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Research Article

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *OSBECKIA STELLATA* BUCH.-HAM. EX D. DON (MELASTOMATACEAE) PREVALENT OF DARJEELING HILLS

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ABSTRACT

Objective: Diversity of plants has attracted a great deal of research interest in search of natural antioxidants and antimicrobials. There are numerous known and wild medicinal plants with a variety of their curative properties in Darjeeling hills of the eastern Himalayan range of India. Methods: Green mature leaves of *Osbeckia stellata* Buch.-Ham. ex D. Don., of Melastomataceae collected from Darjeeling District were tested for presence of phenols, tannins, steroids, flavonoids, saponins, triterpenoids and glycosides. Results: Petroleum ether extract had shown better antimicrobial activities in comparison with ethanol extract. Petroleum ether extract was very effective against fungus *Aspergillus niger* and bacteria like *Escherichia coli, Proteus vulgaris* and *Staphylococcus aureus.* Petroleum ether extract also showed better antioxidant activity by scavenging DPPH free radical. Conclusion: This wild plant has the prospect of being a new clinically efficient antimicrobial or antioxidant compounds.

Keywords: *Osbeckia stellata,* Melastomataceae, Antioxidant, antimIcrobial, Phenolics.

INTRODUCTION

Plants are well-known to have many crucial biological effects including antioxidant, antitumor, anti-mutagenic and antimicrobial properties. For many centuries, extracts of plants have been used in conventional medicine for the treatment of different diseases[2]. Plants even offer protection against different critical diseases like cardiovascular disease, chronic disease and certain types of cancer[3-5]. That is why diversity of plants has attracted a great deal of research interest in search of natural antioxidants and antimicrobials present even in wild and non-conventional plant resources.

Significance of the natural antioxidant based medicine for the prevention and treatment of several diseases was reported by Spigno and De Faveri[6]. Plant antioxidants can avert oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species (ROS) including reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non-radicals such as hydrogen peroxide, hypochlorous etc. Antioxidants scavenge free radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, superoxide and singlet oxygen[7,8].

In recent decades, antibiotic resistance of pathogens is an intensifying problem worldwide[9,10]. This has led to the search for new, safe and effective antimicrobial agents from non-conventional resources like plant products. Antimicrobials are essential not only for development of medicines. At the same time, there is a growing demand among consumers for natural preservative or additives in processed foods[11]. Herbal extracts are fast becoming popular as natural antimicrobial preservatives or additives[12-14]. Antibacterial activities of extracts of different plants against various microorganisms have been reported by many scientists[15-18]. Some medicinal herbs have also been assessed[19,20].

In India, rich biodiversity of wild plants is available. The wild or underutilized plants mainly of relatively unexplored regions of India have recently drawn attention of many researchers as a potential source of natural antioxidants and antimicrobials[8,21,22].

The eastern Himalayan region is one of the main hotspots in plant biodiversity. There are numerous medicinal plants and a variety of their curative properties have been attributed to folk medicine[23]. Darjeeling hills on the eastern part of Indian Himalayan range, lying between $87^0$59' to $88^0$53'E and $26^0$27'to $27^0$13'N is a significant region in this regard[21].

Osbeckia stellata Buch.-Ham. ex D. Don (Melastomataceae) is a prevalent shrub growing in this area. The plants are erect branched shrubs, 0.5-2.5 m, with 4-angled stems covered with strigose or velutinous hairs. Leaves are opposite, entire, subcoriaceous, strigose, 3-5 nerved. Flowers terminal, prominent, pink to purple, sepals and petals four each, stamens eight with beaked anthers, connate carpels with 4-lobed inferior ovary. Fruit capsule with apical pores[24]. This plant is found at altitudes of 600-2300 m. of Darjeeling District flowering in August-November.

Traditionally different species of *Osbeckia* are used for different medicinal purposes. Aqueous extracts of *Osbeckia octandra* DC. and *O. aspera* Blume, have traditionally been used for treatment of viral hepatitis by Ayurvedic practitioners in Sri Lanka[25]. These plant extracts also exhibited hepato-protective effects[26,27]. Another species of *Osbeckia chinensis* L. has long been used as an anti-inflammatory agent and antipyretic in China [28,29]. Phenolics were major antioxidants in *Osbeckia aspera* [30]. There were sparse reports of *Osbeckia nepalensis* on diabetes reporting its antihyperglycemic activity of therapeutic compounds from aqueous and ethanolic extract of *Osbeckia nepalensis* in alloxan induced diabetic rats [31].

The present study has been aimed to understand the antimicrobial and antioxidant activities of *Osbeckia stellata* Buch.-Ham. ex D. Don., of Melastomataceae.

MATERIALS AND METHODS

Plants collection and preparation

Green mature leaves of *Osbeckia stellata* Buch.-Ham. ex D. Don., of Melastomataceae were collected in November 2011, from village of Sillery-Gaon (88π33□E, 27π15□N) at 1830m altitude of Darjeeling District, West Bengal, India. The leaves were cleaned thoroughly to make those completely free from any possible contamination. The leaves were dried at 50 ºC for 3-4 days, then separately ground into fine powder using a mechanical grinder and sieved. The powder was kept in dark coloured glass bottles and subsequently used.

Preparation of solvent extraction

Two types of common solvents were used for extraction. 40 g of each dry powder was mixed either in 100 ml sterile petroleum ether or ethanol (100%) for 48 hours at 24ºC with stirring[32]. The extracts were centrifuged and filtered through Whatman No.1 filter paper and bacterial 0.45µm filter (Millipore). Then extracts were evaporated using vacuum rotary evaporator to near dryness and stored in glass vials in dark at 4ºC. Extractive values were calculated in terms of percentage considering the weight of plant material as 100%. These crude solvent extracts were diluted with either sterile double distilled water or 10% dimethyl sulphoxide (DMSO) which

are to be used as negative control respectively to obtain required concentration before experiments.

Phytochemical evaluations

The extracts were tested to phytochemical evaluation using standard techniques of plant secondary metabolites according to Harborne and Turner [33] and Evans [34]. Extracts were tested for phenolics, tannin, flavonoids, alkaloids, triterpenoids, saponin, steroids, coumarin, anthraquinone and glycosides.

Test micro-organisms

Four enteropathogenic, three food-spoiler and one probiotic bacterial strains were selected for the antimicrobial activities of the extracts. The strains used were *Salmonella enterica* serovar typhimurium MTCC 3224, *Serratia marcescens* MTCC 4822, *Staphylococcus aureus* MTCC 7405, *Escherichia coli* MTCC 3221, *Klebsiella pneumoniae* subsp. pneumoniae MTCC 6644, *Proteus vulgaris* MTCC 7299, *Bacillus cereus* MTCC 6909, *Lactobacillus brevis* MTCC 4460 and those strains were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial cultures were maintained on tryptic soyagar (HiMedia) and subcultured regularly. The fungal strain *Aspergillus niger* was taken from laboratory collection (isolated from bread) and grown on Sabouraud dextrose agar (HiMedia). Standard inoculum was prepared by sub-culturing 4-5 freshly grown isolated colonies of each strain in Tryptic soy broth (TSB) and incubated at 35-37 ºC for 24 hours. Inocula were standardized with sterile TSB to give final cell load of 10^6-10^7CFU/ml.

Disc diffusion bioassay

The disc diffusion test was carried out as described by Jorgensen et al.[35]A 0.5 ml standardized inoculum suspension of each bacterial strain was spread on TSA plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no.1 filter paper discs were aseptically placed on plates. Sample decoctions or extracts of standard concentrations (10 mg dry weight) were aseptically poured on the discs along with sterile double distilled water or 10% DMSO as negative and ampicillin as positive controls. Plates were allowed to stand for 30 minutes at room temperature prior to incubation at 35-37 ºC for 24 hours. The inhibition zone diameters were measured three times and means were represented.

Total phenolic Content (TPC)

The total phenolic contents of extracts were determined spectrophotometrically [36]. One ml of Folin-Ciocalteu's reagent (Merck, India), previously diluted (1:20), was added to one milliliter of sample (250 µg/ml) and mixed thoroughly. To the mixture, 4 ml of sodium carbonate (75 g/L) and 10 ml of distilled water were added and thoroughly mixed. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 min and the absorbance of the supernatant was taken at 760 nm. A standard curve was obtained using various concentrations of gallic acid (Sigma-Aldrich, Germany). Results were expressed as percentage of gallic acid equivalents (GAE) per 100 g of fresh mass. The total phenolic compounds (TPC) was expressed as gallic acid equivalents (GAE)/g of dry weight (mgGAE/g dw) using the following equation obtained from a standard gallic acid graph y= 3.9207x + 1.0607 (R^2 =0.9932).

Total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimetric assay[37]. 1ml of sample or standard solution of catechin (500 µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. After 5 minutes, 2 ml of 1 M NaOH was added and total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared control at 510 nm. Total flavonoid content of the sample was expressed as percentage of catechin equivalent per g dry weight (mgCE/g dw).

Ascorbic Acid Content

Ascorbic acid content in the juice was determined with DCPIP visual titration method described earlier by Ranganna[38]. The DCPIP dye, which is blue in alkaline solution, is reduced by ascorbic acid to a colorless form. About 5 mL of extract solution was titrated with DCPIP dye to a pink color end point. The ascorbic acid content was determined by the titration of the standard ascorbic acid solution with DCPIP dye.

DPPH free radical scavenging activity

The antioxidant or free radical scavenging activity of the extracts was measured on the basis of decrease in the absorbance of methanol solution of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical[39]. DPPH is one of the few stable and commercially available organic nitrogen radicals exhibiting a dark purple color at absorbance 517 nm[40]. When free radicals are scavenged, DPPH will be reduced, producing a light yellow coloration reducing the absorbance. 05 ml of DPPH (25 mg /L) solution was added to 1 ml of sample solution (at different concentrations). Mixture was shaken vigorously and kept at room temperature for 30 min in dark. Then the absorbance was measured at 517 nm and compared with standards. Scavenging activity was calculated as the percentage inhibition (I%) using the following formula:

$$\% \text{ DPPH Anti-radical activity (I\%)} = \frac{\text{Control Absorbance - Sample Absorbance x 100}}{\text{Control Absorbance}}$$

Radical-scavenging potential was expressed as IC_{50} value (calculated from linear regression of the graph of concentration vs. I%) representing the concentration, which scavenged 50% of the DPPH radicals.

Statistical analyses

For all tests three replicates were done and the mean values and standard deviations were determined.

RESULTS AND DISCUSSION

Table 1: Phytochemical qualitative evaluation of *Osbeckia stellata*

Phytochemicals	Pet. Ether extract	Ethanol extract
Phenolics	++	++
Tannin	++	++
Flavonoids	++	++
Alkaloids	- -	- -
Triterpenoids	++	++
Saponin	++	++
Steroids	++	++
Coumarin	- -	- -
Anthraquinone	- -	- -
Glycosides	++	++

The leaves extract was tested to phytochemical evaluation. It was found that alkaloid, anthraquinone and coumarin are completely absent. Phenols, tannins, steroids, flavonoids, saponins, triterpenoids and glycosides are present. Six different types of anti-oxidative tannins were detected in methanol extract of *Osbeckia chinensis*[29].

Table 2: Antibacterial activities, indicated by diameter of inhibition zone (DIZ, mm, for 10 mg dry wt./ disc, Mean±SD) of Extracts of *Osbeckia stellata* against the micro-organisms [- means <7mm DIZ i.e DIZ of negative control]

Extracts	SolventTypes	*E.coli*	*S.aureus*	*S. enterica*	*S. marce-scens*	*K. pneu-moniae*	*P.vulgaris*	*B. cereus*	*L.brevis*	*A.niger*
	Pet Ether	15±1.527	13±1.527	12±1	8±0.577	9±0.577	15±1.527	12±1	10±1	13±1.527
	Ethyl Alcohol	9±0.577	10±1.527	9±1	-	7±1	10±1	10±1.527	8±0.577	9±1.527

The disc diffusion assay revealed that the extracts had different degrees of bacterial and fungal growth inhibition, depending on the microbial strains (Table 2). Petroleum ether extract had shown better antimicrobial activities in comparison with ethanol extract. Petroleum ether extract was very effective against fungus *Aspergillus niger* and bacteria like *Escherichia coli, Proteus vulgaris* and *Staphylococcus aureus.* But ethanol extract showed moderate antimicrobial activities against *Staphylococcus aureus*, *Proteus vulgaris and Bacillus cereus*. *Serratia marcescens* strain is the least sensitive among all strains tested against both extracts. *Melastoma candidum* D. Don of the same family was reported to have potential antibacterial and antifungal activity against medically important microbial strains[41].

Table 3: Phytochemicals estimation and antioxidant activities of *Osbeckia stellata*

Phytochemicals	Pet. Ether extract	Ethanol extract
Phenolics (mgGAE/g dw)	39±6	33±5
Flavonoids (mgCE/g dw)	26.32±1.5	28.54±1.3
Ascorbate (mg/g dw)	0.45±0.04	0.36±0.04
DPPH IC_{50} (µg/ml)	26	31

Phytochemicals estimation and antioxidant activities of leaves extracts were shown in Table 3. Total Phenolic contents were expressed as mg gallic acid equivalent as this compound represents the most simple form of a phenolic compound. While ethanol extract had lesser amount of phenolics or ascorbates, Petroleum ether extract contained higher amounts of phenolics and ascorbates. Ethanol extract contained slightly higher amounts of flavonoids. Of the three phenolic acids isolated by Grayer et al. from another species of *Osbeckia aspera*, gallic and protocatechuic acids were more active at protecting the liver cells from the two toxic compounds than ellagic acid. The same study also identified several quercetin and kaempherol glycosides[30]. These active constituents of the extract showed hepatoprotective and immunosuppressive effects which are favorable in acute hepatitis[42].

DPPH scavenging assay is applied extensively for the determination of free radical scavenging or antioxidant activity of any compound. DPPH assay measures the capability of the extract to donate hydrogen to the radical. In DPPH assay the lower the IC_{50} the better it is able to scavenge the radicals, particularly peroxy-radicals which are the propagators of the autoxidation of lipid molecules and thereby break the free radical chain reaction[43]. Petroleum ether extract showed better antioxidant activity. Plants of the *Osbeckia* family have been shown to possess hepatoprotective properties, which could be due to the presence of antioxidant compounds. A crude aqueous extract of *O. aspera* was shown to have significant activity against the Xanthine Oxidase generated DPPH free radical in a dose-dependent mode and demonstrated a scavenging effect on hydroxyl radical mediated damage to deoxyribose[27]. Plants of the *Osbeckia* family have been shown to possess hepatoprotective properties, which could be due to the presence of antioxidant compounds. The *Osbeckia aspera* extract was shown to inhibit the activities of the DPPH free radical and xanthine oxidase and demonstrate a scavenging effect on hydroxyl radical mediated damage to deoxyribose[27].

Osbeckia stellata leaves have shown potentially superior antimicrobial and antioxidant efficacy. The petroleum ether extract has better antimicrobial efficacy against most of microbes examined. Petroleum ether extract also showed strongest antioxidant activity. Differential antimicrobial or antioxidant activity of extracts against different bacteria might be due to presence of different active phyto-chemicals. Among those antimicrobial compounds, phenolic compounds, terpenoids, and steroids are very important compounds in antimicrobial or antioxidant effects. Further study is required to determine the different active compounds from this plant and their full spectrum of efficacy. This wild plant has the prospect of being a new clinically efficient antimicrobial or antioxidant compounds and the knowledge can be extended for future investigation into the fieldof pharmacology for better drug discovery.

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