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CHEMOTAXONOMIC STUDY OF THREE *ARTEMISIA* SPECIES GROWING IN SINAI, EGYPT.

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By

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ABSTRACT

A comparative chemotaxonomic study of three *Artemisia* species, *A. monosperma*, *A. judaica*, *A. herba alba* is presented. Eight forms of *A. monosperma*, growing in Wadi El- Arish (North Sinai) and the other two species growing in Wadi El-Shiekh and Wadi El-Talaa (Saint Catherine, South Sinai) respectively, were collected in the same growth season to eliminate the effect of ecological factors.

The chemical study comprised the preliminary phytochemical screening, investigation of total, water-soluble and acid-insoluble ash; carbohydrates; total nitrogen and amino acids; lipids; fatty acids and flavonoids. The results revealed that the eight forms of *A. monosperma* were greatly similar in their chemical composition. On the other hand, they differed qualitatively and quantitatively from the other two species, viz. *A. herba alba* and *A. judaica*. Therefore, the phytochemical results fully justified the systematic treatment.

INTRODUCTION

The genus *Artemisia* is of common use in folk medicine and in pharmaceutical preparations (Boulos, 1983), and several compounds were isolated from its tissues. Most of these compounds are of medicinal interest. In this regard Fahmy *et al.* (1960) isolated four crystaline compounds from powdered leaves and the flowering tops of *A. monosperma* . Maksudov *et al.* (1962) determined the essential oils, organic acids, tannins, sugars, ash and tars in blooms of *A. scoparia*

Many authors identified and isolated a great number of flavone compounds from different *Artemisia* species : Rodrigues *et al.* (1972) from seven *Artemisia* taxa ; Segal *et al.* (1973) from *A. herba alba* ; Khafagy *et al.* (1979) from *A. monosperma*; Ghazouly *et al.* (1984) and Bacha (1984) from *A. judaica* and Saleh *et al.* (1985) from *A. monosperma* and *A. herba alba* . Sayed *et al.* (1979) studied the fatty acids of *A. absinthium* , while Laivant and Proskurnikova (1965) studied the amino acids of the protiens of *A. rhodantha* , qualitatively ,quantitatively, and their seasonal fluctuations during the developmental phases. Garrone *et al.* (1973) examined the level of free amino acids in *A. vulgaris* and *A. verlotorm*. Also, Khamdamov and Chamsrkov (1976) studied qualitatively the amino acids in *A. diffusa* , *A. halophila* and *A. turanica* . Hammouda *et al.* (1978) isolated an acetophenone derivative and coumarins from *A. monosperma*.

Alekseeva (1962) studied the metabolism of carbohydrates in *A. turanica* in various soils under desert conditions. Maki (1968) isolated hemicellulose from leaves of *A. capillaris* . On the other hand, Garrone *et al.* (1973) examined the levels of soluble carbohydrates in *A. vulgaris* and *A. verlotorum* .

A critical taxonomical revision was realized by Gazara (1987) for *Artemisia* species growing in Sinai and known earlier by Tackholm (1974). In this revision , it was possible to distinguish between different *Artemisia* species according to vegetative, head as well as floral characters. The following key was made by Gazara (1987).

1.a Heads homogamous, oblong, tapering at base.....*A. herba alba*

1.b Heads heterogenous, hemispherical to oblong-ovate not tapering at base 2

2.a Involucral bracts hairy , bisexual flowers fertile, 15-29 per head ..... *A. judaica*

2.b Involucral bracts glabrous, bisexual flowers sterile, 3-9 per head 3

3 Heads ovate, 4 mm long, female flowers 2-6 per head .....*A. monosperma*

In the present study a chemotaxonomical investigation was carried out to compare the three *Artemisia* species. namely *A. monosperma*, *A. judaica* and *A. herba alba*, and between the eight different forms of the first species.

MATERIALS AND METHODS

The material used in the present investigation was obtained from *A. judaica*, *A. herba alba* and *A. monosperma* growing naturally in Sinai. The two first species were collected from South Sinai (Saint Catherine area) . *A. judaica* was collected from Wadi El-Shiekh and *A. herba alba* from Wadi El-Talaa . The eight different forms of *A. monosperma* (A1-A8) were collected from Wadi El-Arish, North Sinai. The plant samples of the three species were collected at the same growth season. The shoots were manually cleaned, dried in an oven at 50C and reduced to fine powder.

The preliminary phytochemical screening was carried out on the powdered dried shoots of the three different species of *Artemisia*. This included testing for volatile oils and tannins (Balbaa *et al.* 1981), unsaturated sterols (Brieskorn *et al.* 1961) , flavonoids (Wall *et al.* 1954 and Balbaa *et al.* 1981), glycosides and\or carbohydrates (Vogel

1978), and saponins (Wall *et al.* 1954 and Abd El Maksoud 1983).

The total ash, water-soluble ash as well as acid-insoluble ash were determined according to E. P. (1984) methodology, using two grams of the powdered air-dried shoots of the eight different of *A. monosperma* (A1-A8), as well as *A. judaica* and *A. herba alba.*

The total carbohydrates were determined according to Karawya and Afifi (1979) method, and the sugar content was expressed as gram dextrose per 100 gram dry weight. The qualitative investigation of the free and combined sugars was realized according to Karawya and Afifi (1979) methods of analysis.

Allen *et al.* (1974) methodology was followed for the investigation of nitrogen, amino acids and lipid contents. Flavonoids were investigated according to Bacha (1984) methodology. Finally, fatty acids were studied using gas-liquid chromatography according to Karawya *et al.* (1979) method. The analysis was done by GCV chromatograph using the following conditions:

-Column	10% PEGA
-Column temp.	70 ° C (initial temp.)
-Rate	8 ° C/min.
-Final temp.	190 ° C
-Final time	20 min.
-Chart speed	2 min./cm.
N_2	30 ml/min.
H_2	33 ml/ min.
Air	330 ml/ min.

RESULTS AND DISCUSSION

Investigation of Plant Constituents:

The principal chemical constituents were studied in order to compare between the eight forms of *A. monosperma* on one hand and between the three *Artemisia* species on the other hand. It can be concluded from Table (1) that all the three species of *Artemisia* contained volatile oils, carbohydrates and/or glycosides, flavonoids, sterols and saponins. Negative results were obtained for tannins and alkaloids in all of them.

Results presented in Table (2), revealed that the percentage of total ash content were approximately similar in different forms of *A. monosperma* ranging between 7.5 and 5.5 g.% in *A. judaica* and *A. herba alba* respectively. It is clear also that water-soluble ash contents in *A. monosperma were* approximately similar in different forms ranging between 3.5 and 4.0 g.%. Obviously, the records were 4.0 and 2.5 g.% in *A. judaica* and *A. herba alba* respectively. Results also clerified that the acid-insoluble content of different forms of *A. monosperma* ranged between 0.5 and 1.0 g.%. *A. judaica* and *A. herba alba* on the other hand had higher values of 2.8 and 2.3 g.% respectively (Table 2).

It is clear that the eight forms of *A. monosperma* had similar contents of total ash as well as water-soluble and acid-insoluble ash contents . These contents differed from those of the other two *Artemisia* species. Although the total ash and water-soluble ash of *A. judaica* were similar to those of *A. monosperma*, the acid-insoluble ash was much higher. The contents of the three types of ash in *A. herba alba* differed from those of the other two species.

Results presented in Table (2) clearly show that the total carbohydrate contents of the studied *Artemisia* species belonging to *A.*

Table (1): Preliminary phytochemical screening of shoots of three species of *Artemisia*.

Test	Species		
	A. monosperma (A1-A8)	*A. judaica*	*A. herba alba*
Volatile oils	+ve	+ve	+ve
Tannins	-ve	-ve	-ve
Unsaturated sterols	+ve	+ve	+ve
Alkaloids	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Glycosides and/or carbohydrates	+ve	+ve	+ve
Saponins	+ve	+ve	+ve

TABLE (2): Quantitative analysis of the three Artemisia species.

Species	Artemisia monosperma								A. judaica	A. herba-alba
Character	A1	A2	A3	A4	A5	A6	A7	A8		
Total ash (g %)	7.5	7.5	7.5	8.0	7.5	8.0	8.0	7.5	7.5	7.5
Water soluble ash (g %)	4.0	4.0	3.5	4.0	4.0	4.0	4.0	4.0	4.0	2.5
Acid insoluble ash (g %)	0.5	1.0	1.0	0.5	0.5	1.0	1.0	0.5	2.8	2.3
Total carbohydrates (g glucose %)	0.87	0.83	0.83	0.83	0.87	0.87	0.87	0.83	2.42	2.60
Total nitrogen (g/100 g plant material)	0.286	0.276	0.286	0.286	0.286	0.276	0.276	0.276	0.332	0.350
Alcohol extract (crude flavones %)	3.28	3.31	3.37	3.24	3.28	3.28	3.37	3.28	0.50	0.40
Total lipid content (g%)	15.4	15.4	14.2	15.4	14.2	15.4	14.2	14.2	10.2	8.2

monosperma (A1-A8) attained values that ranged between 0.83 and 0.879/100 g. dry matter. On the other hand, data collected for *A. judaica* and *A. herba alba* indicated higher values (2.42 & 2.60 g.% respectively).

The qualitative study of sugers presents in the three studied species using paper chromatography (Table 3) revealed that *A. monopserma* with all different forms contained galactose. The other two species, namely *A. judaica* and *A herba alba* contained mannose. All forms of *A. monosperma* differed from the other two *Artemisia* species.

Results in table (2) also show that the amounts of the total nitrogen content attained their maximum values in *A. judaica* (0.332 g.%) and *A. herba alba* (0.350 g.%) that decreased remarkably in the eight forms of A. monosperma (0.276- 0.286 g.%).

The results of total lipid content (Table 2) estimated quantitatively in the different forms of *A. monosperma* (A1-A8) indicated values that ranged between 14.2 to 15.4 g.%. On the other hand, both *A. judaica* and *A. herba alba* indicated lower values of 10.2 and 8.2 g.% respectively.

The percentages of the alcoholic extract, containing total flavones, were also compared (Table 2). It is evident that the crude total flavonoid content of the eight forms of *A. monosperma* were similar, ranging from 3.24 to 3.31 g.%; however, *A. judaica* contained only 0.5 g.% ; neverthless *A. herba alba* contained the least amount of flavonoids (0.14 g.%).

The results of the qualitative study of amino acids in the three studied species using paper chromatography (Table 3) revealed that *A. monosperma* with all its different forms contained lysine, asparagin, aspartic acid, glutamic acid, alanine, tyrosine, methionine and leucine.

Table (3): Qualitative analysis of carbohydrates, amino-acids, flavonoids and fatty acids of the three Artemisia species.

Species	---- Artemisia monosperma ----								A.	A.
Character	A1	A2	A3	A4	A5	A6	A7	A8	judaica	herba-alba
Carbohydrates										
Galactose	+	+	+	+	+	+	+	+	-	-
Mannose	-	-	-	-	-	-	-	-	+	+
Amino acids										
Cystine	-	-	-	-	-	-	-	-	+	+
Lysine	+	+	+	+	+	+	+	+	+	+
Aspargine	+	+	+	+	+	+	+	+	-	+
Aspartic acid	+	+	+	+	+	+	+	+	+	-
Glutamic acid	+	+	+	+	+	+	+	+	+	-
Serine	-	-	-	-	-	-	-	-	-	+
Alanine	+	+	+	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+	+	+	-
Methionine	+	+	+	+	+	+	+	+	-	-
Valine	-	-	-	-	-	-	-	-	+	+
Leucine	+	+	+	+	+	+	+	+	+	-
Isoleucine	-	-	-	-	-	-	-	-	-	+
Flavonoids ‡										
Quercetin 3- glucoside	+	+	+	+	+	+	+	+	-	+
Quercetin 3- rurinoside	+	+	+	+	+	+	+	+	-	+
Quercetin 5- glucoside	+	+	+	+	+	+	+	+	-	+
Isorhamnetin 5- glucoside	+	+	+	+	+	+	+	+	-	-
Patuletin 3- glucoside	+	+	+	+	+	+	+	+	-	+
Patuletin 3- rutinoside	+	+	+	+	+	+	+	+	-	+
Acacetin 7- glucoside	-	-	-	-	-	-	-	-	-	+
Acacetin 7- rutinoside	+	+	+	+	+	+	+	+	-	-
Isovitexin	+	+	+	+	+	+	+	+	-	-
Vicenin -2	+	+	+	+	+	+	+	+	-	+
Schaftoside	-	-	-	-	-	-	-	-	-	+
Isoschaftoside	-	-	-	-	-	-	-	-	-	+
Lucenin -2	+	+	+	+	+	+	+	+	-	-

‡ Identified by Bacha (1984) and Saleh et. al.

Table 1.3 (contd.

Methylated aglycone	+	+	+	+	+	+	+	+	−	+
Chrysoerial 7- rutinoside	−	−	−	−	−	−	−	−	+	−
Leutulin	−	−	−	−	−	−	−	−	+	−
Cirstakogenin	−	−	−	−	−	−	−	−	+	−
Apigenin	−	−	−	−	−	−	−	−	+	−
Fatty acid esters										
Octanoic	+	+	+	+	+	+	+	+	+	−
Capric	+	+	+	+	+	+	+	+	+	+
Undecanoic	−	−	−	−	−	−	−	−	−	−
Lauric	+	+	+	+	+	+	+	+	+	+
Tridecanoic	+	+	+	+	+	+	+	+	+	+
Myristic	+	+	+	+	+	+	+	+	+	+
Pentadecanoic	+	+	+	+	+	+	+	+	+	+
Palmitic	+	+	+	+	+	+	+	+	+	+
Stearic	+	+	+	+	+	+	+	+	+	+
Oleic	+	+	+	+	+	+	+	+	+	+
Linoleic	+	+	+	+	+	+	+	+	+	+
Linolenic	+	+	+	+	+	+	+	+	−	+
Arachidic	+	+	+	+	+	+	+	+	−	−

On the other hand, *A. judaica* contained lysine, aspartic acid, glutamic acid, alanine, tyrosine, valine and leucine; while *A. herba alba* contained cystine, lysine, asparagin, serine, alanine, valine and isoleucine.

Considering the qualitative investigation of flavonoids (Table 3) *A. monosperma* contained quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 5-glucoside, isorhamnetin 5-glucoside,patuletin 3-rutinoside, acacetin 7-glucoside, acacetin 7-rutinoside, vicenin-2, lucenin and methylated aglycones as reported by Saleh *et al.*(1985). Differently, *A. judaica* contained chrysoerial 7-rutinoside, leutulin, cirstakogenin (Bacha, 1984). Finally, *A. herba alba* contained quercetin 3-glucoside, quercetin 3-rutinoside, patuletin 3-glucoside, patuletin 3-rutinoside, isovitexin, vicenin-2 schaftoside, isoschaftoside and methylated aglycones (Saleh *et al.* 1985). These results clarify the presence of different flavones in the three *Artemisia* species. The results also show that the eight forms of *A. monosperma* contain the same flavones, and differ from the other two species.

The results presented in Table (4) reveal the presence of the following fatty acids in the eight forms of *A. monosperma* : octanoic, capric, lauric, tridecanoic, myristic, pentadecanoic, palmitic, stearic, oleic, linolenic and arachidic acids. It is also clear that *A. judaica* contained a group of fatty acids similar to that of *A. monosperma*, with the difference that undecanoic acid is present, while octanoic and arachidic acids are absent. On the other hand, *A. herba alba* contained octanoic, capric, lauric, tridecanoic, stearic, oleic, linoleic acids, and was free of undecanoic, linolenic and arachidic acids. These results show that the percentage of some fatty acids varies not only in the three

Table (4): Percentages of fatty acid esters of the three studied Artemisia pecies.

Species	Artemisia aonosperma								A.	A.
Character	A1	A2	A3	A4	A5	A6	A7	A8	judaica	herba-alba
Octanoic	5.84	0.82	9.09	22.57	5.75	6.85	4.85	0.82	-	25.87
Capric	26.16	9.33	12.12	4.27	16.09	21.27	25.22	9.33	24.31	7.71
Undecanoic	-	-	-	-	-	-	-	-	2.78	-
Lauric	1.11	0.27	0.22	1.65	0.81	2.95	0.33	0.27	8.10	8.81
Tridecanoic	0.47	1.51	2.98	5.37	2.01	5.32	3.49	1.51	2.31	12.11
Myristic	3.96	0.96	1.50	2.20	2.76	11.82	2.18	0.96	3.86	5.50
Pentadecanoic	4.42	16.58	9.20	34.17	9.58	14.65	4.80	16.58	27.39	4.41
Palmetic	50.49	15.79	17.86	11.30	21.86	24.82	16.38	15.79	1.93	2.75
Stearic	0.32	1.57	2.71	1.65	1.53	0.83	0.76	1.57	3.70	1.47
Oleic	0.26	9.02	8.44	3.05	7.66	0.59	15.07	9.02	4.63	17.61
Linoleic	3.47	36.84	18.99	8.79	25.21	0.89	16.81	36.84	7.41	13.76
Linolinic	2.24	5.26	12.99	3.67	3.58	2.92	8.73	5.26	13.58	-
Arachidic	1.26	2.05	3.90	1.22	3.16	7.09	1.64	2.05	-	-

Artemisia species, but also in the forms of *A. monosperma* (Table 4). It must be noted that the qualitative estimation of fatty acids esters by Gas Liquid chromatography is strict since it depends on comparing the fatty acids by authentic samples. On the other hand, the quantitative estimation of some fatty acids by the same method may differ since the peak shape differs in operating conditions and injection technique.

It can be concluded that the eight forms of *A. monosperma* contain the same fatty acids, which differ from those of the other two species viz. *A. judaica* and *A. herba alba*.

From these results, it is clear that the eight forms of *A. monosperma* are greatly similar in their chemical compisition. They differ qualitatively and quantitavely from the other two species, viz. *A. judaica* and *A. herba alba*. In this regard, the phytochemical study fully justifies the systematic treatment.

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