International Journal of Science, Environment and Technology, Vol. 4, No 5, 2015, 1336 – 1343

ISSN 2278-3687 (O)
2277-663X (P)

PHENOLIC COMPOUNDS IN THE TAXONOMY OF SELECTED MEMBERS OF THE FAMILY ZYGOPHYLLACEAE WITH REFERENCE TO APG III CLASSIFICATION SYSTEM

El Bushra El Sheikh El Nur, Ikram Madani Ahmed* and Hind Ahmed Babeker
Department of Botany, Faculty of Science, Khartoum University, Khartoum, Sudan
E-mail: ikramahmed3@yahoo.com (*Corresponding Author*)

Abstract: Chemotaxonomic relationships between six members of the family Zygophyllaceae were studied on the basis of their phenolic compounds constituents. The selected species are: *Tribulus terrestris, Tribulus Pentandrus, Tribulus bimucronatus, Balanites aegyptiaca, Fagonia indica,* and *Tetraena alba*. Leaves, fruits, and stems were extracted in ethyl acetate and ethanol. Phenolic compounds were separated using thin layer chromatography technique (TLC) two eluent were used Hexane: Ethyl acetate (80:20) and Ethyl acetate: Formic Acid: Glacial acetic acid: Water (100:11:11:29) were selected as developing solvents. Polygonal graphs were constructed on the basis of the paired affinity indices for each species. The highest average paired affinity was observed between *Tribullus* spp.; which support the position of the *Tribulus spp.* within the *Tribullus* genus.

Balanites aegyptiaca shows, more or less, the same pattern of affinity towards *Fagonia indica* and *Tetraena alba*. Between species from different genera, highest affinity was observed between *Tribulus spp* and *Balanites aegyptiaca* ;thus supporting their recent affiliation to the same subfamily *Tribuloideae*. More over *Fagonia indica* and *Tetraena alba* with high paired affinity index place them together in the Zygophylloideae. On the other hand, lower affinity indices were recorded for *Tribulus spp*. and *Fagonia indica* and also *Tribulus spp.* and *Tetraena alba*; which suggest placing them into different subfamilies as evidenced by their chemical set up.

Keywords: TLC, phenolics, paired affinity, polygonal graphs, taxonomy, Zygophyllaceae.

Introduction: The Zygophyllaceae, is a family of trees, shrubs and herbs and are mostly restricted to arid and semi-arid areas in the tropics and subtropics (Beier, *et al*. 2003). The taxonomic position of the Zygophyllaceae in relation to other plant families has been subjected to many revisions. It was placed in five different orders at different times (Sheahan and Cutler, 1993). Soltis *et al*. (2000), APG (1998), Savolainen*et al*. (2000a) and Savolainen*et al*. (2000b) grouped the Zygophyllaceae and Krameriaceae together in their own order Zygophyllales within the eurosid group. This position has recently been changed in APG III (2009) to be within the fabids group. Balanites was added by Engler (1896) to the Zygophyllaceae as a separate subfamily as cited by Sheahan and Cutler (1993). Engler (1931) classified *Balanites* in the subfamily Balanitoideae, while Cronquist (1968) placed it in the

*Received Aug 30, 2015 * Published Oct 2, 2015 * www.ijset.net*

Simaroubaceae. In contrast, Hegnauer (1973) denied the chemical similarity between *Balanites* and Simaroubaceae, since the former lacks the quassia-like alkaloids typical of that family. However, most taxonomists had considered it as monogeneric Balanitaceae family (Hutchinson, 1973; Parameswarn and Conrad, 1982; Takhtajan, 1983).

Recently, molecular work by Sheahan and Chase (1996 and 2000) has led to new revision of this family which confirmed the inclusion of Augeoideae into Zygophyllaceae, while *Balanites sp* was suggested to be embedded within the tribuloid group; in spite of the many morphological and anatomical autopomorphies. The family now consists of approximately 285 species which belong to seven genera, subdivided into five subfamilies: Larreoideae, Morkillioideae, Seetzenioideae, Tribuloideae, and Zygophylloideae.

This study is part of a Ph.D. programme carried out in the Botany Department, Faculty of Science, University of Khartoum, to update and revise the taxonomy of the family Zygophyllaceae combining morphological and phytochemical characters.

Materials and Methods

Leaves, stems and fruits of the selected species, were air dried and ground into a coarse powder. A sample of 50 grams of each plant material was extracted successively using ethyl acetate and ethanol. The plant materials were soaked overnight in the solvents, then filtered and evaporated to dryness. Thin layer chromatography technique (TLC) was used to separate and to roughly identify the different phenolic constituents of the extracts. Hexane: Ethyl acetate (80:20) was used as a developing system for ethyl-acetate extracts while ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:29) was used for ethanolic extracts. Plates were examined under ultra-violet light, sprayed with freshly prepared Anisaldehyde-sulphuric acid reagent and were heated at 100 ˚C for 10 minutes. The retention factor (Rf) values for the detected spots were determined and the Paired affinity (PA) indices were calculated according to Ellison et al. (1962) as follows:

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

The results of the calculates were used for construction of polygonal graphs

Results and Discussion

Screening for phenolic compounds in different parts of the studied species indicated that many compounds of different Retention Factors. (Table 1 and Table 2) are present. 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 studied species. Table (3). According to Ellison et al. (1962), paired affinity index (PA) of 50% and above are considered as a marker of close relationship.

Table1: TLC separation of six ethanolic extracts (Rf. values) of sex members of the family zygophylaceae by ethyl acetate: formic acid: glacial acetic acid: water.

Plant species	Retention factors (Rf.)values of the phenolic compound separated from stem extract																
Tribulus terrestris	0.1		0.18				0.42	0.52				0.72				0.86	
T.Pentandrus	0.1		0.18				0.42	0.52				0.72				0.86	
T.bimucronatus	0.1		0.18		0.25		0.42	0.52		0.61						0.86	0.95
Fagonia indica	0.1	0.15		0.2			0.42	0.52	0.58			0.72	0.77				
Tetraena alba	0.1	0.15		0.2			0.42		0.58			0.72					
Balanites aegyptiaca	0.1		0.18		0.25		0.42					0.72	0.77				
Retention factors (Rf.)values of the phenolic compound separated from Leaves extract																	
Tribulus terrestris	0.1	0.18		0.35		0.45	0.55		0.65			0.72			0.8	0.86	
T.Pentandrus	0.1	0.18		0.35		0.45						0.72			0.8	0.86	
T.bimucronatus	0.1	0.18		0.35		0.45	0.55		0.65							0.86	0.95
Fagonia indica			0.2			0.45	0.55	0.63				0.72		0.76		0.86	
Tetraena alba			0.2		0.4	0.45	0.55					0.72				0.86	
Balanites aegyptiaca	0.1		0.2	0.35		0.45	0.55		0.65			0.72			0.8	0.86	
Retention factors (Rf.)values of the phenolic compound separated from fruit extract																	
Tribulus terrestris	0.1	0.14	0.2			0.37	0.44	0.52	0.59	0.62	0.69			0.78			
T.Pentandrus	0.1	0.14	0.2			0.37	0.44	0.52	0.59	0.62	0.69		0.74	0.78			
T.bimucronatus	0.1	0.14	0.2			0.37	0.44	0.52								0.86	0.95
Fagonia indica	0.1				0.33				0.59			0.71					
Tetraena alba	0.1			0.28					0.59			0.71					
Balanites aegyptiaca	0.1	0.14	0.2				0.44	0.52			0.69						

Table2: TLC separation of six ethanolic extracts (Rf. values) of sex members of the family zygophylaceae by hexane: ethyl acetate.

Plant species	Retention factors (Rf.)values of the phenolic compound separated from stem extract																		
Tribulus terrestris		0.1		0.15	0.22		0.29		0.39	0.43					0.64				
T.Pentandrus		0.1		0.15	0.22		0.29		0.39	0.43					0.64		0.77		
T.bimucronatus	0.04	0.1		0.15			0.29		0.39	0.43									
Fagonia indica		0.1			0.22		0.29	0.35	0.39	0.43	0.50				0.64		0.77		0.89
Tetraena alba		0.1					0.29			0.43			0.56		0.64		0.77		
Balanites aegyptiaca		0.1		0.15	0.22		0.29	0.35	0.39	0.43	0.50				0.64		0.77		0.89
Retention factors (Rf.)values of the phenolic compound separated from Leaves extract																			
Tribulus terrestris		0.1		0.15	0.22		0.29	0.35	0.39	0.43	0.50			0.58	0.64	0.69	0.77		0.89
T.Pentandrus		0.1		0.15	0.22		0.29	0.35	0.39	0.43					0.64	0.69	0.77		0.89
T.bimucronatus	0.04	0.1		0.15	0.22		0.29		0.39	0.43	0.50						0.77		
Fagonia indica	0.04	0.1	0.13	0.15	0.22		0.29	0.35	0.39	0.43				0.58	0.64	0.69	0.77		0.89
Tetraena alba		0.1		0.15			0.29			0.43									0.89
Balanites aegyptiaca	0.04	0.1		0.15	0.22		0.29	0.35	0.39	0.43	0.50			0.58			0.77		0.89
Retention factors (Rf.)values of the phenolic compound separated from fruits extract																			
Tribulus terrestris		0.1		0.15		0,26			0.39	0.43									
T.Pentandrus	0.04	0.1		0.15		0,26			0.39		0.50								
T.bimucronatus		0.1		0.15	0.22		0.29	0.35	0.39										0.89
Fagonia indica	0.04	0.1		0.15		0,26			0.39						0.64	0.69		0.81	
Tetraena alba	0.04	0.1		0.15		0,26		0.35		0.43					0.64	0.69	0.77	0.81	
Balanites aegyptiaca	0.04	0.1		0.15	0.22	0,26			0.39	0.43		0.54							

Table 3: Paired affinity averages between the studied members of the Zygophyllaceae

Species	Average of paired affinity index (PA %).
Between Tribulus terrestris, T.Pentandrus and T.bimucronatus	68.4%
Between Tribulus terrestris, T.Pentandrus, T.bimucronatus and Balanites aegyptiaca	59.7%
Between Tribulus spp and Fagonia indica	46%
Between Tribulus spp and Tetraena alba	36%
Between Fagonia indica and Tetraena alba	55%

In order to assess the taxonomic significance of the previously mentioned separated compounds in the systematics of the studied species with reference to the angiosperm phylogeny group (APG III, 2009), different taxonomic levels were investigated. Comparison of the paired affinity indices between the different genera indicated that some species are closely related.

The highest PA average value (68.4%), for all investigated plants, was detected among the three Tibullus sp. Table (3).

The geometrical shapes of the polygonal graphs of the studied taxa*, Balanites aegyptiaca* reflects the same pattern shown by the phenolic constituents of different analyzed parts extracted in the same solvent figure (1).

Figure (2) shows the polygonal representation of the phenolic constituents within the *Tribulus* species indicating that *T. terrestris* and *T. pentandrus* are more or less similar in their stem and fruit ethanolic extract contents and different from *T. bimucronatus*. The present findings add new evidence supporting the taxonomy of *Tribulus* spp. based on their morphological similarities.

A pplying the paired affinity index between different genera table (3) such as *Tribulus* spp. and *Balanites aegyptiaca*, indicates that two genera are closely related (59.7%). This finding supports their new affiliation, based on molecular similarities, reported by Sheahan and Chase (1996 and 2000) and hence inclusion of *Balanites sp.* into the subfamily Tribuloideae in spite of many morphological and anatomical autopomorphies. Few chemical investigations had been done supporting this relationship (Maksoud and AL-hadiddi, 1988; Narayana et al., 1990). Similarly the relatively high paired affinity index of 55 % between *Fagonia indica* and *Tetraena alba* Figure (3) is in agreement with what was published by Beier et al. (2003) who studied phylogenetic relationships and taxonomy of the subfamily Zygophylloideae which led to the currently recognised genera Augea , Tetraena and Fagonia as members of the subfamily Zygophilloideae. On the other hand the lower affinity indices recorded between *Tribulus* spp. and *Fagonia indica* (46.0%) and between *Tribulus spp.* and *Tetraena alba (36.0%)* might be considered as a chemical evidence for separating them into different subfamilies.

Figure1: Polygonal representation of *Balanites aegyptiaca* Based on phenolic constituents of ethanolic extracts:

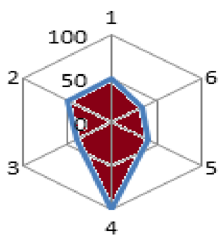
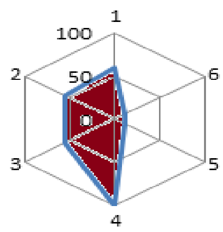
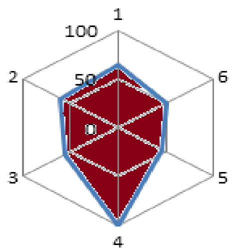
Stem phenolics	Fruits phenolics	Leaves phenolics
		

Figure2: Polygonal representation of the *Triublus spp*. Based on phenolic constituents in ethanolic extracts:

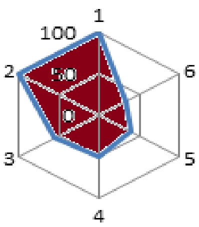
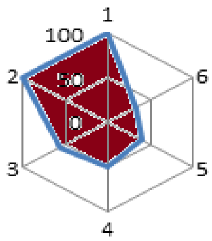
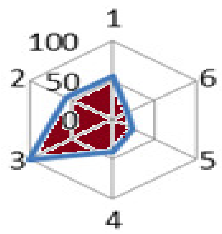
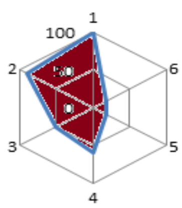
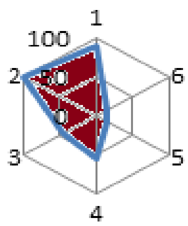
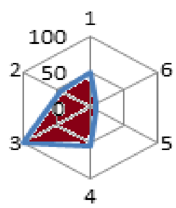
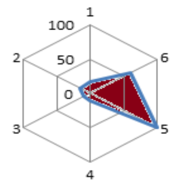
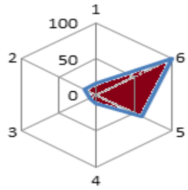
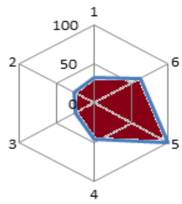
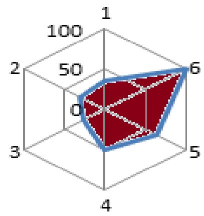
Stem phenolics	*Tribulus terrestris* 	*Tribulus pentandrus* 	*Tribulus bimucronatus* 
Fruits phenolics	*Tribulus terrestris* 	*Tribulus pentandrus* 	*Tribulus bimucronatus* 

Figure3: Polygonal representation of *Fagonia indica* and *Tetraena alba* Based on phenolic constituents of stem and leaves ethanolic extracts

Stem phenolics	*Fagonia indica* 	*Tetraena alba* 
Leaves phenolics	*Fagonia indica* 	*Tetraena alba* 

Similar results have been acheaved by other authors using chromatographic techniques \for different plant species. *Baptisia* (Alston and Turner, 1962,1963), *Collinsia* (Gaber and Strmnaoes, 1964) *Phlox* (Levins and Smith, 1965), *Viola* (Stebbins et al, 1963), *Tragopogon* (Brehm and Ownbey, 1965) and Cucurbits (Baitha and Pandey, 2003).

References

[1] Alston, R. E.and Turner, B. L. 1962. New techniques of complex natural hybridization. Proc. Natl. acad. Sci. U.S. 48: 130-137.

[2] Alston, R. E.and Turner, B. L. 1963. Natural hybridization among four species of Baptisia (Leguminosae). Amer. Jour. Bot. 50:159-173.

[3] APG III, Angiosperm Phylogeny Group (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. APG III. Botanical Journal of the Linnean Society161:105-121.

[4] APG, Angiosperm Phylogeny Group (1998). An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard. 85: 531–553.

[5] Baitha, N. and Pandey, V. (2003) Silica gel chromatographic study of phenolic compound in some cultivated Cucurbits. Him J Sci (2): 123-125.

[6] Beier, B. A., Chase, M.W. & Thulin, M. (2003). Phylogenetic relationships and taxonomy of subfamily Zygophylloideae (Zygophyllaceae) based on molecular and morphological data. Plant Systematic and Evolution 240: 11–39.

[7] Brehm, B. G. and Marion ownbey. 1965. Variation in chromatographic patterns in the Tragopogon dubius-pratensis porrifolius complex (compositae). Amer. Jour. Bot. 52:811-818.

[8] Cronquist, A. (1968). Evolution and classification of flowering plants London & Edinburgh: Thomas Nelson.

[9] Ellison, W.L, Alston, R.E. and Turner, B.L. 1962. Methods of presentation of crude biochemical data for systematic purposes with particular reference to the genus Bahia (Compositae). Am J Bot 49: 599-604.

[10] Engler A. (1931). Zygophyllaceae. In: Engler A, Prantl K, eds. Die naturliche Pjanrenfamilien, 2nd edition, Vol . 19a, Section 2. Leipzig: Engelmann.

[11] Engler, A. (1896). Zygophyllaceae. In: Engler A, Prantl K, eds. Die nat¨urliche Pfl anzenfamilien, 1st edition, vol. 3, Section 4: 74-93. Leipzig, Engelmann

[12] Gaber, E.D. and Stromnaes, O. 1964. The genus Collinsia XXXIII. A paper-chromatographic study of flower extracts from 14 species and 4 interspecific hybrids. (Scrophulariaceae). Bot. Gaz. 125:96-101.

[13] Hegnauer, R. (1973). Chemotaxonomie der Pjanzen, 6. Basel & Stuttgart.

[14] Hutchinson, J. (1973). The families of flowering plants. Oxford: Clarendon Press and taxonomy. IA WA Bulletin, n.s. 3: 75-88.

[15] Levin, D.A. and Smith, D.M.1965. An enigmatic Phlox from I11inois. Brittonia 17:17:254-266.

[16] Maksoud, S. A., El Hadidi M. N. (1988). The flavonoids of Balanites aegyptiaca from Egypt. Plant Systematics and Evolution 160: 153-158.

[17] Narayana L, Satyaaarayana P. and Radhakrishnaiah, H. (1990). Systematic position of Balanitaceae. In: Bilgrami K, Dogra J, eds. Phytochemistry and plant taxonomy. Delhi: C.B.S. Publications.

[18] Parameswaran, N. and Conrad, H. (1982). Wood and bark anatomy of Balanites aegyptiaca in relation to ecology and taxonomy. IAWA Bulletin, n.s. 3: 75-88.

[19] Savolainen V., Fay M. F., Albach D. C., Backlund A., Bank van der M., Cameron K. M., Johnson S. A., Lledo´ M.D., Pintaud J.-C., Powell M.,Sheahan M. C., Soltis D. E., Soltis P. S., WestonP., Whitten W. M., Wurdack K. J., ChaseM. W. (2000a) Phylogeny of the eudicots: a nearly complete familial analysis based on rbcL gene sequences. Kew Bull. 55: 257–309.

[20] Savolainen, V., Chase, M.W., Hoot, S.B., Morton, C.M., Soltis, D.E. and Bayer. C. (2000b) Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. Syst. Biol. 49: 306–362.

[21] Sheahan, M.C. and Cutler, D.F. (1993). Contribution of vegetative anatomy to the systematics of the Zygophyllaceae R. Br. Botanical Journal of the Linnean Society 113: 227–262.

[22] Sheahan, M. C. and M. W. Chase. (1996). A phylogenetic analysis of Zygophyllaceae R. Br. based on morphological, anatomical and rbcL DNA sequence data. Botanical Journal of the Linnean Society 122: 279–300.

[23] Sheahan, M.C. & Chase, M.W. (2000). Phylogenetic relationships within Zygophyllaceae based on DNA sequences of three plastid regions, with special emphasis on Zygophylloideae. Systematic Botany 25: 371-384.

[24] Soltis D.E., Soltis P.S., Chase M.W., Mort M.E., Albach D.C., Zanis M., Savolainen V., Hahn W.H., Hoot S.B., Fay M.F., Axtell M., Swensen S.M., Prince L. M., Kress W. J., Nixon K.C. and Farris J. S.(2000). Angiosperm phylogeny inferred from 18S rDNA, rbcL and atpB sequences. Bot. J. Linn. Soc. 133: 381–461.

[25] Stebbins, G.L., Harvey, B.L., Cox, E.L., Rutger, H.N., Jelemovic, G. and Yagil, E. 1963. Identificatin of the ancestry of an amphiploid Viola with the aid of paper chromatography. Amer.Jour. Bot., 50:830.

[26] Takhtajan, A. (1983). The systematic arrangement of dicotyledonous families. In: Metcalfe CR, Chalk L, eds. Anatomy of the dicotyledons, 2nd edition, Vol. 2. Oxford: Clarendon Press.