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Composition and antioxidant activity of the essential oils of *Thymus trautvetteri* Klokov & Des.-Shost., Thymus *migricus* Klokov & Des.-Shost. and *Thymus caespititius* Brot. from Iran

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ABSTRACT

The chemical composition of the essential oils of *Thymus trautvetteri*Klokov& Des.-Shost., Thymus *migricus*Klokov& Des.-Shost.and*Thymus caespititius*Brot.growing wild in Iran were examined by GC and GC±MS. Elaven components were characterized for *Thymus trautvetteri*with α- Terpinen-7-al (58.62%), P-Cymene (10.9%) andThymol,methyl ether (6.20%) as the major constituents. For *Thymus caespititius*, 12 components were identified with Z- Nerolidoli (17.91%), 1,8- Cineol (13.91%) andThymol (13.33%), as the major constituents and in *Thymus migricus*,16 compounds have been identified. p-Cymen-7-ol (35.98%), cis- Sabinene hydrate (10.45%) and P-Cymene (10.26%) were the main components of this essential oil. Also The essential oils of T. trautvetteri, T. caespititius and T. migricus were subjected to screening for their possible antioxidant activities using 2,2-diphenyl- 1-picrylhydrazyl (DPPH) assay method. *Thymus migricus* showed the best radical scavenging activity with an averaged IC_{50} value of 3.1 ± 0.15 µg/ml.

INTRODUCTION

Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents[1]. Use of essentialoils as antimicrobial agents in food systems may be consideredas an additional intrinsic determinant to increase the safety and shelf life of foods[2-4].

Thymus species are well known as medicinal plants because of their biological and pharmacological properties. The genus *Thymus* L., known as ''Avishan'' in Persian, is a wellknown aromatic perennial herb originated from Mediterranean region. Among 215 species of this genus grown in the world, 14 speciesare distributed in Iranian flora.[5,6]In traditionalmedicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, antiseptic, antitussive and carminativeas well as treating colds[7-8].*Thymus* oils and extracts are widely used in pharmaceutical, cosmeticand perfume industry also for flavoring and preservation of severalfood products[9]. Thyme (*Thymus vulgaris* L.) belonging to the *lamiaceae* family is a pleasant smelling perennial shrub, which grows in several regions in the world[10]. It's well known aromatic plant and its essential oil and aromatic water are used in the mountain regions of the Mediterranean parts of Turkey. Thyme was used by the Greeks as incense in their temples and by the Romans in cooking and as a source of honey. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics[11,12]. Traditionally basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction[13]. Thyme also possesses various beneficial effects as antiseptic, carminative, antimicrobial andantioxidative properties[14]. Compared to reported essential oil compositions of different *Thymus* species, investigations on their biological activities are stillscarce.

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The antibacterial activities of the oils of *T. pubescens* and*T. serpyllum* have been studied and the oils were found to possessbactericidal activities[15]. Bacterial susceptibilityand chemical composition of the oils of *T. kotschyanus*and *T. persicus* have been studied[16].

As part of our studies on essential oil-bearing plantsfrom Iran, we now report the antimicrobial capacityand composition of the essential oils isolated from theaerial parts of three*Thymus* species namely *Thymustrautvetteri*Klokov& Des.-Shost.,Thymus *migricus*Klokov& Des.-Shost. and *Thymus caespititius* Brot. collected during the vegetative phase. To the best of our knowledge, reports on the chemical composition of the essential oil and antimicrobial profiles ofthese plant speciesare scant and there is no report on composition and biological activity of *T. trautvetteri* essential oil. Also a literature review shows that there are a few reports on the phytochemical and biological investigation of essential oils from *T. migricus*and *T. caespititius*. Thus, the present research reports (i) the chemical compositionof the essential oil of aforementioned species that growing in the wild in Iran, (ii)*in vitro* antioxidant activity profiles of these plant essentialoils using 2,2- diphenyl-1 picrylhydrazyl (DPPH) radical scavenging assay and (iii) total phenolic compoundscontent of the plant essential oils as gallic acid equivalents.

EXPERIMENTAL

Plant material and isolation procedure

The plant materials were collected in June 2012 from northwestern Iran. The plants were identified at the Department of Biology, University of Shiraz, Iran and a voucher specimen was deposited at the herbarium of the Medicinal and Natural Products Chemistry Research Centre, Shiraz, Iran. Aerial parts of plants were air-dried at room temperature (25 ºC) in the shade and hydrodistilledusing a Clevenger-type apparatus for 4 h. They were dissolvedin n-hexane, dried over anhydrous sodium sulphate andstored at 4–6 ºC.

Identification of the oil components

GC analysis was carried out using a Agilent 6890N chromatograph (FID) with a HP-5 column (30 m×0.25 mm; 0.25 µm filmthickness). The oven temperature increased from 60 to 240 ºC at3 ºC/min, the injector and detector temperatures were 240 and250 ºC, respectively. Helium was used as the carrier gas with a flowrate of 0.9 ml/min. Relative percentage data was obtained fromelectronic integration of peak areas without the use of correctionfactors. GC–MS analysis was carried out using a Hewlett–Packard 6890machine operating at 70 eV ionisation energy, 0.5 s/scan and themass range: 35–350, equipped with a HP-5 capillary column (phenylmethyl siloxane, 30 m × 0.25 mm; 0.25 µmfilm thickness) programmedas above with helium as the carrier gas with flow rate 0.9 ml/min and a split ratio of 1:20. One of each of the oils was injected for GC and GC–MS. Retention indices were determined by using retention times of n-alkanes that had been injected after the oil under the same chromatographic conditions. The retention indices for all the components were determined according to the Van Den Dool method using n-alkanes as standard[16]. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder three libraries or with the published mass spectra[17].

Assessment of antioxidant activity by DPPH radical scavenging assay

Radical scavenging activity of essential oils was measuredagainst the stable free radical DPPH as described previously.[18]Briefly, three differentdilutions of essential oils, in the range 2.5–20 mg/ml, were incubatedwith a methanolic solution of DPPH 100 µM. After 30 minof incubation at room temperature, the absorbance at 517 nmwas measured by a spectrophotometer. The percentage of inhibition (%I) of the radical was calculatedaccording to the change of absorbance of the DPPH solution foreach dilution of essential oil and IC_{50} values were determined.

Total phenolic content

Total phenolic content in essential oils was determined by theFolin–Ciocalteau colorimetric method as described previously[19]. Briefly, 10 µl of essential oil with 10 µl ofTween-20 were mixed with 0.5 ml Folin–Ciocalteau reagent diluted10 times in deionised water. A methanolic solution of catechin0.25 mg/ml was tested in parallel as reference compound. After 5 min of incu-

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bation at room temperature, 0.4 ml of Na_2CO_3 7.5% in water was added to the samples and they were incubatedat room temperature in the dark. The absorbance at 760 nm wasread after 90 min against a blank of deionised water with a spectrophotometer (Bio-Tek, Model Uvikon XL). The total phenolic content was expressed as mg of catechinequivalent in each g ofessential oil.

Statistical analysis

Multiple comparisons among antioxidant and total phenol valueswere performed by one-way analysis of variance (ANOVA), followedby Turkey post-hoc test using the software SPSS (version11.5.0 for Windows; SPSS Inc., Chicago, IL). Data were considered statistically different at $P < 0.01$.

RESULTS AND DISCUSSION

Constituents were identified by GC-MS analysis of the essential oils,and their retention indices and area percentages are shown in TABLE 1. Chromatographic

TABLE 1 : Chemical composition of the essential oils from three *thymus* species

No.	Constituent	RI	*T. trautvetteri*	*T. caespititius*	*T. migricus*
1	α-Pinene	939	-	-	1.50
2	Comphene	954	-	-	1.49
3	P-Cymene	1025	10.9	-	10.26
4	1,8- Cineol	1031	1.71	13.91	-
5	δ-Terpinene	1060	3.80	-	-
6	cis- Sabinene hydrate	1070	-	-	10.45
7	m-Mentha-4,8-diene	1088	-	-	4.93
8	Linalool	1097	-	2.69	-
9	trans- Sabinene hydrate	1098	-	-	1.34
10	Ipsdienol	1145	1.91	-	-
11	Camphor	1146	1.39	3.12	2.78
12	Isopulegol	1150	-	-	7.69
13	Borneol	1169	-	2.98	-
14	4-Terpineol	1177	-	-	1.13
15	α-Terpineol	1189	-	9.00	1.67
16	Thymol,methyl ether	1235	6.20	-	2.74
17	Thymoquinone	1252	1.73	-	-
18	α- Terpinen-7-al	1285	58.62	-	-
19	Thymol	1290	1.62	13.33	7.78
20	p-Cymen-7-ol	1291	-	-	35.98
21	Carvacrol	1299	-	5.72	-
22	α- Terpinyl acetate	1349	-	2.45	-
23	Linalylisobutanoate	1375	-	-	2.10
24	Z- Caryophyllene	1409	3.79	-	-
25	E- Caryophyllene	1419	1.22	-	3.09
26	Cadinene	1514	-	-	2.82
27	Z- Nerolidoli	1533	-	17.91	-
28	Caeyophyllenoxide	1583	-	4.28	-
29	α- Bisabolol	1686	-	2.74	-
30	Eudesm- 7(11)- en-4-ol	1700	-	7.65	-
31	Taraxeron	2017	3.83	-	-
	Total		96.57	85.78	98.02

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analyses resulted in the identification of 31 components, representing 96.57% in *T. trautvetteri*, 85.78% in *T. caespititius* and 98.02% in *T. migricus* of the essential oils.

Eleven compounds were identified in the essential oil of *T. trautvetteri*. A single compound, α- Terpinen-7-al, accounted for 58.62% of the oil, although 10 compounds were identified. P-Cymene (10.9%), Thymol,methyl ether (6.20%) andTaraxeron (3.83%)Were also found as major components. Regarding *T. caespititius* essential oil, 12 compounds, corresponding to 85.87% of the chemical components in the essential oil, were identified. Among these, the major constituents wereZ- Nerolidoli (17.91%), 1,8-Cineol (13.91%), Thymol (13.33%),α-Terpineol (9.00%), and Eudesm- 7(11)- en-4-ol (7.65%), representing 61.80% of the essential oil. In the T. migricus essential oil, 16 compounds have been identified. p-Cymen-7-ol (35.98%), cis- Sabinene hydrate (10.45%), P-Cymene (10.26%) and Thymol (7.78%) were the main components of this essential oil.

The essential oils of *T. trautvetteri*,*T. caespititius* and *T. migricus*were subjected to screening for their possible antioxidant activities using 2,2-diphenyl- 1-picrylhydrazyl (DPPH) assay method. DPPH shows a maximum ultraviolet and visible (UV–Vis) absorbance at 517 nm. The reduction in the intensity of absorption at 517 nm of methanol solutions of DPPH radical in the presence of antioxidants is usually taken as a measure of their antioxidant activity. In this study, the ability of three essential oils to scavenge DPPH radical was determined on the bases of their concentrations providing 50% inhibition (IC_{50}). The essential oils and positive control (Quercetin) IC_{50}values are given in TABLE 2.

TABLE 2 : Antioxidant activity and total phenolic contents of three *thymus* species

Plant name	DPPH IC_{50} (mg/ml)	Total phenolic content (mg catechin equivalent/g essential oil)
T.trautvetteri	6.77 ± 0.13	4.65 ± 1.00
T.caespititius	4.6 ± 0.19	4.75 ± 1.00
T. migricus	3.1 ± 0.15	3.9 ± 1.86
Quercetin	0.72±0.47	-

Values represent the mean of three experiments ± SD. Quercetin was tested as a reference compound in the DPPH assay. Values with different letters in the same column are significantly different.

T. migricus showed the best radical scavenging activity with an averaged IC_{50} value of 3.1 ± 0.15 µg/ml, about 21% of the potency of synthetic standard Quercetin. DPPH assay results showed good correlations with the total phenolic contents of the plants, measured by the Folin-Ciocalteau assay (TABLE 2).

CONCLUSION

In summary, the results presented here contribute to theknowledge of chemical compositionand antioxidant activities of the tested essential oils obtained from aromatic plants growing in the south-eastern part of Iran. A literature review shows that chemical analysis of *T. trautvetteri* essential oil not previously described for this species of Thymus; and there are a few reports on the chemical composition of the essential oil*T. caespititius* and *T. migricus*. In antioxidant activity assay, despitethe moderate activity of *T. trautvetteri*, the data presented in this studyare also significant given that this is the first time its antioxidant effects assayed for *T. caespititius* and *T. migricus* have been reported.

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