

CHEMOTAXONOMY: THE ROLE OF PHYTOCHEMICALS IN CHEMOTAXONOMIC DELINEATION OF TAXA

Abstract

Chemotaxonomy is the systematic study of chemical variation between plant taxa. Evidence of chemical variation has essentially been used for classification purposes ever since 'folk taxonomies', based on certain obvious plant characteristics were instinctively employed by mankind centuries ago. These categories, such as edibility, taste, colour, smell and medicinal value were founded, however unknowingly, on chemical properties. Awareness of the chemical complexity of plants grew from the desires of Europeans for exotic spices and condiments as well as investigations into their medicinal properties. Early Knowledge about the subject was summarised in herbals, and concentrated on information about physiologically active secondary metabolite such as alkaloids and saponins. During the eighteenth and nineteenth centuries knowledge in the field increased, and some taxonomists made use of several chemical characteristics in attempts to classify plants and to demonstrate their phylogeny. However, although the chemical characters they used were recognised, they were manifestations of processes or compounds not yet completely identified and so their use was based on inadequate knowledge and evidence. Chemotaxonomy has undoubtedly made a big contribution to taxonomic work in the past and will most certainly continue to do so in future. The valuable information it offers is best used in conjunction with other sources of taxonomic evidence and thus a multidisciplinary approach is required in order to establish a system of classification which reflects natural relationships as accurately as possible.

Keywords: Chemotaxonomy, Phytochemicals, Primary Metabolites, Secondary Metabolites.

INTRODUCTION

The application of chemistry to systematics is chemotaxonomy or chemical taxonomy. Natural systems of classification should be based on the analysis and harmonization of evidence from all organs, tissues and parts. The external morphological study alone is inadequate and other branches of study are of considerable value in proper assessment of the systematic status of a taxon and its phylogeny. The taxonomic contributions of chemotaxonomy have made an equally great help to support the ideas of classification and phylogeny. The rise of chemotaxonomy has been the development of sophisticated techniques in chemical analysis which can detect even trace of chemical compounds [1].

Chemotaxonomy is the systematic study of chemical variation between plant taxa. Evidence of chemical variation has essentially been used for classification purposes ever since 'folk taxonomies', based on certain obvious plant characteristics were instinctively employed by mankind centuries ago. These categories, such as edibility, taste, colour, smell and medicinal value were founded, however unknowingly, on chemical properties. As long ago as the first century after Christ the aromatic mints had been recognised and grouped together by Dioscorides [2].

Awareness of the chemical complexity of plants grew from the desires of Europeans for exotic spices and condiments as well as investigations into their medicinal properties. Early Knowledge about the subject was summarised in herbals, and concentrated on information about physiologically active secondary metabolite such as alkaloids and saponins [2]. During the eighteenth and nineteenth centuries knowledge in the field increased, and some taxonomists made use of several chemical characteristics in attempts to classify plants and to demonstrate their phylogeny. However, although the chemical characters they used were recognised, they were manifestations of processes or compounds not yet completely identified [2] and so their use was based on inadequate knowledge and evidence. Gradually the number of recognised natural plant products increased, extending to include proteins, nucleic acids and the major polysaccharide categories. At the same time research into plant metabolism revealed similarities and uniformities in the chemical functioning of plants, while simultaneously highlighting biochemical peculiarities which might be taxonomically or phylogenetically significant. Successful attempts were made to correlate this variation with known classifications, and any claims were made as to the taxonomic merit of various chemical characters [2]. However it is only in recent decades that reasonably rapid surveys of plant extracts have become feasible, due to improved techniques of chemical analysis and the elucidation of the structures of many organic compounds [3]. Technological advances, particularly electrophoresis and chromatography, have simplified and speeded up analyses, and also often made analysis of smaller amounts of material viable. This is particularly valuable when rare herbarium material must be used.

It is now generally accepted that certain compounds and related substances may be characteristic of certain taxonomic groups [4], and certainly chemotaxonomic investigations have been employed at all levels of the taxonomic hierarchy, from sub variety of division [5, 6]. It is thought that when the groups in question differentiated the, ability to form a chemical

substance was retained by virtue of metabolic processes retained by the group or its ancestors. By implication we see that if the pathway of chemical evolution were established then this might offer insight to the Evolutionary history of the group, as well as to the understanding of the present –day relationships within and between groups [4]

Chemotaxonomic Classification

The phenolics, alkaloids, terpenoids, non-protein amino acids, etc. are the important and widely exploited groups of compounds utilized for chemotaxonomic classification [7]. These groups of compounds exhibit a wide variation in chemical diversity, distribution and function [7, 8]. The system of chemotaxonomic classification relies on the chemical similarity of taxon [9, 10].

However, three broad categories of compounds are used in chemotaxonomy:

1. Primary metabolites
2. Secondary metabolites and
3. Semantides

1. PRIMARY METABOLITES

Primary metabolites are the compounds that are involved in the fundamental metabolic pathways. Most of the primary metabolites are of universal occurrence and utilized by the plant itself for growth and development [11, 12]. These compounds are ubiquitous in nature and hence play little role in chemotaxonomic classification. However, these molecules sometimes serve as useful chemotaxonomic behaviour on the basis of their quantities. For example, carbohydrate sedoheptulose is present in genus *Sedum* in large quantity. Therefore, the accumulation of sedoheptulose in the species of genus *Sedum* serves as a useful chemical character in chemotaxonomy [11]. The water soluble polysaccharides (WSP) are also used as chemotaxonomic markers. The gas liquid chromatographic analysis on WSP from annatto tree (*Bixa orellana* L.) showed hemispherical type contained 38% rhamnose, while conical and ovate types contained 17% and 34% glucose, respectively. Thus, glucose and rhamnose content of WSP could be used to distinguish the three landraces of annatto trees [13].

2. SECONDARY METABOLITES

Secondary metabolites are the compounds that usually perform non-essential functions in the plants [11]. They are used for protection and defence against predators and pathogens. These compounds are of restricted occurrence and hence very useful for chemotaxonomic classification.

Some of the major group of secondary metabolites includes glycoside, alkaloid, volatile oil, flavonoid, plant phenols and terpenoids.

a. Glycosides in chemotaxonomy

Glycosides are the compounds in which one or more sugars are combined with non-sugar molecules through glycosidic linkage. Based on the linkage, the glycosides are grouped as O-glycoside, C-glycoside, N-glycoside and S-glycoside. The distribution of O-glycosides like rhein is very common, so it has little chemotaxonomic value. The *R. rugosa* flavonol glycosides were shown to be important chemotaxonomic markers for the classification of species in Cinnamomeae [14]. The use of flavonol glycosides as chemotaxonomic markers could be useful for the identification of *Rosa* species belonging to sections Gallicanae, Cinnamomeae, Caninae, and Synstylae [14]. The C-glycosides like aloin, cascaroside which possess a direct carbon linkage between sugar and non-sugar are not very prevalent in nature. They are found in some plants containing anthraquinone derivatives [15, 16] such as aloin in *Aloe-Liliaceae* [17, 18] cascaroside in *Cascararhamnaceae* [19]. S-Glycosides Sinigrin are exemplified by those produce isothiocyanate on hydrolysis. These compounds are characteristic of the family cruciferae, moringaceae, capparaceae. So these families have phylogenetic relationship [20].

b. Cyanogenic glycoside in chemotaxonomy

The cyanogenic glycosides are the compounds responsible for providing defensive mechanism to plants [11]. Plant species have ability to produce hydrogen cyanide (HCN) by enzymatic hydrolysis of cyanogenic glycosides by the process called cyanogenesis [21]. Cyanogenesis is reported for the first time in the genera *Beilschmiedia*, *Cardwellia*, *Cleistanthus*, *Elaeocarpus*, *Embelia*, *Mischocarpus*, *Opisthiole*, *Parsonsia* and *Polyscias* [22]. Different amino acid like phenyl alanine, tyrosine, valine, leucine, and isoleucine are precursor for the biosynthesis of cyanogenic glycosides, but they are restricted to particular family. For example, a cyanogenic glycoside synthesized from leucine commonly occurs in

the subfamily amygdaloideae (almond) and maloideae (apple) of family rosaceae [11]. The glycosides derived from tyrosine commonly occur in the families of the order mangnoliales and laurales [11].

c. Glucosinolates in chemotaxonomy

Glucosinolates are sulfur- and nitrogen-containing plant secondary metabolites common in the order Capparales, which includes the Brassicaceae family [23]. On the basis of alkyl component of glucosinolate compound, brassica species can be differentiated. For example, *Brassica juncea* (mustard) from Indian subcontinent contain 3-butenyl glucosinolate and allylglucosinolate while those from Asiatic country contain only allyl compound [24, 25]. So, ancestry of Indian species is doubtful, because that is the hybrid of *B. nigra* (allylglucosinate) and *B. compestris* (3-butenyl glucosinate).

d. Alkaloid in Chemotaxonomy

Alkaloids are heterocyclic nitrogen containing basic compounds [26, 27]. But, few non-heterocyclic alkaloids are also present. Chemotaxonomic analysis based on alkaloids depends upon the type of parent base compound present in the alkaloids. The indole alkaloids contain indole as the parent base. More than 2,500 indole alkaloids were isolated mainly from three plant families, Rubiaceae, Loganiaceae and Apocynaceae. These are formed from two building blocks secologanin and tryptamine or tryptophane through a single precursor, strictosidine, and suggesting relationship between these families [28, 29]. Other indole alkaloids like physostigmine obtained from *Physostigma venenosum* (family Leguminosae) [30], yohimbine from *Rauwolfia serpentine* (family Apocyanaceae) [31] and *Corynanthe yohimbe* (family Rubiaceae) [32] and Vinblastine from *vincarosea* (family Apocyanaceae) [33]. The Pyridine and Piperidine alkaloids like Lobeline obtained from *Lobelia inflata* family Lobeliaceae [34]. Nicotine obtained from *Nicotiana tobaccum* family Solanaceae [35]. Anabasine obtained from *Nicotiana glauca* Family Chenopodiaceae [36]. The presence of these alkaloids explains the importance of these alkaloids in taxonomical analysis. Anabasine occurs in tobacco, where it is formed from lysine and nicotinic acid, where as in the legume and chenopod species this can be synthesized from two molecules of lysine [36]. Similarly, the alkaloids like isoquinoline alkaloids, tropane alkaloids, indole alkaloids etc have also been a useful tool for taxonomic classification of plants [37].

e. Plant Phenol in chemotaxonomy

Polyphenols are among the most widespread class of metabolites in nature, and their distribution is almost ubiquitous. It is estimated that 100,000 to 200,000 secondary metabolites exist [38] and some 20% of the carbon fixed by photosynthesis is channeled into the phenylpropanoid pathway, thus generating the majority of the natural occurring phenolics [39]. Flavonoids are largest group of phenolic compounds. They are mostly found in the vacuole of higher plant and absent in lower plant. Different classes of plant phenols include flavones, flavanones, isoflavanones, isoflavonoids, anthocyanidins and chalcones. All flavonoids have common biosynthetic origin and therefore it possess the same basic structural element. For example, 2- phenylchromone skeleton. They may be present in many classes depending on degree of oxidation of pyran ring which may be open and cyclize into furan ring, e.g. 2-phenyl benzopyrilium: anthocyanin and 2-phenyl chromone: flavone, flavanol, isoflavone [39]. A chemotaxonomic study of practically all the species of the genus *Aloe* showed that flavonoids occur as major compounds in 31 out of a total of 380 species investigated [40].

f. Terpenoids in Chemotaxonomy:

Terpenoids occur mostly in higher plants. According to Lawrence they are mostly found in *Myrtaceae*, *Lauraceae*, *Rutaceae*, *Lamiaceae*, *Asteraceae*, *Apiaceae*, *Poaceae*, and *Cupressaceae*.

Monoterpenes: They are the hydrocarbons which are most abundantly present. They have their boiling point range between 140°-180°C. They may be acyclic (example: Myrcene), monocyclic (example: p-cymene), or bicyclic (example: pinenes). Sometimes they constitute 90% of essential oil (example: Citrus oils or in turpentine). Further these may be optically active. The predominance of monoterpene (-) -enantiomers in the emission of some European *Pinus* and *Abies* species was explained by [31]. A monoterpene lactone is nepetalactone, the principle odour constituent of Catmint *Nepeta cataria*, Labiatae, a plant which has a peculiar attraction for the domestic cat because of its odour [31].

Sesquiterpenes: A very large number of Sesquiterpenes are common constituents of the essential oils of higher plants and therefore, may contribute to the pharmacological properties attributed to these volatile fractions [41]. Structural nature as in monoterpenes with

hydrocarbon alcohols and ketones being the most important ones. Examples are β -bisabolene, longifoline, farnesol, santalol, sinesals, cedryl acetate. Recently by GC broad chemodenes were distinguished by the presence of carvone and presence of absence of dill apiole [42].

Diterpenes: The structure of diterpenes is highly variable and strictly dependent on their biogenesis. Diterpene containing drugs have different applications such as anti-hypertensive, co –carcinogenic, anti- oxidant, hallucinogenic properties etc. Diterpenoids occur in the *Garryaceae*, a family difficult to classify on the base of morphological grounds. The chemotaxonomy of *Sideritis* species was evaluated and its acetone extract was shown to possess insecticidal and ascaricidal activity. The extract was found to contain linearol, lineal, isolinearol and siderol [43].

Triterpenes: These show a lot of taxonomic value when combined with other constituents such as phenols and flavanoids. Triterpenes have therapeutic potential in many fields such as cystostatics, insecticides, antiinflammatory agent etc. They play role in confirming the relation of *Pittosporaceae*. This family has more affinity with *Araliaceae* than *Saxifragaceae*. The triterpenes of 5 *lithocarpus* species were examined and they were of friedo unrearranged oleanane group viz, friedelin, friedelan, 3- β -taraxerol and β -amyrin. Glutinol was also present except in Lharlandi where frielan 2- α , 3- β diol was found. In addition, 3 new cycloartane triterpene, lithocarpolone, lithocarpdiol and 24- methylene cycloartane 3- β , 21 diol were found in *L. polystachya* [44].

Tetraterpenes: Carotenoids interfere with photoxidation processes such as treatment of photosensitization linked to porphyria, also ingredient of tanning pills and food technology industry. A qualitative and quantitative examination of carotenoids of pure cultures of four marine micro algae including *Chroomonas salina*, *Vaucheria sassilis*, *Cacolithus* and *Huxleyi* is reported. The latex contains a new natural carotenoid and fucoxanthin [44].

Polyisoprenes: *Erigeron bonariensis* (L.) is a common weed which is traditionally used in urine problems (Asteraceae). *Erigeron* genus has about 390 species of flowering plants. Intercontinental plant inventions resulted in a number of taxonomic problems especially in distinguishing it from *Conyza*. From the investigation on the basis of chemotaxonomy it was concluded that the phenolic content and caffeol derivatives present in it has a closer relationship to *Erigeron* than species of *Conyza* [45].

g. Essential oils in chemotaxonomy:

The following taxonomic discussions intend to illustrate the impact of “Essential Oils” on scientific plant classification.

Rutaceae: Rutaceae family plants are chemically characterised by the synthesis and accumulation of essential oils, furanocoumarins, anthranilic acid derived alkaloids and limonoids. Cneoraceae have represented a taxon in certaesedis for a long time. Their oil cells and the chemical nature of their bitter principles and of their 2- methylchromones leave absolutely no doubts about their intimate rutaceous meliaceous- simaroubaceous affinity. Prenylation of aromatic compounds is common in this family; examples of this tendency are furano and dimethyl pyrano coumarins and a number of essential oil constituents evodionol.

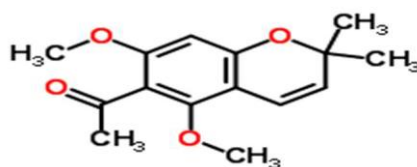


Fig 1: Evodionol

Umbelliflorae: *Umbelliferae* family constitute the plants with furano and dimethyl pyrano coumarins and essential oils which tend to contain phthalides; for example Ligustilide ferulol type monoterpenoids and acetylinic compounds like falcarinone. Essential oils in schizogenous ducts are highly characteristics of Araliaceae and umbelliferae together with other chemical characters they accentuate the overall similarities of these two families and the need for reclassification of other 5 families often included in umbelliflorae, cornaceae and allied families are iridoid producing taxa which seem to have affinities with *Dipsacales* rather than with *Araliaceae* and *Umbelliferae*. Prenylation of aromatic compounds is also common in this family such as Umbelliferone (coumarin).

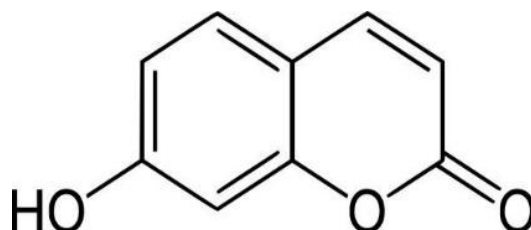


Fig 2: Umbelliferone

Verbenaceae and Labiatae: Many members of these two families are highly aromatic and yield essential oil. In *Labiatae* there is a clear cut vicariism. The group of aromatic plants does not produce iridoid glycosides; at the most some non-glycosylated compounds like myodesertal and myodesertin may be present in essential oils; the non- aromatic group of Labiates is characterised by iridoid glycosides like ajugol, galiridoside, harpagide, lamiol and others. The main taxonomic importance is two chemical groups of *Labiatae* which coincide with classification proposed by [46] for this family. Iridoids are insecticidal and insect deterrent and well described for steam volatile iridoids.

Piperales: Overall presence of oil cells and isolation of aporphine type alkaloids from roots of *Piper auritum* and stems of *Piper sanctum* [45] accentuate affinities between Magnoliales and Piperales.

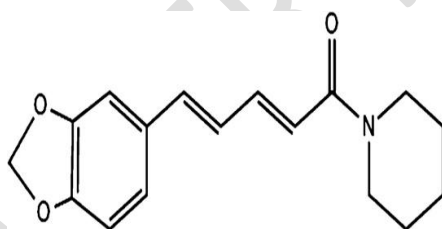


Fig 3: Piperine

Malvales: In this family mucilage cells and cavities are widespread but oleoresin cavities are much more restricted.

Myrtaceae: Myrtaceae family consists of tannin-rich essential oil plants. Methylated, prenylated and acetylated phloroglucinol derivatives occurs frequently in their essential oils; production of this very characteristic of acetogenins (Torquatone, a phloroglucinol derivative; present in some species of *Eucalyptus*) represent a chemical trend of this family.

Dipsacales: With one exception all members of *Dipsacaceae* produce iridoid or secoiridoid glucosides and lack essential oils. The exception is the genus *Morina* whose species does not produce iridoids but have essential oil in oil cells.

3. SEMANTIDES

These comprise DNA, RNA and proteins. Because each of these is so intimately connected with genetic characteristics, many researchers consider them to be of immense taxonomic value, as they potentially are. However, the time equipment and know-how necessary for their effective analysis often limits their usefulness.

Stace (1980) mentions three (3) main methods used in plant protein taxonomy. Viz;

- a. Electrophoresis
- b. Amino-acid sequencing
- c. Systematic serology

Electrophoretic techniques enable proteins to be 'fingerprinted' by establishing their relative size, charge and isoelectric point by separating them in variable gel mixtures across a voltage gradient [6]. Protein profiles produced via electrophoretic separation and subsequent staining have been used in various systematic studies investigating polyploidy taxa, as well as interspecific, intraspecific and population levels. Particular care and expertise are required in the use and interpretation of protein profiles [2].

Amino-acid sequencing attempts to establish the variation in the precise sequence of amino-acids in a single homologous protein throughout a range of organisms. This analysis relies on the fact that a particular protein may vary to a certain extent without altering its essential function. One molecule used extensively for this purpose is cytochrome c, in which 79 out of the approximately 113 amino acids vary interspecifically, but alterations of even a single one of the other 34 destroys the functioning of the molecule. Generally, the number of differences parallels the relational distance between the organisms in traditional classification, but anomalies do present themselves, interfering that a measure of protein structure is not an infallible guide to degree of kinship.

Certain assumptions are made when analysing the results of this technique:

It is assumed that:

- The molecule has evolved via the minimum number of mutations

- No convergent evolution or back-mutation has occurred
- Different positions on the molecule are equally susceptible to substitution.

These assumptions weaken the evidence when the sequencing of a number of homologous proteins yields conflicting evidence [6]. Some researchers have pointed out that to apply cytochrome patterns in chemo-systematics requires that one should take into account the quantitative and qualitative effects of the growth conditions on the cytochrome content [48]. [6] suggests that in order to prevent interpretative mistakes, results from a wide range of proteins, preferably studied by a number of different techniques, and should be pooled rather than placing total reliance on the screening of a single protein.

Synthetic serology is an immunological technique relying on the relative specificity of the immune reaction, and the fact that the degree of cross-reactivity is proportional to the degree of relationship between the organisms.

In plant serology, antisera to antigens from various taxa are raised in animals, using various plant extracts [2], and then the antisera can be used as a standard test against other plant extracts. The degree of coagulation that the other extracts cause in them is used as a measure of their similarity to the original antigen. Refinements in the technique have made this method more specific than it was previously, and serology has been extensively used throughout the taxonomic levels from above family to below species, yielding many valuable data [6].

Nucleic acids have not yet been used very extensively in plant systematics due to the complexity of their analysis. Most techniques are of relatively recent origin, and so the data accumulated thus far are limited [2]. Theoretically these characters should be able to solve many phylogenetic problems, firstly because each organism has DNA with a unique base sequence, and secondly because the theory of evolution is based on the premise that related organisms should show similarities in their DNA which are not shown by unrelated species [46].

The most useful technique in this regard at present is DNA hybridisation in, which DNA double helices are induced to unwind. And then allowed to recombine with each other as well as similarly treated DNA from other species. This results in some hybrid double helices being

formed, the number and fidelity of recombinations theoretically depending on the compatibility of the two DNA base sequences. Some useful results have been obtained which shows the potential value of this method, but techniques have not yet been perfected. [2] point out that variable results have been obtained depending on experimental conditions. Some evidence has suggested that *in vitro* replication of the DNA template is affected by factors such as temperature, and the absence of regulatory phenomena or specific factors that are present *in vivo* [48], and it seems well possible that this might just as well apply to recombination also.

Other techniques have been used to investigate DNA and RNA, but results, according to [6], are of limited application. He suggests that advances in gene cloning and genetic engineering may lead to more extensive use of nucleic acid characters in taxonomy, but a potential drawback to their extensive use in phylogeny is that live material is often a prerequisite.

Chemical variation is of considerable taxonomic value in several ways:

1. Confirmation or support of putative classifications derived from other sources of taxonomic characters, such as morphology.
2. Resolution of problems where relationships based on other evidence are ambiguous or conflicting.
3. Providing evidence to suggest more natural positioning of anomalous taxa, as well as to separate taxa. Often the presence of anomalous taxa in a group is accentuated by their chemical peculiarities.
4. Detection of confirmation of hybridization.
5. Providing additional on/off characters for numerical taxonomy by their presence or absence in taxa.

However, as with all other taxonomic characters, chemical variation must constantly be subject to critical appraisal of techniques and interpretations. Two major problems that appear to need addressing are the lack of standardization of the methodology and the inadequate sampling of groups.

Conclusion

Chemotaxonomy has undoubtedly made a big contribution to taxonomic work in the past and will most certainly continue to do so in future. However, given the lack of fossil evidence and the need for live material in some analyses it seems that its contribution to phylogenetic classification must perforce remain limited. The valuable information it offers is best used in conjunction with other sources of taxonomic evidence and thus a multidisciplinary approach is required in order to establish a system of classification which reflects natural relationships as accurately as possible.

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