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Evaluation of phenolic content and antioxidant capacity in some medicinal herbs cultivated in Iran

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ABSTRACT: In this study the total phenolic content and antioxidant capacity of various medicinal plant species belonging to the Lamiaceae family cultivated in the climatic conditions of Iran were determined and compared. The phenolic content and antioxidant capacity of flowers and leaves of some Lamiaceae species were also compared with those of several non-Lamiaceae species. Total phenolic content and antioxidant capacity of the selected herbs ranged from 6.87 to 33.22 mg GAE/g fresh weight and 0.59 to 3.70 mmol Fe/100 g fresh weight, respectively. The highest phenolic content and antioxidant capacity was found in rosemary (*Rosmarinus officinalis*) and the lowest contents were found in flower-of-an-hour (*Hibiscus trionum*). Although antioxidant capacity and phenolic content in the selected Lamiaceae herbs varied, all of them had useful levels of phenolic compounds and demonstrated considerable antioxidant capacity. It seems that consumption of these herbs could be useful to decrease the damage caused by reactive oxygen species and reduce the risk of diseases. Furthermore, the results showed that flowers as well as leaves of the selected herbs could have a high phenolic content and antioxidant capacity. In this study, Lamiaceae herbs were also shown to have higher amounts of phenolic compounds and antioxidants compared with herbs belonging to other families.

Key words: total phenolic content, antioxidant capacity, Lamiaceae

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INTRODUCTION

Active oxygen and nitrogen species can damage the human body. Unbalanced over-production of reactive oxygen species can lead to oxidative stress (Yildirim *et al*. 2000; Gulcin *et al*. 2002; Ellnain-Wojtaszek *et al*. 2003). The sources for formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be internal or external. Endogenous sources of reactive species generation are mainly normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages and peroxisomes (Fridovich 1986; Halliwell 1994) and exogenous sources include tobacco smoke, ionizing radiation, organic solvents and pesticides (Halliwell & Gutteridge 1989; Davies 1995; Papas 1996)

Damage to lipids, proteins, enzymes, nucleic acids and ultimately, cells and tissues is caused by the overproduction of reactive species leading to aging and a wide range of degenerative diseases including inflammation, cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson, and coronary heart pathologies, among others (Duan *et al*. 2006).

Antioxidant systems are known to scavenge these reactive species. Medicinal plants can be rich sources of antioxidants, especially phenolic compounds such as flavonoids, tannins and phenolic acids and antioxidant vitamins, including ascorbic acid, tocopherol, β-carotene

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and anthocyanins (Salah *et al*. 1995; Saskia *et al*. 1996).

The Lamiaceae family consists of approximately 200 genera with a cosmopolitan distribution. This family is known in Iran by 46 genera and 410 species and subspecies. Many members of this family have traditional and medicinal uses and have been used in folk medicine for many years. They are also applied as culinary and ornamental plants. Eighteen percent of the species are used for medicinal purposes. Lamiaceae species are mainly used for ailments related to the digestive system, especially flatulence and dyspepsia. This plant is also used as a reconstituent and for the treatment of infections (Naghibi *et al*., 2005).

The aim of this research was to determine and compare the total phenolic content (TPC) and antioxidant capacity in extracts of some medicinal plants belonging to the Lamiaceae family from Iran, and to compare them with extracts from several non-Labiateae species.

MATERIAL AND METHODS

Herb sample collection. Selected herbs were collected in August from Urmia university (Northwest of Iran) garden. Selected herbs belonging to Lamiaceae family were *Rosmarinus officinalis*, *Lavandula angustifolia*, *Mentha aquatica*, *Mentha ×piperita*, *Mentha spicata*, *Mentha pulegium*, *Salvia officinalis*, *Salvia sclarea*, *Salvia nemorosa*, *Melissa officinalis*, *Ocimum basilicum*, *Origanum vulgare*, *Thymus vulgaris*, *Thymus kotschyanus* and *Agastache foeniculum*. In addition, the phenolic content and antioxidant capacity of flowers and leaves of *Mentha pulegium*, *Salvia nemorosa*, *Ocimum basilicum*, *Polygonum aviculare* (Polygonaceae), *Hibiscus trionum* (Malvaceae), *Tanacetum balsamita* (Asteraceae), *Cichorium intybus* (Asteraceae) and *Borago officinalis* (Boraginaceae) were evaluated.

Total phenolic content determination. Total phenolics of the methanol extracts were determined colorimetrically using Folin-Ciocalteu reagent (Merck) as described by Seevers & Daly (1970). A standard curve was prepared using different concentrations of gallic acid (Merck) and results were expressed as mg gallic acid equivalent (GAE)/g fresh weight basis. Briefly, 0.5 ml of the methanol extracts were dissolved in 7 ml deionized water and 0.5 ml of Folin-Ciocalteu reagent were added to them in a 10 ml volumetric flask. The contents were mixed and allowed to stand for 5-8 min at room temperature. One ml of a sodium carbonate solution (75 g/l; Na_2CO_3) was then added, followed by bringing the volume to 10 ml with the addition of distilled water. Solutions were mixed and allowed to stand at room temperature for 1 h prior to the determination of total phenol concentration using a spectrophotometer (Pharmacia LKB, Novaspec II) at 725 nm.

Total antioxidant capacity determination. Antioxidant capacity was measured using the Ferric Reducing/ Antioxidant Power (FRAP) assay (Benzie & Strain 1996). FRAP assay measures the change in absorbance at 593 nm because of the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} form by the action of electron donating antioxidants. The FRAP reagent was prepared by mixing 300 mM acetate buffer (3.1 g sodium acetate + 16 ml glacial acetic acid, made up to 1 l using distilled water; pH=3.6), 10 mM tripyridyltriazine (TPTZ, Sigma-Aldrich) and 20 mM $FeCl_3.6H_2O$ in a ratio of 10:1:1 and 3 ml of prepared reagent was poured into test tubes. A total of 100 µl of sample and 300 µl of distilled water was then added to the same test tubes and incubated at 37 °C for 4 min. Absorbance was measured at 593 nm. FeSO4·7H2O was used as the standard and results were expressed in mmol Fe/100 g fresh weight.

Statistical analysis. Data were subjected to analysis of variance. The least significant difference (LSD) test was used for comparison of means (P = 0.05) and data were expressed as mean ± SE. Pearson's correlation test was used to determine the correlations amongst variables.

RESULTS

Significant differences were found amongst the selected herbs for their total phenolic contents and total antioxidant capacities (table 1).Although, there were various values of antioxidant capacity and TPC in selected herbs, all of them have degrees of phenolic compounds and antioxidant potential (table 1, figure 1 and figure 2).

The FRAP levels and TPC of selected herbs ranged from 0.59 to 3.70 mmol Fe/100 g fresh weight and 6.87 to 33.22 mg GAE/g fresh weight, respectively. The highest antioxidant capacity was observed for rosemary (3.70 mmol Fe/100 g fresh weight) followed by orange mint (3.68 mmol Fe/100 g fresh weight*)* and garden thyme (3.47 mmol Fe/100 g fresh weight). The highest TPC was also found in rosemary, (33.22 mg GAE/g fresh weight), followed by knotgrass (31.25 mg GAE/g fresh weight) and oregano (29.67 mg GAE/g fresh weight). The extract of flower-of-an-hour exhibited the lowest TPC (6.87 mg GAE/g fresh weight) and antioxidant capacity (0.59 mmol Fe/100 g fresh weight).

In the present study, among mint species, spearmint had the highest TPC, though there was no significant difference between spearmint and peppermint. Orange mint had the highest FRAP level of the mint species,

Table 1. Total phenolic content and antioxidant capacity in various extracts[a] of selected herbs

Total antioxidant power[c] (mmol Fe/100 g of fresh weight)	total phenolics[b] (mg of GAE/g of fresh weight)	botanical name	common name
3.70±0.11	33.22±1.28	*Rosmarinus officinalis*	rosemary
2.87±0.29	13.31±2.15	*Lavandula angustifolia*	lavander
3.68±0.12	11.26±0.99	*Mentha aquatica*	orange mint
2.50±0.06	19.46±0.35	*Mentha ×piperita*	peppermint
3.35±0.53	22.43±1.13	*Mentha spicata*	spearmint
2.94±0.05	15.95±0.52	*Mentha pulegium*	pennyroyal
2.49±0.36	14.53±0.33	*Salvia officinalis*	garden sage
2.92±0.02	11.95±1.05	*Salvia nemorosa*	meadow sage
1.85±0.05	16.24±0.30	*Salvia sclarea*	mediterranean sage
2.56±0.22	22.49±1.99	*Melissa officinalis*	lemon balm
2.52±0.37	25.69±2.41	*Ocimum basilicum*	sweet basil
2.85±0.06	29.67±1.04	*Origanum vulgare*	greek mountain oregano
3.47±0.38	26.27±0.32	*Thymus vulgaris*	garden thyme
2.94±0.37	16.12±2.09	*Thymus kotschyanus*	thyme
1.15±0.32	7.19±0.27	*Agastache foeniculum*	anise hyssop
2.94±0.05	15.95±0.52	*Mentha pulegium*	pennyroyal (leaf)
3.35±0.08	17.88±0.81	*Mentha pulegium*	pennyroyal (flower)
2.92±0.02	11.96±1.05	*Salvia nemorosa*	meadow sage (leaf)
2.66±0.31	8.55±0.57	*Salvia nemorosa*	meadow sage(flower)
2.52±0.37	25.69±2.41	*Ocimum basilicum*	sweet basil (leaf)
2.89±0.18	15.15±0.95	*Ocimum basilicum*	sweet basil (flower)
2.01±0.25	31.25±1.56	*Polygonum aviculare*	knotgrass (leaf)
1.75±0.17	25.41±1.07	*Polygonum aviculare*	knotgrass (flower)
0.59±0.23	6.87±0.30	*Hibiscus trionum*	flower-of-an-hour (leaf)
0.60±0.25	8.94±0.87	*Hibiscus trionum*	flower-of-an-hour (flower)
0.74±0.07	14.82±0.93	*Tanacetum balsamita*	costmary (leaf)
0.64±0.20	12.52±1.14	*Tanacetum balsamita*	costmary (flower)
1.07±0.15	10.47±0.34	*Cichorium intybus*	chicory (leaf)common
1.03±0.23	10.6±0.51	*Cichorium intybus*	common chicory (flower)
0.87±0.13	8.96±0.79	*Borago officinalis*	common borage (leaf)
0.86±0.09	9.62±0.63	*Borago officinalis*	common borage (flower)
0.28	3.08		LSD at 5%

[a] Data expressed as mean ± SEM. [b] Data expressed as milligrams of gallic acid (GAE) equivalents per gram of fresh weight.
[c]Data expressed as millimoles of Fe equivalents per gram of fresh weight.

though not significantly higher than that of spearmint (table 1).

Among three species of salvia, mediterranean sage had the highest TPC and meadow sage showed the highest FRAP level (table 1).

Comparing the two *Thymus* species showed that TPC and FRAP levels of garden thyme were higher than those of thyme (table 1).

Phenolics and antioxidant power of flowers and leaves were also compared in several herbs belonging to Lamiaceae and non-Lamiaceae (table 1 and figure 3. Phenolic content and antioxidant capacity were sometimes higher in flowers and sometimes higher in leaves (table 1, figure1 and figure 2).

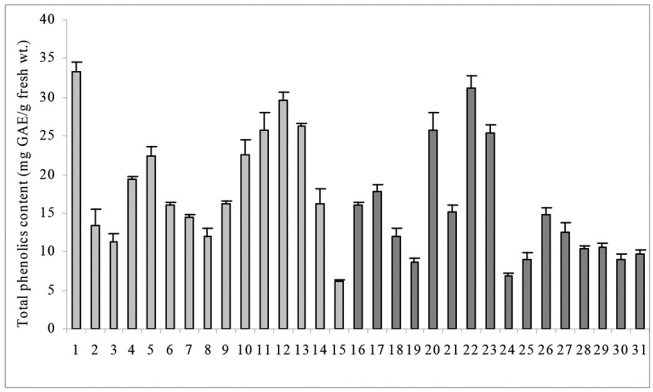

Figure 1. Total phenolic content of selected herbs, Mean ± SEM, (n = 3)
1- rosemary 2- lavender 3- orange mint 4- peppermint 5- spearmint 6- pennyroyal 7- garden sage 8- meadow sage 9- mediterranean sage 10- lemon balm 11- sweet basil 12- greek mountain oregano 13- garden thyme 14- thyme 15- anise hyssop 16- pennyroyal leaf 17- pennyroyal flower 18- meadow sage leaf 19- meadow sage flower 20- sweet basil leaf 21- sweet basil flower 22- knotgrass leaf 23- knotgrass flower 24- flower-of-an-hour leaf 25- flower-of-an-hour flower 26- costmary leaf 27- costmary flower 28- common chicory leaf 29- common chicory flower 30- common borage leaf 31- common borage flower

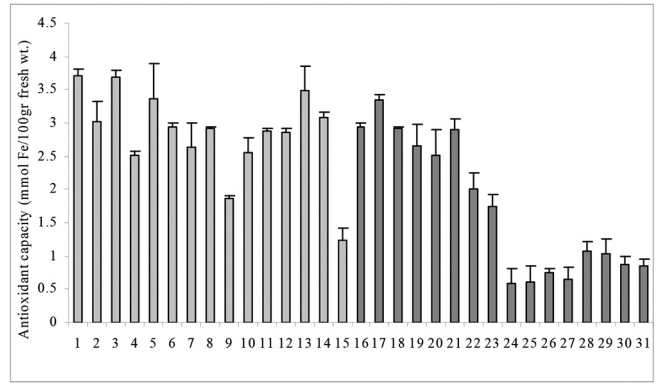

Figure 2. Total antioxidant capacity of selected herbs, Mean ± SEM, (n = 3)
1- rosemary 2- lavender 3- orange mint 4- peppermint 5- spearmint 6- pennyroyal 7- garden sage 8- meadow sage 9- mediterranean sage 10- lemon balm 11- sweet basil 12- greek mountain oregano 13- garden thyme 14- thyme 15- anise hyssop 16- pennyroyal leaf 17- pennyroyal flower 18- meadow sage leaf 19- meadow sage flower 20- sweet basil leaf 21- sweet basil flower 22- knotgrass leaf 23- knotgrass flower 24- flower-of-an-hour leaf 25- flower-of-an-hour flower 26- costmary leaf 27- costmary flower 28- common chicory leaf 29- common chicory flower 30- common borage leaf 31- common borage flower

A positive correlation (r^2=0.52, p≤0.0001) was found between phenolic content and antioxidant capacity of the selected herbs.

DISCUSSION

The Lamiaceae family consists of approximately 200 genera with a cosmopolitan distribution. This family is known in Iran by 46 genera and 410 species and subspecies (Naghibi *et al.* 2005).

Most genera of the Lamiaceae are rich sources of terpenoids and they also contain a considerable amount of various iridoid glycosides, flavonoids, and phenolic acids such as rosmarinic acid and other phenolic compounds (Naghibi *et al.* 2005; Valant-Vetschera *et al.* 2003).

In the present study, selected herbs of the Lamiaceae family demonstrated significant variation in the content of phenolic compounds and antioxidant potential and this is in accordance with previous studies on antioxidant properties of some Lamiaceae plants (Zheng & Wang 2001; Ozgen *et al.* 2006; Nickavar *et al.* 2008).

Hall & Cuppett (1997) reported that rosmarinic acid is known as the main component in Lamiaceae plants with a potent antioxidant activity and thus, the observed antioxidant properties of Lamiaceae plants could depend strongly on rosmarinic acid.

Peng *et al.* (2005) showed rosemary to have the highest antioxidant activity. Several phenolic compounds have been identified from rosemary, including rosmanol, rosmarinic acid, naringin, cirsimaritin and carnosic acid (Zheng & Wang 2001).

The antioxidant potential of mints greatly depends on the presence of phenolics. The major phenolic constituents of mints are caffeic acid derivatives, especially rosmarinic acid and flavonoids, including flavones, flavanones and their glycosidic forms (Gudeon & Pasquier 1994; Janicsak *et al.* 1999; Areias *et al.* 2001). Luteolin, apigenin eriodictyol, hesperetin and their glycosides have been shown to be major flavonoids in the genus *Mentha* (Gudeon & Pasquier 1994; Areias *et al.* 2001).

Salvia species have uses in traditional medicine and they are known as a rich source of flavonoids and phenolic acids. Their main flavonoids are flavones, flavonols, and their glycosides (Lu & Foo 2002). The genus *Thymus* contains flavonoids (luteolin, apigenin, eryodictiol, naringenin, diosmetin, and their glycosides) and phenolic acids (rosmarinic acid, 6-hydroxyrosmarinic acid, caffeic acid, 6-hydroxycaffeic acid, protocatechuic acid, chlorogenic acid, syringic acid, p-coumaric acid, 3,5-dicaffeoylquinic acid, gentisic acid, p-hydroxybenzoic acid and vanillic acid) (Voirin *et al.* 1985; Miura & Nakatani 1989).

Oregano species have high amounts of rosmarinic acid and other hydroxycinnamic acid compounds which have been shown to possess potent antioxidant activity (Larson 1988; Chen & Ho 1997).

Some phenolic compounds have been identified from

Melissa officinalis such as caffeic acid, protocatechuic acid, rosmarinic acid, luteolin-7-glucoside and rhamnazin (Thieme & Kitze 1973).

Rosmarinic acid, *o*-coumaric acid, apigenin-7-O-glucoside, coumarin, herniarin, luteolin, and apigenin have been shown to be lavander phenolic compounds (Areias *et al*., 2001).

The main phenolic compounds of sweet basil are rosmarinic acid, catechol, cinnamyl and caffeoyl derivatives, caffeic and ferulic acids, carnosic acid, catechin and apigenin (Jayasinghe *et al*. 2003).

Some phenolic compounds of knotgrass, belonging to the Polygonaceae, have been identified to be flavonoids and tannins (Samsam Shariat 2007).

Furthermore, some phenolic constituents evaluated in costmary and common chicory (Asteraceae) were tannins and different flavonoids (Samsam Shariat 2007).

Common borage has been shown to contain phenolic acids, especially protocatechuic acid and ferulic acid and tannins (Samsam Shariat 2007; Zadernowsk *et al*. 2002).

There appears to be no published information about phenolic compounds of anise hyssop and flower-of-an-hour.

TPC and FRAP levels of selected herbs showed some heterogeneities and this may be because phenolic compounds possess different antioxidant capacities (Zheng & Wang 2001).

Many different compounds are known to have distinct activities (Larson 1988). Therefore other compounds, without a phenolic structure may be in part responsible for the antioxidant properties of these plants (Nickavar *et al*. 2008).

The results showed that the selected herbs belonging to Lamiaceae family were rich in phenolics and demonstrated considerable antioxidant capacity, despite varying significantly in antioxidant capacity and TPC. Members of the Lamiaceae had relatively higher phenolic contents and antioxidant capacities than other selected herbs. Moreover, the results showed that both the flowers and leaves of selected herbs can include desirable levels of phenols and antioxidant capacity. Therefore, consumption of these herbs (flowers and leaves) as reactive species scavengers could be useful to suppress oxidative damage and reduce the risk of diseases. It was also shown that a major portion of the antioxidant capacity of these herbs was due to phenolic compounds.

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REZIME

Odredjivanje sastava fenola i antioksidativnog kapaciteta nekih lekovitih biljaka iz Irana

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U ovom radu odredjen je sadržaj fenola nekih lekovitih biljaka iz Irana kao i njihov antioksidativni kapacitet. Poredjen je fenolni sadržaj cvetova i listova različitih biljaka.

Ukupni sadržaj fenola kod ispitivanih vrsta varira od 6.87 do 33.22 mg GAE/g sveže mase dok je antioksidativni kapacitet varirao od 0.59 do 3.70 mmol Fe/100 g sveže mase. Najviši sadržaj fenola i antioksidativni kapacitet ruzmarin (*Rosmarinus officinalis*) dok je najniži zabeležen kod *Hibiscus trionum*. Reziltati pokazuju da visok fenolni sadržaj i antioksidativni kapacitet imaju naročito biljke iz porodice Lamiaceae. Rezultati pokazuju jednako dobar antioksidativni kapacitet i kod latica, ne samo listova.

Ključne reči: ukupni sadržaj fenola, antioksidativni kapacitet, Lamiaceae