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Full Length Research Paper

Composition of volatile oils from leaf, stem, root, fruit, and flower of *Ruellia tuberosa* L. (Acanthaceae) from Nigeria

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We report composition of *Ruellia tuberosa* L. (Acanthaceae) leaf, stem, root, fruit, and flower volatile oils from Nigeria. The five volatile oils were obtained by hydro-distillation using all-glass Clevenger apparatus designed to British Pharmacoepia specifications and were procured in 0.09 to 0.36% yields. Each was separately examined using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. Our results revealed leaf oil contain 24 compounds, which make-up 86.95% of it; stem oil has 15 compounds (accounting for 93.96%); root oil with 42 compounds being 91.49%; fruit oil contain 60 compounds which amount to 89.68% and flower oil has 6 compounds representing 95.06% of the oil. Dominant compound sin each essential oil are (%): leaf (E-phytol 21.06, tributylacetyl citrate 19.44, heptacosane 7.55); stem (m-xylene 33.83, heptacosane 16.57, p-xylene 9.67); root (heptane 22.25, heptacosane 12.89, borneol 12.48); fruit (hexacosane 15.43, sextone 13.12, heneicosane 11.14) and flower (tributylacetyl citrate 67.78, 2-methyl-2-pentanol 10.15, 1-methyl-1-cyclopentanol 6.90). Important classes of compounds in Nigerian *R. tuberosa* volatiles are monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, hydrocarbons, aromatics, esters, alcohols, sulphur compounds, ketones and aldehydes.109 compounds were identified in the five essential oils of *R. tuberosa*. These compounds have high therapeutic effects and are characteristic of *R. tuberosa*. The oils are good sources of sextone (methylcyclohexane), β-linalool and alcohols. We present the volatile constituents in the leaf, stem, root, fruit, and flower of *R. tuberosa* which have not been earlier reported in literature.

Key words: *Ruellia tuberosa*, Acanthaceae, essential oil, gas chromatography-mass spectrometry (GC-MS), sextone, monoterpenoids, sesquiterpenoids, linalool, alcohol.

INTRODUCTION

Ruellia tuberosa L. (herb) commonly called 'cracker plant', often grown for ornamental purposes is an Acanthaceae: The 3rd largest tropical family of dicotyledonous plants with about 2500 species, most of which have medicinal values (Burkill, 1985). It has tuberous roots with leaf, stem, fruit and flower parts, is native to America, but widely spread in dry and hilly regions. *R. tuberosa* is traditionally used as diuretic, anti-

pyretic, analgesic, anti-hypertensive, anthelmentic, abortifacient, emetic, in curing bladder disease, kidney disorder, bronchitis, gonorrhoea and syphilis (De Fillpps et al., 2004). There are reports that it possess anti-oxidant, anti-microbial, anti-cancer, anti-nociceptive, anti-inflammatory and gastro-protective activities (De Fillpps et al., 2004; Chothani et al., 2010; Agnihotri et al., 2012; Andhiwal et al., 1985; Alam et al., 2009). The plant has emetic properties and serves as substitute for *ipecacuanha* plants, in the treatment of bladder stones. Its leaf decoction is used in treatment of bronchitis, and its tuberous roots are ingredient in health tonic (De Fillpps et al., 2004; Agnihotri et al., 2012; Samy et al., 2015). It displayed ulcer protective activity in male Wistar rats (Sri Kumar and Pardhasaradhi, 2013). Leaf extracts of *R. tuberosa* L. were reported to control lipid peroxide level and help strengthen antioxidant potential in diabetic rats (Manikandan et al., 2010). *R. tuberosa* root extracts displayed anti-oxidant activity, which were comparable with standards (Chothani and Mishra, 2012).

Preliminary phytochemical screening of ethyl acetate extract of *R. tuberosa* reveals presence of saponin, tannins, and flavonoids (SriKumar and Pardhasaradhi, 2013). Five bioactive flavonoids cirsimaritin, cirsiliol 4′-glucoside, cirsimarin, sorbifolin, and pedalitin, with three other metabolites betulin, vanillic acid, and indole-3-carboxaldehyde were isolated and reported from ethylacetate extracts of *Ruellia tuberosa.* First two flavonoids showed cytotoxicity against KB cell line with the IC_{50} values of 30.05 and 17.91 µg/mL, and the third flavonoid was cytotoxic against HepG2 cell line with an IC_{50} value of 38.83 µg/mL (Lin et al., 2006). Apigeninglucuronides have also been isolated from *Ruellia* (Subramanian and Nair, 1972, 1974). Subramanian and Nair reported that *R. tuberosa* leaves have only traces of apigenin and luteolin, but its flowers contain malvidin-3,5-diglucoside in appreciable amount (Subramanian and Nair, 1974).

Also the flower buds have maximum proportion of flavonoids yielding 3% of apigenin-7-O-glucuronide. Other flavones identified were apigenin-7-O-glucoside, apigenin-7-O-rutinoside and luteolin-7-O-glucoside (Subramanian and Nair, 1974). Aerial parts of *R. tuberosa* yielded 21-methyldammar-22-en-3β, 18, 27-triol atriterpenoid (Singh et al., 2002). Identified and reported essential oil components are sources of important data and information on the plant and its family classifications (Baser and Buchbauer, 2010). Twenty-five compounds were reported from GC-MS analysis of ethanol extract from tuber of *R. tuberosa* (%): Lupeol (68.14), stigmasterol (8.89), α-sitosterol (3.99), sucrose (2.24), 3α-bromo-cholest-5-ene (2.24), 2-methyl-octadecane (2.10), 2-methyl-nonadecane (1.93), 2-methyl-eicosane (1.79), hexacosane (1.43) and heptacosane (1.29) as its prominent compounds (Rajendra et al., 2014).

Previously we examined and reported volatile chemical compositions on three Acanthaceae: *Brillantaisia patula* (leaf and stem), *Hypoestes phyllostachya* (leaf and stem), *Asystasia gangetica* (aerial, seed and root) (Moronkola *et al.,* 2009a, 2009b, 2011). This study examines the composition of volatile content of leaf, stem, root, fruit, and flower of *R. tuberose* L. also an Acanthaceae, from Nigeria, which have not been earlier reported in literature.

MATERIALS AND METHODS

Plant material

Fresh samples of *R. tuberosa* were collected from Ibadan, Oyo State, Nigeria, on 15thOctober 2014. The plant was authenticated in the herbarium, Department of Botany, University of Ibadan, Ibadan, where some voucher samples have been deposited, with voucher number UIH - 22426. Collection of the sample was done during the day time. The plant was separated into leaf, stem, root, fruit, and flower parts.

Extraction of the essential oils

Each separated parts (leaf, stem, root, fruit, and flower) of *R. tuberosa* was crushed and hydro-distilled for 3 to 3½ h in an all glass Clevenger-type apparatus designed to British Pharmacopeia specifications and the oils refrigerated until analyses. Essential oils from the pulverized air-dried plant materials were procured in 0.09 to 0.36% yields (Table 1). Each of the oils had distinct characteristic pleasant smell.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses

Composition of the essential oils was determined by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890N gas chromatography hyphenated with an Agilent system mass detector Triple Quad 7000A in EI mode at 70 eV (m/z range 40 - 600 amu) with an ion source temperature of 250°C and an Agilent ChemStation data system. GC column was equipped with an HP-5MS column (30 m × 250 µm × 0.25 µm) a split-split less injector heated at 200°C and a flame ionization detector (FID) at 230°C. Oven temperature was programmed as follows: Initial temperature 40°C for 5 min, increased 5°C/min to 180°C for 6 min and then 10°C/min to 280°C for 12 min. Helium was the carrier gas at flow rate of 1 mL/min. Injection volume was 2.0 µL (split ratio 1:20).

The components were identified by comparison of their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data (Adams, 2007). The relative amount of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI value

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Table 1. Yields of volatile oils procured from leaf, stem, root, fruit, and flower of *R. tuberosa* L.

S/N	Plant parts	Weight of sample (g)	Weight of essential oil procured (g)	% Yield of essential oil procured	Physical examination (note)
1	Leaf	582	1.78	0.31	Herbal/ leafy
2	Stem	717	2.61	0.36	Slightly choking but pleasant
3	Root	421	0.39	0.09	Woody
4	Fruit	571	0.77	0.14	Fruity
5	Flower	52	0.13	0.25	Floral

of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5MS column.

RESULTS

Results of the yields (0.09 to 0.36%) of extracted essential oils from five parts of *R. tuberosa* L. (leaf, stem, root, fruit, and flower), with their physical examinations are presented in Table 1. GC and GC-MS analysis of the oils afforded the identification of 24 compounds in leaf, 15 in stem, 42 in root, 60 in fruit and 6 in flower. Results of the 109 identified compounds are presented in Table 2. Table 3 has results of the comparison of the eleven classes of compounds found in the five *R. tuberosa* essential oils studied.

DISCUSSION

Volatile oils from five parts of *R. tuberosa* L. (leaf, stem, root, fruit, and flower) were obtained by hydro-distillation. Oils were procured in 0.09 to 0.36% yields, each having characteristic distinctive notes: Leaf oil (0.31% yield) had a pleasant herbal to leafy note; stem oil (0.36%) possessed slightly choking but pleasant smell; root oil (0.09%) had woody odour; fruit (0.14 %) with fruity note, while the flower oil (0.25%) had a floral note (Table 1).

Compounds identified in each are listed in Table 2. A total of 109 volatile compounds had been identified in the five essential oils from the Nigerian *R. tuberosa*. Our results revealed leaf oil contain 24 compounds, which make-up 86.95 % of it; stem oil has 15 compounds (93.96 % of it); root oil with 42 compounds being 91.49% of it; fruit oil contain 60 compounds (89.68% of it); flower oil has 6 compounds responsible for 95.06%. Dominant compounds (%) in each essential oil are: Leaf (E-phytol 21.06, tributylacetyl citrate 19.44, heptacosane 7.55); stem (m-xylene 33.83, heptacosane 16.57, p-xylene 9.67); root (heptane 22.25, heptacosane 12.89, borneol 12.48); fruit (hexacosane 15.43, sextone 13.12, heneicosane 11.14) and flower (tributylacetyl citrate 67.78, 2-methyl-2-pentanol 10.15, 1-methyl-1-cyclopentanol 6.90).

The oils are generally good sources of sextone, β-linalool and alcohols, with monoterpenoids more abundant than sesquiterpenoids. Important classes of compounds in Nigerian *R. tuberosa* volatiles are monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, hydrocarbons, aromatics, esters, alcohols, sulphur compounds, ketones and aldehydes. Percentage of each of this is shown in Table 3, for the five *R. tuberosa* essential oils.

Each of the identified 109 compounds plays important role in the vast ethno-medicinal uses and biological activities demonstrated by *R. tuberosa* (De Fillpps et al., 2004; Chothani et al., 2010; Agnihotri et al., 2012; Andhiwal et al., 1985; Alam et al., 2009, Samy et al., 2015). High content of linalool in *R. tuberose* oils present anticonvulsant and hypnotic activities. This was reported for *Ocimum basilicum* essential oils (Ismail,2006). Limonene, which is in appreciable amount in*R. tuberosa* root and fruit oils, and citral compound (in leaf oil) have sedative and stimulant effects, they may be responsible for *R. tuberosa* application as antinociceptive and anticonvulsant. These activities have been observed and reported in the essential oils of *Lippia alba* and *Satureja hortensis* (Vale et al., 2002; Viana et al., 2000; Hajhashemi et al., 2002). β-caryophyllene and other sesquiterpenes are abundant in *R. tuberose* leaf, root and fruit essential oils, known to be responsible for high anticancer properties, hence responsible for anti-cancer effects of *R. tuberose* (Sylvestre et al., 2005, 2006). Limonene (in root and fruit oils), with perillyl compounds and other monoterpenoids in *R. tuberose* essential oils are agent with special benefit as anti-tumor (Jahangir and Sultana, 2007; Low-Baselli et al., 2000).

Conclusion

Our study on the five volatile oils of *R. tuberosa* L. from Nigeria resulted in the identification of a total of 109 compounds. Monoterpenoids are more abundant than sesquiterpenoids, with the oils rich in esters, alcohols, aromatics and carbonyls. The oils are good sources of sextone, β-linalool and alcohols. We have presented the volatile constituents in *R. tuberosa* (leaf, stem, root, fruit, and flower) which have not been earlier reported in

Table 2. Chemical composition of volatile oils of leaf, stem, root, fruit, and flower of *R. tuberosa* L.

S/N	KI	Compound	% Composition in				
			1.Leaf (LF)	2.Stem (ST)	3.Root (RO)	4.Fruit (SD)	5.Flower (FL)
1	681	2-pentanol	-	-	-	-	4.16
2	688	2,5-Dimethylhexane	-	-	-	0.14	-
3	709	3-Methyl-3-pentanol	-	-	-	0.57	-
4	710	2-methyl-2-pentanol	-	-	-	-	10.15
5	717	Heptane	-	-	22.25	-	-
6	722	Dimethyl disulfide	-	-	-	0.16	-
7	752	3-Methylheptane	-	-	-	0.33	-
8	754	3-Hexanone	-	-	0.25	-	-
9	781	Sextone	2.63	7.92	5.14	13.12	-
10	785	n-Butylacetate	-	-	-	8.36	-
11	806	Hexanal	0.30	-	1.12	-	-
12	811	1-methyl-1-cyclopentanol	-	-	-	-	6.90
13	814	(E)-2-hexenal	5.34	0.93		-	-
14	842	(Z)-1,3-Dimethylcyclohexane	-	-	0.23	1.11	-
15	843	1-hepten-3-one	-	1.37	-	-	-
16	853	2-Heptanone	-	-	-	0.14	-
17	860	Amylcarbinol	-	1.50	-	-	-
18	868	(Z)-hex-3-en-1-ol	2.12	-	-	-	-
19	868	(E)-2-hexan-1-ol	1.04	-	-	-	-
20	880	Ethylcyclohexane	-	-	-	0.32	-
21	893	Ethylbenzene	-	7.77	0.35	0.88	-
22	905	Heptenal	-	-	-	0.49	-
23	906	Heptanal	-	-	0.25	-	-
24	907	m-xylene	-	33.83	-	-	-
25	907	p-xylene	2.51	9.67	-	-	3.22
26	916	Nonane	-	0.45	-	-	-
27	919	(E)-2-Hexenal	-	-	-	0.35	-
28	943	1-octen-3-one	1.97	-	-	-	-
29	952	3-octenone	-	-	2.59	-	-
30	953	3-Octanone	1.90	5.04	-	0.94	-
31	969	1-Octen-3-ol	3.31	-	-	0.71	2.85
32	979	3-Octanol	2.56	1.95	0.90	0.42	-
33	982	Benzaldehyde	0.34	-	1.86	0.17	-
34	995	Ethylhexanol	-	0.73	-	-	-
35	1005	Octanal	-	-	-	0.37	-
36	1015	Decane	-	-	-	0.18	-
37	1018	Limonene	-	-	0.34	0.14	-
38	1030	2,4-Dimethylcyclohexanol	-	-	-	0.26	-
39	1040	2-pentylfuran	-	-	0.41	-	-
40	1081	Phenylacetaldehyde	-	-	0.44	0.63	-
41	1082	β-Linalool	5.71	5.32	2.88	1.27	-
42	1088	Borneol	-	-	12.48	-	-
43	1104	Nonanal	-	0.44	4.07	0.69	-
44	1112	(E)-2-Nonenal	-	-	1.57	0.65	-
45	1121	Fenchone	-	-	0.57	-	-
46	1130	Dihydrocitronellol	-	-	-	0.24	-
47	1136	2-methyl-2-nonen-4-one	0.96	-		-	-
48	1138	Exofenchol	-	-	3.58	0.41	-
49	1140	3-methyl-2-(cyclohexen-1-yl)-acetaldehyde	-	-	2.74	-	-

Table 2. Cont'd.

50	1143	α-terpineol	-	-	0.85	-	-
51	1150	4-Hydroxyl-3-propyl-2-hexanone	-	-	0.38	0.49	-
52	1158	(Z)-β-Terpineol	-	-	-	0.13	-
53	1159	1-nonanol	-	-	0.54	-	-
54	1161	2-Methyl isoborneol	-	-	-	0.45	-
55	1171	p-Anisaldehyde	-	-	-	0.25	-
56	1175	(Z)-piperitol	-	-	0.71	-	-
57	1183	Methyl-nonanoate	-	-	0.60	-	-
58	1186	Safranal	0.53	-	-	-	-
59	1188	2,4-nonadien-1-ol	-	-	0.23	-	-
60	1204	Decanal	-	-	-	0.17	-
61	1205	β-cyclocitral	0.28	-	-	-	-
62	1206	(E)-carveol	-	-	0.50	-	-
63	1207	Perilla aldehyde	-	-	1.23	-	-
64	1215	Decane	-	-	0.82	-	-
65	1218	p-Acetylanisole	-	-	-	0.21	-
66	1218	3-acetylanisole	0.21	-	-	-	-
67	1220	(E,E)-2,4-decadienal	-	-	0.54	-	-
68	1231	Naphthalene	-	0.47	-	-	-
69	1251	2-Undecanone	-	-	-	0.33	-
70	1261	Perilla alcohol	-	-	0.70	-	-
71	1281	Isoaromadendrene epoxide	-	-	1.80	-	-
72	1339	β-Cubebene	-	-	-	0.16	-
73	1371	4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-1H-inden-1-one	0.55	-	-	-	-
74	1398	Cedrene	-	-	-	0.59	-
75	1403	β-Gurjunene	-	-	-	3.41	-
76	1407	4-Acetonylcycloheptanone	-	-	-	0.18	-
77	1410	2-Dodecanal	-	-	-	0.80	-
78	1416	(-)-α-Panasinsen	-	-	-	0.49	-
79	1416	Thujospene	-	-	-	0.15	-
80	1419	α-gurjunene	-	-	0.41	-	-
81	1420	Geranylacetone	-	-	0.49	0.44	-
82	1424	(E)-1,10-Dimethyl-(E)-9-decalinol	-	-	-	1.60	-
83	1432	β-Patchoulene	-	-	-	0.39	-
84	1440	Cadinene	-	-	-	0.46	-
85	1457	β-Ionone	4.01	-	-	0.24	-
86	1465	γ-elemene	-	-	0.60	-	-
87	1471	9,9-Dimethyl-9-silafluorene	-	-	-	0.26	-
88	1474	Eremophilene	-	-		0.65	-
89	1475	α-salinene	-	-	0.46	-	-
90	1490	α-Guaiene	-	-	-	0.13	-
91	1494	β-caryophyllene	0.44	-	1.24	-	-
92	1519	2,6,10-Trimethyltetradecane	-	-	-	0.66	-
93	1524	α-curcumene	-	-	0.29	-	-
94	1558	2,2,7,7-Tetramethyltricyclo(6.2.1.0)(1,6)undec-4-en-3-one	-	-	-	3.26	-
95	1564	(+)-(E)-Nerolidol	-	-	-	0.69	-
96	1580	α-Cadinol	-	-	-	0.88	-
97	1593	β-eudesmol	-	-	0.61	-	-
98	1660	Germacrene D-4-ol	-	-	1.19	0.86	-

Table 2. Cont'd.

99	1754	Hexahydrofarnesylacetone	0.84	-	-	1.47	-
100	1890	2-Methylhexadecan-1-ol	-	-	-	0.35	-
101	1910	Nonadecane	-	-	-	1.53	-
102	1999	Octadecanal	-	-	0.39	-	-
103	2046	E-phytol	21.06	-	-	-	-
104	2109	Heneicosane	-	-	-	11.14	-
105	2336	2-(Z)-9-Octadecenyloxyethanol	-	-	-	0.26	-
106	2594	tributylacetyl citrate	19.44	-	-	-	67.78
107	2606	Hexacosane	-	-	-	15.43	-
108	2705	Heptacosane	7.55	16.57	12.89	-	-
109	2804	Octacosane	1.35	-	-	8.12	-
% Identified			86.95	93.96	91.49	89.68	95.06

Table 3. Classes of compounds in the five *R. tuberosa* volatile oils.

S/N	Class of compound	% in each of the *R. tuberosa* essential oils				
		Leaf	Stem	Root	Fruit	Flower
1	Monoterpenes	-	-	0.34	0.14	-
2	Monoterpenoids	10.53	5.32	25.08	3.18	-
3	Sesquiterpenes	0.44	-	3.00	6.57	-
4	Sesquiterpenoids	0.84	-	1.80	3.9	-
5	Hydrocarbons	11.53	24.94	41.33	52.2	-
6	Aromatics	2.72	51.74	0.76	1.09	3.22
7	Esters	19.44	-	0.60	8.36	67.78
8	Alcohols	30.09	4.18	2.38	4.17	24.06
9	sulphur compounds	-	-	-	0.16	-
10	ketones	5.38	6.41	3.22	5.34	-
11	Aldehydes	5.98	1.37	12.98	4.57	-

literature.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Adams RP (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 4th Edition. Allured publishing corporation, Carol Stream, IL. pp. 1-804.

Agnihotri AG, Raghavan R, Premnath S (2012). Field guide to Herbs. Kaigal Environment Education Programme (KEEP), Krishnamurthy Foundation India (KFI), Baireddipalli Post, Totakanama, Kaigal 517415.

Alam MA, Subhan N, Awal MA, Alam MS, Sarder M, Nahar L, Sarker SD (2009). Antinociceptive and anti-inflammatory properties of Ruellia tuberosa. Pharm. Biol. 47(3):209-214.

Andhiwal CK, Chandra RP, Varshney H (1985). Phytochemical investigation of *Ruellia tuberosa* L. Indian Drugs 23:40-49.

Baser KHC, Buchbauer G (2010). Handbook of essential oils, science, technology, and applications. CRC Press. pp. 1-948.

Burkill HM (1985). The useful plants of west tropical Africa. Families A-D.1:26-30.

Chothani DL, Mishra SH (2012). *In vitro* anti-oxidant activity of *Ruellia tuberosa* root extracts. Free Rad. Antioxid. 2(4):38-44.

Chothani DL, Patel MB, Mishra SH, Vaghasiya HU (2010). Review on Ruellia tuberosa (cracker plant). Pharmacogn. J. 2(12):506-512.

De Fillpps RA, Maina SL, Crepin J (2004). Medicinal plants of the Guianas (Guyana, Surinam, French Guiana). Washington, DC: Department of Botany, National Museum of Natural History, Smithsonian Institution. pp.15-48, 233-485.

Hajhashemi V, Ghannadi A, Pezeshkian SK (2002). Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil. J. Ethnopharmacol. 82(2-3):83-87.

Ismail M (2006). Central properties and chemical composition of *Ocimum basilicum* essential oil. Pharm. Biol. 44(8):619-626.

Jahangir T, Sultana S (2007). Perillyl alcohol protects against Fe-NTA-induced nephrotoxicity and early Tu, promotional events in rat experimental model. Evid. Based Complement. Altern. Med. 4(4):439-445.

Lin CF, Huang YL, Cheng LY (2006). Bioactive flavonoid from *Ruellia tuberosa*. J. Chin. Med. 17(3):103-109.

Low-Baselli A, Huber WW, Kafer M, Bukowska K, Schulte-Herrmann R, Grasl-Kraupp B (2000). Failure to demonstrate chemoprevention by the monoterpene perillyl alcohol during early rat hepatocarcinogenesis: A cautionary note. Carcinogenesis 21(10):1869-1877.

Manikandan A, Arokia V, Doss D (2010). Effect of 50 % Hydroethanolic Leaf Extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on Non-enzymic Antioxidants and other Biochemical Parameters in Liver, Kidney, Serum of Alloxan Induced Diabetic Swiss Albino Rats. J. Biomed Sci. Res. 2(3):190-201.

Moronkola DO (2011). Essential oils from aerial, seed and root of Nigerian *Asystasia gangetica* (L). J. Essent. Oil Bearing Plants 14(5):582-589.

Moronkola DO, Atewolara-Odule OC, Olubomehin OO (2009b). Compositions and comparison of the leaf and stem essential oils from Nigerian Hypoestes phyllostachya 'rosea'p. Beau.[acanthaceae]. Afr. J. Pharm. Pharmacol. 3(9):458-462.

Moronkola DO, Faruq UZ, Osho A (2009a). Essential Oils of Stem and Leaf from Nigerian Brillantaisia patula T. and. var. J. Essent. Oil Bearing Plants 12(5):569-573.

Rajendra KN, Vasantha K, Mohan VR (2014). GC-MS Analysis of Bioactive Components of Tubers of *Ruellia tuberosa* L. (Acanthaceae). Americ. J. Phytomed. Clin. Therapeut. 2(2):209-216.

Samy MN, Sugimoto S, Matsunami K, Otsuka H, Kamel MS (2015). Chemical constituents and biological activities of genus *Ruellia*. Int. J. Pharmacog. 2(6):270-279.

Singh RS, Pandey HS, Pandey RP, Singh BK (2002). A new triterpenoid from *Ruellia tuberosa* linn. Ind. J. Chem. 41B(8):1754-1756.

SriKumar PP, Pardhasaradhi P (2013). Preliminary phytochemical investigation and anti-ulcer activity of aerial parts of *Ruelliatuberosa*L. (Acanthaceae) in male Wistar rats. Int. J. Pharm. Biomed Res. 4(3):145-148.

Subramanian SS, Nair AGR (1972). Flavonoids of *Ruellia prostrate* and *Barleria cristata*. J. Ind. Chem. Soc. 49:825-826.

Subramanian SS, Nair AGR (1974). Apigenin glycoside from *Thunbergia fragrans* and *Ruellia tuberosa*. Curr. Sci. 480.

Sylvestre M, Legault J, Dufour D, Pichette A (2005). Chemical composition and anticancer activity of leaf essential oil of *Myricagalae* L. Phytomedicine 12(4):299-304.

Sylvestre M, Pichette A, Longtin A, Nagau F, Legault J (2006). Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. J. Ethnopharmacol. 103(1):99-102.

Vale TG, Furtado EC, Santos Jr JG, Viana GSB (2002). Central effects of citral, myrcene and limonene constituents of essential oil chemotypes from *Lippia alba* (Mill.) N.E. Brown. Phytomedicine 9:709-714.

Viana GSB, Vale TG, Matos FJA (2000). Anticonvulsant activity of essential oils and active principles from chemotypes of *Lippia alba* (Mill.) N.E. Brown. Biol. Pharm. Bull. 23:1314-1317.

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Full Length Research Paper

Towards elucidating *Eremurus* root remedy: Chemical profiling and preliminary biological investigations of *Eremurus persicus* and *Eremurus spectabilis* root ethanolic extracts

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Plants of the genus *Eremurus* have been used in Kurdish medicine since ancient times. Particularly, the "*Eremurus* roots" sold at the local market (presently known as *Eremurus* spp.) are used as both topical and oral remedies for treating inflammatory disorders. In the present paper, the root ethanolic extracts (EE) of *Eremurus persicus* and *Eremurus spectabilis,* the most common *Eremurus* species in the region, were prepared and their phytochemical profiles drawn with the aim of identifying the plant material sold at the local market. Comparison of the high performance liquid chromatography-ultraviolet/photodiode-circular dichroism (HPLC-UV/PAD-CD) and HPLC-electrospray ionisation-tandem mass spectrometry (HPLC-ESI-MS/MS) fingerprints of both species with that of *Eremurus spp.* showed that the main component of *Eremurus* spp. is *E. spectabilis*, with a minor presence of *E. persicus.* All EEs were then subjected to an exhaustive *in vitro* investigation of anti-inflammatory activity. The assay with cultured human peripheral blood mononuclear cells (hPBMC) activated by phytohaemagglutinin A revealed that *E. persicus* was more effective in inhibiting T-cell proliferation and in reducing the tumor necrosis factor alpha (TNFα) levels *in vitro* than *E. spectabilis* and *Eremurus* spp. Regarding the *Eremurus* spp. extract, its anti-inflammatory effect appeared to be intermediate compared to the other two extracts examined when considering both anti-proliferative and anti-TNF-α results and in accordance with the analytical results, thus validating the ethnomedical use of *Eremurus* roots in traditional Kurdish medicine. Moreover, results herein reported clearly showed the anti-inflammatory potential of *E. persicus* and its capacity to inhibit both *in vitro* hPBMC proliferation and cytokine secretion at non-toxic doses. *E. persicus* root ethanolic extract will undergo further investigations in the near future in order to identify the metabolites responsible for the activity.

Key words: *Eremurus,* Kurdish ethnomedicine, chromatographic profile, anti-inflammatory activity, TNF-α blocker.

INTRODUCTION

In the last two decades, an increasing use of herbal drugs was recorded, probably due to the media contributing to the mass spread of traditional medicine (Mati and de Bore, 2011). In particular, Kurdish folkloric herbal medicine still represents the first choice for primary healthcare in towns, villages and rural areas. Although sometimes, plant remedies could be directly collected in the wild by the final users, only a small number of people retain the knowledge about the correct identification of the plants and their proper use. For these reasons, people usually rely on local markets in order to get rapid insight into the use of plants in traditional medicine and to purchase them (Cunningham, 2001). Nevertheless plants sold in local markets are often not correctly labelled or identified. This is the case of "*Eremurus* roots", which is sold in many kurdish local markets without any indication of the species on the label (from this point on this will be referred to as *Eremurus* spp.) and is used for treating skin inflammatory disorders, both as a topical and an oral remedy (folk medicine).

The plants of the genus *Eremurus* (Xanthorrhoeaceae) comprise nearly 50 species and are mainly restricted to the mountains of central and western Asia (Li et al., 2000). Among them, *Eremurus persicus* (Jaub and Spach) Boiss is widely distributed in the south, east and west of Iran, where it is called "*Sarish*" (Karl, 1982; Wendelibo, 1982; Safar et al., 2009; Vala et al., 2011) and *Eremurus spectabilis* M.Bieb. is mainly distributed in Central Asia (Kurdistan, Turkey, Iran), where it is called "*Ciris*" (Karl, 1982; Ozturk and Olcucu, 2011). Both species have been traditionally used in Kurdish ethnomedicine to cure diseases having a common pathophysiological factor related to inflammation. It has been reported that the aerial parts of the plants, along with the rhizome and root nodules are eaten by native people for treating inflamed eyes, diabetes and eczema (Karaman et al., 2011; Yesil and Akalin, 2009), while their boiled roots are described to be efficacious in relieving rheumatism, gastrointestinal disorders (Ozturk and Olcucu, 2011), scabies (Karaman and Kocabas, 2001) and inflammatory skin conditions (Mamedov and Gardner, 2004) (Table 1).

In this context, the present work was focused on the identification of the species of *Eremurus* possibly present in "*Eremurus* roots" sold at the Sulaymaniyah market (north-western Iraq/Kurdistan) and locally known as *Eremurus* spp. With this aim, since *E. persicus* and *E. spectabilis* are the most common species of *Eremurus* in the kurdish area, both were collected in the Iranian/Iraqi mountains, "*Eremurus* roots" were purchased and a preliminary phytochemical investigation of all drugs was performed. Firstly, different extracts of *Eremurus* spp., *E. persicus* and *E. spectabilis* roots, were prepared and their phytochemical fingerprints were drawn by using high performance liquid chromatography-ultraviolet-photodiode array detector coupled on–line to a circular dichroism detector (HPLC-UV/PAD-CD) as well as HPLC-tandem mass spectrometry (HPLC-ESI-MS/MS) and compared. To the best of our knowledge, no study has been carried out to draw the phytochemical profiles of root extracts obtained from different *Eremurus* species. Indeed, polysaccharides are considered to be the chemical constituents targeting the species, and fructans in the case of *E. spectabilis* (Pourfarzad et al., 2014, 2015), and no scientific investigations into the phytochemical contents of *E. persicus* or *E. spectabilis* root extracts have been reported so far.

MATERIALS AND METHODS

Chemicals

All solvents used were of analytical and high performance liquid chromatography (HPLC) grade, and purchased from Carlo Erba (Milano, Italy). The 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), polyvinylpyrrolidone (PVP), and gallic acid were obtained from Sigma Aldrich (Milan, Italy). Folin-Ciocalteau reagent (FCR) was purchased from Carlo Erba (Milano, Italy). Green tea extract (Green Select®) used as an antioxidant standard was obtained from Indena (Milano, Italy). RPMI-1640 medium was purchased from Gibco, Life Technologies (Monza, Italy), foetal bovine serum (FBS) from EurocloneS.p.A (Milan, Italy) and Phytohaemagglutinin A (PHA) from Roche (Mannheim, Germany). The 3H-thymidine (3HTdR) was obtained from Amersham Pharmacia Biotec (Milan, Italy). MTS reagent was bought from Promega (Milan, Italy).

Plant material

Powdered *"Eremurus* spp." drug was purchased from Sulaymaniyah market, located in north-western Iraq/Kurdistan. *E. persicus* Boiss was collected in an area of the Gulestan Kuh mountain probe Golpayegan at an altitude of 3000 to 3200 m located 120 km from Isfahan/Iran, in August, 2011. This is a spontaneous and ornamental plant, it can reach 30 to 70 cm in height, seeds 8 to 10 mm long, broadly winged. The leaves can be as broad as 1 cm on the base of the stem. Its tuberous roots have a very strange shape; thick fleshy roots fan out in all directions from a central hub. These roots must be handled very carefully. *E. spectabilis* Bieb was collected from the KaniMeran village (KaniMeran mountain), which belongs to the Penjwen-Sulaymaniyah/Kurdistan region, north of Iraq, in July, 2011. This is an uncultivated perennial herbaceous vigorous plant shrub that can reach a height 75 to 200 cm, with shortened rhizome and radically divergent fusiform thickened roots. The leaves can broad 4.5 cm glabrous, margin scabrid or smooth, the roots are short, fleshy and descending. The collected plant materials were identified and classified by Dr. Abdulla Sa'ad at the

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Education Science Department, Faculty of Biology, Salahaddin University, Hawler, Iraq. The voucher specimens (no. 6856) and (no.6873), respectively were deposited in the ESUH (Education Salahaddin University Herbarium), Hawler, Iraq. Freshly cut roots were stored, dried in a drying room with active ventilation at room temperature (about 20 to 22°C) until they reached a constant weight. Roots were cut into a small size and ground with a blade mill to obtain a homogeneous fine powder. The drug was stored in the dark.

Extraction

Eremurus spp. purchased from the market, as well as the dried powdered roots of *E. persicus* and *E. spectabilis* were defatted with petroleum ether 10% (w/v) for 1 h at room temperature under mechanical stirring (Gaggeri et al., 2012), before the extraction was performed. Each dried drug (50 g) was macerated with ethanol (1 L) for 1 h (EE) at room temperature under mechanical stirring. The extraction procedure was repeated three times (each drug), and the filtrates obtained were combined and dried under reduced pressure (Laborota 4000, Heidolph Instruments, Schwabach, Germany). All crude extracts were treated with: 1) charcoal, and 2) polyvinylpyrrolidone (PVP). First, charcoal (0.5 g) was added to crude extract (1 g), dissolved in methanol (500 ml solution) under mechanical stirring for 15 min at room temperature. The solvent was then evaporated under reducing pressure. Second, treatment with PVP was used, as reported by Makkar et al. (1993). Briefly, 1 g extract was suspended in acetone 70% (50 ml) and ethanol (50 ml) before adding 1 g of PVP. After 15 min of mechanical stirring in an ice bath, the solvent was filtered and evaporated at reduced pressure. All obtained dried samples were kept at room temperature in dark conditions.

Phytochemical profile

The ethanolic extracts (EE) were analysed by thin layer chromatography (TLC) on silica gel with a mobile phase of ethyl acetate-methanol-water (100:13.5:10 v/v/v) and revealed with cerium reagent, using UV-365 nm light.

Total phenolics content (TPC)

The TPC of each extract was determined by the method described by Singleton et al. (1999), with some modifications. Briefly, 1 ml Folin-Ciocalteau reagent (FCR) was added to 9 ml of deionized water and kept in a dark bottle. The reaction mixture consisted of 1 ml of sample solubilized in 10% EtOH, 6 ml of deionized water and 500 µl of FCR. The mixture was stirred at room temperature for 3 min, before 1.5 ml of sodium carbonate solution (20% w/v) was added. The mixture was then diluted in water to 10 ml and stored in the dark for 2 h at room temperature. Absorbance of mixtures was measured at 760 nm, against a blank containing 1 ml of EtOH 10%. TPC of each individual sample was expressed as % (w/w) of gallic acid. The analyses were conducted in triplicate and results expressed as mean ± standard error (SE).

HPLC-PAD/UV-CD and HPLC-ESI-MS/MS analysis

EE were analysed using both a high performance liquid chromatography-diode array system coupled on–line to a circular dichroism detector, and high performance liquid chromatography-electrospray-tandem mass spectrometry. Each sample was dissolved in methanol (3 mg/ml) and filtered with a 0.45 µm GH polypro (GHP) membrane before injection into the HPLC-system. The samples were separated on a Chromolith SpeedROD RP-18 endcapped column (50 mm × 4.6 mm, ID 3 mm, macropore size 2 µm, mesopore size 13 nm, Merck, Darmstadt, Germany). The mobile phase consisted of water containing 0.1% (v/v) formic acid (A) and acetonitrile (B), in gradient elution: 2% of B for 5 min, from 2 to 5% B in 5 min, from 5 to 40% B in 20 min, from 40 to 90% B in 10 min, from 10 to 98% B in 10 min, followed by a re-equilibration step of 5 min. The flow rate was set at 1 ml/min and detection was fixed at 297 nm for both UV and CD detectors.

HPLC-UV/PAD-CD analyses were performed on a Jasco system (Japan) equipped with a Jasco AS-2055 plus autosampler, a PU-2089 plus pump and a MD-2010 plus multi-wavelength detector coupled to a CD-2095 plus circular dichroism detector. The HPLC-ESI-MS/MS analyses were carried out on Finnigan LCQ fleet ion trap system, controlled by Xcalibur software 1.4 (ThermoFinnigan, San Jose CA, USA). Mass spectra were generated both in positive and in negative ion mode under constant instrumental conditions. For positive ion mode: ion spray voltage 5 kV, capillary voltage 46 V, capillary temperature 220°C, and tube lens voltage 120 V. For negative ion mode: ion spray voltage 5 kV, capillary voltage -35 V, capillary temperature 220°C, and tube lens voltage -100 V.

Free radical scavenging activity

The free radical scavenging activity (FRS) of the extracts was determined by using a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (Gaggeri et al., 2012). A commercially available standardized green tea extract (Green Select®) was used as a standard. Briefly, both dried extracts and the standard were dissolved in MeOH at a concentration of 10 mg/ml; stock solutions were then serially diluted in MeOH two-fold. The reaction mixture was prepared by adding 100 µl of each extract solution (or standard solution) to 3.9 ml of DPPH solution, freshly prepared by dissolving DPPH in methanol/KH_2PO_4 and NaOH buffer (50/50 v/v) at a concentration of 6×10^{-5} M, giving test solutions with final concentrations of 250, 125, 62.5, 31.25, 15.62, 7.81 µg/ml. After 30 min of incubation at room temperature, the absorbance was measured at 515 nm by a UV-Visible spectrophotometer (Lambda 25 UV/VIS spectrometer, Perkin Elmer instruments, Massachusetts, USA). FRS was expressed as a percent compared with the control, consisting of 3.9 ml of DPPH solution and 100 µl of methanol. The percent inhibition of the DPPH radical by the test solution was calculated using the following formula:

$$FRS\% = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

The analyses were carried out in triplicate and results expressed as mean ± SE. IC_{50} values were calculated by using Graph Pad Prism 4.0.

Anti-inflammatory activity

An *in vitro* proliferation assay on human peripheral blood mononuclear cell (hPBMC) was carried-out as previously described (Gaggeri et al., 2013b). Briefly, hPBMC were obtained from healthy donor peripheral blood and grown in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS), with or without 4 µg/ml of phytohaemagglutinin A (PHA). hPBMC were cultured in the presence of increasing doses of test solutions [dimethyl sulphoxide (DMSO) solutions of *Eremurus spp.*, *E. persicus* and *E. spectabilis* extracts diluted in a 1:2 (v/v) with medium; final concentrations: 800 to 12 µg/ml] or vehicle (control cells). After a

Table 1. *Eremurus* drugs investigated.

Species	Part used in this study	Origin	Folk medicine
E. persicus Boiss.	Roots	Isfahan region (Iran)	Inflammation and skin disorder (folkloric); source of natural glue (Vala et al., 2011)
E. spectabilis Bieb.	Roots	Kurdistan region (Iraq)	Scabies (Karaman and Kocabas, 2001); rheumatism and gastrointestinal disorders (Ozturk and Olcucu, 2011); treatment of inflammatory skin conditions (Mamedov and Gardner, 2004); rheumatism (Cakilcioglu et al., 2011)
Eremurus spp.	Roots	Sulaymaniyah market (Iraqi Kurdistan)	Inflammation and skin disorders (folkloric) (Mamedov and Gardner, 2004)

3-day incubation, 18 h before harvesting, 25 µCi/well of ^{3}H-thymidine (^{3}H-TdR) was added to each well. Radioactivity was measured (TopCount, Packard Instrument) and results were expressed as stimulation index (SI = cpm of simulated cultures/cpm of unstimulated cultures). Cell cultures were carried out in the presence of DMSO alone (at concentrations used in the test solutions) and the effect of DMSO was subtracted. The viability of cultured cells, under the same experimental conditions, was evaluated by the trypan blue exclusion method.

Measurements of cytokines in supernatants

Under the same experimental conditions cytokine measurement was performed. The concentrations of TNF-α, IL-6 and IFN-γ in supernatants were quantified by ELISA using monoclonal antibody pairs (Pierce Endogen, Rockford, IL, USA). Plates were read at 450 nm (Titertek Plus MS 212M).

Cell viability assay

A cell viability test was performed to assess the effect of the three extracts on cell growth. Three tumour cell lines were used: A549 (lung cancer), MCF-7 (breast cancer) and CaCo-2 (colon cancer). The tumour cell lines were grown in RPMI 1640 supplemented with L-glutamine, penicillin, streptomycin and 10% foetal bovine serum in a 5% CO_2 incubator at 37°C. After a proper dilution, separated cells were plated in 96-well flat-bottom microplates at a density of 3×10^3 cells in 100 µl of growth medium. After 12 h, growth medium was replaced with 100 µl of test medium (growth medium plus DMSO extracts). The following concentrations were used: 600, 60, 6, 0.6, and 0.06 µg/ml. DMSO extracts were diluted to a final DMSO concentration of 2.5%. After 72 h incubation, the medium was replaced and 20 µl of MTS reagent was added to each well. After a 2 h incubation, the absorbance was measured at 490 nm wavelength using a plate reader. Five wells for each experimental point were used and each experiment was performed at least twice.

Statistical analysis

Two way analysis of variance considering concentrations and different extracts was performed. Post hoc comparisons between extracts were performed with the Wald test, applying the Bonferroni correction for multiple comparisons. Results where $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Extracts of all three drugs (*Eremurus* spp*, E. persicus* and *E. spectabilis*) were prepared and their biological properties evaluated. First, extractions were performed using water and a water/methanol mixture, according to the literature (Karaman et al., 2011). In both cases, the formation of highly viscous solutions was observed, probably due to the presence of glucomannan, a water-soluble polysaccharide (Smirnova et al., 2001). Therefore, we modified the extraction protocol, avoided the water addition and used ethanol instead of methanol because of its lesser toxicity and more environmentally friendly. This choice is in accordance with the principles of green chemistry and in line with the European directives for products for human use. The ethanolic extracts (EE) were prepared by dynamic maceration (ME) overnight and treated with vegetal charcoal, as previously reported in the literature (Iqbal et al., 2005; Martino et al., 2008; Uddin et al., 2012). After the removal of solvent, this procedure led to well-dried yellow solid. Finally, to remove tannins, which may interfere with some biological assays, a treatment with polivinylpirrolidone (PVP) was performed (Makkar et al., 1993). The extraction yields are reported in Table 2.

The TLC analyses of all EE and the revelation with different reagents showed the presence of polyphenols, flavonoids and naphthoquinones, while coumarins, alkaloids and saponins were not detected. The total phenolic content of the EE of the three samples of *Eremurus*, determined using the Folin-Ciocalteau assay, ranged from a mean (± standard deviation) of 44.93% (±0.28) to 49.84% (±0.54) compared to the standard. These data are very similar to each other.

In order to obtain the phytochemical fingerprint

Table 2. Extraction procedures.

Drug	Extraction method	Time	Yield (%, w/w)
E. persicus	ME	Overnight	**10.0**
E. spectabilis	ME	Overnight	**3.3**
Eremurus spp.	ME	Overnight	**3.7**

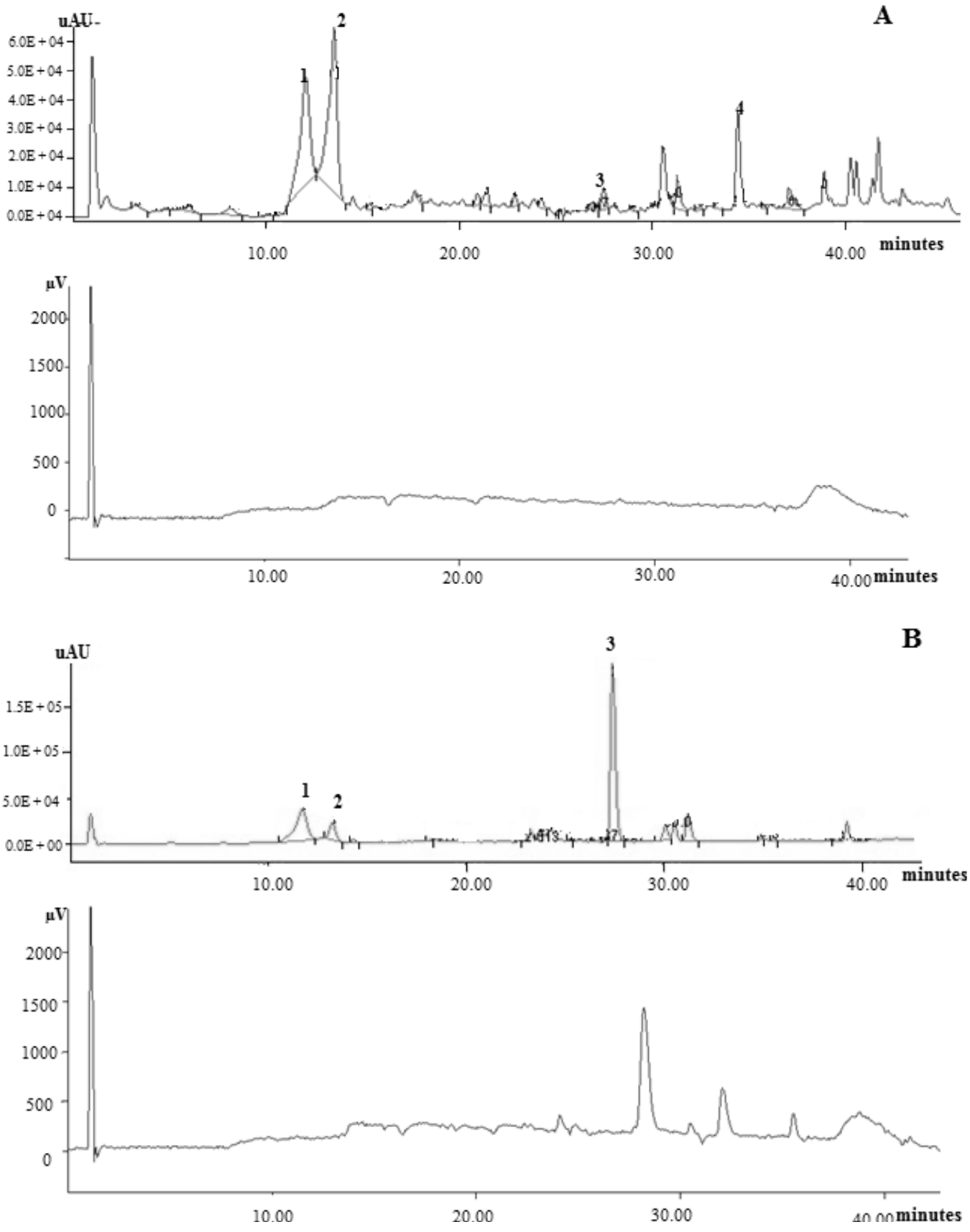
of Eremurus species here studied, a reliable chromatographic method was developed by using HPLC-UV/PAD-CD, a powerful tool for a rapid detection of chiral compounds naturally occurring in crude extracts. By comparing the HPLC-UV/PAD chromatograms of the three extracts acquired at different wavelengths of each main peak, it was found that 297 nm best represented the profile of the major constituents. Representative HPLC-UV/PAD-CD chromatograms (297 nm) for each ethanolic extract are reported in Figure 1. The developed chromatographic method was also applied to HPLC-ESI-MS/MS experiments and the MS experimental parameters were optimized for both positive and negative ion modes. The two early peaks with retention time (RT) ranging from 12.4 to 13.5 min (peak 1 and 2) were present in all chromatograms, with highest intensity in *Eremurus* spp. and *E. spectabilis* extracts (Figure 1). The nearly identical UV spectra profiles (maximum at 220, 295 and 320 nm, Figure 2) of these two peaks suggested that they may belong to the same phytochemical class and their CD traces showed that they are not optically active (Figure 1). ESI-MS spectrum in negative-ion mode of peak 1 showed a quasi-molecular ion $[M-H]^-$ at 179 m/z and an MS^2 spectrum with a prominent 135 m/z. Regarding peak 2, an intense ion at 393 m/z in the negative ion mode and 395 m/z in the positive ion mode allowed us to predict a molecular weight of 394 Da. The ESI-MS and UV spectra of compound 1 (negative ion) and 2 (positive ion) are reported in Figure 2.

Interestingly, both "*Eremurus spp*" *and E. spectabilis* HPLC-UV/PAD profiles showed a prominent peak at 34.4 min (peak 4), which was undetectable in *E. persicus* (Figure 1). The analysis of UV, MS and MS^2 spectra unambiguously showed that the peaks referred to the same compound. This compound (still unknown) has a molecular weight of 540 Da, given that the corresponding MS spectrum clearly display the quasi-molecular ion $[M+H]^+$ at 541 m/z (Figure 3). *E. persicus* chromatographic profile showed an intense peak at 27.4 min (peak 3), also detected in "*Eremurus spp*" with a very low peak area percent This compound was present in *E. persicus* extract and, as suggested by the positive signal in the CD profile, it was optically active (Figure 1B). The corresponding MS spectrum showed the $[M+H]^+$ at 273 m/z and a dimeric ion $[2M+Na]^+$ at 567 corresponding to a molecular weight of 272 Da (Figure 3).

The overall analytical results clearly showed that: i) all three samples analysed possessed the same initial peaks, peak 1 (r.t. 12.4) and peak 2 (13.5); ii) *Eremurus* spp. and *E. spectabilis* EE showed another common peak eluted at 34.4 min (peak 4); iii) the *E. persicus* HPLC-MS/MS profile differed from the other two samples, due to the absence of the compound eluted at 34.4 r.t. (peak 4) as well as the presence of a prominent peak at 27.4 min (peak 3). Peak 3 had to be considered the characteristic peak of *E. persicus.* In the *Eremurus* spp. Chromatogram, it had a very low intensity. Since *Eremurus* spp. and *E. spectabilis* ethanolic extracts possessed a very similar qualitative fingerprint, and were characterized by the same main peaks (1, 2 and 4), it may be concluded that *Eremurus* spp is predominantly *E. spectabilis* with a very small amount of *E. persicus* as evidenced by the presence in *Eremurus* spp. extract chromatogram of a limited amount of peak 3 (Figure 1).

Regarding the biological activity, the free radical scavenging effect, the anti-inflammatory activity and the cytotoxicity were evaluated. Keeping in mind that reactive oxygen species (ROS) are involved in TNFα-induced inflammation (Young et al., 2008) and that some plants of *Eremurus* genus are used in Kurdish folk medicine as treatment of inflammatory states, the antioxidant potential of all extracts was firstly investigated determining their free radical scavenging (FRS) properties through the DPPH assay. The antioxidant potential of extracts was initially evaluated at a stock concentration of 250 µg/ml. Successively, stock solutions were serially diluted into a range of 187.5 to 7.8 µg/ml (in methanol) and their corresponding FRS activity was determined as percentage values. Generally, an interesting FRS activity % was shown for all three plants (Figure 5), since they reached a maximum effect (E_{max}) at a range of 65 to 68% at 250 µg/ml. Over this concentration, the activity did not increase, confirming that the maximum effect had already been reached.

As shown in Figure 4, the three extracts exert a comparable dose-response antiradical effect, since the curves are very similar to each other. Analogously, the IC_{50} values were very close for *Eremurus* spp. (64.03 µg/ml), *E. persicus* (62.12 µg/ml) and *E. spectabilis* (65.52 µg/ml), confirming that these extracts showed similar FRS activities. Moreover, as a part of our ongoing research for novel anti-inflammatory agents, the effect of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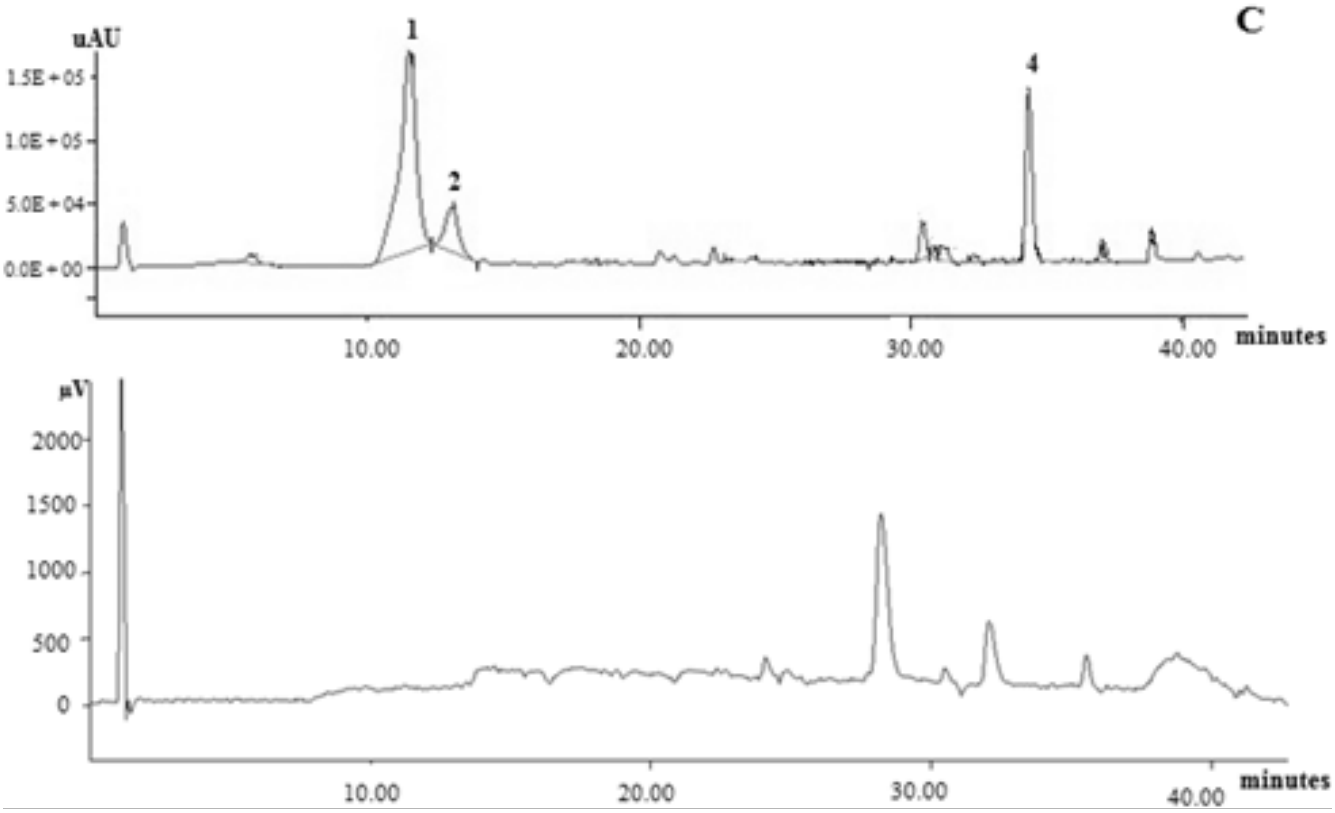

Figure 1. HPLC-UV/PAD/CD chromatograms (up: UV trace; down: CD trace) of ethanolic root extracts of *Eremurus* spp. (A) *E. persicus* (B) and *E. spectabilis* (C). λ: 297 nm.

the three drugs was evaluated using *in vitro* cultured human peripheral blood mononuclear cells (hPBMC). The root EE of *E. persicus* was recently studied by our group, demonstrating an interesting *in vitro* anti-inflammatory effect, inhibiting the PHA-induced hPBMC proliferation as well as TNF-α release (Gaggeri et al., 2013a). The investigation was then extended to the ethanolic root extract of *Eremurus* spp. and *E. spectabilis*. Briefly, hPBMC from three healthy donors were stimulated with phytohaemagglutinin A (PHA) in the presence of increasing doses (50 to 800 µg/ml) of the EE and the cell proliferation was evaluated by monitoring the thymidine incorporation. Since PHA response, expressed as stimulation index (SI), is different among subjects, in order to normalize the data, the results were expressed as the percentage of the corresponding PHA response. As shown in Figure 5, *E. persicus* and *Eremurus* spp extracts exerted a similar effect in the range of 800 to 25 µg/ml, while no inhibition was observed for *E. spectabilis*.

To confirm that the inhibitory effect was specific and to exclude that it was due to cell death, the cell viability was evaluated for PHA stimulated hPBMC by Tripan blue exclusion (Gaggeri et al., 2013a), under the same experimental conditions. Cell viability was 98%, confirming the absence of toxicity (data not shown). Moreover, none of the samples induced any proliferative effect on unstimulated hPBMC, indicating that they are devoid of any adjuvant activity. Additionally, an MTS assay was performed on three different cancer cell lines (A549, MCF-7 and CaCo-2) to evaluate any possible non-specific toxic effect or effects on cell growth. No difference in cell viability was observed between control cells and cells treated with the extracts, even at the highest concentration; these data show that the cell number did not vary in treated cells, compared to controls, thus excluding cytotoxic effects exerted by the extracts.

Given that stimulated hPBMC release cytokines, the observed inhibitory effect could be confirmed by a decrease of the cytokine amount in the supernatants. Hence, the quantification of cytokines (TNF-α, IL-6, and IFN-γ) in the supernatants obtained under the same culture conditions as described earlier, both with or without PHA induction was carried out.

In PHA induced cells, a significant effect ($p < 0.05$) was observed on TNF-α release while no effect was detected on IL-6 and IFN-γ release (data not shown). As shown in Figure 6, TNF-α release was reduced by the three

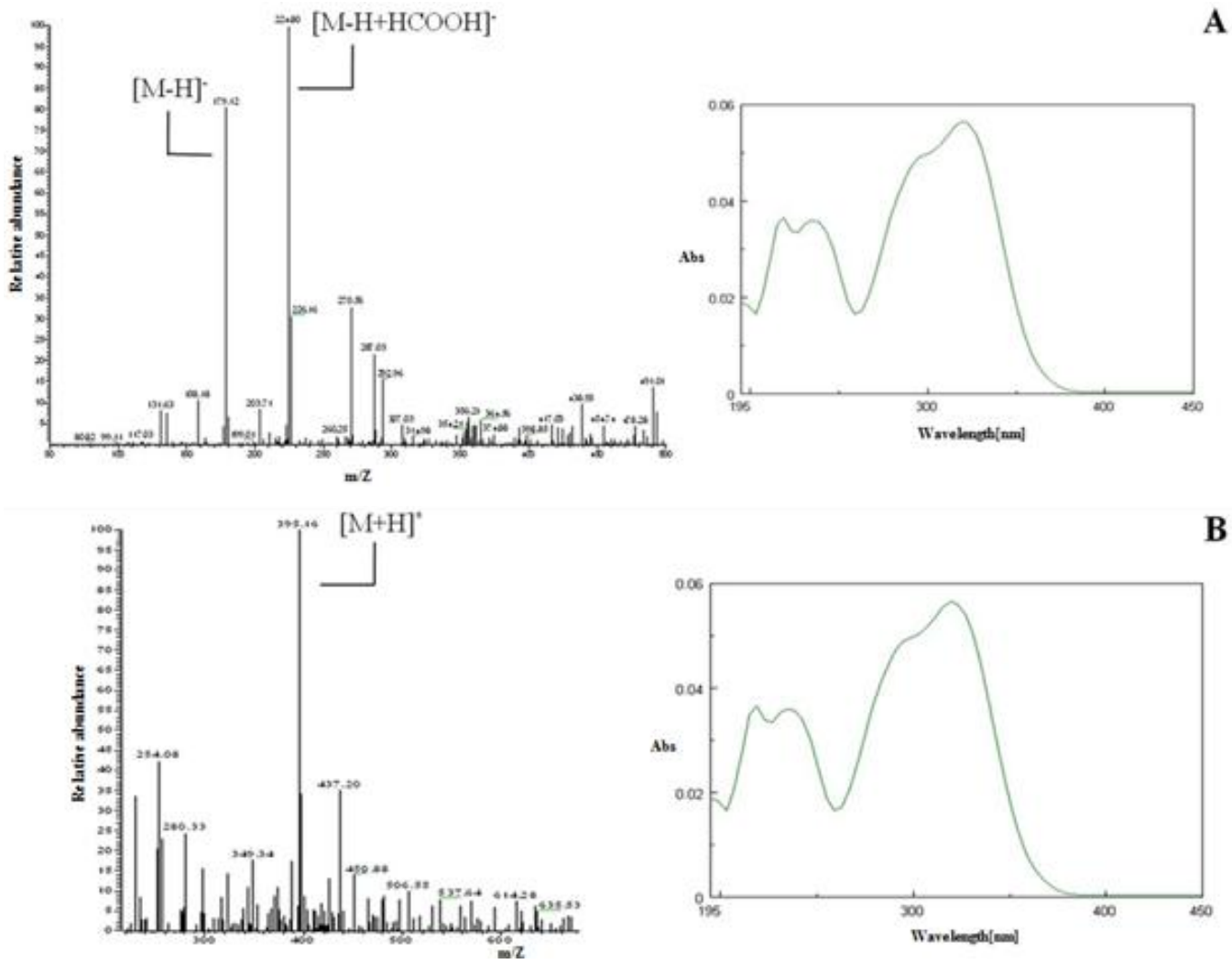

Figure 2. ESI-MS and UV spectra of compound 1(A) and 2 (B).

extracts at 800 and 400 µg/ml, showing a comparable TNF-α blocker effect, while at 200 µg/ml the inhibitory effect on TNF-α release was only determined by *E. persicus.* Moreover, to confirm that the inhibitory effect was specific in parallel experiments where three cancer cell lines were exposed to increasing concentrations of each extract was performed. Obtained data confirmed that the observed inhibition of lymphocyte proliferation cannot be ascribed to a toxic effect leading to cell death but is in fact entirely due to the anti-inflammatory properties of the extracts.

Conclusion

The characterization of *Eremurus* spp. commonly used by native Kurdish people for their anti-inflammatory properties was performed, comparing the phytochemical profiling of *Eremurus* spp ethanolic extract with those of *E. persicus* and *E. spectabilis*. The analytical work demonstrated that the drug commonly sold at the local market is predominantly composed of *E. spectabilis*, since both the HPLC-UV/PAD-CD and the HPLC-ESI-MS/MS profiles of the two extracts are nearly identical. Regarding the biological activity, although *Eremurus* spp. extract exerted an *in vitro* anti-inflammatory effect comparable to that of *E. persicus* extracts in inhibiting *in vitro* PHA-induced lymphocyte proliferation, *E. persicus* EE showed the best TNF-α blocker activity, being the only one effective at 200 µg/ml.

In conclusion, a rapid methodology useful for identifying the *Eremurus* species in the plant material was herein developed. Moreover, taken together, the results support the use of the *Eremurus* plant as anti-inflammatory remedy

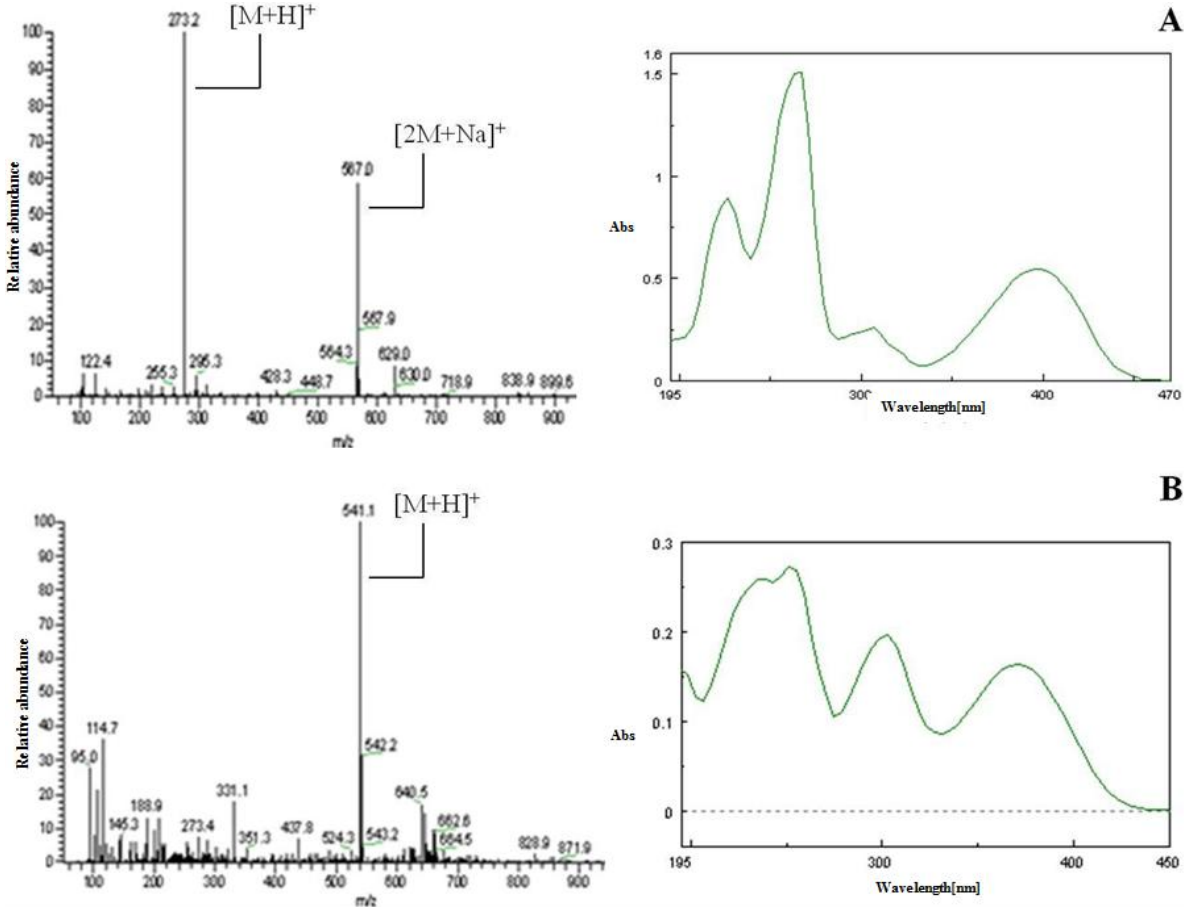

Figure 3. ESI-MS (positive ion mode, full scan) and UV spectra of compound 1(A) and 2(B).

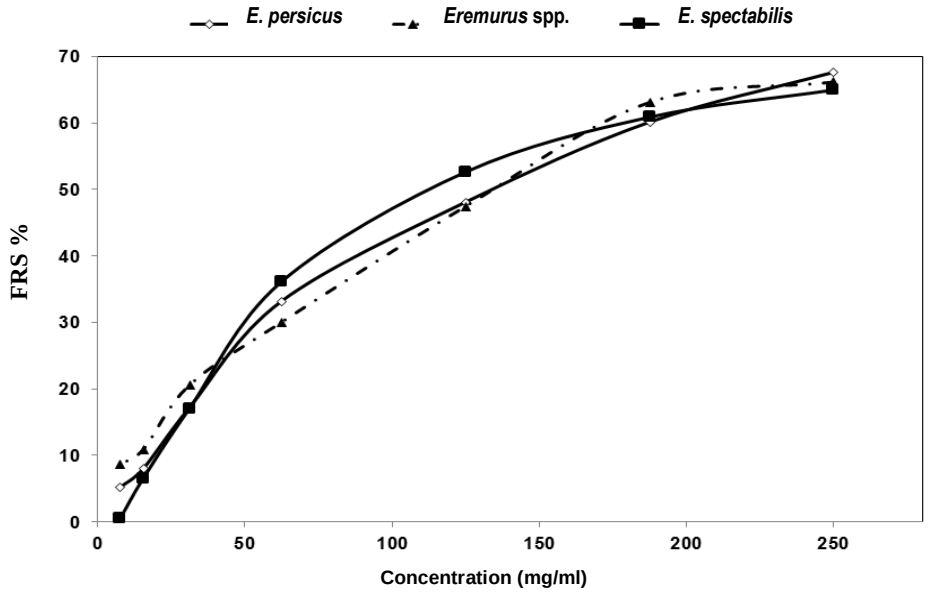

Figure 4. Free radical scavenging activity (FRS) % of *Eremurus* spp., *E. persicus* and *E. spectabilis* extracts.

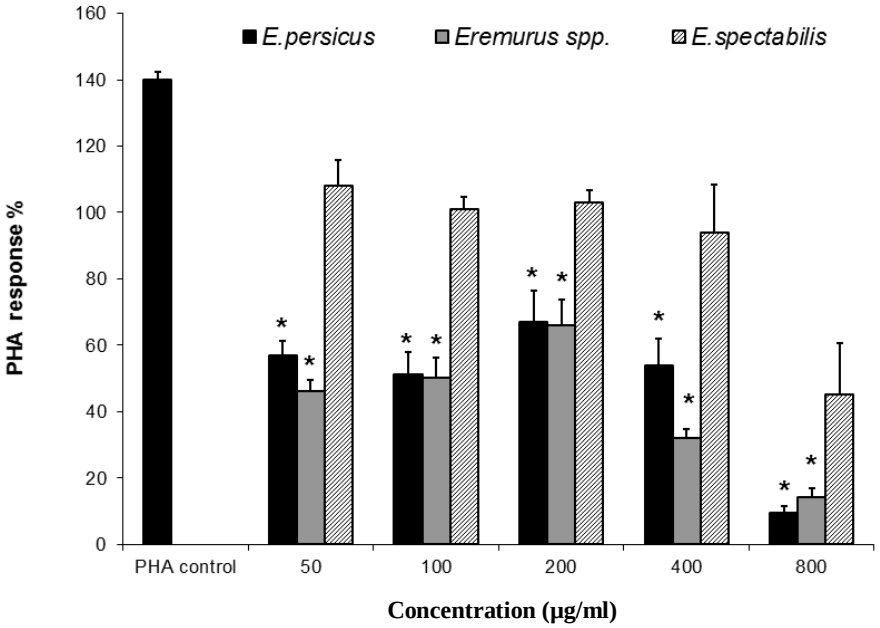

Figure 5. Proliferation of PHA-activated hPBMC in the presence of *Eremurus* spp., *E. persicus* and *E. spectabilis* extracts expressed as percentage of the corresponding PHA response. *P = 0002 vs. PHA.

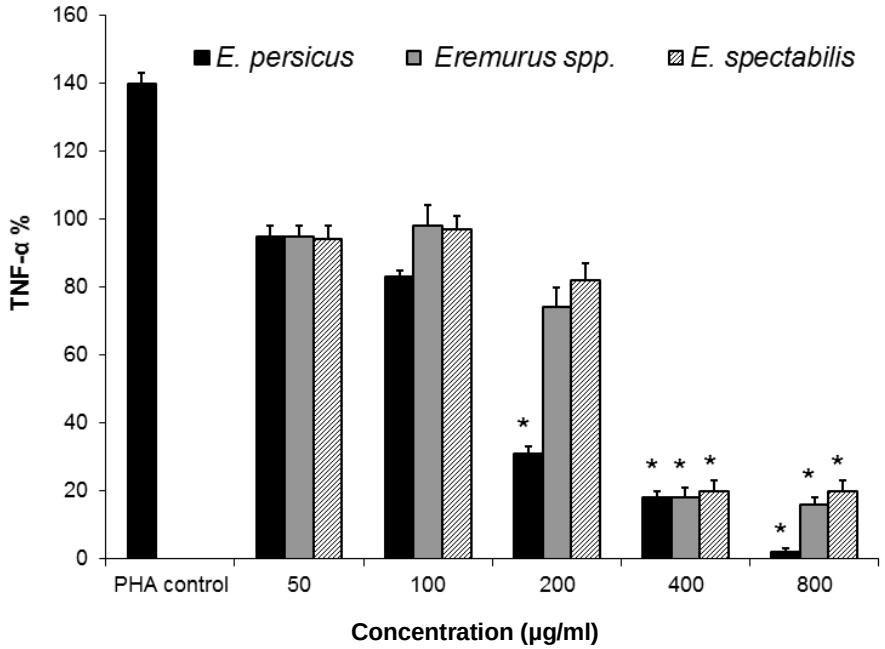

Figure 6. TNF-α levels in culture supernatants of PHA-activated hPBMC after addition of different concentrations of *Eremurus* spp., *E. persicus* and *E. spectabilis*. *P<0.05 vs. PHA.

in Kurdish folk medicine, which exerts its activity through the inhibition of TNF-α. Moreover, and of particular interest, the *E. persicus* roots ethanolic extract, has been shown to be worthy of further investigation to identify the

metabolite(s) responsible for the biological activity and in particular of the anti-inflammatory activity through both *in vitro* and *in vivo* (murine model of endotoxaemia) experiments. Indeed, the inhibition of hPBMC proliferation suggests a possible immunosuppressive action that should be evaluated in future studies.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

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Abbreviations

DPPH, 2, 2-diphenyl-1-picrylhydrazyl; **EE,** ethanolic extract; **FCR,** Folin-Ciocalteau reagent; **FRS,** free radical scavenging; **Hpbmc,** human peripheral blood mononuclear cell; **IL-6,** interleukin-6; **IFN-γ,** interferon-gamma; **PHA,** phytohemagglutinin A; **PVP,** polyvinylpyrrolidone; **TLC,** thin layer chromatography; **TNF-α,** tumor necrosis factor alpha; **TPC,** total phenolics content.

REFERENCES

Cakilcioglu U, Khatun S, Turkoglu I, Hayta S (2011). Ethnopharmacological survey of medicinal plants in Maden (Elazig-Turkey). J. Ethnopharmacol. 137(1):469-86.

Cunningham AB (2001). Applied Ethnobotany: People Wild Plant Use and Conservation. People and Plants Conservation Series. Earthscan Publications, London.

Gaggeri R, Rossi D, Azzolina O, Leoni F, Ahmed KM, Avanzini A, Mantelli M, Gozzini D, Paolillo M, Collina S (2013a). Anti-inflammatory properties of ethanolic root extract of *Eremus persicus* (Jaub and Spach) Boiss, a Kurdish herbal remedy. J. Med. Plants Res. 7:1730-1735.

Gaggeri R, Rossi D, Christodoulou MS, Passarella D, Leoni F, Azzolina O, Collina S (2012). Chiral flavanones from *Amygdalus lycioides* Spach: Structural elucidation and identification of TNFalpha inhibitors by bioactivity-guided fractionation. Molecules 17:1665-1674.

Gaggeri R, Rossi D, Daglia M, Leoni F, Avanzini M, Mantelli M, Juza M, Collina S (2013b). An eco-friendly enantioselective access to (R)-naringenin as inhibitor of proinflammatory cytokine release. Chem. Biodivers. 10(8):1531-1538.

Iqbal M, Khan FA, Farooqui ZH, Ifrahim AFK (2005). Determination of phenol in locally grown fruits and vegetable by spectrophotometric method. J. Chem. Soc. Pak. 27:271-27.

Karaman K, Polat B, Ozturk I, Sagdic O, Ozdemir C (2011). Volatile Compounds and Bioactivity of *Eremurus spectabilis* (Ciris), a Turkish Wild Edible Vegetable. J. Med. Food 20:1-6.

Karaman S, Kocabas YZ (2001). Traditional medicinal plants of *K. maras* (Turkey). Sciences 1(3):125-128.

Karl HR (1982). Lileaceae *Eremurus* species. Iran. Flora. pp. 1-31.

Li C, Shi JG, Zhang YP, Zhang CZ (2000). Constituents of *Eremurus chinensis*. J. Nat. Prod. 63: 653-656.

Makkar HPS, Michael B, Norbert KB, Klaus B (1993). Gravimetric determination of Tannins and their correlation with chemical and protein precipitation methods. J. Sci. Food Agric. 61:161-165.

Mamedov N, Gardner Z (2004). Medicinal plants used in Russia and central Asia for the treatment of selected skin conditions. J. Herbs Spices Med. Plants 11:191-222.

Martino E, Collina S, Rossi D, Bazzoni D, Gaggeri R, Bracco F, Azzolina O (2008). Influence of the extraction mode on the yield of hyperoside, vitexin and vitexin-2''-O-rhamnoside from Crataegus monogyna Jacq. (Hawthorn). Phytochem. Anal. 19:534-40.

Mati E, de Bore H (2011). Ethnobotany and trade of medecinal plants in the qeysare market, Erbil, Kurdish autonomy region Iraq. J. Ethnopharmacol. 133: 490-510.

Ozturk F, Olcucu C (2011). Ethnobotanical features of some plants in the district of semdinli (Hakkari-Turkey). Int. J. Acad. Res. 3(1):117-121.

Pourfarzad A, Habibi Najafi MB, Haddad Khodaparast MH, Hassanzadeh Khayyat M, Malekpour A (2014) Fractionation of *Eremurus spectabilis* fructans by ethanol: Box-Behnken design and principal component analysis. Carbohydr. Polym. 106:374-83.

Pourfarzad A, Habibi Najafi MB, Haddad Khodaparast MH, Hassanzadeh KM (2015). Characterization of fructan extracted from *Eremurus spectabilis* tubers: A comparative study on different technical conditions J. Food Sci. Technol. 52(5):2657-2667.

Safar KN, Osaloo SK, Zarrei M (2009). Phylogeny of the genus *Eremurus* (Asphodelaceae) based on morphological characters in the flora Iranica area. Iran. J. Bot. 15:27-35.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Oxidants and antioxidants Part A Methods in enzymology In: Packer L New York: Academic Press pp. 152-178.

Smirnova NI, Mestechkina NM, Shcherbukhin VD (2001). The Structure and Characteristics of Glucomannans from *Eremurus iae* and *E. zangezuricus*: Assignment of Acetyl Group Localization in Macromolecules. Appl. Biochem. Microbiol. 37:287-291.

Uddin G, Rauf R, Al-Othman AM, Collina S, Arfan M, Ali G, Khan I (2012). Pistagremic acid, a glucosidase inhibitor from *Pistacia Integerrima*. Fitoterapia 83:1648-1652.

Vala MH, Asgarpanah J, Hedayati MH, Shirali J, Bejestani FB (2011). Antibactrial and cytotoxic activity of *Eremurus persicus* (Jaub and Spach) Boiss. Afr. J. Microbiol. Res. 16:2349-2352.

Wendelibo P (1982). Asphodeloideae: Asphodelus, Asphodeline and Eremurus. Flora Iranica 151:3-31.

Yesil Y, Akalin E (2009). Folk medicinal plants in Kurecik area (Akcadag/Malatya- Turkey) Turk. J. Pharm. Sci. 6:207-22.

Young CN, Koepke JI, Terlecky LJ, Borkin MS, Boyd SL, Terlecky SR (2008). Reactive oxygen species in tumor necrosis factorα-activated primary human keratinocytes: Implications for psoriasis and inflammatory skin disease. J. Investig. Dermatol. 128:2606-2614.

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Full Length Research Paper

Ethnobotanical study and phytochemical screening of six medicinal plants used in traditional medicine in the Northeastern Sahara of Algeria (area of Ouargla)

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An ethnobotanical survey was undertaken to collect information from traditional healers on the use of plants (*Atriplex halimus* L., *Searsia tripartita* (Ucria) Moffett, *Limoniastrum guyonianum* Durieu ex Boiss., *Haplophyllum tuberculatum* Juss., *Tamarix gallica* L. and *Nitraria retusa* (Forssk.) Asch.) in folkloric medicine of Ouargla (Algeria), using a predesigned questionnaire. The studied plants were screened for the presence of secondary metabolites. The traditional healers in the study area used the investigated species for the treatment of various diseases. The average of the informant consensus factor (F_{IC}) value for all ailment categories was 0.93, with the highest number of species being used for digestive problems (449) followed by dermatological symptoms (154) and nervous disorders (144). These pathologies were mainly treated by leaves in the form of decoction, representing the dominant formulation. The oral administration which regrouped the major form of usage, which was in form of drink, was most exercised. The phytochemical analysis showed the presence of polyphenolic compounds and saponins in almost all tested plants. For the other metabolites classes, results varied between plants. The multi-uses of species demonstrated the importance of these plants and the diversity of the ancestral knowledge.

Key words: Ethnobotanical study, spontaneous plants, traditional pharmacopeia, phytochemical analysis, Ouargla.

INTRODUCTION

Plants have occupied a prominent place and have been for man, a privileged point of contact with nature and health. Herbal remedies are increasingly popular and used based on sound values. They have been tested by

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our ancestors, of whose virtues confer a significant place in traditional therapy (Tabuti et al., 2003). The World Health Organization (WHO, 2000) defines the traditional medcine as the sum total of the knowledge, skills and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Medicinal plants have provided modern medicine with numerous plant derived therapeutic agents. Most of these plant derived drugs were originally discovered through the study of traditional cures and folk knowledge of native population (Hudaib et al., 2008). A survey was done by USA in the University of the Illinois (Chicago), in 2001 and it was discovered that among medicinal substances found in the market, 122 came from plants (of 94 different species). Among these natural molecules, 80% have been used in combination with the same ones or similar to those for which plants of same origin were used in traditional medicine (Fabricant and Farnsworth, 2000). It shows very well that the uses of plants fact integral part of traditions of all cultures and that the medicinal valorization of these practices is therefore very interesting.

Algeria, with its large area and diversified climate has a varied flora, which is a source of rich and abundant medical matter. On the other hand, Algeria with its history and its strategic location, has benefited from different cultures: Berber, Greco-Roman and Islamic. Important knowledge of plant medicine, currently used in traditional Algerian medicine, originated in the medical heritage of Muslim civilization, transmitted from generation to generation (Chériti et al., 1995; Bellakhdar, 1997). In this light, an ethnobotanical investigation on traditherapeuteses, herbalists and individuals living in contacts with some medicinal plants was done in the goal to exploit the ancestral knowledge of the traditional pharmacopeia, that is transmitted by oral tradition. Indeed, it is very important to translate traditional knowledge to scientific knowledge, in order to help in preserving the knowledge of medicinal plants and should direct biologist and phytochemist in their search to promising species and bioactive constituents.

MATERIALS AND METHODS

Study area

The study was conducted in South-eastern Algeria, in the province of Ouargla, which is located 790 km away from Algiers, and a surface area of 163.230 km². It is limited to North by the Wilayas of Djelfa and El-Oued, to East by Tunisia, to South by the Wilayas of Tamanrasset and Illizi and to West by Ghardaïa (Figure 1). The region of Ouargla is located at the bottom of a very large pan of the low valley of the Oued M'ya, to an altitude of 157 m. The geographical coordinates are: latitude 32° 45' North and 31° 45' South, longitude 5° 20 ' East and 5° 45' West (Rouvillois-Brigol, 1975). The area is characterized by the predominance of dunes all along the valley, Sebkhas and massive dune alterative with funds on which agriculture irrigation was installed. The hydrographic network of Ouargla region mainly consists of three major hydrological elements, Oued M'Ya, Oued N'Sa and Oued M'Zab. These last two Oued participate to some extent, in the water supply of water tables despite low precipitation and their stormy character (Rouvillois-Brigol, 1975).

It has a climate particularly contrasted in spite of the relatively Northern latitude. The average annual temperature, according to the Office National de la Météorologie (ONM) for years (2003 to 2013) range from about 36.74°C in July for the hottest month and 11.50°C in January for the coldest month. Precipitations are very reduced and irregular to crossbar seasons and years. Their distribution is nearly marked by a drought absolute in month of May until the month of August. The yearly middle precipitations are in the order of 3.03 mm, with a maximum of 7 mm for the month of January. The total population residing in the region are estimated at 558 563 inhabitants, with a density of 3.4 inhabitants/km^2.

The inhabitants are ethnic-multiple, with Berber, Arab, African and Mestizo which come from mixed marriages. The main feature of the region is the youth of the population, as the majority of the population is under 25 years old, with a natural growth rate of around 2.15%. Working in the land has always been for the people of the region, who use it as their main source of activity and economy. It has space in agro-pastoral activity, strongly dominated by date palm and cereal in pivot, because of its climate and mobilization of water resources. The total agricultural area amounts to 4877393 ha, about 29.9% of the total area of the province.

Interviews and plant material collection

In order to determine the possible usage of the plant species (*Atriplex halimus* L., *Searsia tripartita* (Ucria) Moffett, *Limoniastrum guyonianum* Durieu ex Boiss., *Haplophyllum tuberculatum* Juss., *Tamarix gallica* L. and *Nitraria retusa* (Forssk.) Asch.) in traditional pharmacopeia of Ouargla region, an ethnobotanical survey was launched since 2011 to 2013, which targeted population groups including; herbalists, traditional healers, herbal practitioners, nomads and physicians. Most of the people interviewed were traditional healers. These plants have attracted serious attention because of their chemo taxonomic criteria. They belong to a botanical family known to frequently contain some secondary metabolites and possess pharmacological potentials. A total of 200 informants (45% of them were female and 55% were male), whose ages ranged from 20 to 80 years participated in this study. The investigation was performed using a predesigned questionnaire which included their age, sex, their job, with questions relating to the plants, the scientific and common names, plant parts in use, methods and forms of preparations and therapeutic indications. All interviews were carried out in the native language (Arabic) of the study area.

Plant samples were collected from the field and were dried and compressed in papers. Papers were changed daily until they remained dry after compression. The voucher specimens of medicinal plants collected was identified based on the "Flora and vegetation of Sahara" (Ozenda, 1983), with the help of the Herbarium specimens available at the Herbarium of the Faculty of Sciences of Nature and Life of the University Kasdi Merbah, Ouargla (Algeria). It should be noted that the new phylogenetic classification APG II (Angiosperm Phylogeny Group, 2003) was adopted in odrer to update the families cited in Table 1.

Data analysis

Informant consensus factor (F_{IC})

Informant consensus factor (F_{IC}) was employed to deduce the

Figure 1. Geographical location of the study area.

homogeneity of the information about a specific plant use to treat a particular category of ailments. All citations were placed into ailment categories for which the plant was claimed to be used. These were either denominated after the affected organ including, skin, auditory, skeletal, visual, poisonous, endocrine, respiratory, nervous system, genital, metabolic, circulatory, digestive, urinary tract, cancerous or parasitic diseases. The F_{IC} results could be useful in prioritizing medicinal plants for further scientific validation of plants and plant products (Moshi et al., 2009; Giday et al., 2009), as pharmacologically effective remedies are expected from plants with higher F_{IC} values (Trotter and Logan, 1986). The informant consensus factor (F_{IC}) was calculated to estimate user variability of medicinal plants (Heinrich et al., 1998; Canales et al., 2005). F_{IC} values ranged from 0.00 to 1.00. High F_{IC} values were obtained when only one or a few plant species was reported to be used by a high proportion of informants to treat a particular ailment, whereas low F_{IC} values indicated that informants disagreed over which plant to use (Heinrich et al., 1998). High F_{IC} values can thus be used to pinpoint particularly interesting species for the search of bioactive compounds (Canales et al., 2005). F_{IC} is calculated using the following formula:

$$F_{IC} = N_{ur} - \frac{N_t}{(N_{ur} - 1)}$$

Where N_{ur} is the number of individual plant use reports for a particular illness category and Nt is the total number of species used by all informants for this illness category.

Frequency of use (F_C)

The frequency of use (F_C), the percentage of informants claiming the use of specie in the treatment of diseases, was calculated according to the formula:

$$F_C\ (\%) = n/N \times 100$$

Where n is the number of informants that claim the use of a plant species to treat a particular disease, and N is the number of informants that use the plants as a medicine to treat any given disease.

Phytochemical analysis

These are tests performed to collect data on the composition of the studied plants (in powder form). Most are based on colorimetric assays and also precipitation, using various reagents. The methods used for the characterization of the chemical groups present in each plant follow the protocols described by Ronchetti et al.

Table 1. Traditional uses of studied species in folkloric medicine of Ouargla (Algeria).

Family	Scientific name	Vernacular name	Area of collection	Additives	Traditional uses
Amaranthaceae	*Atriplex halimus* L.	G'ttaf	Biskra (34° 45' 14.3" N, 005° 24' 43.5" E) Altitude 2 m	Honey, salt with honey, oil, milk, local butter (Dhan), Citrus sinensis (L.) Osbeck (laymoune)	Catarrh stomacal, constipation, diarrhea, gas, bloating, cyst hydatique, fibrome, hypertension, antiseptic, burns, diabetes, fever, jaunasse, anemia, cardiac disease, otitis, rheumatism, cough, obesity, tumor, tiredness, diuretic, vermifuge, involuntary urine, vomiting, wounds and ulcers, tonsillitis, goiters, gallbladder disease, calming, fortify the gums, infertility, prostate, fall of placenta, nephrolithiasis, hypercholesterolemia
Anacardiaceae	*Searsia tripartita* (Ucria) Moffett	Djedari	Ghardaïa (32° 13' 09.1" N, 003° 30' 00.1" E) Altitude 519 m	Honey, oil, milk, *Origanum vulgare* L. (Zâatar)	Diabetes, bloating, fever, kidney disease, cutaneous lesions, migraines, diarrhea, anemia, menstrual disorders, cardiac disease, otitis, inflammation of the mouth, jaunasse, gas, leprosy, catarrh stomacal, cephalalgia, hypertension, loss of appetite, flu, prostate, hemorrhoids, rheumatism, weakness of vision
Plumbaginaceae	*Limoniastrum guyonianum* Durieu ex Boiss.	Zeta	Ouargla (33° 12' 30.9" N, 006° 07' 59.6" E) Altitude 299 m	*Punica granatum* L. (reman)	Antiseptic, burn, leprosy, wounds and ulcers, strengthening, diabetes, jaunasse, anemia, cough, constipation, gas, kidney disease, pains of the head, hypertension, obesity, scorpion stings, tonsillitis and flu, fortify the gum, liver disease
Rutaceae	*Haplophyllum tuberculatum* Juss.	Fidjel	Ghardaïa (32° 13' 19.3" N, 003° 29' 59.9" E) Altitude 314 m	oil, salt with honey, local butter (Dhan), *Punica granatum* L. (reman), *Artemisia campestris* L. (dgouft), *Allium cepa* L. (el-besla), *Carum carvi* L. (karwya)	Antiseptic, injuries and ulcers, calming, hypnotic neurological, infertility, diabetes, bloating, fever, liver disease, otitis, rheumatism, vermifuge, obesity, constipation, colon, diarrhea, gas, hypertension, menstrual pain, cardiac disease, scorpion stings, flu, vomiting, throat inflammation, sweat, tonsillitis, cough, loss of appetite
Tamaricaceae	*Tamarix gallica* L.	Tarfa	Ouargla (33° 17' 12.5" N, 006° 01' 55.5" E) Altitude 1 m	Salt with honey, vinegar	Antiseptic, burn, leprosies, injuries and ulcers, pikes of scorpions and bugs, illnesses of the kidney, diarrhea, anemia, jaunasse, inflammation the gum and of the mouth, gastric ulcer, cephalalgia, hypertension, diabetes, illness of joints, hemorrhage, diuretic, inflammation of the pancreas
Zygophyllaceae	*Nitraria retusa* (Forssk.) Asch.)	Ghardaq	Ouargla (33° 13' 02.9" N, 006° 02' 39" E) Altitude 1 m	Honey, milk, egg white, oil, *Lawsonia inermis* L. (el-hana), *Allium cepa* L. (el-besla)	Antiseptic, cutaneous wound, burn, diabetes, fever, constipation, laxative, diarrhea, cardiac disease, scorpion stings, cough, gastric ulcer, cephalalgia, hypertension, loss of appetite, colon, prostate, articular pains, conjunctivitis, diseases of eyes and eyelids, weakness of vision, fortify the gum.

(1971), Hegnauer (1973), Wagner (1983) and Bekro et al. (2007).

RESULTS

During the ethnobotanical investigation, a total of 200 answers were obtained concerning the use of researched species (Table 1). For each species, scientific and vernacular name, family, therapeutic properties and eventual supplenemts were provided. Plant resources are often used multi-contextually. All species in this study were used in more than one disease category, and about 15 disease categories were identified from the investigated region.

These are more or less involved in the cure of illnesses recorded in the different apparatuses, skin, auditory, skeletal, visual, poisonous, endocrine, respiratory, nervous system, genital, metabolic, circulatory, digestive, urinary tract, cancerous or parasitic diseases. These categories could be considered as a reflection of the cure concept of the local people of Ouargla. The level

Table 2. Informant consensus factor (F_{IC}) for different ailment categories.

Ailment	Skin disease	Auditory disease	Respiratory disease	Nervous disease	Genital disease	Metabolic disease	Circulatory disease	Digestive disease	Urinary disease	Skeletal disease	Visual disease	Endocrine disease	Poisonous disease	Cancerous disease	Parasitic disease
Number of taxa (N*t*)	6	4	6	6	4	6	6	6	6	6	3	4	4	1	1
Number of use reports (N*ur*)	154	35	48	144	83	129	79	449	42	33	17	21	30	10	23
F_{IC}	0.97	0.91	0.89	0.97	0.96	0.96	0.94	0.99	0.88	0.84	0.88	0.85	0.90	1.00	1.00

Table 3. Frequency of use (F_C) (%) values of medicinal plants cited by informants for being used against a given ailment.

Species	Skin disease	Auditory disease	Respiratory disease	Nervous disease	Genital disease	Metabolic disease	Circulatory disease	Digestive disease	Urinary disease	Skeletal disease	Visual disease	Endocrine disease	Poisonous disease	Cancerous disease	Parasitic disease
A. halimus	2.78	0.35	2.43	1.04	15.97	7.99	3.13	38.54	7.29	2.43	1.04	5.56	/	3.47	7.99
S. tripartita	11.27	1.41	2.11	1.41	0.70	2.11	10.56	66.90	0.70	0.70	0.70	1.41	/	/	/
Li. guyonianum	36.91	4.03	18.12	4.70	0.67	20.81	2.68	6.04	0.67	1.34	/	/	4.03	/	/
H. tuberculatum	2.69	7.76	1.49	38.81	10.45	1.79	5.07	26.27	1.49	2.99	/	/	1.19	/	/
T. gallica	5.04	/	1.26	0.42	/	23.95	10.50	47.48	3.78	1.26	/	0.84	5.46	/	/
N. retusa	37.24	/	2.07	0.69	/	6.21	6.21	22.76	3.45	6.90	8.97	0.69	4.83	/	/

of informants agreement was high for most ailment categories (mean F_{IC} = 0.93) and total consensus (F_{IC} = 1.00) was even obtained for cancerous and parasitize diseases, followed by symptoms (154) and nervous disorders (144). For digestive problems we reported *S. tripartita* (F_C = 66.90%), *T. gallica* (F_C = 47.48%) and *A. halimus* (F_C = 38.54%) as the plants with most the frequent use. While for dermatological diseases, *N. retusa* (F_C = 37.24%) and *L. guyonianum* (F_C = 36.91%) were preferred (Table 3). Finally, for nervous disorders, *H. tuberculatum* was the one with the biggest record (F_C = 38.81%) (Table 3).

Various plant parts (leaves, stem, bark, fruits, seeds, root, flowers etc) were used for different therapeutic preparations, with the leaves digestive disease (F_{IC} = 0.99), dermatological symptoms and nervous disorders (F_{IC} = 0.97) in the second and third categories, respectively (Table 2). Endocrine (F_{IC} = 0.85) and skeletal predominating for all species, beginning with 44.26% for *S. tripartita* and arriving at 53.33% for *L. guyonianum* (Figure 2). The studied plants were formulated by the local population using six methods of preparation (decoction, infusion, steeping, powder, excerpt and torrefaction) (Figure 3).

The decoction was the most frequent method of preparation followed by the use of powder and infusion in second place for (*T. gallica*, *S. tripartita*, *N. retusa* and *L. guyonianum*) and (*H. tuberculatum* and *A. halimus*) in this order. For the category (F_{IC} = 0.84) showed relatively low levels of consensus.

The highest plant use citation (449) was cited for digestive problems, followed by dermatological application of different aforementioned symptom treatments, different methods of administration in the form of drinks followed by auditory instillation for *H. tuberculatum* plant, the ingestion concerning *A. halimus* specie, the local application for *N. retusa*, *S. tripartita* and *L. guyonianum* species, these last two fashions nearly occupied second rank, with a percentage similar to *T. gallica* (13 and 12%), successively (Figure 4).

The use of species for different treatments is not always singular, but one often runs to a natural

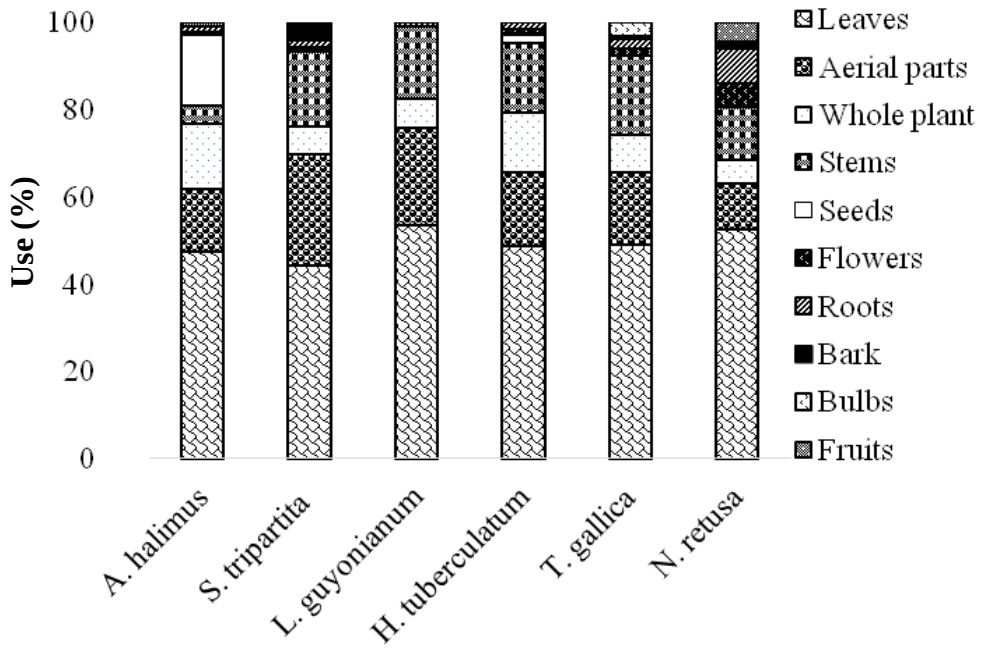

Figure 2. Percentage of plants uses according to the used part.

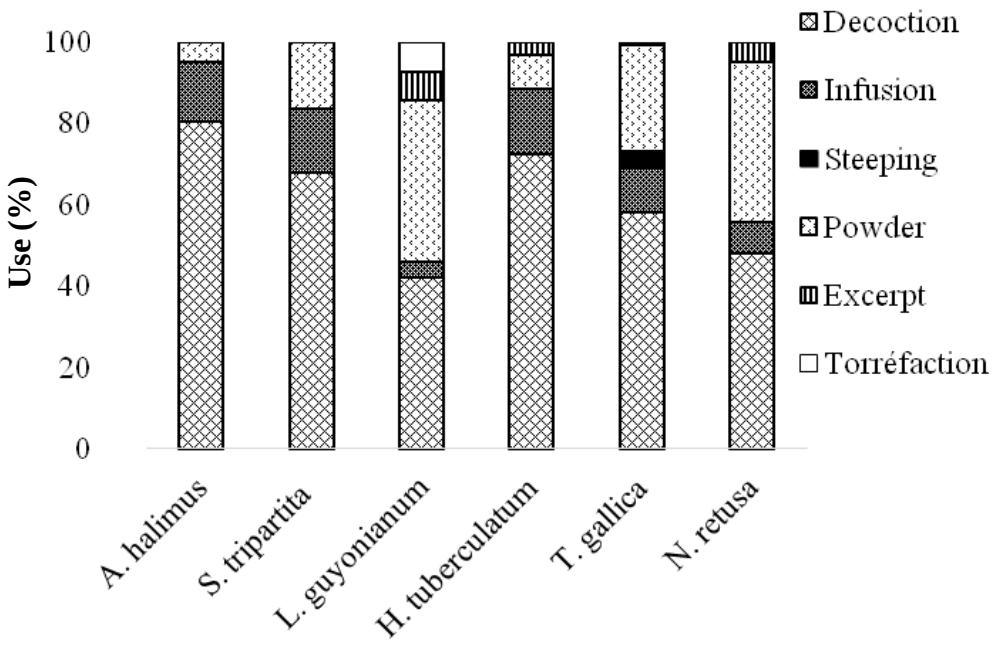

Figure 3. Percentage of plants' uses according to forms of preparation.

supplement addition for a given treatment. To this effect *H*. *tuberculatum* was usually mixed with 07 supplements, *A*. *halimus* with 06, *N*. *retusa* with 05, *S*. *tripartita* with 04, *T*. *gallica* with 02 and *L*. *guyanianum* with 01 (Table 1). Phytochemical analysis of composition aimed to determine the chemical groups responsible for the claimed therapeutic effects. Results are summarized in Table 4. Obtained results indicated the presence of polyphenols and saponins, with an absence of alkaloids, anthocyanins and anthracenosides (free and *O*-combined) in all tested plants. For the other metabolites groups, the results varied. Furthermore, sterols, triterpenes

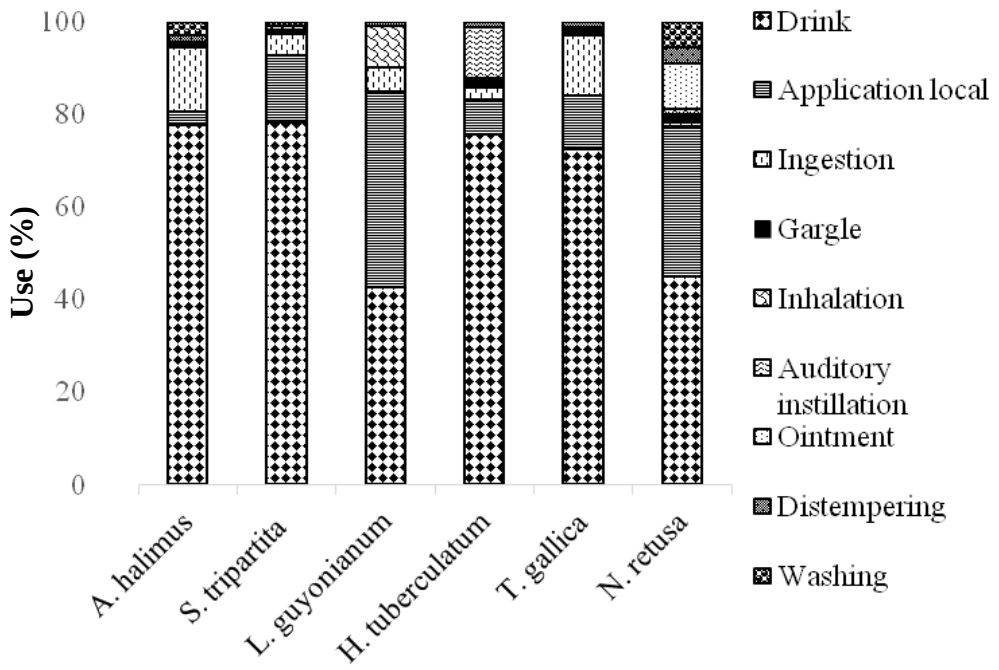

Figure 4. Percentage of plants' uses according to methods of administration.

and free flavonoids were detected in all species except *A. halimus*. The test of tannin gallic was positive only in *T. gallica* plant.

DISCUSSION

The local residents provided information on the researched species and the traditional therapeutic practices of the local population of the region of Ouargla. These medicinal applications spanned a total of 15 disease categories. Each plant might be used to treat various diseases. This can be interpreted as an optimization of natural resources due to tight connection of the people with their local environment (Pieroni et al., 2002). Digestive disease was the most important ailment treated on the basis of number of citations for medicinal uses followed by dermatological symptoms and nervous disorders. For digestive problems we reported *S. tripartita*, *T. gallica* and *A. halimus* as the plants with most frequent use, while for dermatological diseases, *N. retusa* and *L. guyonianum* were preferred (Table 3). The elevated usage of the studied plants for digestive diseases was understandable as this was considered by various authors, local and from other region, as the symptoms with the highest use of medicinal plants for its treatment. Results of the inventory of Ould El-Hadj et al. (2003) led in the region of Ouargla, indicated that digestive problems dominated with a rate of 26.4%. The relative importance assigned to them was similar to that registered by Scarpa (2004) in other geographical areas (Northwestern Argentine Chaco) which indicated that 90 species (26.5% of the total uses) and 58 species (14.6%) were used mainly for diseases of the digestive tract and skin infections, respectively.

Concerning nervous disorders, *H. tuberculatum* had the biggest record. Some traditional uses reported for *Haplophyllum* species suggested that this plant may have activity on central nervous system. For instance, the leaves of this plant infused with vinegar was given to children for the treatement of convulsion and other nervous disorders (Al-Said et al., 1990). Different parts of documented plants were used as medicine by the local traditional healers. Among these, the leaves were most commonly used for the treatment of diseases. The predominance usage of an organ in relation to another in the therapeutic domain can be due to the concentration in active components in this organ. Leaves are the center of most photochemical reactions and are reservoirs of certain metabolites (Chamouleau, 1979). Collection of leaves and then using them as medicine is very easy as compared to roots, flowers and fruits (Giday et al., 2009). Another reason of using leaves could be concerning conservation of the plants, as digging out roots might result in the death of the plant and consequently putting the species in a vulnerable condition (Rehecho et al., 2011).

Preparation of medicinal plants varied, such as decoction, infusion, steeping, powder, excerpt and torrefaction. However aqueous decoction was the most frequent form of preparation which was followed by powder and infusion. These results agreed with the

Table 4. Phytochemical screening of studied plants.

Chemical groups		*A. halimus*	*S. tripartita*	*L. guyonianum*	*H.tuberculatum*	*T. gallica*	*N. retusa*
Polyphenols		+	+++	+++	++	+++	++
Tannins	Catechic	++	+++	+++	-	+++	+++
	Gallic	-	-	-	-	+++	-
Flavonoids	Free	-	++	++	++	+++	+
	Leucoanthocyanes	-	+++	-	-	+++	++
	Catechols	-	-	++	-	-	-
Anthocyanins		-	-	-	-	-	-
Anthracenosides	Free	-	-	-	-	-	-
	O- heterosides	-	-	-	-	-	-
	C- heterosides	-	+	+++	-	+++	-
	G- heterosides	-	++	++	-	++	-
Alkaloids		-	-	-	-	-	-
Sterols		-	++	+++	++	++	+++
Polyterpens		-	++	+++	++	++	+++
Saponins (foaming index)		142.85	500	111.11	250	166.66	500

- (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance).

literature of Kola et al. (2008) and Pascal et al. (2011), where preparations were made with water as a solvent. Infusion was used for delicate plant organs (leaves, bloom heads and flowers) to preserve active principles. For Lori and Devan (2005), infusion was appropriate for delicate and light organs, whereas for hard and compact organs (woods, barks, stems, branches and roots), extraction of active principles required prolonged treatment under heat. The decoction was the beneficial preparation method in order to extract a maximal quantity of the active principles and attenuate or cancel the toxic effect of certain revenues.

Medicinal plants are usually used internally or externally which depends on the illness. The oral consumption of the medicinal plants consisted mainly of remedies used against digestive diseases. Almost all the internal remedies prepared from medicinal plants were prepared in oil, honey, salt with honey, milk, local butter (Dhan), vinegar and egg white. On one hand, the additives like oil, honey, salt with honey, milk, local butter (Dhan), vinegar and egg white are commonly believed to serve as a vehicle to transport the remedies. On the other hand, the combination of studied species with other plants was probably justified by their common therapeutic action and their mixture was perhaps aimed at reinforcing such action. Advices of associations are dictated by a search to increase the efficiency of treatment; it means that taken all together, these constituent will be more efficient than if they were taken separately. Synergy that exists inside a same plant can be improved again and reinforced by the action devised by several natural substances.

The phytochemical investigations revealed the presence of polyphenolic compounds and saponins in almost all tested plants (Table 4). For the other phytocompounds, results varied between plants. Some of the more commonly known phytochemicals, including flavonoids, tannins and saponins were observed in the present study and have all been reported to aid man by creating a preventive barrier against diseases and sicknesses. These metabolites classes have been associated with different pharmacological activities including antibacterial, antioxydant and analgesic properties, which could justify and confirm the therapeutic traditional preparation indications on the basis of plants (Ajibola and Motoyoshi, 1992).

A. *halimus* (Amaranthaceae) known under the name of “G’ttaf” in Ouargla, was mentioned for the treatment of digestive (F_C = 38.54%) and genital (F_C = 15.97%) diseases. Studies on the chemical constituents of *A*. *halimus* have been carried out by many investigators and have shown the presence of various compounds. For exampe, tannins, flavonoids, saponins, alkaloids and resins (Bayoumi and El-Shaer, 1992). In the Arab world, *A*. *halimus* was used to treat chest ailments, as a laxative, to cure stomach pains, for intestinal worns and to regulate gall bladder excretions (Day, 1990; Le Houérou, 1992). According to those surveyed for example *S*. *tripartita* (Anacardiaceae) known locally as “Djedari”, aqueous decoction (67.96%), powder (16.50%) or infusion (15.53%) was considered as the most popular methods of preparation that can address several diseases such as digestive (F_C = 66.90%), skin (F_C = 11.27%) and circulatory (F_C = 10.56%). Concerning the chemical studies of this plant, several compounds such as flavonoids, biflavonoids, isobiflavonoids, catechin, epicatechin-3-*O*-gallate, proanthocyanidin oligomers and polymers, polysaccharides, condensed tannins have been isolated from different parts of the plant (Tebourbi et al., 2006; Alimi et al., 2013). This plant has been used in

Tunisian traditional medicine to treat diarrhoea and dysentery. The root bark extract of this specie has been reported to be beneficial in curing gastric ulcers (Abbassi and Hani, 2012).

In traditional medicine of Ouargla, *L*. *guyonianum* (Plumbaginaceae) known locally as “Zeta” was used as decoction (42.34%) or powder (39, 64%) preparation by the local residents to cure skin (F_C = 36.91%), metabolic (F_C = 20.81%) and respiratory (F_C = 18.12%) diseases. The essential oil of *L*. *guyonianum* from Monastir region (Tunisia) consisted these main constituents: (3Z) hex-3-enylmethanoate (9.18%) in roots, furfural (14.63%) in seeds, methyl-2,4-dimethylbenzoate (14.70%) in leaves and 3-phenylprop-2-enylpentanoate (15.05%) in flowers. Furthermore, evaluation of antibacterial effects against five bacterias: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* NCIMB 8853, *Staphylococcus aureus* ATCC 29213 and *Micrococcus luteus* NCIMB 8166 showed that the indicated oils prevented visible growth of all tested bacteria at a lower concentration (MIC = 0.02 mg/ ml) (Hammami et al., 2011). Recently, it was mentioned that ethyl acetate extract of the aerial parts of *L*. *guyonianum* from Gabes in Tunisia contained gallocatechin, epigallocatechin and epigallocatechin-3- O-gallate which showed anti-oxidant activities (Trabelsi et al., 2012). This endemic species has been used in Algerian folk medicine to treat gastric infections. It has also been employed as an anti-bacterial drug in the treatment of bronchitis (Le Floch, 1983).

H. *tuberculatum* (Rutaceae) known under the name of “Fidjel” in Ouargla was widely used in decoction (72.43%) or infusion (16.22%) forms for nervous (F_C = 38.81), digistive (F_C = 26.27%) and genital (F_C = 10.45) diseases by indigenous groups. Literature survey relayed that a number of phytochemical investigation studies have been reported indicating that *H*. *tuberculatum i*s rich in alkaloids and volatile constituents (Al-Burtamani et al., 2005). The essential oil of the aerial parts of *H*. *tuberculatum* from Beni Abbes in Algeria is characterized by the major components: α-phellandrene (2.1%), β-phellandrene (3.0%), terpinene-4-ol (3.2.%) , p-cymene-8-ol (2.9%), piperitone (17.8), 2,4-bis(1,1-dimethylethyl)-phenol (28.3%), (1E,4E)-germacrene B (2.1%), hexadec-1-ene (3.2%) and octadec-1-ene (2.1%) (Mechehoud et al., 2014). This specie is used in traditional medicine as a remedy for headaches and arthritis, the juice is applied as a wart removal, skin discoloration, infections and parasitic diseases (Al-Burtamani et al., 2005). It is also used to treat nervous system, infertility and fever (Said et al., 2002).

T. *gallica* (Tamaricaceae) known under the name of “Tarfa” in Ouargla, was mainly used in the form of decoction (58.18 %) or powder (26, 06 %) for the treatment of digestive (F_C = 47.48%), metabolic (F_C = 23.95%) and circulatory (F_C = 10.50%) diseases. Studies on the chemical constituents of *T*. *gallica* have been carried out by many investigators and have shown the presence of polyphenolic compounds, such as flavonoids, phenolic acids, tannins and coumarins (Mahmoud et al., 1994, Djurdjević et al., 2006). The *n*-butanol extract of the aerial parts of *T. gallica* from Tebessa in Algeria is characterized by the presence of two flavonoids: 5-Hydroxy-3,7, 4'-trimethoxyflavone and 3,5,7-Trihydroxy-4'-methoxyflavone (Lefahal et al., 2010). In Algeria and surrounding areas, the plant has been used medicinally for rheumatism, diarrhoea and other maladies (Kalpna et al., 2011). The branchlets and the leaves are astringent and diuretic. An external compress of the leaves helps to stop bleeding in wounds. The plants have been reported to be useful in leucoderma, spleen trouble and eye diseases (Sharma and Parmar, 1998).

N. retusa (Zygophyllaceae) known locally as “Ghardaq” in folkloric medicine of Ouargla was used by the traditional healers as decoction (48.04 %) or powder (39, 22 %) preparation to treat skin (F_C = 37.24%) and digestive (F_C = 22.76%) diseases. Chemicl study on *N*. *restusa* have shown the presence of O- glycosides of flavones and flavonols compounds in addition to flafones c- glycosides which exhibits a notable activity in protecting against oxidative stress (Hussein et al., 2009). Belkadhar (1997) indicated that a decoction of fresh leaves of *N*. *retusa* was used in Morocco in case of poisoning, stomach upset, ulcers, gastritis, enteritis, heartburn, colitis, and colonic abdominal pain. In Tunisia and Egypt, the dry leaves of this plant are used in decoction as a substitute to tea and to make cataplasms. Ashes of this plant have the property to withdraw liquids (blood, lymph) from infected wounds (Shaltout et al., 2003).

Conclusions

The population of the survey used the inventoried plants to treat different diseases whose dominants illnesses are digestive diseases, followed by dermatological symptoms and nervous system disorders. The multi-uses of species demonstrated the importance of these plants and the diversity of the ancestral knowledge due to tight connection of the people with their local environment. The traditional medicines constitute in fact a very rich heritage, which should be transmitted to the younger generations. This plant heritage constitute an inestimable treasure that will be able to be valorizes and used subsequently as the therapeutic products of basis to produce medicines improved with the contribution of analyses of the chemical composition and the active principles of these medicinal plants.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Abbassi F, Hani K (2012). *In vitro* antibacterial and antifungal activities of *Rhus tripartitum* used as antidiarrhoeal in Tunisian folk medicine. Nat. Prod. Res. 26(23):2215-8.

Ajibola A, Motoyoshi S (1992). Contribution to the phytochemistry of medicinal plants growing in Nigeria as reported in the 1979-1990 literature. A review. Afr. J. Pharm. Pharm. Sci. 22(3):172-201.

Al-Burtamani SKS, Fatope MO, Marwah RG, Onifade AK, Al-Saidi SH (2005). Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman. J. Ethnopharmacol. 96(1-2):107-12.

Alimi H, Mbarki S, Ben Barka Z, Feriani A, Bouoni Z, Hfaeidh N, Sakly M, Tebourbi O, Ben Rhouma KH (2013). Phytochemical, antioxidant and protective effect of *Rhus tripartitum* root bark extract against ethanol-induced ulcer in rats. Gen. Physiol. Biophys. 32(1):115-27.

Al-Said MS, Tariq M, Al-Yahya MA, Rafatullah S, Ginnawi OT, Ageel AM (1990). Studies on *Ruta chalepensis:* An ancient medicinal herb still used in traditional medicine. J. Ethnopharmacol. 28(3):305-12.

Bayoumi MT, El-Shaer HM (1992). Impact of halophytes on animal health and nutrition. In: Squires VR, Ayoub AT (eds) Halophytes as a resource for livestock and for rehabilitation of degraded lands, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 267-272.

Bekro YA, Bekro JAM, Boua BB, Tra BFH, Ehile EE (2007). Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae). Rev. Sci. Nat. 4(2):217-225.

Bellakhdar J (1997). La pharmacopée marocaine traditionnelle. Médecine arabe ancienne et savoirs populaires, Paris, Ibis press, P 766.

Canales M, Hernandez T, Caballero J, Romo de Vivar A, Avila G, Duran A, Lira R (2005). Informant consensus factor and antibacterial activity of the medicinal plants used by the people of San Rafael Coxcatlán, Puebla, México. J. Ethnopharmacol. 97:429-439.

Chamouleau A (1979). Les usages externes de la phytothérapie. Edition de Maloine S. A., Paris, P 270.

Chériti A, Rouissat A, Sekkoum K, Balansard G (1995). Plantes de la Pharmacopée traditionnelle dans la région d'El Bayadh (Algérie). Fitoterapia 66(6):525-538.

Day C (1990). Hypoglycaemic compound from plants. In: Bailey CJ, Flatt PR (eds) New antidiabetic drugs. London: Smith-Gordon, pp. 267-278.

Djurdjević L, Mitrović M, Pavlović P, Gajić G, Kostić O (2006). Phenolic acids as bioindicators of fly ash deposit revegetation. Arch. Environ. Contam. Toxicol. 50(4):488-495.

Fabricant DS, Farnsworth NR (2000). The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 109:69-75.

Giday M, Asfaw Z, Woldu Z (2009). Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study. J. Ethnopharmacol. 124(3):513-521.

Hammami S, Nguir A, Saidana D, Cheriaa J, Mighri Z (2011). Chemical analysis and antimicrobial effects of essential oil from *Limoniastrum guyonianum* growing in Tunisia. J. Med. Plants Res. 5(12):540-2545.

Hegnauer R (1973). Chemotaxonomie der Pflanzen, band 6, Birkhäuser Verlag, Basel und Suttgart, P 761.

Heinrich M, Ankli A, Frei B, Weimann C, Sticher O (1998). Medicinal plants in México: Healers' consensus and cultural importance. Soc. Sci. Med. 47(11):1859-1871.

Hudaib` M, Mohammada M, Bustanji Y, Tayyema R, Yousef M, Abuirjeied M, Aburjai T (2008). Ethnopharmacological survey of medicinal plants in Jordan, Mujib Nature Reserve and surrounding area. J. Ethnopharmacol. 120:63-71.

Hussein SR, Kawashty SA, Tantawy ME, Saleh NAM (2009). Chemosystematic studies of *Nitraria retusa* and selected taxa of Zygophyllaceae in Egypt. Plant Syst. Evol. 277(3-4):251-264.

Kalpna R, Mital K, Sumitra C (2011). Vegetable and fruit peels as a novel source of antioxidants. J. Med. Plants Res. 5(1):63-71.

Kola KA, Benjamin AE, Danladi NB, Etienne EE, Saburi AA (2008). Ethnobotanical survey of Akwa Ibom State, Nigeria. J. Ethnopharmacol. 115:387-408.

Le Floch E (1983). Contribution to an ethnobotanical study of the flora of Tunisia. Ministry of Higher Education and Scientific Research and the Ministry of Agriculture, Tunis, Tunisia, P 192.

Le Houérou HN (1992). The role of saltbushes (*Atriplex* spp.) in arid land rehabilitation in the Mediterranean Basin: A review. Agroforest. Syst. 18(2):107-148.

Lefahal M, Benahmed M, Louaar S, Zallagui A, Duddeck H, Medjroubi K, Akkal S (2010). Antimicrobial activity of *Tamarix gallica* L. extracts and isolated flavonoids. Adv. Nat. Appl. Sci. 4(3):289-292.

Lori L, Devan N (2005). Un guide pratique des plantes médicinales pour les personnes vivant avec VIH, pp. 3-47.

Mahmoud A, Nawwar M, Sahar A, Hussein M (1994). Gall polyphenolics of *Tamarix aphylla*. Phytochemistry 36(4):1035-1037.

Mechehoud Y, Chalard P, Figuérédo G, Marchioni E, Benayache F, Benayache S (2014). Chemical composition of the essential oil of *Haplophyllum tuberculatum* (Forssk.) L.A. Juss. from Algeria. Res. J. Pharm. Biol. Chem. Sci. 5(5):1416-1419.

Moshi MJ, Otieno DF, Mbabazi PK, Weisheit A (2009). The ethnomedicine of the haya people of bugabo ward, Kagera region, north western Tanzania. J. Ethnobiol. Ethnomed. 5(24):1-5.

Office National de la Météorologie (ONM) (2003-2013). Données métrologiques de Ouargla.

Ould El-Hadj MD, Hadj-Mohammed M, Zabeirou H (2003). Place des plantes spontanées dans la médicine traditionnelle de la région de Ouargla (Sahara Septentrional Est). Courrier du Savoir 03:47-51.

Ozenda P (1983). Flore et végétation du Sahara. 3^{éme} édition. CNRS, Paris, P 622.

Pascal N, Joseph IB, Jean BN, Ferruccio P, Fabiana A (2011). Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: An ethnobotanical study. J. Ethnopharmacol. 133(2):378-95

Pieroni A, Nebel S, Quave C, Münz H, Heinrich M (2002). Ethnopharmacology of liakra: Traditional weedy vegetables of the Arbëreshë of the Vulture area in southern Italy. J. Ethnopharmacol. 81(2):165-85.

Rehecho S, Uriarte-Pueyo I, Calvo J, Vivas LA, Calvo MI (2011). Ethnopharmacological survey of medicinal plants in Nor-Yauyos: A part of the Landscape Reserve Nor-Yauyos-Cochas, Peru. J. Ethnopharmacol. 133(1):75-85.

Ronchetti F, Russo G, Bombardelli E, Bonati A (1971). A new alkaloid from Rauvolfia vomitoria. Phytochemistry 10(6):1385-1388.

Rouvillois-Brigol M (1975). Le pays de Ouargla (Sahara Algérien). Variation et organisation d'un espace rural en milieu désertique. Pub. Dép. Géo. Univ. Paris, Sorbonne. P 316.

Said O, Khalil K, Fulder S, Azaizeh H (2002). Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. J. Ethnopharmacol. 83:251-265.

Scarpa GF (2004). Medicinal plants used by the Criollos of Northwester Argentine Chaco. J. Ethnopharmacol. 91:115-135.

Shaltout KH, Sheded MG, El-Kady HF, Al-Sodany YM (2003). Phytosociology and size structure of *Nitraria retusa* along the Egyptian Red Sea coast. J. Arid Environ. 53(3):331-345.

Sharma SK, Parmar VS (1998). Novel constitutes of *Tamarix* species. J. Sci. Ind. Res. 57:873-890.

Tabuti JR, Lye KA, Dhillion SS (2003). Traditional herbal drugs of Bulamogi, Uganda : plants, use and administration. J. Ethnopharmacol. 88(1):19-44.

Tebourbi O, Trabelsi C, Nasr CB, Sakly M (2006). Antioxidant activity of extract of *Rhus oxyacantha* root cortex. Indian. J. Exp. Biol. 44:246-249.

Trabelsi N, Oueslati S, Falleh H, Waffo-Teguo P, Papastamoulis Y, Merillon JM, Abdelly C, Ksouri R (2012). Isolation of powerful antioxidants from the medicinal halophyte *Limoniastrum guyonianum*.

Food Chem. 135:1419-1424.
Trotter RT, Logan MH (1986). Informants consensus: A new approach for identifying potentially effective medicinal plants. In: Plants in Indigenous Medicine and Diet. Edited by Etkin NL. New York: Redgrave Publishing Company, Bedford Hill, pp. 91-112.
Wagner H (1983). Drogen analyse, Dünschicht chromatogaphische Analyse von Arzneidrogen. Springer- Verlag. Berlin- Heidelberg- New York, P 522.
World Health Organization (WHO) (2000). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva, pp. 1-74.

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