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Evaluation of chemical and phenolic compounds and antiradical properties of *Thymus vulgaris* and *Saturejahortensis* essential oils from Jiroft city

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Abstract

Thymus vulgaris and *Saturejahortensis*L. are popular in folk medicine. In this research Methanolic, Ethanolic, Acetonic and Hexan extracts of *Thymus vulgaris* and *Saturejahortensis*Lplants grown in Jiroft city were evaluated for their antiradical and antioxidant properties also chemical composition, phenolic compounds and antioxidant properties of essential oils of these plants were measured. Theseplants were collected from Jiroft Azad university research farm. After drying the plant materials in shade, essential oils were extracted by hydro-distillation and analyzed by gas chromatography using mass spectrometric detection. Phenolic content was measured by FolinCiocalteau method and antioxidant activity was measured by DPPH method.Essential oils of *Thymus vulgaris* contained higher amount phenolic compounds and antioxidant activity(0.75 mg gallic acid/ml essence and 85.9 % DPPH) than oil of *Saturejahortensis L*(0.4 mg gallic acid/ml essence and 65.18 % DPPH).Chemical composition of the essential oils by GC/MS showed seven common chemical compounds of alpha-Pinene, Alpha-Terpinene, Cymene, gamma-Terpinene, Thymol, carvacrol and Myrcenewere found in both the oils. Major composition oil of *Thymus vulgaris* were Thymol, gamma-Terpinene and Cymene and those of *Saturejahortensis L*were carvacrol, gamma-Terpinene and Cymene.

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Introduction

The main class of substances used in aromatherapy are essential oils, which are obtained from various aromatic parts of plants by different methods such as hydro and steam distillations or cold pressing. EOs and extracts of various species of edible and medicinal plants, herbs, and spices constitute of very potent natural biologically active agents (Nychas*et al.*, 2003).Essential oils are used in perfumery, aromatherapy, cosmetics, medicine, incense, household cleaning products, and for flavoring food and drinks etc. Among the aromatic plant species, *Thymus vulgaris* and *Saturejahortensis L.* occupies a special position.

The *genus Thymus* L., known as "Avishan" in Persian, is a well-known aromatic perennial herb originated from Mediterranean region. Thymus genus plants are mostly woody stem, aromatic, ever green, durable and subshrubs and are usually found in calcic soil and grass fields throughout Europe, Africa, and Asia (Ghahreman, 1994, Mehrgan*et al.*, 2008). All species of Thymus are rich in essential oils and often contain phenolic compounds which are strong antiseptics and antioxidants (NaghdiBadi*et al.*, 2003; Sefidkon and Rahimibidgoli, 2001). Previously, the chemical compositions of the EOs from 11 populations of *T. daenensissubsp. daenensis* were evaluated. carvacrol, thymol and geraniol were found as the main constituents in the oils of the tested populations (Bahreininejad*et al.*, 2010).

Saturejahortensis L. is a medicinal herb recognized by its different effects in drug therapy and traditional medicine, and is widely used in Iran. *Saturejahortensis L.* is a plant, belongs to *Lamiaceae* family (Labiatae) and it is among the species with significant antioxidant effects the most important compounds of *Saturejahortensis* L. essential oil are carvacrol (30 - 40%), thymol(20 - 30%), and some phenolic compounds (Omidbeygi*et al.*, 2007). The objective of this study was Evaluation of chemical and phenolic compounds and antiradical properties of *Thymus vulgaris* and*Saturejahortensis L* essential oils from Jiroft city.Additionally, several studies indicated that different species of *Thymus vulgaris* and*Saturejahortensis*essential oils exhibit the antioxidant and antiradical effects. Since no data exists on polyphenol content and antiradical activity of *Thymus vulgaris* and*Saturejahortensis*essential oils from Jiroft city, present study was conducted in order evaluation of the potential antioxidant activity and also estimation of the phenolic content and chemical composition of these plants.

Materials and methods

Chemicals

All solvents used were of analytical grade; 1, 1-diphenyl -2- picrylhydrazyle (DPPH) was procured from Sigma Chemical Co.; Gallic acid, FollinCiocalteu, Methanol, Sodium carbonate were purchased from Merck Co. (Germany).

Extraction of the essential oil

Air-drying of plant material was performed in ashady place at room temperature for 10 days. Dried aerial parts (100 g) were cut and subjected to hydro distillation for 3 h, using a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4°C.

Estimation of total phenolic compounds

Total phenolic content of each extract was determined by the Folin–Ciocalteu micro method (Slinkard and Singleton 1977). Briefly, 20 μl of extract solution were mixed with 300 μl of Na_2CO_3 solution (20%), then 1.16 ml of distilled water and 100 μl of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min respectively. Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as Gallic acid equivalents by using the following linear equation were obtained from calibration curve:

$$A= 0.98 \quad C \quad + \quad 9.321 \quad \times \quad 0.001$$

R2= 0.9965 (1)

Where A is the absorbance and C is concentration as Gallic acid equivalents (μg/ml).

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the Blios (1958) method. Briefly, 1 ml of a 1 mM methanol solution of DPPH was mixed with 3 ml of extract solutions in methanol (containing 50–400 µg of dried extract). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were carried out using Agilent 5975C Series GC-MSD system (7890A GC and 5975C inert MSD) operating in the EI mode at 70 eV, equipped with a HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.50 µm).Screener method (Agilent application 5988-6530EN, 2010) using freely available Flavor2 screener compound database for retention time locking (RTL) to n-pentad cane (at 27.500 min) was used for analysis. 1 µl of diluted essential oil was injected in split mode at two different split ratios (25:1 and 12.5:1), and inlet temperature was held at 250 °C. Helium was used as carrier gas in constant pressure mode at 9.4 psi. The oven temperature was programmed as follows: 60 °C rose to 240 °C (5 °C/min) and not held. MSD was operated in scan mode in 40-400 m/z range, with ion source and transfer line temperatures held at 230 and 275°C, respectively. The identification of the compounds was based on comparison of their retention times (RT) and mass spectra with NIST/Flavor2 /Adams libraries spectra and literature (Adams, 1995).

Statistical analysis

All these experiments were replicated three times, and the average values are reported. The phenolic compounds and antioxidant activity of *Thymus vulgaris* and*Saturejahortensis*Jiroft cities were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at P<0.05 significant level using the SAS software (2001) program

Results and discusion

Total phenolic compounds

The averages of total phenolic compounds of *Thymus vulgaris* and *Saturejahortensis*based on Folin-Ciocalteau methods were shown in table 1.

Table 1. Total phenolic compound (mg gallic acid/ml essence) of *Thymus vulgaris* and *Saturejahortensis.*

sample	Total phenolic compounds (mg/ml)
Thymus vulgaris	0.75^{a}
Saturejahortensis	0.4^{b}

Each observation is a mean ± SD of 3 replications. Means with same superscripts had no significant difference with each other (P >0.05).

As can be seen from table 1, two plants have significant differences (P < 0.05) in total phenolic content. Among studied plants, *Thymus vulgaris* contained the highest amount of total phenolic, followed by*Saturejahortensis*.

Table 2. DPPH radical scavenging activity of *Thymus vulgaris* and *Saturejahortensis*essential oils at various concentrations.

Essential oils concentration (ppm)	*Thymus vulgaris*	*Saturejahortensis*
50	66.76^{d}	9.72^{g}
100	75.05^{c}	18.33^{f}
250	80.65^{ab}	21.45^{f}
500	83.34^{a}	34.22^{e}
1000	85.09^{a}	65.18^{d}

Each observation is a mean ± SD of 3 replications. Means with same superscripts had no significant difference with each other (P >0.05).

Table 2 showed that *Thymus vulgaris* essential oilshad higher DPPH radical scavenging activity (IC_{50}= 42.25 ppm) than *Saturejahortensis*(IC_{50}= 896.87 ppm). DPPH radical scavenging increased by increasing concentration and significant differences (P <0.05) were found between DPPH radical scavenging of *Thymus vulgaris* essential oils and *Saturejahortensis*.

Chemical compositions of essential oils

Fifteen components were identified in the essential oil of *Thymus vulgaris*, which represented 100% of the oil components. The chemical constituents of oils are presented in (Table 3). The components are listed in order of their retention time on the HP-5MS column.

Table 3. Composition of *Thymus vulgaris* essential oils detected by GC/MS.

NO.	Compound	Formula	RT	Amount of composition (%)
1	alpha-Phellandrene	$C_{10}H_{16}$	10.275	0.79
2	alpha-Pinene	$C_{10}H_{16}$	10.568	0.69
3	1-Octen3-ol	$C_{10}H_{16}$	12.181	0.9
4	Myrcene	$C_{10}H_{16}$	12.561	1.54
5	Alpha-Terpinene	$C_{10}H_{16}$	13.673	1.95
6	Cymene	$C_{10}H_{14}$	13.983	13.49
7	1,8-cineole	$C_{10}H_{18}O$	14.282	0.52
8	gamma-Terpinene	$C_{10}H_{16}$	15.214	14.59
9	-	$C_{10}H_{18}O$	15.648	0.57
10	linalool	$C_{10}H_{18}O$	16.622	2.86
11	Berneol	$C_{10}H_{18}O$	19.297	0.56
12	Terpinene-4-ol	$C_{10}H_{18}O$	19.547	0.88
13	Thymol	$C_{10}H_{14}O$	22.996	56.23
14	carvacrol	$C_{10}H_{14}O$	23.208	3.44
15	Trans-Caryophyllene	$C_{10}H_{14}O$	27.055	0.98
				100

The major components of the *Thymus vulgaris* essential oils were Thymol (56.23%) and gamma-Terpinene (14.59%); the quality of *Thymus vulgaris* is generally determined by its Thymol content.

Twelve components were identified in the essential oil of *Saturejahortensis*, which represented 100% of the oil components. The chemical constituents of oils are presented in (Table 4).

Table 4. Composition of *Saturejahortensis* essential oils detected by GC/MS.

NO.	Compound	Formula	RT	Amount of composition (%)
1	1-phellandrene	$C_{10}H_{16}$	10.212	0.37
2	Alpha-Pinene	$C_{10}H_{16}$	10.526	1.44
3	Beta-Pinene	$C_{10}H_{16}$	12.222	0.56
4	Myrcene	$C_{10}H_{16}$	12.528	1.63
5	Alpha-Terpinene	$C_{10}H_{16}$	13.645	2.61
6	Cymene	$C_{10}H_{14}$	13.942	8.74
7	limonene	$C_{10}H_{16}$	14.103	0.3
8	gamma-Terpinene	$C_{10}H_{16}$	15.207	28.87
9	4-Terpineol	$C_{10}H_{18}O$	19.526	0.36
10	Carvacrol	$C_{10}H_{14}O$	23.272	54.16
11	Thymol	$C_{10}H_{14}O$	25.167	0.48
12	Caryophyllene l	$C_{15}H_{24}$	27.041	0.47
				100

The major components of the*Saturejahortensis* essential oils were Carvacrol (54.16%) and gamma-Terpinene (28.87%).

The ability of essential oils to scavenge 2, 2′-diphenyl 1-picrylhydrazyl radical (DPPH•) was assessed by spectrophotometer. The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Porto *et al.*, 2000).

phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, … . (Gao*et al*, 2000). As it can be seen *Thymus vulgaris* essential oils that contained the highest amount of total phenolic, was found to be the most active radical scavenger followed by *Saturejahortensis*. A high correlation between free radical scavenging and the phenolic contents has been reported for fruits (Gao*et al.,* 2000; Jimenez-Escrig, 2001, Arabshahi, Delouee, Urooj 2007, Benzie and Szeto, 1999). So less Antioxidant activity may be due to less phenolic compounds in *Saturejahortensis*essential oils.

Thymol and Carvacrol essencesare phenoliccomplex that have very strong antibacterial and antioxidant effects (Yahyazadeh*et al.,* 2008).

Radonic and Milos (2003) showed that the major compound of *Thymus vulgaris* essential oilwas phenolic monoterpenethymol (45.2%). Other important compounds were monoterpenic hydrocarbons *p*-cymene (6.4%) and γ-terpinene (5.9%) and oxygen-containing compounds carvacrol methyl ether (5.8%), thymol methyl ether (5.1%), carvacrol (5.3%), geraniol (5.0%) and borneol (3.9%). Mihajilov-Krstev*et al.* (2010) Thirty six components (86.14%) identified asconstituents of *Saturejahortensis* essential oil by combinedGC/FID and GC/MS analyses. The majorcomponents were carvacrol (67.00%), γ-terpinene (15.3%), and p-cymene (6.73%) which is in accordance with our results.

It is well known that the chemical composition and yield of essential oils are affected by exogenous factors such as geographical position, altitude, climate and soil composition Baser *et al.* (2004).

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