CHEMOTAXONOMICAL STUDIES OF SOME GENERA OF TUBIFLORAE

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Sayyada Khatoon

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CERTIFICATE

This is to certify that the dissertation entitled " Chemotaxonomical studies of some genera of Tubifi[illegible]rae" submitted to the Aligarh Muslim University, Aligarh, in partial fulfilment of the requirements for the award of the degree of Master of Philosophy, is a bonafide work carried out by Miss Sayyada Khatoon. No part of the dissertation has been published or submitted for any other degree or diploma.

(Wazahat Husain).

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INTRODUCTION

Taxonomy is a science that includes identification nomenclature and classification of objects, and is usually restricted to objects of biological origin; when limited to plants, it is often referred to as Systematic Botany. The early history of development of botanical science is nothing but a history of development of plant taxonomy. The classification of Angiosperms presents to the taxonomist the greatest problem in Systematic botany. This group is so large and there are so many variations that at first it seems almost impossible to arrange the hetrogenous collection in a satisfactory order. Long, long ago, some men gave for the first time certain name to some plants and classified it as 'suitable for human food'.

The primitive men learnt the uses of more and more plants especially, those which are suitable for food, and gave some sort of classification thethem. In the early stages of civilization man soon recognized the kinds of plants he could eat, and those which he could use as fuel and which would poison him. These early groupings were practical and determined largely by their economic importance. They were not based entirely on morphology but also on smell, taste and nutritive value.

From that period to present day, the development of taxonomy has witnessed a series of changes. Many different classifications of plants have been proposed. They are recognizable as being or approaching one of three types: artificial, natural and phylogenetic. An artificial system classifies organisms for convenience primarily as an aid to identification, and usually by means of one or a few characters. A natural system reflects the situation as it is believed to exist in nature and utilizes all informations available at the time. A phylogenetic system classifies organisms according to their evolutionary sequence, it reflects genetic relationships, and it enables one to determine at a glance the ancestors or derivatives of any taxon.

We are still a long way from a perfect arrangement, but the most approved modern system differs from the ancient grouping of plants by Aristotle and Theophrastus into trees, shrubs and herbs mainly in the subordination of the obvious to the really important. Continued observations forced certain facts on the observers' minds, and the genius of individual workers by supplying a broad general view brought the facts more and more into a system.

Engler and Prantl (1887-1899) in their famous book "Die Naturlichen Pflanzen familien" classified all dicotyledonous plants into two series i.e. Archechlamydae and Metachlamydae.

The latter series also named as Sympetalae. Sympetalae possess ten orders and Tubiflorae constitute one of them, occupying 6th position and comprise of highest number of families (i.e. 20 families) under 7 sub-orders.

1.	Convolvulineae	-	Convolvulaceae, Polemoniaceae
2.	Boraginineae	-	Hydrophyllaceae, Boraginaceae
3.	Verbenineae	-	Verbenaceae, Labiatae
4.	Solaninae	-	Nolanaceae, Solanaceae, Scrophulariaceae, Bignoniaceae, Pedaliaceae, Martyniaceae, Orobanchaceae, Gesneriaceae, Columeliaceae, Lentibulariaceae, Globulariaceae.
5.	Acanthineae	-	Acanthaceae
6.	Myoporineae	-	Myoporaceae
7.	Phrymineae	-	Phrymaceae

Plants of this order are usually herbaceous, rarely woody. Leaves mostly simple, opposite or alternate. Flowers bisexual, actinomorphic or Zygomorphic, hypogynous, rarely epigynous, tetracyclic. Petals united to form a tube, induplicate valvate in bud. Stamens 4, epipetalous, ovary bicarpellary with as many cells; Ovules many or few in each cell with one integument only; placentation marginal.

According to Randle, Tubiflorae from a natural group illustrating the development from a regular tetracyclic isostemonous flowers of a highly specialised zygomorphic type with reduction in the androecium to four or two numbers.

Several lines of development may be traced from the regular isostemonous flowered families. In Polemoniaceae the tendency to Zygomorphy is light; the position of micropyle resembles that in Convolvulaceae. Hydrophyllaceae recall Polemoniaceae in the plan of the regular flower, but the Ovary is bicarpellary and bilocular, instead of tricarpellary as in Polemoniaceae. On the other hand they approach Boraginaceae in the tendency to arrange the flowers in a scorpioid cyme, the frequent presence of a ring of scales on the corolla-tube and the position of the Ovule with an upwardly directed microphyle. In Boraginaceae there is a tendency to Zygomorphic development of corolla, but the chief point of interest is the great and constant reduction in the pistil, the originally bilocular Ovary is divided by a later formed partition into 4 one-Ovuled portions.

The two closely allied families Verbenaceae and Lamiaceae illustrate the perfection of Zygomorphic type. In Verbenaceae the two carpels unite to form an originally bilocular Ovary; each chamber has two ovules, and later is divided by a septum into 4 chambers. In Lamiaceae the reduction and specialization of ovary has become complete. The bilocular ovary with a gyno-

basic style becomes divided into 4 one-ovuled chambers.

Another line of development within the series may be traced from Solanaceae with generally regular isostemenous flowers and a bicarpellary, generally bilocular, ovary with numerous ovules. The tribe Salpiglossideae forms a link with Scrophulariaceae. Gesneriaceae, a mainly tropical family, has a marked tendency to epigyny; the closely allied Orobanchaceae, also with a unilocular many ovuled ovary, represents the genera of Scrophulariaceae; while the family Lentibulariaceae, with generally a markedly 2-lipped corolla and reduction to 2 stamens. Pedaliaceae show specialisation of the fruit. Bignoniaceae are evidently allied; the plan of the flower is that of Scrophulariaceae, but the woody habit, often climbing, and 2-valved capsular fruit with flattened winged seeds suggest an affinity with Apocynaceae.

Acanthaceae, while showing a similar floral plan have a highly specialized flower and fruit, as indicated by the great variety in the development of the stamens and a remarkable mechanism for the dispersal of the seeds in the most of the genera. It is difficult to suggest a point of origin among polypetalous dicotyledons for the Tubiflorae.

There has always been a controversy and diversity of opinion regarding the assemblage of large number of families

in this order. According to Randle (1938) "Tubiflorae is a natural assemblage of related families". Wettstein (1935) considered the Tubiflorae with much the same circumscription as given by Engler with the exception that Fouquieraceae were placed in Parietales and Plantaginaceae were included here. Randle also treated in the same way except that Convolvulaceae were segregated as Convolvulales and neither Fouquieraceae nor Lennoaceae were treated in Tubiflorae. This segregation of Convolvulaceae is due to the presence of latex and placentation; the large embryo with folded cotyledons is also a notable feature. Takhtajan (1958) treated these families under 3 orders namely Polemoniales, Scrophulariales and Lamiales. Cronquist (1957) had placed these families under Polemoniales, Lamiales and Scrophulariales and pointed out that Lamiales and Scrophulariales originated from Polemoniales.

Hutchinson (1959) splitted the Tubiflorae into 7 orders viz. Bignoniales, Verbenales, Solanales, Personales, Polemoniales Boraginales, Lamiales. Bignoniales and Verbenales are placed in Lignosae while the other five in Herbaceae. Verbenales and Lamiales culminate their respective lines of evolution. Boraginaceae is splitted by Hutchinson into Ehretiaceae and Boraginaceae, the former having been placed in Verbenales and the latter family in a separate order of its own i.e. Boraginales This order Boraginales in the Herbaceae is sandwitched between Polemoniales and Lamiales.

Hutchinson also splitted Convolvulaceae into Convolvulaceae and Cuscutaceae, the former being included in Solanales and the latter in Polemoniales. The splitting of Convolvulaceae has been supported by many taxonomists but the separation of Ehretiaceae from Boraginaceae on account of woody and herbaceous habit has been a subject of much controversy and debate.

It is evident from the foregoing introductory remarks that there has been a considerable diversity of opinion regarding the systematic and phylogenetic position of most of the families included in Tubiflorae.

The subject of phytochemistry, has developed in recent years as distinct discipline, somewhere inbetween natural product organic chemistry and plant biochemistry and is closely related to both. Petiver (1699) stated that morphologically similar plants produce constituents (chemical) with similar therapeutical effects. Hoffman (1846) discussed the chemical characteristic features of flowering plants and said that phytochemistry offered the opportunity to check the artificial classification. Greshoff (1909) asserted that Biochemists and Phytochemists had to investigate evolutionary tendencies of metabolic pathways and groups of chemically related plants. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis turnover and metabolism, their natural

distribution and their biological function. One of the challanges of phytochemistry is to carry out all the above operations on vanishingly small amounts of material.

The range and number of discrete molecular structures produced by plants is huge and such is the present rate of advance of our knowledge of them that a major problem in phytochemical research is the collation of existing data on each particular class of compound. It has been estimated that there are now over 5500 known plant alkaloids and such is the pharmacological interest in novel alkaloids that new ones are being discovered and described, possibly at the rate of one a day.

Chemotaxonomy is and applied branch of Botany in general and plant taxonomy in particular with extremely useful and particular aims. It is well known and established fact that chemotaxonomy provides a wide range of new source of characters and thus biochemical systematics has come to occupy a major role. This has been possible due to the development of rapid and efficient screening techniques such as chromatography and electrophoresis resulting in quick and authentic identification of a large number of organic compounds. There are a nu

There are a number of chemical constituents in plants such as phenolic compounds, terpenoids, organic acids, lipids, carbohydrates and their derivatives, the macromolecule of plants,

nucleic acids, proteins and polysaccharides.

The term phenolic compound embraces a whole range of plant substances which possess in common an aromatic ring bearing one or more hydroxyl substituents. Phenolic substances tend to be water soluble since they most frequently occur combined with sugar as glycosides and they are usually located in cell vacuole. Among the natural phenolic compounds, of which over a thousand structures are known, the flavonoids form the largest group but simple monocyclic phenols, phenyl propanoids and phenolic quinones all exist in considerable numbers. Several important groups of phenolic materials in plants e.g. the lignins and tannins are polyphenolic and occasional phenolic units are encountered in proteins, alkaloids and among the terpenoids.

The function of some classes of phenolic compounds are well established (e.g. the lignins as structural material of the cell wall; the anthocyanins as flower pigments and authroquinones as root pigments). Flavonols appear to be important in regulating the growth in the pea plant (Galston, 1969) and their presence in Spinach chloroplasts (Oettmeier and Heupel, 1971) has suggested that they may be involved in photosynthesis; neither of these fuctions, however has yet been firmly established.

Phenols are very susceptable to enzymic oxidation and phenolic materials may be destroyed during isolation procedure, due to action of specific 'phenolase' enzymes present in all plants.

The major phenolic constituents of plants will be divided into two main groups:

(1) Phenolic Acid and Coumarins; (C6–C1 and C6–C3 structures).

(2) Flavonoid compounds, including anthocyanidins; (C6–C3–C6 structures).

The families of phenolic acids are commonly found in plants, a range of substituted benzoic (C6–C1) acid derivatives and those derived from cinnamic (C6–C3) acids which are very wide spread. Both types of phenolic acid usually occur inconjugated or esterified form.

The flavonoid compounds include by far the largest and most diverse range of plant phenolics. Flavonoids include the red and blue anthocyanin pigment of flowers, the yellow flavones, and less common, the aurones, chalcones and isoflavones. These compounds have special interest since they have been used ancient times to dye cloth and more recently provided one of the earliest introductions of biochemical genetics and chemical plant taxonomy.

The plant amino acids are conveniently divided into two groups, the 'protein' and 'non protein' acids, although the line of demarcation between the two groups is not very sharp and methods of identifying and separating both groups are essentially the same. The protein amino acids are generally recognised to be twenty in number and are those found in acid hydrolysates of plant proteins. In general, glutamic and aspartic acids, and their acid amides glutamine and asparagine, tend to be present in large amount than the other since they represent a storage form of nitrogen.

The detailed chemotaxonomical investigations on Tubiflorae are missing in the literature. It is, therefore, considered profitable to undertake a detailed chemotaxonomical study on some selected genera of Tubiflorae in order to judge phyletic and systematic relationships with the ultimate aim to resolve the existing diversity.

REVIEW OF LITERATURE

In the begining chemotaxonomy was used as an applied science with extremely practical aims and benefits. Nemiah Grew (1673) gave a modern shape to the grouping of plants by writing "An Idea of a Phytological History Propounded". Later in 1696 James Petiver, emphasized that herbs of same make work the same effects. Rudolph Jacob Camaratius (1696) gave the same hypothesis as that of James Petiver and also mentioned the connection between forms of plants and their properties in his book "De Convenientia plantarum in fructification et viribus".

In 1804, A.P. de Candole published the "Essai Sur les proprieties medicales des plantes comparees avec leurs formes exterieures et leur classification naturelle", which assumed the status of next milestone in the history of chemotaxonomy.

His second edition of 'Essai' appeared in 1816. He remarked that composition of plants do not have much impact of soil when grown on different soil. There was no distinction between Jasmineae and Oleineae in the first edition. He treated them in a single group but in his second edition he remarked that even insects can detect the difference of Jasmineae from Oleineae.

In 1830, Lindely worked on Hydrocyanic acid (HCN) and regarded them as valuable tools for taxonomy. He examined that

the fruit being drupe, their bark yielding gum and the presence of hydrocyanic acid gave some results. On the basis of these character he distinguished Amygdalae from Rosaceae and Pomaceae. He also mentioned that the cyanogenetic plants are toxic.

In 1847, Rochleder published a treatise on Phytochemistry in the form of "Beitrage zur Phytochemie" and in 1854 "Phyto-chemie". In a paper Rochleder emphasized that both chemist and botanist should know the chemistry and botany. Without the knowledge of these two sciences it would not be possible to work on chemotaxonomy.

A long series of papers was published by Gulliver from 1861 to 1880. On the basis of raphides distribution, he pointed out that there are 3 orders (families) of British dicotyledons families which can be characterized as raphis bearer, and these are Balsaminaceae, Onagraceae and Rubiaceae.

Helen C. des Abott (1880) pointed out that "There has been comparatively very little study of chemical principles of plants from a purely botanical view. It promises to become a new field of research". According to her theory of evolution in plants, life may best be illustrated on the basis of chemical constituents of plant form.

Greshoff (1891) observed that alkaloid Laurotetanine is always present in the members of Lauraceae. He noted the presence of alkaloid in Hernandia, Illegera, Gyrocarpus, Cassytha.

Baker and Smith (1897), during their investigation of essential oil of Eucalyptus and its relatives, found that there is a close connection between chemistry of the oils and the taxonomy of the plants yielding them.

Trueß (in 1904, 1907 a, b, 1910) published 4 papers pertaining to the role of HCN.

Greshoff (1909) noted the occurance of tannins, alkaloids, cyanogenetic compounds and saponins in a wide variety of plants. He found a high conc. of HCN in the leaves of (Plantanum acerifolia Willd.). He also defined a "comparative phytochemistry as the knowledge of the connection between the natural relationship of plants and their chemical composition", and said that "every accurate description of a genus or a new species should be accompanied by a short chemical description of a plant".

In the year 1910, Gorter investigated chlorogenic acid and its distribution in nature.

Twenty six papers have been published by Mc Nair from 1916 to 1965 and recently been reprinted in 1965. He was an eminent scientist who published papers on taxonomic usefulness of variations of fat or oil contents in plants. In a paper which appeared in 1929 he said that 83 families contain more than 300 oils, fats and waxes in relation to climate and taxonomy. He classified oils into 3 categories such as drying oil, semi drying oil and non-drying oil. He also pointed out the other features such as

iodine numbers, saponification values and specific gravities. Mc Nair drew the conclusion on the plants of tropical habitat store fats or non-drying oils of higher melting points than the plants of temperate regions.

In 1935 he switched over to the alkaloids and noted that each species of the same genus has different groups of closely related alkaloids. Many members of same family may contain a single alkaloid but its occurence may be rarely observed in more than one family (eg. Protopine in Papaveraceae).

The alkaloids, flavonoids, Glyceroides and volatile oils have been used as an aid to taxonomy. Mc Nair (1935), while discussing "Angiosperm phylogeny on the chemical basis" said that "Plants can be classified chemically in accordance with the substance made by them. Such a chemical classification may be compared with or used as supplement to morphological classification and may be of some importance in the development of the true natural system of Angiosperm Phylogeny". He also said that chemical products of more highly evolved plants have larger molecules, and the iodine number of glyceroids is higher in the more highly evolved groups. The above stated facts support the argument that herbaceous plants are more advance in origin and trees are more primitive. The Ranunculaceae are more advance than Magnoliaceae and Barberidaceae.

On the basis of his chemical investigation of plants he

argued that dicotyledons are more advance than monocotyledons and group sympetalae is the most advanced.

Bate Smith (1761-74) studied the systematic distribution of phenolic constituents of plants and their taxonomic significance in dicotyledons and monocotyledons.

Riley and Bryants (1961), by using paper chromatography separated 9 species of 4 genera of family Iridaceae without identifying the chemical substances present in it.

Harborne (1965) wrote a paper entitled "Comparative biochemistry of flavonoids-I Distribution of chalcone and aurone pigments in plants". In this paper he described the distribution of Aureusidin 6-glucoside (aureusin) in the Scrophulariaceae. He concluded that the yellow flavonoid pigments (eg. chalcones and aurones), because of their relatively infrequent occurence in nature, are of much greater phytochemical interest than are the widely distributed carotenoid pigments. Pigments of chalcones or aurone types were noted in the petals of eight plants in the course of a systematic chemical survey of nearly two hundred sps. belonging to angiosperm families, particularly in the order Tubiflorae. The discovery of isosalipurposide as the yellow colouring matter in four unrelated plants is therefore of some note. It is interesting that Paeonia trollioides contains this pigment while another sps. of the same genus i.e. P.lutea contain only carotenoids as yellow flower pigment.

He reported for the first time the aurone cerunoside in Limonium (Plumbaginaceae) and Chirita (Gesneriaceae). The occurence of an aurone in Chirita is not unexpected since the Gesneriaceae and Scrophulariaceae are very closely allied, being placed close together in the order Tubiflorae by most systematics. Other chemical similarities between these two families are known eg. the occurance of 4'- methoxylated flavones and of quinones.

In the same year, 1965, Desborough and Raloquin carried out disc electrophoresis on 26 species of Solanum for separation of tuber proteins. Comparison of resulting protein patterns were used to detect possible differences between species. In some instances, interspecific hybrids appear to have a greater number of bands than either parental species. The availability of a large number of tuber bearing Solanum Sps., interspecific hybrids, and their derivatives representing different ploidy levels provides an excellent source of material for both genetic and taxonomic studies.

Wellburn and Hemming (1966) published a paper on the occurence and seasonal distribution of higher Isoprenoid alcohols in the plant kingdom. Mixture of higher Isoprenoid alcohols have been detected in leaves of eleven angiosperms examined. In four of these plants, the total amount of these isoprenoid alcohols was found to rise as the leaves aged.

Higher isoprenoid alcohols were also found in the algae, Chlorella pyrenoidosa, but not detected in the eleven Cryptogames examined.

Desborough and Peloquin (1966) isolated the esterase isozymes from Solanum tubers.

Another paper on comparative biochemistry of Flavonoids was published in 1967 by Harborne. He examined the flavonoid patterns in the Bignoniaceae and the Gesneriaceae.

3-Desoxanthocyanins which characterize the subfamily Gesnerioideae of the Gesneriaceae have been reported once in the closely related Bignoniaceae, in Arrabidaea. A research of 16 representative sps. has now failed to reveal any further occurences of these rare plant pigment in the Bignoniaceae. An examination of the other flavonoids in leaves and petals of bignoniads showed that most sps. contained flavones rather than flavonols. This result links the Bignoniaceae with families such as the Acanthaceae and Gesneriaceae, which also have flavones as regular leaf or petal constituents and separates its clearly from predominantly flavonol containing families, such as Solanaceae and Convolvulaceae. 6-hydroxyflavon is clearly a common structural feature of Bignoniaceae flavonoids. This flavonoid is also present in the Labiatae, Scrophulariaceae and Verbenaceae.

In this paper he worked out the 27 of Gesneriaceae and Concluded that Fieldia, Jerdonia Rhabdothamnus and Titanotrichum are taxonomically "difficult" i.e. they are difficult to place with certainly in either of the two sub-families. In case of Jerdonia, it is not clear whether it has been placed in the right family (it could be a member of Scrophulariaceae). The chemical data regrettably do not help much in placing these anamolous taxa. Thus Titanotrichum has yellow carotenoid petal pigments characteristic of the Gesnerioideae, but lacks 3-desoxanthocyanins, the most taxonomically significant pigment in the subfamily. Similarly Jerdonia contain cyanidin 3- sambubioside found also in Aeschynanthus, which places it in the Cyrtandroideae, but this glucoside type is also known in Scrophulariaceae.

In 1968, Abraham and Kirson et.al. published their work in the form of a chemotaxonomic study of withania somnifera (L.) Dun. According to their description there exists three chemical types of withania somnifera, Solanaceae, each containing different steroidal lactones of the withanolide type, they have been called types I, II and III. Morphological differences could not be detected between the 3 types, although each of them has a definite and separate area of distribution. No quantitative ontogenetic changes in the withanolide content could be observed.

According to Jamieson and Reid (1969) the leaf lipids of 5 members of family Boraginaceae contain relatively high proportions of α-Linolenic and Octadecatetraenoic acids. Variations in the proportions of polyunsaturated acids are found during the growing season. A large number of branched-chain saturated acids are found as trace components of M. scorpioides leaf lipids. Herbin and Robins (1969) described the patterns of variations and development in leaf wax alkanes. Alkane production in Solandra grandiflora continues throughout the life of the leaf, the chain length increasing with age. No relationship is apparent between the chain length of alkanes and alkanoic acids contained in the cuticular wax of a series of Aloe.

In 1969, Baker and Holloway compared the composition of the cutin from the cuticle of 24 Angiosperm leaves and fruits by GLC. Monobasic acids, specially hexadecanoic, are important constituents of cutin. Wide varieties of cutin composition occur among different species, within species and between leaves and fruits of the same species.

Smith et. al. (1971) carried out the identification of Eupalitin, Eupatolitin and Patuletin glycosides in Ipomopsis aggregata (Polemoniaceae). The 6-methoxylated flavonols are important taxonomic markers in the family, occuring as they do in the 18 known genera. The most significant systematic point about the presence of patuletin in this family Polemoniaceae not the obvious link with the compositae(most of the

members contains 6-hydroxyflavonols), but, instead the implied link with those families in the Tubiflorae which contain 6-hydroxyflavones (notably the Bignoniaceae, Globulariaceae, Buddleiaceae, Labiatae and Scrophulariaceae). According to Takhtajan and Cronquist Polemoniaceae placed in a separate order Polemoniales while Engler included it in Tubiflorae and the chemical evidence is now more in favour of this tradional system.

In the same year Harborne carried out the examination of few such families, eg. Umbelliferae, Bignoniaceae, Gesneriaceae and Acanthaceae for flavones such as luteolin. The present survey of 6-hydroxylutiolin and Scutellarein in the Tubiflorae is also of some taxonomic interest in that it confirms the inclusion of two families, the Buddleiaceae and Plantaginaceae previously separated from this order, within the group. The reason for including Plantaginaceae within the order is the presence of 6-hydroxyluteolin in Plantago.

Lowry (1972) said that the occurence of 'normal' 3-hydroxylated anthocyanins in 8 Malaysian species of the Gesneriaceae supports the important chemotaxonomic results for this family. New compounds found in Chirita, Disissandra and Didymocarpus are the glucosides of cyanidin pigments which may have some systematic value.

Gilbert's paper on Chalcone glycosides of Antirrhinum majus was published in the year 1973. He pointed out that three chalcones have been found in yellow flowers of A. majus, two of which have been identified.

Anjaneyulu et. al. (1974) described the lignans of Gmelina asiatica.

Gibbs (1974) comprised his work in the form of a book entitled "Chemotaxonomy of Flowering Plants". In this book he concluded the Tubiflorae in the following way:

a. Fequiraceae is the only family with ellagic acid, otherwise it fits well here.

b. Convolvulaceae is the only family with ergoline alkaloids.

c. Boraginaceae is the only family in the order that has Pyrrolizidine alkaloids, and in great number L-Bornesitol is widely distributed in it. The seed fats of this family have some unusual fatty acids.

d. Verbenaceae shares with Lamiaceae in a number of terpenoid substances.

e. Lamiaceae appears to have the 'raffinose-family' of oligosaccharides well represented, he feels sure that further investigations of other families in the Tubiflorae would show them to occur widely in the group.

f. Solanaceae is particularly rich in alkaloids, at least twelve groups being known to occur in it.

g. Scrophulariaceae is having the plants with cardiotonic substances specially the two closely related genera Digitalis and Isoplexis.

h. The seed fats of several members of the family Bignoniaceae have been shown to have unusual fatty acids.

i. A careful study of 'raphides' and/or 'acicular fibres' of the family Acanthaceae using fresh material is desirable.

j. Gesneriaceae has most interesting flavonoids. One wonders how widely some of them are distributed.

Keeton and Koogh (1975) isolated a mixture of n-alkanes, four pentacyclic triterpenes, and the butyrate of germanical from the Asclepiadaceae. The structure of these have also been established.

Chen and Chen (1976) identified 6-Methoxybenzolinone and triterpenoids from roots of Scoparia dulcis. These are important from taxonomic point of view.

Powell and his co-workers (1976) described the structure of a unique lignan diester from Salvia plebeia seeds. In the same year Goetz et. al. identified a new C-Glycosylflavone from Gentiana asclepiadea.

The distribution of the first 8 triterpene alcohols in the seeds of eleven plants belonging to seven genera of Solanaceae family was determined by Itoh and Co-workers in 1977.

Nikolin et. al. (1978) identified Ipopurpuroside, a new glucoside from Ipomoea purpurea. The results of acid hydrolysis together with chemical and spectroscopic analysis have shown that it consists of glucose, rhamnose and 6-deoxy-D-glucose glycosidically linked to ricinoloic acid. The acyl group removed by alkaline hydrolysis was identified as methyl butyric acid. The carboxyl group of ipopurpuroside is esterified.

In 1978, Murphy carried out the immunochemical comparisons of Ribulose biphosphate carboxylases using antisera to Tobacco&Spinach enzymes. There were close antigenic similarities between tobacco enzymes, enzymes from other members of the Solanaceae, and enzymes from members of the Nolanaceae, Cuscutaceae, and Convolvulaceae. There were relatively close similarities between spinach enzyme and enzymes from two other members of the Chenopodiaceae. There were relatively great differences between tobacco enzyme, spinach enzyme, and most other enzymes tested. The enzymes from most of the Angiosperms tested were as different from tobbaco enzymes and almost as different from spinach enzymes as were enzymes from the gymnosperms.

Boulter et. al. (1979) found out the relationships between the partial amino acid sequences of plastocyanin from members of ten families of flowering plants. They also included Scrophulariaceae and Solanaceae here. In the several topologies generated during the course of this investigation, the amino acid sequences from members of Solanaceae were always separated from those of Asteraceae. Furthermore, it was always possible to separate some of the families into three groups: (1). Asteraceae; (2). Solanaceae, Scrophulariaceae, Plantaginaceae and Caprifoliaceae; (3). Fabaceae and Rosaceae.

The chemical analysis of the neutral extracts of Satureia calamintha and S. graeca aerial parts afforded besides calaminthadiol, a new triterpene, isocalaminthadiol which belongs to the 3, 4 - seco - 12 - ursene class. The isolation of isocalaminthadiol and calaminthadiol may constitute a valid index for the chemotaxonomic characterisation of the genus Satureia.

In 1979, samples of leaves, flowers and whole plants were taken from clonal stock of Hedeoma drummondii by Firmage and Irving to determine the effect of developmental stage on the monoterpene profile. Thd results are discussed in relation to biogenetic pathways and implications for taxonomic work. In the same year Khosa et. al. carried out the isolation of bergenin from the roots of Viburnum nervosum.

Sendra and Cunat (1980) analysed the phenolic fraction of Coridothymus capitatus essential oil (Spanish origanum oil) and indicated seven major components. One of them is well known Carvacrol and among the others there are one di-isopropylphenol and 5 methyldi-isopropylphenols, which were found for the first time from a natural source.

Kakeo et. al. (1980) identified the structure of Solanaviol, a new steroudal alkaloid from Solanum aviculare. In the same year Ghosal and colleague isolated a new furofurano lignans from Justicia simplex (Acanthaceae). The composition of the essential oil of Salvia stenophylla was investigated by Jaquier et. al. (1980). The oil contains a high percentage of --bisabolol and mannol which are mainly responsible for its characteristic smell.

A chemical analysis of the natural extract of Satureia calaminthe and S. graeca aerial parts afforded, besides Calaminthadiol, a new triterpene, isocalaminthadiol. The isolation of these was done by Romeo et. al. (1980) and may constitute a valid indicator for the chemotaxonomic characterisation of the genus Satureia.

Escamilla and Rodrigues (1980) isolated two new diterpenoid acetates from Sideritis serrata. Piozzi et. al. isolated kaurronoid diterpenes from Stachys lanata.in 1980.

Jain *et. al.* (1980) isolated a novel-nor-harmal alkaloid from the roots of *Adhatoda vasica*. In the same year Rodriques and Savona gave the structures of 2 new diterpenoids, galeopsin and pregaleopsin, isolated from aerial parts of *Galeopsis angustifolia* (Lamiaceae). Tomas and Ferreres (1980) isolated two new flavone glucosides from *Sideritis javalambre* and their structures were also established.

According to Xaasan *et. al.* (1980) the occurence of the unusual flavones from *Ocimum canum* have a taxonomic value. Both nevadesin and salvigenins are rare flavones.

Evans and Somanabandhu (1980) worked on some species of Solanaceae. In 49 species of *Solanum* they found that Cuscohygrine have been detected in 25, Solanine and related amines in 17 and Solanine derived amides in 16. 5 species of *Cyphomandra* were examined and all contained both amines and amides. From roots of *Markgrafiantha solanaceus* cuscohygrine has been isolated which too probably occurs, in the roots of *Lycianthes rantonnettii*. The distribution of these compound throughout the taxa could be of chemotaxonomic value.

In 1980, Homberg and Geiger reported the Flouresecence spectra of 100 flavones, absorbed on cellulose, with and with-

out Shift reagents. The correlation between Fluorescence and structure of these compounds is also discussed. Salama et. al. (1981) identified a lignan glucoside from Euphrasia rostkoviana. Its structure was elucidated by spectroscopic means.

Appareo et. al. (1981) extracted allien from dried leaves of Adenocalymma alliaceum (Bignoniaceae), previously known from Allium species. Husain and Markham (1981) in a leaf flavonoid survey of Origanum, discovered that flavone C-glucoside, vicenin-2, was restricted to the section Majorana. This being the first report of a glycoflavone in the Lamiaceae.

Elliger and colleagues (1981) found that the Caffiec acid esters of a mixture of glucaric acid and lactone forms occur in tomato (Lycopersicon esculentum) leaves. Hydrolysis of these materials yeilded only caffiec acid and glucaric acid. The esters described are inhibitory to the growth and development of the tomato fruitworm (Heliothis zea) and represent one of the several resistance factors of the plant.

Bianco et. al. (1981) identified 5 iridoids from the whole plant of Odontites verna. Goswami et. al.(1981) isolated 4 new compounds in trace amount from the leaves and characterised them by spectral studies. Only eleven Flavonoids

have been isolated previously from Didymocarpus pedicellata. Recently in 1981, Rathore et. al. reported a new flavone from the leaves of this plant. This led to a chemical reinvestigation of D. pedicellata to identify didymocarpin and other related minor components. A new flavoneglucoside was isolated by Ripperger (1981) from the aerial parts of Hierochloe hirtosensica. Two new quinones, echinone and echinofuran, have been isolated by Inouye et. al. (1981) from callus cultures of Echium lycopsis alongwith several acyl esters of shikonin. The structures of both quinones have been established by spectroscopic methods and by chemical degradations.

Husain and Markham (1981) published a paper entitled 'The glycoflavone Vicenin-2 and its distribution in related genera within the Lamiaceae'. This flavone C-glycoside, vicenin-2, was found to be restricted to the section Majorana.

Evans and Ramsey (1981) examined the alkaloids of the Solanaceae tribe Anthocercideae. All 20 sps. of the tribe contained tropane alkaloids. Anthocercis was characterized by a wide range of alkaloids. The taxonomically reinstated genus Cyphanthera showed phytochemical similarities to Duboisia in producing tobacco alkaloids (Nicotine and Anabasine) and tropane esters involving C_3 and C_4 acids. Anthotroche species all produced hyoscymine and accumulate

its apo-and nar-derivatives. In accordance with the new taxonomic revision of the tribe, the alkaloid spectra of Grammogolon and Cremidium resembled those of Cynhanthra spe.

Kubo et. al. in 1982 described the chemotaxonomic significance of Ent Kaurene diterpenes in varieties Rabdosia umbrosus. In this quantitative identification of six ent-kaurene diterpenes, by reserve phase HPLC, in crude ether extracts of a single leaf of 5 major Rabdosia umbrosus varieties are described. These diterpenes are significant chemosystematic markers among these plants.

In 1982, four of the six electrophoretically distinguishable isc-enzymes of the L-lactate dehydrogenase from potato tubers were purified by Mayr et. al., from crude extracts. They are composed of mixture of different subunits. Since the isoenzymes agree hargely with respect to their enzymatic properties and to their primary structure as suggested from fingerprinting and amino acid analysis, it was suggested that the variations of the subunits were caused by proteolytic processing in-vivo rather than by different genetic coding. The amino acid sequence of the substrate binding region (Arg. 6 peptide) shows a homology to that of L-lactate dehydrogenases of animals and bacteria

indicating a common origin of plant, animal and bacterial enzymes.

Brouchs *et. al.* (1982) reported three highly oxygenated flavones from the leaves of *Thymus vulgaris* for the first time in this genus. Their structures were determined by spectroscopic methods. Huixing *et. al.*(1982) carried out a chemotaxonomical investigation of the *Symphytum officinale* polyploid complex and *S. asperum* (Boraginaceae). Mateo *et. al.* (1983) found out 56 components in the essential oils from 12 samples of *Sideritis hirsuta*. The correlations among their concentration in the oils are also discussed in their paper.

The compositions of leaf surface gums of various *Nicotiana* spe., grown under similar field conditions, have been analysed by Heeman and colleague in 1983 and these are compared with some *Nicotiana tobaccum* cultivers. Diterpenes could only be detected in the extracts of two of the 16 *Nicotiana* species; labonoids have been dtected for the first time in *N. tomentosa*. The relative quantitative distributions of n- and methyl - branched alkanes in the leaf surface gums proved to be specific for different species. De Pascual *et. al.* (1983) identified the twenty components in *Lavandula latifolia* essential oil (spike oil). One of the compounds, espliegol (α-terpineol), is a new natural product.

Koiwai and colleague (1983) carried out fatty acid analysis in seeds of 62 Nicotiana spe. and leaves in 56 Nicotiana spe. The total fatty acid content on dry wt. basis ranged from 25 to 40% of seeds and from 2.1 to 4.4% of green leaves. Linolenate was the dominant fatty acid in the leaves of all spe. studied, comprising 50-63% of the total fatty acid content. In seeds of most spe. Linoleate predominated, constituting 69-79% of the total fatty acid content. 14 of 21 spe. in the section Suaveolentes and one species in the section Noctiflorae had relatively high proportions (10-30%) of linolenate.

Jones and Hughes (1983) recorded the ascorbic acid (Vit. C) content of the leaves of 213 Angiosperm spe. The nutritional and taxonomic significance of the results was also discussed in their paper "Foliar ascorbic acid in some Angiosperms".

Perez and Nequeruela (1984) obtained essential oils from three population of Thymus villosus subsp. lusitanicus by using vapour phase chromatographic patterns. Two infra-specific chemovars and two chemoforms, could be distinguished.

Kodama et. al. (1984) reported glucosides of Ionone-related compounds in several Nicotiana species. α-glucosides were isolated from fresh leaves of Nicotiana rustica, two or more of the glucosides were shown to be present and the amounts

measured in *M. alata*, *M. repanda*, *M. rustica*, *M. undulata*, *M. acuminata*, *M. sylvestris* and *M. tabacum*. No glucosides were detected in *M. paniculata*.

Demtoft *et. al.* (1985) worked out iridoid glucosides from *Utricularia australis* and *Pinguicula vulgaris*. They concluded that the iridoid found in lentibulariaceae belonged to structural types common in Scrophulariaceae and related families.

Akhila and Nigam in 1985 published a paper related to the essential oil of *Pogostemon cablin*. In this paper they pointed out that Gas chromatographic and mass spectro-scopic analysis of the Indian patchouly oil revealed the presence of 39 volatile compounds among which 4 monoterpene hydrocarbons, 9 sesquiterpene hydrocarbons, 4 epoxides, 5 sesquiterpene alcohols, 1 norsesquiterpene alcohol, 2 sesquiterpene ketones and 3 sesquiterpene ketoalcohols were identified. Nine peaks remained unidentified.

Aquino *et. al.* (1985) determined the free amino acids from the aqueous extract of *Erythraea centaurium*. They also described the chemical composition and biological properties of the same plant.

A new pregnane ester genin, plocigenin, and a new pregnane ester diglycoside, plocin, were isolated from the

dried twigs of Berlioza carophylla by Deepak and colleague in 1985.

Khomova et. al. (1985) determined the composition of waxes from waste products of the manufacture of Lavender and sage oils by using TLC, GLC, UV, IR and mass spectra. Seven classes of compounds were found. The total acids of sage waxes contained 21 components and the Lavender oil 19 components. The treatment of the waxes with H_2O_2 in an acid medium during the manufacture resulted in the destruction of chlorophylls and in a change in the degree of unsaturation of the total fatty acids.

Singh and Tandon (1985) determined the diterpenes from Coleus forskohlii and carried out the stereochemistry of the carbonyl chromatophore. They concluded that the stereochemistry around the cabonyl chromophore in coleonol, coleol, coleosol, coleonol D, coleonol E and coleonol F is the same but there are differences in the stereoelectronic environment of the carbonyl chromophore.

A phytochemical study on 12 species of the genus Sideritis growing in Spain was carried out by Villar et. al. (1985). The essential oil content was determined and the presence of alkaloids, tannins, anthraquinones, saponins, steroids, triterpenes and coumarins was investigated.

Semenenko and colleague (1965) found out the composition of the essential oil of Salvia garedshii. Data were presented on the basis of quantitative and qualitative composition of the oil. Nineteen compounds (90.3%) were identified and an assumption was made as to the classification of 6 others.

El-Negoumy et. al. (1986) extracted the leaves of Phlomis aurea for flavonoids and observed that they contain the 8-glucosides, 7-rutinosides and 7-p-coumaryl glucosides of naringenin, apigenin, luteolin and chrysoeriol, hispidin 7-glucoside, luteolin 7-diglucosides, vicenin-2 and lucenin-2. The microscopic hairs on the leaves only contained the 7-monoglucosides and their acylated derivatives. P. floccosa showed a similar flavonoid pattern, but with no flavonones.

Harbone and his co-workers in 1986 carried out a chemotaxonomic study of flavonoids from European Teucrium sps. They concluded that a survey of aerial tissues of 42 European taxa of the genus Teucrium has revealed the widespread presence of five surface flavonoids cirsiliol, cirsimaritin, cirsilineol, salvigenin and 5 hydroxy-6, 7, 3', 4'-tetramethoxyflavone. The latter two compounds are useful taxonomic markers in that salvigenin is characteristic of sps. of section Polium, while 5-hydroxy-6, 7, 3', 4'- tetramethoxyflavone is completely confined to sps. of the other 5

sections surveyed. Eleven flavone glycosides, four flavonol glycosides and the glycoflavone vicenin-2 were found to occur as vacuolar constituents. One of the flavone glycosides, cirsimaritin 4'-glucoside, only occur in the species T. arduini, while two others, hypolaetin and isoscutellarein 7-acetyl-allosylglucosides, are characteristic of the closely related T. chamaedrys and T. webbianum. 6-Hydroxyluteolin is widely present as the 7-glucoside and 7-rhamnoside, the latter compound being a new glycoside. In general, the chemical results are correlated with sectional classification and usefully indicate that at last one taxon, T. compactum, is misplaced within the genus. Phyletically the restriction of flavonol glycosides mainly to section Teucrium suggests that this may be basic group within the genus.

Barberan and colleague in 1986 published a paper entitled 'A chemotaxonomic study of flavonoids in Thymbra capitata. According to them the occurence of the compounds, 5, 6, 4'-trihydroxy-7, 3'-dimethoxyflavone, 5, 6,-dihydroxy-7, 3', 4'- trimethoxyflavone, luteolin, diosmetin, vicenin-2 and luteolin 7-rutinoside supports the inclusion of this plant in the genus Thymbra, rather than Thymus or Coridothymus.

PLAN OF WORK

Chemotaxonomy is a field of scientific investigation which has attracted students and researchers from diverse academic disciplines. It can be said that chemotaxonomic investigations are hybrid in nature, since they provide a point where chemical and botanical researches converge. Hybrids are often characterized by hybrid vigour but unfortunately nately hybrid sterility is quite as common. Both the contributors to this discipline taxonomy and chemistry need to be thoroughly understood and well planned before a productive chemotaxonomic exercise could be undertaken.

It may be useful before, dealing with applications and methods of chemotaxonomy in detail, to present a plan of an idealized chemotaxonomic investigation, which indicates where problems of understanding and methodology may arise.

Seven main stages can be recognized in a chemotaxonomic investigation which reconcil s the necessity for informed chemical procedure and a project design which satisfies formed taxonomic requirements. These are:

a) Choice of group, taxonomic survey and sound sampling.

b) Choice, mastery and modification of suitable chemical techniques in a pilot survey.

c) Complete analysis of all samples.

d) Selection of suitable group/ group of chemical compounds.

e) Interpretation and comparison with data from all other sources.

f) Readjustment of classification if necessary.

g) Establishment of any evolutionary relationship if indicated by the new data

These all stages are rarely incorporated in chemotaxonomic work, but the results of such investigation are not very much reliable.

The proposed work will be carried out according to the plan laid down in the following pages:

THE PLANT MATERIAL:

Ideally, fresh plant tissue should be used for phytochemical analysis and the material should be killed by immersing in alcohol within minutes of collection. This is not possible for the materials which are not available at hand and may have to be supplied by a collector from a long distance. In such a situation the shade dried material stored in plastic bags remains suitable for analysis for several days. Plants may also be dried before extraction. This drying procedure should be carried out under controlled conditions to avoid too many chemical changes. The plants should be dried as quickly, as possible without using high

temperature or sun ligh, in a good air draft or in shade. These, thoroughly dried, plants can be stored before analysis for a long period.

It is important to observe that the material should not be contaminated with other plants. The plants should also be free from all type of diseases. Totally misleading results can not be ruled out, if these precautions are not strictly observed. This is because of simple reason that the metabolic by-products of micro organisms are much different from those of angiosperms.

In chemotaxonomical studies the plant must be identified by an acknowledged authority. The identity of the material shoul either be beyond question or it should be possible for the identity to be established by a taxonomic expert. So, it is now usual practice to deposit voucher specimen of a plant examined, in a recognized herbarium for future reference.

EXTRACTION:

Mode of extraction depends on the texture and water content of the plant material being extracted and on the type of substance to be isolated. In general, it is desirable to 'Kill' the plant tissue, i.e. prevent enzymic oxidation or hydrolysis occuring. Alcohol, is a good all purpose solvent for preliminary extraction.

When isolating substances from green tissue, the success of the extraction with alcohol is directly related to the extent

of chlorophyll removal from the tissue. When the tissue debris is completely free of green colour, all the low molecular weight compounds are supposed to have been removed.

The classical chemical procedure for obtaining organic constituents from dried plant tissue is to continuously extract powdered material in a Soxhlet apparatus with a series of solvents starting in turn with ether, petroleum and chloroform and then using alcohol and ethyl acetate. An extract obtained is clarified by filtration through celite on a water pump and then concentrated in vacuo.

The usual method of extraction for different chemical groups is summarised below:

EXTRACTION OF FLAVANOIDS:

For non-flavanoidic and resinous matter the fresh plant tissue or dried powder of the plant tissue is treated with petroleum ether (60-80°C) and benzene; till the solvent is colourless. After this, the extracted material is completely exhausted with hot acetone (56-56.5° C) or alcohol ( ) and the extract is concentrated first at atm. pressure and then under reduced pressure. The gummy mass obtained is subjected to thin layer chromatography (TLC).

EXTRACTION OF AMINO ACIDS:

Dried powder of seeds is washed with chloroform or benzene. Then the treated powder is completely exhausted with

hot acetone or alcohol. The acetone extracts are concentrated first at atmospheric pressure and then under reduced pressure. To the dry extract 4% isoprophyl alcohol is added and decanted. The residue is subjected to TLC.

METHODS OF SEPARATION:

The separation and purification of the plant constituents is mainly carried out by using one or the other or a combination, of three chromatographic techniques:

CHROMATOGRAPHY:

The term chromatography denotes a procedure in which a solution of substances to be separated is passed in a direction determined by the arrangement of the apparatus, over a more or less finely divided insoluble organic or inorganic solid as stationary phase, resulting in retention of the individual components at different distances.

Chromatography is based upon the principle that the components of the mixture can be separated from one another and concentrated into zones, by passing the mixture through two phase systems, one of the phase acts as a carrier for the mixture and the other exerts a differential restraining effect on the component The different types of chromatography may be grouped according to the phases, (liquid/solid chromatography, liquid/liquid chromatography and gas/liquid chromatography), physical principles or the

same feature of the technique such as:

1. Paper chromatography (PC)
2. Thin layer chromatography (TLC)
3. Gas liquid chromatography (GLC)
4. Column chromatography

TLC, GLC and PC will be used in this project. The choice of the techniques depends largely on the solubility properties and volatilities of the compounds to be separated. PC is particularly applicable to water soluble plant constituents, namely the carbohydrates, amino acids, nucleic acid bases, organic acids and phenolic compounds. TLC is the method of choice for separating all lipid soluble components, i.e. the lipids, steroids, carotenoids, simple quinones and chlorophyll. By contrast, the third technique GLC finds its main application with volatile compounds, fatty acids mono and sesquiterpenes, hydrocarbons and sulphur compounds. Finally it may be pointed out that there is considerable overlap in the use of the above techniques and often a combination of PC and TLC or TLC and GLC may be the best approach for separating a particular class of plant compounds.

All the above techniques can be used both on a micro and macro scale. The techniques to be used are outlined in the coming

THIN LAYER CHROMATOGRAPHY (TLC)

PREPARATION OF THE SUSPENSION AND FILLING THE SPREADER:

25 gms. of silica gel G is evenly mixed with 50 ml.distilled

water by vigorously shaking in a stoppered conical flask for about 5 to 10 min. Owing to its plaster of paris contents the fairly thin suspension sets within a few minutes. It is then immediately transferred to the open spreader which has already been set for a desired layer thickness.

COATING OF THE PLATES:

After preparing the suspension, it is poured into the spreader and the slurry can be seen coming out, the process of spreading is begun. For this it is best to stand by the centre of the tray, hold the spreader with both hands and draw it across the plates to the further end plate without applying much pressure To test the evenness of the plates, the spreader can be drawn across them before the suspension is added. When the end plate has been reached, the lever is again turned to the right, which prevents any liquid still remaining in the spreader from running over.

At the time of coating of the plates, the cover screw by the side of the arrow is undone and the tipping mechanism is taken for clearing the parts of the apparatus.

All the parts of the apparatus are thoroughly brushed under running water and then rinsed with distilled water, it is particularly important not to knock the surfaces that form the coating slit, thereby damaging or scratching them. They have to be perfectly smooth.

Narrow plates can be coated more evenly if they are laid lengthwise in the direction of coating.

TREATMENTS OF PLATES AFTER COATING:

DRYING:

For the process of drying the plates are left as such until their surface has become completely dry (about 10 min.). It is advisable to leave them overnight to dry in air.

ACTIVATION:

These completely dried plates are placed in a drying cabinet. Time and temperature of heating are determined by the required activity of layer. Generally, 120° C temperature is required to activate the plates and about half an hour is sufficient for this activation.

STORAGE:

Since active plates become deactivated in moist air, they are stored over a desiccant in a desiccator of 30 Cm. internal diameter or in a plate cabinet. If hot plates are placed, in a desicator the tap must be left open, so this is provided with a short drying tube filled with silica gel.

The coated plates must be protected as much as possible against laboratory fumes and mechanical damage.

TESTING THE SILICA GEL G PLATES:

The layers should be uniform in appearance by both transmitte

and reflected light. The surface must be smooth with no coarse grains visible. They must adhere sufficiently well so that they are not markedly damaged if rubbed lightly with the finger. To obtain a sharp boundary, about 2 mm. of each edge of the layer can be scrapped off with a spatula or with a thumb and fore finger.

SPOTTING:

The first requirement for a good chromatogram is that the size of the spot should be as small as possible. The solution of the sample to be analyzed is put on the film about 15 mm.from the edge of the film by gently dropping the liquid from a capillary. The process of spotting should be carried out in a gentle current of air to incfease the rate of evaporation of the solvent It is also better to keep on blowing the spot gently so as to minimize the size of the spot.

SPOT SIZE:

Measurement of the spot area is a simple semiquantitative method, since this area is related to the amount applied.

In order to measure the spot, the chromatogram is transferred to transparent graph paper and the area is determined in $m.m.^2$.

The weight of a substance is then plotted against area on a calibration curve. Since the absolute areas are affected by the thickness and activity of the layer, the most accurate

results are obtained by running known amounts on the same chromatogram and comparing amounts on this single chromatogram.

However, it is generally sufficient to derive mean values for each concentration from determinations under standard conditic on several identical treatment plates of equal thickness.

Templates can also be prepared for each substance instead of calibration curves. Holes of suitable sizes are cut out of foil or graph paper and the corresponding weights of compound are written along sie. For evalyation, the template is laid over the developed chromatogram so that the area of the hole corresponds as closely as possible to that of the spot.

SOLVENTS:

Only very pure solvents must be used in chromatography otherwise the results obtained are not free from errors. Different solvent systems will be first tried on microscopic slides evenlly coated with silica gel. These slides will be coated by dipping technique. The slurry will be prepared in chloroform/Methanol (70/30). These plates may be used after drying over a hot plate from 1-3 minutes. This rapid and simple techniqqe does not require the use of applicator, glass plates of specific dimension or prolonged drying period. Some of the solvent systems that will be used are given in table-I.

The rate of migration of a compound on a given absorbent

depends upon the solvent used, the solvent can be arranged in order of eluting power. Mixture of two or more solvents of different polarity often gives better results.

All solvents must be dry and pure. Benzene or chloroform (containing ethanol) are the best initial solvents for mixture of unknown substances. If the substances remain near the baseline when chromatographed, either a more strongly elutive solvent is chosen or a more strongly elutive component is added to the solvent being used. If the substance migrates too rapidly (near the solvent front) a weaker eluotive solvent is used. The series of solvents in order of increasing eluotropic power is given below.

ELUOTROPIC SERIES OF SOLVENTS

Increasing Eluotropic Power	Petroleum ether
	Cyclohexane
	Trichloroethylene
	Toulene
	Benzene
	Chloroform
	Diethylether/ethyl acetate/
	Pyridine
	Acetone
	n-propanol
	Ethanol
	Methanol
	Water

DEVELOPMENT:

There are many techniques of the development of chromatogram. The technique which is generally used is ascending techniqu In this technique after spotting, the chromatoplates are placed in developing chamber containing sufficient amount of suitable solven The lower edge of the film should be dipped in the solvent and the atmosphere in the jar should also be saturated with solvent vapour To avoid irregularities in migration of the solvent, the chromatoplates should always be developed against the direction of applica tion of the layer and to obtain straight solvent fronts, the adsorbent should be scrapped off the edges of the plates before chromatography, and the chromatoplates should not touch the sides of the jar.

TIME AND DISTANCE OF DEVELOPMENT:

It is obvious that the time required for development will depend on the distance to be travelled by the front. Generally, t solvent front ascends very rapidly in the begining and then slows down. The optimum distance for the front to travel is about 10 Cm There are many factors which influence the rate of advancement of solvent front such as particle size of adsorbent, thickness of adsorbent film, the atmosphere in the developing jar, the angle of the chromatoplates and the solvent.

SPRAYING:

There are many compounds which are not characterized by

the possession of colour or strong absorption in U.V. fluorescence They have to be rendered visible by special detection reagents which are usually applied to the layer by means of spray. The resultant products thus formed often fluoresce or absorb in U.V. light.

Highly aggressive reagents such as conc. sulfuric acid, chromic acid, nitric acid, perchloric acid etc. can be sprayed onto inorganic layers and any organic compound charred by finally heating at high temperature.

Rf VALUE (Retention factor):

The next step involves the calculation of Rf value. The Rf value is the relation between the distance of the centre of the spot from starting point and the distance of the solvent front from the starting point so,

$$\text{Rf value} = \frac{\text{Distance of the substance from the start}}{\text{Distance of the solvent from the front}}$$

The difference in the rate of movement of the components are caused by their various partition coefficients i.e. their differential solubility in the mobile and starting phases.

The Rf value is constant for each compound only under identical experimental conditions. It depends upon a number of factors of which the following may be mentioned.

- Quality of the layer material
- Activation grade of the layer
- Layer thickness
- Quality of the solvent
- Chamber saturation
- Chromatographic technique
- Distance of the starting point from the solvent front
- Presence of Impurities
- Concentration of the substance applied
- Other substance present

TABLE - I

Different Type Of Solvent Systems For Chromatography:

Solvents	Proportions	Applications
Acetic Acid	2-60%	All types.
Acetic Acid-HCl-Water (Forestal Solvent)	30:3:10	Anthocyanidines, flavones, flavonones, phenolic acids.
Amyl Alcohol-Acetic Acid-Water	4:1:5	All types.
Amyl Alcohol-Ammonia or Methyl Amine	Saturated with 2-6-8 N base	Phenolic amines, phenolic amino acids.
Benzene-Acetic Acid-Water	6:7:3 or 125:72:3	Phenolic acid, phenolic amino acid.

Solvents	Proportions	Applications
Butanol-Acetic Acid-Water	4:1:50 (upper layer homogenous mixture eg.6:1:2)	All types both glycosides and aglycones.
Butanol-Ethanol-Water or Amonia	5:1:2 or 1:1:1	Phenols, acids, cinnamic acid esters.
Chloroform-Acetic Acid-Water	2:1:1	Flavonoids, glycosides, phenolic acids, phenolic amines.
Ethanol-Ammonia-Water	35:2:13	Phenolic acids.
Ethyl Acetate-Formic or Acetic Acid-Water	3:1:3 or 10:2:3	Phenols, anthocyanins, flavonols, glycosides, amino phenols.
Formic Acid-HCl-Water	5:2:3	Anthocyanins, flavones.
Pet. Ether alone or mixed with Methanol	Saturated	Anthraquinones.
Phenol-Water and dil. acid(0.1-2N Acetic or HCl)	Saturated	Flavonoids, anthocyanins, phenolic amines.
Isopropanol-Water or Dil.acid(Formic or HCl)	3:2 or 1:3	Many flavonoids and anthocyanins.
Benzene-Pyridine-Formic Acid	36:9:5	Biflavonyls.
Toulene-Ethyl Formate-Formic Acid	5:4:1	Biflavonyls.
Benzene-Dioxane-Acetic Acid	9:25:4	
Benzene-Methanol-Acetic Acid	45:8:4	Phenols.
Benzene-Methanol	95:5	

TABLE - II (Harborne, 1973)

PROPERTIES OF THE DIFFERENT FLAVONOID CLASSES:

Flavonoid Class	Distribution	Characteristics Properties
Anthocyanins	Scarlet, red, mauve and blue flower pigments, also in leaf and other tissues.	Water soluble, visible max.525 to 545 nm., mobile in BAW on paper.
Leucoanthocyanidins	Mainly colourless, in heart woods and in leafes of woody plant.	Yeild anthocyanidine (colour extractable into Amyl Alcohol when tissue is heated for 0.5 hrs. in 2M. HCl.
Flavonols	Mainly colourless co-pigment in both cyanic and acyanic flowers, widespread in leaves.	After acid hydrolysis bright yellow spots in UV light on Forestal chromatograms, spectral max. 350 to 386 nm.
Flavones	As flavonols.	After acid hydolysis bright yellow spots on Forestral chromatograms, spectral max. 330 to 350 nm.
Glycoflavones	As flavonols.	Contain C-C linked sugar, mobile in water unlike normal flavones.
Biflavonyls	Colourless almost entirely confined to the gymnosperms.	On BAW chromatograms dull absorbing spots of very high Rf.
Chalcones and Aurones	Yellow flower pigments, occasionally present in other tissues.	Give red colour in Ammonia (colour change can be observed in situ, visible max. 370 to 410 nm.

Flavonoid Class	Distribution	Characteristic Properties
Flavenones	Colourless in leaf and fruit (especially in Citrus).	Give intense red colours with Mg/HCl, occasionally an intense bitter taste.
Isoflavones	Colourless, often in root only common in one family, the Leguminosae.	Mobile on paper in water, no specific colour tests available.

PHENOLIC COMPOUNDS:

The term phenolic compounds comprises a wide range of plant substances which possess in common an aromatic ring bearing one or more hydroxyl substituents. Phenolic substances are water soluble and most frequently occur combined with sugar as glycosides and they are usually located in the cell vacuole. Among the natural phenolic compounds, the flavenoids form the largest group but simple monocyclic phenols, phenyl propanoids and phenolic quinones all exist in considerable number. The lignins, melanins and tannis are polyphenolic materials in plants while the phenolic units are encountered in proteins, alkaloids and among the terpenoids as well. Phenols are very subseptibbe to enzymic oxidation and phenolic material may be lost during isolation proceduras, due to the action of specific 'phenolase' enzyme present in all plants. Extraction of the phenols from plants with boiling

alcohol normally prevents enzymic oxidation and this procedure should be adopted routinely.

The majority of phenolic compounds (and especially the flavonoids) can be detected on chromatograms by their colours or fluorescences in UV light, the colours being intensified or changed by fuming the chromatograms with ammonia vapour. The phenolic pigments are visible and coloured and are thus particularly easy to monitor during their isolation and purification.

All phenolic compounds are aromatic in nature, so that they all show intense absoption in the UV region of the spectrum. The main emphasis will be given to the flavonoids of some genera of Tubiflorae.

TABLE - III (Harborne, 1973)

COLOUR PROPERTIES OF FLAVONOIDS IN VISIBLE AND ULTRAVOILET LIGH

Visible Colour	Colour in UV light Alone	Colour in UV light with NH_3	Indication
Orange	Dull orange, red or mauve	Blue	Anthocyanidines 3-glycosides.
Red mauve	Fluorescent yellow cerise or pink	Blue	Most anthocyanidin 3, 5-diglycosides.
Bright yellow	Dark brown or black	Dark brown or black	6-Hydroxylated flavonols and flavones; some chalcone glycosides.
		Dark red or bright orange	Most chalcones.

Visible Colour	Colour in UV light		Indication
	Alone	With NH_3	
	Dark yellow or yellow green	Bright orange or red	Aurones.
Very pale yellow	Dark brown	Bright yellow or yellow brown	Most flavonol glycosides.
		Vivid yellow green	Most flavone glycosides.
		Dark brown	Biflavonyls and unusually substituted flavones.
None	Dark mauve	Faint brown	Most isoflavone and flavonols.
	Faint blue	Intense blue	5-Deoxyiso-flavones and 7, 8-dihydroxy-flavanones.
	Dark mauve	Pale yellow or yellow green	Flavanones and flavanonol 7-glycosides.

DETECTION OF FLAVONOIDS:

The flavonoids are a group of some 750 naturally occuring plant constituents whose structures are derived in one way or another from the aromatic nucleus of flavan or 2-phenyl benzopyran. Flavonoids are usually divided into classed depending on the oxidation level of the central pyran ring. The two most important classes being the flavonols and the anthocyanidins. Flavonoids occur in plants in combined or protected form; the aglycones, being phenolic, are presumably toxic to the living cells and may be normally isolated only from dead tissues (eg. heart wood). When analysing flavonoids,

it is usually better to examine the aglycones present in hydrolysed plant extracts before considering the complexity of glycosides that may be present in the original extract.

Attachment of sugar renders these compounds water soluble, and flavonoids are mostly located in the cell vacuoles. Less frequently, flavonoids occur with the majority of hydroxyl groups protected by methylation (eg. in citrus fruits) and such compounds are lipid soluble and are presumably contained in the cytoplasm.

The anthocyanidins are as a rule intensely coloured substances and their glycosides, the anthocyanins, are responsible for most scarlet, red, mauve, purple and blue colours in flowers, fruits and leaves of higher plants. Other flavonoid classes, particularly chalcones, aurones and certain flavonols make a significant contribution to yellow colouration in many species.

Flavonoids contain conjugated aromatic systems and thus show intense absorption bands in U.V. and visible regions of the spectrum. Flavonoids are present in all vascular plants, but some classes are more widely distributed than other; while flavones and flavonols are universal, isoflavones and biflavones are found in only few plant families.

EXAMINATION OF FLAVONOIDS IN ALCOHOLIC PLANT EXTRACT:

The majority of flavonoids being water soluble, are

isolated from fresh plant tissue by extracting with hot alcohol or from dried material with hot aqueous alcohol; the few which are lipid soluble require separate treatment. Alcoholic extracts, after washing to remove chlorophylls, lipids and so forth, are concentrated and flavonoids are then obtained by crystallization or following chromatographic separation.

Anthocyanines, cations probably present in cell sap in association with organic acid anions, are unstable as the chlorides. By using methanolic or ethanolic acid HCl as the extracting medium, this group of compounds is extracted.

An inestimable advantage of the flavonoids is that they are readily detected on chromatogram, without the use of chromogenic sprays. Many are coloured and the remainder can be viewed by means of U.V. light. In addition, most undergo characteristic reversible colour changes when treated with NH_3..

Partition chromatography is carried out on paper with solvent such as butanol-acetic acid-water, while absorption chromatography is carried out on a paper and uses aqueous solvents (water alone or with varying amounts of acetic acid). Thin layer chromatography is also commonly employed, but the most popular adsorbent is cellulose, a material wich gives

very similar separations to those on paper. Other TLC adsorbents are silica gel (for methylated flavonoids, isoflavones and so on) and Polyamide (for flavonoid glycosides).

EXAMINATION OF FLAVONOID AGLYCONES IN HYDROLYSED PLANT EXTRACT:

PROCEDURE:

1. A small amount of plant tissue (usually)leaf or flower) is immersed in 2 N HCl and heated in a test tube for 30-40 min. at 100°C.

2. The cooled extract is then filtered if necessary and extracted with ethyl acetate.

3. If the solution is coloured (either because the original tissue was coloured with anthocyanins or because colour has formed from leucoanthocyanidin during acid treatment) then the aqueous extract is further heated to remove the last traces of ethyl acetate and re-extracted with a small volume of amyl alcohol.

4. The ethyl acetate extract is concentrated to dryness, taken up in 1-2 drops ethanol and the aliquot chromatographed one-dimensionally, alongwith the authentic markers, in five solvents: Forestal (acetic acid-conc. HCl-water; 10:3:30), 50% HOAc (50% aqueous acetic acid), BAW (n-butanol-acetic acid-water;5:1:5, top layer) PhOH (Phenol saturated with water) and water.

5. The amyl alcohol extract, which should be coloured, is concentrated to dryness, taken up in a few drops of 1% methanolic HCl and aliquot chromatographed in Forestal and in Formic acid-conc. HCl-water; 5:2:3.

GAS LIQUID CHROMATOGRAPHY (GLC):

The chemotaxonomic problems that will not be solved by TLC alone, GLC will be used. In GLC the adsorbent liquid on an inert material served as stationary phase while gas serves as mobile phase and the substance to be separated are are in the form of vapours.

In GLC a mixture of vapours is resolved into its components as it is carried in a stream of gas through a column filled with either an adsorbent or an inert material coated with a liquid stationary phase.

The gas filled chromatography is essentially a piece of long narrow (1/4" id) glass, copper or stainless steel tubing filled with stationary phase. The carried gas under constant pressure passes through the column, carrying along the infected vapours. As the separated components leaves the column, they are carried through a detecting device and thence are vented into the atmosphere or passed into collecting vessels. The whole unit is maintained at a desired operating temperature by use of heaters or vapour jackets.

DETECTION OF AMINO ACIDS:

Nitrogen first appears in organic form as glutamic acid, the key reaction being the transfer of ammonia to - ketoglutarate, catalysed by glutamic dehydrogenase. The plant amino acids are conveniently divided into two groups, the 'protein' and''non protein' acids, although the division between the two two groups is not sharp enough and methods of identifying and separating both groups are essentially the same.

The protein amino acids are generally recognised to be twenty in number, and are those found in acid hydrolysates of plant proteins. They also occur in the free amino acid pool of plant tissues at concentration varying between 20 to 200 g fresh weight; there are considerable quantitative variations from tissue to tissue, depending on the metabolic status of the plant in question.

Only one of the 'non-protein' amino acid is regularly present in plants that is ubiquitous α-amino-butyric acid. The remainder of which over 200 structures are known, are of more restricted occurance. Most are structural analogues of one or other of the twenty 'protein' amino acid.

Amino acids are colourless ionic compound. They are all water-soluble, although the degree of solubility varies. Their melting points are very high. They can be esterified.

The esters are more volatile than the free and thus can be separated by GLC.

'Neutral' amino acids are those in which the amino groups are balanced by an equal number of acidic group. Basic amino acids have an additional free amino group while acidic amino acids have an additional acidic group.

RECOMMENDED TECHNIQUES FOR PROTEIN AMINO ACIDS:: THIN LAYER CHROMATOGRAPHY(TLC) AND PAPER CHROMATOGRAPHY(P

A great variety of different procedures are described in the literature, although the fundamental approach has changed little from that used in the very early days of PC.

In spite of the fact that it is possible to separate the 20 protein amino acids by one dimensional chromatography (Hanses et. al.1961), the normal practice is to employ two dimensional separations. It is most frequently carried out on paper or on thin layers of silica gel G, cellulose or silica gel-cellulose mixture. For PC the best solvent pair is n-butanol-acetic acid-water (BAW) and phenol water. The same pair may be use for TLC on silica gel G, but for TLC on microcrystalline cellulose, replacement of BAW by chloroform-methanol - 2M NH_4OH (2:2:1) as the first solvent is recommended (Brenner et. al. 1969).

One advantage of using PC is that a concentrated aqueous

alcoholic plant extract can be applied directly to the paper for separation. By contrast, TLC systems are sensitive to the presence of salts and sugars that may contaminate a crude plant extract, and purification on ion exchange resins is a normal pre-requisite for good separations (Brenner, et. al. 1969). But, in such purification, care must be taken to avoid losing material on the acidic and basic ion exchange resins which are used.

One procedure which eliminates the necessity of preliminary purification is conversion of the amino acids to their dinitrophenyle (DNP) derivatives and their subsequent separation on TLC. These derivatives are yellow compounds.

DEVELOPMENT:

The standard reagent for amino acids is ninhydrin, which is commercially available in a form ready for spraying on the chromatograms or plates. Alternatively, it may be prepared fresh as a 0.1% solution of acetone. After spraying the paper or plate is heated for 10 minutes at 105°C when most amino acids give purple or grey blue colours. Development of colour may be achieved without heating the plates, if Cadmium acetate is added to ninhyd rin. 112 ml. of a soln. of Cadmium acetate (1 g.) in water (100 ml.), acetic acid (20 ml.) and acetone (1 ml.) is used for dissolving 1 g. ninhydrin and the paper or plate is dipped in this reagent. On leaving the

chromatogram over-night in the dark in a closed vessel cotaining H_2SO_4, the amino acids appear as dark red spots on a white backgroud.

TABLE - IV (Harborne, 1973)

NINHYDRIN COLOURS OF PROTEIN AMINO ACIDS;

Amino Acid	Ninhydrin Colour
Glycine	Red-Violet
Alanine	
Serine	
Crysteine	
Theonine	Violet
Valine	
Leucine	
Iso-Leucine	
Methionine	
Aspartic acid	Blue-Violet
Asparagine	Orange Brown
Glutamic acid	
Glutamine	Violet
Arginine	
Lysine	
Prolein	Yellow
Phenyl-alanin	
Tyrosine	Grey-Violet
Tryptophane	
Histidine	

HIGH VOLTAGE ELECTROPHORESIS AND THIN LAYER CHROMATOGRAPHY:

The best procedure for achieving sharp separations of common amino acids is the combined use of electrophoresis and TLC.

In this procedure the crude extract is applied as a narrow band (2.5 Cm.) near one corner of a plate spread with cellulose MN300, which has been dried at room temperature. A marker spot of thionin (Michrome dye no.215) is placed at the opposite end of the plate.

The plate is sprayed lightly with formic acid-acetic acid buffer (pH 2.0). Using a wick of dialysis tubing and Whatman 3 MM paper (held in place by glass strips), the plate is developed horizontally in the same buffer at 1000 V. (10-20 mA) in a Shandon cooled plate electrophoresis tank for 25-35 min. (thionin marker moves ca. 4-8 Cm.).

The plate is blown dry and the bands which have separated are reduced to spots by dipping the plate (turned through 90°) in distilled water and allowing it to develop to 2.5 Cm., the plate is re-dried.

The plate is then developed twice in the second direction with methyl ethyl ketone-pyridine-water-n-propyl acetate-acetic acid pyridine (120:60:20:4:1) for 4 hours.

The plate is developed with the ninhydrin-cadmium reagent. A typical separation is achieved by this procedure.

GAS LIQUID CHROMATOGRAPHY (GLC):

This technique can not be applied directly to amino acids because they are so involatile. In recent years, successful separations by GLC have been obtained using temperature programming on their derivatives, for example, on the N-acetyl-n-amyl esters. These are prepared from the amino acids by successive treatment with amyl alcohol and anhydrous HBr and then with acetic anhydride. GLC is carried out on a column of Chromosorb W (60-80 mesh), coated with 1% polyethene glycol (carbo wax 1546 or 6000), with temperature between 125° and 155° and flow rates of 60 to 240 ml./min.

QUANTITATIVE MEASUREMENTS:

The high resolution provided by PC or TLC makes them an attractive method to be used for quantitative determination. It is simple to stain the paper or plate with ninhydrineunder standardised conditions, elucte or scrape off the various coloured components and measure their concentration separately from their visible colour in the spectrophotometer. A good alternative is first to prepare the Dinitrophenol (DNP) derivatives of the amino acids in the crude extract, separate the derivatives by two-dimentional chromatography and since they

are yellow in colour, determine their concentration directly without further staining.

RECOMMENDED TECHNIQUES FOR NON-PROTEIN AMINO ACIDS EXTRACTION:

Non-protein amino acids frequently occur in high concentration in seeds and many srveys have carried out on seed extracts. The typical extraction procedure used by Dunnill and Fowden (1965), is as follows:

Finely grounded seed powder (1g.) is shaken with 75% ethanol (25 ml.) for one day. The supernatant, after centrifugation is applied to a small column (12x0.8 Cm.) of Zeokarb 225 (H^+ form in 75% ethanol) to retain organic acids, it is washed with aqueous ethanol and then eluated with aqueous ethanol containing 2 M NH_4OH (25 ml.).

This eluote is concentrated and applied to a 3 mm. sheet of filter paper for chromatography, the volume applied being equivalent to 0.25 g. seeds.

PAPER CHROMATOGRAPHY (PC):

Two-dimensional PC is the most widely used technique for detecting non-protein amino acids in plants. 75% phenol-water (ammonia vapour), followed by butanol-acetic acid-water (80:10:29) as one pair of solvents; ethyl acetate-pyridine-water (2:1:2) and butanol - 3 M NH_4OH (top layer) as a second pair are used. One-dimensional PC in several solvents is

sometimes sufficient to separate different non-protein amino acids.

DETECTION:

One of the easiest ways of recognizing many non-protein amino acids is by their typical colour response to ninhydrin. Instead of giving the usual purple colour, they may turn green, brown or deep red. This is often how they are first detected during two-dimensional chromatographic screening programmes. It is often useful, with certain types of amino acids, to employ more specific colour reagents. The Sakaguchi reagent, for example is specific for arginine and its derivatives while Fearon's PCAF reagent is specific for the guanidine compound, canavanine and deaminocanavanine.

DETECTION OF SAPONINS AND SAPOGENINS:

The formation of persistent foams during plant extraction or during the concentration of plant extracts is reliable evidence of presence of saponins. Indeed, if large large quantities of saponin occur in a plant, it is difficult to successfully concentrate aqueous alcoholic extracts, even when using a rotary evaporator. A simple test for saponins is therefore, to shake up an aqueous alcoholic plant extract in a test tube, and note if a persistent foam is formed above the liquid surface. Saponins can also be tested for in crude

extracts bytheir ability to haemolyse blood cells. However, it is usually preferable to confirm such simple tests by TLC and by spectral measurements.

To test for sapogenins, dried tissue is hydrolysed with molar HCl for 2-6 hr, neutralised and the solid dried and extracted with petroleum. This extract is taken to dryness, and the residue dissolved in chloroform and the IR spectrum determined. The same solution is then concentrated and subjected to TLC on silica gel in solvents such as acetone-hexane (4:1), chloroform-carbon tetrachloride-acetone (2:2:1). Sapogenins are then detected as pink or purple spots by spraying the plates with antimony chloride in conc. HCl and heating at 100°C for 10 min. The different sapogenins are not easily separated from each other by TLC. For separating diosgenin from yamogenin, it is necessary to carry out continuous development with methylene dichloride-ether (4:1) for 8 hrs.

Saponins are much more polar then the sapogenins because of their glycosidic attachments and they are more easily separated by PC on by TLC on cellulose. However, TLC on silica gel is successful in such solvents as butanol saturated with water or chloroform-methanol-water (13:7:2; lower layer).

Besides these techniques some very simple and quich tests, which were used by Gibbs, can also be applied to many

chemotaxonomic problems. These tests can be employed to detect the presence or absence of certain groups of compounds in a number of families, genus and even some species. Some such quick tests are as follows:

1. The presence or absence of polyenolases in plants can be detected by cigarette test as well as hot water test.

2. The leucoanthocyanins may be detected by using the HCl/Methanol test.

3. Cyanogenic glycosides can be detected by HCN test. If a cyanogenic glycoside containing plant is hydrolysed it gives free HCN which may be detected by HCN test.

4. Juglone Test A Band can be used to detect the naphthaquinone flavonoids and aesculin or similar coumarin respectively from the family Juglandaceae.

5. According to Bate-Smith, Leucoanthocyanin Test A, can detect the presence or absence of aucbin type glycosides in plants. Tunmann (1931) noticed that presence or absence of syringin (eglycoside) can be detected by Syringin Test A.

6. Ehrlich test can indicate the presence or absence of succubin or similar substances.

7. Aurones in many families can be detected by Aurone Test A (NH_3).

8. The presence or absence of syringaldehyde can be detected by using Maule test. Schinoda test can be used to detect leucoanthocyanin, flavonoids, alkaloids.

9. Anthraquinone test can be used to detect anthraquinone.

10. Oxalic acid can be detected by Oxalate test.

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