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Antioxidant activity in *Ocimum sanctum* Linn, *Ocimum bascilicum*

INAMPUDI SAILAJA[1] **AND** IVVALA ANAND SHAKER[2]
[1]Department of Biochemistry and Molecular Biology, College of Agricultural Biotechnology, Loni, AHMEDNAGAR (M.S.) INDIA
[2]Department of Biochemistry, Rural Medical College, P.I.M.S, Loni, AHMEDNAGAR (M.S.) INDIA

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Ocimum sanctum Linn., *Ocimum bascilicum* Linn., which have been normally used in traditional puja at every Indian Hindu home, was estimated by three different methods: ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolorization assay and DPPH free radical scavenging assay. Additionally, their total phenolic contents were analyzed by Folin-Ciocalteau micro method. The result showed that *Ocimum bascilicum* seemed to be better source of antioxidant compounds, followed by *Ocimum sanctum*. Total phenolic content analysis, were significantly related to those of FRAP, ABTS, DPPH.

Key words : Antioxidant status, Total phenolic content, *O.bascilicum, O.sanctum*

INTRODUCTION

Plants are one of the most important sources of medicines. *Ocimum basilicum* L. (labiatae) is a grassy and annual plant. The leaves of this plant are oval with a sharp tip and the flowers are yellow, white and pink. It is a native plant of Iran, Afghanistan and India (Mirheidar, 1990, Volak and Jiri, 1997; Zargary, 1990). This plant is a popular culinary herb and its essential oils have been used for many years in food perfumery and dental products (Suppakul *et al*., 2003) in Iranian traditional medicine (Dasgupta *et al*.,2004; Javanmardi *et al*., 2002). According to the literature, about 45 compounds are found in volatile oil of this plant and the major compounds are linalol, eugenol, methylchavicol, methylcinnamat, linolen, ocimene, pinene, cineol, anethol, estragol, thymol, citral and comphor (Mirheider, 1990; Wang *et al*., 2003; Grayer *et al*., 1996). Different parts of *Ocimum basilicum* have diuretic (Mirheider, 1990; Zargary, 1990) antipyretic (Mirheider, 1990) and antitussive effects (Mirheider, 1990; Volak and Jiri, 1997) and have been used to treat gastritis, stomach-ache, flatulence and constipation (Mirheider, 1990; Zargary, 1990, Avesina, 1990; Singh, 1999; Akhtar and Munir, 1989), vomiting and hiccup (Mirheider, 1990). The seeds and the boiled extract are used for treatment of diarrhea. This plant also has therapeutic effects for nasal polyps (Mirheider, 1990), upper respiratory tract diseases and dyspnea (Mirheider, 1990; Volak and Jiri, 1997), inflammation of the urinary tract (Volak and Jiri, 1997) and it has been used as a bathing solution for treatment of ulcers (Volak and Jiri, 1997; Avesina, 1990) and increase in lactation (Zargary, 1990). Inhalation of the fragrance of this plant is useful to alleviate headache (Mirheider, 1990), and also it produces a favorable impression on the mental activity (Satohand Sugawara, 2003). In the previous studies, it was used as anti-inflammatory (Singh, 1999; Singh, 1999; Singh, 1998), anti-HIV (Yamasaki *et al*., 1998), antioxidant (Dasgupta *et al.*, 2004; Jayasinghe *et al.*, 2003; Yun *et al.*, 2003; Lee and Shibamoti, 2002), antibacterial and antifungal (Suppakul *et al*., 2003; Edris and Farrag, 2003; Opalchenova and Obreshkova, 2003; Lachowicz *et al.*, 1998). In Ayurveda Tulsi (*Ocimum sanctum* L.) has been well documented for its therapeutic potentials and described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna) (Sirkar, 1989). Although the traditional medical practitioners in India have been widely using this medicinal plant for management of various disease conditions from ancient time, not much is known about the mode of action of Tulsi, and a rational approach to this traditional medical practice with modern system of medicine is also not available. In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils and eugenol in therapeutic potentials of *Ocimum sanctum* L. (Sen, 1993; AOAC, 1997). Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of Tulsi plant. The therapeutic potential of Tulsi has been established on the

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basis of several pharmacological studies carried out with eugenol and steam distilled, petroleum ether and benzene extracts of different parts of Tulsi plant (Sen, 1993; Khanna and Bhatia, 2003). Holy basils (*Ocimum sanctum*, *Ocimum bascilicum*), available from fresh markets of Shridi, Taluka Rahata, Distt. Ahmednagar, Maharashtra by three different methods, *viz.*, ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolorization assay, and DPPH free radical scavenging assay. The total phenolic contents of the above culinary plants were also estimated and related to their antioxidant capacity results.

MATERIALS AND METHODS

Sample extraction:

Fresh samples of plants were collected in and around Loni. Their moisture contents were analyzed by the method of AOAC (1997). The sample extraction method of Leong and Shui (2002) was modified for sample preparation. Edible portion of the fresh sample was homogenized using a blender. A mixture of deionized water and 95% ethanol was used as extraction solvent and 95% ethanol was selected for preliminary studies. From preliminary studies, 60% (v/v) of 95% ethanol was selected as extraction solvent for chilies and holy basils, while deionized water and 95% ethanol were chosen for garlic and pumpkin, respectively. Two grams of homogenized sample were added with 10 ml of the selected solvent. The extraction was done by using a vortex mixer for 60 seconds. The mixture was filtered and the filtrate was collected and used for FRAP, ABTS, DPPH, and total phenolic content assayed by spectrophotometer.

Ferric reducing antioxidant power (FRAP) assay:

The FRAP was assessed according to Benzie and Strain (1999). Briefly, 6 ml of working FRAP reagent prepared daily was mixed with 20 - 100 µl of the extract. The absorbance at 593 nm was recorded after 30-min incubation at 37^0 C. Absorbance increases were calculated as FRAP values by comparing with standard curves created by vitamin C (0 - 15 µg), and reported as mg vitamin C equivalent per gram of fresh weight.

ABTS radical cation decolorization assay:

The ABTS method of Re *et al.* (1999) was modified. ABTS radical cation (ABTS+) was produced by reacting 7 mM ABTS stock solution with 2.45 mM postassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 - 16 hours before use. The ABTS+ solution was diluted with deionized water and 95 % ethanol (1:1) to an absorbance of 0.70 (+ 0.02) at 734 nm. The extract (20-100ìl) was mixed with 6 ml of diluted ABTS+ solution. The decrease of absorbance was recorded at 1 min after mixing. Absorbance decreases were calculated as ABTS values by comparing with standard curves created by vitamin C (0 - 20 ìg), and the results were reported as mg vitamin C equivalent per gram of fresh weight.

DPPH free radical scavenging activity:

The method of Brand-Williams *et al.* (1995) was used with some modifications. DPPH radical solution (0.8 mM) in 95% ethanol was prepared. The extract (100-1000µl) was diluted to 5.4 ml by deionized water and 95 % ethanol (1:1) before 0.6 ml of the DPPH solution was added and mixed. The decrease of absorbance was recorded at 1 min after mixing. Absorbance decreases were calculated as DPPH values by comparing with standard curves created by vitamin C (0 - 40 µg), and the results were reported as mg vitamin C equivalent per gram of fresh weight.

Total phenolic content:

The Folin-Ciocalteau micro method of Waterhouse (2005) was used to estimate total phenolic content (TPC). The extract (60-300 µl) was diluted with deionized water to 4.8 ml, and 300 ìl of Folin-Ciocalteau reagent was added and shaken. After 8 min, 900 µl of 20% sodium carbonate solution was added with mixing. The solution was left at 40^0C for 30 min before reading the absorbance at 765 nm. Gallic acid (0 - 50 µg) was used as standard, and the results were reported as mg gallic acid equivalent per gram of fresh weight.

Calculation and statistical analysis

The values of FRAP, ABTS, DPPH, and TPC (mg standard equivalent per gram of fresh weight) were calculated using the equation below:

Values of FRAP, ABTS, DPPH, and TPC

$$\left\{\begin{array}{c}\text{Mg standard equivalent per}\\ \text{gram of fresh weight}\end{array}\right\} = \frac{(\text{SA} - \text{BA}) / (\text{Slope} \times 2) \times 10}{1000}$$

where:

SA = Sample absorbance for FRAP value and TPC or absorbance decrease of sample for ABTS and DPPH values.

BA = Blank (no extract) absorbance for FRAP value

and TPC or absorbance decrease of blank for ABTS and DPPH values (extract was substituted by demonized water for blank).

Slope = Slope of standard curve.

[10/U] = Total volume of extract (10 ml)/used volume of extract (ml).

[2] = Weight of used sample (g).

[1,000] = Factor for changing μg to mg.

Each experiment was performed in triplicate on different purchased samples. Mean comparisons were performed by Duncan's new multiple range test (DMRT). The correlations between all antioxidant status assays and total phenolic contents were analyzed.

RESULTS AND DISCUSSION

The results of the antioxidant capacity assessment of the studied plants as determined by FRAP, ABTS, and DPPH assays are shown in Table 2. These differences could be explained by different mechanisms of analytical methods. FRAP assay measures the ability to reduce a ferric tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}-TPTZ) of samples (Leong and Shui, 2002) ABTS and DPPH assays are based on the reduction of ABTS (Re *et al*., 1999) and DPPH free radicals (Benzie and Strain, 1999) of samples, but values from DPPH assay might be lower than those from ABTS assay. Wang *et al*. (1998) showed that some compounds which have

Table 1: Moisture content of plant

Plants	Moisture content (%)
Ocimum sanctum	86.44 + 0.46
Ocimum bascilicum	85.90 + 0.61

ABTS scavenging activity may not show DPPH scavenging activity and Arts *et al.*(2004) found that some products of ABTS scavenging reaction may have a higher antioxidant capacity and can further react with remained ABTS radicals.

Table 2 shows that *O. bascilicum* had the highest antioxidant capacity analyzed by FRAP assay, followed by *O. sanctum*. In ABTS assay, the plants with the highest antioxidant capacity were *O. bascilicum*, followed by *O. sanctum*, respectively. However, results of DPPH assay showed that *O. sanctum*, and then followed by *O. bascilicum*, respectively. For overall results, it might be concluded that *O.bascilicum* was better sources of antioxidant compounds in this study, followed *O.sanctum*, respectively. In case of total phenolic content analysis, it was found that *O.bascilicum*, contained the highest amount of phenolic compounds, followed by *O.sanctum*, respectively.

Table 2: Antioxidant status and total phenolic content

Plants	FRAP*	ABTS*	DPPH*	TPC**
Ocimum sanctum	3.05 + 0.73 ^{a}	5.21 + 0.79 ^{a}	0.42 + 0.22 ^{b}	1.25 + 0.47 ^{b}
Ocimum bascilicum	1.49 + 0.55 ^{b}	4.03 + 0.39 ^{b}	0.52 + 0.21 ^{b}	2.38 + 0.46 ^{c}

* mg Vitamin C equivalent / g fresh weight.

** mg Gallic acid equivalent / g fresh weight.

Means (+ S.D.) with different superscripts in each column are significantly different ($p<0.05$). Superscript a indicates the group with the highest value and e belongs to the lowest value group.

The correlations between results of antioxidant capacity and TPC analysis are highly significant ($p<0.01$) as shown in Table 3. These correlations indicate that the phenolic compounds could be the main cause of antioxidant power of *O.bascilicum* plant, in accordance with the previous findings that many phenolic compounds in plants are good sources of natural antioxidants (Satoh and Sugawara, 2003). In this study, the contribution of the phenolic compounds content to ABTS values was greater than to FRAP and DPPH values. Thus, ABTS assay may preferably be a better method to study the antioxidant power which is mainly caused by phenolic compounds, than FRAP and DPPH methods, in that order.

Table 3: Correlation results of three antioxidant capacity assays and total phenolic content (TPC) from all extracts

	Correlation of coefficient	Significance
ABTS	0.825	< 0.01
DPPH	0.973	< 0.01
FRAP	0.683	< 0.01
TPC	1.000	-

Conclusion:

This work shows that *O.bascilicum* and *O.sanctum* are good sources of natural antioxidants, therefore, they should be cultivated in large quantities.

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