Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Pelagia Research Library

Der Pharmacia Sinica, 2012, 3 (5):536-541

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Evaluation of antinociceptive and antioxidant properties of the ethanolic extract of *Sonneratia caseolaris* leaves

Md. Sariful I. Howlader[1*], Shubhra K. Dey [2], Arpona Hira[2], Arif Ahmed[2]

[1]Department of Pharmacy, World University of Bangladesh, Dhaka-1205, Bangladesh
[2]Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh

---

ABSTRACT

The ethanolic extract of leaves of Sonneratia caseolaris exhibited statistically significant (p>0.01 & p>0.001) writhing inhibition in acetic acid induced writhing model in white albino mice (Swiss-webstar strain). The crude extract produced 24.37% inhibition of writhing at the dose of 250 mg/kg body weight & 43.15 % inhibition of writhing at the dose of 500 mg/kg body weight while the standard drug diclofenac inhibition was found to 65.48 % at a dose of 25 mg/kg body weight. The antioxidant property of ethanolic extract of S. caseolaris was assessed by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity. In DPPH scavenging assay the IC_{50} value was found to be (68 µg/ml) which was comparable to the standard ascorbic acid (13 µg/ml). Phytochemical nature (group determination of plant constituent) and selected phytochemical analysis of the ethanolic extract of the leaves of S. caseolaris indicated the presence of flavonoid, reducing sugars, tannins & saponins types of compounds. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation. The present study tends to suggest the antinociceptive and antioxidant activities of the crude ethanolic extract of the leaves of S. caseolaris and justify its use in folkloric remedies.

Key words: *Sonneratia caseolaris*, Antinociceptive, Antioxidant.

INTRODUCTION

Sonneratia caseolaris is a mangrove [1] species belonging to family Sonneratiaceae [2].It is collected from Karamjal, Sundarban, Bangladesh. It is a species of the lowland rainforest. *S. caseolaris*, known as Mangrove apple or Crabapple mangrove. This species is widespread and can be found in Bangladesh, Brunei Darussalam, Cambodia, China (Hainan Island), India, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Viet Nam, Northeast Australia, Papua New Guinea, Solomon Islands, Vanuatu, New Caledonia, and the Maldives.

Twenty-four compounds including eight steroids, nine triterpenoids, three flavonoids, and four benzene carboxylic derivatives have been isolated and identified from stems and twigs of medicinal mangrove plant Sonneratia caseolaris [3] (SC).The plant contains Phenolic compound, such as gallic acid, and flavonoids, e.g. luteolin and luteolin-7-O-glucoside [4]. Some of the constituents responsible for moderate cytotoxicity are (-)-(R)-nyasol , (-)-(R)-4'-O-methylnyasol and maslinic acid from SC [5]. The fruits of *S. caseolaris* have antidiabetic activity [6].

Pain is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [7]. Analgesic activities are commonly exhibited by the non-steroidal anti-inflammatory drugs (NSAIDS). These NSAIDs exert anti-inflammatory effect principally by inhibiting the synthesis of prostaglandin [8].

Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases in human. Reactive oxygen species (ROS) have a tendency to donate oxygen to other substances. Many such reactive species are free radicals and have a surplus of one or more free-floating electrons rather than having matched pairs and are, therefore, unstable and highly reactive includes the hydroxyl radical (OH.), the superoxide radical (O_2), the nitric oxide radical (NO.) and the lipid peroxyl radical (LOO.) cause severely deleterious effects on the human body [9]. Enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, cytochrome P450 system and oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria [10]. ROS are the products of normal cellular metabolism, having both deleterious and beneficial effect in the body. The balance between the production of free radicals and the antioxidant defenses in the body has important health implications. If there are too many free radicals produced and too few antioxidants, a condition of "oxidative stress" develops which may cause chronic damage body [11]. Antioxidants play an excellent role in preventing cell damage. They donate their own electrons to free radicals. Free radical accepts the electron from antioxidant and they do not attack the cell and the chain reaction of oxidation is inhibited [12]. Phenolic compounds, flavonoid and triterpenoids containing foods and beverages with antioxidant activity have been reported [13]. Very recent, health risks and toxicity have been reported using synthetic antioxidants restricted [14]. Some well known natural antioxidants like rosemary and sage are already exploited commercially either as antioxidant additives or as nutritional supplements stipulating the antioxidant potential of plant species [15]. In recent years, the interest in natural antioxidant, especially of plant origin, has greatly increased [16].

Since no literature is currently available to substantiate antinociceptive & antioxidant activities from ethanolic extract of leaves of *S. caseolaris*, therefore the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive and antioxidant activities that also confirm its use as pain killer and as an antioxidant.

MATERIALS AND METHODS

Collection and identification of plant materials: *S. caseolaris* was collected from Karamjal, Sundarban Khulna, Bangladesh. A specimen copy was deposited to Bangladesh National Herbarium for identification & the accession number DACB-29787.

Preparation of ethanolic extract: The leaves of *S. caseolaris* were freed from any of the foreign materials. Then the leaves were air-dried under shed temperature followed by drying in an electric oven at 40º C. The dried plant materials were then ground into powder. About 350g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 900ml of 95% ethanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). Which was concentrated with rotary evaporator at bath temperature not exceeding 40˚ to have gummy concentrate of extract (yield approx. 2.77%).

Phytochemical screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent. Reducing sugars with benedict's reagent [17-19].

Test Animals & Drug: Young Swiss-albino mice male sex,3-4 weeks of age, weighing 20 -25g, were used for in vivo pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were housed in standard environmental conditions at animal house of Khulna University animal lab and fed with rodent diet and water ad libitum. All

experimental protocols were in compliance with BCSIR Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care. The standard drug Diclofenac Na was used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

Antinociceptive activity: The antinociceptive activity of the crude ethanolic extract of *S. caseolaris* was studied using acetic acid induced writhing model in mice [20-21]. The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Positive control group was administered with Diclofenac Na (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

Anti-oxidant Activity: Quantitative assay was performed on the basis of the modified method of [22]. Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 25, 50, 100, 200 and 500 mg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against concentration and from the graph IC_{50} was calculated. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control. (DPPH) free radical scavenging activity was determined by the method described by (Choi et al., 2000; Desmarchelier et al., 1997) [23-24]. Plant extract (0.1 mL) was added to 3 mL of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition was calculated from [(A0–A1)/A0] x 100, where A0 is the absorbance of the control and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg mL-1) versus % inhibition.

Statistical Analysis: For analgesic determination, data were presented as mean ± Standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. *p* values < 0.01 & < 0.001 were considered to be statistically significant (*p* indicates probability).

RESULTS AND DISCUSSION

Chemical group test: Results of different chemical tests on the ethanolic extract of *S. caseolaris* leaves showed the presence of reducing sugars, saponin, tannins & flavonoids. (Table1).

Table 1: Results of different group tests of ethanolic extract of *S. caseolaris* leaves.

Phytoconstituents	Ethanol extract of *S. caseolaris*
Alkaloid	-
Reducing sugar	+
Tannins	+
Gums	-
Flavonoids	+
Saponin	+
Steroid	-

+: Positive result; - : Negative result;

Antinociceptive activity: Table 2 showed the effect of the ethanolic extract of *S. caseolaris* on acetic acid induced writhing in mice. At the dose of 250 mg/kg & 500 mg/kg of body weight, the extract produced 24.37% & 43.15 %writhing inhibition in test animals respectively. The results were statistically significant (P <0.01 & P <0.001) and was comparable to the standard drug Diclofenac Na, which showed 65.48 % at a dose of 25 mg/kg weight.

Table 2: Effects of the ethanolic extract *S. caseolaris* on acetic acid induced writhing of mice (n=5).

Group	Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Control	1% tween 80 solution 10 ml/kg, p.o.	19.70± 0.96 (100)	---
Positive Control	Diclofenac Na 25 mg/kg, p.o.	6.80 ± 0.66** (34.52)	65.48
Test Group- 1	Et. Extract of *S. caseolaris* 250 mg/kg, p.o.	14.90± 0.90 * (75.63)	24.37
Test group- 2	Et. Extract of *S. caseolaris* 500 mg/kg, p.o.	11.20 ± 0.72 ** (56.85)	43.15

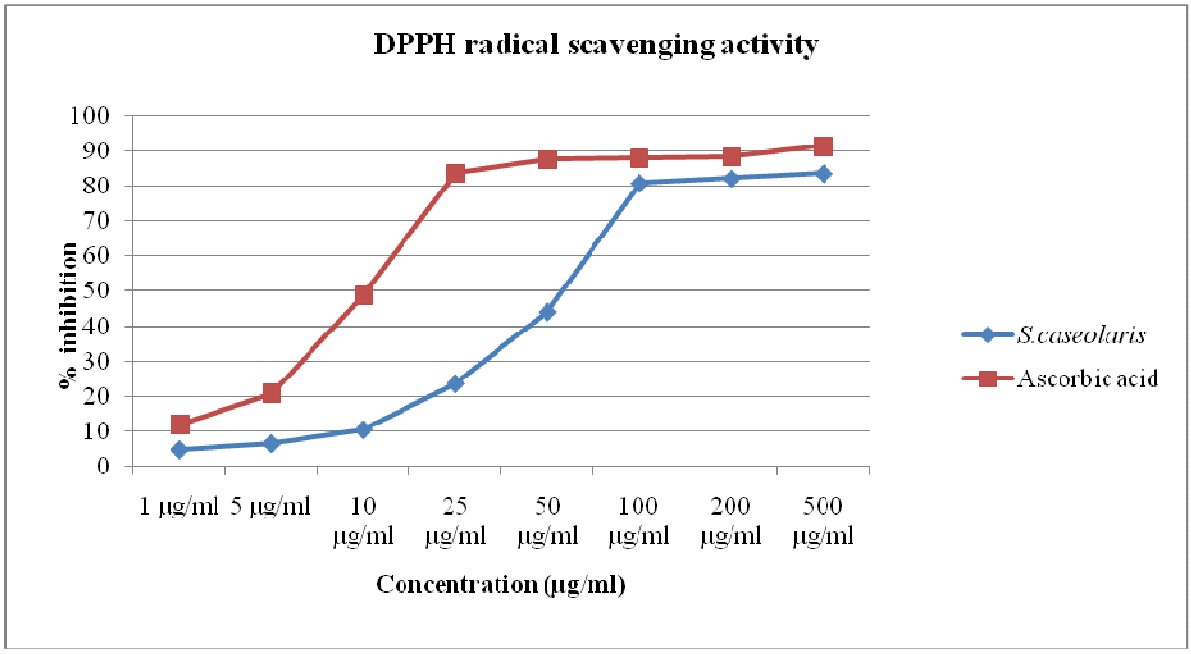
*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; *indicates P < 0.01; **indicates P < 0.001; one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral.*

Anti-oxidant: DPPH applied TLC plates ware observed under UV detector both in short (254 nm) and long (360 nm) wavelength. Antioxidant components in the ethanolic extract of *S. caseolaris* were identified. The extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose. Ethanolic extract of *S. caseolaris* showed potential antioxidant activity where the IC_{50} was 68±0.82 µg mL-1 (P < 0.001), as compared to that of ascorbic acid (IC_{50} 13 ± 0.21 µg mL-1) (P < 0.001) which is a well known antioxidant (Table-3, Fig.-1).

Table 3: Percent inhibition and IC_{50} (50%) inhibition of Crude extract of *S. caseolaris.* and ascorbic acid for DPPH radical scavenging activity

Extracts/Standard	% Inhibition at different concentration(µg/mL)								IC_{50} (µg/ml)
	1	5	10	25	50	100	200	500	
Extract of _Sonneratia caseolaris	4.84	6.57	10.55	23.70	44.11	80.89	82.19	83.56	~68
Ascorbic acid (Standard)	11.94	20.93	48.79	83.65	87.54	87.89	88.24	91.34	~13

Fig:1 Percent inhibition and IC_{50} (50%) inhibition of Crude extract of *S. caseolaris.* and ascorbic acid for DPPH radical scavenging activity.



DISCUSSION

Antinociceptive activity of the ethanolic extract of *S. caseolaris* leaves was tested by acetic acid induced writhing model in mice. The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the

extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Koster *et al*.[25] and Williamson *et al*.[26] who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. With respect to the writhing test, the research group of Deraedt *et al*. described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid [27]. These authors found high levels of prostaglandins PGE_2 and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. On the basis of the result of acetic acid induced writhing test, it can be concluded that the ethanolic extract of *S. caseolaris* might possess an antinociceptive activity.

Preliminary phytochemical screening showed the presence of flavonoid, tanin, alkaloid in the plant extract. Multiple biological effects, including antioxidant activity commonly found in plants containing polyphenolic compounds, like flavonoids, tannins and phenolic acids [28]. Tannic acid present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action. It was shown that the percentage (%) scavenging of DPPH radical was increased significantly with increasing dose, $P< 0.001$. IC_{50} value of the extract was found to be very fairly significant (68 ± 0.49 µg/ml) when compared to the IC_{50} value of the reference compounds ascorbic acid (13 ± 0.21 µg/ml) respectively.

CONCLUSION

In conclusion it can be revealed that the crude ethanolic extract of *S. caseolaris* leaves possess significant antinociceptive as well as antioxidant activities. The potential extract of *S. caseolaris* as antinociceptive and antioxidant agents may be due to the presence of phytoconstituents like tannins, phenolics etc and might be responsible for its activity and justify its use as a traditional folk remedy. However, extensive researches are necessary to search for active principles responsible for these activities.

REFERENCES

[1] Keng H., Orders and Families of Malayan Seed Plant, University of Malaya Press, Kuala Lumpur, Malaysia, **1969**.
[2] Wan Jusoh WFA, Hashim NR, Mapping firefly distribution in Negeri Sembilan and Melaka mangrove forests, Proceedings of the 8th International Annual Symposium on Sustainability Science and Management, Terengganu, Malaysia. **2009**.
[3] Minqing T, Haofu D, Xiaoming L, Bingui W, *Chin J Oceanol and Limnol*, **2009**, 27(2), 288-296.
[4] Phaechamud T, Yodkhum K, Limmatvapirat C and Wetwitayaklung P. *Res J Pharm Bio Chem Sci*, **2012**, 3(1), 725.
[5] Bandaranayake WM, *Mangroves and Salt Marshes*, **1998**, 2, 133-48.
[6] Rahmatullah M, Azam MNK, Pramanik S, Sania, Rahman S, Jahan R. *Inter J Pharm Tech Res*, **2012**, 4(1), 125-129.
[7] Mate GS, Naikwade NS, Chowki CS, Patil SB, *Int J Green Pharm*, **2008**, 2, 118-121.
[8] VANE JR, *Nature: New Biology*, **1971**, 231, 232–235.
[9] K Bagchi, S Puri, *Eastern Mediter Health J*, **1998**, 4(2), 350-360.
[10] Tiwari AK, *Current Science*, **2004**, 86, 1092-1102.
[11] Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J, *Molecul. and Cel. Biochem.* **2004**, 266,37–56.
[12] Dekkers JC, van Doornen LJP, Han CG, Kemper. *Sports Med*, **1996**, 21, 213-238.
[13] Brown JE, Rice-Evans CA, *Free Radical Res*, **1998**, 29, 247-255.
[14] Buxiang S and Fukuhara M, *Toxicology*, **1997**; 122: 61.
[15] Rahman MH, Alam MB, Chowdhury NS, Jha MK, Hasan M, Khan MM, Rahman MS, Haque ME, *Inter J Pharmacol*, **2011**, 7, 257-262.
[16] Jayaprakasha GK, Jaganmohan R, *Zeitschrift Fur Naturforscung*, **2002**, 55, 1018-1022.
[17] Trease GE, Evans WC,Trease and Evan's Pharmacognosy, 13th ed., University Press, Cambridge, **1989**, 546.
[18] Ghani A, Medicinal plant of Bangladesh with Chemical Constituent and Uses. 2nd Edn., Asiatic society of Bangladesh, Dhaka, Bangladesh, **2003**, 603
[19] Harborne JB,. Phytochemical Methods (A Guide to Modern technique to plant Analysis) 3rd Edn., Chapman and Hall, London, **1984**.

[20] Whittle BA, *Br J Pharmacol Chemother*, **1964**, 22, 246-249.
[21] Ahmed F, Selim MST, A.K. Das AK, Choudhuri MSK, *Pharmazie*, **2004**, 59, 329-333.
[22] Gupta M, Mazumdar UK, Sivahkumar T, Vamis MLM, Karki S, Sambathkumar R, Manikandan L, *Nig J Nat Prod Med*, **2003** 7, 25-29.
[23] Choi Y,Jeong HS, Lee J, *Food Chem*, **2007**, 103(1): 130-138.
[24] Desmarchelier C, Mongelli E, Coussio J, Ciccia G, *Phytother Res*, **1997**, 11: 254-256.
[25] Koster R, Anderson M, De-Beer EJ, *Fed Proceed*, **1959**, 18:412–418.
[26] Williamson EM, Okpako DT, Evans FJ, Pharmacological Methods in Phytotherapy Research: Selection, preparation and pharmacological evaluation of plant material; Vol. 1, 1st ed., (John Willey and Sons), England, **1996**:131-184.
[27] Derardt R, Jougney S, Delevacee F, Falhout M, *Eur J Pharmacol*, **1980**, 51:17-24.
[28] Brown JE, Rice-Evans CA, Luteolin rich artichoke extract protects low-density lipoprotein from oxidation in vitro. *Free Radical Res*, **1998**; 29: 247-255.