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Biochemical studies using PAGE technique and phytochemical screening by HPLC method among some taxa of Acanthaceae S.L. in Saudi Arabia

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ABSTRACT

Twenty-one species belonging to 13 genera of Acanthaceae obtained from Saudi Arabia were studied using electrophoretic analysis for protein patterns and two isoenzymes polymorphism in addition the phytochemical screening by TLC and HPLC. A total number of twenty-three protein bands observed between the species ranged 10 and 250 K Da, nine esterase and six peroxidase polymorphisms were detected. The biochemical data (protein profiles and isoenzymes polymorphisms) considered a significant tools for the relationships between the studied species. Furthermore, the phytochemical screening including the total contents of alkaloids, flavonoids, terpenoids and saponins were demonstrated the presence of alkaloids, flavonoids in all studied taxa with a variable contents and absence of total terpenoides and saponins in some species. As well, fourteen phenolic acids have been screened by high-performance liquid chromatography (HPLC) method. Gallic acid, phenol, protocatechuic acid, p-coumaric acid and o-coumaric acid in substantial amounts. Both the biochemical and the phytochemical data were conducted by means of the numerical analyses based on in total 38 electrophoretic characters and 18 phytochemical contents. On the basis of UPGMA clustering analysis, the two recognition distinct taxonomic groups and several clusters were distinguished. The existent results are useful for evaluating the relationships between the studied Acanthaceae species both at subfamilies and tribe levels.

Keywords: *Acanthaceae,* Protein profiles, Isoenzymes polymorphism, Phytochemical assay, Numerical analysis

INTRODUCTION

Acanthaceae Juss. ex Bercht and J. Presl are a relatively large family comprising of 3900 species related to 200-205 genera [1]. It is currently placed in order Lamiales close to the Bignoniaceae [2]. Bentham and Hooker [3] recognized Ruellieae, Justicieae and Acantheae tribes in the family, moreover [4] recognized these lineages but compined the first two as subfamily Ruellioideae. Acanthaceae represented in Saudi Arabia by 14 genera and 35 species, many of them are economically important for both traditional medicine and horticulture according to Al-Farhan *et al*. [5]. Phylogenetic variations and taxonomic relationships among the Acanthaceae were formally investigated using chloroplast DNA sequences by Lucinda *et al*. [6] and using iridoids and quaternary amines by Henrik *et al.* [7]. A conventional key and tabular version to the 36 taxa related to 21 genera of the Acanthaceae s.l. are provided by El-Gazzar *et al*. [8]. Seed protein and isozyme electrophoresis have been the most widely employed molecular genetic markers during the last quarter century [9,10]. Some taxa of this family are studied phytochemically by several authors, Daniel and Sabnis [11], Lucinda *et al.* [6], Wamtinga *et al*. [12] and Vijayalakshmi and Kripa [13]. The main objective of this study is to clarify the relationships among 21 taxa related to two Acanthaceae subfamilies through polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC) and to discuss whether the obtained studies can provide an additional fundamental tool which helps in the future the explanation of the taxonomic trends at specific and infra-specific level within the family.

MATERIALS AND METHODS

Twenty-one species that have been assigned to Table 1 were collected from the natural habitats in Jazan of Saudi Arabia and identified according to Chaudhary [14], Al-Farhan *et al*. [5] and Masrahi [15].The voucher specimens were deposited at the Jazan University Herbarium, KSA (JAZUH).

PAGE (polyacrylamide gel electrophoresis) analysis

Seed protein. 2.0 g of extracted seeds homogenized in 0.2 ml of tris-Hcl buffer containing 2% SDS and 2% β-mercaptoetahnole (pH 6.8) centrifuged at 6000 rpm for 10 minutes. 100 µl were injected onto gel (12% thickness) with bromophenol dyes as tracking dye, separating buffer containing Tris-gylycin buffer pH 8.3. After electrophoresis process; the gel stained with commassie brilliant blue-R250. Characterizations of seed protein were carried using one dimensional sodium dodecyle sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [16]. **isoenzymes analysis**. Extracted seeds were separated on PAGE and conducted as the method outlined Wendel and Weeden [17]. The gels (8 % thickness) were stained after the electrophoresis process; the staining solution used according to Graham *et al*. [18] and Jonathan and Wendel [19] as follows: In **esterase isoenzymes** (EC.3.1.1.1), gel stained with 1 ml of 1% α- naphthyl acetate dissolved in 60% acetone were added to 25 ml 0.1 phosphate buffer (pH 6.5). 20 mg of fast blue RR were added to 25 ml of the same buffer. In **peroxidase isoenzymes** (EC.1.11.1.7), gel soaked in o-dianisidine dissolved in 100 ml of 95% ethanol, 20 ml of acetate buffer (0.88 M sodium acetate, 0.65 glacial acetic acid, pH 4.7), 20 ml of distilled water and 5 ml of 3% hydrogen peroxide are added just before using. The gel incubated at room temperature, washed and filtered [8]. The electrophoretic bands are scanned by using Hoefer Scanning Densitometer GS 300.

Phytochemical studies

Total alkaloids, flavonoids, saponins and terpenoids were estimated as follows: 20 g of extracted leaves are carried out according to method of Ben-Hammouda *et al.* [20]. Alkaloids determined according to methods of Harborne [21]. Flavonoid contents excluded according to methods of Boham and Kocipai [22]. Saponin carried out according to Obadoni and Ochuko methods [23]. Terpenoid contents estimated according to Olayiwola [24]. Dried flavonoids extracts dissolved in chloroform: methanol (95:5) and subjected to thin layer chromatography (TLC) which performed on silica gel plates (DC-Alufolien 60 F254). Different spots appeared on silica gel plates under UV before and after sprayed with aluminum chloride (AlCl3). Extraction and identification of phenolic acids by HPLC according to methods of Amarowicz *et al*. [25]. Developing solvent systems carried out according to Nicola [26]. Dried flavonoids dissolved in ethyl acetate: methanol: water (30: 5: 4). Identification of phenolic compounds was performed on a Hewlett-Packard HPLC (Model 11 00), using a hypersil C18 reversed- phase

Table 1. The distribution of the studied species and their distribution in the family according to Hansen [27].

No.	Taxa	Sub family	Tribe	Place
01	*Anisotes triculcus (Forssk.) Nees*	Acanthoideae	Acantheae	Wadi Razan
02	*Blepharis maderaspatensis (L.) Hayne*			Jabal Abadil
03	*Blepharis ciliaris (L.) B.L. Burtt*			Abu Arish
04	*Crossandra wissmanii Schwartz*			Jabal Fayfa
05	*Lepidogathis scariosa Nees*			Wadi Al Abadil
06	*Justicia flava (Vahl) Vahl*	Ruellioideae	Justicieae	Wadi Abadil
07	*Justicia heterocarpa T. Anders*			Jabal Fayfa
08	*Justicia caerulea Forssk*			Wadi Abadil
09	*Monechma debile (Forssk.) Nees*			Jabal Abadil
10	*Ecbolium gymnostachyum (Nees) Milne-Redh*			Jazan
11	*Ecbolium viride (Forssk.) Alston*			Al- Ardha
12	*Asystasia gangetica (L.) Anders*		Ruellieae	Al -Aridah
13	*Barleria hochstetteri Nees in DC*			Jazan
14	*Barleria trispinosa (Forssk.) Vahl*			Jabal Abadil
15	*Barleria bispinosa (Forssk.) Vahl*			Jabal Abadil
16	*Hypoestes forskalei (Vahl) Soland.*			Baysh
17	*Peristrophe cernua Nees*			Jabal Abadil
18	*Peristrophe paniculata (Forssk.) Bru*			Ad Darb
19	*Phaulopsis imbricata (Forssk.) Sweet*			Bani Malik
20	*Ruellia patula Jacq*			Jabal Fayfa
21	*Ruellia grandiflora (Forssk.) Blatt*			JAZUH

(2.5 x 4.5 mm) Injection by means 5µm of a Rheodynesizevalve (Model - 7125) with 50 fixed loop was used, the chromatograms of the phenolic compounds were observed and analyzed.

Data analysis

Different protein profiles, isoenzyme polymorphisms by PAGE technique and the phytochemical analysis were scored as either presence (1) or absence (0) (Table 2 and Table 5). Reproducible and clear bands were scored for the construction of the data matrix. The data matrix thus prepared was the input file for using the NTsys-Pc program [28]. The similarity matrices were used for the construction of dendrograms with unweighted pair-group method on arithmetic averages (UPGMA).

RESULTS and DISCUSSION

All the studied taxa were investigated using electrophoretic process by means of PAGE techniques and the phytochemical screening including a total content of alkaloids, flavonoids, terpenoids and saponins were carried out using TLC and HPLC. The examined species listed in Table 1 with family classification of the by Hansen [27].

Protein profiles

Figure 1 illustrated the total number of 23 protein bands ranged between 10 K Da and 250 K Da. A crowded protein bands noticed at 10 and 37 k Da reion. Two bands around 37 kDa and 10 kDa observed among all studied species (monomorphic). 13 protein bands found in *A. triculcus*. species a total 15 protein profiles were detected In *Blepharis,* both two species shared in 12 bands. In *C. wissmanii,* 13 protein profiles are observed. Also, 15 protein bands are present in *L. scariosa.* Three species of *Justicia* have 17 bands, the lowest number of 13 bands found in *J. flava* and *J. heterocarpa,* the highest one (14 bands) present in *J. caerulea*; three *Justicia* taxa shared in 9 bands. As well, 13 bands are detected In *M. debile*. In *Ecbolium* species 15 protein profiles bands were observed, *E. gymnostachyum* has 12 bands while *E. viride* has 13 bands. Also, In *A. gangetica* 12 bands of protein profiles. Likewise, in the three *Barleria* taxa 14-15 bands of protein profiles have been detected, the highest number of 15 bands found in *B. trispinosa* whereas the lowest one of 14 bands offered in *B. hochstetteri* and *B. bispinosa. H. forskallei* has 14 bands. Likewise, 13 bands found In *Peristrophe* taxa. Both species shared in 11 bands. In the same sense,12 band found in *P. imbricata.* 11-12 protein profiles present in the two *Reullia* species, 12 bands noticed in *R. patula* and 11 bands detected in *R. grandiflora.*

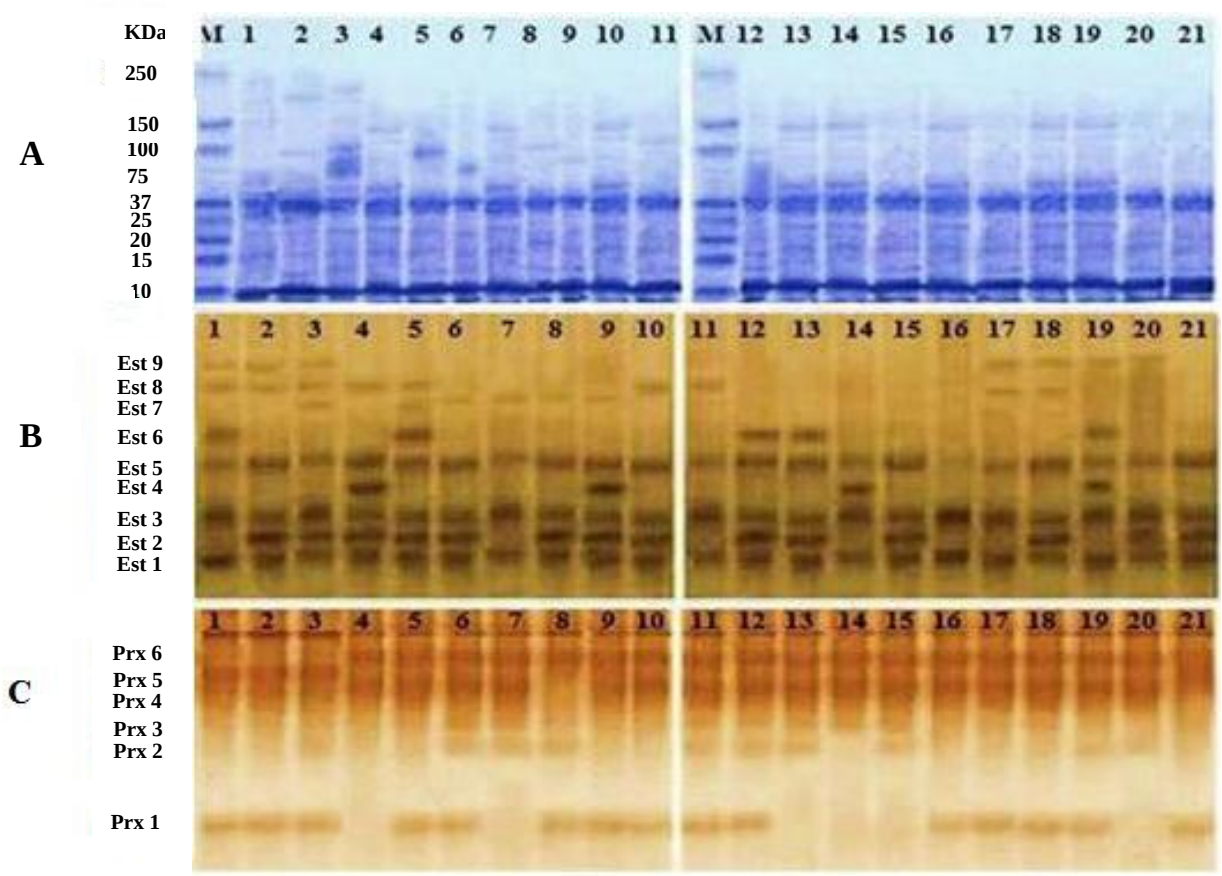

Figure 1: Electrophoretic gels of (a) protein profiles and (b) Esterases bands (c) peroxidase bands of the studied species as numbered in Table 1. M: Protein marker; Est: Esterase; Prx: Peroxidase.

Isoenzyme polymorphisms

Table 2 showed nine esterase and six peroxidase profiles, two esterase profiles of Est 1 and Est 3 found in all the studied taxa. It is clear that, six esterases and three peroxidase bands found in *A. triculcus,* as well, a total seven esterases and two peroxidase isoenzymes were displayed in two *Blepharis* species. Likewise, *C. wissmanii* and *L. scariosa* have eight esterase and four peroxidase profiles. Also, three taxa of *Justicia* have five esterase and six peroxidase groups, the highest number of Est bands found in *J. flava* and *J. caerulea,* the lowest one estimated in *J. hetercarpa;* their three species shared in a total four esterase and two peroxidase isoenzyme bands. *M. debile* has six esterase and three peroxidase profiles. In two *Ecbolium* species, a total five esterase and six peroxidase polymorphisms present in *E. gymnostachyum* and ten isoenzymes bands showed in *E. viride*, both two species shared seven bands (Table 2).

Table 2. Presence (1) and absence (0) of 38 electrophoretic profiles between the taxa (as numbered list in Table 1) used in the numerical analysis.

No.	Species																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Seed Protein profiles																				
01	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
02	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
03	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	0	1	1
04	1	1	1	1	1	0	1	1	1	1	0	0	1	0	0	1	0	0	1	0	0
05	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
06	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
07	1	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0
08	0	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0
09	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
10	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0
11	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	0	0
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0
14	1	1	1	0	1	0	0	0	0	1	0	1	0	1	0	1	0	0	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
18	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
20	0	1	1	1	1	1	0	1	1	0	0	1	1	1	0	1	1	1	1	1	1
21	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
22	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
23	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Pro. bands	**13**	**14**	**13**	**13**	**15**	**13**	**13**	**14**	**13**	**12**	**13**	**12**	**14**	**15**	**14**	**14**	**12**	**12**	**12**	**12**	**11**
	Esterase isoenzymes																				
01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
02	0	1	1	1	1	1	0	1	1	1	0	1	1	0	1	0	0	1	0	1	1
03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
04	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0
05	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1
06	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0
07	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0
08	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
09	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
Total Est bands	**6**	**6**	**7**	**6**	**7**	**5**	**4**	**5**	**6**	**5**	**4**	**5**	**5**	**3**	**4**	**2**	**5**	**6**	**5**	**4**	**4**
	Peroxidase isoenzymes																				
01	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	0	1
02	0	0	0	0	0	1	1	1	0	0	1	1	1	0	1	0	0	0	0	1	0
03	0	0	0	0	0	1	0	0	0	0	1	1	1	1	1	0	0	0	0	1	0
04	1	0	0	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1
05	1	1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
06	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total Prx bands	**3**	**2**	**2**	**2**	**3**	**5**	**3**	**3**	**3**	**3**	**6**	**5**	**4**	**3**	**4**	**3**	**3**	**3**	**3**	**4**	**3**

In *A. gangetica,* five esterases and five peroxidases were estimated. In the three *Barleria* species a total six esterase profiles and four peroxidase were exhibited, also three *Barleria* species shared in Est 1, Est 3, Prx 3, Prx 4 and Prx 6. *H. forskalei,* two esterase and three peroxidase have been detected. Six esterase and three peroxidase found in two species of *Peristrophe* and shred in fives esterases and three peroxidases profiles.

Likewise six esterase and three peroxidase bands were estimated in *Phaulopsis*. *Reullia* species have a four esterase profiles.

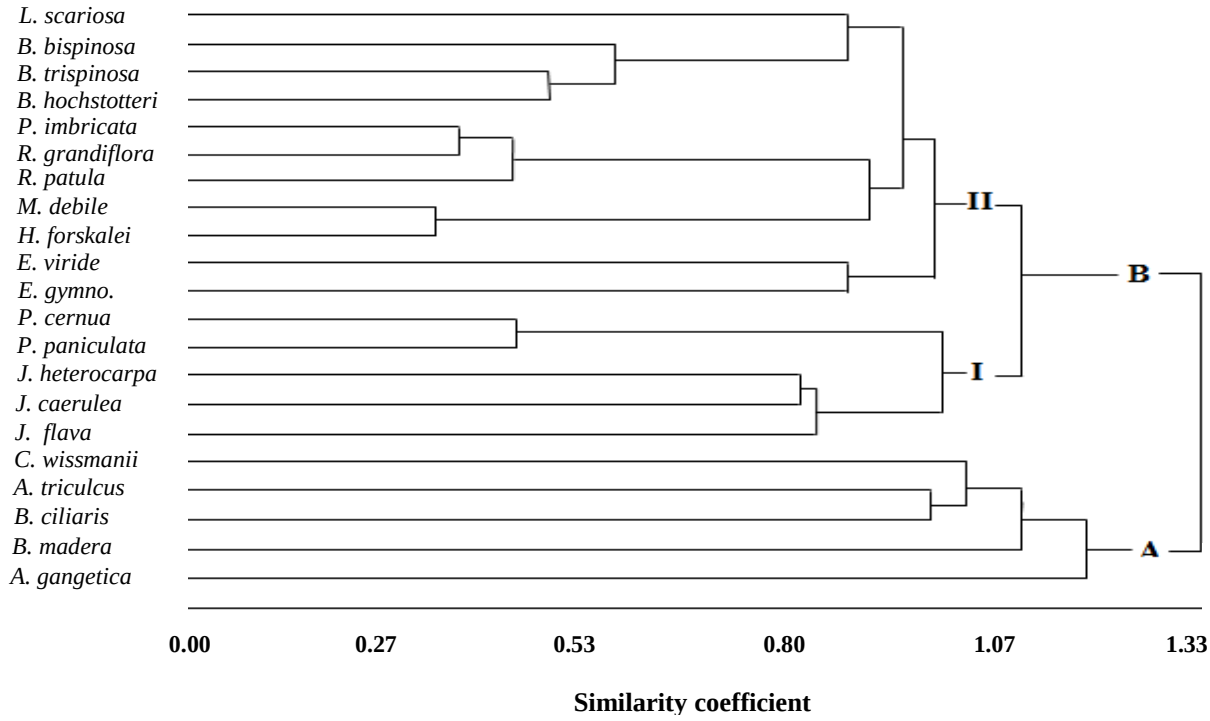

Fig. 2. Dendrogram based on 38 attributes of seed protein and isoenzymes patterns between the studied species.

The current dendrogram (concerning the electrophoretic data) exhibited two main groups delimited at the taxonomic level of 1.33. The upper (group A) contains the studied taxa of *A. gangetica, B. maderaspatensis, B. ciliaris, A. triculcus* and *C. wissmanii* whereas the lower one (group B) has two subgroups (sub level), the first inclusive the studied taxa of *Justicia* linked with the two species of *Peristrophe.* The second one included the two species of *Ecbolium, Barleria* taxa in addition the two species of *Reullia* linked with *P. imbricata* (Figure 2).

Phytochemical Analysis

Thin layer chromatography (TLC) technique revealed a large number of flavonoid spots spraying by aluminum chloride (ALCL3) solution appeared in *L. scariosa* while the lowest one appeared in *E. gymnostachyum*, *E. viride A. gangetica* and *P. paniculata* (Table 3, Figure 3). TLC plate analysis gave a positive reaction for the flavonoid compounds and gave purple, yellow to brown colors between the species because the aluminum chloride (AlCl3) served as a developer to make visible the secondary metabolite on the TLC plate. Totals flavonoids and alkaloids were detected in all the studied taxa with a variable contents whereas totals terpenoids and saponins are absent in the minor taxa, the highest value of alkaloids found in three species of *Justicia* also the high flavonoids contents have been estimated in three species of *M. debile, B. trispinosa* and *P. cernua*. From Table 3, no saponins are observed in *C. wissmanii* and two *Peristrophe* species. Also, no terpenoids was detected in *A. triculcus* and *P. paniculata.* Most the studied species contain a variable values of flavonoids, the highest flavonoid content of 2.085 mg/g and 2.292 mg/g were noticed in *M. debile* and *B. trispinosa,* respectively while the lowest one was estimated in *J. heterocarpa, J. caerulea and B. bispinosa*. 14 phenolic contents assayed by HPLC in which three unknown compounds were appeared (Table 4). Meanwhile the highest content of alkaloids was found in three taxa of *Justicia*. Phenolic acids assayed showed a five phenolic compounds are found In *A. triculcus,* synapic acid, phenol, *p*-coumaric, o-coumaric and unknown compounds. In two *Blepharis* species, most analyzed compound were presented such as gallic acid, phenol, protocatechuic acid, o-coumaric acid and two unknown contents. *C. wissmani* has ferulic acid, gallic acid, phenol and two unknown phenolic contents. In *L. scariosa* the majority of phenolic contents were presented except sinapic acid, protocatechuic acid, p-hydroxy benzoic acid and unknown one. As well, *Justicia* species have the most studied phenolic acids, their taxa shared in presence of gallic acid, phenol compounds, p - coumaric acid and o - coumaric acid. Also, five phenolic acids (Phenol, protocatechuic acid, phloretic acid, o - coumaric acid and *p*-coumaric acid) determined in *M. debile*.

Table 3: Flow rates (RF) and total contents of alkaloids, saponins and terpenoids (mg/g d. wt) in the studied taxa as numbered list in Table 1.

No.	Species	Flavonoids		Alkaloids	Saponins	Terpenoids
		RF spots	Contents			
01	*Anisotes triculcus*	3	0.890	0.432	0.340	----
02	*Blepharis maderaspatensis*	3	0.403	0.306	0.324	0.567
03	*Blepharis ciliaris*	3	0.500	0.072	0.435	0.730
04	*Crossandra wissmanii*	3	0.203	0.430	---	0.543
05	*Lepidogathis scariosa*	4	0.762	0.053	0.043	0.880
06	*Justicia flava*	3	0.464	1.047	0. 055	0.046
07	*Justicia heterocarpa*	3	0. 021	1. 032	0. 590	0. 0.32
08	*Justicia caerulea*	3	0. 055	1. 054	0.032	0. 080
09	*Monechma debile*	3	2.085	0.032	0. 008	0.821
10	*Ecbolium gymnostachyum*	2	0. 060	0. 018	0.022	0. 356
11	*Ecbolium viride*	2	0.203	0.045	0. 054	0.721
12	*Asystasia gangetica*	2	0. 654	0. 050	0.0091	0. 050
13	*Barleria hochstetteri*	3	0.301	0.054	0.058	0. 540
14	*Barleria trispinosa*	3	2.292	0.023	0. 036	0.023
15	*Barleria bispinosa*	3	0. 020	0. 03	0.531	0. 039
16	*HypoEstes forskalei*	3	0. 904	0.870	0.653	1.875
17	*Peristrophe cernua*	3	1.342	0.530	---	0.340
18	*Peristrophe paniculata*	2	0. 711	0.042	----	---
19	*Phaulopsis imbricata*	3	0.890	0.521	0.800	0.324
20	*Ruellia patula*	3	0.403	0.620	0.9 70	0.651
21	*Ruellia grandiflora*	3	0.504	0.416	0.730	0.871

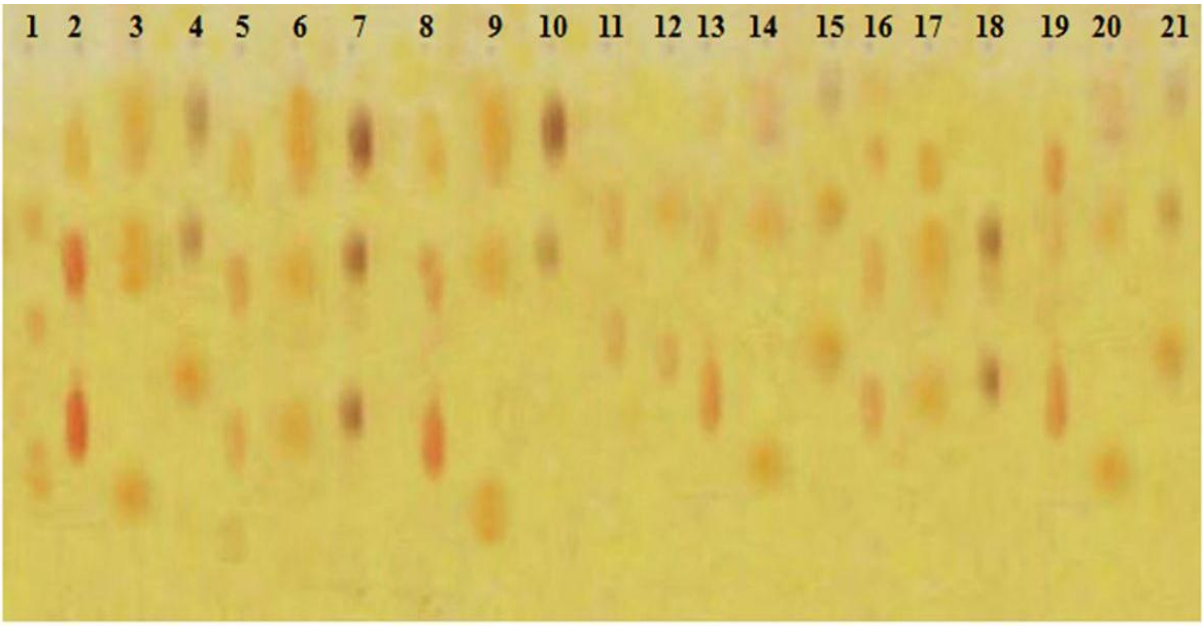

Fig. 3: TLC of methanolic extract sprayed by aluminum chloride (AlCl3) detected by UV light; 1: *A. triculcus,* 2: *B. maderaspatensis,* 3: *B. ciliaris,* 4: *C. wissmanii*, 5: *L. scariosa*. 6: *J. flava*, 7: *J. heterocarpa*, 8: *J. caerulea*, 9: *M. debile,* 10: *E. gymnostachyum,* 11: *E. viride ,* 12: *A. gangetica,* 13: *B. hochstetteri,* 14: *B. trispinosa,* 15: *B. bispinosa,* 16: *H. forskalei,* 17: *P. cernua ,* 18: *P. paniculata,* 19: *P. imbricata,* 20: *R. patula* and 21: R. *grandiflora.*

Table 4: Relative percent of the 14 phenolic acids (µg/mg d.wt) using HPLC in the studied species as numbered list in Table 1.

Species No.	Phenolic acids													
	ferulic acid	sinapic acid	gallic acid	phenol	vanillic	protocatechuic	Phloretic	*p*- OH- benzoic	*p*-coumaric	o-coumaric	chlorogenic	Unknown	Unknown	Unknown
01	--	0.72	--	0.29	--	--	---	--	0.23	0. 02	--	--	--	0.71
02	--	0.34	,32	0.39	--	0.86	--	--	--	0. 05	--	--	0.99	0.64
03	--	--	0.36	0.89	--	0.70	--	0.23	--	3.08	--	--	0.71	0.91
04	0.14	--	0.81	0.63	--	--	--	--	--	--	--	--	0.44	0.63
05	0.21	--	0.66	0.79	0.76	--	0.33	--	0.32	0.22	0.06	0.11	--	0.82
06	--	--	0.86	0.87	--	0.66	--	--	0.87	0. 33	--	--	--	--
07	--	--	0.75	0.43	--	--	--	--	0.94	0.54	--	0,87	--	--
08	--	--	0.52	0.22	--	--	0.33	--	0.86	0.55	--	--	--	--
09	--	--	--	0.54	--	0.034	0.21	--	0.43	0.44	--	--	--	--
10	---	0.70	--	0.41	--	--	--	0.89	--	--	0.08	--	--	0.77
11	--	0.54	--	0.51	--	--	--	1.00	0.65	--	--	--	--	0.59
12	0.23	--	1.17	0.42	--	--	--	--	--	--	1.09	--	--	--
13	--	--	--	0.85	--	--	0,76		--	--	--	0.26	--	--
14	--	--	--	1.17	--	--	0,85	--	--	--	--	0.67	--	--
15	--	--	--	0.91	--	0.28	--	--	--	--	--	0.43	0.83	--
16	0.34	--	--	0.61	0.64	0.98	--	0.62	0.28	0.79	0.07	--	0.74	--
17	--	--	--	0.72	--	--	0.76	0.75	0.73	0.59	--	--	--	0.89
18	--	--	0.68	0.62	--	--	0.65	--	0.88	1.12	--	0.093	--	--
19	--	--	--	0.62	0.94	--	--	--	0. 53	0.34	--	--	--	--
20	0.96	0.82	--	0.83	--	0. 88	0.77	--	0.24	0.05	--	0.063	--	--
21	0.43	0.43	--	0.94	--	0.20	--	0.91	--	--	--	--	--	--

Likewise, the *E. gymnostachyum* has a synapic acid, phenol content, p-hydroxy benzoic acid*,* chlorogenic acid and unknown one. *E. viride* has a synapic acid, phenol, p-hydroxy*benzoic acid, p*- coumaric acid and unknown one. Both two *Ecbolium* species shared in four contents, sinapic acid, phenol, p-hydroxy benzoic acid and unknown one. As well, *A. gangetica* has four phenolic acids, ferulic acid, gallic acid, phenol and chlorogenic acid. It is clear that, three *Barleria* species have a different phenolic amounts and shared in phenol and unknown one. Also, *B. hochstetteri* and *B. trispinosa* shared in phenol, phloretic acid and unknown compound while protocatechuic acid and unknown one estimated only in *B. bispinosa.* It is clear that, *H. forskalei* has a most phenolic acids such as ferulic acid, phenol, protocatechuic acid, vanillic acid, P - hydroxy benzoic acid, p-coumaric acid, o- coumaric acid, chlorogenic acid and unknown one. In *Peristrophe* species*,* four phenolic acids

of Phenol, phloretic acid, *p*- coumaric acid and *o*- coumaric acid were determined. Also phenol compound, vanillic acid, *p*- coumaric acid and o- coumaric acid were restricted In *Phaulopsis imbricata.*

Table 5. Presence (1) and absence (0) of 18 phytochemical characters between the taxa (as numbered list in Table 1) used in the numerical analysis.

Phenolic **Contents**	**Species**																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Alkaloids	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
flavonoids	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
terpenoids	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
Saponins	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
ferulic	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1
sinapic	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1
gallic	0	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0
Phenol	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
vanillic	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Protoca.	0	1	1	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	1
Phloretic	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	0	1	1	0	1	0
p-hydroxy benzoic	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1
p-coumaric	1	0	0	0	1	1	1	1	1	0	1	0	0	0	0	1	1	1	1	1	0
o-coumaric	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0
chlorogenic	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0
Unknown	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0
Unknown	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Unknown	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0

Table 4 illustrated that, ferulic acid, sinapic acid, phenol, protocatechuic acid, phloretic acid, *p*- coumaric acid, o-coumaric acid and unknown compound have been determined in *R. patula*. In *R. grandiflora,* ferulic acid, sinapic acid, phenol, protocatechuic acid and p-hydroxy benzoic acid have been detected. Both two species shared in four phenolic acids contents (ferulic acid, sinapic acid, phenol, protocatechuic acid). Furthermore, the studied species of *A. triculcus, B. maderaspatensis, B. ciliaris,* and *C. wissmanii* were related to the three species of *Justicia.* The current phytochemical studies confirmed that, gallic acid and p-hydroxy benzoic acid in addition to the unknown compounds were more common in Acantheae and Justicieae tribes of sub family Acanthoideae than subfamily Relluoideae tribes. As well, *p*-coumaric acid was common in the most studied species of tribe Ruellieae of subfamily Relluoideae.

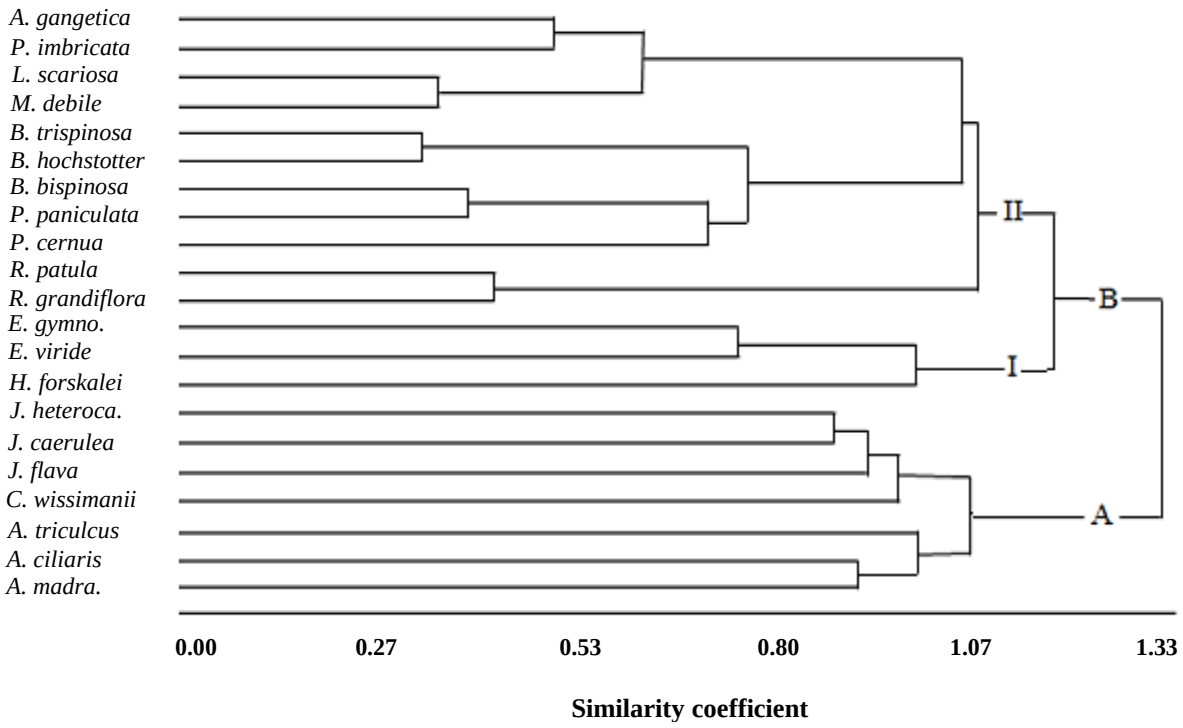

Fig. 4. The dendrogram based on 18 attributes of phytochemical screening between the studied taxa.

Javid *et al*.[29] and Iqbal *et al*. [30] decided that, a storage proteins and isoenzymes are not affected by the environmental fluctuations, PAGE technology especially SDS-PAGE is particularly considered as a reliable tool for economic characterization of germplasm. From the present data, two protein bands around 37 kDa and 10 kDa and two esterase profiles namely Est 1 and Est 3 noticed among all taxa i.e. monomorphic whereas the remainders considered polymorphic because of they are present in some species and absent in the others. A total of thirty-eight electrophoretic characters (protein and two isoenzymes of esterases and peroxidases) are used in the statiscal program of the numerical analysis in addition the eighteen phytochemical contents. The electrophoretic data considered a good support for the inclusion of the modern classification of four major lineages within the studied species of Acanthaceae as suggested formally by Hansen [27] and Scotland *et al.* [31]. The phenogram of Figure 2 which regards to electrophoretic protein and isoenzymes polymorphisms exhibited a distinctive two main clusters. The upper cluster (group A) inclusive the studied taxa of subfamily Acanthoideae meanwhile the lower one (group B) included most studied taxa of subfamily Reulloideae, as well three *Barleria* species clustered in same group with *Lepidagathis,* this inclusion in accordance with modern results of Lucinda and Michael [6]. Furthermore, the phenolic acids data considered intermediate between the studied species of the subfamily Acanthoideae and the subfamily Rulleoideae. Presence of flavonoids, saponins and different phenolic acids in some taxa of the family which in accordance with Vijayalakshmi and Kripa [13]. It is obvious that, the resulted Phonogram of Figure 4 (with regard to phenolic contents) exhibited two main groups delimited at taxonomic level of 1.33, the first group (A) included the studied taxa of sub-family Acanthoideae linked with three species of *Justicia* whereas the second one (group B) included two subgroups, the first subgroup had the three species of genus *Ecbolium* meanwhile the second subgroup in which three sublevels have been recognized; first sublevel comprised the two *Reullia* species have been separated in alone cluster at taxonomic level of 0.45. The second sub level comprising of the two species of *Peristrophe* and the three *Barleria* species at taxonomic level of 0.89; the last of sublevel in the subgroup B inclusive the studied taxa *of M. debile, L. scariosa, P. imbricata* and *A. gangetica.* Analysis of the fourteen phenolic acids which have been estimated by HPLC technique revealed that, phenol were estimated in all the studied species, furthermore, o-coumaric acid and p-coumaric acid offered in more than 70 % of the examined species. Also, p-hydroxy benzoic acid and gallic acid have been estimated in about 50 %. Both sinapic acid and vanillic acid represented in less than 25 % of the species, such conclusion fairly agreed with Daniel and Sabnis [11].

Conclusion

Twenty-one species related to family Acanthaceae were collected from the natural habitats in Jazan of Saudi Arabia, the modern classification of the family included two sub families Acanthoideae and Ruellioideae. The present work carried out using electrophoretic analysis for protein patterns by Sodium dodecyl sulphate- Poly acrylamide gel electrophoresis (SDS-PAGE technique) and two isoenzymes polymorphism using Poly acrylamide gel electrophoresis (PAGE technique) in addition the current studies including the phytochemical screening using TLC (thin layer chromatography) and HPLC (high-performance liquid chromatography) techniques. 38 attributes of seed protein and isoenzyme patterns (nine esterase and six peroxidase profiles) and 18 phytochemical characters including total of alkaloids, flavonoids, terpenoids and saponins and 14 phenolic acids have been analyzed between the species. A total results for biochemical and phytochemical data were subjected to the numerical analysis program. The electrophoretic data gives a .positive results correspond to the modern classification of four major lineages within the family which in turn agreed with the modern classification. Furthermore, the phenolic acids considered to be intermediate between the two subfamilies and needs further, updated studies to confirm the relationships between the Acanthaceae taxa.

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