treat intestinal, joint, liver and skin diseases. Routine random efforts are not likely to increase the desired success rate of discovery, while experience indicates that a modified collection policy offers the best chances for the discovery and development of agents for the treatment of AIDS (acquired immune deficiency syndrome) and cancer.

Searching for new biologically active compounds from natural sources starts obviously, in the field. Plant, microbial or animal materials to be sought and investigated may be selected through a number of approaches. No matter what selection criterion (a) is (are) used, the first step in obtaining the organism concerned is to undertake field collecting work to search for and to collect the organism. Good knowledge on the eco-geographic distribution and precision in the taxonomic identification of the organism(s) sought are crucial if the field work involves the search for a pre-determined organism or set of organisms. Such knowledge and precision during field work are of secondary importance, however, if the search and collection are based on biodiversity or ethnomedical uses, since accurate taxonomic identification may be made at a later date, in a Museum or Herbarium environment.

If we do well for a moment on our hoary past, Rigveda, one of our oldest repositories of human knowledge written between 4,500-1,500 B.C. mentions the use of 67 plants for the therapeutic purposes and Yajurveda enlists 81 plants whereas Atharvaveda written somewhere 1,200 B.C. describes 290 plants. India unquestionably occupies the top position in the use of herbal drugs. It is one of the foremost countries exporting plant drugs or their derivatives and excels in home consumption too. According to Indian mythology, when the illness and diseases got rampant on the earth, the sages learnt the science of healing from Lord Indra and recorded them in scriptures. It has been estimated that about 75,000 species of higher plants exist on the earth. A reasonable estimate of about 10% has been used in traditional medicine. However, perhaps only about 1% of these are acknowledged through scientific studies to have therapeutic value when used in extract form by human.

Traditional healers and pharmacists in developing countries are in important source of information about plant sources of new drugs. Only fractions of the earth's natural pharmacopoeia have been analyzed with modern techniques. The threat of imminent extinction of many plant species, especially in tropical areas, makes it urgent that scientists learn as much as possible before old remedies are forgotten or their raw materials are destroyed. This process requires the observation and recording of medical techniques, identification of plant materials and experimental investigation of the ingredients and their effects. Ethnopharmacology can also be an important element of a developing nation's medical and economic system. Third World governments are being encouraged to seek a synthesis between modern and traditional medicine. Although developing countries are providing many of the raw materials needed in drug manufacturing, the final products are often returned as high-priced medicines. As more plants are needed for large-scale production, over harvesting has led to stock depletion. Chemists have so far been unable to reproduce the complex structure of many plant compounds. Further coordinated research into folk traditions, plant species, growing conditions and local medical needs is urged. Care must be taken, however, to preserve the main advantages of traditional medical care: low cost and easy access.

Many higher plants produce economically important organic compounds such as oils, resins, tannins, natural rubber, gums, waxes, dyes, flavors and fragrances, pharmaceuticals and pesticides. However, most species of higher plants have never been described, much less surveyed for chemical or biologically active constituents and new sources of commercially valuable materials remain to be discovered. Advances in biotechnology, particularly methods

for culturing plant cells and tissues, should provide new means for the commercial processing of even rare plants and the chemicals they produce. These new technologies will extend and enhance the usefulness of plants as renewable resources of valuable chemicals. In the future, biologically active plant-derived chemicals can be expected to play an increasingly significant role in the commercial development of new products for regulating plant growth and for insect and weed control.

Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. Interest in natural products research is strong and can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify and structurally characterize these active constituents and advances in solving the demand for supply of complex natural products. Opportunities for multidisciplinary research that joins the forces of natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry and pharmacology to exploit the vast diversity of chemical structures and biological activities of natural products.

Special attention is paid to the present role of natural products in therapy: as biologically active compounds as such, as starting materials for (semi)synthetic drugs and, last but not least, as source of inspiration or as models for the synthesis of new drugs with better therapeutic, chemical or physical properties than the original compounds.

Herbal Wealth of India

Now-a-days natural products are an integral part of human health care system, because there is popular concern over toxicity and resistance of modern drugs. India is one of the 12 leading biodiversity centers with presence of over 45,000 different plant species, 15000-18000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes and 13 million marine organisms. From this flora, 15,000 to 20,000 have good medicinal value. Among those only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani and 30 in modern medicines.

Herbal Drug Market

The global herbal products market is worth of US $32 billion and is growing at a rate of about 9-15%. The average turnover of Indian herbal medicine industry is about 2,300 crore

rupees. However, to achieve the goal of major exporter of herbal remedies, several steps need to be taken.

- Systematic study of world market demand and short listing of medicinal herbs with good potential.
- Systematic cultivation of medicinal herbs on a large scale.
- Encouragement for agro-based photochemical and pharmaceutical industries to manufacture value added herbal products.
- Strict legislation to control quality and purity.
- Upgradation of cultivation and collection process.
- Documentation of research work and standardization for quality.

Steps Necessary for Promoting Herbal Drugs

Phytochemistry or natural product chemistry research is the backbone of herbal industry. For promoting use of herbals in modern medicine, phytochemistry should be envisaged for:

- Isolation, purification and characterization of new phytoconstituents.
- Use of newly isolated phytoconstituents as “lead” compound for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity.
- Conservation of lead phytoconstituents into medicinally important drugs.

Ethnopharmacological Approach to Herbal Drugs

The term ethno-pharmacology refers the interdisciplinary scientific observation, description and experimental investigation of indigenous drugs and biological activities. There are 119 drugs of known structure that are still extracted from higher plants and used globally in allopathic medicine. About 74% of these were discovered by chemists who were attempting to identify the chemical substances in the plants that were responsible for their medical uses by humans. These 119 plant-derived drugs are produced commercially from less than 90 species of higher plants. Since there are at least 250,000 species of higher plants on earth, it is logical to presume that many more useful drugs will be found in the plant kingdom if the search for these entities is carried out in a logical and systematic manner. The first and most important stage in a drug development programme, using plants as the starting material, should be the collection and analysis of information on the use(s) of the plant(s) by various indigenous cultures. Ethnobotany, ethnomedicine, folk medicine and traditional medicine can provide information that is useful as a 'pre-screen' to select plants for experimental pharmacological studies. Examples are given to illustrate how data from ethnomedicine can be analyzed with the aim of selecting a reasonable number of plants to be tested in bioassay systems that are believed to predict the action of these drugs in humans. The ultimate goal of ethnopharmacology should be to identify drugs to alleviate human illness via a thorough analysis of plants alleged to be useful in human cultures throughout the world.

Practical Aspects of Herbal Drug Discovery

The following scheme represents a summary of the stages involved in the development of pure drug from a plant source.

- Collection and identification of the plant and deposition of voucher sample in herbaria.
- Literature survey on the plant species selected for studies.
- Extraction with solvent and preparation of non-polar and polar extracts for initial biological testing.
- Evaluation of plant extract against a panel of biological test methods, as exemplified by receptor binding, enzyme inhibition and /or cytotoxicity assays.
- Activity guided fractionation on the extract showing activity, by monitoring each chromatographic fraction with bioassay chosen from the panel available to the investigation.
- Structure elucidation of pure active isolate(s) using spectroscopic techniques and chemical methods, if necessary.

- Test each active compound (whether of novel or known chemical structure) in all in vitro and in vivo biological test methods available, in order to determine potency and selectivity of the drug.
- Molecular modeling studies and preparation of derivatives of active compound.
- Large-scale isolation of interesting active compounds for toxicological, pharmacological and for mutation studies, when total synthesis is not practical.
- Clinical trials (Phase I – III).

Current Status of Herbal Drugs

The executive board of WHO (World Health Organization) recently passed a resolution calling on countries

1. To promote the role of traditional practitioners in the health care systems of developing countries and,
2. To allocate more financial support for the development of traditional medical systems.

The board also urged the medical profession not to undervalue the traditional medical system. WHO recognizes that modern medical care is unavailable to the majority of the world's poor residents and that traditional birth attendants deliver 2/3 of the world's babies. To fulfill the primary health needs of all the world's inhabitants it will be necessary to utilize both the Western and the traditional medical system. In some countries, such as Sri Lanka, India and China the traditional health system is legally recognized. WHO also advocates utilizing those medicinal plants and remedies used by traditional practitioners to effectively treat their patients. Example of some of these plants are *Ammi visnage*, a Mediterranean plant, used to treat angina pectoris, *Cymbopogan proximus*, an Egyptian plant, used to remove urinary tract stones, the root of *Combretum*, used in Ghana to treat guinea-worm, *bitter leaf*, a Nigerian plant which kills mouth bacteria and *Desmodium adcendens*, *Thonningia sanguinea* and *Deinbollia pinnata* used in various combinations to treat bronchial asthma.

An early objective of the World Health Organization's (WHO) traditional medicine program was to promote a realistic approach to the subject. The realism with which countries around the world, both developed and developing, examine their own traditional practices suggests that progress is being made towards this goal. The current challenge is to pursue action along 3 lines: evaluation, integration and training. In traditional medicine it is necessary to separate myth from reality so that valid practices and remedies can be distinguished from those that are patently ineffective and/or unsafe. Thus, WHO will

continue to promote the development, teaching and application of analytical methods that can be used to evaluate the safety and efficacy of various elements of traditional medicine. Traditional practitioners also require training. They need to be provided with additional skills. It is essential to make practitioners of traditional medicine allies rather than competitors. The training of traditional birth attendants in aseptic delivery techniques and simple antenatal and postpartum care provides a good example of the possibilities that exist for collaboration between the traditional and modern health care sectors.

In the past 2 years WHO has carried out numerous activities in the field of traditional medicine. For example, among the activities coordinated by WHO headquarters was the continuing search for indigenous plants for fertility regulation in men and women. In 1983, WHO collaboration centers for traditional medicine continued to strengthen national efforts in research and development. A prerequisite for the success of primary health care is the availability and use of suitable drugs. It is reasonable for decision makers to identify locally available plants or plant extracts that could usefully be added to the national list of drugs or that could even replace some pharmaceutical preparations that need to be purchased and imported. NAPRALERT (for national products alert) is a computerized database derived primarily from scientific information gathered from the world literature on the chemistry, pharmacology and ethnopharmacology of natural plant products. It can provide both a general profile on a designated plant and a profile on the biological effects of a chemical constituent thereof. A valuable feature of the NAPRALERT database is its ability to generate information on plants from a given geographical area.

Plant-derived drugs have an important place in both traditional and modern medicine. For this reason a special effort to maintain the great diversity of plant species would undoubtedly help to alleviate human suffering in the long term. Proven agro industrial technologies should be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines. About 80% of the world's people depend largely on traditional plant-derived drugs for their primary health care (PHC). Medicinal plants serve as sources of direct therapeutic agents and raw materials for the manufacture of more complex compounds, as models for new synthetic products and as taxonomic markers. Some essential plant derived drugs are atropine, codeine, morphine, digitoxin/digoxinand and quinine/artemisinin.

Use of indigenous medicinal plants reduces developing countries reliance on drug imports. The Napralert database at the University of Illinois establishes ethnomedical uses for

about 9200 of 33,000 species of monocotyledons, dicotyledons, gymnosperms, lichens, pteridophytes and bryophytes. Even though many people use medicinal plants, pharmaceutical firms in industrialized nations do not want to explore plants as sources of new drugs. Scientists in China, Germany and Japan are doing so, however. Screening, chemical analysis, clinical trials and regulatory measures are needed to ensure safety of herbal medicines. W.H.O. has hosted interregional workshops to address methodologies for the selection and use of traditional medicines in national PHC programs. W.H.O., the International Union for the Conservation of Nature and Natural Resources and the World Wide Fund for Nature developed guidelines for conservation of medicinal plants. Their 2-pronged strategy includes prevention of the disappearance of forests and associated species and the establishment of botanical gardens. W.H.O's Traditional Medicine Programme hopes that people will apply known and effective agro industrial technologies to the cultivation and processing of medicinal plants and the production of herbal medicines and the creation of large-scale networks for the distribution of seeds and plants.

Alternative medicine use and expenditures in the United States is increased substantially between 1990 and 1997, attributable primarily to an increase in the proportion of the population seeking alternative therapies, rather than increased visits per patient.

Natural products research continues to provide a tremendous variety of lead structures which are used as templates for the development of new drugs by the pharmaceutical industry. Advances in bioassay technology and in chemical methodology have combined to make natural products a cost effective source for new leads. While microbial products have been the mainstay of industrial natural products discovery, in recent years phytochemistry has again become a field of active interest. Drug discovery programs based on microbial products and phytochemical are discussed and contrasted.

Glaxo PLC has had a significant involvement with Natural Product Source Materials for all of its commercial history and, most recently, has pursued this interest by use of such materials as templates for new lead discovery. Through the expertise and facilities in its Natural Products Discovery Department, Glaxo extracts relatively small quantities of plant material (typically 200-250 g dry weight) and cultures microorganisms from environmental samples (typically 10-50 g). Extracts and fermentation broths are screened in order to detect bioactive principles (BPs). If the potency, selectivity and specificity of the BP are acceptable, isolation, purification and structural elucidation follows. It is most unlikely, that the BP itself will become a drug; it is much more likely to initiate a medicinal chemistry synthesis

program in order to try to produce a molecule that has both the essential biological and desirable chemical properties to become a drug development candidate.

Plants have been used as medicine for millennia. Out of estimated 2,50,000 to 3,50,000 plant species identified so far, about 35,000 are used worldwide for medicinal purposes. It has been confirmed by WHO that herbal medicines serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of developing countries. Meanwhile, consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives. The recent resurgence of plant remedies results from several factors: 1) the effectiveness of plant medicines; 2) the side effect of most modern drugs; and 3) the development of science and technology. It has been estimated that in the mid-1990s over 200 companies and research organizations worldwide are screening plant and animal compounds for medicinal properties. Actually, several important drugs used in modern medicine have come from medicinal plant studies, e.g., taxol/paclitaxel, vinblastine, vincristine, topotecan, irinotecan, etoposide, teniposide, etc. As for drugs derived from orchids, some novel discoveries, both in phytochemical and pharmacological properties, were reported by some universities. However, studies on plants are very limited. Only about a third of the million or so species of higher plants have been identified and named by scientists. Of those named, only a tiny fraction has been studied. Nowadays the linking of the indigenous knowledge of medicinal plants to modern research activities provides a new approach, which makes the rate of discovery of drugs much more effective than with random collection.

Future Prospects in Herbal Medicines

At the moment, scientific research on medicinal plants is being carried out most intensely in research institutes, universities and pharmaceutical laboratories as well as in the clinics of many developed countries. This research is oriented mainly in two directions. Firstly, the active ingredients of plants that have long been known for their healing properties are investigated. The second sphere of basic research is directed towards the discovery of new kinds of medicinal plants and new drugs from the more remote regions of the world, which have not been explored so far. Drugs of each and every traditional medicine, like Ayurveda, Unani and Siddha need to be tested and validated scientifically. Council for Scientific and Industrial Research (CSIR), New Delhi, is already involved in this field and validated about 350 formulations for different activities. This is a welcome trend since it attempts to marry traditional practice with modern knowledge for the betterment of health.

WHO emphasized on the need to ensure the quality control of herbs and herbal formulations by using modern techniques. Several countries have herbal pharmacopoeias and lay down monographs to maintain their quality. Ayurvedic Pharmacopoeia of India recommends basic quality parameters for 80 common herbal drugs.

Chemical Classes of Natural Products

Scientific validation of the medicinal activity of plants by chemical analysis has led to the isolation and identification of various classes of natural products. The important classes are as follows.

Sesquiterpenoids

The sesquiterpenoids in general, are the higher boiling fractions of the essential oils. Wallach (1987) was the first to suggest that the sesquiterpenoid structure is built up of three isoprene units. The sesquiterpenoids are classified into four groups according to the number of rings present in the structure. The structure of eremophhilone [Fig.1 (I)] and the related hydroxyeremophilone [Fig. 1 (II)] both found in the wood oil from *Eremophila mitcbelli* are of particular interest since they present exceptions to the isoprene rule i.e. their structure cannot be built up from three isopentene residues. Eremophilone is an α , β- unsaturated ketone, as shown by its ultraviolet spectrum and by reduction with sodium and alcohol to dihydroeremophilol [Fig.1 (III)].

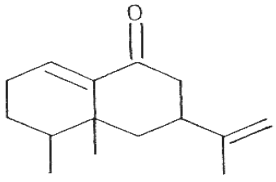
Biological Properties of Sesquiterpenes

In spite of the large number and wide variety of naturally occurring sesquuiterpene lactones (over 3,500) that have been chemically characterized, little work has been done on their biological activity and ecological significance, antibacterial, antifungal, cytotoxic, allergenic, deterrent activity, toxicity and antifeedant activity, The sesquiterpene diplophyllin from the hepatic *Diplophyllum albicans* , for instance, showed an anticarcinogenic activity in KB cell cultures against the skin cancer found in man. The sesquiterpene norpiinguisone from Porella speciHs acted fungitoxically against the mould Aspergillus niger.

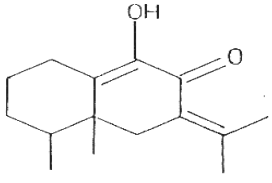
Triterpenoids

The term triterpenoid refers to a group of natural products containing thirty carbon atoms based on six isoprene units. Triterpenoids are generally found in two forms, tetracyclic and pentacyclic, on the basis of number of rings they possess. Unlike steroidal sapogenins, these on selenium dehydrogenation yield a mixture of naphthalene and phenanthrene.

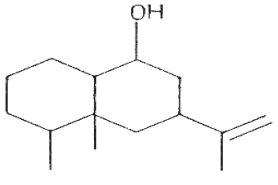
Basic skeletons of some triterpenic compounds are shown in (Fig. 2 and 3). The position of double bond and hydroxyl group varies in different sapogenins. Papyriogenin D is an oleane type sapogenin with 21-hydroxy-3-oxo-olean - 11, 13 (18)-diene-28-carboxylic acids. A new triterpenoid characterized as 3, 11-dihydroxy-23-oxo-lup-20-(29)-en-28-oic acid belongs to lupane type.



I



II



III

Fig. No. 01

Structure of eremophhilone (I), hydroxyeremophilone (II)] & dihydroeremophilol (III)

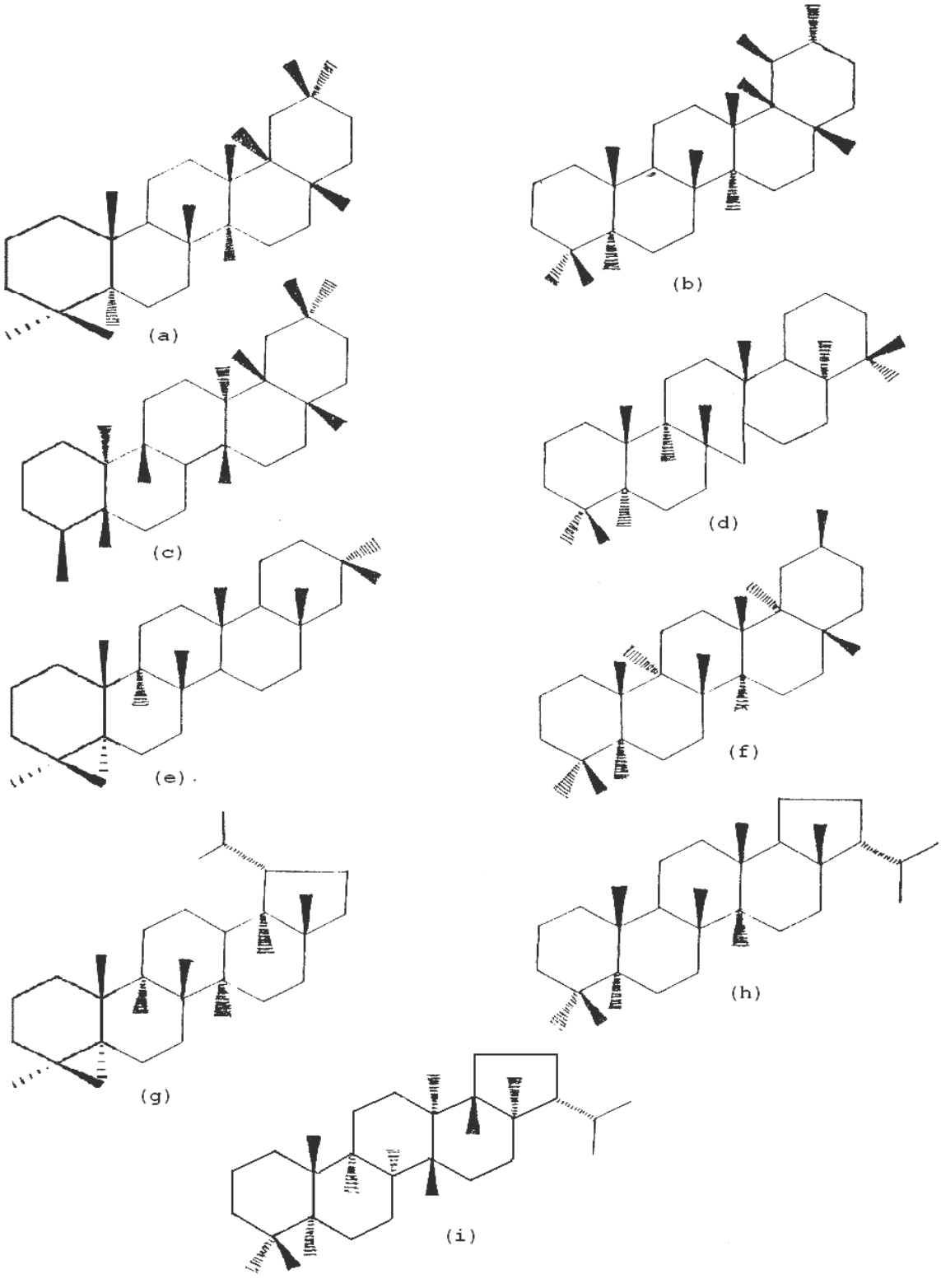


Fig. No. 02

Basic skeletons of some triterpenic compounds

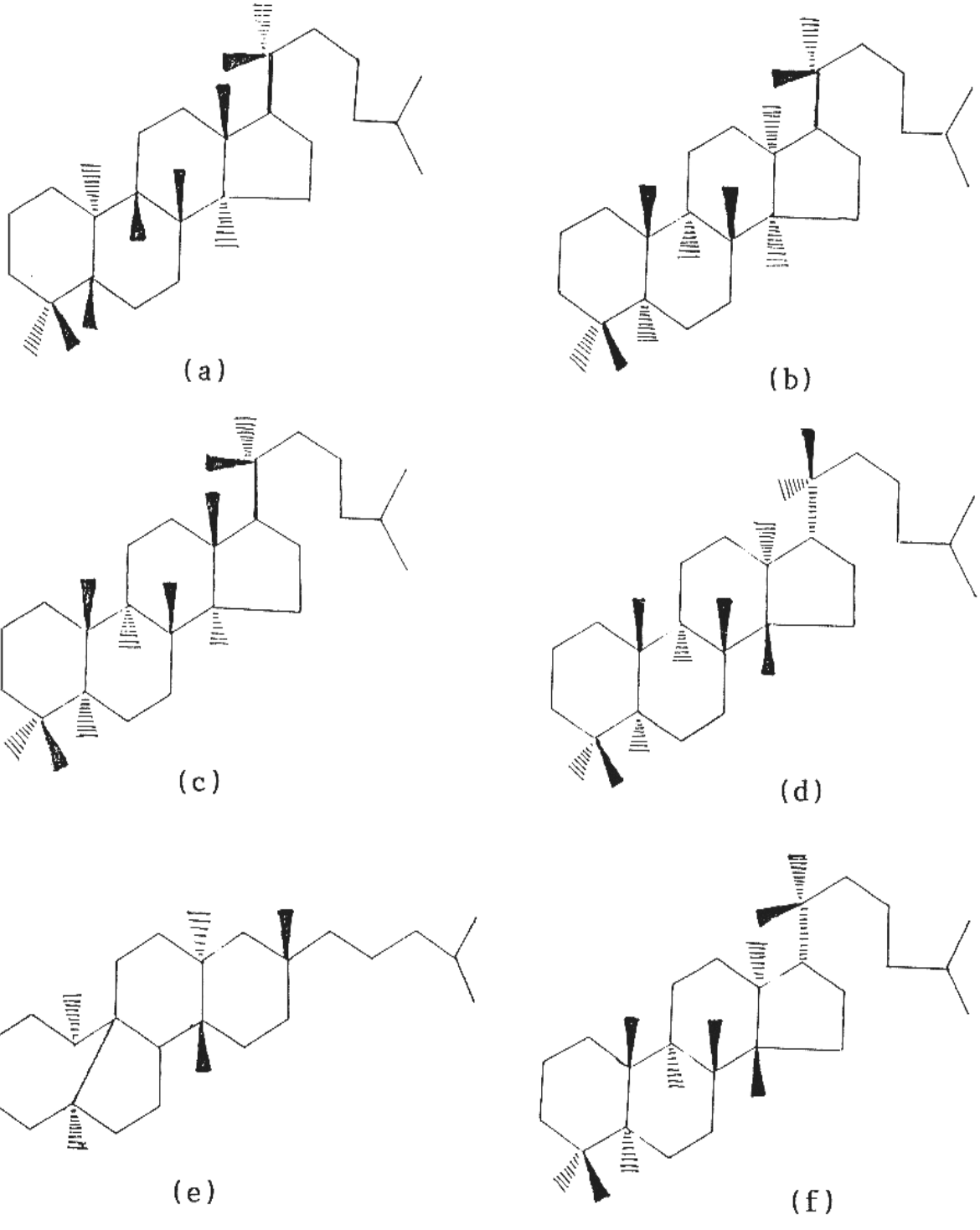
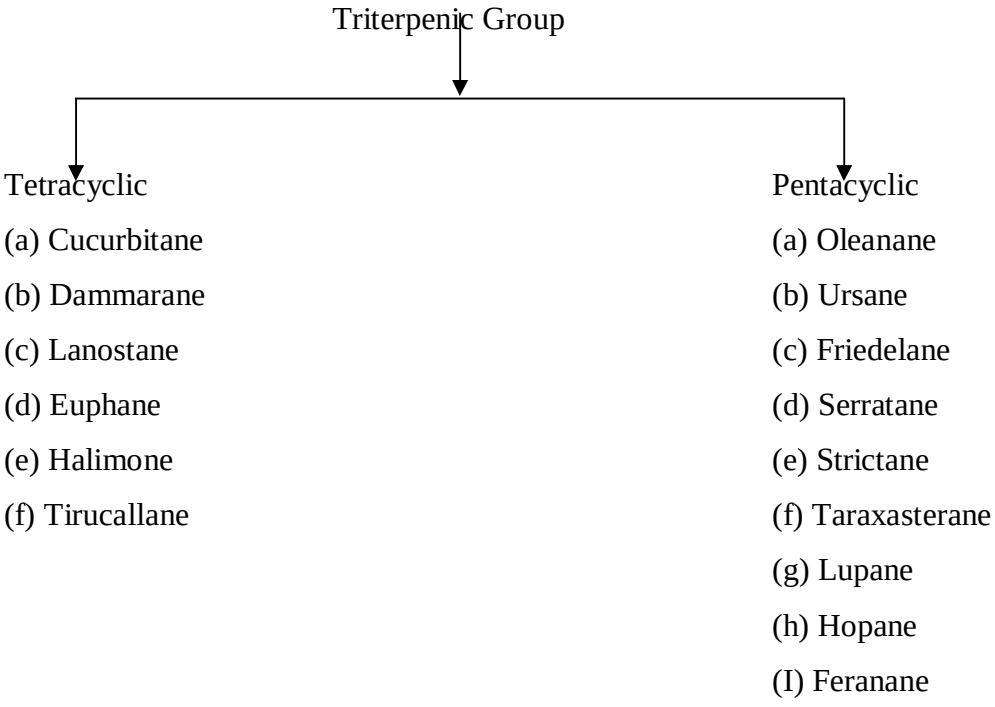


Fig. No. 03

Basic skeletons of some other triterpenic compounds

Malabaricanediol (3, 20-diol) with double bond at C-24 joins halostane type. A sapogenin, sechind, carrying 3-hydroxy and 26-carboxylic group with double bonds C-7, 25 joins the lanostane type. Further classification of these compounds is done as below:-



Biological Properties of Triterpenoids

The wide occurrence in nature and structural diversity of triterpenoid has evoked considerable interest in their biological activity.

A new highly cytotoxic meliacin type triterpene has been isolated from *Aphanamixis grandifolia.* The antifungal activities of 49 "pentacyclic tritepenoids were tested in vitro using Saccharomyces carlsbergenesis as a test organism and it was found that the pentacyclic triterpene glycoside of oleanolic acid and hederagenin with a free carboxylic group at C-28 or C-27, possess the highest fungicidal activity.

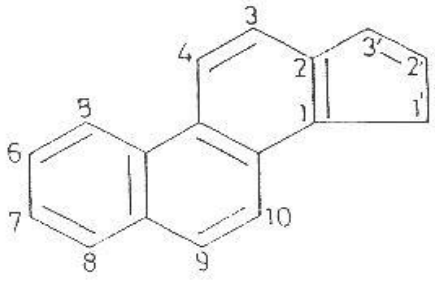
Carbenoxolone, the succinic acid, derivative of glycyrrhetinic acid, also possesses the antiulcer activity. The effect of ursolic acid and its derivatives on lipid metabolism in experimental atherosclerosis was studied, and this revealed that their derivatives decreased the blood cholesterol, β - lipoprotein and phospholipid concentration in rabbit. Two triterpenoid aglycones showed anti-inflammatory, analgesic and antipyretic activities in experimental animals. Tritepenoid glycosides foetoside C and cyclofoetoside B from *Thalictrum foetidum* and thalicoside A from *T. minus* were studied for their anti-tumor activity in rats with implanted tumor. Triterpenoidal saponins of oleane group show spermicidal activity. The lanostane type nortriterpene oligosides isolated from *Asteropus sarasinosum* were studied for their ichthyotoxic and antifertility activities.

Steroids

Steroids are based on the 1,2-cyclopentenophenanthrene skeleton [Fig. 4 (I)] and form a group of structurally related compounds which are widely distributed in -.2 S and plants.

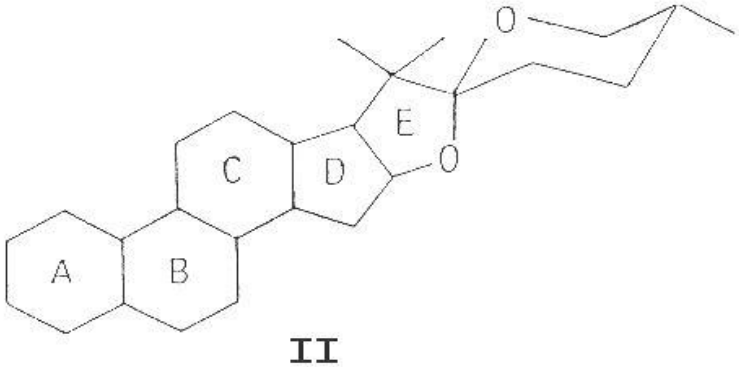
On dehydrogenation with selenium at 420°C, all steroids give chrysene as the main product with small amount of picene. They all give Diel's Hydrocarbon among other products. α -Spinasterol, ergosterol, campasterol, stigmasterol and β-sitosterol are common plant steroids. These steroids are some times present in glycosidic forms and as acetate derivatives. Some are cholane type saponins. The aglycones of this group, possessing spirostane nuclei having rings A B C D E and F were isolated first [Fig. 4 (II)]. Many natural sterols are unsaturated and called 'stanols'. The major compound i.e. β - sitosterol, has been isolated invariably from almost all the plant species. Some steroidal glycosides have open 'F' rings [Fig. 4 (III)] and known as furostanol glycosides or bisdesmoside.

Due to their diverse pharmacological activities like anti-inflammatory, anti-ulcerogenic, anti-bacterial, antifungal, anti-rheumatic etc. the vast majority of steroids play an important role in the field of medicines.

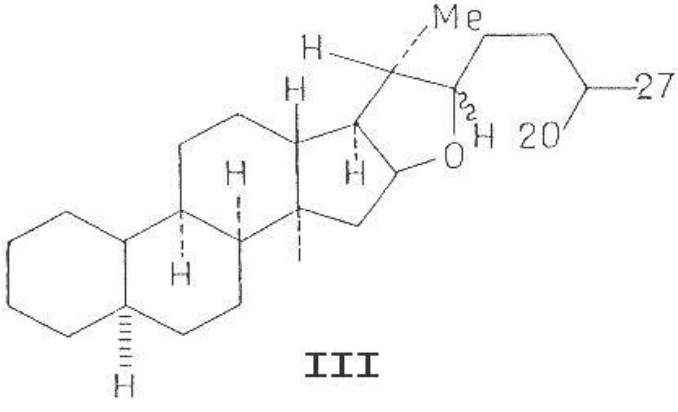


1,2-Cyclopentenophenanthrene

I



II



III

Fig. No. 04

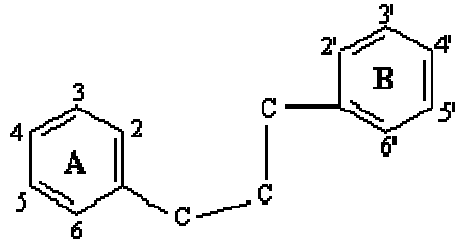
Basic skeletons of Steroids

Flavonoids

Flavonoids are a class of phenolic compounds widely distributed in plants. They occur either as free molecules or as glycosides. Over 1000 individual types are known, and the list is constantly expanding .The term flavonoid is used to include all of the pigments that possess structure based upon C6-C3-C6 carbon skelton found in flavones, chalcones, anthocyanins, etc. Examples of yellolow flavonoids (chalcone, aurones and yellow flavonols) and red, blue or purple anthocyanins (Fig. 5). When they are not directly visible they contribute to the color by acting as copigments. Flavonoids have shown potential health benefits arising from the antioxidative effects of these phytochemicals whose properties are attributed to phenolic hydroxyl group attatched to the flavonoid structure. Scavenging of free radical seems to play a considerable part in the antioxidant activity of flavonoid compounds. In very rescent years flavonoids as potent radical scavengers have attracted a tremendous interest as possible therapeutic against free radical mediated disease.

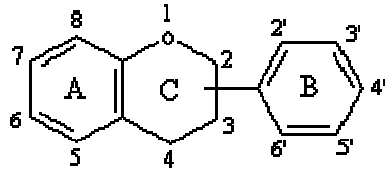
Chemistry of Flavonoids

The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain.

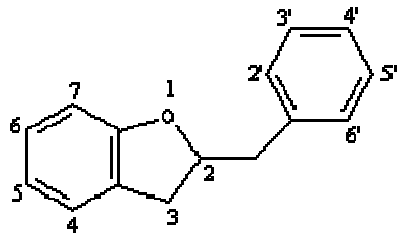


The skeleton above can be represented as the C6 - C3 - C6 system.

Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognized as flower pigments in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers but include all parts of the plant. The chemical structure of flavonoids are based on a C15 skeleton with a CHROMANE ring bearing a second aromatic ring B in position 2, 3 or 4.



In a few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five - membered ring.



AURONES (2-benzyl-coumarone)

The oxygen bridge involving the central carbon atom (C2) of the 3C - chain occurs in a rather limited number of cases, where the resulting heterocyclic is of the FURAN type.

Classification of Flavonoids:

Various subgroups of flavonoids are classified according to the substitution patterns of ring C. Both the oxidation state of the heterocyclic ring and the position of ring B are important in the classification. Examples of the 6 major subgroups are:

1. Chalcones:

Chalcone do not have a central heterocyclic nucleus and are characterized by the presence of a three carbon chain with a ketone function and an α, β unsaturation, substitutions on the A ring are most often identical to those of other flavonoids, where as the B ring is fairly often unsubstituted. Isoprenyl and pyranochalcones seem rather common, especially in the Fabaceae. Aurones are characterized by a 2 benzyllidenocoumarone structure.

2. Flavone

Generally found in herbaceous families, e.g. Labiatae, Umbelliferae, Compositae. Apigenin (Apium graveolens, Petroselinum crispum). Luteolin (Equisetum arvense)

In this ring A in over 90% of the cases is substituted by two phenolic hydroxyl groups at C-5 and C-7.These hydroxyl groups are either free or etherified, and one of them may be engaged in a glycosidic linkage. Other substitutions are possible, free or etherified hydroxyl groups at C-6 or C-8 or both in a carbon – carbon bond with a saccharide.

The ring B, substituted in the 4'-position in 80% of cases, may be 3',4'di- substituted or, less frequently, 3',4',5'-trisubstituted; the substituents are OH or –OCH3 groups. The other positions (2' and 6') are substituted only exceptionally.

3. Flavonol

Generally found in woody angiosperms. Quercitol (Ruta graveolens, Fagopyrum esculentum, Sambucus nigra) Kaempferol (*Sambucus nigra, Cassia senna, Equisetum arvense, Lamium album, Polygonum bistorta*).

These flavonols and their glycosides are universally distributed, but some of the substitution patterns are restricted to some families. Lamiaceae, Rutaceae and Asteraceae.

4. Flavanone

These molecules are characterized by the absence of a 2,3-double bond and by the presence of atleast one assymetric center. In natural flavanones C-2 is normally in the 2S configuration. These flavonoids are some what less common than their unsaturated homologs, and it is notworthy that some families tend to accumulate their C-alkylated derivatives (Asteraceae, Fabaceae).

5. Anthocyanins

Anthocyanin pigmentation is almost universal in the flowering plants and provides scarlet to blue colors in flowers, fruits, leaves and storage organs. It continues to provide a challenge to plant biochemists because of the intricate chemical variation and the complexity of biosynthesis, metabolism and regulation.

The term anthocyanins initially coined to designate the substance responsible for the color of the corn flower, applies to a group of water soluble pigments responsible for the red, pink, mauve, purple, blue, or violet color of most flowers and fruits. These pigments occurs as glycosides (the anthocyanins), and their aglycones (the anthocyanidins) are derived from 2-phenylbenzopyrylium cation. Anthocyanins are present in all of the angiosperms, although they are genrally characeristics of flower petals and of the fruits, anthocyanins can also be found in the bracts (bromeliaceae).

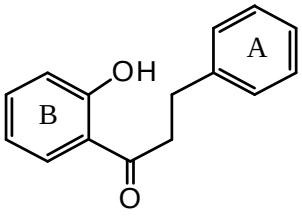
Anthocyanins whose vivid color attracts insect and birds play a major role in pollinaton and seed dispersal. A high coloring power and the absence of toxicity lend to these natural coloring glycosides the potential to replace synthetic color in food technology. Therapeutical appliations of anthocyanins are limited to treatment of vascular disorders; the drug containing them are used for the extraction of anthocyanins and the preparation of galanicals designed to treat the symptoms linked to capillary and venous fragility.

Anthocyanins are natural plant pigments; they are glycosides and their aglycone i.e., the sugar free pigments, are known as the anthocyanidins. The fundamental nucleus in anthocyanidins is benzopyrylium chloride [Fig. 6 (I)], but the parent compound is 2-phenyl benzopyrylium chloride or flavylium chloride [Fig. 6 (II)].

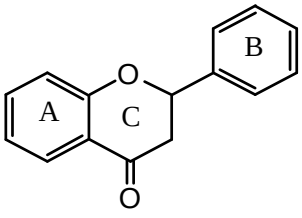
Most of the anthocyanidins are derivatives of 3,5,7-trihydroxyflavylium chloride. Thus, the hydroxylation patterns in the natural anthocyanidins fall into the three basic groups

of pelargonidin, cyaniding and delphinidin. Table No. 1 lists the common anthocyanidins (as chloride).

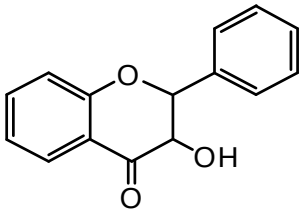
A new anthocyanidins, 6-hydroxycyanidin has been found in the red flowers of *Alstroemeria* (Alstroemeriaceae) where it occurs as the 3-glucoside and 3-rutinoside. The first report of 5-methylcyanidin as a new anthocyanidin was in *Egeria densa* (Elodeaceae). Some known anthocyanidins have been listed in Table No. 2.



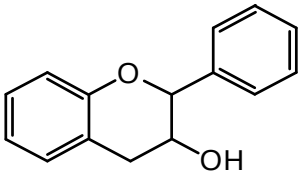
Chalcone



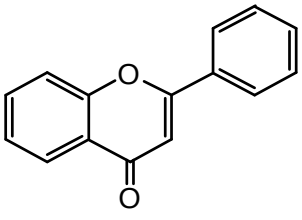
Flavanone



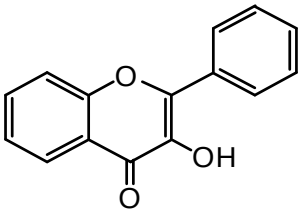
Dihydroflavonol



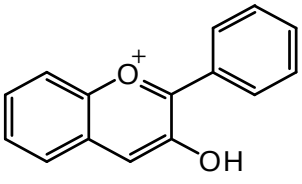
Flavan-3-ol



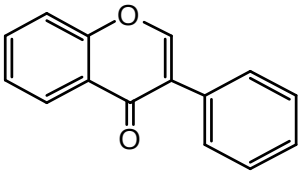
Flavone



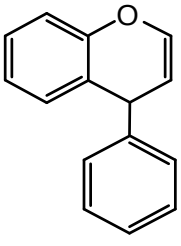
Flavon-3-ol



Anthocyanidin



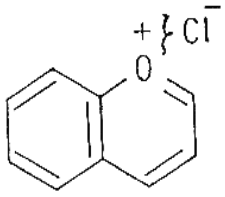
Isoflavone



Neoflavone

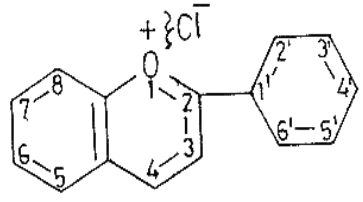
Fig. No. 05

Basic Skeletons of Flavonoids



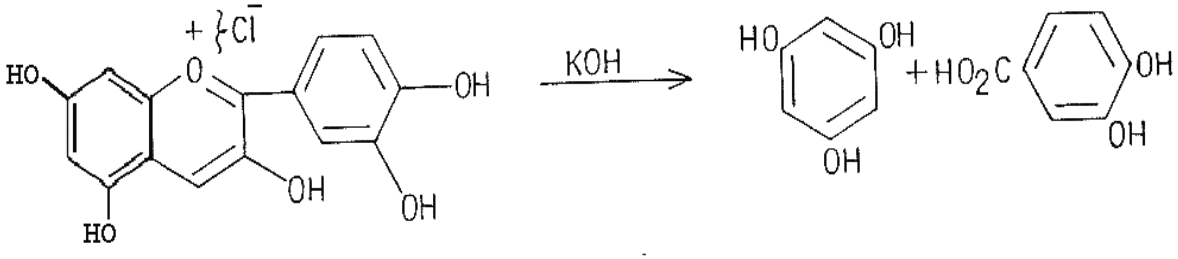
Benzopyrylium Chloride

I



Flavylium Chloride

II



Cyanidin Chloride

III

Fig. No. 06

Fundamental Nucleus of Anthocyanidins

Table No. 01

List of Common Anthocyanidins (As Chloride)

S. No.	Aglycone Trivial Name	Chemical Name	Occurrence
1	Pelargonidin	3,4',5,7-tetrahydroxy flavylium chloride	Present in orange red to scarlet flowers, eg., Scarlet Pelargonium, Orange red dahlia.
2	Cyanidin	3,3',4',5,7-pentahydroxy flavylium chloride	Present in crimson to bluish red flowers, eg., deep red dahlia, red roses, blue cornflowers.
3	Delphinidin	3,3',4,5,5',7-hexahydroxy flavylium chloride	Present in violet to blue flowers, eg., Delphinium
4	Hirsutidin	3,4',5-trihydroxy-3',5',7-trimethoxy flavylium chloride	Present in Primula hirsute.
5	Malvidin	3,4',5,7-tetrahydroxy-3',5'-dimethoxy flavylium chloride	Present in flowers less blue than the Delphinidin group eg., Primula viscose.

Table No. 02

List of Some Known Anthocyanidins

S.No	Name	Structure
1	Apigenindin	5,7,4' tri OH
2	Lutieolindin	5,7,3',4'-tertra OH
3	Tricetinidin	5,7,3',4',5-penta OH
4	Pelargonidin	3,5,7,4'-tera OH
5	Aurantinidin	3,5,6,7,4'-penta OH
6	Cyanidin	3,5,7,3',4-penta OH
7	5-Methylcyanidin	5-methyl ether
8	Peonidin	3'- methyl ether
9	Rosinidin	7,3'-dimethyl ether
10	6-Hydroxycyanidin	3,5,6,7,3',4'-hexa OH
11	Delphenidin	3,5,7,3',4',5'-hexa OH
12	Petunidin	3'-methyl ether
13	Malvidin	3',5'-dimethyl ether
14	Pulchellidin	5-methyl ether
15	Europinidin	5,3'-dimethyl ether
16	Capensinidin	5,3',5'-trimethyl ether
17	Hirsutidin	7,3',5'-trimethyl ether

6. Isoflavonoids

All molecules in this group can be related to skelton of 3-phenylchromane.they are present in Dicotyledons, they are infact almost specific to Fabaceae only. Nearly 700 isoflavonoids are known they are classified in to dozens of types, in all types we can note the high frequency of isoprenylated derivatives, and consequently of furan, – dihydrofuran, and pyran type structures.

The most common compound are isoflavone, which occur in the free state, or more rarely as glycosides isoflavonoids have an additional ring such as in case of pterocarpans and their derivatives and also in coumaranochromones. Other isoflavonoids have a coumarinic structure induced by the oxidation of an isoflavene. Some polycyclic compounds have an additional carbon atom, for example rotenoids arising from the oxidative cyclization products of a 2'- methoxyisoflavone.

Most of these (flavanones, flavones, flavonols, and anthocyanins) bear ring B in position 2 of the heterocyclic ring. In isoflavonoids, ring B occupies position 3. The Isoflavonoids and the Neoflavonoids can be regarded as abnormal flavonoids.

Structure of the Anthocyanidins

The anthocyanin is first hydrolysed with hydrochloric acid and the anthocyanidin is isolated as the chloride. The usual analytical methods are applied to determine the number of hydroxyl and methoxyl groups present in the molecule. The structure of the anthocyanidin is ascertained by the nature of the products obtained by fusing the anthocyanidin with potassium hydroxide, phloroglucinol or a methylated phloroglucinol and a phenolic acid are obtained, [e.g., cyanidin chloride gives phloroglucinol and protocatechuic acid is depicted in Fig. 6 (III)].

Proanthocyanidins

Proanthocyanidins, the oligomers and polymers long been referred to as “condensed tannin or non-hydrolyzable tannins", fall into three distinct classes. One of these consists of flavon-3-ol units linked singly through carbon-carbon linkages at C (4)-C (8) or C (4)-C (6). The compounds of this class are readily convertible by common anthocyanidins, pelargonidin, cyanidin and delphinidin. [The dimers and trimers of this class are designated as proanthocyanidin B [Fig. 7 (I, II)] and C types [Fig. 7 (III)] respectively] and are most commonly present in the vegetative tissues of the plants. Another class possesses structures in which two flavan-3-ol units are joined doubly by ether and carbon linkages, and it is decomposed by acid treatment with the production of a mixture of uncharacterized flavylium salts (the dimers are designated as proanthocyanidin A type). The third class related to the second in complex anthocyanidin formation, includes a series of compounds containing a substituent, such as a C6-C3 or a chalcon moiety, which is attached to the flavan-3-ol framework [e.g., cinchonain, kandelins and gambiriins].

According to the biogenetic mechanism proposed by Haslam, most proanthocyanidins are derived from the condensation units via a 4-C/8-C bond. Hence, the over all formula of the water soluble proanthocyanidins obtained by fermentation is depicted (Fig. 8). The catechin units may be (+)-catechin or (-)- epicatechin and gallocatechin units may be (+)-gallocatechin or (-)-epigallocatechin. (+)-Catechin and (+)-gallocatechin both have a trans configuration while their epimers have a cis-configuration. The structure of the proanthocyanidin depends on three main variables, the relative proportions of procyanidin

and prodelphinidin units i.e. the PC : PO ratio, the stereochemistry of the heterocyclic nucleus of the monomers units, i.e. *cis-trans* ratio and the degree of polymerization.

Nomenclature

A system of nomenclature for naming proanthocyanidins was introduced by Hemingway et al. (1982) and outlined by Haslam (1982). Subsequently developments have shown the merit of this system, especially as it relates to the naming oligopyranoanthocyanidins. However, it is evident that this system must now be extended to encompass the more complex structures of this type and a greater variety of flavanoid monomer (configurational base) units.

Briefly, the original form of this system was inspired by the realization that the previous trivial system of nomenclature used to distinguish procyanidin dimmers and trimers was unsuitable for naming oligomers containing monomer units with differing oxidation patterns. Moreover, use of a systematic system based on IUPAC rules for absolute stereochemistry aromatic ring substitution patterns is excessively cumbersome and potentially misleading.

The system is as follows: proanthocyanidins are named in a similar way to polysaccharides where C-4 of the flavan monomer unit is equivalent (in the nomenclature sense) to C-1 of a monosaccharide in an oligo- or polysaccharide chain. The interflavanoid linkage is indicated in the same way as polysaccharides, the bond and its direction being contained in brackets (4→). The configuration of the interflavonoid bond at C-4 is indicated by the α,β- nomenclature (IUPAC, 1979) within the above brackets. The flavanoid monomer units are defined in terms of the trivial names of monomeric flavan-3-ols, the names catechin, epicatechin, etc. being reserved for those units with a 2R- configuration, whereas those with a 2S- configuration are distinguished by the enantio prefix. Typical examples are the dimer structure [Fig. 9] which is named epicatechin-(4β→8)-catechin and the dimer [Fig. 9)] named ent- epicatechin-(4α→β)-epicatechin.

This system is extended so that it is as generally applicable as possible. This firstly requires that an agreed system of nomenclature is adopted to name all monomer units likely to be encountered in proanthocyanidins. These are listed in Table. No. 3.

The names for the monomers units (a)-(k) are those already established for those flavan-3-oIs with (2R, 3S) absolute stereochemistry with the particular A- and B-ring hydroxylation patterns listed in Table No. 3. New names are prosopin (k) after its isolation - from *Prosopis glandulosa* and oritin (j) which is named from the fact that the first flavan-3,4-

diol isolated with this phenolic hydroxylation pattern was obtained from *Acacia orites*. The names for (a) - (c) are also new and stress their relationship to flavan (i.e., they lack a 3-hydroxy group) and also relates them to the corresponding (2R, 3R) or 3α-hydroxy isomers of the unit (d) - (k) are distinguished by adding 'epi' to the ·beginning of each monomer name, i.e., epiafzelechin, etc. This does not arise for (a) - (c) as they lack a 3-substituent. The (2R), (2S, 3R) or (2S, 3S) isomers are indicated by adding ent to the beginning of the appropriate monomer name (IUPAC, 1979), i.e., ent-Iuteoliflavan, ent-epigallocatechin, etc. Many examples of the use of this nomenclature follow.

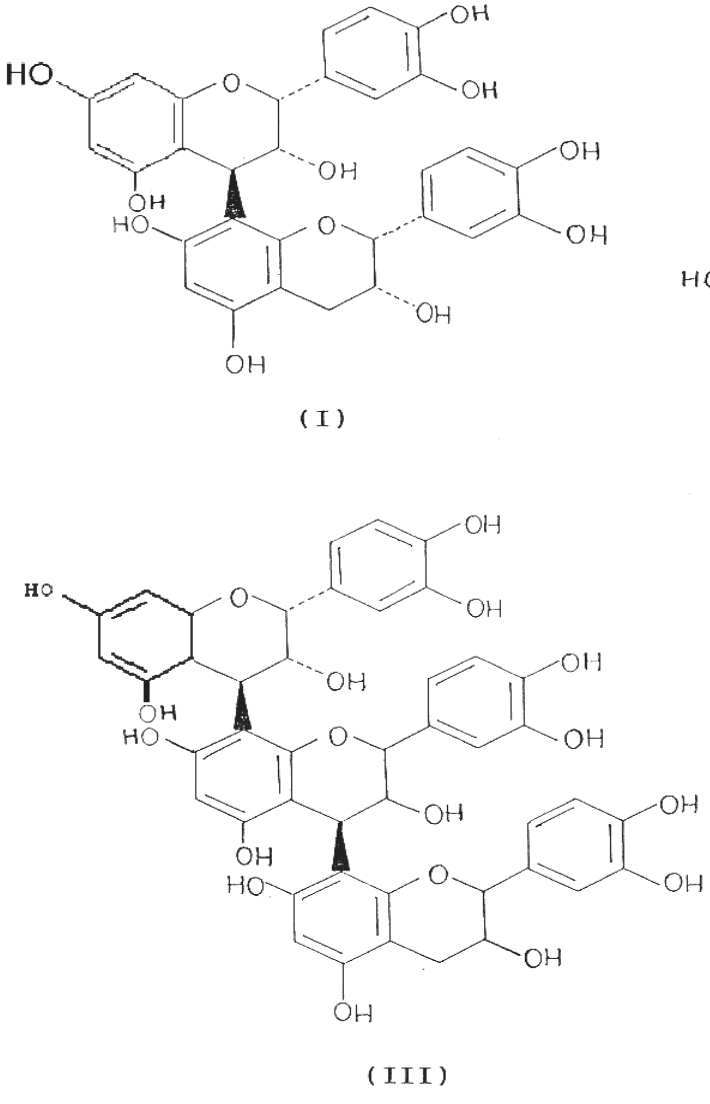
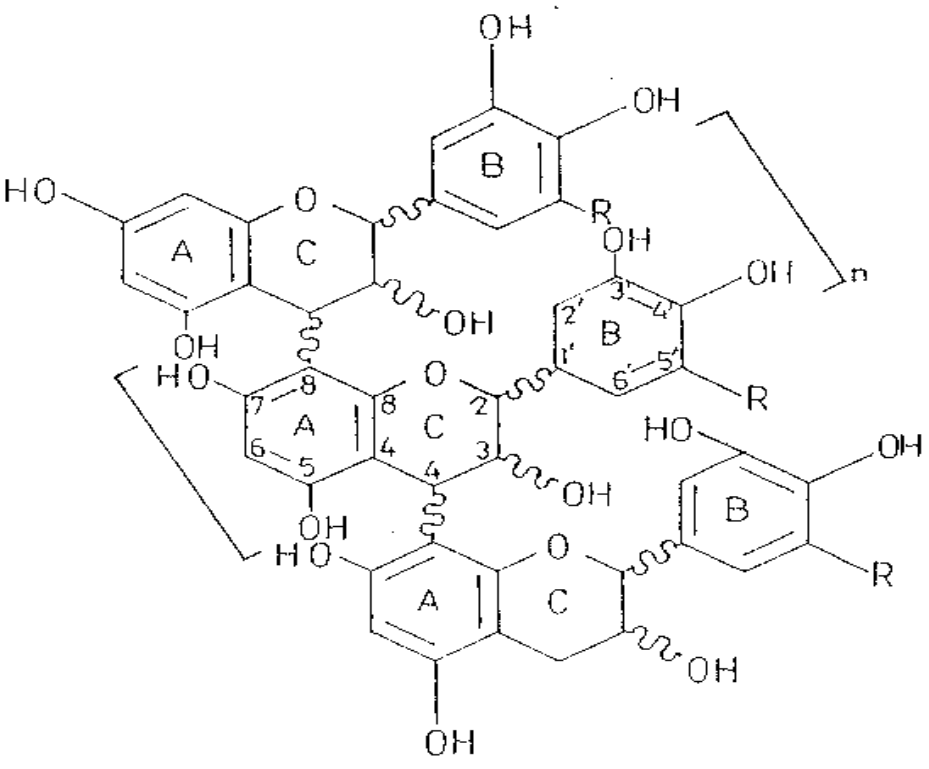
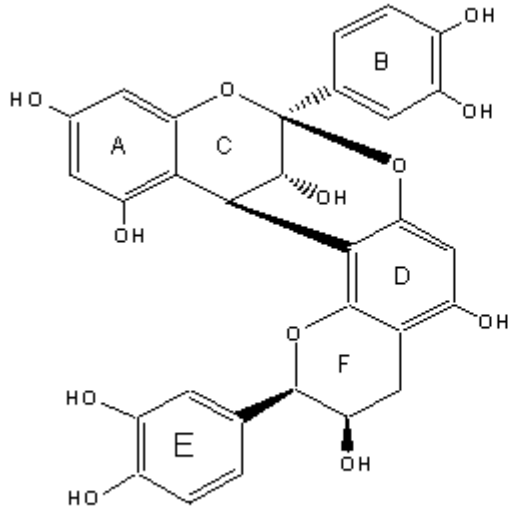


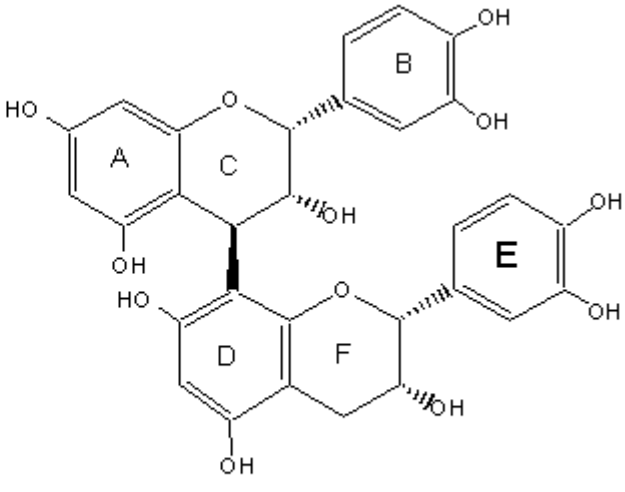
Fig. No. 07

Different Types of Proanthocyanidins B (I, II) & C types (III)





Procyanidin A2(PC-A)



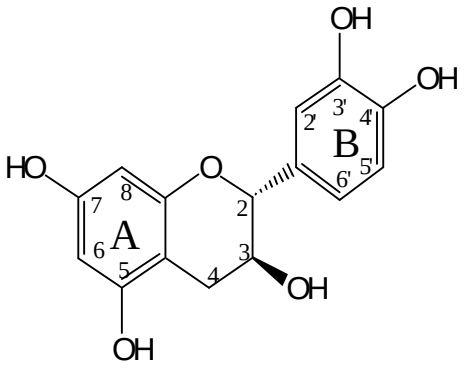
Procyanidin B2(PC-B)

Fig. No. 08

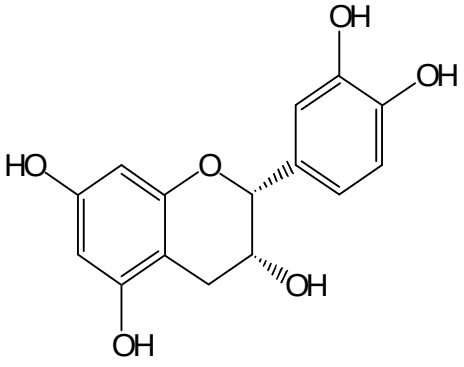
Proanthocyanidin

R=H: Catechin or Procyanidin moiety

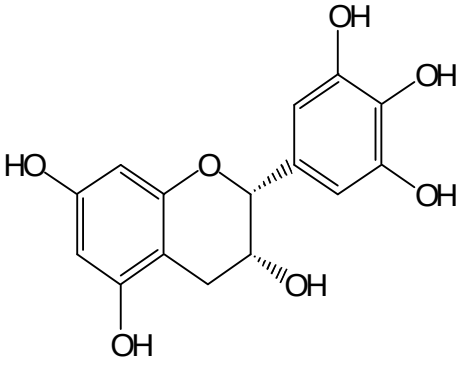
R=OH: Gallocatechin or Prodelphinidin moiety



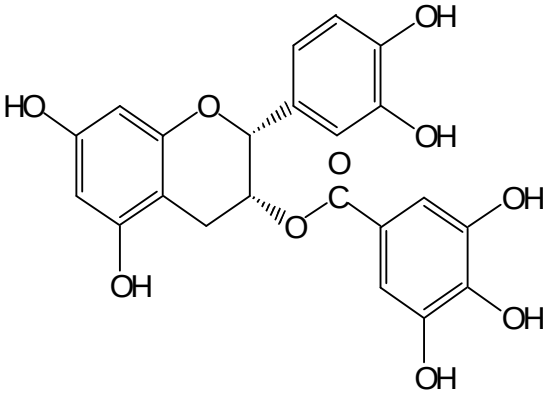
(+)-catechin (CAT)



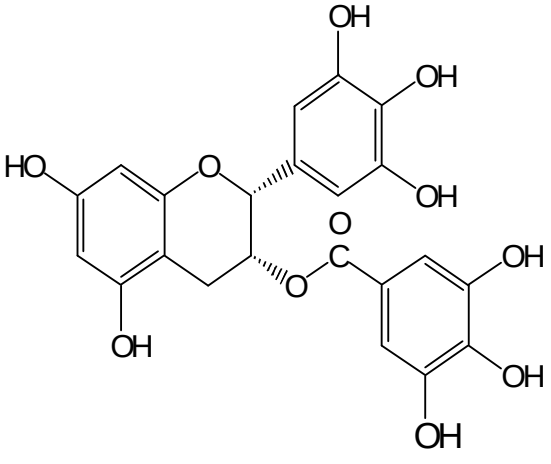
(-)-epicatechin (EC)



(-)-epigallocatechin (EGC)



(-)-epicatechin gallate (ECG)



(-)-epigallocatechin gallate (EGCG)

Fig. No. 09

Types of Proanthocyanidin

Table No. 03

List of Monomer Units in Different Class of Proanthocyanidin

Proanthocyanidin Class	Monomer Unit	Substitution Pattern						
		3	5	7	8	3’	4’	5’
Proapigeninidin	Apigeniflavan (a)	H	OH	OH	H	H	OH	H
Proluteolinidin	Luteoliflavan (b)	H	OH	OH	H	OH	OH	H
Protricetinidin	Tricetiflavan ©	H	OH	OH	H	OH	OH	OH
Propelargonidin	Afzelechin (d)	OH	OH	OH	H	H	OH	H
Procyanidin	Catechin (e)	OH	OH	OH	H	OH	OH	H
Prodephinidin	Gallocatechin (f)	OH	OH	OH	H	OH	OH	OH
Proguibourtinidin	Guibourtinidol (g)	OH	H	OH	H	H	OH	H
Profisetinidin	Fisetinidol (h)	OH	H	OH	H	OH	OH	H
Prorobinetinidin	Robinetinidol (i)	OH	H	OH	H	OH	OH	OH
Proteracacinidin	Oritin (j)	OH	H	OH	OH	H	OH	H
Prometacacinidin	Prosopin (k)	OH	H	OH	OH	OH	OH	H

A considerable number of doubly linked (so called A-type) proanthocyanidins are now known, often this type of linkage co-occurring with the above single linkages in the same molecule. Typical is proanthocyanidin A2 [Fig. 10 (III)] where two epicatechin unit are linked through a normal 4β→8 linkage and also through C-2 to O-7 of the adjacent epicatechin unit. The naming of such compounds is readily accommodated by the proposed system by including both types of linkage within the brackets, as follows: epicatechin-(2β→7, 4β→8)-epicatechin. As in the case of initerglycosidic linkage, there is no need to name O in the 2β→7 linkage as this is obvious from the epicatechin substitution pattern.

The system may also be used to name the leucoanthocyanidins. The flavan-3,4-diols are currently named by a confusing system of trivial names, some actually having more than one. It is proposed for example, that (+)-mollisacacidin is called fisetinidol 4α-ol and (-)-melacacidin is called epiprosopin-4α-ol.

A-Type Proanthocyanidin

The structure of proanthocyanidin A-2 was known with any certainty, and this was thought to be structurally correlated with epicatechin-(4β→8)-epicatechin. Proanthocyanidin A-2 [(-)-epicatechin-(2β→7, 4 β→8)-(-)-epicatechin)] was first isolated from the seeds of

Aesculus hippocastanum. The structure was deduced by Haslam and his collaborators via spectroscopic and chemical evidence and has, more recently been unequivocally established by X-ray crystallography. A new variety of proanthocyanidin possessing the doubly linked unit of either 2β, 4β [Fig. 10 (III)] or 2α, 4α-configuration [Fig. 10 (IV)] has since been reported. Constituent unit other than (+)-catechin and (-):- epicatechin have also been encountered e.g. a flavonol, a flavan C ring, (-)-epigallocatechin and the afzelechin. As with the procyanidins and other classes of condensed tannins., the group of Nishioka made considerable contribution to the chemistry of the A-type analogues containing both A and B type linkages, e.g. triflavanoid [Fig. 10 (VI)].

Owing to the close structural relationship between proanthocyanidin A-2 [Fig. 10(II)] and procyanidin B-2 [Fig. 10(I)] a biosynthetic pathway for the conversion of B- to A-type procyanidins has been proposed which involves an enzyme mediated hydroxylation at C-2 (C ring) [Fig. 10 (I)]. Despite the considerable progress in the semi-synthetic approach towards condensed tannins over the last fifteen years, similar efforts aimed at the oxidative conversion of B to A-type procyanidins are much more limited. These methods are restricted to the use of $H_2O_2/NaHC0_3$ and molecular oxygen, both sets of conditions, however, given low yields of the A-type proanthocyanidins. It seems reasonable to assume that the transformation of procyanidins B-2, into the A-type analogue involves the oxidative removal of hydride ion at C-2 (C) as the initial step. The nature of the oxidizing species is, however, not clear when using oxygen. Although this reagent may effect the transformation [**Fig**. 10 (1-11)], it seems more reasonable that the prevailing conditions include oxidation of the O-dihydroxy functionality of the procatechol B- or E-rings to an O-quinone which subsequently serves as oxidants for the conversion.

Proanthocyanidins with A - type linkages invariably display ^{3}JHH = 3-4 Hz for 3- and 4H (C-ring), a phenomenon which by reference to X-ray data for procyanidin A-2 [Fig. 10 (III)] and ^{13} C NMR comparisons, has consequently been accepted to indicate 3, 4-trans relative configuration for all known compounds in this class of naturally occurring condensed tannins. The recent synthesis of the first A – type analogue [Fig. 10 (V)] with 3, 4-cis configuration of the C-ring, however, indicated that these compounds exhibit identical ^{1}H NMR coupling constants (J3,4 = 3.6 Hz) irrespective of the relative configuration of their C - rings. Consideration of the structure of the prorobinetinidin related compound [**Fig**.10 (V)], with the conformational rigidity of the bicycling ring system indicates very similar dihedral angles between 3- and 4-H (C) in both 3, 4-trans [Fig. 11 (1)] and 3, 4-cis [Fig. 11(II)]

homologues which leads to almost identical coupling constants for these protons. A method based on the selective ^{1}H NOE association of 3-H (C) permitting such a differentiation the A-series of (4, 8)-linked proanthocyanidins was also described.

Biological Properties of Proanthocyanidins

A novel flavano-flavonol, ephedrannin A showing the hypotensive activity has been isolated from the crude drug "mao-kon" the roots of *Ephedra plants*. Tannins, both condensed and hydrolyzable, were found to cause bursting of the second-stage larvae of dog roundworm (*Toxocara canis*), when combined with an appropriate larvicidal compound such as decanoic acid or tetradecanol. This bursting activity of tannins increased with increase of the degree of condensation for condensed tannins and with increase of the proportion of phenolic moieties for hydrolysable tannins. Bate- Smith has concentrated largely on the predominating proanthocyanidin polymers and has used their solubility and protein-precipitating ability. The synergistic action of proanthocyanidins on the anthelmintic activity of known anthelmintic has been demonstrated.

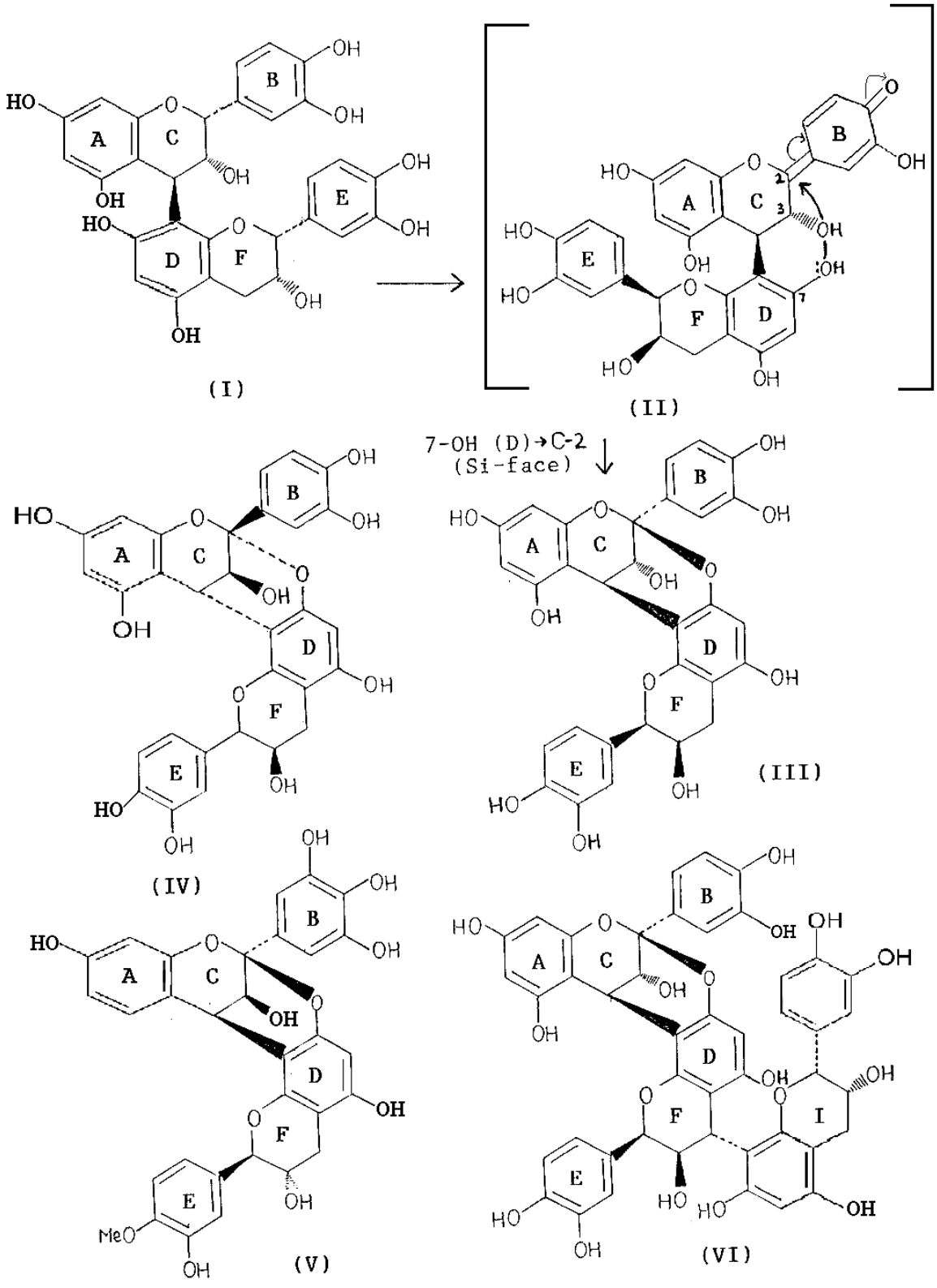


Fig. No. 10

Formation of A-Type Proanthocyanidin

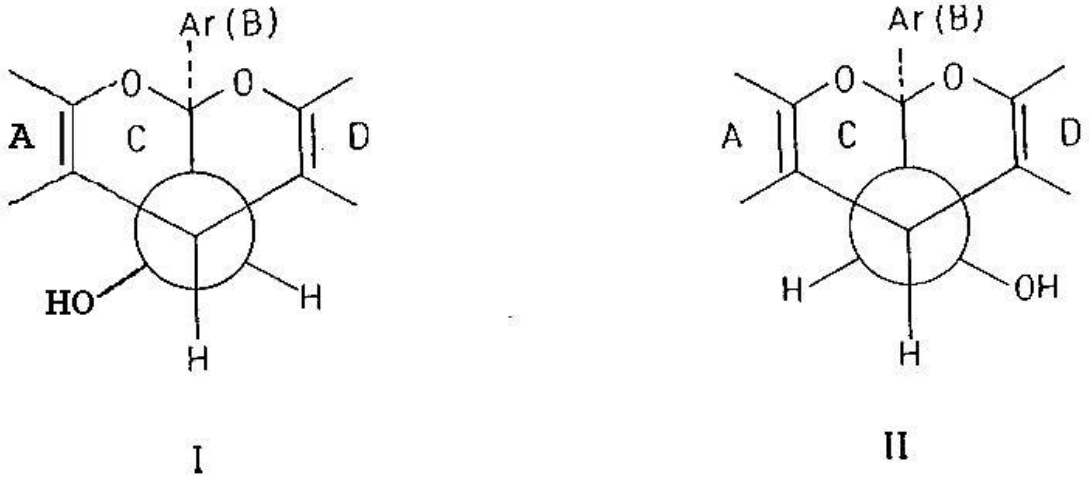


Fig. No. 11

Bicyclic Ring System Indicates Similar Dihedral angle between 3, 4-trans (I) and 3, 4-cis (II)] Homologues

Ligans

A group of natural products characterized by the presence of the 2,3-dibenzylbutane skeleton in the molecules called lignans. Evidence that a stereocontrolled phenolic oxidative coupling process operates during lignan biosynthesis, rather than the random free radical coupling that occurs during lignan formation, has been presented. Pinoresinol, (-)-olivil, and (+)-cyclo olivil are the common lignans (Fig. 12).

Biological Properties of Lignans

Pharmacological screening of these lignans revealed significant CNS activity in animals. The prostalidins A-C produced a mild antidepressant action in albino mice and rats. The action was potentiated by carpacin that itself showed only a weak sedative action. The combined active constituents have a low toxicity. Reversal of sickling and crenation in erythrocytes by plant extracts containing ligans is reported. Dihydrodicaffeic acid dilactone (DDCAD) is an inhibitor of phosphodiesterase, isolated from microbial cultures. DDCAD showed an antihypertensive effect in spontaneous hypertensive rats and (+)-pinoresinol-di-β-D-glucoside was isolated as an antihypertensive constituent contained in the bark of *Eucommia ulmoides*, which has been used as an hypertensive drug among Chinese people (173). (+)-Pinoresinol and (+)-pinoresinol-β-D-glucoside showed high inhibitory activity against cyclic adenosine monophosphate (cAMP)- phosphodiesterase *in vitro*. Lignans have

a considerable number of therapeutic agents used as antipychotics, antianxiety agents, antihypertensive and so on showed inhibitory effect against phosphodiesterase.

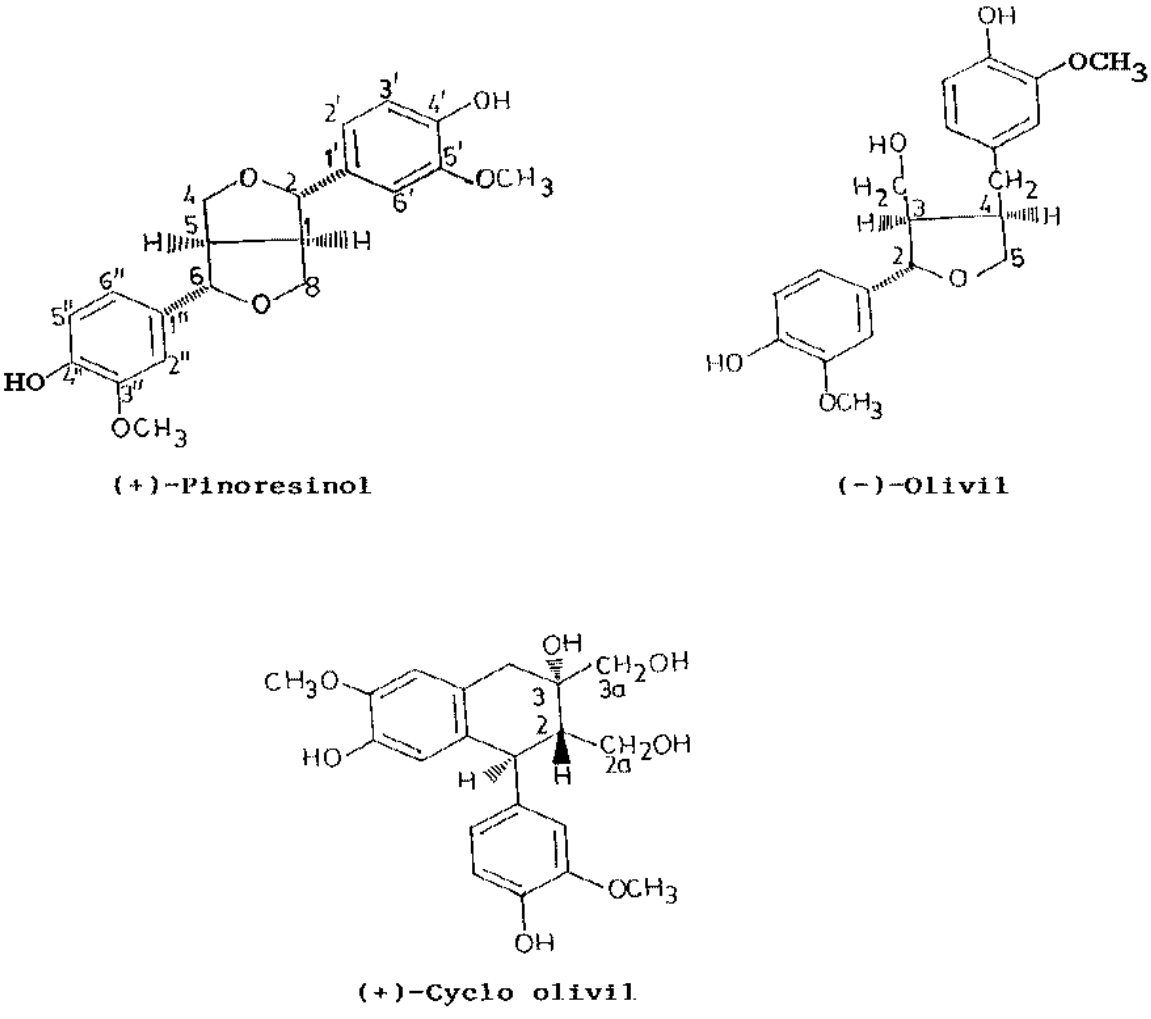


Fig. No. 12

Some common Lignans

Coumarins

Coumarins are the δ-Iactone of coumarinic acid. The classical synthesis of coumarin from salicylaldehyde and acetic anhydride has been improved by the use of anhydrous sodium fluoride as catalyst. Coumarin has been found to be distributed extensively in varied types of flora and in all parts of the plants. They have also been reported from microorganism and animals. About 300 coumarins have been listed from plants sources and parts of the plant associated with coumarins isolated from *Gramineae, Orchidaceae, Labiateae, Leguminosae, Umbelliferae, Guttiferae, Rutaceae* etc..

The survey covering the development in the field of natural coumarins during five year period 1971-1975 has been reviewed. A consolidated account of the newer developments in the isolation methods and. the use of spectroscopic techniques in structure elucidation studies along with a tabulation of the new coumarins characterized during the period 1976-1980 has been put forward. Plant coumarins discovered between 1978 and 1989 have been tabulated thus giving information on their trivial names, year of isolation, structure, formula, melting point, optical rotation and plant source. The coumarins discovered during 1988-1994 are also reviewed. The coumarin are classified into the following groups -

1. Simple Coumarins
    (a) Hydroxylated coumarins
        (i) Oxygenated in heterocyclic rings
        (ii) Oxygenated in carbocyclic ring
    (b) Alkylated coumarins
        (i) Alkylated in heterocyclic rings
        (ii) Alkylated in carbocyclic rings
        (iii) Prenylated coumarin

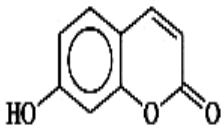
2. Furanocoumarins
    (a) Linear furanocoumarins
    (b) Angular furahocoumarins
3. Pyranocoumarins
    (a) Linear pyranocoumarins
    (b) Angular pyranooumarins
4. Phenyl coumarins
5. Biscoumarins
6. Triscoumarins
7. Coumarin-lignoids or Coumarin ligans
8. Coumestane

The coumarins being classified according to the ring oxygenation pattern. Largely within each section, phenols are presented before their ethers and glycosides, and carbon substituents are considered in order of increasing number of carbon atom and in increasing oxidation level within each group.

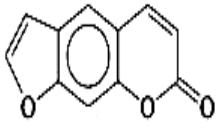
Simple Coumarins

The coumarins are typical metabolic products of higher plants. The simple ones are formed from the corresponding substituted trans-cinnamic acid derivatives. Hydroxylation of the ortho position of the particular cinnamic acid in question takes place first and the resultant ortho-coumaric acid derivatives are subsequently glycosylated. These are then rearranged in a spontaneous reaction to the corresponding coumarinic acid glucoside, which are structurally derived from cis-cinnamic acid.

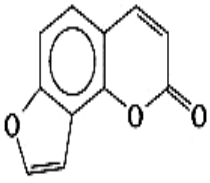
The vast majority of coumarins carry on oxygen substituent at C-7 position and consequently 7-hydroxycoumarin (umbelliferone) is often regarded as the parent coumpound [Fig. 13 (I)]. The other examples of this group are esculetin (6, 7- dihydroxy coumarin), osthol (7 -OCH_3 and 8- $CH_2CH{=}CMe_2$) and delbergin (4-C_6H_5, 5- OCH_3), isofrazetin (5,6 -OH, 7 -OMe) and fraxetin (7,8 -OH, 6 -OMe) etc.



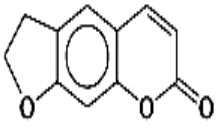
Simple Coumarin(I)



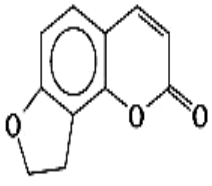
Linear Furanocoumarin(II)



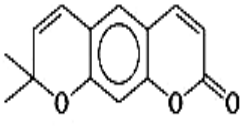
Angular Furanocoumarin(III)



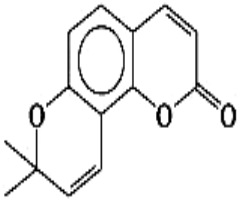
Dihydro Furanocoumarin(IV)



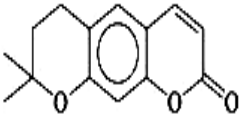
Dihydro Angular Furanocoumarin(V)



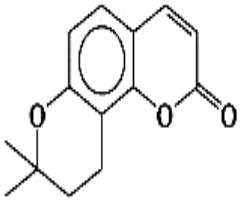
Linear Pyranocoumarin (VI)



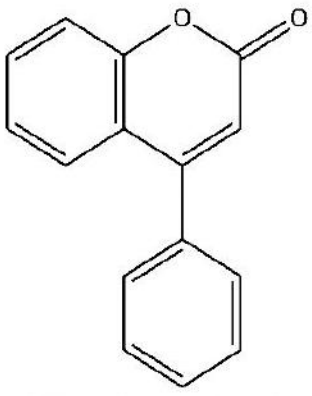
Angular Pyranocoumarin (VII)



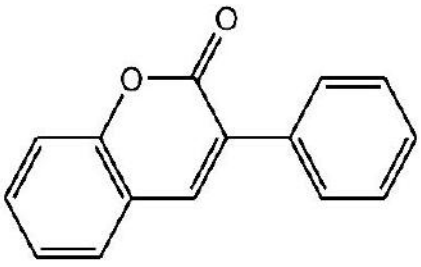
Dihydro Pyranocoumarin (VIII)



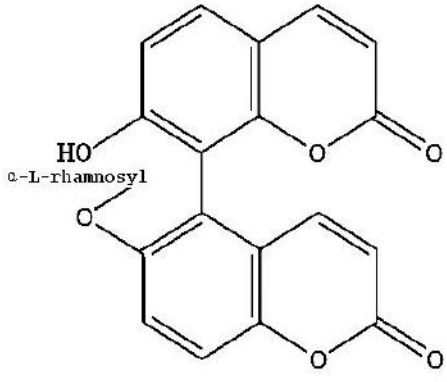
Dihydro Angular Pyranocoumarin (IX)



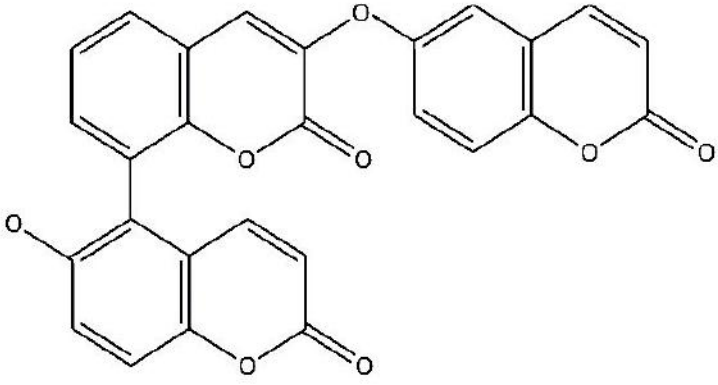
4-Phenylcoumarin(X)



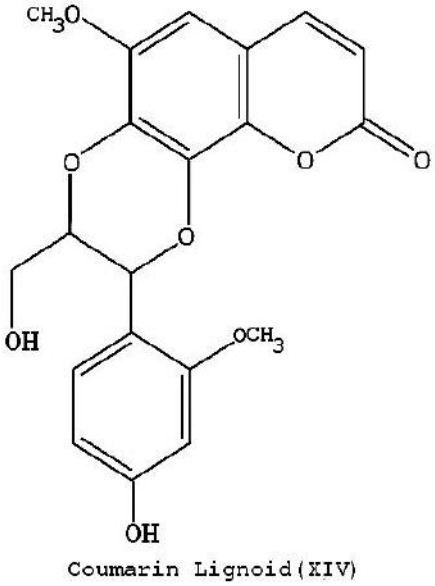
3-Phenylcoumarin(XI)



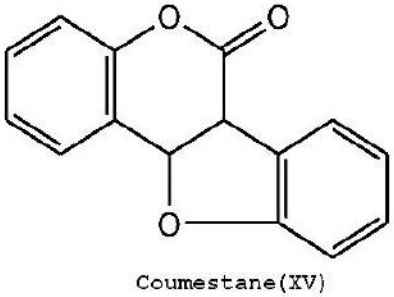
Biscoumarin(XII)



Triscoumarin(XIII)



Coumarin Lignoid(XIV)



Coumestane(XV)

Fig. No. 13

Classification of Coumarins

Furanocoumarins

These are compounds with a furan ring fused with the benzene ring of coumarin. Furanocoumarins occur as linear or angular analogs, which differ in the coumarin moiety. Over 100 such compounds have been isolated from plant sources; most of these arise by alkyl or alkoxy substitution at the available aromatic positions, at either of the two olefinic carbon of the furan ring, or, less frequently at either of the two olefinic carbons of the coumarin lactone ring. On the basis of fusion of the ring through different positions, these are grouped as linear, angular, dihydrofurano and dihydroangular furanocoumarins [Fig. 13 (II-V)]. The common compounds of the linear furanocoumarins type are psoralen, bergaptol (5-OH), bergepten (5-OCH_3)., imperatorin (8-$OCH_2CH=CMe_2$), isoimperatorin (5-CH2CH=CMe2), isopiminellin (5,8-OCH_3) and xanthotoxin (8-OCH_3). The marmasin is the example of dihydrofuranocoumarin. Angular furanocoumarin type has common examples i.e., angelicin (R=H), oroselol (R=-C(OH)Me2), pimpinellin (5-OCH_3, 6-OCH_3). Dihydro angular furanocoumarin is exemplified by columbianadin.

Pyranocoumarins

In pyranocoumarin, a pyran ring IS there in place of furan ring in furanocoumarin. They are categorized into different groups on the mode of fusion of the ring. These are linear (xanthyletin type), dihydro pyrano, angular (seselin type) – and angular dihydropyranocoumarin [Fig. 13 (VI-IX)]. The examples of linear pyranocoumarin type racemosin, poncitrin, (+)-methyl decursidinol, 3',4'- dihydroxynthyletin, peuarenln, isofloroselin and decursin. The angular pyranocoumarins are exemplified as avicennol and dipetalin. Angular type dihydropyranocoumarins are peucedanocoumarin I, peucedanocoumarin II, peucedanocoumarin and pteryxin.

Phenylcoumarins

This group has varied type of structures as 3-phenyl coumarin and 4-phenyl coumarin Fig. 13 (X-XI)]. Among the structural type of 3-phenyl coumarin are erasnin and isobustin, whereas 4-phenyl coumarin have examples such as stevenin, demethyl derrusnin, 2',4',5'-trihydroxy-4-phenyl coumarin and nivetin.

Biscoumarins

Biscoumarins are a rather rare group of coumarins and so far only eleven simple biscoumarins are known. In these, the two coumarin units are condensed through a C-C or an ether bond and one of these. The chirality of the biscoumarin, edgeworoside C [Fig. 13 (XII)] was shown to be R. Some examples of this group are as daphnoretin, jayantinin, O-demethyl-3,8'-bisiderin and aflavarin. Furanobinordentalin is the first biscoumarin linked with formation of a dihydrofuran ring between the pyran rings of the two linear pymnocoumarin units.

Triscoumarins

Triscoumarins are also found in some plants. A coumarin moiety is attached through one or more carbon-carbon bonds to another structural entity have special interest. The chirality was deduced to be S for the triscoumarin, edgeworoside A [Fig. 13 (XIII)]. The common examples of this group are as triimbelletin and wikstorosin.

Coumarin lignoids or Coumarin lignans

In most of coumarin lignoids or ligans a C6-C3 unit is linked with a coumarin nucleus through a dioxane bridge. The common examples are as cleomiscosin A (C-A) [Fig. 1 (XIV)], hemidesmin-1, hemidesmin-2 and aquillochin.

Coumestans

Several new coumestans have been identified together with previously known compounds. The common compounds of this group are cournestan, wedelolactone, 4-methylcoumestan and coumesterol. 3-Aryl-4-hydroxycoumarins derived from 4-hydroxycoumarin and O-quinone may be oxidatively cyclized to coumestans [Fig. 13 (XV) using potassium ferricyanide.

Biological Activity of Coumarins

Coumarins are known to have a wide range of biological properties, such as anticoagulant, cytostatic, antibacterial and fungicidal activity. Coumarins are a large group of compounds with full range of physiological activity. A large number of coumarins have been reported for their haemorrhagic activity. The 4-hydroxy coumarin group is generally

responsible for this activity. The dicumastrol and warfin [3- (α-acetonyl benzyl)-4-hydroxy coumarin] are the most effective haemorrhagic rodenticides.

Plant juices and extracts are well known for dermal photosensitizing properties and used in Ayurvedic treatment of leucoderma. A large number of furano coumarins were responsible for this activity. The xanthotoxin, bergapten and imperatorn showed significant activity against leucoderma. It was shown that unreduced linear furano coumarins were more active while the free ': group reduces the activity.

Coumarin has a very low antibacterial activity, other members, such as ammoresinol, novobiocin, coumermycin, chartreusin and anthamantin, exhibited significant activity. Ostruthin and ammoresinol were most active against a wide spectrum of bacteria, e.g., *Staphylococcus aureus, Micrococcus luteus, M. lysodeicticus, Bacillus megatherium.* Dicoumarol showed inhibitory action on the growth of certain bacteria, e.g., *S. aureus, S. albus, Streptomyces pyrogenes* and *Bacillus anthracis.*

The most important coumarin antibiotic, novobiocin, was isolated as a fungal metabolite from *Streptomyces niveus* and *S. spheroides* and its antibacterial spectrum was found to be different from those of other known antibiotics. It acted against Gram-positive, e.g., *S. aureus, Streptomyces pyogrmes, S. pneumoniae,* Corinebacterium diphtheria and Gram-negative bacteria e.g., *Haemophyllus influenzae, Neisseria meningitidis* and *Pasteurella.*

The antifungal activity of coumarins was determined and psoralen, ostruthin and imperatorin were found as most active. Coumarin lactones have antihelmentic activity. The 7- and 3-methyl coumarins were found as the most effective. The dihydro coumarins were inactive. The hypnotic and sedative activity of cumarins given by oral administration in mice has been reported. The 3-4 double bonds are necesssary for this activity. A strong effect was shown by ethyl coumarin carboxylate. The inhibitory effect of umbelliferone and a number of its alkyl and acyl derivatives on the growth of a variety of bacteria, yeast and molds have been studied. Herniarin and similar alkyl ethers are more effective antimicrobial agents than umbelliferone. Antimicrobial properties of some hydroxycoumarin have been examined and compared with *Fraxinus omus* bark extract. The present data suggest that the hydroxy coumarin, fraxin, esculin and fraxetin are mainly responsible for the antimicrobial properties of *Fraxinus omus* bark extract, The coumarins prenyletin, haplopinol and aesculetin were shown to be responsible for the antimicrobial activity of *Haploppus multifolius.*

Antitumor-promoting activities of naturally occurring substances, 17 umbelliferous material extracts, an angular pyranocoumarin, Pd-II, isolated from the Chinese crude drug "Bia-Hua Qian-Hu (204) and six linear furanocoumarin, i.e., imperation, isoimperation, oxypeucedanin, pabulenol, neobyakangelicol and byakangelicin isolated from "Tang-Bia-Zhi (203), were tested for their effect on the tumour promoter enhanced phospholipids metabolism of cultured cells.

Molluscicidal activity related to coumarin appears to depend on the nature of the substituents and the ring system. Reduction of the ring system led to a decrease of mollucicidal activity. It is noteworthy that 6-methyl coumarin is more effective than 7-methyl coumarin in contrast, 7-methoxy and 7-chlorocoumarin showed greater mollucicidal activity than 6-substituted analogues. Among the natural coumarins, those containing a chromene and a furan ring are the most potent. With respect to the furan ring, it seems that it is important that a linear fusion of the ring (3-(αα-dimethyl alkyl) psoralen Vs angelicin, hortinone and 5-O-methyl hortidone is necessary for mollucicidal activity.

The coumarins, decuroside showed the strongest inhibitory activity against the primary and secondary wave aggregation human platelet. The antipyretic and analgesic activities of coumarins were also noticed. The coumarin has an ability to reduce the body temperature. The vasodilator activities of coumarin have also been reported. Scopoletin and isoscopoletin and aesculin isolated from *Gundelia toumefortin* and its aqueous extract have shown anti inflammatory activity. Several of the 4-n propylcoumarins isolated from Mummea Africana have been shown to possess considerable insecticidal activity. Coumarins are known to have anticoagulant, cytostatic, antibacterial and fungicidal activities.

The nomenclature for the flavan-3-ols is rather confusing, as it is based both on the actual structure, the chemical identification, and derivations thereof. For the proanthocyanidin oligomers, a highly systematic nomenclature exists, based on the structures of the monomers and the attachment sites.

Flavonoid Types and Examples

Type	**Compounds**
Flavone	Chrysin, Butin, Apigenin, Luteolin, Fistin
Flavonol	Quecetin, Kaempferol
Flavonone	Eriodictyol, liquiritigenin
Chalcones	Unstable isomes of flavonones

Xanthone	Gentisin
Isoflavone	Formononetin, Genistein
Biflavone	Amentaflavone

Solubilities and extraction of flavonoids

Although as a general rule, glycosides are water soluble and soluble in alcohol a fair number are sparingly soluble. (rutin, hesperidin), aglycones are soluble in a polar organic solvents when they have at least one free phenolic group, they dissolve in alkaline hydroxide solutions.Lipophilic flavonoids of leaf, tissues are directly extracted by solvents of medium polarity.Glycosides can be extracted, at high temperature, by acetone or alcohol (ethanol, methanol) mixed with water (20 to 50% depending on whether the drug is fresh or dried). Solvent evaporation under vacuum can be next followed, when only the aq. phase is left, by a series of liquid liquid extraction by non miscible solvents petroleum ether which eliminates chlorophyll and lipids; diethylether which extracts free aglycones and ethyl acetate which dissolves the majority of glycosides. The free saccharides remain in the aqueous phase with the most polar glycosides when these are present.

The separation and purification of the different flavonoids is based on the usual chromatographic techniques (on polyamide, cellulose or sephadex gel) as in case of the most of the secondary metabolites, in the last few years HPLC has taken a place of choice in the battery of isolation techniques for glycosylflavonoids.

Characterization of Flavonoids

Although several color reactions allow the characterization of aglycone and glycosides in crude extracts, preliminary work on these extracts is classically dominated by TLC analysis (but paper chromatography has not been abandoned). The chromatogram can be studied:

- Directly, since chalcones and aurones are usually visible, and turn orange and red, respectively in the presence of ammonia vapors.
- By examination under UV light before and after spraying with alluninium trichloride, and before and after exposure to ammonia vapors.
- After spraying with a 1% solution of the ester of 2-amino ethanol and diphenylboric acid, in other words the “Naturstoff ReagenzA”, by examination under UV light then under visible light.
- After spraying with ferric chloride, anisaldehyde diazotized, sulfanilic acid and other general reagents for phenols.
- By utilizing more or less specific reactions or properties such as: reaction with magnessium powder–for flavanones and dihydroflavanones, or with zinc for

flavonoids, both in presence of hydrochloric acid, reaction of dihydrochalcones, first with sodium borohydrides, then with 2,3- dichloro-5,6-dicyano-1,4-benzoquinone.

- Structural ellucidation, Mass spectrometry and NMR techniques are generally emphasized; UV also can provide very useful information. The usefulness of UV data extends to the use, in routine HPLC analysis, of diode array detectors.

Quantification of Flavonoids:

The classic quantification methods are colorimetric or spectrophotometric. HPLC now makes possible, a rapid and precise estimate of all flavonoids present in a drug, therefore it is widely used.

Antioxidant Flavonoids:

Flavonoids or bioflavonoids, are biquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruits, skin or peels, bark and flowers. A great number of plant medicine contain flavonoids, which have been reported by many authors as having antibacterial anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilator actions.

The structural componants common to these molecule includes two benzene rings on either side of a 3-carbon ring, multiple combinations of hydroxyl groups, sugars, oxygen, and methyl group attached to these structures create the various classes of flavonoids: flavonols, flavanones, flavones, flavan-3-ols(catechins), anthocyanins and isoflavones, flavonoids have been shown in no. of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals.

Free radicals are responsible for many diseases .These radical oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increases exposure to environmental and /or dietary xenobiotics. ROS are also beneficial component of the immune response, hepatic cytochrome P450-mediated detoxification processes (oxidative stress) that is thought to cause the subsequent cellular damage which leads to the disease processes.

The body’s anioxidant system including superoxide dismutase, catalase and glutathione, should keep the oxidative process in check, however deficiencies of nutritional antioxidants (flavonoids, vitamins A, C, E, the minerals selenium and zinc coenzyme Q10, lipoic acid, and L-cysteine), and / or an overwhelming oxidant stress can overload this system. In one study 41 flavonoids of the flavone and flavonol types were investigated for their antioxidative property using a lipid peroxidation generation system, the results showed

that both types specifically and markedly reduced the proportion of peroxidants induced by H_2O_2, Fe^{2+}, or a fenton type reaction**.**

Epidemiological Studies

Two recent epidemiological studies reveal an inverse correlation between dietary flavonoids intake and coronary heart disease mortality. Study found that those with the highest intake of flavonoids (mostly from onions and apples) had a reduced risk for coronary disease. The mechanism of free radical damage includes ROS induced peroxidation of polyunsaturated fatty acids in the cells membrane, lipid bilayer, which cause a chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane lipids and proteins. Subsequently, cell contents, including DNA, are damaged. It is this free radical induced damage which is thought to precede these overt disease processes.

Mechanism of action of Quercetin

Flavonoids as a rule are antioxidant and a number of Quercetin's effects appear to be due to its antioxidant activity. Quercetin scavengers oxygen radicals, inhibit xanthine oxidase and inhibits lipid peroxidation *in vitro*, as another indicator of of its antioxidant effects, quercetin inhibits oxidation of LDL cholesterol in in vitro, probably by inhibiting LDL oxidation itself, by protecting vit. E in LDL from being oxidized or by regenerating oxidized vit E, by itself and pair with ascorbic acid, quercetin reduce the incidence of oxidative damage to neurovascular structures in skin, and inhibited damage to neurons caused by experimental glutathione depletion.

Quercetin's anti-inflammatory activity appears to be due to its antioxidant and inhibitory effects of inflammation producing enzymes cycloxygenase, lypoxygenase and the subsequent inhibition of inflammatory mediatiors Quercetin exerts antiviral activity against reversetranscriptase of HIV and other retroviruses.

RESEARCH METHODOLOGY FOR HERBAL DRUGS

The overall aim of herbal drug study is to explore the application of traditional medicinal plants of India. The specific objectives aimed in work are as follows:

- Standardization of plant drugs as per Indian Herbal Pharmacopoeia.
- To explore the possibilities of traditional uses of plant drugs with proper chemical and pharmacological profiles.
- To identify most potent plants and to extract potent plants parts in different solvents.
- To check the medicinal activity of all extracts.
- To analyze the active extracts and to find out active phytoconstituents by screening them for medicinal activity.
- To make separation and isolation of active phytoconstituents of active extracts and characterization of active phytoconstituents.
- To make the various formulations of active extracts of the selected plants and screening of those formulations for *in-vivo* or *in-vitro* for medicinal activities.

However, for analyzing and correlating the data obtained, correctly and more precisely, the study is designed and carried out in different steps, which are schematically represented as follows in figure.

Methodology

1. Literature survey on the basis of ethnomedicinal or folklore observations.
2. Study of plants parts used.
   a) Pharmacognostic Investigations
   - ✶ Collection and authentication.
   - ✶ Organoleptic evaluation.
   - ✶ Physicochemical evaluation.

   b) Phytochemical Investigations
   - ✶ Extraction in different solvents.
   - ✶ Preliminary qualitative chemical analysis.
   - ✶ Separation and isolation of active phytoconstituents of active extracts.

- ✶ Characterization of active phytoconstituents.

3. Pharmacological Screening

- ✶ *In-vivo* or *in-vitro* studies of various formulations of active extracts of the selected plants.

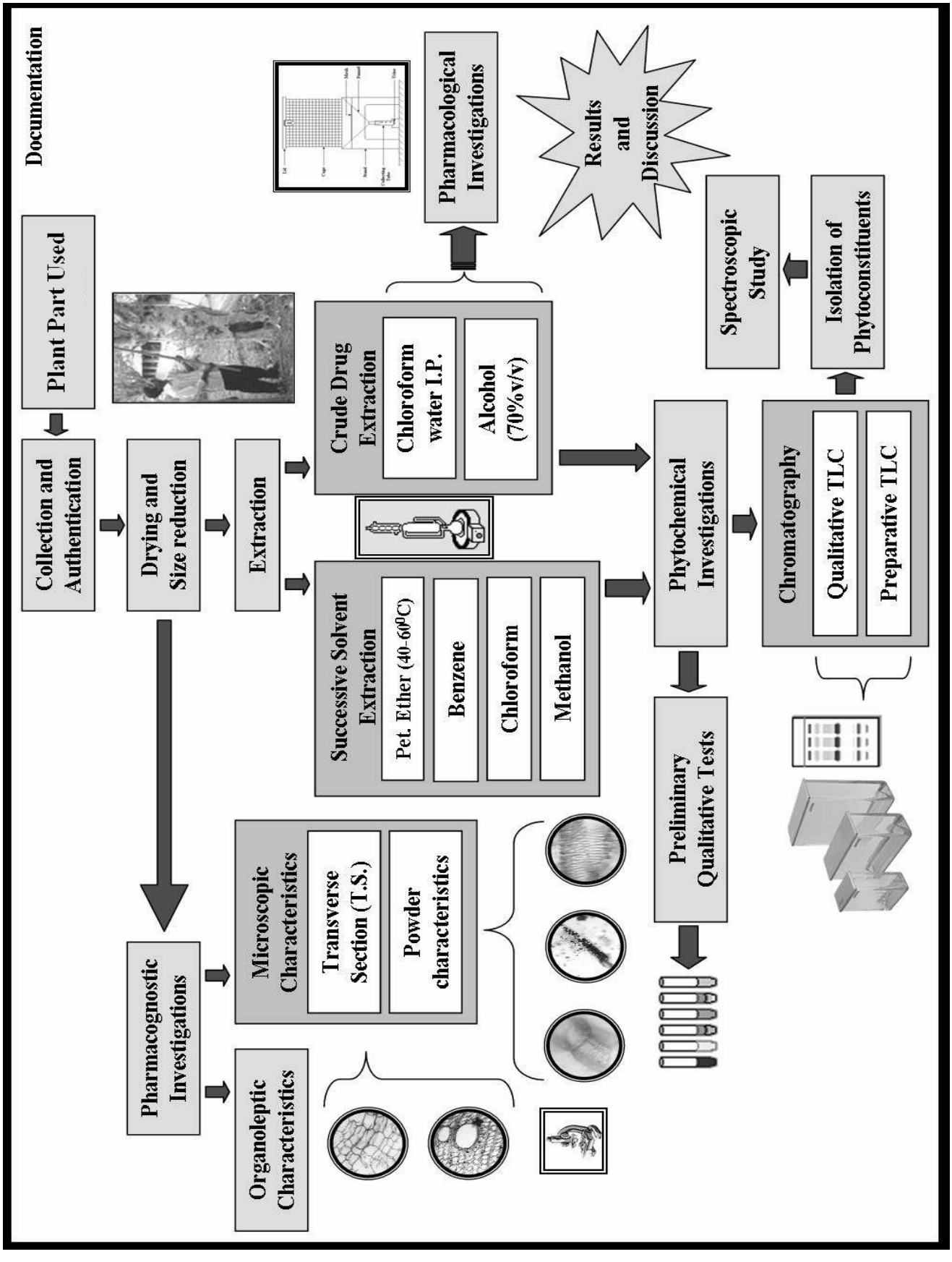


Fig. No. 14: Schematic Layout of the Study of Herbal Drugs.