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Total phenolic content and antioxidant capacity of basil (*Ocimum basilicum* L.) leaves from different locations

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Abstract

The present study was carried out to determine the antioxidant activity and total phenolic content of *Ocimum basilicum* collected from different regions of the world. The accession V1 is from Sudan, V2 from Iraq, V3 from Germany, V4 from Thailand, V5 from Russia and V6 from Maldives. The extracts from six basil accessions were analysed for their DPPH free radical scavenging activity and their total phenolic content (TPC). The results suggest that the highest antioxidant activity was found in V6 (from Maldives) and the lowest antioxidant activity was found in V4 (from Thailand). The highest amount of phenolic content was found in V6 (from Maldives) and the lowest phenolic content was found inV4 (from Thailand). This study shows that basil is a good source of free-radical scavenging compounds that have their traditional medicinal applications.

Introduction

Free radicals, namely reactive oxygen species (ROS) are known to cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury implicated in the process of ageing (Alma *et al*., 2003). Besides natural antioxidants, some synthetic antioxidants are also reported such as butylatedhydroxytoluene and butylatedhydroxyanisole. But these synthetic antioxidants possess greater risks of side effects; therefore, investigations on identifying the natural antioxidants have become a very important issue (Alma *et al.*, 2003). In the past few years, natural antioxidants have generated considerable interest in preventive medicine (Govindarajan *et al*., 2003). Medicinal plants have been reported to possess antioxidant activities (Yanishieva *et al*., 2006) suggesting that they might have potential human health benefits. Basil is a known medicinal plant; it is called "Reyhan" in Sudan. This genus is of the Lamiaceae family and its dried leaves, as well as its essential oil, are used in the food industry as aromatic and flavoring ingredients. Also, basil is commonly used to treat fever, inflammatory, stomach ache, flatulence, constipation and is also used as an antibacterial and antifungal agent (Özcan and Chalchat, 2002; Rafieian *et al*., 2015). In addition, extracts of the leaves displayed powerful antioxidant activity in various assay models (Jayasinghe *et al*., 2003; Gulcin *et al.*, 2007). *O. basilicum* had been found to contain linalool, eugenol, methyl chavicol, methyl cinnamate, ferulate, methyl eugenol, triterpenoids and steroidal glycoside known to exhibit antioxidant activities (Pietschmann *et al*., 2005; Siddiqui, Aslam, Ali *et al*., 2007; Siddiqui, Ali, Begum *et al*., 2007; Zheljazkov *et al*., 2008)

The aim of this research is to determine the antioxidant activity and total phenolic content of the plant basil (*Ocimum basilicum)* varieties collected

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from different regions of the world.

Materials and methods

Collection and preparation of plant materials

Basil seeds were obtained from the Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleber, Germany. The seeds of six basil accessions were planted at the experimental farm of the National Oilseed Processing Research Institute (NOPRI), University of Gezira, Wad Medani, Sudan. Each accession was grown in three replicates. The accession V1 is from Sudan, V2 from Iraq, V3 from Germany, V4 from Thailand, V5 from Russia and V6 from Maldives. Leaves were dried at room temperature for one week and ground into a fine powder using a blender (LB20E, USA).

Extraction of plant material

The extraction of plant material was performed at the International Institute for Halal Research and Training (INHART) of the International Islamic University Malaysia. One gram of each sample was weighed in vials, each with a 20 mL capacity, using a Mettler Toledo, MS304 sensitive balance. All vials containing samples and solvent (ethanol) were allowed to macerate for three days. Finally, the supernatants were collected for further analysis.

Diphenyl-1-Picrylhydrazyl (DPPH) antioxidant assay

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) according to the method described by (Musa *et al*., 2013). Approximately 100 µL of basil leaves crude extract were mixed with 1 mL of DPPH solution and kept in the dark for 15 minutes. The ascorbic acid solution was prepared by dissolving 2 mg ascorbic acid in 100 mL methanol. Amount of 300 µL of Ascorbic acid was added to each well at six different concentrations (0, 5, 20, 40, 60, 80, 100 µL). Figure 1 shows the standard curve of ascorbic acid. Plates were mixed, covered and incubated in the dark at room temperature for 1 hour and then the absorbance was measured at 517 nm using Multi-microplte reader (Spectrostar Nano, BMG Labtech, Australia). All determinations were performed in triplicate. The DPPH scavenging activity was determined based on the following equation:

$$DPPH\ scavenging\ activity\ (\%) = \frac{(Absorbance\ of\ control - Absorbance\ of\ Sample)}{Absorbance\ of\ control} x100$$

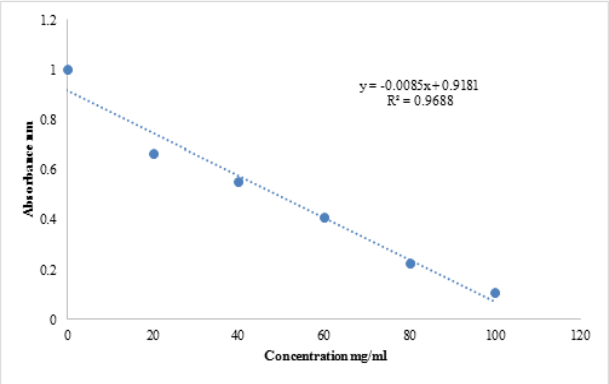

Figure 1. Ascorbic acid standard curve

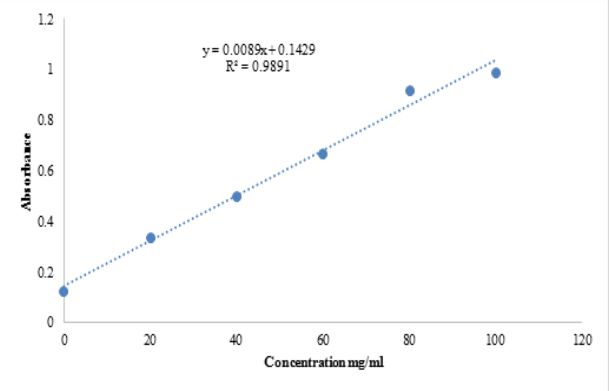

Figure 2. Standard calibration curve of gallic acid

Determination of total phenolic content (TPC)

Antioxidant activity was determined using TPC based on the method of (Abdelhady *et al*., 2011). Amount of 100 mL distilled water and 1 mL diluted Folin–Ciocalteu reagent was added to 100 µL sample extracts. The samples were set aside for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was taken at 765 nm wavelength using a spectrophotometer after 2 h. Three replicate assays were performed. Fig. 2 shows the standard curve of gallic acid. The total phenolic content was calculated as gallic acid equivalent (GAE) by the following equation:

$$T = CxV/M$$

Where:

T: is the total phenolic content in mg Trolox Equivalent/g of Extract.

C: is the concentration of gallic acid established from the calibration curve in mg/mL

V: is the volume of the extract solution in mL and M is the weight of the extract in grams.

Results and discussion

In this study, antioxidant activity and total phenolic contents of *Ociumum basilicum* obtained

Table1. DPPH scavenging assay to assess the antioxidant of basil

Extract	DPPH radical scavenging activity (%)
V1	81.30 ± 2.4
V2	73.55 ± 3.0
V3	82.70 ± 1.6
V4	69.33 ± 3.4
V5	77.44 ± 3.1
V6	89.22 ± 2.5

*The result is a mean of three replicates ± SD (standard deviation)
*Where V1= Sudan, V2 =Iraq, V3 =Germany, V4 = Thailand, V5 = Russia and V6 = Maldives.

Table 2. Total phenolic content of basil *(Ocimum basilicum)*

Sample	Average mg GAE/100g DW
V1	13485 ± 919
V2	16684 ± 1203
V3	18171 ± 251
V4	2086 ± 74
V5	16138 ± 818
V6	25593 ± 1335

*Result is mean of three replicates ± SD (standard deviation)
*Where V1= Sudan, V2 =Iraq, V3 =Germany, V4 = Thailand, V5 = Russia and V6 = Maldives.

from different regions of the world were determined.

Determination of antioxidant activity

Antioxidant properties by using2,2-diphenyl-1-picrylhydrazyl (DDPH) assay are shown in Figure 3. Ascorbic acid was used as a standard for this experiment. DPPH values of different samples used varied from 89.22% to 69.33%. The highest antioxidant activity was found in V6 (from Maldives) and the lowest antioxidant activity was found in V4 (from Thailand). The antioxidant effectiveness is probably due to a relatively high content of methyl-eugenol in basil (3.68%). The same result was previously showed by Politeo *et al*. (2006).

Determination of total phenolic content

The total phenolic (TPC) content of the different extracts of *Ocimum basilicum* was determined using the Folin–Ciocalteu phenol reagent. In the present study, the total phenolic content of six different extracts of basil varied from 0.408 mg GAE/g DW to 0.881 mg GAE/g of dry material. Maldivian basil had the highest phenolic content and Thai basil the lowest.

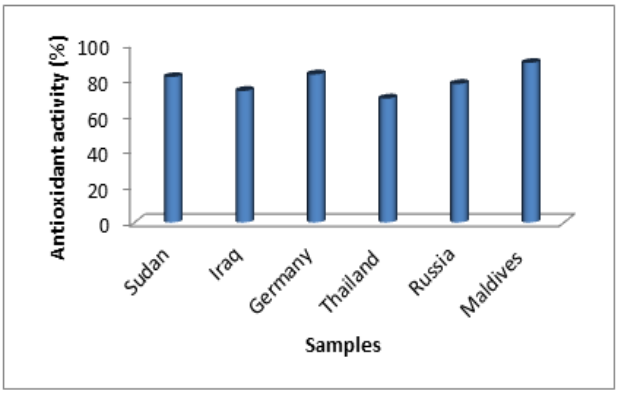

Figure 3. DPPH radical scavenging activity

The presence of a phenolic compound in the essential oil increases its antioxidant power (Pripdeevech *et al*., 2010). Total phenolic compounds in basil accessions were higher than the other Lamiaceae plants (Zheng and Wang, 2001). Uyoh and others (2013) determined total phenolic contents of *Ocimum basilicum* and *Ocimum gratissimum* grown in Nigeria extracts ranged from 9.09 – 27.41 mg GAE/g DW and the DPPH radical scavenging activities ranged from 58.43% - 92.37% and 6.27% - 16.67% respectively. Katsube *et al.* (2004) and Katalinic *et al.* (2006) reported that relationship between the content of total phenolic compounds and their antioxidant capacity.

Conclusion

Basil is a good source of free radical scavenging compounds that have their traditional medicinal applications, that may be successful for future modern medical applications and personal care as well.

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