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Essential oil composition, total phenolic content and antioxidant activities of Iranian *Zataria multiflora* Boiss

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Abstract

The essential oils obtained by hydrodistillation from the aerial parts of wild and cultivated *Zataria multiflora* Boiss, were analyzed by Gas Chromatography (GC) and Gas Chromatography – Mass Spectrometry (GC/MS). Forty eight components were identified in the essential oil of *Z. multiflora*. The major components were Carvacrol (63.51%), Linalool (5.78%), *p*-Cymene (5.31%), Thymol (4.60%), β-caryophyllene (3.15%) and γ-Terpinene (2.62%). The total phenolic content and the antioxidant activity of plant extract was determined by Folin-Ciocalteau and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays respectively. This study confirms that the extracts of *Z. multiflora* possesses high phenolic content and antioxidant activities. The results support the traditional usage and also possible use of *Z. multiflora* essential oil and extracts in the food, pharmaceutical and cosmetic industries.

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Introduction

The use of traditional herbs and medicinal plants has recently become very popular because they contain large amounts of natural products with biological properties. Plants are now one of the important sources of new pharmaceuticals and healthcare products (Mulabagal and Tsay, 2004).

Medicinal plants contain antioxidant compounds called polyphenols that are known to reduce oxidative stress. The antioxidant properties of these compounds are responsible for their anticancer, antiviral and anti-inflammatory properties (Ninfali *et al.*, 2005).

The labiatae family include about 250 generaand 6700 species and plants spread in the warm and temperate regions all over the world (Mabberley, 1997). They are mainly grasses and shrubs, very fragrant and rich in medicinal properties and widly used in spices, perfumes, and medicinal products (Omidbaigi, 2000).

Zataria multiflora Boiss. (Avishan-e-Shirazi in Persian and Sa'atar or Zaatar in the old Iranian medical books) is a plant belonging to the laminaceae family that geographically grows only in Iran, Pakistan and Afghanistan (Hosseinzadeh, Ramezani, & Salmani, 2000). This plant grows wild in the warm regions of central and southern parts of Iran (Ali, Saleem, Ali, & Ahmad, 2000). *Z. multiflora* is extensively used as a flavor ingredient in a wide variety of food and drinks in Iran. Also this plant is used in traditional folk remedies as antiseptic, antispasmodic, sedative, carminative and antimicrobial properties (Fazeli *et al.*, 2007; Sharififar, *et al.*, 2007). The main components of the essential oil of this plant are phenolic compounds such as carvacrol and thymol (Shaffiee & Javidnia, 1997; Saei-Dehkordi, *et al.*, 2010).

This study focuses on essential oil composition, phenolic content and antioxidant activities of *Z. multiflora* as possible new source for valuable components and phenolic content and natural antioxidant.

Materials and methods

Plants material

The aerial parts of *Z. multiflora* were harvested in pre-flowering stage from wild grown plants in the Bash Mountain from Estahban, Fars province, at an altitude of 1860 m. Voucher specimen was deposited at the herbarium of medicinal and aromatic plants of Islamic Azad University, Estahban branch, Estahban, Iran. The harvested plants were dried at room temperature (25°C) for 2 weeks, then, air-dried plants (in each habitat) were ground and powdered with mixer for essential oil extraction and other experiments.

Essential oil extraction

The dried aerial parts of *Z. multiflora* were subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus. The essential oil obtained was separated from water and dried over anhydrous sodium sulfate and stored in sealed amber flasks at 4 °C till analysis.

Gas Chromatography (GC)

Gas Chromatography analysis was performed on an Agilent technologist model (7890A) equipped with flame ionization detector and capillary column DB-5 (30 m 0.32 mm, 0.25 µm film thicknesses). The chromatographic conditions were as follows: The oven temperature increased from 60 to 210°C at a rate of 3°C/min then 210 to 240 °C at a rate of 20°C/min. The injector and detector temperatures were 280 and 290°C, respectively. N2 used as the carrier gas (1 ml/min).

Gas Chromatography-Mass spectrometry (GC-MS):

Essential oil was also analyzed by Hewlett- Packard GC-MS (model 6890 series II) operating at 70e V ionization energy. Equipped with a DB-5 capillary column (phenyl methyl siloxane (30 m, 0.25 mm, 0.25 µm film thickness) with He as the carrier gas and a split ratio of 1:50. The retention indices for all the components were determined according to the Van

Den Doll method using nalkanes as standard (Van Den Dool and Kratz, 1963). The compounds were identified by comparison of retention indices (RRI-AP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra (Adams, 2001).

Preparation of the methanolic extracts

Grounded air dried plant material (7.5 g) was weighed in a glass and then defatted with light petroleum for 3 h. Each Sample was extracted with 200 ml of 90% aqueous methanol, for 2 days with one change of solvent after 1 day. Supernatants were combined and evaporated to dryness using a rotary evaporator to a volume of about 1 ml. These concentrated extracts were freeze-dried and weighed to determine the yield, and kept at -20°C until used. Shortly before each experiment, the lyophilized powder was dissolved in methanol at the desired concentration, and tested for antioxidant activity and total phenolic content.

Total phenolic content

Phenolic contents were determined by a Folin-Ciocalteau reagent using a method described by Spanos and Wrolstad (1990) to 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na2CO3 (7.5%, w/v) were added and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a spectrophotometer (LABOMED. INC. UV/VIS double beam PC. UVD. 2980). A methanolic solution of gallic acid was tested in parallel to obtain a standard curve. The values were expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g dry weight).

Free Radical Scavenging Capacity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging effects were evaluated according to the method employed by Brand-Williams *et al.*, 1995. Briefly, 4 different concentrations of the plant extract dissolved in methanol were incubated with a methanolic solution of DPPH 100 μM in a total volume of 4 ml. After 30 min of incubation at room temperature in the dark, the absorbance at 517 nm was measured using a spectrophotometer (LABOMED. INC. UV/VIS double beam PC.UVD.2980). After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm. Methanol was used as blank and all measurements were carried out in triplicate. Quercetin was used as reference compounds. All solutions were made daily. The percent inhibition of DPPH free radical was calculated by the formula:

Percentage inhibition (%I) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

where, A_{blank} is the absorbance of the control reaction (DPPH alone), and A_{sample} is the absorbance of DPPH solution in the presence of the plant extract. IC_{50} values denote the concentration of the sample, required to scavenge 50% of DPPH free radicals.

Results and discussion

Chemical composition of the essential oil

The essential oils isolated by hydrodistillation from the aerial part of *Z. multiflora* were found to be Yellow oil, (2.80%) based on dry weight (Table 1). The essential oil was analyzed by GC and GC-MS. In total, forty eight components were identified in the essential oil of *Z. multiflora* that represented 99.82% of the oils. The major components were Carvacrol (63.51%), Linalool (5.78%), *p*-cymene (5.31%), Thymol (4.60%), β-caryophyllene (3.15%) and γ-terpinene (2.62%). The composition of the essential oil of *Z. multiflora* has been reported previously by other researchers. Mahmoodi *et al.*, (2012) reported, phenolic monoterpene carvacrol (71.1%) and γ-terpinone (7.34%) were the main components of *Z. multiflora* essential oil. Dehkordi *et al.*, (2010) reported, monoterpen and thymol were the main components of *Z. multiflora* essential oil. Alipour-eskandani, (2011) found that the major components of *Z. multiflora* obtained from Shiraz Province of Iran was Carvacrol (71.1%). Rastegar *et al.*, (2011) reported, thymol (30.72%) and carvacrol (29.95%) were the main components of *Z. multiflora* essential oil.

Table 1. Essential oil constituents of Iranian *Z. multiflora*.

No	Component	RI	% in essential oil
1	α-Thujene	923	0.16±0.05
2	α-Pinene	930	1.40±0.24
3	Camphene	944	0.11±0.05
4	Sabinene	969	0.01±0.01
5	β-pinene	973	0.23±0.11
6	3-Octanone	981	0.70±0.13
7	Myrcene	987	0.95±0.24
8	3-Octanol	991	0.04±0.01
9	n-Decane	995	0.11±0.06
10	α-Phellandrene	1002	0.15±0.04
11	δ-3-Carene	1008	0.06±0.02
12	α-Terpinene	1014	0.90±0.14
13	*p*-Cymene	1022	5.31±0.75
14	Limonene	1025	0.32±0.12
15	β-Phellandrene	1026	0.13±0.04
16	1,8-Cineole	1028	0.68±0.15
17	(Z)- β-Ocimene	1032	0.01±0.01
18	Benzene acetaldehyde	1039	0.01±0.01
19	(E)- β-Ocimene	1043	0.04±0.01
20	γ-Terpinene	1055	2.62±0.35
21	cis-Sabinene hydrate	1063	0.30±0.14
22	trans-Linalool oxide	1068	0.16±0.06
23	Terpinolene	1084	0.39±0.12
24	Linalool	1100	5.78±0.85
25	Hotrienol	1102	0.13±0.04
26	Borneol	1162	0.17±0.05
27	Terpinene-4-ol	1174	0.86±0.19
28	α-Terpineol	1187	0.26±0.08
29	γ-Terpineol	1201	0.58±0.12
30	trans-Dihydro carvone	1210	0.22±0.08
31	Thymyl methyl ether	1237	1.02±0.21
32	Carvacrol methyl ether	1240	----
33	Thymol	1290	4.60±0.75
34	Carvacrol	1300	63.51±2.25
35	α-Terpinyl acetate	1349	0.04±0.02
36	Thymol acetate	1354	0.05±0.02
37	Eugenol	1357	0.11±0.04
38	Carvacrol acetate	1372	0.74±0.17
39	α-Gurjunene	1406	0.04±0.01
40	β-Caryophyllene	1418	3.15±0.86
41	γ-Elemene	1431	0.07±0.02
42	Aromadendrene	1436	0.96±0.24
43	α-Humulene	1450	0.22±0.09
44	allo-Aromadendrene	1457	0.14±0.07
45	γ-Gurjunene	1468	0.03±0.01
46	Viridiflorene	1492	0.64±0.20
47	δ-Cadinene	1519	0.03±0.01
48	Germacren-D	1571	----
49	Spathulenol	1574	0.70±0.24
50	Caryophyllene oxide	1579	0.98±0.32
	Oil Yield (% w/w)		2.80
	Total		99.82

The monoterpene phenolic components, Carvacrol and thymol and their precursors, γ-terpinene and *p*-cymene are the main components in *Z. multiflora* essential oil in our research and previous studies. But the amounts of these components are differences in other studies as a correlation are between these components. This results according to thymol and carvacrol biosynthesis pathway. Mikio and Taeko (1962) and Yamaura *et al.* (1992) proposed that thymol biosynthesis pathway renders as follows: γ-Terpinene is the component involved in the aromatization process which results in the formation of *p*-cymene, the precursor of possible oxygenated derivatives, thymol or carvacrol. Thymol and carvacrol are structurally very similar, having the hydroxyl group at a different location on the phenolic ring. It may be assumed that the sequence in this process is as follows: γ -terpinene, *p*-cymene, thymol or carvacrol (Figure 1).

Table 2. Total phenolic content and radical scavenging activity of Iranian *Z. multiflora*.

Growth conditions	Total phenolic content [a] (mg GAE/g DW)	IC_{50} [b] (µg/ml)
Z. multiflora	38.64 ± 1.45	38.54 ± 0.43 b
Quercetin (artificial antioxidant)	-----	28.23± 0.42 a

[a] Data expressed as mg of gallic acid equivalents per g dry weight (DW).

[b] IC_{50}: Data expressed as µg per millilitre. Lower IC_{50} values indicated the highest radical scavenging activity. Means with different letters were significantly different at the level of $p<0.05$.

Each value in the table was obtained by calculating the average of three experiments ± standard deviation. Means with different letters were significantly different at the level of $p<0.05$.

Total phenolic content and antioxidant activity

Phenolic compounds are commonly found in both edible and non edible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Many plants extracts containing bioactive compounds including phenolics and flavonoid possess efficient antioxidant properties preventing free radical damages (Koleva, *et al.*, 2002 & Larson, 1998).

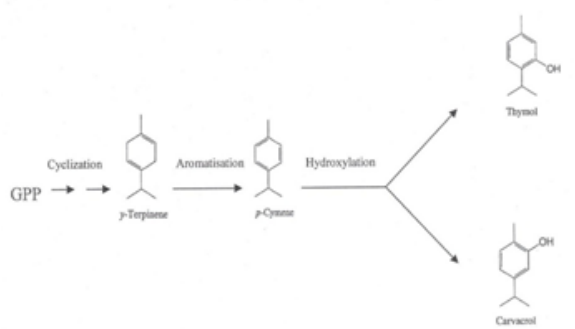

Fig. 1. Thymol and carvacrol biosynthesis pathway (Mikio and Taeko, 1962).

Free radicals may cause many disease conditions such as cancer and coronary heart disease in human (Javanmardi, *et al.*, 2003 & Löliger, 1991).

Phenolic compounds are a class of antioxidant agents which act as free radical scavengers and are responsible for antioxidant activity in medicinal plants (Shahidi and Wanasundara, 1992). The total phenolic content in *Z. multiflora* was measured by Folin Ciocalteau reagent and expressed as gallic acid equivalent (standard curve equation: y = 0.6035x - 5.4306, R^2 = 0.9922 data not shown). According to the findings presented in the table 2, *Z. multiflora* extract has high phenolic content (38.64 mg GAE/g DW). The antioxidant activity of the aerial parts of *Z. multiflora* extracts were assessed by the DPPH (2, 2-diphenyl-1-picryl hydrazyl) free radical scavenging. The DPPH assay determines the scavenging of stable radical species of DPPH by antioxidant (Othman, *et al.*, 2007).

The capacity of reducing DPPH radical by antioxidants was determined by monitoring the decrease in its absorbance at 517 nm (Firuzi *et al.*, 2005).

According to the Table 2. The *Z. multiflora* extracts

has high antioxidant activity (38.54µg/ml) compare Quercetin as standard artificial antioxidant. The total phenolic contents of *Z. multiflora* showed negative linear correlation with the results of DPPH ($Y=0.8539x + 42.639$, $R^2 = 0.7434$, data not shown). In fact, with increasing in total phenolic content, IC_{50} value in DPPH radical scavenging decreased and antioxidant activity was increased. These results suggest that the major part of the antioxidant activity in *Z. multiflora* results from the phenolic compounds. This is in line with the observation of other authors who found similar correlations between total phenolic content and antioxidant activity of various plants (alizadeh *et al.*, 2010 and 2011, Javanmardi *et al.,* 2003; Nencini *et al.,* 2007).

Conclusions

The present study showed that, essential oil composition in *Z. multiflora*,is rich in phenolic compounds as carvacrol and thymol and his precursors, also the extracts of *Z. multiflora* indicating good phenolic content and antioxidant activity. The results showed that, Iranian *Z. multiflora* are strong radical scavengers and can be considered as a good source of natural antioxidant for traditional and medicinal uses.

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