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Full Length Research Paper

Essential oil composition and antioxidant activity of aerial parts of *Asperula oppositifolia* collected from Darkesh, Iran

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The chemical compositions of the essential oil of *Asperula oppositifolia* aerial parts were examined by gas chromatography/mass spectrometry (GC/MS); forty-two compounds were identified. Analysis of this essential oil revealed the presence of 2-(6,6-Dimethyl bicyclo[3.1.1]hepta-2en-2-yl) ethanol (17.16%), Decane (8.47%) , Dibuthyl phthalate (5.59%) and 1-Bromo Naphthalene(4%). The antioxidant activity of aerial parts of methanolic extract was studied by *in vitro* 2'2'-diphenylpicrylhydrazyl (DPPH) radical – scavenging activity, revealing that this plant could be used as new medicinal resource for antioxidant agent.

Key words: Chemical composition, essential oil, 2-(6,6-Dimethyl bicyclo[3.1.1]hepta-2en-2-yl)ethanol, *Asperula oppositifolia* ,antioxidant activity.

INTRODUCTION

Asperula oppositifolia rechingeri is described from North-Khorasan Province, Iran. *A.oppositifolia* comprises six subspecies mainly distributed in E. Afghanistan, Pakistan, and Middle Asia. Describing these taxa originating from Iran demonstrates the extent of diversity of *Asperula* species in this country.

Morphological evidence supports taxonomic position of these taxa in *A.oppositifolia*, and the subspecies appear to be most closely related to subsp pseudo-cynanchica Ehrend (Ghahramaninezhad et al., 2006).

Phytochemical studies on this plant have been carried out, and flavonoids were identified (Borisov et al., 1972). This present communication, for the first time, did an analysis of arial parts essential oil and antioxidant activity of methanolic extract of A. oppositifolia is reported.

EXPERIMENTAL

Plant material

Aerial parts of A.oppositifolia were collected at the flowering stage from Darkesh protected area (Iran), in June 2010 and identified at the Research center for plant sciences, Ferdowsi university of Mashhad. Collected specimen has been deposited in the

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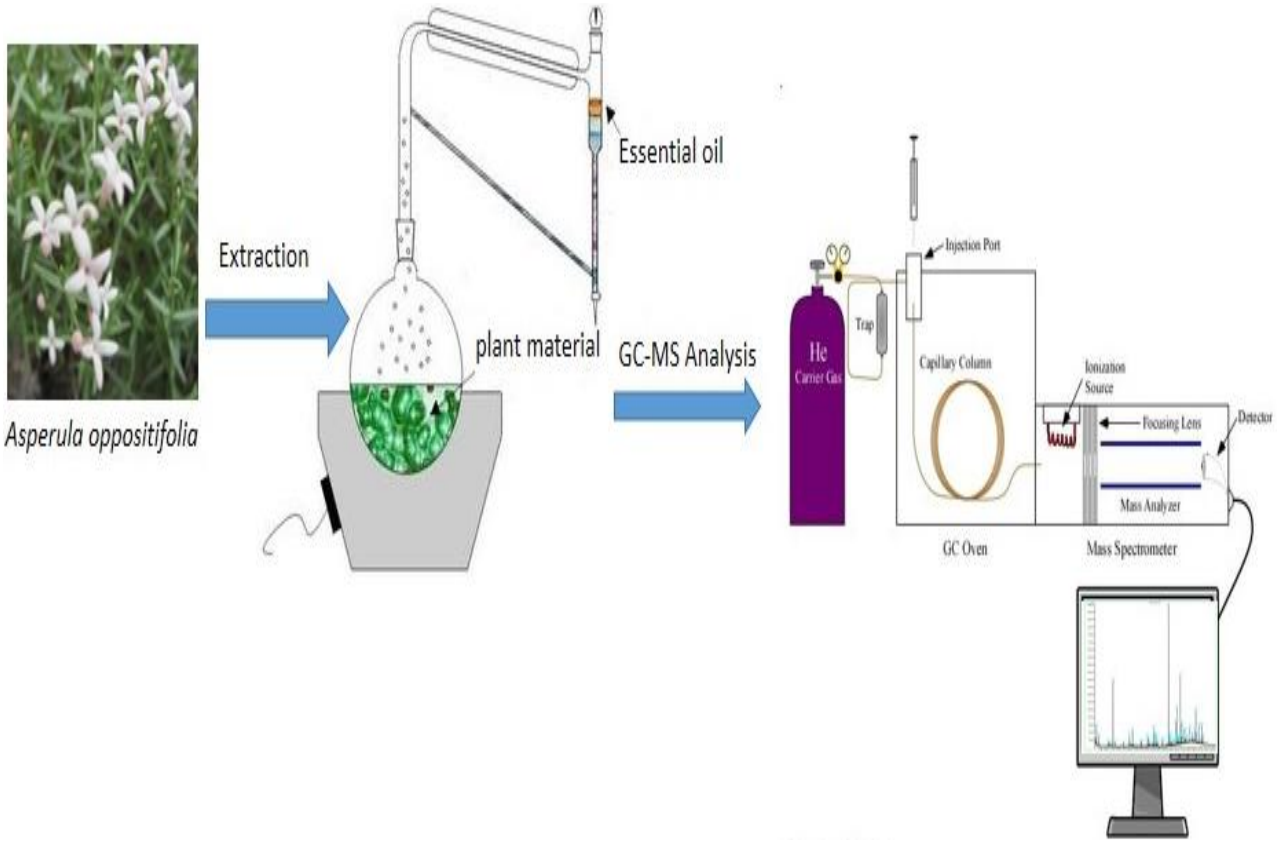

Figure 1. Aerial parts of *A.oppositifolia* collected at the flowering stage from Darkesh protected area, Iran.

Herbarium of Research Center (Figure 1).

Isolation of the essential oil

Aerial parts of *A. oppositifolia* were air-dried for 3 days before essential oil distillation. The plant material (100 gr) was cut into small pieces and the essential oil was obtained by hydrodistillation method, using a Clevenger apparatus. The temperature and pressure of hydrodistillation were 120ºC and 560 mmHg respectively. The distillation time was five hours. The resulting pale yellow oil was then dried over anhydrous sodium sulphate and 30 µL was solubilized in 1 mL of dichloromethane before the GC/MS (Gas Chromatography and Mass Spectroscopy) injection. 1 µL of this solution was directly used for analysis (Boland et al., 1991).

Gas chromatography and mass spectrometry (GC/MS)

Gas chromatographic analyses were performed using a HP 5890 series II gas chromatograph (Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a HP-5 (5% phenyl/95% dimethylpolysiloxane) fused silica capillary column (30 m× 0.25 mm; film thickness 0.25 Mm). Hydrogen was the carrier gas (1.0 mL min^{-1}) (Bianchi et al., 2007).

The injector temperature was kept at 250°C and the oven temperature program was from 60 to 240°C at a rate of 3°C min^{-1}. Detector (FID) was operated at 280°C. Pure oils (1 µL) were injected in split mode (100:1). The GC-MS analyses were performed in an Agilent 5973N mass selective detector coupled to an Agilent 6890 gas chromatograph (Palo Alto, CA), equipped with a HP5-MS capillary column (30 m × 0.25 mm × 0.25 µm). It operated in electronic ionization mode at 70eV, with transfer line maintained at 260°C; while quadrupole and ion source temperature were held at 150 and 230°C, respectively. Helium (1.0 mL min^{-1}) was used as carrier gas. Oven temperature program, injector temperature and split rate were the same as stated for GC analyses (Kohl et al., 2001; Dos Santos et al., 2001).

A standard solution of *n*-alkanes (C_8-C_{24}) was used to obtain the retention indices (Vandendool and Kratz, 1963). Individual volatile components were identified by comparison of their mass spectra (MS) and retention indices (RI) with those reported in literature (Adams, 2001; Davies.,1990) and also to the Wiley Registry of Mass Spectral Data,6th Edition (Wiley Interscience, New York).Component relative percentages were calculated based on GC/MS peak areas without using correction factors (Pino et al., 2005; Bianchi et al., 2007).

Antioxidant activity

The antioxidant potential of the methanolic extract was evaluated in term of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability. The determination was performed in triplicate.

DPPH radical scavenging activity

The scavenging effect on the DPPH radical was determined according to the methods reported previously (Singh et al., 2005).

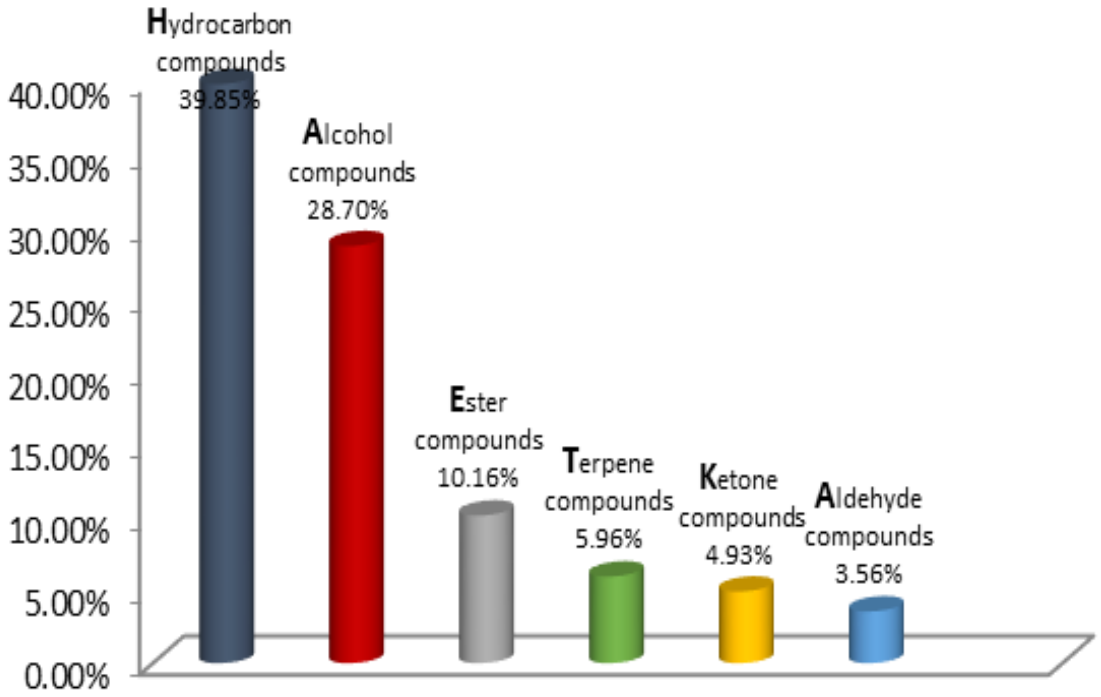

Figure 2. Distribution of *Asperula oppositifolia* compounds.

50 µl of various amounts of methanolic extracts (10, 5, 2.5, 1.125, 0.625, 0.312, 0.156 and 0.078 mg/mL) was mixed with 5 mL of 0.004% methanolic solution of DPPH. Each mixture was incubated for 30 min in the dark and the absorbance of the samples was calculated at 517 nm using the UV-Vis spectrophotometer. The DPPH solution was freshly prepared and kept in the dark at 4°C in between the measurements. Both control and standard were subjected to the same procedure except that of the control.

A lower absorbance indicated a higher radical scavenging power and these data were calculated according to the equation: DPPH scavenging activity (%I)=[1–(A_t /A_o)]x100, where A_t is the absorbance of the sample at 517 nm and A_o is the absorbance of the control at 517 nm.

RESULTS AND DISCUSSION

Essential oil analysis

The average yield of essential oil obtained after hydrodistillation of the leaves of *A.oppositifolia* was about 0.3%. Table 1 reports the chemical composition. Forty-two components were identified, accounting for 67.94% of the total oil.

The various compounds were identified by comparison of their Kováts retention indexes, determined utilizing a non-logarithmic scale on nonpolar (Rtx-5MS) columns , and by comparison of the mass spectra of each GC component with those of standards and reported literature (Jennings et al.,1980).

High resolution gas chromatography-mass spectrometric (HP GC-MS) analysis and Kováts Index values showed that its principal components are:2-(6,6-Dimethyl bicyclo[3.1.1]hepta-2en-2-yl)ethanol (17.16%), Decane (8.47%), Dibuthyl phthalate (5.59%) and 1-Bromo naphthalene(4%) and Dodecane (3.08%). Figure 2 shows the distribution of *A. oppositifolia* essential oil compounds.

Antioxidant activity

DPPH radical scavenging activity

Figure 3 shows the dose-response curve of 2'2'-diphenylpicrylhydrazyl radical scavenging activity of the methanolic extract of *A.oppositifolia* .It was observed that highest concentration showed the highest inhibitory effect.

Conclusion

Owing to the undesirable problems and side effects arising from the consumption of artificial chemical compounds, essential oils from various plant species, especially those edible and medicinal ones, have attained appreciable interest among the research community. This is the first study on the essential oil compounds and antioxidant activity of ethanolic extract of *A.oppositifolia.* Our data indicate that the polar compounds are the major ones in essential oils and possess a moderate antioxidant activity. These results suggest that essential

Table 1. Percentage composition of the essential oil distillated from aerial parts of *Asperula oppositifolia.*

S/N	Compound	Experimentally determined KI[a]	HP GC-MS Peak area [%]	Method of identification
1	Ethylbenzene	868	0.62	GC-MS,Ms
2	Z-3-Hexene-1-ol	849	1.03	GC-MS,Ms
3	Deca111 1,3-Dimethyl benzen	883	2.28	GC-MS,Ms
4	1,4-Dimethyl benzene	883	1.48	GC-MS,Ms
5	5-Methyl nonane	960	0.39	GC-MS,Ms
6	3-Methyl nonane	971	0.44	GC-MS,Ms
7	Decane	999	8.47	GC-MS,Ms
8	p-Cymene	1076	0.6	GC-MS,Ms
9	Linalool	1100	0.7	GC-MS,Ms
10	Nonanal	1108	0.27	GC-MS,Ms
11	α- Terpineol	1055	0.49	GC-MS,Ms
12	Dodecane	1095	3.08	GC-MS,Ms
13	Decanal	1206	0.29	GC-MS,Ms
14	Pulegone	1217	0.4	GC-MS,Ms
15	Thymol	1298	0.96	GC-MS,Ms
16	β – Damascenone	1382	0.36	GC-MS,Ms
17	Tetradecane	1399	1.07	GC-MS,Ms
18	Neryl acetone	1454	0.37	GC-MS,Ms
19	1-Bromo naphthalene	1463	4	GC-MS,Ms
20	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadienone	1473	0.9	GC-MS,Ms
21	β- Ionone	1485	0.77	GC-MS,Ms
22	Pentadecane	1499	0.54	GC-MS,Ms
23	2,6-di(t-butyl)phenol	1511	1.6	GC-MS,Ms
24	Hexadecane	1599	0.78	GC-MS,Ms
25	Z-14-methyl-8-hexadecane-1-ol	1668	0.35	GC-MS,Ms
26	Cyclotetradecane	1684	0.4	GC-MS,Ms
27	2-(6,6-Dimethyl bicycle[3.1.1]-2en-2-yl)ethanol	1694	17.16	GC-MS,Ms
28	Heptadecanal	1716	2	GC-MS,Ms
29	Nonyl phenol	1727	0.5	GC-MS,Ms
30	Anthracene	1765	0.53	GC-MS,Ms
31	Octadecane	1799	0.64	GC-MS,Ms
32	6,10,14-trimethyl -2-pentadecanone	1847	1.83	GC-MS,Ms
33	Nonadecane	1898	0.55	GC-MS,Ms
34	(E,E)-6,10,14-trimethyl -5,9,13-pentadecatrien-2-one	1919	0.44	GC-MS,Ms
35	Methyl hexadecanoate	1928	0.35	GC-MS,Ms
36	Dibuthyl phthalate	1966	5.59	GC-MS,Ms
37	Eicosane	2000	0.63	GC-MS,Ms
38	n-Heneicosane	2098	1.22	GC-MS,Ms
39	(Z,Z,Z)-9,12,15-Octa-decatrienoic acid methyl ester	2101	1.35	GC-MS,Ms
40	Docosane	2200	0.64	GC-MS,Ms
41	Tricosane	2300	1.14	GC-MS,Ms
42	Tetracosane	2400	0.73	GC-MS,Ms
	Total	-	67.94	-

a: Retention; indices on RTX-5MS(based on homologous series of n-alkanes;C_8-C_{24}).

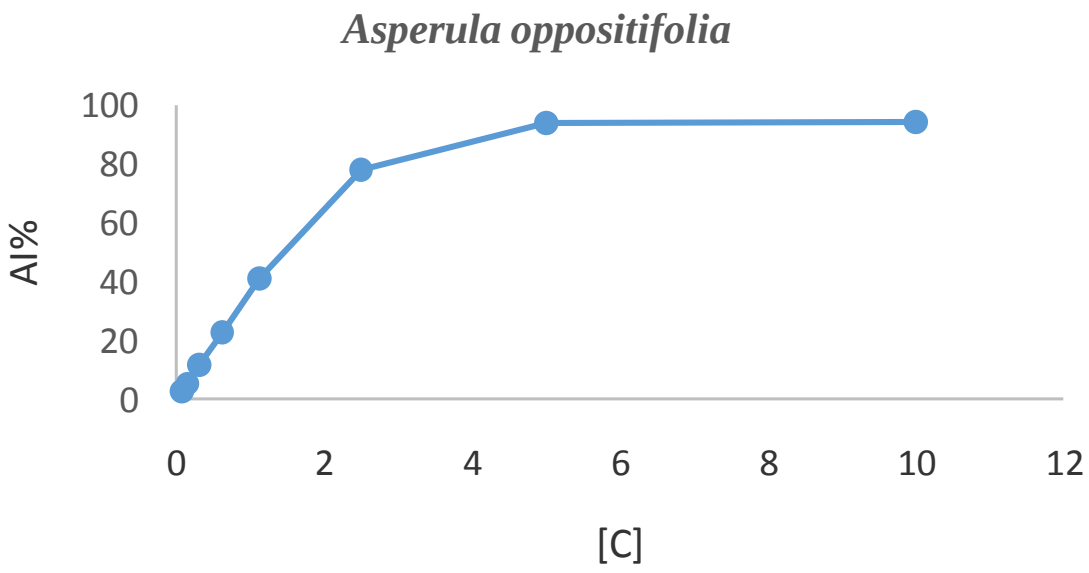

Figure 3. Radical scavenging of methanolic extract of *A. oppositifolia* on DPPH.

oil of *A. oppositifolia* could be used as new medicinal resource for antioxidant agent.

Conflict of Interests

The authors have not declared any conflict of interests.

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