

Bioactivities of *Sonneratia Caseolaris* (Linn) Leaf and Stem Using Different Solvent Systems

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ABSTRACT

This study was aimed to report the antioxidant, cytotoxic and antibacterial potentials of different fractions of crude ethanol extract of leaf and stem of *Sonneratia caseolaris* Linn. The liquid-liquid fractionation was conducted among Ethyl Acetate, chloroform and carbon tetrachloride and was designated as EAFS, CFS, CTFS for stem and EAFL, CFL, CTFL for leaf respectively. Antioxidant activity of individual fraction was then evaluated by DPPH free radical scavenging assay; whereas cytotoxic activity was investigated by brine shrimp lethality assay and antibacterial activity by disk diffusion method. In antioxidant assay, the EAFL was found to be more potent (IC_{50} $12.0 \pm 0.12 \mu\text{g/ml}$) whereas in cytotoxicity test both the EAFS and CTFL demonstrated lowest LC_{50} (25.0 ± 0.05 and $25.0 \pm 0.07 \mu\text{g/ml}$, respectively). Among all the fractions, EAFS and CTFS were found to have highest inhibitory effect against salmonella bacterial strains (zone of inhibition 8-11.5 mm). Promising results from this study support the ethnomedical uses of the plant as antioxidant, cytotoxic and antibacterial agents and further exploration for isolating active drug lead.

Abbreviations: DPPH; 2,2-diphenyl-1-picrylhydrazyl; IC_{50} : 50% Inhibitory Concentration; DMSO: Dimethylsulfoxide

Introduction

Sundarbans is the largest single block of tidal halophytic mangrove forest in the world and located in southern part of Bangladesh and India near the Bay of Bengal. From the ancient time different plants of this forest have been widely used in traditional medicines. The Mangrove plants have to undergo extensive physiological and morphological adaptations due to high salinity, tidal effects and ecological variations. As a result plants here are rich of different secondary metabolites possessing varied biological activities [1]. Many of these secondary metabolites can be used as it is or modified structurally in managing human ailments. Thus, the diverse chemical compounds resulting from the mangrove plants may be novel alternative for discovering new drug molecules [2]. *Sonneratia caseolaris* L (Family: Sonneratiaceae) is locally known

as ora, choila, etc. It is a small evergreen tree distributed in the tidal creek and mangrove swamps of Bangladesh, India, Ceylon, Malay. This small evergreen tree rises up-to 8 m; occasionally reaching 20 m. Branches are horizontal, twig slender with round and opposite leaves of 7 cm long. It has underground roots as well as breathing roots (pneumatophores) at its base up to 1.5 m-tall upright.

The red flowers with 6 valvular lobes containing green sepal tube have offensive smell and are opened for only one night. The flower has 6 red petals overshadowed by long showy, numerous white to reddish stamens. Its green, star-shaped leathery berry type fruit is quite large (about 4 cm across) and are eaten raw or cooked on riping. Traditionally, Bangladeshi people use different parts of this plant as antiseptic, vermifuge against intestinal worms and

also in sprains and swellings, hemorrhage, and in the treatment of smallpox, cough, kidney failure, leucorrhoea, urinary tract infection and piles [3,4]. So far from this plant, some bioactive constituents including fatty acids, hydrocarbons, steroids, pectin, sugars, flavone, luteolin and its 7-O- β -glucoside (cynaroside) have been isolated [5-7]. Previous researchers have reported antinociceptive and antidiarrhoeal activities of this plant [8,9]. However, no cytotoxic and antibacterial activity has yet been reported. As novelty, present study was designed to investigate the antioxidant, cytotoxic and antibacterial activity of different fractions *S. caseolaris*.

Methods

Chemicals and Reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) whereas ethanol, carbon tetrachloride, chloroform and ethylacetate were obtained from Merck (Darmstadt, Germany). Standard drug vincristine sulfate were purchased from Beacon Pharmaceuticals Ltd. Bangladesh.

Collection of Plant Material

Leaf and stem of the plant were collected from Mangrove Forest Sunderbans (Mongla, Khulna, Bangladesh) during December, 2011 and was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No.: DACB - 36540) and a voucher specimen was also deposited there.

Preparation of Crude Extract

The collected stem and leaves were separated from undesirable materials and shade-dried followed by grinding into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder samples (600g of stem and 500g of leaf) were then kept wetting in 2.0 litres and 1.5 litres ethanol (98%) respectively, for a period of 14 days accompanying periodical shaking or stirring. After filtration using cotton and Whatman filter paper the filtrate was concentrated by evaporation of solvent in a rotary evaporator and the weight of the crude extract obtained from stem was 8.7g and 7.5g for leaf.

Solvent-Solvent Partition of Crude Extract

The crude extracts (Leaf and stem) was then fractionated in three solvents (carbon tetrachloride, chloroform and ethyl acetate) of varying polarities using separating funnel, and the obtained six samples were designated as EAFS, CFS, CTFS for stem and EAFL, CFL, CTFL for leaf respectively. The amount was found to be 0.35g, 0.43g and 1.44g for EAFS, CFS, CTFS respectively and 0.40g, 1.88g and 0.30g for EAFL, CFL, CTFL respectively.

Microorganisms

For antibacterial assay five strains (*Shigella dysenteriae*, *Escherichia coli*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus aureus*) were collected from International Centre

for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) and used.

Antioxidant Activity

Free radical DPPH scavenging capability was used as indicator of anti-oxidant potential [10]. Different concentrations (400-1.57 $\mu\text{g/ml}$) of fractionated extracts were prepared and in 2mL of each of the different concentration, 6mL of freshly prepared DPPH solution (0.004%) was added. The reaction was allowed to complete by keeping the tubes in dark place for 30 minutes followed by measuring absorbance in a UV spectrophotometer at 517 nm against suitable blank. Here ascorbic acid was used as standard. IC_{50} value (the concentration of sample required to scavenge 50% of the DPPH free radicals) was determined by using the following formula:

$$\% \text{ inhibition} = \left\{ \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \right\} \times 100$$

Cytotoxic Activity

Following previously reported Meyer method [11] *Artemia salina* leach (brine shrimp eggs) were hatched in simulated sea water with constant oxygen supply to get matured nauplii. Different concentrations of extracts (400-0.781 $\mu\text{g/ml}$) were prepared in simulated seawater using dimethylsulfoxide (DMSO) as co-solvent where the concentration of DMSO did not exceed 10 $\mu\text{l/ml}$. The test solution was then added in 5 ml simulated seawater containing ten live nauplii in a glass vial. The vials were inspected after 24h using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percentage of lethality of the nauplii for each concentration and control was calculated. The LC_{50} of the test samples were also computed from that data. In this assay vincristin sulfate was used as standard.

Antibacterial Activity

Disc diffusion method was followed here [12,13]. The required amount of the test samples was dissolved in definite volumes of solvent to prepare solutions of desired concentration ($\mu\text{g/ml}$). The sterile Matricel (BBL, Cocksville, USA) filter paper discs were impregnated with known amount of test substances (Extract, control, standard) using micropipette and air dried. The discs were then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop. The plates were then heated at 40 $^{\circ}\text{C}$ for ensuring maximum diffusion and then incubated for 12-18 hour. The diameter of the zone of inhibition was measured in term of millimeter. Ciprofloxacin (5 $\mu\text{g/disc}$) was used as standard drug.

Statistical Analysis

All values are expressed as mean \pm SD of three parallel measurements. The results of cytotoxic assay were statistically analysed by Finney 27 using the log-probit software program Ldp Line $^{\circledR}$ model "Ehabsoft".

Results

Antioxidant Activity

In this investigation, EAFL showed excellent free radical scavenging activity (IC₅₀=12.0 µg/ml). Between the other two fractions of leaf, the free radical scavenging activity in chloroform extract (IC₅₀=19.0 µg/ml) was superior to that of the carbon tetrachloride extract (IC₅₀= 49.0 µg/ml). One the other hand, among the fractions of the stem extract, chloroform extract (IC₅₀=69.0 µg/

ml) showed better radical scavenging activity than the rest of the two (Table 1).

Cytotoxic Activity

The standard drug (vincristine sulphate, LC₅₀: 0.156 µg/ml) showed significant mortality compared to control to the tested shrimp nauplii. Among the fractions, the EAFL and CTFL were found to be more lethal (LC₅₀:25.0 µg/ml) (Table 2).

Table 1: IC₅₀ values of standard and test samples in antioxidant assay.

Test Sample	Sample Code	IC ₅₀ (µg/ml)
Ascorbic acid	Standard	8.0±0.12
Ethyl Acetate soluble fraction of <i>S. caseolaris</i> stem	EAFS	138.0±0.8
CHCl ₃ soluble fraction of <i>S. caseolaris</i> stem	CFS	69.0±0.21
CCl ₄ soluble fraction of <i>S. caseolaris</i> stem	CTFS	201.0±0.15
Ethyl Acetate soluble fraction of <i>S. caseolaris</i> leaf	EAFL	12.0±0.12
CHCl ₃ soluble fraction of <i>S. caseolaris</i> leaf	CFL	19.0±0.07
CCl ₄ soluble fraction of <i>S. caseolaris</i> leaf	CTFL	49.0±0.05

Table 2: LC₅₀ value of the test samples of *S.caseolaris* in cytotoxic assay.

Test Sample	Sample Code	LC ₅₀ (µg/ml)
Vincristine Sulphate	Standard	0.156±0.09
Ethyl Acetate soluble fraction of <i>S. caseolaris</i> stem	EACS	25.0±0.07
CHCl ₃ soluble fraction of <i>S. caseolaris</i> stem	CFCS	50.0±0.13
CCl ₄ soluble fraction of <i>S. caseolaris</i> stem	CTCS	100.0±0.16
Ethyl Acetate soluble fraction of <i>S. caseolaris</i> leaf	EACL	50.0±0.11
CHCl ₃ soluble fraction of <i>S. caseolaris</i> leaf	CFCL	50.0±0.18
CCl ₄ soluble fraction of <i>S. caseolaris</i> leaf	CTCL	25.0±0.05

Antibacterial Activity

The EAFS and CTFS showed good antibacterial activity against *Salmonella* strains (zone of inhibition 8-11 mm) at the dose of 500

µg/disc compare to standard drug ciprofloxacin (zone of inhibition 12-15 mm at the dose of 5 µg/disc). Other fractions showed varying degrees of antibacterial activity on different tested strains. Only the CTFL failed to show any inhibition against the tested organisms (Table 3).

Table 3: Average zone of inhibition of various fractions of *S. caseolaris* leaf and stem against different bacterial strains.

Average Diameter of zone of inhibition (mm)													Bacterial Strains
Ciprofloxacin (5*)	CTFL (500)	CTFL (250)	CFL (500)	CFL (250)	EAFL (500)	EAFL (250)	CTFS (500)	CTFS (250)	CFS (500)	CFS (250)	EAFS (500)	EAFS (250)	
11.5±0.07	0	0	7.5±0.13	0	7.5±0.08	5.5±0.11	5.5±0.07	0	7.5±0.09	5.5±0.06	0	0	<i>Escherichia coli</i>
13.5±0.05	0	0	5.5±0.20	0	8.5±0.06	5.5±0.07	7±0.09	5.5±0.09	7.5±0.16	0	6.5±0.07	5.5±0.07	<i>Shigella dysenteriae</i>
12±0.09	0	0	7.5±0.13	0	8.5±0.12	5.5±0.05	8±0.11	7±0.13	5.5±0.06	0	8.5±0.11	7±0.11	<i>Salmonella typhi</i>
15±0.13	0	0	5.5±0.07	0	6.5±0.17	0	9±0.09	8.5±0.16	6±0.07	0	11.5±0.1	7.5±0.1	<i>Salmonella paratyphi</i>
11±0.15	0	0	8±0.09	6±0.07	7.5±0.11	0	8.5±0.13	6±0.07	7.5±0.09	5.5±0.09	6±0.11	0	<i>Staphylococcus aureus</i>

Note: *µg/mL.

Discussion

In Ethnopharmacology, traditionally used medicinal plants are scientifically screened for diverse biological activities. As different plant parts can be used to cure different life threatening diseases, they are confirmed here to be an important source of novel pharmacophore [14]. Antioxidants prevent the degradation of any chemicals including foodstuffs or drugs and that's why they are widely used in those formulations since ages to prolong the shelf life of food or drugs. However, some issues have limited their uses e.g. some synthetic antioxidants butyl hydroxyl anisole and butyl hydroxyl toluene (BHA and BHT, respectively) need to be replaced with natural antioxidants because of their potential health risks and toxicity. Thus, the search for antioxidants from natural resources has received much attention now a days [15]. Phenolic natural products such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals and inhibiting peroxidation. Antioxidants that scavenge free radicals play an important role in prevention of cardiovascular disease, aging, cancer, and inflammatory disorders [16]. In addition, essential nutraceuticals can be formulated using these naturally occurring antioxidants which can help to prevent oxidative damage in the body. It has been reported that in plant diverse compounds with different polarity and structure are present and their solubility may vary in solvents having different polarity. In our experiment, among all the fractions, EAFL showed the highest free radical scavenging activity ($IC_{50}=12.0 \mu\text{g/ml}$). The difference in the DPPH radical scavenging activity in different fractions implies towards the preference of the solvents for extraction of targeted bioactive compounds.

Brine shrimps lethality bioassay has been considered as pre-screening assay for antitumor, antimalarial and insecticidal activities due to its simplicity and low cost. Therefore it is proposed to be a convenient probe to evaluate bioactivities of plant extracts [12,17]. Various fractions of crude ethanol extract of *S. caseolaris* leaf and stem exerts notable cytotoxic activity in different dose level. The difference in mortality (Table 2) may be due to the diversity in the amount and type of cytotoxic compounds present in the extracts and it hypothesize that the cytotoxicity revealed by these fractions might have mild antitumor activity which may be useful as chemotherapeutic agents to kill the cancerous cells. Natural products are capable to manage bacterial infections efficiently and now a days they are considered as better alternative especially when antimicrobial resistance is a concerned issue. The test microorganisms used in this study cause different forms of human infections. From a medical perspective, *E. coli* results into septicemia and infection of lungs, skin lesions, gall bladder and meninges, and also a number of food related diseases. *S. aureus* are also responsible for food poisoning, boil, ulcers, toxic shock and pneumonia etc. [13]. Disc diffusion method applied here is widely acceptable for the preliminary screening of antibacterial

activity indicating the sensitivity or resistance of microorganisms to the test materials. Plant derived secondary metabolites such as alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins are reported to exhibit antimicrobial potentials [18,19]. Plant containing Quercetagenin-7-arabinosyl-galactoside, a flavonoid has been used extensively to treat infectious disease [20]. The flavone baicalein is reported to be largely responsible for antimicrobial effects [21]. Flavonoid rich plant extracts from species of *Hypericum* [22] and *Chromolaena* [23] have been reported to possess antibacterial activity. Many other phytochemical preparations with high flavonoids or saponin content have also been reported to exhibit antibacterial activity [24-26].

Conclusion

Present study reports various fractions of *S. caseolaris* leaf and stem having different level of antioxidant, cytotoxic and antibacterial activities which show a relationship with the ethnomedicinal uses of this plant. Thus, further studies on this plant may to identify the active principles responsible for these activities.

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Competing interests

The authors declare that they have no conflict of interest.

Availability of Data and Materials

The datasets supporting the conclusions of this article are included within the article.

Ethics Approval

Not applicable

Consent for publication

Not applicable

Authors' Contributions

This work has been carried out in collaboration among authors. First and second authors have equally contributed to perform the experiments and write manuscript. SKS supervises the whole works and other co-authors supported corresponding author equally. All authors read and approved the final manuscript.

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