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Original Article

Chemical Composition and Antioxidant Properties of some Species of Lamiaceae Family from Iran

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Abstract

Spearmint (*Mentha spicata* L.), oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) are used as traditional medicines, culinary herbs and food flavorings. All samples collected from Khuzestan province, Iran and plant extracts prepared using Clevenger apparatus. Antioxidant capacity and major parameters affecting antioxidant properties including total phenolics, flavonoids and vitamin C were evaluated using aqueous extract. Chemical composition of essential oil was evaluated be GC/MS analysis. Results showed high antioxidant capacity of thyme (IC_{50}=103.78), followed by spearmint and oregano (IC_{50} of 143.87 and 164.01 respectively). As previous studies confirmed high correlation of total phenolics, flavonoids and ascorbic acid with antioxidant capacity, thyme had the highest bioactive compounds comparing to other extracts. The highest chemical compounds in thyme, oregano and spearmint were menthol (58.3%), menthol (40.1%) and thymol (39%) respectively. By increasing public interests to natural components, the aim of our study was evaluating antioxidant and bioactive components of plants used locally and try to find and screen new and natural compound for substituting synthetic antioxidant like, BHT.

Keywords: Spearmint, Thyme, Oregano, Antioxidant, DPPH, Total phenolics

Introduction

Reactive oxygen species and free radicals such as superoxide anion, hydrogen peroxide and hydroxyl radical are constantly formed in the human body by normal metabolic action and have been implicated in the pathogenesis of certain human disease, including cancer, aging, diabetes and atherosclerosis [1,2]. Antioxidants are known as molecules capable of inhibiting oxidation process in body, so preventing forming free radicals that can be found naturally in biological materials or foods [3].

in recent years plant-derived antioxidant have raised considerable interest among food scientist, manufacturers and consumers. Many spices and culinary herbs are common sources of phenolic compounds which have been reported to show superior antioxidant capacity [4].

Lamiaceae family is one of the largest families of flowering plant and comprises of about 3200 species. The family is noteworthy for the number of species producing and essential oil, spice or both of them [5].

Mentha spicata L. or common spearmint is a perennial hardy branched plant with height of 25-75 cm. It is now cultivated throughout Asia, Europe, Middle East and USA [6, 7]. There are 25-30 species within *Mentha* L. genus, including spearmint, peppermint, wild mint, corn mint, curled mint, bergamot, American mint, Korean mint, etc [8]. *Mentha* oil is among the 10 most traded in the word and is used in many industries including pharmaceuticals, cosmetics, food and chemicals. Spearmint is a stimulant that improves memory and

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has several biological uses, such as insecticide, antimicrobial, antioxidant and antispasmodics [9,10].

Oregano is native to Europe and central Asia, now cultivated all over the world. It's a hardy, bushy, herbaceous perennial plant up to 90 cm high [6, 11]. Oregano is reach in phenolic compounds with strong antioxidant and antibacterial and is widely used to extend the shelf life of foods [12,13]. Oregano has therapeutic properties (diaphoretic, carminative, antispasmodic, antiseptic, tonic) being used in traditional medicine system in many countries [14,15].

thyme is native to southern, southeastern Europe and western Mediterranean area and now widely cultivated a spice throughout temperate climates. It is a perennial, herbaceous shrub up to 45 cm height with woody root [6, 16]. thyme is an aromatic plant that has been long used in foods for culinary purposes. It's essential oil contains more than 60 ingredients, most of which posses important antioxidant and antimicrobial properties [17].

According to WHO majority of the world's population in developing countries still rely on herbal medicine to meet their health needs [18], so the purpose of this study is evaluating antioxidant capacity and chemical compositions of spearmint, oregano and thyme native to Khuzestan province at southwest of Iran.

Material and Methods

Plant Material

Aerial parts of wild growing flowering plants of *Mentha Spicta* L., *Origanum vulgare* L. *and Thymus vulgaris* L. were collected from Izeh, Khuzestan Province, Iran. Each plant considered as a separated experiment. Samples were air dried, grounded and the resulting powder subjected to hydrodistillation for 3 hours in a Clevenger-type apparatus according to European Pharmacopoeia method [19]. Essential oil and plant extracts were separated and essential oil were dried over anhydrous sodium sulphate and stored at 4 °C for further analysis [20].

Antioxidant Capacity

The ability of plant extracts for scavenging free radicals were evaluated using DPPH method. 50 µl of various dilutions of plant extract (0-300 µg/ml) were added to 5 ml methanol solution of DPPH (0.004%) and incubated for 30 min at ambient temperature. then sample absorbance recorded at 517 nm against control. Antioxidant activity or Inhibition (scavenging) percent calculated using following equation:

Antioxidant activity: $(Abs_{control} - Abs_{sample})/Abs_{control} \times 100$

The IC_{50} value is defined as the amount of the antioxidant necessary to inhibit (scavenge) DPPH radical by 50%. According to IC_{50} plant extracts with higher antioxidant activity have lower IC_{50} value. Synthetic antioxidant of BHT was used for comparing antioxidant capacity of plant extracts.

Determination of Total Phenolic Contents

Total phenolic content determined according to Folin-Ciocalteu method. 200 µL fo distilled water, 50 µL of diluted extract and 50 µL of Folin-Ciocalteu reagent were mixed together. After 6 min, 500 µL of 7.5% sodium carbonate solution were added to mixture and using distilled water to 1.3 mL and allowed to stand at room temperature for 60 min. then absorbance was read at 765 nm. Calibration curve constructed using Gallic acid and the results reported as mg of gallic acid equivalents (mg GAE)/100g DW [21].

Determination of Flavonoid Concentration

Total flavonoid content were determined based on formations of flavonoid-aluminum complex having absorption at 415 nm. Briefly 0.5 ml of each extract was dissolved in methanol (1.5 ml) and then 0.1 ml aluminum chloride 10% and 0.1 ml sodium acetate were added to the solution. Finally 2.8 ml distilled water added to the solution and after 30 min incubation at room temperature, absorbance was read at 415 nm. Standard curve constructed using different concentrations of catechin solution (0-100 mg/L) and total flavonoid expressed as catechin equivalent (mg CE/100g DW) [22].

Determination of Vitamin C

5 g of plant were ground in the dark for 20 min with 5 mL metaphosphoric acid (3% w/v). the mixture centrifuged at 2000 g for 5 min and the supernatant was collected. 1 ml of supernatant was mixed with 9 ml of EDTA solution (3.15 g oxalic acid and 0.0274 g EDTA in 500 mL distilled water), 1 ml metaphosphoric acid acetic solution, 2 mL sulfuric acid and 4 mL ammonium molybdate solution (5%). The mixture left to settle for 3 min and then absorbance was read at 705 nm. Ascorbic acid used for constructing standard curves and

results reported as mg ascorbic acid/g Fresh weight (FW) [2].

GC/MS analysis Conditions

For identification of chemical compounds in essential oil extracted using Clevenger apparatus, Agilent gas chromatography model 6890 N, equipped with MSD model 5973 N and fused silica capillary column were used for qualitative and quantitative analysis. The GC oven temperature was held at 50 °C for 5 min, then programmed from 50 °C to 240 °C at a rate of 3 °C/min and from 240 °C to 290 °C at a rate of 5 °C/min, held 2 min at 290 °C using He gas as carrier (1 ml/min). the temperature of injector and detector were 240 and 280 °C respectively. The percentage composition of the essential oils was computed form GC peak area without using any correction factors. Qualitative analysis was based on comparison of retention times and indices on both columns and mass spectra using computer mass spectra libraries model Agilient technologies 5973 Network and corresponding data available in the literature.

Statistical Analysis

The data collected in this research statistically analyzed using ANOVA and significant means differences were further evaluated using Duncan multiple range test at 5% probability level.

Results and Discussion

Antioxidant Activity

The scavenging effect of plant extracts investigated using DPPH radicals are shown in figure 1 and 2.

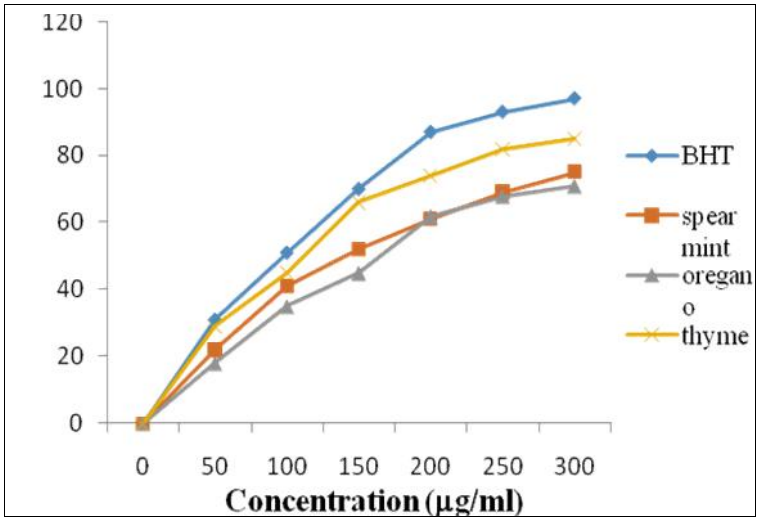

Fig. 1 Free radical scavenging activity of some species of Lamiaceae family

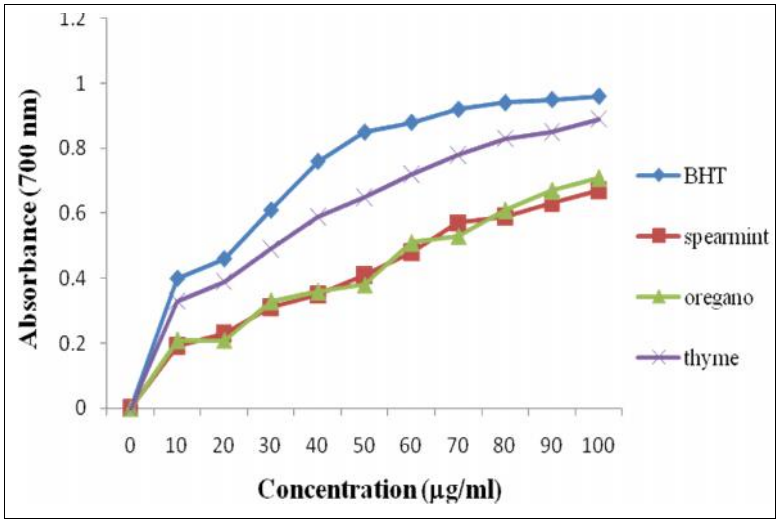

Fig. 2 Reducing power of some species of Lamiaceae family

Table 1 Bioactive compounds of thyme, oregano and spearmint

Plant type	Total phenol mg GA/100g DW	Flavonoid mg CE/100g DW	Vitamin C mg/g FW
Thyme	23±0.89	3.71±0.65	0.25±0.04
Oregano	12.2±0.92	1.81±0.09	0.17±0.08
Spearmint	17.2±0.65	2.1±0.11	0.22±0.1

Table 2 Essential oil composition of organo, spearmint and thyme growing in southwest of Iran

Oregano (*origanum vulgare* L.)		Spearmint (*menthe spicatha* L.)		Thyme (*thymus vulgaris* L.)	
Compound	percent	Compound	percent	Compound	percent
Menthol	58.3	Menthol	40.1	Thymol	39.0
p-cymene	38.1	Carvacrol	23.9	p-cymene	24.7
Thymol	4.8	Thymol	16.8	Sabinene	15.6
β-pinene	1.1	Neomenthol	3.7	Myrcene	3.8
γ-terpinene	0.58	E-caryophyllene	2.4	Limonene	2.1
α-pinene	0.57	Borneol	2.1	γ –terpinene	1.4
Limonene	0.44	β-bourbonene	1.6	Neomenthol	1.3
Camphene	0.41	*Trans*-sabinene hydrate	1.1	Mentone	1.1
Mentone	0.35	α- terpinene	1.1	β-Pinene	0.88
α-terpinene	0.35	Farnesene-E-β	0.88	Pulegone	0.73
Borneol	0.29	Eucalyptol	0.79	E-caryophyllene	0.64
Sabinene	0.22	Sabinene	0.75	α-copaene	0.22
Terpinene-4-ol	0.18	δ-3-Carene	0.55	*Cis*-sabinene hydrate	0.18
β-E-Farnesene	0.17	α-Cadinene	0.33	Camphene	0.16
caryophyllene oxide	0.17	D-Germacrene	0.21	terpinene-4-ol	0.13
1,8-Cineole	0.14	γ-Eudesmol acetate	0.17	α-bisabololoxide	0.12
Cis-sabinene hydrate	0.10	-	0.16	α-Cadinene	0.12
Cis-β-Ocimene	0.09	1,8-Cineole	0.13	Fenchyl acetate	0.09
β-Myrcene	0.09	Pulegone	0.13	α-Terpineol	0.07
α-Phellandrene	0.08	α-Terpineol	0.09	δ-3-Carene	0.5
Trans-sabinene hydrate	0.05	Pentadecanoic acid	0.07	Chrysanthenone	0.03
Total	95.5	-	96.3	-	91.6

Results showed all plant extract were capable of scavenging DPPH radical dose-dependent. The highest scavenging activity belonged to thyme with 85% percent, followed by spearmint (75%) and oregano (71%). Comparing scavenging activity result with synthetic antioxidant BHT at the same concentration (97%) shows high ability of some Lamiaceae family species for scavenging free radicals and acting as a good substitute for synthetic antioxidants. Based on IC_{50} values the same results were observed. The lowest IC_{50} which indicates the highest antioxidant activity belongs to thyme (103.78) followed by spearmint and oregano (143.87 and 164.01 respectively). Results show than thyme could be a good alternative for synthetic antioxidant of BHT (IC_{50}=88.7). In a study in Poland Radical Scavenging activity of fresh and dried peppermint, lemon balm and oregano were evaluated. After 5 minutes scavenging activity for dry samples were 95, 95 and 84 respectively [23]. Sahin *et al*., evaluated antioxidant activity of methanolic extract and oil of oregano in Turkey. Results showed high scavenging activity of methanolic extract (IC_{50}=9.9) [11].

Total Phenolic and Flavonoid Content and Correlation to Antioxidant Capacity

The amount of total phenolic content measured by Folin-Ciocalteu method varied significantly among plant extracts. The highest level of total phenolic content was found in thyme (23 mg GA/100g DW), followed by spearmint (17.2 mg GA/100g DW) and oregano (12.2 mg GA/100g DW) (table 1).

Same pattern as phenolic content was observed in flavonoid content of plant extracts.th highest level of flavonoid was observed in thyme (3.71 mg CE/100g DW) followed by spearmint (2.1 mg CE/100g DW) and oregano (1.81 mg CE/100g DW). Flavnoids are polyphenolic secondary

methabolites widely dispersed throughout the plant kingdom. They are the most widespread and diverse phenolic compounds [10].

Phenolic compounds are among the important constituents in plants that participate in the cell defense system against free radicals [23]. Researchers are interested in phenolic acid and flavonoids due to their pharmacological behavior and exerting protective effects against oxidative stress and reverse correlation with atherosclerosis and cardiovascular disease [8, 24]. Many studies have shown good positive linear relationship between total phenolic content and antioxidant activity[4]. Based on our data there is a great correlation between phenolic content and antioxidant activity based on DPPH method (R^2=0.96).

Vitamin C content

Vitamin C (ascorbic acid) content of thyme, spearmint and oregano were 0.25, 0.22 and 0.17 mg/g FW respectively. In addition to phenolic compounds, ascorbic acid is one the important constituents participating in defense system of plants [23]. Ascorbic acid is one of the major water soluble free radical scavengers found in biological tissues and is effective at scavenging free radicals and forming low energy radicals. It is considered to be one of the most powerful, least toxic natural antioxidants [6].

Chemical Compositions

Qualitative and quantitative composition of thyme, spearmint and oregano are shown in table 2.

In thyme identified compound represent 91.6% of total oil. The highest compound were thymol (39%) followed by para-cymene (24.7%), sabinene (15.6%), myrcene (3.8%), limonene (2.1%), γ-terpinene (1.4%). In oregano identified compounds represent 95.5% of total oil. The highest compound was menthol (58.3%) followed by p-cymene (38.1%), thymol (4.8%), β-pinene (1.1%) and γ-terpinene (0.58%). In spearmint identified components represent 96.3% of total oil. The highest compound was menthol (40.1%), followed by carvacrol (23.9%), thymol (16.8%), neomenthol (3.7%), E-caryophyllene (2.4%), borneol (2.1%) and β-bourbonene (1.6%).

Saei-Dehkordi et. al., evaluated chemical composition of essential oil of *Zataria multiflora* Boiss (Shirazi thyme) from different parts of Iran. In all samples thymol was the most abundant component ranging from 27 to 64% [25].

Tyagi Kumar & Malik evaluated chemical components of *Mentha× piperita* L. oil. The identified 47 compound that highest levels belonged to Menthol (19.1%), isomenthone (14.8%), limonene (10.6%), iso-menthanol (8.8%), menthyl acetate (6.6%), β-pinene (5.6%) and α-pinene (4.8%) [26].

Scherer *et al*., evaluated composition of Brazilian spearmint. They identified 37 compound that dominant constituent was carvone by 67.1%. the other important components were limonene (14.34%), myrcene (2%), dehydro-carvone (1.1%), bourbonene (1.8%) and caryophyllene (1.76%) [9].

In essential oil of oregao collected from Eastern Anatolia region of Turkey a total 62 constituent were identified representing about 89% of the oil. Caryophyllene (14.4%) and spathulenol (11.6%) were the main constituents followed by germacrene-E (8.1%) and α-terpineol (7.5%) [11].

In a study conducted in Lithuania essential oil of oregano growing wild were evaluated. The main constituents of the essential oil from 8 localities were β-ocimene (14.9-21.6%), germacrene D (10-16.2%), β-caryophyllene (10.8-15.7%) and sabinene (6.6-14.2%) [27].

In another study oregano essential oil contained carvacrol (57.71%) as the most prevalent compound, followed by p-cymene (10.91%), γ-terpinene (7.81%), terpinen-4-ol (6.68%) and thymol (3.83%) [13].

Conclusion

Plant secondary metabolite highly influenced by region. Evaluating local plants and their metabolites open a new era for substituting synthetic antioxidant for preserving food material and broaden their use as medicine and nutraceuticals. Our results indicated highly correlation of antioxidant properties with total phenilics, flavonoids and ascorbic acid. Among three plants of Lamiaceae family, including spearmint, thyme and oregano, the highest antioxidant activity was observed in thyme followed by spearmint and oregano.

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