Antioxidant Properties of *Ocimum sanctum* L.

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Abstract

The antioxidant properties of the methanol and ethanol extracts from various parts of O*cimum sanctum L.* were examined in vitro. Antioxidant activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The free radical scavenging activity of methanol fraction was superior to all other fractions (IC_{50} = 12.35 µg/ml), which was higher than synthetic antioxidant butylated hydroxy anisole, BHA, (18.27 µg/ml). Furthermore, the amount of total phenolic compounds was determined and its content in methanol fraction (73.61mg GAE/g dw) was the highest as compared to other extract or fractions. Our study suggests that natural antioxidants derived from *Ocimum sanctum* L. have the potential to be used as food preservatives.

***Keywords*:** *Ocimum sanctum L.*, Antioxidant activity, DPPH, Phenolic compounds

1. Introduction

There is a growing interest in research on food components such as phenolic compounds because of their possible linkage to health benefits, e.g., reduction of heart disease and cancer, based on their antioxidant activity (Seeram et al., 2002). Many medicinal plants contain large amounts of antioxidants such as polyphenols, which have an important role in preventing a variety of stress-related diseases and aging because these are closely related to the active oxygen and lipid peroxidation (Noguchi, & Niki, 1999). Antioxidants have been used for the prevention and treatment of free radical-related disorders (Middleton, Kandaswami & Theoharides, 2000). However, there have been concerns about synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis (Barlow, 1990). Consequently, there is a scientific interest to find naturally occurring antioxidants for using as natural preservative ingredients in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Velioglu et al, 1998).

Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to family Lamiacea are very important for their therapeutic potentials. *O. sanctum* L. known as Black Tulsi in Bengali, 'Krishna/Shyama Tulsi' in Hindi, and 'Holy Basil' in English, is an erect softy hairy aromatic herb or undershrub found throughout India and Bangladesh. It commonly known as the 'tree of heaven,' is used in traditional medicine in many parts of Asia including Bangladesh and India.

Several ethnobotanical surveys show that *O. sanctum* was among the plants reported to be used traditionally to treat bacterial infections such as enteric diseases i.e., diarrhea, dysentery and other gastrointestinal infections; upper respiratory tract infections associated with coughing pneumonia,

asthma and bronchitis; urogenital infections including sexually transmitted diseases, skin infections (dermatitis, eczema, scabies), wounds and ulcers; headache, ophthalmic, insect bites, nasal bleeding, stroke, measles, paludism; and bacterial fevers such as typhoid fever and diabetes and veterinary problems[9,11]. It is also used in the treatment of epilepsy, shigellosis, trypanosomiasis, convulsion, pile and anaemia[12]. It is also implicated in the oral hygiene and veterinary[13,14]. Comprehensive biological activities of *O. sanctum* have been reviewed[11] and it is associated with a wide variety of biological activities[15-18].

For the production of energy to fuel through biological processes oxidation is essential in many living organisms. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. The role of oxygen radicals has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, ageing, etc.[1]. Antioxidant has been used for the prevention and treatment of free radical related disorders. Antioxidants halt the free radical chain reactions. Some antioxidants are themselves free radicals, donating electrons to stabilize and neutralize the dangerous free radicals. Other antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage (Prakash 2001). However there have been concerns about synthetic antioxidants such as ascorbic acid because of possible activity as promoters of carcinogenesis. Synthetic antioxidants, such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT), also have restricted to use in foods as they are suspected to be carcinogenic[2]. Therefore, the importance of searching natural antioxidants has greatly increased in recent years[3,4]. The pharmaceutical industry is undertaking the rapid development and use of natural antioxidants, especially those of plant origin, to replace synthetic drug. Moreover, essential oils are plant secondary metabolites, mainly monoterpenes, sesquiterpenes and their corresponding oxygenated derivatives, which have been showed various pharmacological effects, such as antimicrobial, antioxidant, spasmolytic, carminative, hepatoprotective, antiviral and anticarcinogenic effects[5,8]. Although it remains unclear which of the compounds, of medicinal plants are the active ones, phenolics recently have received increasing attention because of some interesting new findings regarding their biological activities[7]. From pharmacological and therapeutic points of view, the antioxidant properties of phenolics, such as free radical scavenging and inhibition of lipid peroxidation, are the most crucial. Even though a variety of herbs and plants are known to be the sources of phenolic compounds, studies isolating phenolics and evaluating their antioxidative effects have rarely been carried out[7,8].

2. Materials and methods

2.1. Preparation of extracts

Tulsi (O*cimum sanctum* L) was collected from Kushtia city area of the Bangladesh during February to April, 2014. The 50 g of coarse powder of the air-dried leaves of *Ocimum sanctum* L. was weighted with the help of a rough balance, and then the weighted out coarse powder was placed in a large conical flask. Then the powder (50 g) soaked in the suitable solvent at room temperature for 7 days in a large conical flask. The mixture was stirred by glass rod every day an hour. After 7 days the extract was filtered through a filter paper in several times. The solvent is added according to the polarity. The leaves powder (50 g) was extracted with ethanol and methanol separately at room temperature for 7 days and the solvents were evaporated by vacuum rotary evaporator. The extraction process yielded in methanol (6.7) g and ethanol (6.6) g extracts. After filtration each of the extracts was concentrated to dryness in a rotary evaporator at 40^0 C under Vacuum.

2.2. Chemicals and reagents List

Gallic acid, Folin-Ciocalteau's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxy anisole (BHA), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) were obtained from Sigma-Aldrich (St.Louis,MO). Nitrotetrazolium-20-blue chloride (NBT) was purchased from Fluka (Buchs, Switzerland). All other chemicals and solvents were of highest commercial grade.

2.3. **Antioxidant assay**

2.3.1. **Free radical scavenging capacity**

The antioxidant activity of the methanol and ethanol extract from various parts of *ocimum sanctum L.* were measured on the basis of the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Cuendet, Hostettmann, & Potterat, 1997). Various concentrations of 100 µl of test extract or fractions were added to 3 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation period in the dark at room temperature, the absorbance was measured against a blank at 517 nm. Inhibition of free radical DPPH in percent (%) was calculated by the formula:

$$\text{Percentage inhibition (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}})] \times 100$$

Where, A_{blank} is the absorbance of the control reaction (containing all reagents except test compound), and A_{sample} is the absorbance of the test compound. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the extract or fractions and percentage inhibition of free radical formation/ percentage inhibition DPPH was assayed. Synthetic antioxidant reagents, butylated hydroxy anisole (BHA) and L-ascorbic acid, were used as positive controls and all tests were carried out in triplicate.

2.3.2. Determination of total phenolics

Total phenolic constituent of the aforementioned extracts were determined by Folin-Ciocalteu reagent in alkaline medium (Lister, & Wilson, 2001) and was expressed as gallic acid equivalents (GAE). Different concentrations of gallic acid were prepared in 80% methanol. 100 µl test sample (from a range of concentrations) was taken in a cuvette, then 1 ml of distilled water and 500 µl (1/10 dilution) of the Folin– Ciocalteu reagent was added, and cuvette was shaken thoroughly. After 1 min, 1500 µl of 20% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of samples was measured at 760 nm and the results were expressed in mg GAE/g dw of dry weight of samples.

2.4. Statistics Values are given as the mean ± S.D. of triplicate experiments. Statistical analysis was done by Student's *t*-test.

3. Results and discussion

Results:

Table 1. Total phenolic compounds of *Ocimum sanctum* L. leaves.

Extracts	Total phenolic [a] (mg GAE/g dw)
Methanol extract	73.61 ± 2.1
Hexane fraction	25.83 ± 1.5
Chloroform fraction	41.47 ± 0.6

Values are given as the mean ± S.D. of triplicate experiments.

Table2. DPPH scavenging activities of *Ocimum sanctum L*. in methanolic and ethanolic extract.

Extract	Used part	Conc.µg/ml	Inhibition%	IC_{50} Value
Methanol Extract	Leaf	100	73.51±1.3	12.35
		150	86.23±1.4	
		200	97.13±1.2	
	Stem	100	70.13±1.3	15.27
		150	81.49±1.5	
		200	93.11±1.2	
	Root	100	56.13±1.7	72.24
		150	70.58±0.6	
		200	78.52±1.8	
Ethanol Extract	Leaf	100	68.13±1.5	22.41
		150	83.35±1.5	
		200	91.32±1.8	
	Stem	100	66.17±1.5	36.05
		150	82.12±1.3	
		200	91.33±0.6	
	Root	100	49.19±0.4	104.43
		150	62.12±1.3	
		200	69.12±1.1	

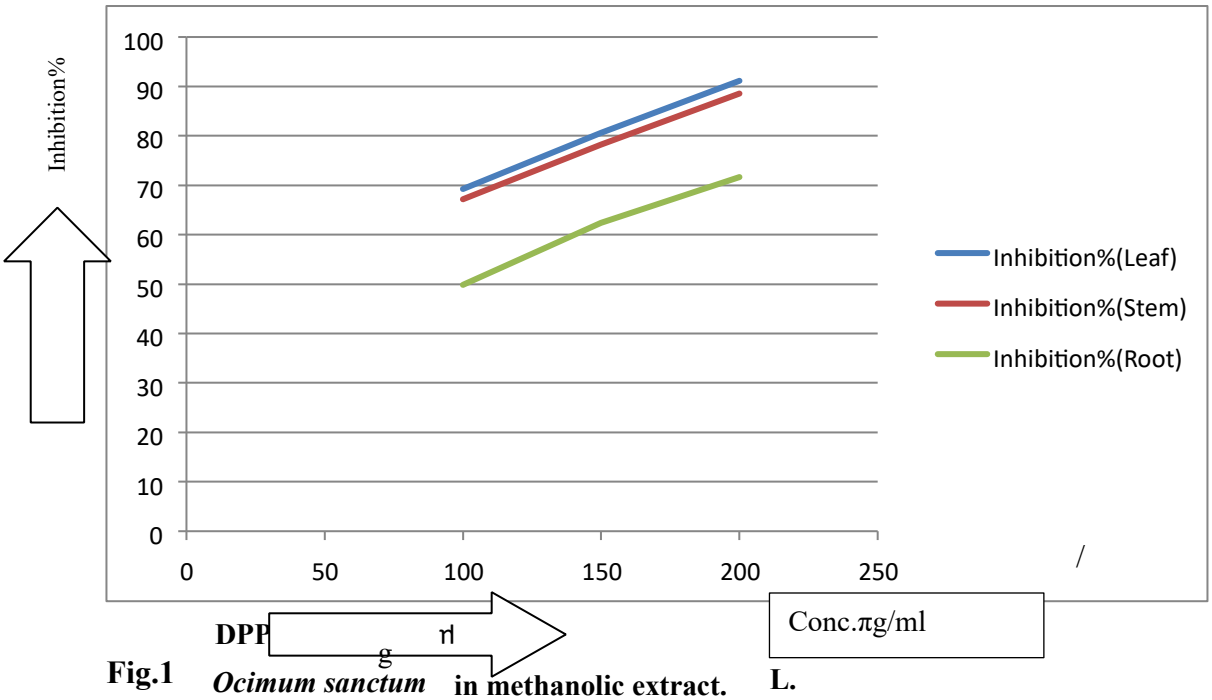

Fig.1 ***Ocimum sanctum*** **in methanolic extract.** **L.**

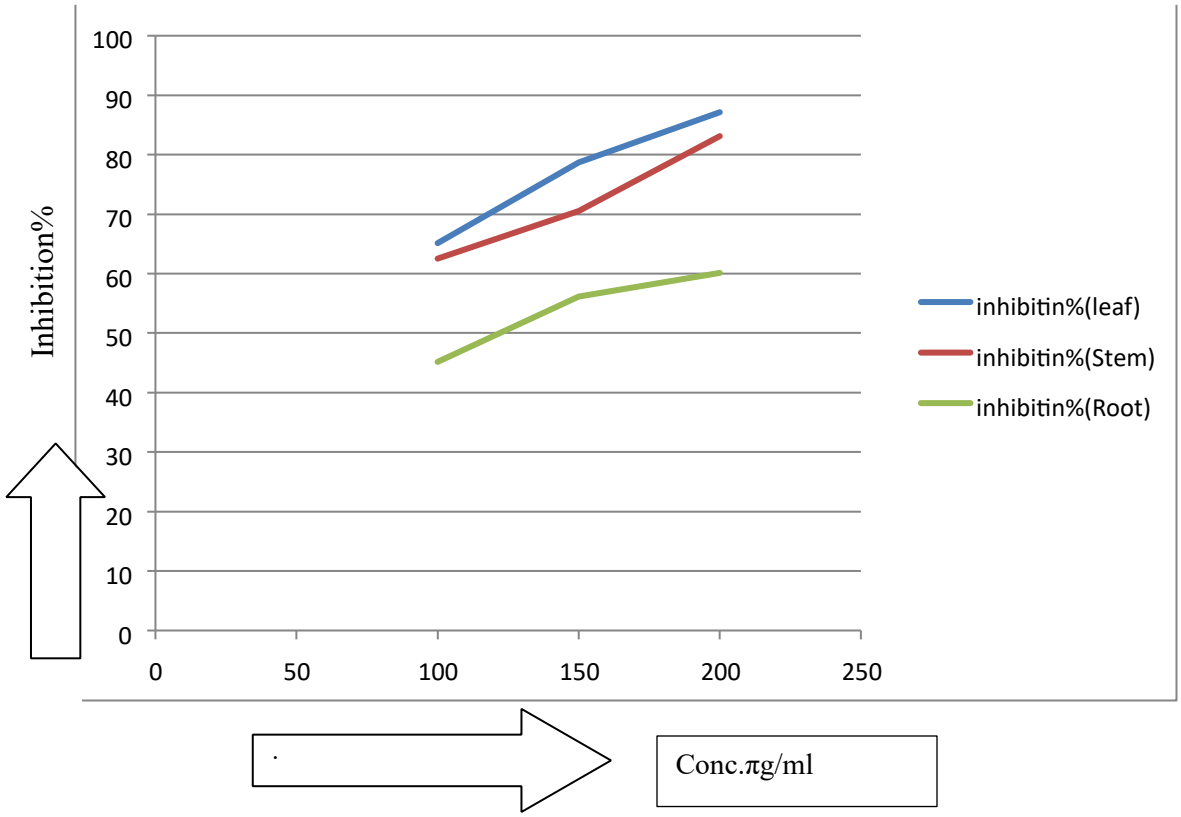

Fig.2:DPPH Scavenging activities of *Ocimum sanctum* L. in ethanolic extract.

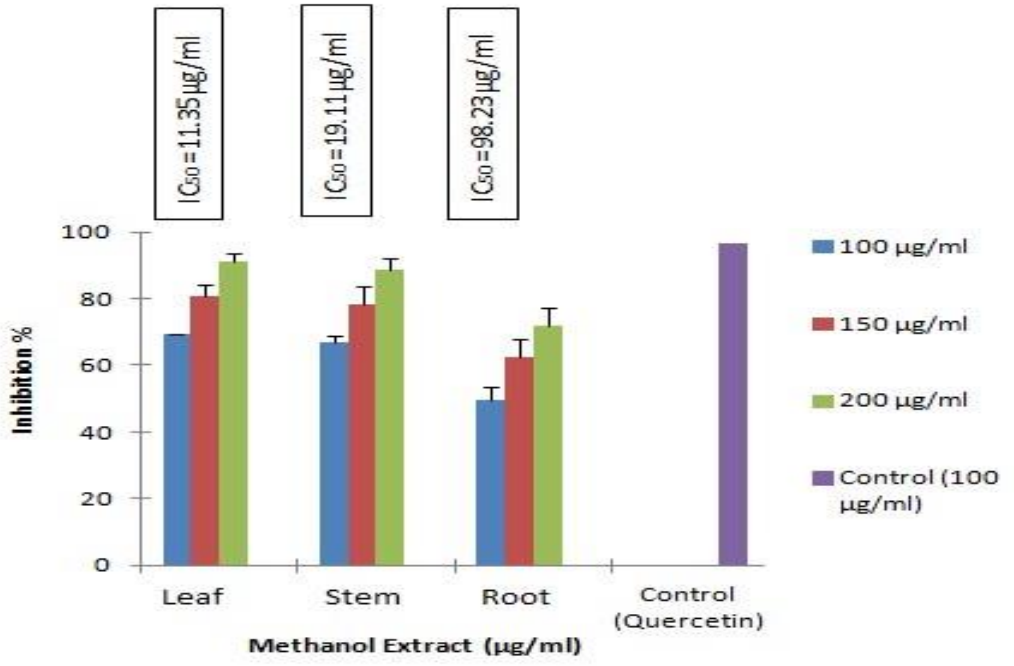

Fig.3. DPPH Scavenging activities of *O. sanctum* L. in methanolic extracts.

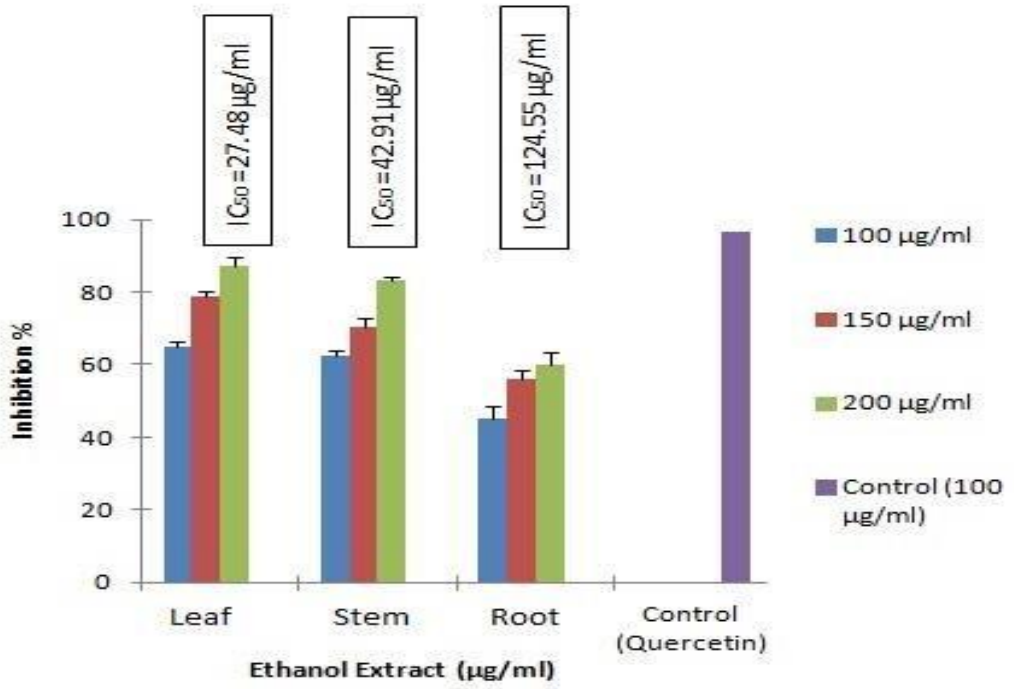

Fig.4. DPPH Scavenging activities of *O. sanctum* L. in ethanolic extracts.

Discussion

Antioxidant has been used for the prevention and treatment of free radical related disorders. However there have been concerns about synthetic antioxidants such as ascorbic acid because of possible activity as promoters of carcinogenesis. There is a scientific interest to find naturally occurring antioxidants for use in foods to replace synthetic antioxidants.

In this study we found that that the leaves of *Ocimum sanctum* L. enrich in phenolic compounds (table 1.) have strong DPPH scavenging activities. The antioxidant effect of these extracts is due to the presence of phenolic or alcoholic components (Karamanoli, 2002). The antioxidant activity is to be mainly due to their redox properties (Galato et al., 2001) which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The DPPH free radical scavenging activity of leaves, stems and roots extracts have been shown in Fig.1.2,3 and Fig.4. The IC_{50} values were compared with the IC_{50} value of Quercetin (positive control). A lower IC_{50} value indicates a greater antioxidant activity. In methanol and ethanol, the IC_{50} values of leaves of *Ocimum sanctum* L. were recorded 12.35 µg/ ml and 22.41 µg/ ml, respectively (table2.).

The total phenolic content of MeOH extract and its fractions was tested, and occurred in the range from 25.83 to 73.61 mg GAE/g of dry sample (Table 1). The total phenolic content of MeOH extract was 73.61 mg GAE/g and hexane and chloroform were 25.83 and 41.47 mg GAE/g respectively. These results showed that the total phenolic content in MeOH extract was the highest as compared to all other extracts. This may be due to the presence of high bioactive compounds in MeOH extract. The aforesaid high total phenolic constituent of the MeOH extract also supports the strongest radical scavenging activity of MeOH extract suggesting that activity is mostly related to its water-soluble phenolic compounds.

Free radical scavenging activity of methanol and ethanol extracts measured by DPPH assay, is shown in Fig. 1. Their activity of the plant extracts is concentration dependent and lower IC_{50} value reflects better protective action. The IC_{50} values of methanol and ethanol extracts were recorded in the range of 12.35 to 104.43 µg/ml. Methanol extract exhibited stronger DPPH scavenging activity than ethanol extract. The free radical scavenging activity of methanol extract (IC_{50} = 12.35 µg/ml) was superior to other extract. The IC_{50} value of methanol extract (IC_{50} = 12.35 µg/ml) was lower than synthetic antioxidant, butylated hydroxy anisole (BHA) (18.27 µg/ml). Therefore, methanol extract showed higher activity than butylated hydroxy anisole. The strongest activity of methanol extract may be related to its higher phenolic content (73.61 mg GAE/g) as measured by gallic acid test (Table 1).

Antioxidative and antimicrobial properties of the plant extracts are of great interest in both academia and the food industry, since their possible uses as natural additives emerged from a growing tendency to replace synthetic chemicals by natural ones. The results demonstrated that the extracts from *ocimum sanctum L* exerted a significant antioxidant property. Owing to its strong inhibitory activity and excellent protective features exhibited in antioxidant activity tests, the extracts from the *o. sanctum* could be concluded as a natural source that can be as used as food in the food industry. Our findings introduce a unique natural source which possesses strong antioxidant substances with potential applications in food industry. Further studies are required to assess the efficacy of extracts in foods and their toxicity to humans because medicinal plants do not have ‘generally regarded as safe’ (GRAS) status.

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