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Research Article

Chromatography Analysis of Fatty Acids, Volatile Compounds and Alkaloids of *Ephedra alata* Growing Wild in Southern Tunisia and Evaluation of their Antioxidant Activity

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ABSTRACT

In order to provide more information about phytochemical composition of Tunisian *Ephedra alata* known for their medicinal uses, fatty acids, volatile compounds and alkaloids have been analyzed in fresh or dry plant materials whether powdered or not. Extraction was performed with hexane and ethanol in soxhlet extractor and all extracts were analyzed by GC-MS. Phosphomolybdenum, DPPH and FRAP methods were used to evaluate their antioxidant activities. The dry matter present a more diversified fatty acids composition dominated by palmitic acid, oleic acid and linolelaidic acid. Hydrocarbons represent the largest group of volatile compounds, followed by alcohols. The most known detected ephedrine-type alkaloid characteristic of the *Ephedra* genus are dominated by pseudoephedrine, norephedrine, ethylephedrine. Some other compounds are mentioned for the first time in *E. alata* alkaloids such as azetidine, 1,2-dimethyl-3-phenylaziridine, N-ethyl benzamide and N-methyl-mandelamide. Lower total antioxidant and anti-radicular effects were shown with hexane extracts and alkaloid fractions instead the ferric reducing antioxidant power test showed the highest results with obtained alkaloid from powdered fresh and dry plant materials.

Lipids, volatile compounds and alkaloids in *E. alata* that have been assessed could provide possible roles in case of human breast cancer and health benefits and therefore a research on this plant might be of value in drug industry.

Keywords: *Ephedra alata*; fatty acids; volatile compounds; alkaloids; GC-MS; Tunisia.

INTRODUCTION

Ephedra (Ephedraceae) is a genus of nonflowering seed plants regrouping nearly, fifty species wordwide known for the most of them as shrubs adapted to arid and semiarid conditions[1]. In the past, *Ephedra* extracts obtained from the dried stems boiled in water, are used on the treatment of fever, nasal congestion, allergies and asthma and added as dietary nutritional supplements for their pronounced stimulant effects[2-4]. Today, major interests are given for the application of *Ephedra* available in bulk herb, capsules or hydro-alcoholic extract, in appetite suppression by inducing hypophagia[5-7] and energy formulas in enhancing muscle performance by increasing the metabolic rate of adipose tissue promoting consequently a body weight loss[8-11]. Although the phytochemical composition of various *Ephedra* species is not completely elucidated. Some phenolic compounds (coumaric acid, chlorogenic acid, rutin, catechin, quercetin), flavonoid (vicenin II, lucenin III, kaempferol 3-rhamnoside, quercetin 3-rhamnoside, herbacetin 7-glucoside, herbacetin 8-methyl ether 3-O- glucoside-7-O-rutinoside and herbacetin 7-O-6″-quinylglucoside and furanofuran (syringaresinol, digalloylglucose, nilocitin, *p*coumaric acid) were identified in the whole plant of *E.alata*[12,13]. The ephedrine-type alkaloids used for their sympathomimetic actions are the popular components of many nutritional supplements. The metabolites ephedrine and some of other compounds structurally related to it such as pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, ethylephedrine and methylpseudoephedrine are the most alkaloids of biological relevance found in the aerial parts of different *Ephedra* species with yields ranging from 0.02 to 3.4% of plant material. In addition to the ephedrine-type alkaloids, some other compounds like ephedroxane, ephedradine (A, B, C and D), N-methylbenzealamine, cyclopropyl-α-amino acids, 6-methoxykynurenic acid and tetramethylpyrazine have been isolated[3].

Almost all commercial applications of the plant extracts derive from these stimulant compounds. The two isomers ephedrine and pseudoephedrine considerate as precursors of methamphetamine, act similarly by stimulating the heart rate, increasing blood pressure, promoting bronchodilatation, and by exhibiting pronounced effects on the central nervous system[14]. Norephedrine and norpseudoephedrine are used as stimulant, decongestant and anorectic agents[15]. The isomers methylephedrine and methylpseudoephedrine are derivative forms of pseudoephedrine known by their more prolonged and less potent action than adrenaline and may be used for treating neuropathic pain[16].

E. alata is a range plant with medicinal application, distributed in Africa (Algeria; Egypt, Libyan, Morocco,

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Tunisia, Mauritania, Chad and Mali) and in Asia (Saudi Arabia, Iraq, Iran, Palestine, Lebanon, Jordan and Syria). This shrub dispersed in mobile and stable sand dunes with sandy ground in southern Tunisia (Rjim Maâtoug and El Borma) and have a long history of local traditional medicinal use of the dried stems for its bronchodilator and anti-asthmatic effects, especially and disposes foliage with acceptable aroma that can be used as food stuff during animal grazing in drought periods.

To date, the available literature does not report about chemical composition of *E.alata* found mainly in underpopulated area of Tunisian sahara desert. It is the first attempt to study volatile compounds, fatty acids and alkaloids obtained from *E.alata* aerial part and determine their potential antioxidant activities. Therefore, this study will constitute a valuable addition to the scientific literature concerning *E. alata*.

MATERIALS AND METHODS

Plant material

Fresh aerial part of *E. alata* collected from Sabria-Faouar (Kebili) in june (Full vegetative growth stage). Voucher specimens of the plant were kept in the Range Ecology laboratory of the Arid Lands Institute. The plant material was used fresh or dried in shade, finely powdered if necessary, with an electric mill and kept for the extraction process.

Alkaloids isolation

Extraction of stable alkaloids was made for 12 hours in hot hexane in soxhlet extractor and then with a sufficient amount of ethanol renewed three times every four hours. The obtained hexane and ethanol fractions are filtered and thereafter concentrated with rotary evaporator. The recovered ethanolic filtrate was macerated over night with 200 mL of acetic acid (10%). After filtration, the alkaloid extract was alkalinified with ammonia to Ph10 and placed in separatory funnel where we added a measured quantities of chloroform until the last fraction extract was found negative to Dragendorff's reagent. The combined chloroform extract was filtrated, dried with Na_2So_4 and concentrated in rotary evaporator. The residue was weighed and percentage yield was calculated using the formula:

% yield = 100 x weight of the alkaloidal residue/ weight of plant material

Methylation

In screw cap test tube we added to the hexane fraction (2 mL) 0.2 mL of a methanolic solution of potassium hydroxide (2N). The tube was stirred vigorously for 30 seconds and then allowed to stand until the upper part of the solution becomes clear. This fraction contains the methyl esters and will be available for injection into the chromatograph.

GC-MS analysis

The hexane extracts containing volatile compounds and the alkaloid fractions were analyzed by GC-MS-QP2010 Ultra apparatus, equipped with RTX-5MS (5% diphenyl/95% dimethyl polysiloxane) capillary column (30m x 0.25mm x 0.25µm film thickness) combined with an EMAF620 detector (quadrupole) with electron impact ionization of 70 eV. The oven temperature was programmed from 80 to 280°C at 10°C/min; carrier gas, He (linear velocity of 36.8 cm/s); scan time: 2.2 s; mass range: 35-500 Da. Samples (20µl) were injected with a split ratio of 1.0.

For the analysis of fatty acids we used Supelcowax Tm 10 capillary column (30m x 0.25mm x 0.25µm film thickness) with the following conditions: oven temperature from 50 to 250°C at 10°C/min; carrier gas is He with linear velocity of 39.7 cm/s; scan time 2.2 s; mass range: 35-500 Da and samples (20µl) injected with a split ratio of 1.0.

Compounds identification

Alkaloids and volatile compounds were identified by comparing their retention time with those of known compounds and also by comparing their mass spectra with those stored in computer library (Wiley 275 library and NIST MS Search 2.0 spectral library) provided by the instrument software. Some of the identification was confirmed by comparing the literature data and the calculation of retention index. A serie of n-alcane (C_9-C_{29}) was injected under the same conditions of samples into the GC-MS system.

The fatty acids were identified by comparing their retention times with those of the internal FAMEs standards (AOAC 996.06 standard) injected under the same conditions.

Antioxidant activity

The antioxidant activity of various *E. alata* extracts was tested by three methods such the total antioxidant capacity by phosphomolybdenum method, scavenging ability on DPPH radical and chelating effect on ferric reducing antioxidant power. BHA, BHT and ascorbic acid (AA) were used as standards and all determinations were performed in triplicate.

Evaluation of total antioxidant capacity by Phosphomolybdenum method

The total antioxidant capacity of the plant extracts was evaluated by the method of Prieto et al[17]. An aliquot of 0.2 ml of the sample solution was mixed with 1.8 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Incubation was then carried out for 90 min in a water bath at 95 °C. After cooling to room temperature, the absorbance of the solutions was measured using a UV-visible spectrophotometer at 695 nm against a blank (0.2 ml methanol was mixed with 1.8 ml of the reagent). The absorbance of the test sample was measured at 695 nm. The antioxidant activity was expressed for the samples as gallic acid equivalents (mg/g of methanol extract).

Scavenging ability on DPPH radical

Antiradical activity was evaluated by measuring the scavenging activity of *E. alata* extracts on the 2,2-diphenyl-l-1-picrylhydrazil (DPPH) radical, using the method described by Braca et al[18] with slight modifications. The diluted extracts (0.2, 0.4, 0.6, 0.8 and 1 mg/mL) were prepared in methanol. We prepared 0.004% DPPH in methanol. Then 1mL of this solution was mixed with 50 µL of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at

Table 1: Fatty acids composition of *Ephedra alata* hexane extracts.

Peak	R. Time	Compound Name	% area (GC-MS) F NP	F P	D NP	D P
1	9.348	Lauric acid	0.25	*n.d*	*n.d*	0.81
2	11.455	Tetradecanoic acid	1.56	3.84	1.59	1.29
3	12.893	Pentadecanoic acid	0.47	*n.d*	0.49	1.16
4	14.628	Palmitic acid	9.94	7.47	12.66	19.97
5	16.619	Heptadecanoic acid	*n.d*	*n.d*	*n.d*	0.45
6	18.942	Oleic acid	12.88	5.90	7.74	15.53
7	19.539	Linolelaidic acid	2.87	4.96	12.81	21.08
8	20.638	Linoleic acid	*n.d*	1.68	3.86	4.49
9	24.245	Docosanoic acid	6.17	3.45	*n.d*	*n.d*
10	25.695	Eicosanoic acid	*n.d*	1.64	2.15	4.37
11	26.580	7,10,13-Eicosatrienoic acid	*n.d*	0.57	*n.d*	*n.d*
12	28.571	cis-11,14,17-Eicosatrienoic acid	*n.d*	*n.d*	*n.d*	0.21
13	30.889	Heneicosanoic acid	*n.d*	*n.d*	7.82	0.31
14	34.080	Tetracosanoic acid	*n.d*	10.72	8.01	4.64
% non-fatty acid compounds detected by GC-MS			65.86	59.77	42.87	25.69

n.d: not detected; F: Fresh, D: Dry, P: Powdered, NP: Not Powdered

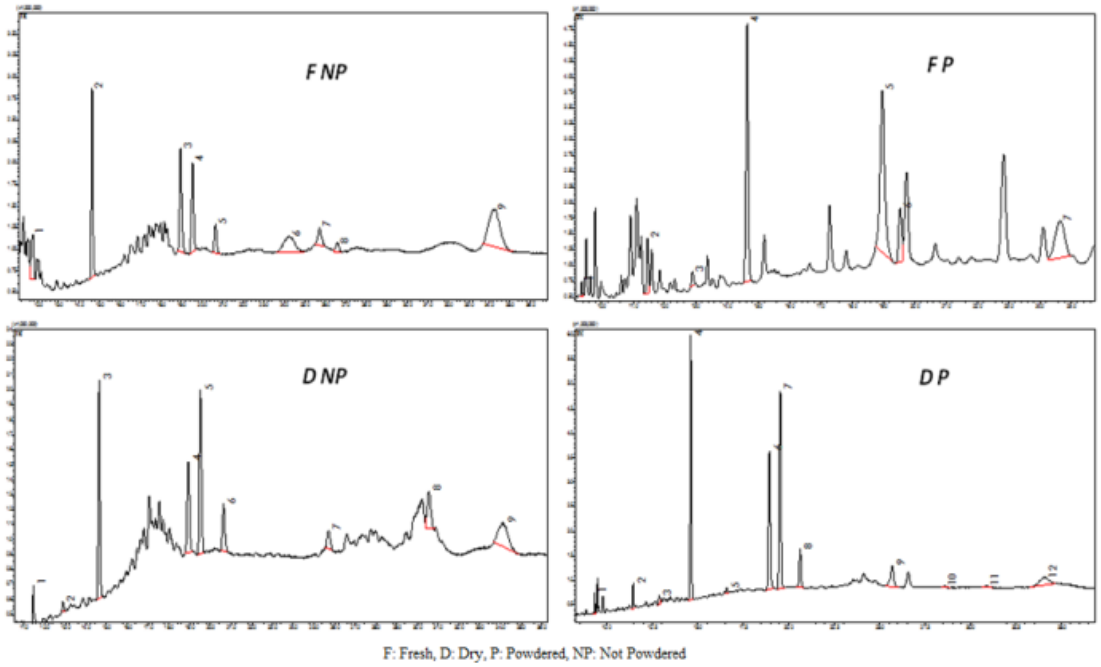

Figure 1: Total ion chromatograms (TIC) of GC-MS *Ephedra alata* hexane extract constituents.

517 nm using spectrophotometer against methanol. The blank was used as 1mL of methanol with 1mL of DPPH solution. The optical density was recorded and the IC_{50} (concentration of sample required to scavenge 50% of DPPH radicals) values were determined as follows: IC_{50} (%) = 100 x $(A_0-A_t)/A_0$, where A_0 is the optical density of the blank and A_t represent the optical density in the presence of the plant extract.

Ferric reducing antioxidant power

The ability to reduce ferric ions was measured using the method described by Benzie and Strain with some modifications[19]. The FRAP reagent was generated by the mixture of 300 mM sodium acetate buffer (pH 3.6), 10 mM (tripyridyl triazine) TPTZ solution and 20 mM $FeCl_3,6H_2O$ solution (volume ratio of 10:1:1). FRAP reagent (950 µl) was mixed with sample (50 µl) and the mixture was incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working trolox solutions (1mM) were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mM trolox equivalents/g DW.

RESULTS AND DISCUSSION

Fatty acids

The GC-SM analysis of the *E. alata* hexane extracts results in several peaks as shown in figure1. In the Table1 are listed the identified compounds and their quantification presented according to their retention times. A total of 14 compounds that represent 35 to 40% of the fresh samples

and more than 60 to 75% of the driest ones of the totality of analyzed extracts, were identified. The dry powdered matter appears the most riche sample in fatty acids. The palmitic acid and oleic acid were the major compounds

Table 2: Volatile compounds from the hexane extracts of *Ephedra alata.*

N°	R_t (mn)	Compound Name	Relative peak area %			
			F NP	F P	D NP	D P
1	4.192	3-Hexanol	0.79	0.25	0.13	0.33
2	4.272	2-Hexanol	0.55	0.26	-	0.36
3	4.590	Hexamethylcyclotrisiloxane	-	12.79	-	6.06
4	5.102	Diacetone alcohol	0.18	-	0.13	-
5	5.586	1,2-Dimethylbenzene	0.19	-	-	-
6	6.037	Ethenylbenzene	0.32	0.08	-	0.18
7	7.268	2-Nitrohexane	0.26	0.03	0.27	-
8	7.487	2,3,5-Trimethylhexane	0.18	0.03	0.18	-
9	7.626	Benzaldehyde	0.11	0.04	-	-
10	8.373	Oktamethylcyklotetrasiloxane	-	-	-	1.70
11	8.427	Decane	0.39	5.87	-	0.39
12	9.119	β-Methylstyrene	0.21	0.10	-	-
13	9.173	7-Methyl-3-methylene-7-octenyl propionate	1.47	0.15	-	0.15
14	9.295	Eucalyptol	-	1.84	-	-
15	9.366	p-Menthane-1,8-diol	-	0.25	-	-
16	9.601	cis-β-Ocimene	0.14	-	-	-
17	9.789	Dodecane	0.27	-	-	-
18	10.153	1-Octanol	1.67	0.13	-	-
19	10.773	Undecane	0.21	0.13	-	-
20	11.066	β-Thujone	-	1.20	-	-
21	11.226	Benzene ethanol	4.74	0.55	1.20	1.66
22	11.315	α-Thujone	-	2.14	-	-
23	11.851	(-)-cis-Sabinol	-	1.28	-	-
24	12.003	Camphor	-	3.12	-	0.48
25	12.481	Endo-Borneol	-	0.50	-	-
26	12.717	Terpinen-4-ol	-	0.59	-	-
27	15.287	Tetramethylbisphenol A	-	0.28	-	-
28	15.708	Cyclohexasiloxane, dodecamethyl-	-	2.35	-	0.91
29	16.938	3,4-dimethyl-5-phenyloxazolidine	3.01	3.23	-	1.75
30	17.070	Unknow	1.18	-	-	1.17
31	17.210	Tetradecane	0.40	0.10	-	0.34
32	19.124	Pentadecane	0.23	5.84	-	1.95
33	19.637	Dodecanoic acid, methyl ester	-	-	0.47	-
34	20.931	Heptadecane	0.46	0.25	0.18	0.28
35	22.064	Hexadecamethyl-cyclooctasioxane	-	1.82	-	1.00
36	22.884	Cyclohexylmethyl hexyl sulfite	0.95	0.14	-	0.24
37	23.174	2-Hexyldecanol	-	0.22	0.97	-
38	24.268	Nonadecane	0.67	0.33	0.27	0.35
39	24.656	Octadeamethyl-cyclononasiloxane	-	0.58	0.29	0.32
40	24.915	(E)-Phytol	1.14	0.11	0.20	-
41	25.041	Hexahydrofarnesyl acetone	0.35	0.19	0.21	0.73
42	25.586	Neophytadine	0.66	0.17	-	-
43	25.815	Octacosane	0.54	0.16	-	-
44	25.922	2,2-Dimethyl-3-phenyl-N-tert-butyl aziridine-1-carboxamide	0.57	-	1.05	-
45	26.253	Hexadecanoic acid, methyl ester	0.42	0.12	8.65	0.35
46	26.518	2-methylhtetracosane	-	0.20	-	-
47	26.909	Tetratetracontane	-	3.90	0.13	-
48	27.062	1,2-Dimethyl-3-phenylaziridine	4.09	-	-	-
49	27.204	1-Nonadecene	0.55	-	-	-
50	27.291	dotriacontane	1.17	0.18	0.16	0.23
51	27.428	(14β)-Pregnan	-	0.11	0.19	0.16
52	27.684	Unknow	-	-	-	0.20

53	27.857	(+)-3-Carene, 2-α-isopropenyl-	1.80	-	0.20	0.19
54	28.571	Nonadecanol	0.76	0.87	1.00	0.66
55	28.620	1,2-Octadecanediol	0.34	-	-	-
56	28.700	Heneicosane	1.73	0.14	12.3	1.81
57	28.999	Phytol	6.37	9.18	19.79	30.16
58	29.142	Unknow	-	-	2.00	-
59	29.421	Unknow	-	0.28	0.13	0.63
60	29.535	Unknow	-	-	-	0.77
61	29.690	2-methylhexacosane	0.50	0.52	-	0.66
62	29.978	1-Docosanol	0.90	0.62	-	-
63	30.048	2-Hexyldecanol	2.90	0.16	0.30	0.19
64	30.233	1-Hexacosanol	-	0.71	-	2.42
65	30.450	Stearaldehyde	0.32	0.68	1.60	1.66
66	31.005	5-Butyl-5-ethylheptadecane	0.52	1.64	0.35	-
67	31.129	Tetrateracanoate	0.86	8.79	2.54	1.70
68	31.311	Pentadecanol	2.24	1.79	-	4.09
69	31.388	Tetracosane	6.57	-	2.52	-
70	31.691	triatetracontane	-	-	7.94	-
71	31.871	Heneicosanoic acid, methyl ester	-	-	4.84	-
72	32.057	Unknow	-	-	7.37	5.62
73	32.179	Octacosanol	-	-	16.1	-
74	32.310	Hexacosane	0.42	0.65	-	-
75	32.467	2-methyltetracosane	0.33	3.36	-	14.87
76	32.626	1,54-Dibromotetrapentacontane	0.37	-	-	-
77	32.710	17-Pentatriacontene	1.46	-	-	-
78	32.830	Eicosyl trifluoroacetate	0.58	0.49	-	-
79	32.924	Docosane	10.50	-	0.22	0.31
80	33.524	Octacosyl heptafluorobutyrate	-	12.01	0.5	0.22
81	33.930	7-Hexyldocosane	0.20	-	0.42	-
82	34.025	Triacontane	0.90	-	-	-
83	34.225	2-methyloctacosane	0.62	-	0.16	0.33
84	34.593	Unknown	2.94	-	-	-
85	34.780	Pentatriacontane	25.80	4.12	4.07	10.11

R_t: Retention time; -: Not detected; F: Fresh, D: Dry, P: Powdered, NP: Not Powdered

Table 3: Relative content of functional groups in identified volatile compounds from *Ephedra alata.*

		Relative area %			
Functional groups	Number	F NP	F P	D NP	D P
Hydrocarbons (without terpenes)	38	58.43	45.34	29.66	41.99
Alcohols	15	22.58	14.85	39.82	39.87
Miscellaneous (including unknowns)	13	13.00	12.60	13.49	12.73
Terpenes	10	0.14	11.20	0.00	0.48
Esters	7	3.42	12.91	14.46	0.96
Aldehydes	2	0.43	0.72	1.60	1.66
Total	85	98.00	97.62	99.03	97.69

F: Fresh, D: Dry, P: Powdered, NP: Not Powdered

present in all analyzed samples with higher amounts ranged between 7.47 to 19.97% and 5.90 to 19.97%, respectively. Others major compounds detected in the fresh samples whether powdered or not such as docosanoic acid (6.17 and 3.45%, respectively) and tetradecanoic acid (1.56 and 3.84%, respectively). The tetracosanoic acid which appears in higher amount of 10.72% in the fresh powdered matter is not detected in the not powdered fresh matter and is present in appreciable amounts of 8.01 and 4.64% in the dry powdered or not powdered matters, respectively. The linolelaidic acid contents present in all samples reached a high content in the dry matter specially when powdered (21.08%) on the contrary to docosanoic acid that is present in the fresh matter if powdered or not (6.17 and 3.45%, respectively) and completely absent in the dry samples.

There is no available information in the open literature concerning *E. alata* fatty acids and the current study describes for the first time the fatty acids composition and content of this species growing wild in Tunisia. High

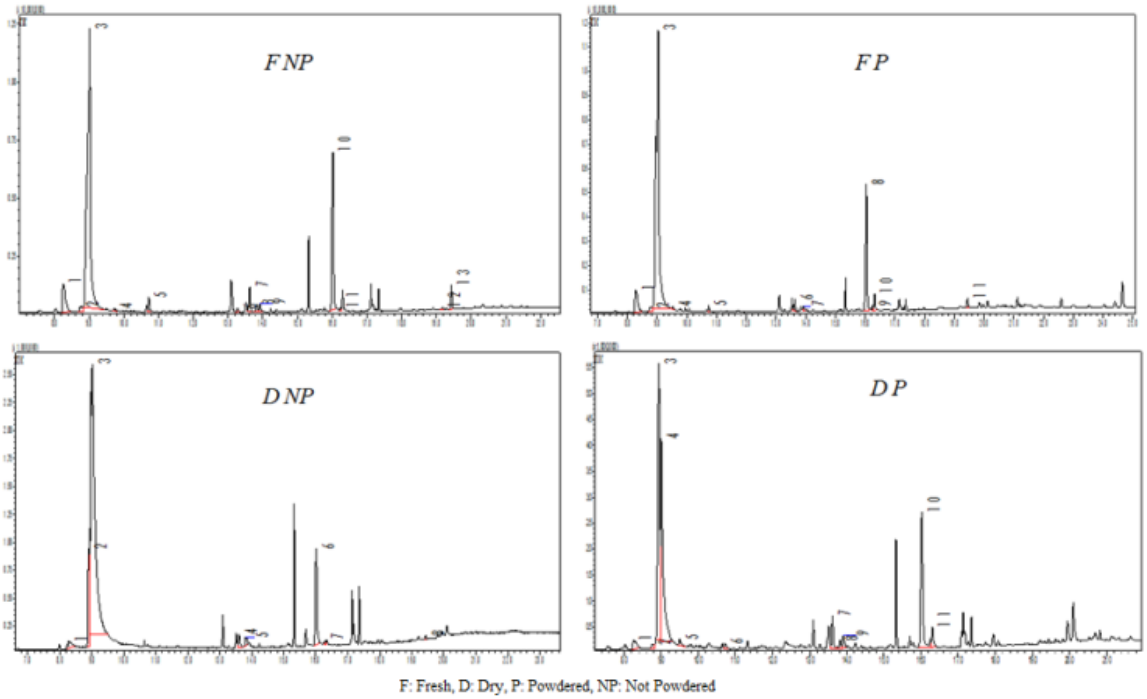

Figure 2: GC-MS chromatograms of the alkaloids in *Ephedra alata*.

variability was observed in the quantitative results for all analyzed fatty acids. As reported by different investigators, this variability may be due to several parameters such as the age of the analyzed organs, growing and climate conditions, genetic history, nutrition, harvest time and the pretreatment operations (quantitative analysis, storage methods, drying and powdering), any of which can profoundly alter the composition of the endogenous lipids of plants[20,21].

In the dry matter, the content of linolelaidic acid reaches a maximum of 21.08% if powdered. This acid was known by its effects to induce apoptosis, cell cycle arrest and inflammation[22]. In the same way, the acids (palmitic and oleic) well-known by their important health attributes becomes maximum in dry powdered samples (19.97 and 15.53%, respectively). These findings may explain the traditional use of the dried stems of the plant and suggest that the contents of acids in use should be taken into consideration.

Volatile compounds

The identified volatile compounds of *E. alata* hexane extracts analyzed by GC-MS are shown in Table 2. A total of 85 compounds were identified (in parenthesis, the number of identified compounds), which included hydrocarbons without terpenes (38), alcohols (15), miscellaneous including unknown compounds (13), terpenes (10), esters (7) and aldehydes (2) (Table 3).

Enormous changes occur in the chemical composition of the plant after drying or powdering matter and no logical order explaining this qualitative and quantitative variation. Hydrocarbons that represent 58.43 and 45.34% in the fresh powdered and not powdered matter, respectively, were the largest group of volatile compounds in *E. alata*, followed by alcohol (22.58 and 14.85%, respectively) and miscellaneous (13.00 and 12.60%, respectively). After powdering the fresh matter the content of terpenes reached to 11.2%. In fact, camphor, α-Thujone and eucalyptol are the major detected terpene compounds (3.12, 2.14 and 1.84%, respectively).

The ester fractions have reached high values of 12.91 and 14.46% in powdered fresh matter and dry matter, respectively.

When dried, the hydrocarbon fractions decrease in *E. alata* hexane extract (29.66% if not powdered and 41.99% in powdered matter) followed by alcohols that represent about 39%. A similar study in Algeria desert, has reported the presence of five main compounds of *E. alata* leaves in dichloromethane extract such as 2-propenoic acid, 3-phenyl (18.19%), phenol, 4-(3-hydroxy-1 propenyl) (7.88%), benzoic acid (8.52%), benzaldehyde, 4-hydroxy-3,5-dimethyl (7.04%) and benzaldehyde, 4-hydroxy- 3-methoxy (4.38%)[23].

Alkaloids

Gas chromatography and mass spectroscopy analysis of compounds was carried out in alkaloid extracts of *E. alata* as shown in Table 4. The GC-MS chromatograms of the fourteen peaks of the detected alkaloids were given in Figure 2.

With hot ethanol in soxhlet extractor method, the alkaloid fractions constitute between 71.55
to 86.10% of obtained extracts. Quantitatively, no difference between not powdered fresh or dry plant materials (around 14mg/g) but these yields increase to reach 20.37 and 22.19 mg/g in powdered material, respectively. From the literature data, the total alkaloid content in *Ephedra* genus that depends on species, the harvesting/growing conditions and the geographic origin varies considerably between 0.5 and 49.9 mg/g[3].

Qualitatively, fourteen alkaloid compounds were detected, among them ten compounds were well identified and four peaks showed dubious suggestions from the MS instrument libraries with synthetic compounds that derive all from the ephedrine/pseudoephedrine isomers. Among the identified compounds, ephedrine, the most known characteristic compound of this *Ephedra* genus is not detected in this study but some associated ephedrine-type

Table 4: GC-MS alkaloids data in *Ephedra alata*..

S. N°	Compound Name (Rt)	S (%)	RI (Rtx-5MS)		Compound MS fragment-ions (% Intensity)		Relative area % (mg/g)**			
			Cal.	Lit.	Analysed	Suggested	FNP	FP	DNP	DP
1	Norephedrine (8.620)	97	1345	1350	44(100), 77(8.7) 79(5.9), 51(3.4)	44(100), 77(5.2) 79(4.4), 51(2.8)	5.12 (0.85)	4.56 (1.25)	1.24 (0.21)	1.70 (0.53)
2	Azetidine (8.745)	84	1378	n.a	57(100), 56 (5.6) 29(5.1), 58(4.2)	57(100), 58 (28) 56(9.2), 29(4.0)	0.79 (0.13)	0.85 (0.23)	-	0.57 (0.18)
3	Deoxyephedrine (8.941)	83	1393	n.a	71(100), 58(77) 56(31), 43(10) 91(8.4), 77(7.8)	71(100), 42(34.4) 43(30), 56(30) 77(6.8), 70(6.4)	-	-	15.47 (2.64)	26.36 (8.17)
4	Pseudoephedrine (9.015)	97	1399	1400	58(100), 30(8.2) 77(4.1), 59(4.0)	58(100), 30(7.6) 59(4.0), 56(2.8) 77(2.8)	56.15 (9.29)	51.85 (14.19)	54.57 (9.31)	22.98 (7.13)
5	Methylephedrine (9.723)	97	1452	1430	72(100), 44(6.6) 42(5.0), 73(4.8) 77(3.7), 70(3.5)	72(100, 44(4.8) 73(4.4), 42(3.2) 56(2.8), 77(2.0)	0.25 (0.04)	0.20 (0.06)	-	0.51 (0.16)
6	N-methyl-mandelamide (10 .715)	87	1528	1555	79(100), 107(81) 77(64), 118(56) 51(19), 105(17)	107(100), 79(96) 77(64), 51(22) 27(16), 39(16) 50(11), 105(10.8)	1.15 (0.19)	0.42 (0.12)	-	0.42 (0.13)
7	Ephedroxane (13.272)	88	1735	n.a	57(100), 42(51) 58(41), 191(20) 56(20), 117(18) 132(17), 91(15)	57(100), 58(33) 42 (25), 191(17) 56(16), 77(10) 117(10), 91(8.0)	0.25 (0.04)	-	-	-
8	Ethylephedrine (13.624)	84	1765	*1765	87(100), 86(92) 58(71), 72(29) 30(19), 77(16) 79(12), 56(10)	86(100), 58(65) 42(19), 77(14) 56(11), 51(9.0) 87(6.8), 44(5.6)	2.01 (0.33)	0.96 (0.26)	0.99 (0.17)	2.25 (0.70)
9	Unknown 1 (13.816)		1782		58 (100), 100(45)		0.64 (0.11)	-	0.76 (0.13)	0.63

					101(17), 43(12) 43(8.0), 28(7.8) 77(7.5), 42(6.4)					(0.20)
10	Unknown (13.914)	2	1790		71(100), 56(22) 107(22), 42(12) 117(9.9), 79(9.7) 43(8.1), 77(7.6)		0.67 (0.11)	0.54 (0.15)	-	1.01 (0.31)
11	1,2-dimethyl-3-phenylaziridine (16.018)	82	1984	n.a	146(100),147(92) 105(34), 132(24) 148(23), 91(18) 77(16), 117(15)	146(100),105(48) 132(24), 91(21) 42(18), 77(18) 147(14),117(12)	16.90 (2.80)	12.74 (3.49)	7.89 (1.35)	13.64 (4.23)
12	Unknown (16.236)	3	2004		132(100),133(42) 105(36), 28(15) 77(15), 43(10) 79(9.9), 91(9.5)		0.25 (0.04)	0.38 (0.10)	0.26 (0.04)	1.48 (0.46)
13	N-ethyl benzamide (19.172)	87	2311	n.a	105(100), 77(41) 148(34), 149(31) 43(28), 28(24) 45(24), 41(17) 43(17), 207(16)	105(100), 77(70) 149(46), 148(40) 51(26), 50(9.6) 106(7.6), 78(6.8)	0.26 (0.04)	1.29 (0.35)	-	-
14	Unknown (19.434)	4	2341		86(100), 44(96) 105(58), 77(29) 43(13), 191(13) 238(9.0), 51(7.2)		1.58 (0.26)	0.62 (0.17)	0.19 (0.03)	-
% Non-alkaloid compounds detected by GC-MS							13.9	25.59	18.62	28.45
Yield (g/100g of plant materials)							1.65	2.74	1.71	3.10
Alkaloid fraction (mg/g plant material)							14.24	20.37	13.88	22.19

Rt: retention time RI: retention index; S: similarty; n.a: not available; Cal.: Calculated; Lit.: Literature
*column and program data non specified
**In parentheses below was given the real concentration of each compound expressed in mg/g

alkaloids or derivatives such as pseudoephedrine, norephedrine and ethylephedrine are detected in all analyzed samples as major compounds.

Pseudoephedrine has reached a highest amount of 14.19 mg/g in powdered plant material used as fresh (51.85%) followed by 1,2-dimethyl-3-phenylaziridine (3.49 mg/g) and norephedrine (1.25mg/g). Azetidine, methyephedrine, N-methyl-mandelamide and unknown2 compound are present in low content (ranged between 0.04 and 0.31 mg/g) found in all samples except the not powdered dry

Table 5: Total antioxidant capacity, DPPH scavenging activity and ferric reducing antioxidant power of *Ehedra alata* hexane extracts and alkaloids.

Property	Plant extract								
	Hexane extract				Alkaloids				Standards
	FNP	FP	DNP	DP	FNP	FP	DNP	DP	
Total antioxidant capacity (mg GAE/g)	6.5 ±0.12	38.9 ±1.44	4.0 ±0.10	34.0 ±1.40	10.4 ±0.02	155.2 ±1.40	140.4 ±3.09	132.0 ±1.45	BHA 851±25 BHT 1004±13
DPPH % activity (5mg/ml)	38.09 ±2.01	19.41 ±1.56	41.99 ±3.20	16.49 ±1.18	17.58 ±1.20	13.29 ±1.04	12.65 ±2.36	10.83 ±2.19	AA (IC_{50}) 7.93±0.28µg/ml
FRAP (µM TE/g)	463 ±11	101 ± 4	440 ±8	110 ± 2	1112 ±9	1433 ±17	926 ±7	1200 ±6	BHA 2380±62 BHT 1570±36 AA 1232±12

Values are expressed as mean of triplicate determinations ± standard deviation

plant material. Solely, from the fresh not powdered analyzed sample considerate as the most riche in alkaloid compounds, ephedroxane is found in low amount (0.04mg/g). The deoxyephedrine is the only compound that appears after drying with appreciable amounts of 2.64 mg/g if the plant material is not powdered and 8.17 mg/g if powdered.

Ephedrine and pseudoephedrine considerate as the two main alkaloids, account in the most *Ephedra* species more than 70% of the total alkaloid content[2,24-27]. In addition to findings that depend largely, on the sensitivity of the analysis used, the results of our study are in agreement with the suggestion of Wang et al which propose that *Ephedra* species growing in more alkaline soil and in more arid conditions contain more total ephedrine-type alkaloids and high pseudoephedrine content[28]. The other alkaloids and amino compounds which are not closely structurally related to ephedrine, such as ephedroxane and N-ethyl benzamide has been reported in the aerial parts of some *Ephedra* species[29]. From the *E. alata* growing wild in southern Tunisia, 1,2-dimethyl-3-phenylaziridine (1.35-4.23 mg/g), azetidine (0.13-0.23 mg/g) and N-methyl-mandelamide (0.12-0.19 mg/g) are mentioned for are mentioned for the first time in this study, as alkaloids obtained from the *E. alata* aerial part.

Antioxidant activities

The antioxidant capacity obtained through the phosphomolybdenum, DPPH and FRAP methods for hexane extracts and alkaloids of the aerial part of *E. alata* in comparison with synthetic antioxidant BHA, BHT and ascorbic acid (AA) used as references, are shown in Table 5. Overall, the hexane extracts (from 4.0±0.10 to 38.9±1.44 GAE/g) and alkaloid fractions (from 10.4±0.02 to 155.2±1.40 GAE/g) showed a much lower total antioxidant when compared to standard BHA (851±25 GAE/g) or BHT (1004±13 GAE/g). These results are consistent with the lowest antiradical effects determined by the DPPH assay tested with a 5mg/ml concentration and the percentage activity has not acceded 45% for hexane extracts and 19% for the alkaloids. In contrast, all alkaloid fractions were found to possess a significant reducing power when compared to used standards. In fact, the obtained alkaloid from powdered fresh and dry *E. alata* plant materials were the best antioxidant as demonstrated by the highest values (1433±17 and 1200±6 µM, respectively) of antioxidant activities compared to hexane extracts (from 101.4± 4 to 563±11 µM TE/g) and ascorbic acid, BHA and BHT (1232±12, 1570±32 and 2380±62 µM TE/g, respectively).

Reports on antioxidant activity of *E. alata* hexane extract besides alkaloids are limited. It is known that in addition to pronounced inhibition of human breast cancer by the plant extract, *Ephedra* genus is a source of various phenolic compounds and therefore possesses a high antioxidant capacity[30-32]. Similar results are given with the hydroethanol extracts of Palestinian and Jordanian *E. alata* that exhibited a strongly and comparable antioxidant activities [31, 33, 34].

CONCLUSION

In conclusion, the volatile compounds, fatty acids and alkaloids obtained from Tunisian *E.alata* aerial part have been characterized and their antioxidant activity have been assessed

for the first time in this study. The chroamatography analysis indicated a strange complex phytochemistry of distinct natural products that may explain the diversified uses of the plant. The presence of pseudoephedrine as a major alkaloid compound which can be used for medical purposes or as a powerful and highly addictive stimulant, therefore a research on this plant might be of value in drug industry.

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