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Antioxidant and Antibacterial Activities of Four Local Medicinal Plants

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Abstract

Potential antioxidant and antibacterial activity of methanolic, chloroformic and n-hexane leaf extracts of four local important medicinal plants like *Ocimum americanum*, *O. basilicum, O. gratissimum* and *Centella asiatica* was investigated**.** The methanolic leaf extracts of these plant species exhibited the potent DPPH free radical scavenging activity (IC_{50} value, 2.67 ± 0.01, 14.17 ± 0.11, 60.22 ± 0.01 and 2.39 ± 0.025 µg/ml, respectively). Methanolic leaf extract of *C. asiatica* showed strongest antioxidant activity. Chloroformic leaf extracts possessed moderate antioxidant activity (IC_{50} value of 79.44 ± 0.05, 110.56 ± 0.02, 54.95 ± 0.05, 101.0 ± 1.0 µg/ml, respectively) in all samples. The lowest antioxidant activity was recorded from *n*-hexane leaf extracts of *O. americanum, O. gratissimum*, *C. asiatica* and *Ocimum basilicum* (IC_{50} value 147.87 ± 0.06, 378.19 ± 2.65, 104.65 ± 0.39, 467.58 ± 0.52 µg/ml, respectively***)***. Methanolic and chloro-formic leaf extracts showed antibacterial activity against both Gram-positive and Gram-negative pathogenic bacteria, namely *Bacillus megaterium*, *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*. Methanolic leaf extract of *O. americanum* and chloroformic extract of *C. asiatica* showed excellent antimicrobial activity.

Introduction

Natural antioxidants are being extensively studied for their ability to protect organisms and cells from damage caused by oxidative stress. Medicinal plants are in general, harmless sources for obtaining natural antioxidants. There is an increasing demand to

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evaluate the antioxidant properties of the plant extracts and in the last years, the attention has been focused on the antioxidant products from natural sources (Lobo et al. 2010 and Amiri 2010). Many pharmacological studies have shown that extracts of some antioxidant plants possess anti-inflammatory, anti-allergic, anti-tumor, anti-bacterial, anti-mutagenic and anti-viral activities to a greater or lesser extent. Today the large numbers of drugs are derived from medicinal plants, like morphine from *Papaver somniferum*, ephedrine from *Ephedra vulgaris* etc. The medicinal plants are rich in secondary metabolites which are potential sources of drugs and essential oils of therapeutic importance (www.intechophen.com).

Ocimum spp. and *Centella asiatica* are widely used in Bangladesh by the traditional medical practitioners in day to day practice. Generally, *Ocimum* spp. of the Lamiaceae family rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties (Klaudija et al. 2016). In this regard, *O. basilicum*, *O. americanum* and *O. gratissimum* are very important members of this family for their medicinal value. *Ocimum* sp. is extensively used in traditional medicine (Javanmardi et al. 2002) and exhibits phytotherapeutic properties (Maria et al. 2008), antimicrobial (Ram et al. 2011), antifungal (Zhang et al. 2009), as well as antioxidant and insect repellent activities (Telci et al. 2009 and Kweka et al. 2008).

C. asiatica is a prostrate stoloniferous plant that belongs to Apiaceae and indigenous to Bangladesh (Varrier 1997). The therapeutic use of *C. asiatica* with its wide range of application has been documented in South East Asia and Bangladesh in particular for centuries. *C. asiatica* is effectively being used in the treatment of fever, jaundice, dysentery, diarrhea, mental illness within the frame of traditional medicine of Bangladesh (Ahmed 2009).

Despite the popular use of these medicinal plants in Bangladesh there is no reliable data available about the antioxidant and antimicrobial potential of these plants. So, efforts should be made to evaluate the antioxidant and antimicrobial properties of these plants. The aim of this work was to analyze antioxidant and antibacterial activity of three widely used species of *Ocimum,* namely *O. americanum, O. basilicum, O. gratissimum* and *Centella asiatica.*

Materials and Methods

Leaves of four local important medicinal plants like *Ocimum americanum, O. basilicum, O. gratissimum* and *Centella asiatica* were collected from medicinal plant garden of BCSIR Laboratories Chitttagong, Chattogram. After collection leaves were dried under shade at 25 - 27°C and powder was made using an electric blender. The powder was stored at 4°C in air tight container. The air-dried powdered leaf materials (200 g) of four plants were extracted with 550, 600 and 650 ml of solvents *n*-hexane, chloroform and methanol separately in succession. The plant extracts were then evaporated, dried and stored in a beaker at 4°C till use. 2, 2-diphenyl-1-picryl hydrazyl **(**DPPH) free radical scavenging

assay was used for determining antioxidant activity. The antioxidant potential of the crude extracts were determined on the basis of their scavenging activity of the stable dark-colored crystalline powder 2, 2-diphenyl-1-picryl hydrazyl free radical that has significant applications in laboratory research. The free radical scavenging property of extracts were analyzed by 1, 2-diphenyl 1-picryl hydrazyl assay developed by Brand-Williams et al. 1995. In determining DPPH free radical scavenging activity, different concentrations (1.75, 3.13, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml) of the extracts and the positive control (Quercetin) were prepared with pure ethanol. Then 2 ml of 0.004% DPPH solution was added in test tube of different extracts. The test tubes were allowed to stand at dark for 30 min to complete the reaction and then absorbance was recorded at 517 nm. The decrease in absorbance with blank was also measured. Negative control was prepared in the same way as the sample except addition of sample or standard. Percent scavenging activity was calculated using the formula: scavenging activity = (A0 − A1)/A0 × 100%, where A0 is the absorbance of control, and A1 is the absorbance of sample or standard. The experiment was carried out in triplicate. By using the equation y = mx + c (where c is intercept and, m is slope); IC_{50} value of extract was calculated.

Crude leaf extracts (*n*-hexane, chloroformic and methanolic) of *O. americanum, O. basilicum, O. gratissimum* and *C. asiatica* were used to evaluate the antibacterial activity by using disc diffusion method. One Gram-positive pathogenic bacteria (*Bacillus megaterium* ATCC 18) and three Gram-negative pathogenic bacteria *Escherichia coli*; ATCC 8739**,** *Pseudomonas aurigonisa*; ATCC 2783318 and *Salmonella typhi*; ATCC 13311 were used in this test. A single bacterial colony was cultured in 25 ml LB broth for 24 hrs at 37ºC and the liquid culture was spread uniformly on nutrient agar plates using a sterile cotton swab. The plates were kept for 15 minutes and then used for the sensitivity test. Gentamycin (10 mcg/disc) were used as standard antibiotic. The sterile filter paper discs were prepared by adding 500 µg extracts per disc. The negative control was 100% ethanol.

One Petri dish was arbitrarily divided in four parts where one negative control disc, one antibiotic disc and two extract discs were placed. The plates were then incubated at 37°C for 18 to 24 hours. After the incubation, the plates were observed for inhibition zone and were measured using scale in millimeter. The tests were repeated three times to ensure reliability.

Results and Discussion

Antioxidant activity of leaf extracts of *Ocimum americanum, O. basilicum, O. gratissimum*, and *Centella asiatica* were examined by DPPH free radical scavenging assay. Quercetin was used as a standard. Among three leaf extracts of the plants methanolic leaf extract showed lowest IC_{50} value (2.67 - 60.22 µg/ml) compared to standard Quercetin (2.28 ± 0.01 µg/ml) (Table 1). Chloroformic leaf extract exhibited lower IC_{50} value (54.95 - 110.56 µg/ml) and *n*-hexane leaf extract showed the least antioxidant activity (147.87 -467.58

µg/ml). Comparison of IC_{50} values of different plant extracts with standard Quercetin are presented in Fig. 1. It was previously reported that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds and aromatic amines could reduce and decolorize DPPH by their hydrogen donating ability (Blois 1958,

Table 1. DPPH-free radical scavenging activity.

Solvent system	Plant species	IC_{50} value µg/ml
Standard	Quercitin	2.28 ± 0.01
Methanolic	*O. americanum*	2.67 ± 0.01
	C. asiatica	2.39 ± 0.025
	O. basilicum	14.17 ± 0.11
	O. gratissimum	60.22 ± 0.01
Chloroformic	*O. americanum*	79.44 ± 0.05
	O. basilicum	110.56 ± 0.02
	O. gratissimum	54.95 ± 0.05
	C. asiatica	101.0 ± 1.0
n-hexane	*O. americanum*	147.87 ± 0.06
	O. basilicum	467.58 ± 0.52
	O. gratissimum	378.19 ± 2.65
	C. asiatica	104.65 ± 0.39

Results are the average of triplicate measurements ± Sd.

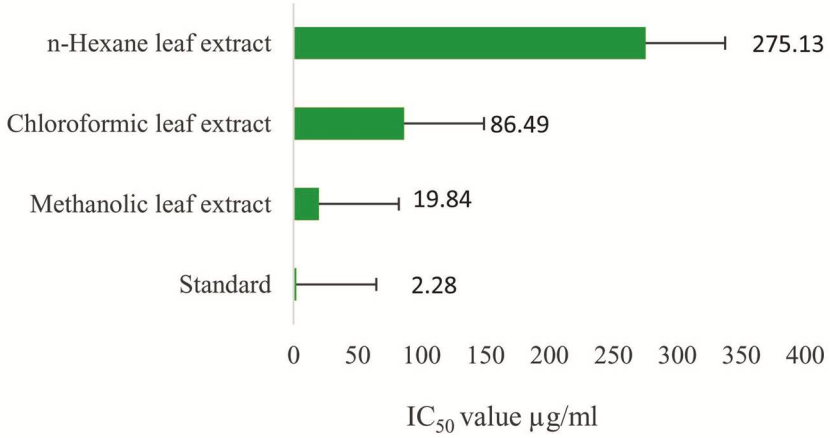

Fig. 1. Comparison of average IC_{50} values of methanolic, chloroformic and *n*-hexane leaf extracts of *O. basilicum*, *O. americanum*, *O. gratissimum and C. asiatica* with (Quercitin).Values are taken by three replicate determinations (n = 3) ± Sd. IC_{50} values of methanolic leaf extracts of all the plants are close to IC_{50} value of standard.

Hossain et al. 2015). Therefore, methanolic leaf extracts seem to possess hydrogen donating capability as they tend to extract mostly polar compounds from the plant material and showed potent antioxidant activity.

The IC_{50} value of methanolic leaf extracts of *O. americanum*, *O. basilicum* and *C. asiatica* were found 2.67 ± 0.01, 14.17 ± 0.11, 2.39 ± 0.025 µg/ml, respectively (Table 1). On the otherhand, methanolic leaf extract of *O. gratissimum* showed the least antioxidant activity. DPPH-free radical scavenging activity of methanolic leaf extracts of *C. asiatica, O. americanum* and *O. basilicum,* respectively are presented in Fig. 2. The IC_{50} value of methanolic leaf extracts of *O. americanum, O. basilicum* and *C. asiatica* clearly indicated that the concentration of leaf extracts was needed to scavenge 50% of the free radical which was very close to the IC_{50} value of standard (2.28 µg/ml, where regression equation, y = 23.07 x + 41.64, R_2 value = 0.566, data not shown). Therefore, methanolic leaf extract showed the potent antioxidant activity. *C. asiatica* showed the highest antioxidant activity (Regression equation, y = 25.75 x + 40.18, R_2 value = 0.524, data not shown) from methanolic leaf extract (Fig. 2). Yadav et al. 2017 also reported that *C. asiatica* showed

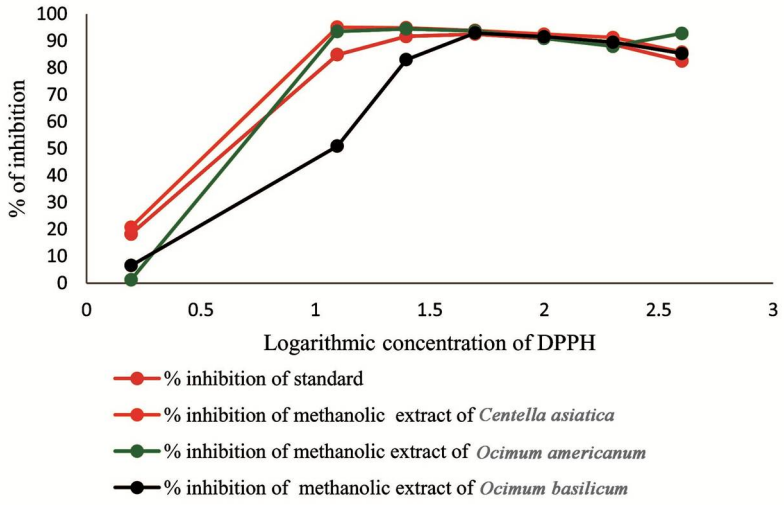

Fig. 2. DPPH radical scavenging assay of methanolic leaf extracts of *C. asiatica, O. americanum* and *O. basilicum*.

potential antioxidant activity using different solvent extracts. It was also in good agreement with the findings of Afrin et al. (2016), who reported that methanolic extract of *Caesalpinia crista* leaves acts as an antioxidant. Agarwal et al. (2017) also reported that methanolic root extract of *O. kilimandscharicum* and *O. sanctum* showed potent antioxidant activity. Sunitha and Rani (2017) reported that methanolic seed extracts of *O. americanum* showed the highest antioxidant activity. Strong antioxidant activity of methanolic extact of *O. basilicum* was also reported by Jayasinghe et al. (2003).

On the other hand, chloroformic leaf extract of all plants used in this study showed moderate antioxidant activity by comparing the IC_{50} value may be due to the presence of non-polar or less polar compounds**.** During this study**,** *O. gratissimum* and *O. americanum* showed moderate antioxidant activity of chloroformic leaf extract in comparison with other plants*.* IC_{50} value of chloroformic leaf extracts of *O. gratissimum* and *O. americanum* were 54.95 ± 0.05 and 79.44 ± 0.05, respectively. Afrin et al. (2016) reported that the leaf extract of chloroformic solvent system exhibited 50% inhibition (IC_{50}) at a concentration of 537.03 and 97.72 µg/ ml by *Cynometra ramiflora.* Hakkim et al. (2008) also reported the antioxidant activity of *O. gratissimum* and *O. americanum* leaf extract.

N-hexane leaf extracts of all the medicinal plants showed the lowest antioxidant activity**.** The IC_{50} value of leaf extracts were found to be 147.87 ± 0.06**,** 378.19 ± 2.65, 104.65 ± 0.39 and 467.58 ± 0.52 µg/ml for *O. americanum, O. gratissimum*, *C. asiatica* and *O. basilicum,* respectively. Patil et al. (2011) also reported that *n*-hexane extract of *Ocimum* sp. showed the lowest antioxidant activity**.** N-hexane extract of *C. asiatica* also showed the lowest in both antibacterial and antioxidant activity reported by Rattanakom and Yasurin (2014).

Table 2. Antibacterial activities of *O. gratissimum*, *O. americanum, O. basilicum* and *C. asiatica* leaf extracts.

Bacteria	Plant species	Antibiotic gentamycin (mm)	Methanolic leaf extract (mm)	*n*-hexane leaf extract (mm)	Cholroformic leaf extract (mm)
Escherichia coli (ATCC 8739)	*O. gratissimum*	21	25	-	10
	C. asiatica	25	-	14	16
Salmonela typhi (ATCC 13311)	*O. americanum*	18	26	-	16
Bacillus megaterium (ATCC 18)	*C. asiatica*	25	20	-	19
	O. basilicum	28	-	-	9
Pseudomonas aeruginosa (ATCC 2783318)	*O. basilicum*	19	25	9	-
	O. americanum	21	-	10	10

In case of antibacterial screening, all the leaf extracts (methanolic, chloroformic and *n*-hexane***)*** of all medicinal plants used in this study showed potent to moderate antibacterial activity against *B. megaterium*, *S. aureus*, *E. coli, P. aeruginosa* and *S. typhi* (Table 2). Methanolic and chloroformic leaf extracts showed notable zone of inhibition compared to *n*-hexane extract. Methanolic leaf extract produced zone of inhibition was in between 20 and 26 mm. *Ocimum. basilicum* showed the highest inhibition zone (25 mm) compared to antibiotic gentamycin (19 mm) against *Pseudomonas aeruginosa* using methanolic leaf extract (Fig. 3a). *Ocimum gratissimum* also showed similar inhibition zone (25 mm) against *E. coli* using methanolic leaf extract (Fig. 3b). In case of methanolic leaf extract maximum zone of inhibition was obtained 26.0 mm (Fig. 3c) against *Salmonella*

typhi by *O. americanum* for 500 µg/disc compared to standard gentamycin (18.0 mm inhibition). In this study, methanolic leaf extracts showed most potential antibacterial activity. Venugopal et al. (2015) reported that methanolic extracts of *Ocimum* sp. showed maximum inhibition zone against *S. aureus* and *B. subtilis* which supports the present

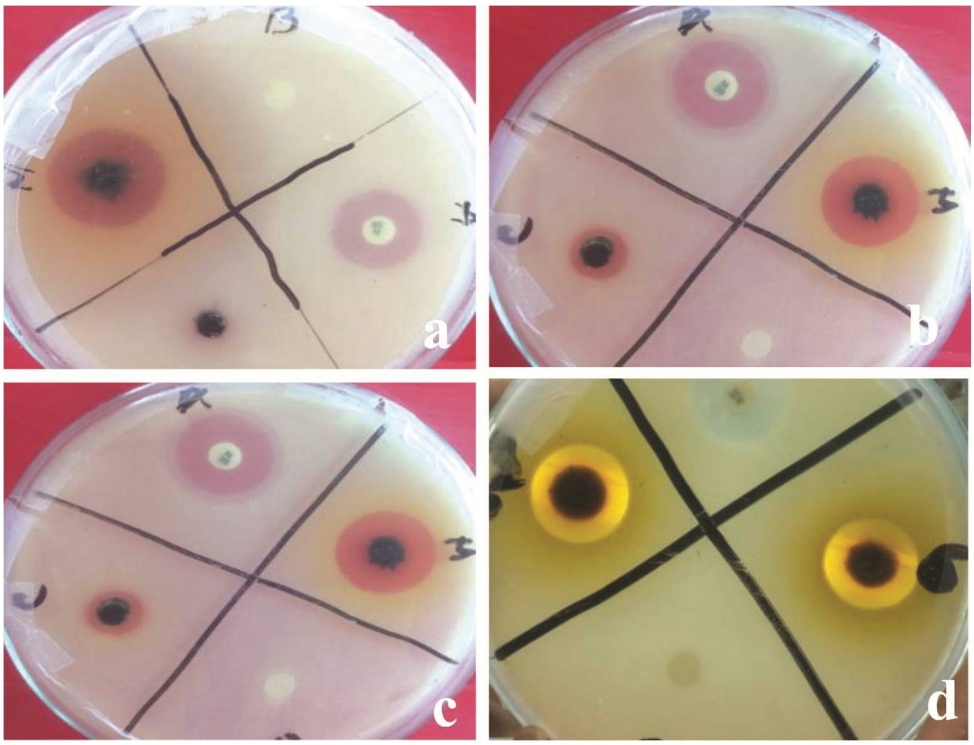

Fig. 3(a-d). Antibacterial activity of three species of *Ocimum* and *C. asiatica*. a. *O. basilicum* showing inhibition zone against *Pseudomonas aeruginosa* using methanolic leaf extract. b. *O. gratissimum* showing inhibition zone against *E. coli* using methanolic and chloroformic leaf extract c. *O. americanum* showing inhibition zone against *Salmonela typhi* using methanolic and chloroformic leaf extract. d. *C. asiatica* showing inhibition zone against *Bacillus megaterium* using methanolic and chloroformic leaf extract.
*Note: Methanolic leaf extract indicated as M**,** cholroformic leaf extract indicated as C in Petri dish.

observation. In chloroformic leaf extract inhibition zone ranged in between 9.0 - 19.0 mm (Table 2). Maximum zone of inhibition was 19.0 mm case of *C. asiatica* leaf extracts (Fig. 3d) obtained against *Bacillus megaterium* compared to standard gentamycin (25.0 mm inhibition). According to Anjana et al. (2016), chloroform extracts exhibited wide range of antibacterial activity than that of methanolic extract in *Ocimum.* On the other hand similar antioxidant activity *n*-hexane leaf extracts produced lower zone of inhibition against the tested microorganisms. *n*-hexane leaf extract of *O. basilicum, O. americanum* and *C. asiatica* produced inhibition zone was in between 9 and 14 mm.

Investigating all the results of antibacterial activity, it was found that the methanolic leaf extract of *O. americanum* showed excellent activity against both Gram-positive and

Gram-negative bacteria. Deepak et al. (2015) investigated the antimicrobial activity of six plant species which are used in Indian folklore medicine traditionally against bacterial and fungal infections.

The present study indicated that the methanolic leaf extracts of *O. americanum, O. basilicum, O. gratissimum* and *C. asiatica* have potent antioxidant activity. Therefore, it could be concluded that these medicinal plants are good sources of natural antioxidants and antibacterial activity and good source for natural drugs.

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