SECTION-III

PHYTOCHEMICAL STUDIES

CHAPTER-1

INTRODUCTION
AND
REVIEW OF LITERATURE

Introduction and Review of Literature :

The welfare of mankind is served by the species that share the earth along with them. The use of plants is as old as humanity. A large number of plant products were used in India, Egypt, China and Greece before the begining of Christian era. India's flora is very rich from medicinal point of view. Rural and tribal people of our country are very well informed about the plant properties and their utlization for food, medicine and various other uses. It is not improper to say that modern drug has its root in the folk medicine of remote past.Present day researches or investigations have been necessitated due to rapid depletion of natural resources on one hand and dwindling traditional ethnic culture on the other hand.

During the last century a lot of work has been done in the field of medicinal plants resulted in the discovery of various chemical constituents. The medicinal properties of plants depend upon the presence of active principles which possess the pharmacological activity. The medicinal plants and their successful utilization to elliviate human ailments have encouraged researchers to continue the investigation for finding out new drugs from natural sources. The systematic scientific evaluation of medicinal plants has proved that there are some active principles like carbohydrates, glycosides, alkaloids, saponins, tannins, steroids, phenols etc. and they are being used in medicines from ancient time upto now. The active principles are those substances which exert an influence on the structure and function of animal body.

A large number of reports and information available in literature, in relation to the general idea about the medicinal uses, distribution and other uses of plants have been given. But all these information are not well crystallized and sufficient. It is for this reason, various pharmaceutical and phytochemical studies have been carried out to find out the potential therapeutic properties of various plants. The crude drugs have been used from times immemorial in various systems of medicine, however clinical and pharmacological evaluation of these drugs has to the taken up systematically. Phytochemistry which is systematic study of plants for their chemical components has been a subject of fascinating study for chemist and medicinal man. Phytochemistry has developed in recent years as a distinct discipline. It is concerned with various organic substances accumulated in the plants and deal with the chemical structures of these substances and their biological functions etc.

The study of organic chemistry began from the study of substances obtained from living organisms viz, human being, animals and plants. The various products/extracts obtained from them were named as plant products or natural products. Such study is more important in India, because in our country the medicinal herbs or Greece system of medicine is based on the rich medicinal flora of the country.

'Pharmacopoeia' which forms the basis of modern allopathic system of medicine was originally prepared in the form of Rigveda by our ancestors which

compiles the curative values of different plants for different ailments. Discovery of drugs and diseases are two simultaneous processes going on in all the creatures who have come into existence on the earth. The history of drugs and their relative utilization can be realized from different practitioners. Many compounds have been adopted by modern pharmaceutical industries after long therapeutic and chemical investigations. Yet a large number still need detailed study. The therapeutic activity of plants does not depend upon a single readily identifiable constituent but upon the total chemical nature of the plant extract used in the product. A number of chemical constituents have been found to be of great use in the treatment of diseases and improvement of health.

Most of the present day knowledge and information of therapeutically active principles has developed from study of herbal and folk medicines. Many earlier workers have done considerable investigations on the medicinal flora of India, which ultimately contributed to a wide variety of active constituents and plant drugs to modern therapy. To find out chemical constituents of medicinal importance is a prime object of pharmaceutical research which can be modified through chemical procedure into useful drugs. A few new drugs of herbal origin have successfully passed test of commercial screening paving the way for the development of pharmaceutical and phytochemical industries. A large number of active principles from medicinal and food plants having various medicinal values have been reported by various workers from time to time. Among plants, carbohydrates, protein land fats are primary plant constituents in most of the

groups where as secondary plant constituents differ from plant to plant having no relation to the metabolism or function of organism.

It is not possible to give a uniform procedure for isolation of natural products because plant material produces a mixture of heterogenous compounds. The extraction, therefore is subsequently carried out with solvent of increasing polarity ranging from petroleum ether to water. The widely encountered active principles obtained from plant may be broadly classified under the following groups (1) Carbohydrates (2) alkaloids (3) glycosides (4) flavonoides (5) saponins (6) amino acids, proteins (7) fixed oils, (8) Sterols and others. The utilization of plant is possible when chemist acts as a bond between the botanist and user. Various workers did considerable work in this field. Rosenthaler (1930) and Bonner *et. al.* (1965) gave chemistry of plants. Agrawal (1978) had introduced a chemistry of organic natural product. Gupta *et. al.* (1970) has observed phytochemical screening of ethnomedicinal plants. Gopakumar *et. al.* (1989) tried to correlate medicobotany with phytochemical constituents. All these efforts involve callaborative and concerned participation of ethnobotanists pharmacologists and phytochemists.

Some of the active constituents studied by various workers are reviewed below :

The chemical composition of a plants is completely ascertained only when the nature and quality of all the chemical individuals of which it is composed are known. The medicinal value of plants have been ascribed due to the presence of

certain active chemical principles contained there in. These active principles/ constituents may be put under the following group :

(1) Carbohydrates : The name of carbohydrates reflects the fact that a group of non-nitrogenous compounds, composed of carbon, hydrogen and oxygen and represented by a general formula $Cn(H_2O)n$. Carbohydrates provide a basic need of life. They form important structural material for plants and occur in all living cells. Carbohydrates play an important role in the metabolism all living organism. These represent a great store house of energy for human beings. Carbohydrates includes those sugars which behave as polyhydroxy aldehydes or polyhydroxy ketones and compounds like starch, glycogen and cellulose which yield sugars on hydrolysis. Carbohydrates are amongst the most abundant and widely distributed in both the plant and animal kingdoms. Carbohydrates are further classified into Sugar and non-sugar on the basis of their physical properties. Sugars are monosaccharides, disaccharides and oligosaccharides. They form major constituents of our diet and are widely used in manufacture of articles of daily use like paper, textile industries and drugs etc. Carbohydrates have been analysed by several scientists (Somogyi, 1954; Peach and Tracy, 1955; Haryey, 1969; Larm *et. al.* 1973; Grover and Rao, 1976-77,81).

Karawya *et. al.* (1977) reported that roots of Moghut, leaves and flowers of Malva contain sucrose, glucose and fructore, *Althaea* roots contain sucrose only and leaves contain sucrose and glucose.

Gowdwa (1984) isolated mucilage from the seed coat of *Hyptis suaveolens* containing L-fucose, D-xylose, D-mannose, D-galactose, D-glucose and 4-0-methyl 1-D glucoronic acid. Fractionation of the mucilage of seeds of *Hyptis suaveolens* with the fehling solution gave a neutral and an acidic polysaccharide.

Amino acids : Amino acids are derivative of carboxylic acid in which one or more hydrogen atoms of alklyle groups have been replaced by corresponding number of amino groups. They are designated as α, β, γ etc. Out of all these type α, amino acids are physiologically important as they are the building blocks of animal and plant proteins. So for about 25α amino acids are known, out of which 10 are essential. e.g. lysine, valine, leucine etc. deficiency of any one of these in animal check the growth and may even cause death. These are important in the plant resistance mechanism and correlation between amino acid contents and susceptibility or resistance have been suggested by various workers (Gassner and Hassbrauk, 1933; Laxminarayan 1955; Hadwiger and Hall; 1963).

The name proteins was introduced by Mulder 1839 who derived it from Greek word Protos (primary) i.e. compound of primary importance. Proteins are complex, nitrogenous organic substances which occur in all animals and plants, especially in seeds. Protein are the most abuondant macromolecules in living cell and consist of 50% of their dry weights. They are formed in all cells and cell inclusions. Proteins act as enzymes for the maintenance of cell life and mainly used in foods. A number of proteins have been reviewed by several scientist,

Subramanium *et. al.* 1962; Wright and Baulter, 1973; Shri Laxmi and Rao, 1981; Grover and Rao 1981; Ali and Sadry; 1986 and Dinda and Guha 1987 etc. Sassi and Soleh (1970) reported the isolation of amino acid from the leaves of the four different varieties of *Magifera Indica.* The amino acids were found to be alamine, glycine, leucine, tyrosine and valine. Tanker and Ulubelen (1975) reported thirteen amino acids, In the bulb of *Merendera caucasica*, prolin being the highest. Hitz and Hanson (1980) analysed twenty two species of cereals and grasses to determine glycine levels. In the root nodule of *Lotus tenuis*, Shaw *et. al.* (1981) reported the presence of amino acids; 2, 4-Diamino-3 methyl butanolic acid.

Alkaloids : The term "Alkaloids" was coined by W. Meissner in 1819. Accoding to him "Alkaloid" which mean alkali like (alk-alkali oid-like) were defined as basic nitrogen compounds isolated from plants. Scientists such as Konigs (1880) and Landerburg tried to define alkaloids, keeping their point in view alkaloids can be defined as "A basic nitrogenous plant product mostly optically active and possessing nitrogen heterocycles as their structural units with a pronounced physiological action". The function of alkaloids in plant is not clearly understood but it is clearly understood that they are produced in plants for many functions like, they may act as reservoirs for protein synthesis and act as protective substances against the animal or insect attack. They may act as reserve substances to supply nitrogen. They may function as plant stimulants or regulators in activities like growth, metablism and reproduction.

Phenolic compounds : The term "Phenolic compounds" means a wide range of naturally occuring substances, catechol, resorcinol, phenolic acid, syringic acid, cinnamic acids and the lactone derivatives the coummorins are widely distributed common phenols. Thin layer chromatography is most versatile and has proved especially valuable for separating the classes of phenols. Gaind and Gupta, (1973) reported that leaves of *Kalanchoe pinnata* furnished coumoric, ferulic, syringic and caffeic acid. Newby *et. al.* (1980) also analysed the distribution of free and covalently bond phenolic acid from. Kamal *et. al.* (1983) reported the siolation of plumbagin, droserone, isoshirnanolone and a new napthalenone 1, 2 (3)-tetrahydro 3, 3-biplumbagin from the phenolic fraction of light petrol extract of the roots *Plumbago zeylanica* L. Ozo *et. al.* (1984) isolated phenols from five different (Yam) species. Shibuya (1984) reported ferulic acid, p-coumaric acid and deferulic acid from the alkaline extract of the rice endosperm cell wall.

Saponins : The term saponin is applied to plant glycosides that form soapy solution in water by foam test. They are haemolytic in action when injected intravenously into the blood stream of animals. They are highly toxic. The toxicity of saponin appears to be due to their activity in lower surface tension. The formation of molecular complex with cholestrol and other 3 β-hydroxysteroid is another property of saponins. The saponins are of two types e.g. steroidal saponins and Triterpenoidal saponins.

Steroids : Steroids contain a cyclopantanophenanthrene ring system on which some shorter side or longer side chain can be attached. It is widely distributed in nature. They occupy the central position in human body and eliminated through urine increasing and decreasing level cause many syndrome.

Glycosides : Glycosides are a group of compounds which occur in leaves, bark and seeds of plant in low concentration and on hydrolysis with acids, bases on enzyme give one or more sugars and a aglycone part. They are laevorotatory crystalline and colourless solid having important physiological properties. They are bitter and poisonous in nature. They are stored deposits of constituents, needed for the developement of plant.

Glycosides are classified into as a glycosides, N-glycosides, S-glycosides and O-glycosides, on the basis of attachment of sugar to the glycone part at glycosides. Several workers have reviewed them. (Ramchandran *et. al.* 1987; Thakkar, 1986; Khabil, 1986) etc.

Today we are in that position that we can apply a good number of new techniques and technologies which are available in all the allied disciplines of modicine and have resulted in providing new dimension for the discovery of large number of drugs of plant origin and ultimately their synthesis in the laboratory. The physical separation technique such as column, paper, thin layer, gas liquid and high pressure liquid chromatography coupled with modern spectroscopic techniques of UV, IR, NMR and mass spectroscopy have been utilized for the isolation and structure elucidation of various complex organic malecules. In all

the different types of separation methods chromatography has unique position of being applicable to all type of problem in the area of science.

Chromatography : The term chromatography (Greak-Khromatus-Colour, and graphos-written) and its principle were first discovered by Mikhail tswett, a botanist in 1906, for the separation of coloured substances into individual components. Now a days tremendous modification have been applied to the technique. So it can be used to separate different components of any given mixture.

The technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium (Called stationary phase) under the influence of some solvent or gas (Called moving phase). Based on the nature of the fixed and moving phase different types of chromatography are as follows -

(a) Partition chromatography

(b) Absorption chromatography

(c) Gas chromatography

(a) In partition chromatography the stationary phase is a liquid, frequently water, held on a suitable inert porous solid. The mobile phase can be a gas or liquid mixture e.g. paper chromatography.

(b) The technique in which the stationary phase is solid (e.g. alumina or silica gel) and the mobile phase is either a gas or liquid is known as adsorption in different parts of the adsorbent column. The adsorbed component are then

eluted by passing suitable solvent through the column e.g. column chromatography.

(c) When the moving phase is a mixture of gases, it is called gas chromatography.

There are mainly four types of chromatography :

Paper chromatography : It is very simple form of chromatographic tool to the organic chemist and biochemist. In this technique a drop of solution containing the samples is introduced at some point of the paper, migration then occur, as a result of flow by a mobile phase called developer is caused by capillary forces. When it reaches the spot of test solution the various substances are moved at various speed according to their partition coefficient. After solvent has travelled to suitable height the paper is dired and the various spots are visualised by suitable reagent called visualising reagents. The movement of substances relative to the solvent is expressed in term of Rf-value i.e. migration paprameters.

Types of paper chromatography :

(a) Descending chromatography

(b) Ascending paper chromatography

(c) Circular paper chromatography

(d) Two dimentional paper chromatography

Paper chromatography is now a days mostly used for the separation of amino acids. (Evon and Mohar 1962; Grover and Rao 1981), Carbohydrates (Jain and Nishkin, 1965; Shrivastava and Pande 1978; Shivram *et. al.* 1979), Steroids and saturated and unsaturated fatty acid (Buckman, 1959).

Thin layer chromatography : The technique of TLC was introduced by Izmailov and Shraiber (1930). It is also knwon as surface chromatography. In this technique a chromatoplate of a glass plate strip, coated with a uniform layer of the various coating material, silica gel (acidic), alumina (basic) Kieselguhr (neutral) and cellulose powder (neutral) as the adsorbents according to the nature of separating material. In this technique, slurry of the adsorbent in a suitable solvent and spread uniformly on a glass plate (strip). After drying the plates at room temperature for 30°C activated at 100-110°C for 5 minutes in oven. Samples of appropriate solvent is applied through a capillary and development into a solvent system on the chromate plate and placed vertically in a glass tank containing the solvent. After developement and spraying with suitable reagent the chromatograms reveals the number of components present in the mixture which are identified with the help of Rf values.

This technique has several advantages over other techniques because it is simple, faster and separated compounds can easily be recovered by scraping the adsorbent layer and dissolved in a suitable solvent. It consititute a versatile analytical tool for separation compounds like amino acids (Fahny et al., 1961; Ikan, 1969) alkaloids, glycosides, steroids, methyl esters of saturated unsaturated fatty acid (Kricohner, 1967) and others.

Column chromatography : Column chromatography is also known as adsorption chromatography. It is known that rate of adsorption varies with a given adsorbent

for different materials. This principle of selective adsorption is used in column chromatography. In this method, the mixture to be separated is dissolved in a suitable solvent and allowed to pass through a tube containing the adsorbent. Adsorbents used for column are starch, calcium, magnesium, carbonate, activated silicic acid, activated magnesium silicate and activated alumina.

Size of column and adsorbent are used according to the sample to be separated. The component which is of greater adsorbing power is adsorbed in the upper part of the column. The next component is adsorbed in the lower portion of the column. This process is continued. As a result.the material are separated and adsorbed in the various parts of the column. The column is eluted by a suitable solvent.

Alumina columns are used for separating glycosides and saponins. Silver nitrate coloumn is used for the separation of mythyl esters of fatty acids. Srarch and cellulose coloumns are employed for the separation of amino acids and carbohydrates.

Hesses *et. al.* 1952 have established activity of adsorbents.

Gas chromatography : Gas chromatography is an efficient and rapid method for the separation of the component from small batches of mixture of volatile compounds. This self make it a useful technique in organic chemistry. However the method is also used to identify the components separated from mixture and to

measure quantitatively the amount of each component. It is applied for measuring the rancidity in vegetable oils for studying the essential oils etc. One feature that makes it particularly useful is that, on the completion of one analysis the system is immediately ready for the use again.

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