

Antioxidant Property, Cytotoxicity and Antimicrobial Activity of *Lasia spinosa* Leaves

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Abstract

This study was undertaken to evaluate antioxidant property, cytotoxicity and antimicrobial activity of *Lasia spinosa* leaves. Ethyl acetate soluble partitionate of the methanolic extract of the plant material showed significant antioxidant property using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay with IC₅₀ value of 16.42 µg/ml. In the brine shrimp lethality bioassay, LC₅₀ values obtained from the best fit line slope were 0.544, 3.181, and 4.096 µg/mL for standard (vincristine sulphate), dichloromethane and ethylacetate soluble partitionates of the methanolic extract respectively. The leaves extracts showed mild antimicrobial property.

Key words: antimicrobial, antioxidant, cytotoxic, *Lasia spinosa*

Introduction

Lasia spinosa (Bengali name: Kankachhu, Family: Araceae) is a stout, intensely prickly marsh plant with creeping rootstock, found in low-lying shady areas, near water bodies. It is a spinous perennial herb that is mostly distributed in Southeast Asia. The plant usually grows surrounding ponds, and its shoots are consumed as traditional food in many communities of Southeast Asia. It is an ethnobotanically important medicinal plant. Different parts of the plant are used for various ailments, e.g., colic, rheumatism and intestinal diseases. In Khagrachari, an area of Bangladesh, the corm is used as a remedy for throat affections, whereas leaves and corms together are used for cure of piles (Yusuf *et al.* 2009). The stem is used as antitussive, expectorant, is boiled and used in baths for relieving itching from roseolar infantum, measles, rubella and other skin disorders. The tubers are used for the treatment of rheumatoid arthritis, constipation and to purify blood in Rajshahi and Natore district of Bangladesh (Rahmatullah *et al.* 2010). In Naga tribes of India, the leaves are used for intestinal disorders,

and its anticestodal effect was determined by Yadav and Temjenmongla (Yadav & Temjenmongla 2006). The rhizomes of the plant possess antioxidant, antimicrobial and cytotoxic activities (Hasan *et al.* 2011, Shefana & Ekanayake 2009). Deb *et al.* demonstrated antinociceptive, anti-inflammatory and antidiarrhoeal activities of the root extract (Deb *et al.* 2010).

Methodology

Collection of the plant

Lasia spinosa leaves were collected from Sundarban, Khulna, Bangladesh during May 2011. The plant was identified from the Department of Botany, Dhaka University, Dhaka and a specimen has been deposited in the Dhaka University Herbarium, Dhaka (Accession No. DUSH 1940).

Extraction and isolation

The leaves of the plant were collected in fresh condition. The dried and coarse powder (650 g) was extracted with methanol (2.5 l) in an air tight flat-bottomed container for 15 days at room temperature

with occasional stirring. The extract was then filtered through a cotton plug followed by a Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at low temperature and pressure to afford crude methanolic extract (23 g). The crude extract (5 g) was dissolved in 10% aqueous methanol which was subsequently extracted first with petroleum ether (PE), then dichloromethane (DCM) and finally with ethyl acetate (EA). The aqueous methanolic extract was preserved as aqueous fraction (AQ) (Kupchan & Tsou 1973, Van Wagenen *et al.* 1993). All the fractions were evaporated to dryness by using a rotary evaporator and kept in airtight containers for further analysis.

Test organisms

Five Gram positive, 8 Gram negative bacteria and 3 fungal species were collected as pure cultures from the Institution of Nutrition and Food Science (INFS), University of Dhaka. The microorganisms were maintained on nutrient agar medium (Merck, Germany). *Artemia salina* (brine shrimp) eggs were collected from an aquarium shop (imported from Thailand) and hatched to get mature nauplii for brine shrimp lethality bioassay.

Screening of antioxidant activity

The free radical scavenging activity of the extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined following standard procedure (Feresin *et al.* 2002). Two milligrams of each of the partitionated test sample was dissolved in methanol and solution of varying concentrations such as 500, 250, 125, 62.50, 31.25, 15.62, 7.81, 3.90, 1.95 and 0.97 µg/ml were prepared by serial dilution technique. Then 2 mL of each of the test sample was mixed with 3 mL of a DPPH-methanol solution (20 µg/ml) and the mixture was allowed to stand for 20 minutes for reaction completion. The absorbance was determined at a wave length of 517 nm and the corresponding percentage of inhibition was calculated by using the following equation:

$$\% \text{ Inhibition} = \frac{1 - (\text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{control}}) \times 100}{1}$$

Then % inhibitions were plotted against respective concentrations used and IC_{50} was calculated from the graph using butylated hydroxy toluene (BHT), a potential antioxidant, as positive control. The experiment was repeated thrice and the mean IC_{50} value was taken to compare with BHT.

Screening of cytotoxic activity

In brine shrimp lethality bioassay, dimethyl sulfoxide (DMSO) solutions of the four partitionates (PE, DCM, EA and AQ) were applied to *A. salina* for 24 hours (McLaughlin & Rogers 1998, Meyer *et al.* 1982). For the experiment, 4 mg of each of the extracts were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.562, 0.781 µg/ml) were obtained by serial dilution technique. The solutions were then added to the pre-marked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the % lethality of brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration.

Screening of antimicrobial activity

The antibacterial activity was carried out by the disc diffusion method using nutrient agar medium (Bauer *et al.* 1966). The sterile Matricel (BBL, cocksville USA) 6.0 mm filter paper discs, impregnated with 400 µg of petroleum ether, dichloromethane, ethyl acetate and aqueous extract, were placed gently on the previously marked zones in the agar plates. Standard Ciprofloxacin discs (50 µg/disc) were used as positive control to ensure the activity against test organisms. The zones of inhibition produced by the extracts were measured thrice and its mean value was taken to compare with the standard.

Statistical analyses

Regression analysis was carried out for analyzing the data obtained from brine shrimp lethality bioassay to study the relationship between different samples and vincristine sulphate.

Results and Discussion

The study was performed to determine the antioxidant, cytotoxic and antimicrobial activity of *L. spinosa* leaves. The antioxidant property of the plant was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging test following previously described procedure (Pietta *et al.* 1998, Velioglu *et al.* 1998). The cytotoxic activity of plant materials was performed by using brine shrimp lethality bioassay (Meyer *et al.* 1982). The

antimicrobial activity was performed against different gram-positive, gram-negative bacteria and fungi using disc diffusion technique (Wilkinson 2007).

The partitioning of crude methanolic extract yielded 750 mg, 590 mg and 305 mg partitionate of PE, DCM and EA respectively.

The antioxidant activities of various extracts of leaves of *L. spinosa* are shown in Table 1. The ethyl acetate fraction (EA) showed the highest free radical scavenging activity with IC₅₀ value 16.42 µg/ml in comparison to positive control (BHT). At the same time the aqueous fraction (AQ) also exhibited moderate antioxidant potential having IC₅₀ value of 73.20 µg/ml. Table 2 shows the result of the brine shrimp lethality testing of various extractives of *L. spinosa* after 24 hours of exposure to the samples and the positive control, vincristine sulphate (VS). The LC₅₀ values were found to be 16.69, 3.18, 4.09, 98.66 and 0.544 µg/

mL for petroleum ether, dichloromethane, ethyl acetate, aqueous and vincristine sulphate respectively. In comparison to positive control (Vincristine sulphate), the cytotoxicity exhibited by dichloro methane (DCM) soluble partitionate of methanolic extract was promising. On the other hand, ethyl acetate (EA) partitionate demonstrated comparable cytotoxicity in comparison with dichloro methane (DCM).

Table 1. The result of antioxidant activity of petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EA) and aqueous (AQ) soluble partitionate of methanolic extract and positive control butylated hydroxy toluene (BHT)

Sample	IC ₅₀ (µg/mL)
BHT	23.19
PE	174.78
DCM	248.82
EA	16.42
AQ	73.20

Table 2. The result of cytotoxicity of petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EA) and aqueous (AQ) soluble partitionate of methanolic extract and positive control vincristine sulphate (VS) on brine shrimp nauplii

Sample	LC ₅₀ (µg/mL)	Regression equation	R ²
VS	0.544	y = 33.22x + 58.78	0.958
PE	16.696	y = 40.06x + 1.022	0.931
DCM	3.181	y = 32.21x + 33.81	0.858
EA	4.096	y = 34.62x + 28.80	0.848
AQ	98.663	y = 30.80x - 11.42	0.708

Table 3. Antimicrobial activity of petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EA) and aqueous (AQ) fraction of *L. spinosa* leaves and positive control Ciprofloxacin (CF)

Test bacteria and fungi	Diameter of zone of inhibition (mm)				
	PE	DCM	EA	AQ	CF
Gram positive					
<i>Bacillus cereus</i>	8	7	8	-	45
<i>Bacillus megaterium</i>	-	-	-	-	48
<i>Bacillus subtilis</i>	9	9	9	9	43
<i>Staphylococcus aureus</i>	7	7	8	-	46
<i>Sarcina lutea</i>	7	7	7	-	45
Gram negative					
<i>Escherichia coli</i>	7	-	8	-	45
<i>Pseudomonas aeruginosa</i>	7	7	-	-	46
<i>Salmonella paratyphi</i>	8	8	9	-	45
<i>Salmonella typhi</i>	7	7	-	-	46
<i>Shigella boydii</i>	7	7	-	-	45
<i>Shigella dysenteriae</i>	10	-	-	-	45
<i>Vibrio mimicus</i>	7	7	-	-	46
<i>Vibrio parahemolyticus</i>	-	-	-	-	45
Fungi					
<i>Candida albicans</i>	10	10	10	-	45
<i>Aspergillus niger</i>	10	10	8	-	45
<i>Sacharomyces cerevacaee</i>	7	7	-	-	44

The antimicrobial effects of *L. spinosa* leaves against various test organisms are shown in Table 3. The aqueous extract exhibited no activity against the tested microorganisms at a concentration of 400 µg/disc. The petroleum ether extract showed mild inhibitory activity against various Gram-negative bacteria such as *Shigella dysenteriae* (10 mm). It showed mild antifungal activity against *Candida albicans* (10 mm) and *Aspergillus niger* (10 mm). The dichloromethane extract revealed mild antifungal activity against *Candida albicans* (10 mm) and *Aspergillus niger* (10 mm) and the ethyl acetate extract showed mild antifungal activity against *Candida albicans* (10 mm).

The extracts of leaves of *L. spinosa* showed significant antioxidant and cytotoxic properties with non significant antimicrobial properties. Further investigation is required for isolating the specific bioactive constituents responsible for antioxidant and cytotoxic activities.

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