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An Approach to the Chemosystematics of the Genus *Cucumis* L

A. J. A. PETRUS

Department of Chemistry, Kanchi Mamunivar Centre for Post-Graduate Studies (Autonomous), Puducherry - 605 008, India.

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ABSTRACT

Phylogenetic relationships in the order Cucurbitales as well as the phylogeny and classification of its taxonomically most problematic family, Cucurbitaceae, have been the focus of several studies. Taxonomists over the years have differed on the delimitation of *Cucumis* L. and numerous taxonomic treatments have been proposed since the pioneering work of Linnaeus (1753). Using maximum parsimony, maximum likelihood, and Bayesian inference analyses of sequence data from the nuclear and chloroplast genomes, the genus *Cucumis* has recently been recircumscribed. Among the various chemical classes elaborated in plants, the foliar phenolics express greater stability in general and contribute significantly to the chemosystematics of both, angiosperms and gymnosperms. Hence, it is felt that an evaluation of the available literature on the foliar flavonoid constitution of the recently defined *Cucumis* would be relevant. This paper, therefore, analyses the distribution of the phytophenols in the taxa to ascertain the characteristically common foliar marker biochemical of the genus in addition to an attempt to justify the inclusion of the genus *Mukia* within *Cucumis*.

Key words: Cucurbitaceae, *Cucumis*, *Mukia*, Phytophenols, Chemosystematics.

INTRODUCTION

Cucurbitaceae is a family of about 120 genera and over 900 species, widely distributed in tropical and subtropical regions of Africa including Madagascar, South, Southeast and East Asia, Australia as well as Central and South America[1,2]. The cucurbits are also among the most diverse plant families, cultivated and naturalised worldwide in a variety of environmental conditions. These include some of the earliest cultivated food plants in both the Old and the New World tropics and subtropics. Examples of some of the World's most important vegetable crops are cucumber (*Cucumis sativus*), melon (*Cucumis melo* L.), West Indian Gherkin (*Cucumis anguria*), pumpkins and squashes (*Cucurbita moschata* and *C. mixta*), red pumpkin (*Cucurbita maxima*), water melon (*Citrulus vulgaris*), loofah (*Luffa acutangula*, *L. cylindrica*), bottle gourd (*Lageneria siceraria*), snake gourd (*Trichosanthes cucumerina* var. *anguina*), bitter gourd (*Momordica charantia*) and pepitos (*Cucurbita pepo*). A number of them are used since antiquity not only as food, but also in folk medicine, health-care and cosmetology. Notable medicinal applications include their use in the treatment for diabetes, bronchial and respiratory ailments as well as in the oral health care and as an abortifacient[3,4]. Gourds also find use as containers and as resonators in musical instruments such as the sitar.

The genus *Cucumis* (Cucurbitaceae) comprises of some 38 African/Indian Ocean species, 19 Asian, and six Australian/ New Guinean species. Most of the *ca.* 63 species of *Cucumis*, presently known are monoecious annuals, but dioecious mating systems and a perennial habit evolved several times within the genus[5]. Formerly, *Cucumis* L. was a more narrowly defined genus[6]. Based on the recent molecular-phylogenetic findings[7,8], the species of the genera *Cucumella* Chiov., *Dicaelospermum* C.B.Clarke, *Mukia* Arn., *Myrmecosicyos* C.Jeffrey, and *Oreosyce* Hook.f. are now transferred into a more broadly defined *Cucumis*[9,10]. In the context of the present nuclear and chloroplast DNA data-based recircumscribtion of *Cucumis*, an examination of the relevance of the chemotaxonomic significance of the marker phenolics has been attempted for the first time in this paper.

Biosystematics

In the traditional systems of taxonomy, morphological traits are commonly used for classifications. However, for delimiting those taxa that exhibit morphological/cytological similarities or whose morphological characters show reticulate relations, chemical data in conjunction with those drawn from other approaches (say, morphological, cytological, embryological, anatomical and molecular) are frequently resorted to resolve the issue and to establish phylogenetic relations at interspecific, intergeneric and family levels[11,12].

Chemical characters may be broadly categorized into visible traits, micromolecular, and macromolecular. Compared to the very few visible characters, such as starch grains, raphides, lapachols and silica that have limited usage in taxonomy, the micro- and macro-molecules prove to be important taxonomic tools[13]. Micromolecular characters relate to the low molecular weight secondary metabolites belonging to various chemical classes, such as phenolic, alkaloid, terpenoid, cyanogenic, free amino acid, lipid and others. Examples of macromolecules offering such informations include certain proteins, DNA and RNA. Though chemical characters offer themselves as important taxonomic tools, some of them, for instance, alkaloids and proteins are susceptible to change with regard to changing environmental conditions or ontogeny. Plant parts of the same physiological age should, therefore, be used for comparative analysis.

Phytophenols

Phytophenols are the ubiquitous class of bioactive constituents of the human diet, manifesting both nutritional and health benefits, mediated largely by their redox property, reactive species-scavenging and metal chelating capacities[14-15]. They are thus capable of mitigating oxidative stress-induced tissue damage associated with chronic diseases. Structurally, plant phenolics are a diversified group of plant-derived molecules, originated from the pentose phosphate, shikimate and phenylpropanoid pathways. This class of secondary metabolites are produced by the plant kingdom, including certain algae and specific insects. In plants, they are distributed throughout most tissues, including the flowers, roots, seeds and leaves, with the foliar phenolics expressing greater stability[16]. Plant phenolics are characterized by the presence of at least one aromatic ring with one or more hydroxyl groups directly bonded to the ring. They range from the naturally occurring simple, low molecular weight, single-aromatic-ring compounds to the large and complex tannins and derived-polyphenols. The most productive plant metabolic route, in terms of the number of phenolic substances it produces, is the one that leads to the flava-/flavo-noids, a C_6 -C_3-C_6 framework with close to 10,000 structures characterised to date. Flavonoids, because of their easy isolation, identification, stability and occurrence in different structural forms, have been used extensively for differentiating various species, establishing relationships at inter-generic and sub-familial levels and solving taxonomic problems[13].

Phylogenetic systematics of *Cucumis*

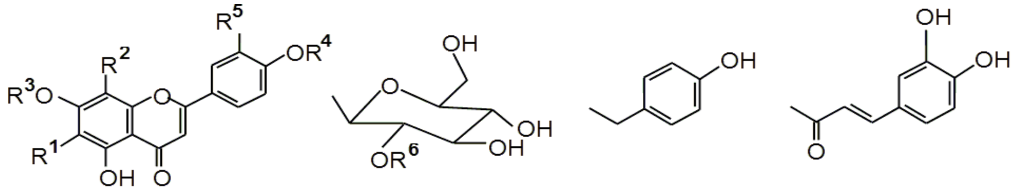
Phylogenetic relationships in the order Cucurbitales as well as the phylogeny and classification of its taxonomically most problematic family, Cucurbitaceae, have been the focus of several studies of the recent past[17-20]. Taxonomists over the years have differed on the delimitation of *Cucumis*, which was first described by Linnaeus in *Species plantarum* (1753). Numerous taxonomic treatments of *Cucumis* have been proposed since the pioneering work of Linnaeus[8]. Using maximum parsimony, maximum likelihood, and Bayesian inference analyses of sequence data from both the nuclear and chloroplast genomes, Ghebretinsae, Thulin and Barber have provided a comprehensive phylogeny of *Cucumis* and the traditionally related genera, *Cucumella* Chiovenda, *Dicaelospermum* C.B. Clarke, *Mukia* Arnott, *Myrmecosicyos* C. Jeffrey and *Oreosyce* Hooker f.[8,9]. Accordingly, the genus *Cucumis* from its erstwhile paraphyletic circumscription is recircumscribed by including the five genera of the subtribe cucumerinae Pax in compliance with the monophyly of *Cucumis*[8-10].

Foliar phenolics of *Cucumis*

Two major chemical classes, viz., cucurbitacins and flavonoids dominate the phytochemistry of cucurbits. Cucurbitacins are bitter-tasting triterpenoids that are present in high concentrations in root tissue and are toxic to mammals. In contrast, the fruits of many species in this family are edible and used as foods, including pumpkin, watermelon, melon and cucumber[21]. Since the foliar phenolics generally contribute significantly to chemosystematics of both, angiosperms and gymnosperms, it is felt that an evaluation of the data available on the foliar flavonoid constitution of the recently defined *Cucumis* would be relevant. Survey of the flavonoid constitution of *Cucumis* has probably originated from the U.S. Vegetable Breeding Laboratory in 1969 during the screening for nematode resistance by Brown *et al.,*[22]. Twenty six compounds have been identification by paper chromatography from 15 *Cucumis* species. Based on the similarities in the distribution of flavonoids, they had grouped the studied species into two large complexes. The first consisted of *C. africanus*, *C. heptadactylus*, *C. melo* and *C. myriocarpus* and the second one composed of *C. anguria*, *C. dipsaceus*, *C. ficifolius*, *C. prophetarum*, *C. pustulatus*, and *C. zeyheri*. *Cucumis hirsutus*, *C. humifructus*, *C. metuliferus*, *C. sagittatus*, and *C. sativus* were each considered to be distinct from all the other species studied as they possessed distinct patterns unlike any other species.

Table 1 summarises the distribution of the naturally occurring foliar flavonoids of the genus recorded in literature. A conspicuous feature in all the studied *Cucumis* species is the biosynthesis of *O*-glucosylated-*C*-glucoflavones (Fig. 1). These compounds are also reported to be elaborated in eight further species of the genus, besides those noted in Table 1, according to Brown *et al.*,[22]. In such glycoflavonoids occurring in nature, however, the hydrolysable *O*-glycosyl moiety can have two possible modes of linkage: either to a phenolic hydroxyl (X-/X'-*O*) or to one of the hydroxyl functions of the *C*-glycosyl residue (X"-*O*). Consequently, two possible isomeric products of *C*-glycosylflavonoid-*O*-glycoside can result. Despite the fact that careful assignment of the characteristic UV, ^{1}H- and ^{13}C-magnetic resonance data can distinguish the two isomers, there are occasions when investigators have concluded wrongly about the structure of their isolates[29]. For instance, 7-*O*-β-D-glucopyranosyl-6-*C*-β-D-glucopyranosylapigenin (saponarin) has been reported to occur in mature barley (*Hordeum vulgare*) leaves, along with lutonarin and its 3'-hydroxylated analogue[29-31]. A number of publications have described the structure of this glycoflavone, isolated from young green barley leaves, as 2"-*O*-β-D-glucopyranosyl-6-*C*-β-D-glucopyranosylapigenin (**5**)[32-37]. Markham and Mitchell have however subsequently established the major isolate from young green barley leaves also as saponarin and not compound 5, using authentic saponarin[38]. With little concern over the true structure of the isolates and admitting the literature reports *in toto* and also ignoring the site of glycosylation (*X/X'/X"*), *O*-glucosylated-*C*-glucoflavones, in general, appear to be the characteristically common foliar marker biochemical of the newly defined genus *Cucumis* L.

This hypothesis may be illustrated taking the following example. Three taxa are reported in literature as species of *Melothria* L.[39], for which the flavonoid profile are available to date, in the context of their wide use in the traditional systems of medicines. viz., *Melothria maderaspatana* (L.) Cogn.[3,4], *M. heterophylla* (Lour.) Cogn.[40-42] and *M. perpusilla* (Blume) Cogn.[43]. However, based on seed and stamen morphology, Jeffrey has divided *Melothria* into four genera, viz., *Melothria* L., *Mukia* Arn., *Solena* Lour., and *Zehneria* Endl.[44], resulting in many species of *Melothria* getting transferred to *Mukia*, *Solena* and *Zehneria*. All these genera have three stamens. In *Zehneria*, the stamens are two-thecal whereas in *Mukia* and *Solena*, two stamens are two-thecal and the remaining stamen is one-thecal. *Mukia* has straight anther-thecae



β-D-glucopyranosy (GL) | 4-hydroxy-1-ethyl-benzene (HEB) | cafeoyl moiety (Ca)

	Compound	R^1	R^2	R^3	R^4	R^5	R^6
1	6-*C*-β-D-glucopyranosylapigenin (isovitexin)	Gl	H	H	H	H	H
2	8-*C*-β-D-glucopyranosylapigenin (vitexin)	H	Gl	H	H	H	H
3	7-*O*-β-D-glucopyranosylisovitexin (saponarin)	Gl	H	Gl	H	H	H
4	4'-*O*-β-D-glucopyranosylsaponarin	Gl	H	Gl	Gl	H	H
5	2"-*O*-β-D-glucopyranosylisovitexin	Gl	H	H	H	H	Gl
6	4'-X-di-*O*-glucopyranosylisovitexin	Gl	H	H	Gl-Gl	H	H
7	8-(4-hydroxy-1-ethylbenzene)-isovitexin	Gl	HEB	H	H	H	H
8	6-(4-hydroxy-1-ethylbenzene)-vitexin	HEB	Gl	H	H	H	H
9	6,8-di-*C*-β-D-glucopyranosylapigenin (Vicenin-2)	Gl	Gl	H	H	H	H
10	6-*C*-β-D-glucopyranosylluteolin (homoorientin)	Gl	H	H	H	OH	H
11	8-*C*-β-D-glucopyranosylluteolin (orientin)	H	Gl	H	H	OH	H
12	7-*O*-β-D-glucopyranosylhomoorientin (lutonarin)	H	Gl	Gl	H	OH	H
13	6-C-β-D-glucopyranosyl-3',4',5-trihydroxy-7-methoxy-flavone-4'-X-di-O-glucopyranoside (4'-X-di-O-glucosylswertiajaponin)	Gl	H	CH_3	Gl-Gl	H	H
14	6-*C*-diglucopyranosylapigenin	Gl-Gl	H	H	H	H	?
15	6-*C*-diglucopyranosylorientin	Gl-Gl	H	H	H	OH	?
16	X'''-cafeoyl-6-*C*-diglucopyranosyl-apigenin	Gl-Gl	H	H	H	H	?
17	X'''-cafeoyl- 6-*C*-diglucopyranosyl-orientin	Gl-Gl	H	H	H	OH	?

? = linkage not established

Fig. 1: Name and Chemcial structures of the folair flavonoids reported from the genus *Cucumis*

Table 1: Natural distribution of flavonoids in species and cultivar varieties of the genus *Cucumis*

Species/ cultivar varieties	Compound*																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
C. maderaspatanus[23-24]	+	+	+							+	+	+					
C. melo var. Doublon[25]														+	+	+	+
C. metuliferus[26]					+												
C. myriocarpus[26]	+				+					+							
C. sativus[26,27]	+	+			+	+	+	+		+	+		+				
C. sativus Mustang[28]	+		+	+					+								
C. sativus Cezar[26]	+				+	+				+			+				
C. sativus Hela[26]					+	+							+				
C. sativus Monastyrski[26]					+					+							
C. sativus Olimp[26]					+												
C. sativus Polan[26]					+	+							+				
C. sativus Delicius[26]	+				+	+				+			+				

*The names and chemical structures of the compounds are described in Fig. 1

and verrucose seeds, whereas *Solena* has oblique, curved anther-thecae and smooth seeds. Thus, Jeffrey has re-established the genera: *Zehneria* Endl., characterized by its three 2-thecous stamens, *Solena* Lour., by its peculiar obliquely triplicate anther-thecae, and *Mukia* Arn., by its tumid seeds and clustered flowers, as distinct from *Melothria* L. into which they had been sunk by Cogniaux[39]. *Melothria* is presently an entirely New World genus of plants with long-stalked fruits and male racemes, compressed seeds, and 3 stamens, two of which are 2-thecous and the other, 1-thecous. According to this revision, *M. heterophylla* (Lour.) Cogn. is now *Solena heterophylla* Lour. subsp. heterophylla[45] and *M. perpusilla* (Blume) Cogn. is *Zehneria bodinieri* (H. Lév.)[45]. *O*-glucosylated-*C*-glucoflavones, *viz.*, 7-*O*-β-D-glucopyranosylisovitexin (**3**) and 7-*O*-β-D-glucopyranosylhomoorientin (**12)** are reported only from *C. maderaspatanus* L.[23-24] and has not been reported yet from either *S. heterophylla* Lour. or from *Z. bodinieri* (H. Lév.). From the leaf-extract of *S. heterophylla* Lour., 1,2,4,6-tetra-*O*-galloyl-β-D-glucopyranose, 3,4,5-trihydroxybenzoic acid (gallic acid), 3-*O*-rutinosylquercetin (rutin), 7-*O*-β-D-glucopyranosylluteolin and 7-*O*-β-D-glucopyranosylapigenin alone and no *C*-glycoflavone has been reported until now. Similarly, 4'-*O*-β-D-galactosylquercetin, 3-*O*-β-D-glucosyl (1→2)- β-D-glucosylkaempferol and 3-*O*-β-D-glucosyl-(1→2)-β-D-galactosylquercetin7-*O*-β-D-glucoside alone have been isolated till date from *Z. bodinieri* (H. Lév.).

CONCLUSIONS

The occurrence of compounds 3 and 12 in *C. maderaspatanus* L. is chemosystematically in harmony with the recent revision of the genus *Cucumis* L. The present study is however subject to the limitations that the flavonoid profile available in literature to date is only limited and requires more study reports to gain larger sample size. Further, the reports of partially/incompletely characterized compounds 14-17 as well as those reported from eight other species by Brown *et al.*[22] has restricted the specificity of the marker compound.

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