Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(1):449-454

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Characterization of chemical groups and determination of total phenolic content and *in-vitro* antioxidant activities of ethanolic extract of *Ocimum sanctum* leaves growing in Bangladesh

Mohammad Nurul Amin[1], *Syed Masudur Rahman Dewan[1], Wahiduzzaman Noor[1] and A. F. M. Shahid-Ud-Daula[1,2]

[1]*Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh*
[2] *Faculty of Science, University Brunei Darussalam, Brunei BE141*

ABSTRACT

The present study was conducted to evaluate the possible chemical groups present, total phenolic content, and antioxidant activity of ethanolic extract of leaves of Ocimum sanctum. In the study, presence of tannin and flavonoid were found significant in the crude extract. For investigating the antioxidant activity, three complementary test systems, namely DPPH free radical scavenging, reducing power assay and ferrous ion chelating ability were conducted. In DPPH free radical scavenging test, IC_{50} value for the crude extract was found fairly significant (7.23±0.05 µg/ml) while compared to that of the reference standards ascorbic acid and BHA (2.12±0.02 & 4.87±0.05 µg/ml respectively). In reducing power assay, the maximum absorbance for the extract was found 2.155 ±0.018 at 100 µg/ml while compared to 2.811 ± 0.013µg/ml and 2.031± 0.019 µg/ml for standard ascorbic acid and BHA respectively. The IC_{50} value of the extract as percentage of Fe^{++} ion chelating ability was determined as 19.05±0.08 µg/ml where EDTA showed 8.87±0.035 µg/ml. The total phenolic amount was also calculated as quite high in the extract 279.05±0.73 mg/g of gallic acid equivalent). Therefore, the obtained results tend to suggest potent antioxidant activities of the crude ethanolic extract of the leaves of O. sanctum and justify its folkloric use.

Key words: *Ocimum sanctum*, Antioxidant activity, DPPH, Total phenolic content, Reducing power assay.

INTRODUCTION

Ocimum sanctum Linn., commonly known as *Holy basil* in England and *Tulsi* in Bangladesh, is a herbaceous plant famous for its significant role in traditional medicine [1]. Recent studies with *O. sanctum* have suggested that the plant has hypoglycemic [2], adaptogenic[3], anticancer [4], radioprotective[5], analgesic and anti-inflammatory properties [6]. Earlier studies have indicated that leaves of *O. sanctum* also possess antizygotic, amti-implantation and early abortification effect in women and in experimental animals [7]. Fresh leaves of *O. sanctum* taken with black pepper are also applied as a prophylactic measure for malaria [8]. Mostly the leaves and sometimes the seeds of *O. sanctum* are used for medicinal purposes [9].

Antioxidants are molecules which are capable of hindering oxidation by counteracting reactive oxygen species [10]. Reactive oxygen species (ROS) are generated during cellular metabolism. Oxidative stress which is involved in many acute and chronic diseases is mediated by ROS. So the balance between antioxidation and oxidation is believed to be critical for proper maintaining of a healthy biological system [11-13].

Only few studies are presently available that documents its antioxidant potential. In recent times, focus on plant research has increased for the determination of their phenolic concentration and related total antioxidant potential [10, 12, 14]. It is suggested that some medicinal plants contain various types of natural antioxidants, such as phenolic acids, flavonoids and tannins. Therefore, this investigation was conducted to study the antioxidant activities and content of total phenolics of *O. sanctum.*

MATERIALS AND METHODS

Chemicals

In the study, 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Trichloro acetic acid (TCA), L- Ascorbic acid, Butylated Hydroxy Anisole (BHA), Gallic acid, Folin-ciocalteu phenol reagent, phosphate buffer (pH 6.6), Potassium ferricyanide [$K_3Fe(CN)_6$] (1%), distilled water, EDTA, Ferrozine, $FeCl_2$ and $FeCl_3$ (0.1%) of analytical grade (Merck, Darmstadt, Germany) were used for antioxidant activity assay.

Plant material and Extract Preparation

For this present investigation the leaves of *Ocimum sanctum* were collected by the authors from the surrounding area of Noakhali, a coastal region of Bangladesh, in November, 2010. The plant was identified and authenticated by the expert botanist of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 39694).

500 gm of the dried and powdered samples were soaked each in 2500 ml of 80% ethanol (Merck KGaA, Darmstadt, Germany) in clean, sterilized and flat-bottomed glass container. The container with its contents was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material and Whatman® filter paper no. 1. The resultant filtrate was then evaporated in water bath maintained 45°C to dryness and thus reddish black gummy concentrate of the extract was obtained. The gummy concentrate was designated as crude extract of ethanol.

Preliminary Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents [15]. In each test 10% (w/v) solution of the extract in ethanol was taken.

Determination of Total Phenolic Content

The total phenolic content of the extracts was determined by the modified Folin-Ciocalteu method [16]. Briefly, 0.5 mL of each extract (1 mg/ml) was mixed with 5 ml Folin-Ciocaltu reagent (1:10 v/v distilled water) and 4 ml (75g/L) of Sodium carbonate. The mixture was vortexed for 15 second and allowed to stand for 30 min at 40°C for color development .The absorbance was read at 765 nm with a spectrophotometer (UV-1800, Shimadzu, Japan). Total phenolic content was determined as mg of Gallic acid equivalent per gram using the equation obtained from a standard Gallic acid calibration curve.

DPPH free radical scavenging assay

The stable DPPH radical-scavenging activity was measured using the modified method described by using the modified method described by Chang *et al.,* 2001 [17]. Stock solution (1mg/ml) of the different solvent fractions of ethanol extract of the leaves of *O. sanctum* prepared in respective solvent systems from which serial dilutions were carried out to obtain the concentrations of 5, 10, 20, 40, 60, 80, 100 µg/ml. In this assay, 2 ml of 0.1 mM ethanolic DPPH solution was added to 2 ml of extract solution at different concentrations and the contents were stirred vigorously for 15 seconds. Then the solutions were allowed to stand at dark place at room temperature for 30 min for reaction to occur. After 30 min, absorbance was measured against a blank at 517 nm with a double beam Analykjena UV-Visible spectrophotometer. The percentage of DPPH free radical-scavenging activity of each plant extract was calculated as:

$$\text{DPPH free radical-scavenging activity (I \%)}, = [(A_0 - A) / A_0] \times 100,$$

Where, A_0 is the absorbance of the control solution (containing all reagents except plant extract); A is the absorbance of the DPPH solution containing plant extract.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration (µg/ml) to determine the concentration of extract necessary to decrease DPPH radical-scavenging by 50% (called IC_{50}). The IC_{50} value of each extract was estimated by sigmoid non-linear regression, using SigmaPlot 2000 Demo (SPSS Inc., Chicago, IL, USA). All determinations were performed in triplicate. Ascorbic acid was used as positive control standard.

Reducing power assay

The method of Dehpour *et al*., [18] was followed to determine the reducing power of *O. sanctum* leaves of ethanolic extract. 1 ml of extract solution of different concentrations (5, 10, 20, 40, 60, 80, 100 µg/ml) was mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). The mixture was incubated at 50°C for 20 minutes. The reaction was terminated by adding 2.5 ml of Trichloroacetic acid (10%, w/v), then the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1% w/v) solution. Then the absorbance was measured at 700 nm against a blank using UV spectrophotometer. Increased absorbance value of the reaction mixture indicates increased reducing power. Three replicates were made for each test sample and average data was noted. Here, Ascorbic acid and BHA were used as positive control standard.

Ferrous ion chelating ability

The ferrous ions chelating activity of ethanolic extract of *O. sanctum* and standards were investigated according to the method of Dinis *et al.,* [19]. Briefly, different concentrations of the extract (5-100 µg/ml) were added to 0.1ml solution of 2 mM ferrous chloride ($FeCl_2$). Then, the reaction was initiated by the addition of 0.2ml of 5 mM Ferrozine and mixture was shaken vigorously and kept at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in spectrophotometer, wherein the Fe^{+2} chelating ability of extracts was monitored by measuring the ferrous ion-Ferrozine complex. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given in the following formula.

$$\text{Ferrous ions chelating ability (\%)} = [(A_0 - A) / A_0] \times 100$$

Where A_0 is the absorbance of the control solution (containing all reagents except extract); A is the absorbance in the presence of the sample of plant extracts. All the tests were carried out in triplicate and EDTA was used as standard.

RESULTS AND DISCUSSION

Chemical Group Test

Results of different chemical tests on the ethanolic extract of *O. sanctum* leaves ensured the presence of steroids, alkaloids, saponins, gums and significantly presence of flavonoids and tannins (Table 1).

Table 1: Presence of possible chemical groups

Serial No.	Chemical Constituents	Tests	Extract	Result
1	Test for Reducing Sugar	Benedict's Test	Ethanolic	_
		Fehling's Test	Ethanolic	_
		Alpha Napthol Solution Test	Ethanolic	_
2	Test for tannins	Ferric Chloride Test	Ethanolic	++
		Potassium dichromate Test	Ethanolic	++
3	Test for Flavonoids	Hydrochloric Acid Test	Ethanolic	++
4	Test for Saponins	Foam Test	Ethanolic	+
5	Test for Gums	Molish Test	Ethanolic	+
6	Test for Steroids	Libermann-burchard Test	Ethanolic	+
		Sulphuric acid Test	Ethanolic	+
7	Test for Alkaloids	Mayer's Test	Ethanolic	+
		Wagner's Test	Ethanolic	+
		Dragendroff's Test	Ethanolic	+
		Hager's Test	Ethanolic	+

+: Presence; - : Absence; ++: significantly presence

Total Phenolic Content

The amount of total phenolic content was determined as quite high in the ethanolic crude extract of *O. sanctum* (279.05±0.73 mg/g of gallic acid equivalent) (Table 2). Phytochemical compounds especially phenolic compounds such as flavonoids, phenyl propanoids, phenolic acids, tannins etc. are very important components for the free radical scavenging and antioxidant activities of plants. So, the relationship between phenolic content of medicinal plants and antioxidant activity is well documented [20, 21]. The result of present study revealed that the presence of high concentration of phenolic components in the extract may effectively eliminate radicals and they contribute directly to antioxidant effect of the system [22].

Table 2: Total phenolic content determination of ethanolic extract of leaves of *Ocimum sanctum*

Extract	Avg. absorbance at 765 nm	Total phenolic content of ethanolic extract of *Ocimum sanctum*
Ethanol extract of *Ocimum sanctum*	1.39±0.085	279.05±0.73 mg gallic acid equivalent (GAE) per gm of dry extract

The values are expressed as mean ± standard deviation (n=3).

DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity of the *O. sanctum* was found to be increased with the increase of concentration of the extract (Table 3). The extract showed 90.36±0.04 % radical inhibitions at 100 µg/ml whereas at the same concentration the standards ascorbic acid and BHA showed 95.82±0.09 and 93.09±0.06% inhibitions respectively. The IC_{50} value of extract of *O. sanctum* was determined as 7.23±0.05 µg/ml where Ascorbic acid & BHA showed 2.12±0.02 and 4.87±0.05 µg/ml respectively showed at table 3. A method which is based on the scavenging of the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively for the prediction of the antioxidant activities of extracts of plants [23, 20]. The high inhibition value of *O. sanctum* ethanol extract may be due to the presence of phytochemicals such as tannins and flavonoids in the extract. It has been reported that tannins and flavonoids, commonly found in plants have significant antioxidant activity [24].

Table 3: DPPH radical scavenging activity of the ethanolic extract of *O. sanctum* leaves and standards

Concentration (µg/ml)	% Inhibition of different solvent extract and Standard		
	Ocimum sanctum	Ascorbic Acid (AA) (Standard)	BHA (Standard)
5	45.34±0.07	68.53±0.03	53.63±0.05
10	60.76±0.08	79.00±0.04	77.23±0.09
20	75.12±0.03	83.81±0.05	90.18±0.07
40	80.40±0.05	91.94±0.03	91.11±0.09
60	83.05±0.07	95.23±0.09	92.03±0.04
80	88.85±0.09	95.59±0.07	92.31±0.08
100	90.36±0.04	95.82±0.09	93.09±0.06
IC_{50} (µg/ml)	7.23±0.05	2.12±0.02	4.87±0.05

Values are expressed as mean±SD (n=3).

Table 4: Reducing power assay of the ethanolic extract of *O. sanctum* leaves and standards

Concentration (µg/ml)	Average absorbance at 700nm of extract and Standards at different concentration		
	Ehtanolic extract of Ocimum sanctum leaves	AA (Standard)	BHA (Standard)
5	0.376 ±0.014	0.370 ±0.013	0.435 ±0.011
10	0.726 ±0.016	0.820 ±0.017	0.776 ±0.013
20	1.019 ±0.027	1.447 ±0.011	1.598 ±0.012
40	1.279 ±0.016	1.929 ±0.014	1.749 ±0.017
60	1.486 ±0.019	2.624 ±0.015	1.842 ±0.013
80	1.798 ±0.011	2.772 ±0.012	1.976 ±0.015
100	2.155 ±0.018	2.811 ±0.013	2.031 ±0.019

Values are expressed as mean±SD (n=3)

Reducing Power Assay

Ascorbic acid and BHA were used as positive control for the determination of reducing power of ethanolic extract of *O. sanctum*. The maximum absorbance for the ethanolic extract of *O. sanctum* was found to be 2.155 ±0.018 at 100 µg/ml while that of standard ascorbic acid and BHA was found as 2.8111 and 2.031, respectively at the same concentration (Table 4). With the increase of concentration, the absorbance of the extract and standards were found to be increased gradually. Recently it has been reported that there is a direct correlation between antioxidant

capacity and reducing power of certain plant extracts [21]. The reducing properties are generally related with the presence of reductones, which are responsible to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [22].

Fe^{++} Ion Chelating Ability

Fe^{++} ion chelating ability of ethanol extract of *O. sanctum* is shown in table 5. The extract showed 85.89±0.08% Fe^{++} ion chelating ability at 100μg/ml where as the standard EDTA showed 99.75±0.011% at the same concentration. The IC_{50} value of the extract was also found significant while compared to the IC_{50} value of the reference standard EDTA. In this assay, crude extract and standard compounds interfered with the formation of ferrous and ferrozine complex which suggest that they have chelating activity and are capable of capturing ferrous ion before the formation of ferrozine. Iron is responsible to generate free radicals through the Fenton and Haber–Weiss reaction. But metal ion chelating activity of an antioxidant compound retard this oxyradical generation and the consequent oxidative damage. So, metal ion chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalysing transition metal [25].

Table 5: Fe^{2+} ion chelating ability of ethanol extract of *O. sanctum* leaves and EDTA (Standard)

Concentration (μg/ml)	% Chelating Ability of different solvent extract and Standard	
	Ethanol extract of *Ocimum sanctum*	EDTA (Standard)
5	30.88±0.06	36.97±0.032
10	35.99±0.04	57.71±0.027
20	55.47±0.07	81.69±0.037
40	74.32±0.09	91.35±0.019
60	79.36±0.03	99.19±0.020
80	82.78±0.06	99.30±0.021
100	85.89±0.08	99.75±0.011
IC_{50} (μg/ml)	19.05±0.08	8.87±0.035

Values are expressed as mean±SD (n=3)

CONCLUSION

In conclusion it can be revealed that the crude ethanolic extract of *O. sanctum* leaves contain significant antioxidant activities. The significant potency of the extract of *O. sanctum* as antioxidant agents may be due to the presence of phytochemicals like tannins, flavonoids, phenolics etc and might be responsible for its activity and justify its use as a traditional folk remedy for toothache. However, extensive researches are required to search for active principles responsible for these activities.

Acknowledgement

The authors are thankful to BNH to identify the plant. The authors are also grateful to all the teachers of Dept. of Pharmacy, Noakhali Science and Technology University for their cooperation.

REFERENCES

[1] Glosh GR, Tulasi (N. O. Labiatae,Genus-Dcimum) New approaches to medicine and health (NANAH)., **1995**, 3: 23-29.
[2] Giri J, Suganthi B, Meera G, *Indian J Nutr Dietet*, **1987**, 24: 193-198.
[3] Bhargava KP, Singh N. *Indian J Med Res*, **1981**, 73: 443-451.
[4] Aruna K, Sivaramakrishnan VM. *Food Chem Toxicol*, **1992**, 30: 953-956.
[5] Devi PU. Ganasoundari A. *Indian J Exp Bio*., **1995**, 33: 205-208.
[6] Singh S, Majumdar DK, Rehan HM. *J Ethanopharmacol*, **1996**, 54: 19-26.
[7] Vora SB, Garg SK, Chaudhary RR. *Indian J Med Res*, **1969**, 57: 893-899.
[8] Dastur JF. *Medicinal plants of India and Pakistan,* Bombay, India: DB Taraporevala Sons & Co., **1962**.
[9] Gupta SK, Prakash J, Srivastava S. *Indian J Exp Biol*, **2002**, 40: 765-773.
[10] Wong C, Li H, Cheng K, Chen F. *Food Chem*, **2006**, 97: 705-711.
[11] Hong H, Liu G. *Life Sci*, **2004**, 74: 2959-2973.
[12] Katalinic V, Milos M, Kulisic T, Jukic M. *Food Chem*, **2006**, 94: 550-557.
[13] Montuschi P, Barnes PJ, Roberts LJ. *The Faseb J Review*, **2004**, 18: 1791-1800.
[14] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, *Food Chem,* **2006**, 97: 654-660.

[15] Evans WC. *Trease and Evan's Pharmacognosy*. 13th ed. University Press, Cambridge, **1989**, pp 546.
[16] Wolfe K, Wu X, Liu RH. *J Agric Food Chem,* **2003**, 51:609-614.
[17] Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF *J Agric Food Chem*, **2001**, 49:3420–3424.
[18] Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi *Grasas Aceites*, **2009**, 60(4): 405-412.
[19] Dinis TC, Madeira VM, Almeida LM. *Arch Biochem Biophys,* **1994**, 315(1):161-169.
[20] Kulisic T, Radonic A, Katalinic V, Milos M. *Food Chem,* **2004**, 85:633–640.
[21] Tanaka M, Kuie CW, Nagashima Y, Taguchi T. *Nippon Suisan Gakkaishi*, **1988**, 54:1409-1414.
[22] Duh PD, *J Agric Food Chem,* **1994**, 42:629–632.
[23] Yen GC, Duh PD. *J Agric Food Chem,* **1994**, 42:629–632.
[24] Vinson JA, Dabbagh YA, Serry MM, Jang J. *J Agric Food Chem,* **1995**, 43:2800-2802.
[25] Duh PD, Tu YY, Yen GC. *Lebensmittel- Wissenschaft und Technol*, **1999**, 32: 269-277.