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Chemtaxonomic Relationship of Roots Phenolic Compounds for Selected Species of Four Families Recently Grouped in Brassicales by APGIII

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Abstract: Chemotaxonomic relationship between five members recently grouped in the order Brassicales by APGIII were studied on the basis of their roots phenolic compounds constituents. The selected species are; *Schimpera arabica* from Brassicaceae*, Maerua crassifolia and Maerua oblongifolia* from Capparacea, *Cleome gynandra* from Cleomaceae and *Salvadora persica* from Salvadoraceae. Roots were extracted in ethanol (80%). Phenolic compounds were separated using Thin Layer Chromatography (TLC) technique. The developing solvent which is a mixture of Toluene- Chlorophorm – Acetone (in a rate of 10:50:7). The paired affinity between the species was constructed base on roots phenolic compounds, most of the species revealed closeness but The highest paired affinity was observed between *Schimperaarabica and Maeruaoblongifolia.* Polygonal graphs were constructed on the basis of the paired affinity which reflects the obvious closeness between studied species. This investigations Provided evidence that support grouping the selected species in the order Brassicales. More chemicals investigation for different members and different parts of the order will be better to demonstrate their closeness.

Keywords: TLC, Phenolics, Paired Affinity, Polygonal Graph, Chemotaxonomy, APGIII, Brassicales

1. Ntroduction

Plant chemotaxonomy is the study of chemistry of plant products to provide taxonomic characters which may help in plant classification or solve taxonomic problems. Chemical markers, such as phenolic compounds, have been still extensively used in botanical chemosystematic studies [1]. These have largely concerned the high or middle taxonomic levels: order, family, genus, section [2]. Brassicales classification had undergone considerable revision in the past decade particularly in recent years [3]. Some newer systems were suggested in which many genera were moved to different families, some families were merged and others were split. According to the Cronquist system of classification, the order Brassicales is called the Capparales. The only families included are the Brassicaceae and Capparaceae which are treated as separate families [4]. Many other authors agreed that Capparaceae and Brassicaceae are closely related and should be considered as one family [5], [6], [7]. In his second edition of the classification of flowering plants, Cronquist included members of the family Cleomaceae to the family Capparaceae [8].

Judd [9] conducted a morphological cladistic analysis in the order Brassicales. They considered the Cleomaceae genera to be more closely related to Brassicaceae than Capparaceae and allowed Cleome and the other members of Cleomaceae to be included in Brassicaceae. They also suggested that Capparoideae form a paraphyletic grade sister to a monophyletic Cleomoideae plus Brassicaceae. Based on these analyses, the two families Capparaceae and Brassicaceae have been merged into one family: the Brassicaceaesensulato (s.l.) [10]. Other classifications have continued to recognize Capparaceae but with a more

restricted circumscription, either including Cleome and its relatives in Brassicaceae or recognizing them in the segregate family Cleomaceae [11].

Brassicaceae for all taxonomist recognized by the cruciform corolla, tetra dynamous stamens, and characteristic silique fruit type. In addition to these floral and fruit features, there is strong molecular evidence supporting Brassicaceae as a monophyletic group [12]. Although Brassicaceae is one of few families of higher plants to have been recognized as such throughout recorded history [6]. However, according to several authors many of the tribal relationships within the family are unnatural and intergeneric relationships within the family remain difficult and unresolved [12] [13], [14]. Although the majority of flowers in Brassicaceae conform to the same basic floral formula, there are deviations [6], [15], [16]. Several putative basal members of Brassicaceae share floral features with Capparaceae such as the woody habit and presence of a gynophore and lack of the tetra dynamous stamens. There has been considerable debate whether these shared features indicate shared ancestral states or convergent evolution [4], [6], [17].

most taxonomists have agreed that Brassicaceae and Capparaceae are closely related families, a relationship that is supported by morphological and chemical data [6], [7], [11], [18], [19], [20]. Molecular and morphological phylogenetic analyses of the family and relatives reveal that Capparaceae has traditionally circumscribed are paraphyletic, with the larger, mostly temperate family Brassicaceae embedded within it [7], [9], [11], [19], [20], [21]. Chloroplast sequence data strongly support the monophyly of the three lineages: Brassicaceae s. str., Capparaceaesubfam. Capparoideae, and Capparaceaesubfam. Cleomoideae, with strong support for a sister relationship of Cleomoideae to Brassicaceae [11], [21], [22]. Thus, phylogenetic relationships inferred from chloroplast sequence information (ndhF, trnL-trnF, matK, and rbcL) and the criterion of monophyly permit three alternatives for familial recognition: (i) three families, Brassicaceae s. str., Cleomaceae, and Capparaceae s. str.; (ii) two families, Brassicaceae (including Brassicaceae s. str. And subfamily Cleomoideae) and Capparaceae (represented only by subfamily Capparoideae); and (iii) one single family, Brassicaceaes.l. [11]. Some authors merge all three into one, all-inclusive Brassicaceae [9, 10,23].

In pre-molecular classifications the family Salvadoraceae has always been considered an outsider, either as 'incertasedis' [24], or dumped in or close to Celastrales [4] or Oleales [25], [26]. Dahlgren [27] placed Salvadoraceae in an expanded order Capparales and he later segregated the family in the separate order Salvadorales [28]. The presence of mustard oils in Salvadoraceae was recognized early as an indicator of affinity with Capparales [25], [26], and it was only through the molecular support of studies based on several gene regions that an association with all mustard oil-producing families was finally confirmed [7], [19], [20], [29].

Recently and on the base of molecular studies [30] and [31] system has adopted treating Cleomaceae and Capparaceae as segregate families; Salvadoraceae and other taxa to be included in the order Brassicales.

The present investigation on the distribution of phenolics was carried out in five species of Barssicales to examine their relative phyletic distance as evidenced from their biochemical picture.

The chemotaxonomic results were subjected to numerical taxonomic treatment as an aid to establish phenolic relationship in the different species of the order.

2. Material and Methods

2.1. Plant Material

Five species belonging to four different families of the order Brassicales were selected for this study. They were collected from their natural habitats. They were taxonomically identified and authenticated by comparing the collection with the available specimens deposited in the Herbarium of the Botany Department -Khartoum University and the Herbarium of Medicinal and Aromatic Plants Research Institute. The species were deposited in the Herbaria and their names were updated consulting the relevant publications.

2.2. Methods

2.2.1. Distribution of Phenolic Compounds

Thin Layer Chromatography (TLC) technique was applied to determine the distribution of phenolic compounds of roots of the studied species.

30g of silica gel Dissolved in 60 distilled water was coatedto the TLC plate (200 x200mm) with thickness 0.5mm.

2.2.2. Procedure

For each dry Sample (3.00 g) was extracted by macerating them in 15ml of 80% Ethanol in test tubes, the test tubes were tightly closed by foil to prevent the evaporation of the solvent. The roots extractswere kept for 3days in room temperature in order to maximum possible extraction of phenolic compounds. Using a capillary tube, 3 drops of each extract was placed on each spot at the starting point of TLC plate so that they line up with the notches etched for each plant species. The developing solvent which is a mixture of Toluene- Chlorophorm - Acetone (in a rate of 10:50:7) was prepared. Enough volume of the developing solvent was poured in a jar in order to reach 1 cm deep at the bottom and a piece of filter paper was placed into the jar to saturate the atmosphere with the solvent. Then the plate was dipped carefully in the developing solvent and the chromatogram was allowed to develop.

Plates were removed from the chamber and the highest solvent level on them was traced with pencil. Also the spots were traced while held under a UV lamp. Then the spots were marked with a pencil. The R_f values were then calculated for each spot.

2.2.3. Analysis of Phytochemical Data

Ratio of fronts (R_f) values:

Ratio of fronts (R_f) values for each spot were calculated

according to the equation:

$$\text{Rf} = \frac{\text{Distance from start to center of substance spots}}{\text{Distance from start to solvent front}}$$

The method adopted by Ellison [32] was followed to make the suitable comparisons in the form of qualitative relationships. Species were compared on the basis of their biochemical affinities. Values of paired affinity (PA), group affinity (GA) and isolation value (IV) were calculated as follows:

$$PA = \frac{-Spots\ common\ in\ species\ A\ and\ B}{\text{Total spots of A and B}} \times 100$$

$$GA = Total\ PA\ value\ +\ 100$$

$$IV = \frac{\text{Number of unique spots in a species}}{\text{Total number of spots in all species}}$$

3. Result

Screening for phenolic compounds on TLC, resulted in many spots of different Retention Factor (Rf) values. The relative distribution of all spots and Retention Factor (Rf) of phenolics in the roots of the selected species separated by thin layer chromatography are tabulated in Table 1. A total number of 13 spots representing Different phenolic compounds were detected in roots part of the selected species. Some of the separated compounds are common to different species which might suggest possible closeness of the species. This is verified by calculating the paired affinity index between different pairs of the selected species. According to Ellison et al. (1962), paired affinity index (PA) of 50% and above are considered as a marker of close relationship. The PA value calculated on the basis of presence and absence of the phenolics is shown in Table 2. Some of the selected species revealed high PA as shown in Figure 1, but The highest PA value 75% was observed between *Schimpera Arabica* and *Maerua oblongifolia.* The lowest PA value 25% was found between *Cleome gynandra* and *Maerua crassifolia*. These values showed that *Maerua oblongifolia and Schimpera arabica were* closely related.

Group affinity values also showed the close relationship between *Maerua oblongifolia* (365) and *Schimpera arabica* (320) as shown in Table 3.

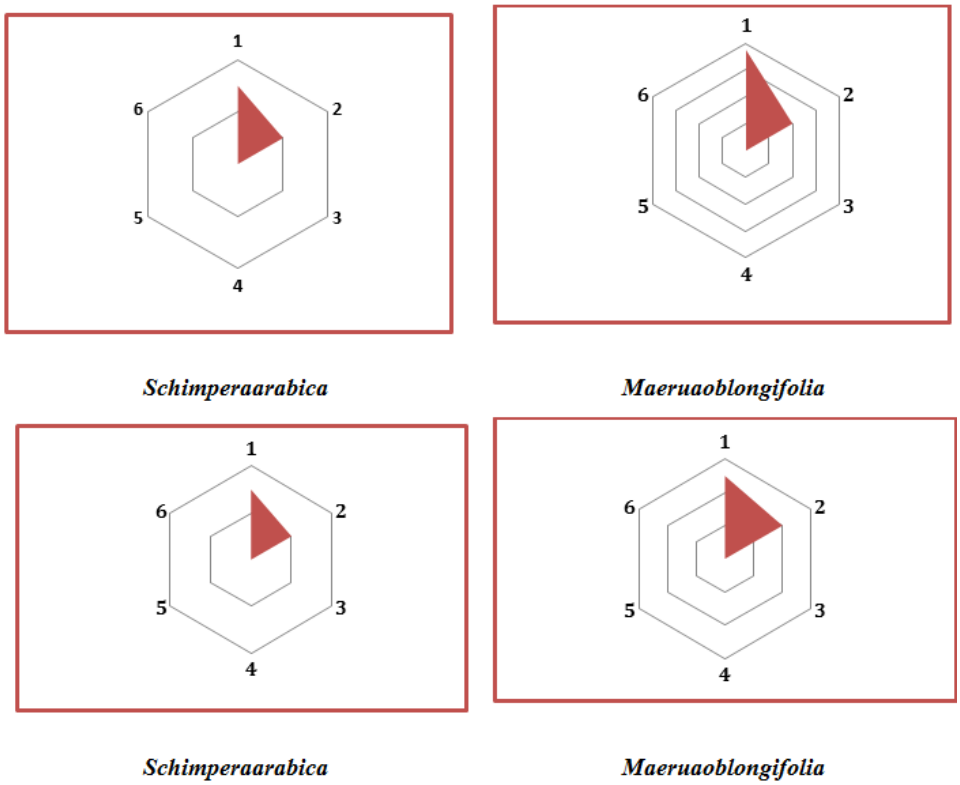

Figure 1. *Polygonal graphs show the closeness between studied species based on their paired affinity (PA).*

Table 1. *Thin layer chromatography separation of phenolics in the roots of the studied species revealing their R_f values.*

No.	RF values T/C/A	*Cleome gynandra*	*Maeruacrassifolia*	*Maeruaoblongifolia*	*Schimperaaarabica*	*Salvadorapersica*
1	0.13	-	-	+	+	+
2	0.25	-	-	+	+	+
3	0.67	+	+	-	-	-
4	0.68	-	-	+	+	+
5	0.92	-	+	+	-	-

Table 2. *Paired Affinity values (PA) of studied specie.*

	Cleome gynandra	***Maeruacrassifolia***	***Maeruaoblongifolia***	***Schimperaaarabica***	***Salvadorapersica***
Cleome gynandra		25%			
Maeruacrassifolia	25%		50%		
Maeruaoblongifolia				75%	40%
Schimperaarabica			75%		50%
Salvadorapersica			40%	50%	

Table 3. *Group affinity, number of unique spots and isolation value of phenolic compounds of the roots of studied species.*

No.	**Species**	**GA**	**No. of unique spots**	**Isolation value (%)**
1.	*Cleome gynandra*	225	0	0
2.	*Maeruacrassifolia*	225	0	0
3.	*Maeruaoblongifolia*	365	0	0
4.	*Schimperarabica*	320	0	0
5.	*Salvadorapersica*	290	0	0

4. Discussion

Phenolics as a chemical marker can be used as one of the substantial criteria for decisions in plant taxonomy [2]. The main concept of the classification of the Brassicales had been started by the inclusion of all mustard oil containing plants together in one order. by early 1970s, 15 plants families had been considered as members of Brassicales [27], and more mustard producing families had been included by [27], [28]. Most of the classifications of the order Brassicales had been evolved in 20th century. They differ in their placement of the families and the genera which included in each family. The most considerable revisions were those concerning the three families Brassicaeae, Capparaceae and Cleomaceae.

Based on some morphological characters, Brassicaceae and Capparaceae were treated as two separate families [4], [8], [33]. Based on molecular studies members of the two families were firstly treated as one family Brassicaceae [23] but the [34] system has adopted treating Cleomaceae and Capparaceae as segregate families; Salvadoraceaeand other taxa to be included in the order Brassicales [34]. Higher PA value was considered as an indication of close affinity between different species. PA value of 50% and above was considered as a marker of closeRelationship. In this regard, the two species of *Schimperaarabica and Maerua oblongifolia* (PA value of 75%) were most closely related, this result support the claim that Brassicaceae and capparaceaeare closely related and should be considered as one family [5], [7], [23]. *Cleome gynandra* has the lowest paired affinity comparing with other species but it was close to *Maerua crassifolia*, thing that support its placement with capparaceae in one family [8]. Plogonal graphs reflect the closeness of studied species, thing that support the grouping the studied species in one order Brassicales by [30], [31].

5. Conclusion

Plant chemotaxonomy can provide taxonomic characters which may help in plant classification or solve taxonomic problems beside the classical and molecular plant taxonomy. This study provided some important biochemical basis for the chemotaxonomy of the order Brassicales. As phenol compounds have been still extensively used in botanical chemosystematic studies, this investigation revealed that Phenolic compounds can be of taxonomic important factor in chemotaxonomy of the order, because they demonstrated the chemotaxonomy relationship between the selected species. The recommendation of this study is that, further investigation of phenolic compounds on other parts of the selected species or other species from the order are needed as confirmatory evidences.

References

[1] Singh, Ram, *Chemotaxonomy: A tool for plant classification.* Journal of Medicinal Plants, 2016. 4 (2): p. 90-93.

[2] Míka, V, V Kuban, B Klejdus, V Odstrcilová and P Nerusil, *Phenolic compounds as chemical markers of low taxonomic levels in the family Poaceae.* Plant Soil and Environment, 2005. 51 (11): p. 506.

[3] Hohmann, Nora, Eva M Wolf, Martin A Lysak and Marcus A Koch, *A time-calibrated road map of Brassicaceae species radiation and evolutionary history.* The Plant Cell, 2015. 27 (10): p. 2770-2784.

[4] Cronquist, Arthur, *An integrated system of classification of flowering plants*. 1981: Columbia University Press.

[5] Hauser, Larry A and Theodore J Crovello, *Numerical analysis of generic relationships in Thelypodieae (Brassicaceae).* Systematic Botany, 1982: p. 249-268.

[6] Rollins, Reed Clark, *The Cruciferae of continental North America: systematics of the mustard family from the Arctic to Panama*. 1993: Stanford University Press.

[7] Rodman, James, Robert A Price, Kenneth Karol, Elena Conti, Kenneth J Systma and Jeffrey D Palmer, *Nucleotide sequences of the rbcL gene indicate monophyly of mustard oil plants.* Annals of the Missouri Botanical Garden, 1993: p. 686-699.

[8] Cronquist, A, *The evolution and classification of flowering plants Second Edition.* New York Botanical Garden, New York, 1988.

[9] Judd, Walter S, ROGER W SANDERS and MICHAEL J DONOGHUE, *Angiosperm family pairs: preliminary phylogenetic analyses.* Harvard papers in Botany, 1994: p. 1-51.

[10] Group, The Angiosperm Phylogeny, *An ordinal classification for the families of flowering plants.* Annals of the Missouri botanical Garden, 1998: p. 531-553.

[11] Hall, Jocelyn C, Kenneth J Sytsma and Hugh H Iltis, *Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data.* American Journal of Botany, 2002. 89 (11): p. 1826-1842.

[12] Price, Robert A, Jeffrey D Palmer and Ihsan A Al-Shehbaz, *1 Systematic Relationships of Arabidopsis: A Molecular and Morphological Perspective.* Cold Spring Harbor Monograph Archive, 1994. 27: p. 7-19.

[13] Al-Shehbaz, Ihsan Ali, *The tribes of Cruciferae (Brassicaceae) in the southeastern United States.* Journal of the Arnold Arboretum, 1984. 65 (3): p. 343-373.

[14] 14. Koch, Marcus, Bernhard Haubold and Thomas Mitchell-Olds, *Molecular systematics of the Brassicaceae: evidence from coding plastidic matK and nuclear Chs sequences.* American Journal of Botany, 2001. 88 (3): p. 534-544.

[15] Endress, Peter K, *Evolution and floral diversity: the phylogenetic surroundings of Arabidopsis and Antirrhinum.* International Journal of Plant Sciences, 1992: p. S106-S122.

[16] Bowman, John L, Holger Brüggemann, Ji−Young Lee and Klaus Mummenhoff, *Evolutionary changes in floral structure within Lepidium L. (Brassicaceae).* International Journal of Plant Sciences, 1999. 160 (5): p. 917-929.

[17] Al-Shehbaz, Ihsan A, *The biosystematics of the genus Thelypodium (Cruciferae).* Contributions from the Gray Herbarium of Harvard University, 1973 (204): p. 3-148.

[18] Iltis, Hugh H, *Studies in the Capparidaceae. III. Evolution and phylogeny of the western North American Cleomoideae.* Annals of the Missouri Botanical Garden, 1957. 44 (1): p. 77-119.

[19] Rodman, JE, KG Karol, RA Price, E Conti and KJ Systma, *Nucleotide sequences of rbcL confirm the capparalean affinity of the Australian endemis Gyrostemonaceae.* Australian Systematic Botany, 1994. 7 (1): p. 57-69.

[20] Rodman, James E, Kenneth G Karol, Robert A Price and Kenneth J Sytsma, *Molecules, morphology, and Dahlgren's expanded order Capparales.* Systematic Botany, 1996: p. 289-307.

[21] Hall, Jocelyn C, Hugh H Iltis and Kenneth J Sytsma, *Molecular phylogenetics of core Brassicales, placement of orphan genera Emblingia, Forchhammeria, Tirania, and character evolution.* Systematic Botany, 2004. 29 (3): p. 654-669.

[22] Hall, Jocelyn C, *Systematics of Capparaceae and Cleomaceae: an evaluation of the generic delimitations of Capparis and Cleome using plastid DNA sequence data This paper is one of a selection of papers published in the Special Issue on Systematics Research.* Botany, 2008. 86 (7): p. 682-696.

[23] II, A, *An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II.* Botanical Journal of the Linnean Society, 2003. 141 (4): p. 399-436.

[24] Thorne, Robert F, *An updated phylogenetic classification of the flowering plants.* Aliso (USA), 1992.

[25] Melchior, Hans, *A. Engler's Syllabus Der Pflanzenfamilien... II, Band, Angiospermen*. 1964: Borntraeger.

[26] Aaron, Goldberg, *Classification, evolution, and phylogeny of the families of Dicotyledons.* Smithsonian contributions to botany (USA). no. 58., 1986.

[27] Dahlgren, Rolf, *A system of classification of the angiosperms to be used to demonstrate the distribution of characters.* Bot. Noti. ser, 1975. 128: p. 119-47.

[28] Dahlgren, Gertrud, *The last Dahlgrenogram. System of classification of the dicotyledons*. 1989, The Davis and Hedge Festschrift: plant taxonomy, phytogeography and related subjects. Edinburgh, University Press.

[29] Rodman, J, P Soltis, D Soltis, K Sytsma and K Karol, *Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies.* American Journal of Botany, 1998. 85 (7): p. 997-997.

[30] Chase, Mark W and James L Reveal, *A phylogenetic classification of the land plants to accompany APG III.* Botanical Journal of the Linnean Society, 2009. 161 (2): p. 122-127.

[31] Allantospermum, Anarthriaceae, Aristolochiaceae Apodanthaceae, Borthwickia Boraginales, Capparaceae Buxaceae, Cornaceae Centrolepidaceae, Dasypogonaceae Cynomoriaceae, Dioscoreales Dilleniales, Euphorbiaceae Dipterocarpaceae, Francoaceae Forchhammeria and Haptanthaceae Gesneriaceae, *An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV.* Botanical journal of the Linnean Society, 2016. 181 (1): p. 1-20.

[32] Ellison, William Lee, Ralph E Alston and Billie Lee Turner, *Methods of presentation of crude biochemical data for systematic purposes, with particular reference to the genus Bahia (Compositae).* American Journal of Botany, 1962: p. 599-604.

[33] Hutchinson, John, *The Genera Of Flowering Plants (Angiospermae) Vol-2*. 1967.

[34] Bremer, Birgitta, Kåre Bremer, Mark Chase, Mike Fay, James Reveal, Douglas Soltis, Pamella Soltis and Peter Stevens, *An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III.* Botanical Journal of the Linnean Society, 2009.