International Journal of Agricultural Technology 2015 Vol. 11(7):1635-1643
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Free Radical Scavenging Potential and Phytochemical Analysis of Leaf Extract from *Ocimum Sanctum* Linn

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Rana, M., Sayeed, A., Nasrin, S., Islam, M., Rahman, M. and Firoz Alam, M. F. (2015). Free radical scavenging potential and phytochemical analysis of leaf extract from *Ocimum sanctum* Linn. International Journal of Agricultural Technology 11(7):1635-1643.

Abstract Plants produce many important antioxidant compounds which are considered as safer than synthetic antioxidants. Plants also contain some bioactive compounds which have therapeutic activities. In this study, free radical scavenging activity and phytochemical constituents of leaf extract of *Ocimum sanctum* were assessed. Free radical scavenging activity was analyzed by DPPH method. The IC_{50} values of ascorbic acid (positive control), methanol, ethanol and chloroform extracts were 99.25, 214.84, 253.55 and 261.11µg/ml, respectively. Phytochemical analysis of studied plant extracts showed the presence of alkaloids, tannins, flavonoids, and saponins.

Keywords: Free radical scavenging activity, DPPH, phytochemicals, ascorbic acid, *Ocimum sanctum*

Introduction

Free radicals are atoms or molecules with one or more unpaired electrons. They are highly unstable and cause damage to other molecules by taking electrons from them toward attains stability. They are continuously produced in the human body, because they are essential for energy supply, detoxification, chemical signaling and immune function (Gulcin, 2005). They can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which lead to cell injury and can induce numerous diseases such as cancer, diabetes, cardiovascular diseases, atherosclerosis, arthritis, aging and metabolic syndrome (Hsu *et al*., 2003; Hosseinimehr *et al*., 2007; Raghuveer and Tandon, 2009). Free radicals can be removed by the protective role of natural and synthetic antioxidant agents (Lobo *et al*., 2010). In recent years, the use of natural antioxidant has acquired much concentration from consumers as they

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are considered safer than synthetic antioxidants (Mbaebie *et al*., 2012). Plants produce many important antioxidant compounds that includes carotenoids, flavonoids, vitamin C and E, etc. (Qusti *et al*., 2010). Thus, plants were potential sources of antioxidant.

Generally, medicinal plants are of great significance to the health of individuals and communities (Mir *et al*., 2013). In recent years, there has been an increasing consciousness about the importance of medicinal plants as drugs from the plants are easily available, less expensive, and safe and rarely have side effects (Yadav and Agarwala, 2011). These plants contain some bioactive compounds like tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols which provide definite physiological action on the human body (Mandal *et al*., 2013). Most of these compounds have therapeutic activities such as insecticidal (Kambu *et al*., 1982), antibacterial, antifungal (Lemos *et al*., 1990), anticonstipative (Ferdous *et al*., 1992), spasmolytic (Sontos *et al*., 1998), antiplasmodial (Benoit-vical *et al*., 2001) and antioxidant (Kahkonen *et al*., 2003) activities etc.

Ocimum sanctum Linn. has been widely known for its medicinal value for thousands of years (Soni and Sosa, 2013). *O. sanctum* leaf contains a variety of constituents including saponins, flavonoids, triterpenoids, and tannins that may have biological activity (Jaggi *et al*., 2003). It has been shown that *O. sanctum* bear anti-carcinogenic, antirheumatic, anthelmintic, anti-septic, antistress, anticancer, antioxidant and antibacterial properties (Karthikeyan *et al*., 1999; Duke, 2008; Soni and Sosa, 2013; Rama and Sundar, 2013). Therefore, this present work was undertaken to evaluate the free radical scavenging activity and phytochemical constituents of leaf extract of *O. sanctum*.

Materials and methods

Plant material

Fresh leaves of *O. sanctum* Linn. (Family: Lamiaceae) were collected from Rajshahi University campus during November-December, 2013. Plants were identified and authenticated by Dr. A.H.M. Mahbubur Rahman, Associate Professor and Plant taxonomist, Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extracts

Collected plant materials were washed with clean sterile distilled water and dried for 3 days in oven under 60 ℃ to reduce water content. Then the dried

plant materials were crushed into fine powder using mortar-pestle and electric blender (Nokia, Osaka-Japan). Fifty gram powder was dipped into 250ml solvent in a conical flask with rubber corks and left for two days on orbital shaking (IKA Labortechnik KS 250 Basic Orbital Shaker, Staufen, Germany). Filtration was done through teton cloth and Whatman No. 1 filter paper. The filtrate was taken into glass beaker and kept into water bath (4 holes analogue, Thermostatic water bath, China) at 60 ℃ for evaporation of excess solvent and stored at 4 ℃ (Sayeed *et al*., 2014a; Akueshi *et al*., 2002). Particular concentrations of the plant extracts were prepared for experimentation.

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid.

Free radical scavenging activity

Free radical scavenging activity of leaf extracts of *O. sanctum* was carried out using DPPH. DPPH is a molecule containing a stable free radical. Free radical scavenging potential of the extracts was tested against solution of 1,1-diphenyl-2-picrylhydrazyl. Antioxidants react with DPPH and convert it to 1,1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant (Kumar and Tyagi, 2013).

Procedure of free radical scavenging activity test

DPPH radical scavenging activity of the extract was measured by the method described by Mannan *et al*. (2013) and Hsu *et al*. (2007) with some modifications. The antioxidant activity was compared with ascorbic acid. 3ml of 0.1mM DPPH solution was mixed with 2ml of various concentrations (25 to 275μg/ml) of extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0 \times 100]$$

Where, A_0 is the absorbance of a DPPH solution without tested sample and A_1 is the absorbance of the tested sample. All measurements were

performed in triplicate and data presented as mean ± standard deviation (SD). Finally, the DPPH radical scavenging activity (%) was plotted against respective concentrations used and IC_{50} was calculated from the graph. IC_{50} value is the effective concentration of sample at which the antioxidant activity is 50% (Mandal *et al*., 2009).

Phytochemical analysis

The following tests were carried out to detect the presence of active chemical constituents like alkaloids, tannins, glycosides, flavonoids, terpenoides and saponins.

1. Test for Alkaloids

To detect the presence of alkaloids, few drops of Mayer's reagent were added to the extract, cream colored precipitate indicates the presence of alkaloids (Siddiqui and Ali, 1997).

2. Test for Tannins

1ml of 5% $FeCl_3$ is added to the extract, presence of tanning is indicated by the formation of bluish black or greenish black precipitate (Siddiqui and Ali, 1997).

3. Test for Glycosides

To the solution of the 2ml extract in glacial acetic acid, few drops of $FeCl_3$ and concentrated H_2SO_4 were added, and reddish brown color at the junction of two liquid layers and upper layer appears bluish green indicates the presence of glycosides (Trease and Evans, 1989).

4. Test for Flavonoids

Few drops of 10% concentrated H_2SO_4 was added to the extract, followed by 1ml of ammonia, formation of greenish yellow precipitate indicates the presence of flavonoids (Siddiqui and Ali, 1997).

5. Test for Terpenoids

In 2ml of extract, 5ml chloroform and 2ml concentrated H_2SO_4 was added. Reddish brown colorations of interface indicate the presence of terpenoides (Harborne, 1973).

6. Test for Saponins

20ml water is added to 150mg extract and shaken vigorously, layer of foam formation indicates the presence of Saponins (Siddiqui and Ali, 1997).

Results

Free radical scavenging activity

The free radical scavenging activity was observed at 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275µg/ml of ascorbic acid and methanol, ethanol, chloroform extracts of *O. sanctum* (Fig. 1). The IC_{50} values were calculated after plotted the data on graph paper (Table 1). Fig. 1 shows the dose dependent results of extracts comparing with ascorbic acid. Both plant extract and ascorbic acid reduced the radical with increasing concentrations. The IC_{50} values of ascorbic acid, methanol, ethanol and chloroform extract were 99.25, 214.84, 253.55 and 261.11µg/ml, respectively.

Table 1. Free radical scavenging activity of different samples

Sample	IC_{50} (µg/ml)
Ascorbic acid	99.25 ±0.09
Methanol extract	214.84 ±0.33
Ethanol extract	253.55 ±0.17
Chloroform extract	261.11 ±0.23

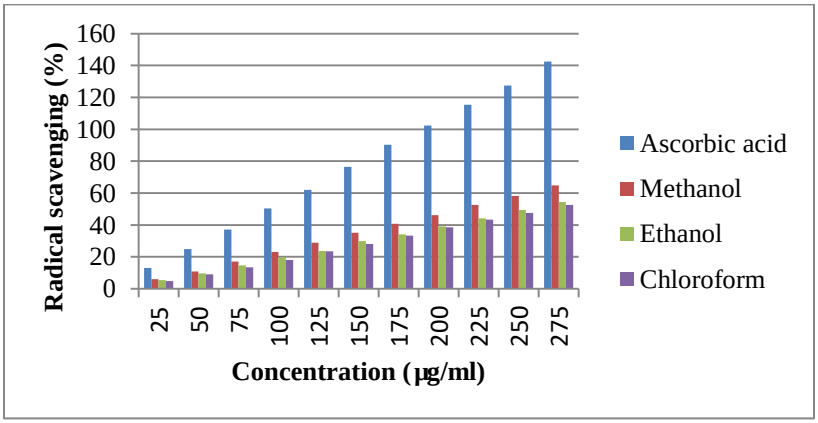

Figure 1. Free radical scavenging activity of leaf extract of *O. sanctum.*

Phytochemical analysis

Phytochemical analysis of studied plant extract suggests the presence of alkaloids, tannins, flavonoids, and saponins (Table 2). Methanol extracts showed good result compared to ethanol and chloroform extracts. Methanol extract showed the presence of alkaloids, tannins, flavonoids, and saponins, whereas, ethanol extracts contained alkaloids, tannins, flavonoids and chloroform extracts contained tannins only.

Table 2. Phytochemical analysis of leaf extract of *O. sanctum*

Phytoconstituents	**Methanol**	**Ethanol**	**Chloroform**
Alkaloids	+	+	-
Tannins	+	+	+
Glycosides	-	-	-
Flavonoids	+	+	-
Terpenoids	-	-	-
Saponins	+	-	-

Discussion

The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1,1-diphenyl-2-picrylhydrazyl, a stable free radical, to decolorize in the presence of antioxidants (Kumarasamy *et al*., 2007). DPPH has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical (Jao and Co, 2002). Antioxidants react with DPPH and convert it to 1-1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant (Kumar and Tyagi, 2013). This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants (Jao and Co, 2002). From the results of free radical scavenging activity of leaf extracts of *O. sanctum*, it reveals plant extracts showed antioxidant activity. Higher IC_{50} value indicates the lowest antioxidant activity, whereas, lower indicates the highest activity. Methanol extract showed highest antioxidant activity compared to other extracts. DPPH radical scavenging activities of the extracts depend on the extraction solvent as well as plant type. Antioxidant activities may increase with increasing of phenolic components (Soni and Sosa, 2013). Many studies have reported about antioxidant activity of this plant. Soni and Sosa (2013) support our results. Antioxidant activity has

also been demonstrated by other researchers (Sethi *et al*., 2004; Nair *et al*., 2009; Mishra *et al*., 2011; Rama and Sundar, 2013).

Phytochemical constituent was analyzed in another study. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993). Analysis of plant extract showed the presence of alkaloids, tannins, flavonoids, and saponins. The similar result is revealed by Soni and Sosa (2013). It has also been shown the results by some other researchers which support our results (Pathmanathan *et al*., 2010; Rama and Sundar, 2013). Methanol extracts showed highest results and it may be for better solubility of active components in methanol (Sayeed *et al*., 2014b). It has been reported that different phytoconstituents have different degrees of solubility in different types of solvents depending on their polarity (El-Mahmood and Doughari, 2008). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc (Ali *et al*., 2008). They are of great importance to the human health. Phenolics have been known to possess a capacity to scavenge free radicals. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors (Soni and Sosa, 2013). They play an important preventive role in the development of cancer, heart diseases and ageing related diseases (Larsomn, 1988).

Conclusions

In conclusion, *O. sanctum* leaf extracts possess free radical scavenging activity and contain alkaloids, tannins, flavonoids, and saponins.

Acknowledgments

We are grateful to department of Botany, University of Rajshahi, Bangladesh for providing necessary chemicals and equipments for the completion of research.

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(Received: 7 September 2015, accepted: 25 October 2015)