General Introduction

CHAPTER 1

GENERAL INTRODUCTION

The classical plant taxonomy is mainly founded upon comparative external morphological characters. It is only because it acts as a general reference system, into which data from other disciplines can be fed. Inspite of the 'synthetic' approach which requires evaluation of all types of characters related to form, structure and function in taxonomy, this traditional method continues to have a larger say in classification than any other type of taxonomic characters. The information from other disciplines of botany ensures that morphology in general would continues to act taxonomically supreme for many more years to come (Ogura, 1964; Cronquist, 1975).

There are several plus points for morphological characters, when compared to taxonomic characters from other disciplines of botany. First of all, they are easily observed, secondly, these characters have innumerable variants that would help in delimitation and identification and thirdly, there is a well-knit terminology to describe the variations. So, morphology continues to enjoy an edge over other sources of characters in plant classification (Holttum, 1967).

Chemical systematics is a branch of botany which deals with the investigation of distribution of chemical compounds or groups of biosynthetically related compounds in series of related or supposedly related plants (Swain, 1963) and its bearing on the classification of that group. This is comparatively a new discipline of taxonomy, where chemical data are used in refining the natural classification. Hence, if morphological characters are believed to be genetically controlled external expressions, then chemical characters can be regarded as hidden or cryptic characters, expressing the internal biochemical complexity. The chemical characters, therefore, should also be considered as equally important as the morphological features. The chemical characters have the advantage over morphological ones in that they can be exactly described in terms of definite structural and chemical formulae.

The present day chemical systematics has assumed as much importance as any other discipline of botany in solving taxonomic riddles. It has also been increasingly used in understanding plant relationships. This does not mean that a new system of classification solely based on chemical characters is to be framed. It only suggests that chemical data along with data from various other disciplines could be utilized as supplementary to the basic morphological skeleton which forms the basis of existing systems of classification.

The potential importance of phytochemical evidences in taxonomy started attracting the attention of plant taxonomists since early 1960. With the advancement in the field of isolation techniques and characterisation of organic compounds (PC, TLC, GLC, coupled with UV, NMR, IR spectroscopy) enabled screening of large number of plants within very limited time and utilisation of such data in plant taxonomy. Information gathered during the past two decades have very well shown that chemical characters correlated well enough with other type of characters and that they deserve consideration in taxonomic matters. Alston and Turner (1963), Swain (1966), Harborne, Boulton and Turner (1971), Bendz and Santesson (1973), Heywood (1973 a), Gibbs (1974) and Cognin _et_ _al_. (1977) have put this young and promising sub discipline of taxonomy on a sound footing. The innumerable papers that now appear in various journals of biological sciences are indications of the interest that has been generated among biologists working in this area of systematics. Of late, attempts have also been made to evaluate chemical evidences by statistical methods. They call it numerical chemotaxonomy (Runemark, 1968; Weimark, 1972; Crawford and Dorn, 1974).

Any chemical compound present in the plant is a potential chemical marker, which may be used at the higher or lower levels of hierarchy. The chemical characters which are having structural variability, stability, wide-spread distribution, which are under genetic control and which are reasonably easy to isolate and identify have been now accepted to be of high taxonomic value (Harborne, 1973, 1975) and Swain (1975).

The various chemical constituents were arbitrarily divided into primary and secondary metabolites. The primary metabolites include constituents like sugar, amino acid etc. which takes part actively in various metabolic pathways and which are in constant circulation. The secondary metabolites comprise those compounds, which are produced by the plant but do not involve themselves in metabolic activities of various tissues (Alston and Irwin, 1961). In second system of classification, these chemical constituents are grouped on the basis of their biosynthetic pathways such as shikimic, mevalonic, fatty acid and polyketide pathway. In a third type of classification (proposed by Davis and Heywood, 1967), the various chemical characters have been treated under three headings such as :

a. Directly visible :- Starch grains and Raphides.

b. Plant products :- Alkaloids, Flavonoids and Terpenoids.

c. Serological and electrophoresis studies of proteins.

d. DNA, RNA, Somatic hybridization.

Chemical characters which are visible directly under a microscope provide ample scope for chemotaxonomist to use the distribution pattern of these characters in understanding taxonomy of groups. Starch grains and raphides are the visible characters which have attracted the attention and subsequent use by the anatomist for taxonomic purpose.

Reichert (1913) and Czaza (1978) have utilised the differentiation and specificity of occurrence of starches as characters for evaluating taxonomic systems. Gibbs (1958 a) on the basis of his study on the raphides, opined that these compounds may have some value as evidence of relationship. It is interesting to note the almost uniform presence of raphides in the members of the well-knit group, the Centrospermae.

The secondary metabolites are all those compounds produced by the plant but are not ultimately involved in basic energy transfer or assimilatory activity of all tissues (Alston and Irwin, 1961). This general term is almost discarded now and the component groups are referred to in terms of their specific structures (eg. alkaloids, steroids, etc.). The functions of most of these compounds in the plants are not known at present. But it is believed that all these compounds formed after long biosynthetic pathways are not made for fun sake but have definite functions, which are unknown at present. Earlier these compounds were considered to be "excretory products", "waste products" or "side products" and Heslop-Harrison names them as "luxury diversifications" which reflect the chemical virtuosity of the plant concerned. All these compounds are genetically controlled and probably are fixed at low selection pressure.

Of all these secondary constituents, flavonoids are the most widely studied with respect to plant systematics (Gardner, 1977; Cronquist, 1977). The use of flavonoids in evaluating contemporary classificatory systems has mainly been based upon their distribution pattern, presence or absence. The many positive correlations that they display have encouraged chemotaxonomists to consider them as evolutionary markers. Recently Harborne (1977) and Gornall *et al*. (1979) have brought together information on flavonoids and angiosperm evolution.

1:1 Phenolic compounds

Phenolic compounds include a wide range of plant substances, which possess in common an aromatic ring bearing one or more hydroxyl substituents. They include a wide range of organic compounds and are of frequent distribution in plants. Much work has been done particularly by Bate-Smith, and Harborne on the systematic distribution of these compounds. They have stressed the utility of phenolics as reliable chemical marker. Phenolics are divided into two rather arbitrary groups. 1. Simple benzene derivatives (Hydroxy benzoic acids, cinnamic acids, coumarins etc.). 2. Flavonoids, which have C_{15} skeleton. Quinones and phenolic acids belonging to 1st group and flavones, flavonols and leucoanthocyanins to the IInd, have been selected for detailed chemical screening of the Bignoniaceae and Verbenaceae reported in the present work.

Flavonoids

Flavonoids, by far are a group of natural products (polyphenols). This group includesall the C_6-C_3-C_6 compounds related to a flavone skeleton. The skeleton may be considered as consisting of (i) a C_6-C_3 fragment (phenyl propane unit) that contains the "B" ring and (ii) a C_6 fragment, the "A" ring. These two are of different biosynthetic origin (Geissman, 1962). The flavonoids are sub divided based on the oxidation level of C_3 fragment as flavones, flavonols, chalcones, aurones etc.

Flavonoids have been one of the most exploited phytochemical characters in relation to the classification. The flavonoid data are being incorporated together with data from other discipline into phylogenetic schemes of angiosperm classification (Harborne, 1977).

In classical system, the evolution pattern of many morphological characters, along with the aid of fossil data is generally presented in the form of morphogenetic series. In the same way, the flavonoid sequence or so-called chemogenetic series in phylogenetic terms could be studied by two approaches. viz. The use of various informations gathered from biosynthetic pathways; and correlation of flavonoid structure with progressive specialization in plant structures (Harborne, 1967, 1972, 1977; Swain, 1975).

Harborne (1967) concluded that because flavonoids can be placed in a biosynthetic sequence, $A \rightarrow B \rightarrow C \rightarrow D$, we could assume that an organism with A has fewer enzymes than one with D, and therefore, that A is the simpler (more primitive) organism. He concluded that, "The difficulty in assessing whether one morphological character is advanced over another is the lack of information regarding the development of one from the other; this difficulty vanishes in the case of chemical features which are biosynthetically related."

Nothing much is known about various enzymes which take part in flavonoid biosynthesis. Therefore, it is necessary to consider, whether the formation of end product in different organisms is due to homologous enzymatic pathways or not. However, there is some indication that there may be more than one pathway to the same compound or a single compound may be synthesized via different pathways. This has significant implication for the use of flavonoid distribution in a phylogenetic sense.

The correlation approach (Sporne, 1956) states that if one character is shown to be statistically correlated in its occurrence with another character, already known to be primitive then it too may be considered primitive. For eg. Harborne (1977) has pointed out the correlation of pro-anthocyanidin with the woody habit as there is loss of these compounds in herbaceous plants. Therefore, it will be inappropriate to assume that a particular taxon is primitive because of the absence of a single compound viewed as a primitive character.

Primitive and advanced characters

As it is done with morphological characters, the chemical characters are also sought to be arranged under two headings; primitive and advanced (Harborne, 1967, 1972). Although it is difficult to judge whether a chemical character is primitive or advanced, various attempts have been made to arrange them according to their structural configuration or on the basis of their occurrence in the supposedly primitive or advanced plant families. These characters are also not free from the usual drawbacks like parallelism, convergence and retrogressive evolution. Harborne (1967, 1972) has come up with an orderly arrangement of chemical characters into primitive and advanced states (Table 1).

Further study on the phylogeny of flavonoids resulted in a re-thinking of ideas put forward by Harborne (1977). The dominant reduction trend prevalent in flavonoid evolution resulted in a change of understanding primitive and advanced characters. Some of the characters which were considered primitive earlier are now considered as highly advanced (Table 2), due to the reduction sequence which could be explained in terms of retrogressive evolution (Gornall and Bohm, 1978).

Flavonoid surveys themselves appear to fall into three major classifications :

1. Chromatographic patterns or profiles without identification of flavonoids.

Table : 1. Evolution of some flavonoid characters in the angiosperms according to Harborne (1967, 1972).

Character	Primitive state	Advanced state
Anthocyanin in petal	cyanidin	delphinidin or pelargonidin
	3-deoxygenation (1967)	3-oxygenation
	3-oxygenation	3-deoxygenation (1972)
	O-methylation absent	O-methylation present
	O-acylation absent	O-acylation present
	simple glycosylation	complex glycosylation
Proanthocyanidin in leaf	present	absent
Flavonols/flavones in leaf	flavonols incl. myricetin	quercetin & kaempferol only
	flavones absent	flavones present
	O-methylation absent	O-methylation present
	simple glycosylation	complex glycosylation
Extra A-ring hydroxylation	absent	present
...if present	at 8-position	at 6-position
2'-oxygenation	absent	present
C-glycosylation in leaf	present	absent
Biflavonyls in leaf	present	absent
Flavanones	present	absent
Yellow anthochlors in flower	chalcones	chalcones & aurones
C-acylation	present	absent

In addition, three "isolated" (advanced?) characters were recognized:

1) replacement of anthocyanins by betalains
2) presence of isoflavones
3) presence of 5-deoxygenation

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cf. Angiosperm Flavonoid Evolution:A Reappraisal by R.J.Gornall and B.A.Bohm.

Table : 2. Different character states for angiosperm flavonoids (including anthocyanidins), illustrating possible phylogenetic conditions and the difficulty of distinguishing between primitive and highly advanced types.

Character	Primitive	Advanced	Highly advanced
Deoxygenation	OH	H	--
O-methylation	OH	OMe	OH
Extra A-ring oxygenation	absent	present	absent
...if present	6-H,8-O	6-H,8-H	6-O,8-H
2'-oxygenation	absent	present	absent
O-glycosylation	simple (gluc., &/or rhamn.)	complex (gluc.,gal., arab., xyl., & rhamn.)	simple (gluc., &/or rhamn.)
Proanthocyanidins	present	absent	absent
O-acylated glycosides	absent	present	absent
C-glycoflavones	absent	present	absent
Biflavonyls	absent	present	absent
Isoflavones	absent	present	absent
Anthochlors	absent	present	absent
...if present	chalcones	aurones	chalcones
Flavonoid bisulphates	absent	present	?
C-acylation	absent	present	absent
Anthocyanins	present	replaced by betalains	--

cf. Angiosperm Flavonoid Evolution: A Reappraisal by R.J.Gornall and B.A.Bohm.

2. Hydrolysis studies surveying differences in aglycones.
3. Complete identification of whole glycosides.

1. Chromatographic profiles/patterns

In most surveys, paper chromatographic profiles still represent the initial step. Such biochemical profiles not only lineup all the phenolic compounds, but also present mirror images of chemical complexity of the plant. The clear-cut differences in chromatographic profiles corresponding to similar taxonomic dichotomies based on morphological and/or other characters are quite common in literature (cf. Alston, 1967). However, this can be considered as a crude method. It has its own limitations and at the same time there are few difficulties in assessing real or apparent differences or similarities between spots based on crude Rf values and colour reagents. Secondly it is very much necessary to do co-chromatography before similar or different spots are adjudged. Therefore, without knowing the identity and structural simplicity/complexity of the flavonoids, it would be impossible to determine if a single flavonoid profile with few spots, was primitively simple or advanced, through loss of spots along with biosynthetically advanced modifications of the remaining compounds.

2. Hydrolytic studies

This is the major type of flavonoid survey where acid hydrolyze crude flavonoid extract is subjected to chromatography of flavonoid aglycones and leucoanthocyanidins.

The 3rd type of survey include complete sugar analysis, using hydrolytic enzyme and/or sugar recovery followed by identification using PC, TLC, GLC techniques (Crawford and Mabry, 1978; Bohm and Wilkins, 1978).

1:2 Phenolic acids

They are simple phenols having a functional acid group and varying number of hydroxyl groups at different positions. The angiosperms show a wide distribution of various phenolic acids. Tomoszewski (1960) found p-hydroxy benzoic acid and gentisic acid in the leaves of 97% of the 122 plants belonging to 86 families. In the similar way Griffith (in Biochemistry of phenolic compounds by Harborne, 1964) found gentisic acid in 73/80 families screened. The gentisic acid has an unusual hydroxylation pattern and which is not directly related to either the commonly occurring flavonoids nor the lignin, is also reported in many gymnosperms (Ibrahim *et al*., 1962).

Three of the acids, p-hydroxybenzoic, vanillic and syringic, are present in combined form as ester groups in lignin (Smith, 1955). Syringic acid is particularly found in plants of which the lignin contain condensed syringyl residue. Thus syringic acid is present only in angiosperm lignin while gymnosperm lignin is devoid of this acid. Salicylic and the related o-pyrocatechuic acid only seem to be characteristic constituent of certain groups like Ericaceae and to other genera like Populus, Asclepias, Calotropis and Vinca. Ellagic acid is an infrequent constituent of the sympetalae but is absent from other taxa of vascular plants.

1:3 Quinones

They are the aromatic diketones, which form the largest class of natural colouring matters. They are generally known from higher plants and fungi. In higher plants they play a subsidiary or secondary role. They are generally present in the bark or underground parts. In leaves they are masked by other pigments. They are classified into Benzo-, Naphtha-, and Anthraquinone depending on the mono, bi or tricyclic ring system.

Benzoquinone - They are common fungal pigments, but rarely encountered in higher plants in noticeably high concentration. They possess an aromatic ring with or without aliphatic side chain. A number of hydroxy or methoxy benzoquinones are found in a few higher, economically important plants. The plastoquinones of chloroplast are functionally important benzoquinones and serve as redox carriers in photosynthesis. The ubiquinone of mitochondria closely resembles plastoquinone and acts as hydrogen carrier in respiratory chain.

Naphthaquinone - These quinones are yellow/red in colour. Vitamin K and related vitamins are structurally naphthaquinones. Some of them are toxic and antimicrobial and therefore, used as drugs and poisons. Others are equally important as dye stuffs. eg. Lapachol, Lawsone etc. Vitamin K and its related compounds have a very specific role in electron transport system.

Anthraquinone - These constitute the largest group of natural quinones. They are common in the Rubiaceae, Rhamnaceae and Polygonaceae. However, they are found to be present in both higher plants and fungi. Most of the anthraquinones from higher plants are hydroxylated at C_1 and C_2 position. 2-methylanthraquinone is known to be a constituent of Teak wood. Most of the known anthraquinones occur in Rubiaceae, where its presence in leaves is directly related to distribution of flavonoids. The pharmacological action of anthraquinone is due to its conversion to anthranols by intestinal bacteria. In plants their function is not properly understood. It is assumed that they play some role in oxidation - reduction processes.

1:4 Iridoids

They are a group of monoterpenoid compounds which are present as constituents of a number of orders of the dicotyledons. These monoterpene lactones most frequently occur in plants combined with sugar (glucoside) in the cytoplasm. The presence of these compounds in a given group of plants is considered by many taxonomists (Hegnauer, 1966 b, 1969, 1971; Kubitzki, 1969; Meeuse, 1970; Bate-Smith, 1972; Bate-Smith and Swain, 1966; Jensen et al., 1975) to be a valuable chemical character. It is used together with other characters so as to correlate all iridoid containing taxa and thus suggest a common origin for them.

1:5 Saponins

In general, saponins are those compounds which show ability to haemolyse blood cells. The distribution of saponins is not completely known. They possess steroid or triterpenoid aglycone. The steroidal saponins are more common in monocots while the triterpenoid saponins are found in dicots. Their taxonomic value is less at a higher level of hierarchy although they may be used as useful chemical characters at lower level.

1:6 Tannins

They are polyphenolic compounds. Bate-Smith and Metcalfe (1957) during their chemical survey found that presence of tannins, especially condensed tannins, is associated with the woody habit and most of the non-tanniniferous families are herbaceous. Tannins being antimicrobial, they have a protective function in plants.

The association of leucoanthocyanin and tannin with the woody habit and therefore, can be taken as a supporting evidence of the classification of angiosperm into woody lignosae and herbaceous Herbaceae as done by Hutchinson (1959). It is because, the presence of tannin shows correlation with other primitive characters. This is taken as a primitive feature, which is often lost in the course of evolution. Thus the highly advanced herbaceous taxa are devoid of these compounds.

1:7 Alkaloids

Alkaloids comprise the largest single class of secondary substances. They are basic plant products having a nitrogen containing heterocyclic ring system and high pharmacological activity. They are restricted to a particular group of plants and therefore, often used as a criterion in classification of only those groups which contain them. The various types of alkaloids and even their occurrence are used effectively in classifying plants (Manske, 1944; Price, 1963; Gibbs, 1974; Daniel and Sabnis, 1979).

1:8 Present work

In the present study, leaf phenolics especially phenolic acids and flavonoids (flavones, flavonols and glycoflavones) have been studied in detail. The quinones have been grouped into 3 broad categories depending upon the spectroscopic analysis, whereas rest of the markers are used as groups and their presence or absence is noted.

Two sympetalous families, the Bignoniaceae and Verbenaceae are taken up for chemotaxonomical studies with a view to assessing the utility of selected chemical markers in their classification.

In the second chapter 31 members belonging to 4 tribes and 25 genera of the Bignoniaceae have been screened for various chemical markers and attempt is made to understand the evolutionary trends within the family. The position of controversial genus Paulownia is also taken into account and its position in family is discussed on the basis of chemical findings.

The third chapter deals with family Verbenaceae and a similar type of chemical treatment is given to 35 members belonging to 3 tribes and 13 genera. The taxonomic controvercy surrounding the genus Avicennia is also discussed in light of flavonoid chemistry.

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