Introduction:

A. Historical Perspective

The study of Indian indigenous drugs first began in the early part of the last century and it was then confined chiefly to the collection of available information with regard to various medicinal plants growing in different parts of the country. The earliest contributions were from the writings of Sir William Jones whose memoir entitled "Botanical Observations on Select Plants" is well known. This was followed by John Fleming's "Catalogue of Medicinal Plants" in 1810. Ainslie's "Materica medica of Hindustan" in 1813, and Roxburgh's "Flora Indica" in 1820. In 1844 O Shaughnessys published his "Bengal Pharmacopoeia" which was the first book of its kind which dealt exclusively with the properties and uses of the medicinal plants used in Bengal. In 1868 a "Pharmacopoeia of India" was published under the able editorship of Waring. It symbolized a new epoch in establishing and recording the value of indigenous medicinal products on modern lines. The more important drugs were officially recognised with a view to their eventual adoption in the British pharmacopoeia. While a large number of the drugs, especially those in local use by practitioners and as household remedies, were not included in this work. Mohideen Sheriff published his "Supplement to the Pharmacopoeia" in the year 1869. He is also the author of "Materia Medica of Madras" which was edited and published after his death by Hooper. V.C. Dutt's translation of Sanskrit Materia Medica brought into prominence the drugs used in the old Hindu medicine even now largely practised in India. Then Fluckiger's and Hanbury's "Pharmacographia and Materia Medica of Western India" 1883 was published. These were
followed by the publications of that very comprehensive book on the Indian Medicinal plants the "Pharmacographia Indica" in 1855 under the joint editorship of Worden and Hooper.

This treatise contains a mass of information regarding the uses of the indigenous materia medica in the Eastern and Western medicine. The most elaborate and applaudable work of all is "A dictionary of the Economic Products of India" published in 1895 by Sir George Watt, the Reporter on the Economic Products to the Government of India. This monumental work, compiled with the help of a large team of workers, refers to all the previous work on medicinal plants and other plants of economic importance. Its pages are full of information of every description regarding the use of different barks, roots, flowers, leaves and woods for different medicinal and other purposes. Works published still later such as Kanti Lal Dey's "Indigenous Drugs of India" and Kirtikar and Basu's "Indian Medicinal Plants" are largely summaries and compilation from the above mentioned literature in a more systematised and elaborated form. In the latter book, plates illustrating various important medicinal herbs are given which greatly help the reader in differentiating them from plants with which they are often confused (Chopra & Chopra, 1955).

According to them though all these attempts were admirable, the pharmacology of most of the indigenous remedies remained an unexplored field till recent years. The reason for this is not far to seek. Investigations of this nature require a considerable outlay of money in the form of well equipped chemical laboratories; and pharmacologists is another essential prerequisite. Medicine we have observed is now intimately related to chemistry, and the ultimate solution of most problems, whether physiological
or biological, rests on some physical or chemical basis. This is forcibly presented to us in the study of the action of drugs. The importance of the cooperation of chemists at every stage of research work can only be realised by the workers themselves. If satisfactory results have to be achieved and if the work is to be carried out on the same standard as in other effluent and advanced countries, the co-operation of competent chemists with experience is the first essential.

It must be pointed out that the time and labour required to work out the chemical composition of a single drug are enormous. This may be judged from the fact that it would take an experienced chemists several months, perhaps a year or more, to isolate in a pure state and roughly describe the nature of different chemical constituents of a single crude drug. The determination of the chemical constitution of the active principles concerned would take a considerably longer time. The isolation of a sufficient quantity of the active principles and the testing of them pharmacologically would occupy many months. The magnitude of the task of working out all the drugs used in the indigenous systems of medicine in detail transcends all imagination. There is such an enormous scope for research in this field, and so little has been done, that it is impossible for any one individual or any one institution to cope with it adequately. The cooperation and intimate associations of a large number of sincere and devoted workers of ability is needed to find the truth. This is now being gradually done. Chairs in Pharmacology have been founded by various universities and Medical Colleges and facilities for research work on modern scientific lines are now being made increasingly more available.
As the action of these drugs or their active principles can only be established by a careful chemical, pharmacological and clinical studies. The investigation in all the three aspects should be carried on side by side. The experimental work on the pharmacological side can only be done in laboratories well equipped with all modern appliances. The first laboratory of its kind established in this country was at the School of Tropical Medicine, Calcutta in 1921. One of the main duties of the Professor of Pharmacology laid down was investigation of the indigenous drugs on scientific lines. The chemical department of this institution had a small team of chemists who worked out the chemical composition of drugs, isolated the active principles and handed them to the pharmacologists for determination of their action on the animal organisms. The clinical testing of drug was made possible by the Carmichael Hospital for Tropical diseases, a research hospital attached to this institution.

The staff of the Department of Pharmacology and Chemistry of this institution was only a nucleus to start this work. Although a modest beginning was made in 1921, when the school started functioning, it was not really 1926 when the Indian Research Fund Association, now known as the Indian Council of Medical Research gave a grant to develop this work. Investigations were then taken up in right earnest and on proper and systematic lines. In 1935 the Imperial Council of Agricultural Research (now Indian Council of Agricultural Research) appreciating the importance of basic research of this type that was being done gave a grant for investigation of the closely allied group of poisonous plants and food poisons of India which are such a menace to men and livestock in this country.
The sustained work carried out during this period that have followed, the research work and indigenous drugs have received considerable encouragement and has made satisfactory progress. The example of the Indian Council of Medical Research and Indian Council of Agricultural Research was followed by the Council of Scientific and Industrial Research. The Council gave very generous grants to various medical institutions and other research bodies for this work. It also established in 1950, the Central Drug Research Institute at Lucknow as one of the eleven major National Laboratories of India. One whole division of this great institution is devoted entirely to the Indian indigenous drugs. After the independance this research has been put on a sound and firm basis. Much has been accomplished in Indian Medicinal plants on systematic and scientific lines.

It is obvious from the foregoing that the Indian Council of Medical Research and its predecessor the Indian Research Fund Association have been the pioneers in encouraging the study of Indian Indigenous Drugs on scientific lines. It was the example of this body which stimulated interest on this important subject of vital importance to the country. (Chopra and Chopra, 1955).

B. Modern Research on Medicinal Principles of Plants:

Modern medicine which is considered as an advanced medicine has its roots in Europe where experimental science was born and developed. Medicinal principles or hallucinatory principles of plants were curiously looked into as chemical principles. It required the birth of organic chemistry before 19th century. In 1870 Friedrich Wilhelm Surternur isolated morphine from opium extract and characterised it as an alkoloid. This was the first
active principle isolated from the plant as pure crystals (Patwardhan Ranade, 1989). Followup of this work led to the discovery and characterisation of many alkoloids, flavonoids in pure form in consistant qualities and tested their efficiencies e.g. the isolation of quinine from cinchona bark which came as a life saving drug in the 19th century perhaps till recently, where malaria prevailed, is a classical example. Another example is the similar active principle derived from Digitalis which has been widely used since the time of its discovery by the German botanist Leonhard Fuchs in 1542 and subsequently William Withering in 1785 publishing a book describing the beneficial effects of Digitalis in the patients of dropsy. Today it is used for curing number of diseases, like insanity, pulmonary, T.B. pneumonia, diptheria etc., and more predominantly, in the recent years, for its wonderful action on heart.

The isolation of quinine and many other alkoloids boosted the development of pharmacology. In second half of the 19th century pharmacology departments were established coordinating with organic chemistry in the universities over Europe in the West. This led to the rapid progress. Side by side, development in the field of synthetic organic chemistry enabled chemists to consider the possibility of synthesizing substitutes for plant products and studying their therapeutic properties.

The first drug for which synthetic substitute was sought was quinine. In 1875 John Mc-Kendrick, Professor of Physiology of the University of Glasgow and an eminent chemist James Dowar derived quinoline bases from quinine and showed them to have biological activity. Six years later in 1881 tetrahydroquinoline base was synthesized at the University of Munich. This was marketed under the name "kairin" and was very promising
antipyretic. In 1884, a safer derivative of quinine named quinazone was synthesized which was marketed under name "antipyrine". This was shown to be very potent antipyretic drug but devoid of antimalarial activity. The commercial success of quinazone stimulated similar investigation in the preparations of analogue of morphine and cocaine. Today cocaine is rarely used as a local anaesthetic as other safer and suitable analogues are in therapeutic use. This process of improving upon nature has resulted in the valuable substitute for salycin, atropine ephelerine, papavarins etc. More and more introduction of these synthetic drugs not only transcended the traditional medicines derived from plant but pervaded throughout the medical world and the people started using these drugs invariably without allowing the system to make their own adjustment. These medicines were introduced in India by British and with a quick and instant relief have made people rely on these medicines. However, it did not give in many causes, the lasting effect, but often carried one or the other side effects. The chemists today therefore have not been successful in developing truely superior substitutes for the most efficacious drug of plant origin such as digitalis glycoside, caffeine, eugenol, colchicine, ergomerine, pilocarpine, reserpine, vinca alkoloids etc. Because of this the modern medical world today has started looking into the herbal medicine.

Today the British pharmacopoeia lists about 30 active principles extracted from plant of which only 4 have been introduced in last 50 years (Patwardhan & Ranade 1989).

Ayurvedic medicines connect several active principles of more than one plant. They are administered in the form of Quath, Chura, Leha, Vatika, Bhasma, Swaras, Phant etc. The modern medicine and chemistry demand
the chemical nature of these recipes. This needs to be investigated under the light of modern science by applying modern methods of plant analysis. The most important concept of chemical analysis is the chemical extraction used in various solvent systems which will bring out the extract polar, non polar, hydrophilic, compounds with different properties. This depended upon the properties of the solvent which is able to extract the bound form of compounds and released. Followed by this is the powerful technique developed in the analytical chemistry. It is the exploitation of the properties of analytical compounds such as the solubility the partitioning principle, the changes possessed by the compound and so on. These properties have been exploited in chromatographical technique.

There are several chromatographic techniques which are employed to separate compounds in microquantity to large amounts. Paper chromatographic technique is a pioneering technique developed for the first time where separation of compounds occur based on their solubility and mobility in the solvents. All chromatographic systems consist two phases. One is the stationary phase which may be solid, liquid, gel or solid-liquid mixture. The second mobile phase may be liquid or gaseous and flows over or through the stationary phase. The choice of stationary or mobile phase is made such that the compounds to be separated have different distribution coefficient. This could be achieved by various modes of setting up on systems.

**Application of Thin layer chromatographic technique:**

*a historical perspective*

Thin layer chromatography was developed first in 1938 by two Russian scientists Ismailov and M.S. Shraiber who described the basic
principles of the procedure in an article under the title, "Analysis by drop chromatography and its application in pharmacy". They applied the method of separation and characterization of medicinal plants. These workers described the method based on their observation of division of substances in the zones of a thin layers of adsorbent using one drop of the substance. The results obtained by the method proposed by them are qualitatively the same, as those obtained by the usual chromatographic adsorption method of analysis. The method enabled to obtain satisfactory results using one drop of the substance under test with very small quantities of the adsorbent in a minimal time.

During the same period constant efforts were made to achieve micro-chromatography where a small quantity of the extract would tell what are all the ingredients that it contained. It was true that during those days the column chromatography to separate organic compounds from the mixture in large quantity had already existed, but there were no methods to identify in a precise way a small quantity of adsorbed substances. This problem was solved by the development of thin layer chromatographic technique by the Russian workers. In 1958 the general simplicity and wide applicability was demonstrated. This can be considered as the beginning of the era of thin layer chromatography which facilitated separation of biologically active compounds of plant origin at microquantity.

This was a period of column chromatography which heralded the pharmaceutical and other industries for separation of organic compounds. However, it did not meet the requirement for the compounds that are in microquantity, nor did it meet the requirement of separating various organic compounds having different properties to separate when existed together
say in plant. In order to carry out partition chromatography on microscale filter paper i.e. paper chromatography which is regarded as an "open" column was then used. The results in amino acid field found wholesale recognition and the method was generally adopted. This is regarded as golden era of paper chromatography began in 1956 and used as a universal method for separating organic compounds of varied properties, solubility, extractibility and over and above existed in varied quantity. Attempts were continuously made under the influence of these successes to overcome the difficulties that were still arising by changes of degree of imprignation by using new solvent combination, by phase reversal and by chemical modification of cellular fibres. This led to develop use of paper imprignated with silica gel, which was also found to be unsatisfactory. This led to the replacement of the paper with Kisselgur the silica gel with a binder to make a thin layer on the glassplate. Kirchner et al. (1951) were the first to investigate the separation of terpine derivative on thin layer adsorbents. The efficacy of this technique largely replace in many qualitative analysis of organic compounds with paper chromatography.

D. High Performance Thin Layer Chromatography: As a sophisticated technique in pharmacology of modern period

This technique is oftenly called HPTLC is of more recent origin. It is ingenously developed to combine the efficiency of separation of organic compounds that existed in microquantity by what is called an "open column" of TLC and its quantification by densitomeric scanning. With a software attached to that it is able to give the scales of different samples that are separating on the TLC in microquantities. It is an offline technique which is described as a chromatographic seperation of samples on high
performance layer used in advanced technique of scanning and data acquisition system for detection of silver gel or polyride. For that purpose any other appropriate bed have a particle solvent size of 0.5 microns and a layer thickness of 100 to 200 microns. The samples can be applied with automated sample application as spots or bands on a precoated high performance plate. Approximately 15 samples can be applied and separated simultaneously utilising a very few millilitres of mobile phase.

The advantages of this technique are many over HPLC or GLC or GC, for these techniques are performed by single layer calibration without application. Both these techniques being on-line allow only one single injection at a time. The co-chromatography of samples and standards are not possible. The HPTLC is the fastest chromatographic method. It has a lowest cost per analysis. The test population of 10 or more samples along with standards can be analysed simultaneously on one single bed and by one single run. The resolution precision and accuracy is compatible with other high performance chromatographic technique. In the data acquisition and evaluation mode the software is built in for computing results for content uniformity test (C.U.T.) in compliance with G.L.P. Archival as well as visualization with photo documentation is possible with a plant chromatography like HPTLC and lastly the choice of the type of calibration function depended upon the dosage concentration may be high or low. The scheme of the principle of HPTLC is as under: