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Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions

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Abstract

Basil (*Ocimum basilicum* L.) is used in traditional medicine, as a culinary herb and a well-known source of flavouring principles. Total antioxidant activity in 23 Iranian basil accessions was determined as Trolox equivalent antioxidant capacity (TEAC). Total phenolic contents were determined using a spectrophotometric technique, based on the Folin-Ciocalteau reagent, according to the method of Spanos and Wrolstad [Journal of Agricultural & Food Chemistry, 38 (1990) 1565] and calculated as gallic acid equivalents GAE/g dw. Total antioxidant activity varied from 10.8 to 35.7 μM Trolox, and total phenolic content ranged from 22.9 to 65.5 mg gallic acid/g dw in "Dezful I" and "Babol" accessions, respectively. A linear positive relationship existed between the antioxidant activity and total phenolic acids content of the tested basil accessions ($R^2 = 0.71$). Iranian basils possess valuable antioxidant properties for culinary and possible medicinal use.

Keywords: *Ocimum basilicum*; Antioxidant activity; Total phenolic content; Culinary herb

1. Introduction

Among the various medicinal and culinary herbs, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits (Exarchou et al., 2002). Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.

The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues (Hirasa & Takemasa, 1998). Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids (Velioglu, Mazza, Gao, & Oomah, 1998). Many herb spices, especially those belonging to the Lamiaceae family, such as sage, oregano, and thyme, show strong antioxidant activity (Hirasa & Takemasa, 1998). The genus *Ocimum*, a member of the Lamiaceae family, contains between 50 and 150 species of herbs and shrubs (Simon, Morales, Phippen, Vieira, & Hao 1999). A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts (Nakatani, 1997).

The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects (Löliger, 1991). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu et al., 1998). The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi, Janitha, & Wanasundara, 1992). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet

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oxygen, or decomposing peroxides (Osawa, 1994). Many of these phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations (Velioglu et al., 1998).

The purpose of this study was to evaluate Iranian *Ocimum* accessions as new potential sources of natural antioxidants and phenolic compounds. Our study also demonstrates a possible relationship between phenolic content and antioxidant activity.

2. Materials and methods

2.1. Plant material

Seeds of 23 accessions of basil (*Ocimum basilicum* L.) were collected from small farmers, home gardens and open markets in Iran and were grown to the flowering stage at Tehran University Experimental Research Station, Iran (Table 1). Seeds of all *O. basilicum* accessions were grown in a sterilized soil mix. Three seedlings of each accession were transplanted into 8-l pots and arranged in a randomized complete block design, in three replicates. Greenhouse plants were irrigated to pot capacity daily and maintained at day/night temperatures of 26–30 and 18–21 °C, respectively. The above-ground biomass of each plant was harvested at full bloom, weighed, bulked and placed in a paper bag and dried in a forced air drier at 32 °C for 15 days for analyses.

Voucher specimens of each accession were also collected, dried and stored at the Tehran University Herbarium, Iran (Table 1). Taxonomic identification of each accession was conducted by T. Masumi (Plant Taxonomist at Tehran University).

Table 1
List of different basil (*O. basilicum* L.) accessions from Iran, depicting variable total phenolic contents and antioxidant activity

Accession No.	Origin (city)	Total phenolic (mg GAE/g dw)	Antioxidant activity (μmol TE/g dw)
140-05	Babol	65.5	35.7
143-01	Birjand	41.4	16.1
133-15	Brujerd	41.5	19.8
125-17	Dezful I	23.0	10.8
125-12	Dezful II	29.5	12.9
133-15	Isfahan	64.6	26.0
133-19	Isfahan (green)	44.9	19.8
133-12	Isfahan (purple)	48.9	22.7
170-13	Kerman	57.1	30.6
171-04	Kermanshah	39.2	26.9
151-11	Khorram abad	42.3	20.6
155-08	Khuzestan	59.2	28.1
174-07	Mahallat	63.8	35.2
178-14	Malavi	42.9	19.7
116-21	Orumieh	32.8	15.1
168-08	Qazvin	38.6	19.1
163-11	Qom	54.1	26.3
157-06	Sanandaj	55.9	28.4
148-16	Shahr rey	40.6	19.7
183-27	Yazd	42.6	15.8
121-12	Zabol I	42.6	32.1
121-18	Zabol II	32.3	15.0
121-14	Zabol III	47.9	27.3
LSD $P<0.01$		8.11	1.33
LSD $P<0.05$		6.07	0.99

Data of total phenolic contents are expressed as milligrams of gallic acid (GAE) equivalents per gramme dry weight. Data of antioxidant activity are expressed as micromoles of Trolox equivalents per gramme dry weight.

2.2. Chemical reagents

The chemical reagent ABTS [2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] was purchased from CALBIOCHEM (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were of analytical and HPLC grade and obtained from Sigma Co. (St. Louis, MO).

2.3. Sample preparation for total antioxidant activity (TAA) measurement

Two hundred and fifty milligrammes of dried plant material of each accession were ground and dissolved in 10 ml of 80% acetone. Sample extracts were rotated for 1 h in the dark and centrifuged at 5400 *g* for 10 min. One millilitre of supernatant was centrifuged to dryness at 45 °C using a Vacufuge™, "Eppendorff" (Westbury, N.Y. Germany) and stored at −80 °C for further experiments. Each sample (with three replicates) was dissolved in 1 ml acetone prior to analysis for total phenolics and radical scavenging capacity.

2.4. Total phenolic compound analysis

The amount of total phenolics in 23 Iranian *Ocimum* accessions extracts were determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (1990), as modified by Lister and Wilson (2001). To 50 μl of each sample (three replicates), 2.5 ml 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na_2CO_3 (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 SPECTRAmax-PLUS384 UV–vis spectrophotometer. Results were expressed as milligrammes of gallic acid equivalent per gramme of dry weight (mg GAE/g dw).

2.5. Measurement of total antioxidant activity

The TAA values were estimated by the Trolox equivalent antioxidant capacity (TEAC) assay (Miller & Rice-Evans, 1996). We measured the relative capacity of antioxidants to scavenge the $ABTS^{+}$ radical compared to the antioxidant potency of Trolox (water-soluble Vit E) as a standard. The method used was based on Miller and Rice-Evans (1996) modified by Lister and Wilson (2001). The absorbance at 734 nm was measured using a SPECTRAmax-PLUS384, UV–vis spectrophotometer. The antioxidant capacities of samples, were measured against a Trolox standard and expressed as TEAC.

2.6. Statistical analysis

Three replicates of each sample were used for statistical analysis. Correlation analyses of antioxidant activity (Y) versus the total phenolic content (X) were carried out using the correlation and regression programme in MINITAB 13.2 (Minitab 2002 Software Inc., Northampton, MA). Data were subjected to analysis of variance, and means were compared by least significant difference (LSD). Differences at $P<0.05$ were considered to be significant.

3. Results and discussion

3.1. Total phenolic content

The amount of total phenolics varied in different accessions and ranged from 22.9 to 65.5 mg GAE/g of dry material. The highest total phenolic levels were detected in "Babol", "Isfahan" and "Mahallat", and the lowest in "Dezful I" and "Dezful II" (Table 1). The amount of total phenolic compounds in all tested basil accessions was higher than the other Lamiaceous plants reported such as *Thymus vulgaris* (Kähkönen et al., 1999), *Mentha piperita, Melissa officinalis* and *Rosmarinus officinalis* (Zheng & Wang, 2001). Some selected phenolics of these Iranian basil accessions, have previously been separated and identified by comparison with authentic standards using reversed-phase high-performance liquid chromatography (HPLC), and rosmarinic acid was the predominant phenolic acid in these accessions (Javanmardi et al., 2002). Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Kähkönen et al., 1999). Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom (Larson, 1988). Rosmarinic acid, an important phytochemical, has been found to be a potent active substance against human immunodeficiency virus type 1 (HIV-1) (Mazumder et al., 1997).

3.2. Antioxidant activities

Antioxidant activities of Iranian *Ocimum* accessions extracts in acetone are shown in Table 1. The TAA ranged from 10.8 to 35.7 μM Trolox equivalents in "Dezful I" and "Babol", respectively (Table 1). Analysis of variance of means showed that there was no significant statistical difference between "Babol" and "Mahallat" (Table 1). Our results compared favourably with previous studies on Lamiaceous plants *Mentha piperita, Melissa officinalis* and *Rosmarinus officinalis* (Zheng & Wang, 2001), and showed equivalent or higher antioxidant activity.

The correlation between TAA (Y) and total phenolic contents (X) of Iranian basils had a correlation coefficient of $R^2=0.71$, ($Y=0.51\ X-0.931$) (Fig. 1) . This result suggests that 71% of the antioxidant capacity of Iranian *Ocimum* accessions results from the contribution of phenolic compounds. Also, it can be concluded that antioxidant activity of plant extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids, and vitamins, among others, that in this case contributed to 29% of the antioxidant capacity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also have a metal chelating potential (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Besides flavoring purposes, spices and herbs have also been used for their medical or antiseptic properties (Kähkönen et al., 1999).

The present study showed that Iranian *Ocimum* accessions, which are often present in Iranian dishes, are strong radical scavengers and can be considered as good sources of natural antioxidants for side dishes, medicinal and commercial uses. However, due to the diversity and complexity of the natural mixtures of phenolic

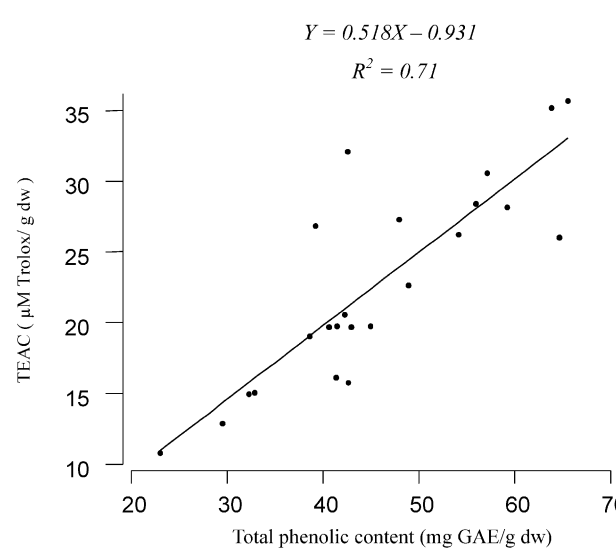

Fig. 1. Linear correlation of Trolox equivalent antioxidant capacity (TEAC) (Y) versus the total phenolic content (X) of 23 Iranian *Ocimum basilicum* accessions, using regression program in MINITAB 13.2 (Minitab 2002 Software Inc., Northampton, MA).

compounds in the *Ocimum* accession extracts of Iran (Javanmardi et al., 2002), it is rather difficult to characterize every compound and assess or compare their antioxidant activities. Each herb generally contained different phenolic compounds, and each of these compounds possesses differing amounts of antioxidant activity.

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