

Phytochemical and Pharmacological Evaluation of Fruits of *Sonneratia apetala*

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Abstract

Sonneratia apetala-Buch.-Ham is a mangrove tree from the family, Lythraceae. The aim of the study is to evaluate analgesic, antidiarrhoeal, anthelmintic, cytotoxic activity of the fruits of *S. apetala*. The extract showed dose dependent analgesic activity using acetic acid induced writhing inhibition of the Swiss albino mice. The extract produced 46

Index terms— *sonneratia apetala*-Buch.-Ham. analgesic activity, antidiarrheal activity, anthelmintic test, brine shrimp lethality bioassay.

1 Introduction

Plants and herbs have been in use for both cure and prevention of diseases. Man has been experimenting with the plants; some used for food, others for dress, and stills others for treatment of diseases and to keep personnel in a state of health. Researchers are searching for bioactive molecules responsible for specific pharmacological effect in medicinal plants. But edible food can also be a potent source of drug molecules. This leads for the searching of medicinal value of edible fruit. popularly known as mangrove apple plant belonging to the Lythraceae family and it grows as tree or shrub, have the anti-HIV, Antibacterial, antiproliferative and antiestrogenic activities and for the treatment of insanity, epilepsy and asthma (Field, 1995). The literature also reports that the leaf part of the plant is widely used for dysentery, sprain & bruises, in treatment of eye troubles (such as cataract) and open sores in children ears and also in heart troubles (Bandaranayake, 1995). The aerial analgesic activity using acetic acid induced writhing inhibition of the Swiss albino mice. The extract produced 46% and 69% writhing inhibition at the doses of 250 mg/kg and 500 mg/kg body weight respectively while the standard drug (Diclofenac Sodium) produced 82% writhing inhibition at a dose of 25 substantiated by significant prolongation of latent period and decrease in total number of stools at four dose level as compared to standard loperamide. In anthelmintic test the extract showed significant and dose dependent decrease in There is a number of traditional uses and medicinal benefits that is why this plant part was selected for pharmacological investigations.

2 II.

3 Materials and Methods

4 a) Sample Collection

The fruits of *S. apetala* were collected from the world largest mangrove forest-The Sundarbans.

Collected fruits were dried by shade drying. Then these were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

5 *S. apetala*

Keywords: *Sonneratia apetala*-Buch.-Ham analgesic activity, antidiarrheal activity, anthelmintic test, brine shrimp lethality bioassay.

9 H) SCREENING FOR CYTOTOXIC ACTIVITY

(61µg/ml) and LC 90 (616 µg/ml).

mg/kg body weight. In vivo antidiarrheal activity was distributed through out saline area. The mangrove plant

b) Cold Extraction terpenoids. To the alcoholic extract, sodium bicarbonate solution was added and observed for the production of effervescences. Production of effervescences indicates the presence of acidic compound (Amer, Abou-Shoer , Abdel-Kader, El-Shaibany, Abdel-Salam, 2004) e) Evaluation of Analgesic Activity

The analgesic activity of extract was studied using acetic acid induced writhing model in mice (Whittle,1964;Ahmed, Selim, Das, Choudhuri, 2004). The animals were divided into control, standard 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. Standard 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 hind legs) for 5 min. First extraction-150gm grinded fruits powder was soaked in 600 ml of ethanol in a glass container for seven days accompanying regular shaking and stirring. After fifteen days the extract was separated from the debris by filtration by a piece of clean, white cotton cloth.

Second extraction-The residue was again soaked in 250 ml of ethanol for three days and then was separated from the debris by filtration through by a piece of clean, white cotton cloth.

Total weight of the dried extract of *S. apetala* was 22gm where percent yield was 14%. (Agoha, 1981;Barakat, 1973). For the presence of tannins was indicated by ferric chloride and lead acetate test. Presence of gum was evaluated by taking extract and then molish reagent and sulphuric acid were added. Red violet ring was not produced at the junction of two liquids which indicated the absence of gums. To 1 mL of extract, few drops of nitric acid were added by the sides of the test tube and observed for formation of yellow color. This indicates the presence of xanthoprotein. To 0.5 g of the extract, 2 mL of chloroform was added; Conc. H₂SO₄ (3 mL) was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of III.

6 Results and Discussion

7 a) Phytochemical Screening

The ethanolic extract of was subjected to qualitative phytochemical tests for detection of different classes of chemical compounds. Alkaloid, reducing sugar, tannin, steroid, glycoside, flavonoids and acidic compounds were present in the extract.

8 b) Evaluation of Analgesic Activity

At the dose of 250 mg/kg and 500 mg/kg the extract showed inhibition of writhing inhibition by 46.54% and 69.62% respectively while the standard drug Diclofenac Na inhibition was found to be 82.31% at a dose of 25 mg/kg b.w. The result was statistically significant at the level of $p < 0.001$ (Table ??). The active principle responsible for this analgesic activity may be acidic compounds, terpenoids, reducing sugar, gums, xanthoprotein, flavonoids and tannins ?? *Haemonchus contortus* (Nematode), was considered and isolated because it has similarity with parasites living in human body. After cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.4 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na₂HPO₄ and 0.27 g KH₂PO₄ in 1L of distilled water at 37±1 °C. The parasites were divided into different groups consisting of six parasites in each group. Extract at the concentrations of 25, 50, 100 and 200 mg/mL and standard albendazole (info) at the concentrations of 15 mg/mL and 10 mg/mL of 10 mL in PBS were prepared and transferred to petri dishes. Control group was treated with 0.1% tween-80 in PBS. Six parasites were placed in each petri dish and observed. The time of paralysis was recorded when no movement was observed unless shaken vigorously. The death time was recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50°C) or subjected to external stimuli. Anthelmintic activity was expressed as the time required for paralysis and death of parasites as compared to control.

9 h) Screening for Cytotoxic Activity

The cytotoxicity assay was performed on brine shrimp nauplii using the method described by Mayer, experiment, 4 mg of methanol and ethyl acetate extracts were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.123, 1.563, 0.781 µg/ml) were obtained by serial dilution technique. The solutions were then added to the premarked 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 percent of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC₅₀ and LC₉₀) of the test samples were obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration and compared with the standard vincristine sulphate.

10 c) Evaluation of Antidiarrhoeal Activity

In the castor oil induced diarrheal mice, extract at various doses lessened the total number of faeces as well as delayed the onset of diarrhea in a dose dependent manner (Table ??). % Inhibition of defecation at doses 62.5mg/kg, 125mg/kg, 250 and 500 mg/kg b. wt. was 74. ??9, 82.26, 87.90, 94.35 respectively. Standard loperamide also showed decrease in total number of feces and 95.15% inhibition of defecation. It is known that the active component of castor oil is the ricinoleic acid, which is liberated from the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins (Yoshio et al., 1999) and stimulating peristaltic (decreasing Na + and K + absorption) activity and diarrhoea (Zavala, Perez, Vargas & Perez. 1998). Loperamide is a opiate/alkaloid analogue ??Tripathi K.D. 2008). It Inhibits prostaglandin synthesis and delay diarrhoea induced with castor oil (Sunil, Bedi, Singla & Johri, 2001). Thus alkaloids of *S. apetala* may follow the same pathway as Loperamide does. Moreover, antidiysenteric and antidiarrhoeal properties of medicinal plants were found to be due to alkaloids as well as tannins, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Havagiray, Ramesh & Sadhna. 2004). These constituents are also present in *S. apetala* responsible for antidiarrhoeal activity.

11 d) Evaluation Of Anthelmintic Activity

In the present study the *S. apetala* was found to show anthelmintic activity when compared to albendazole used as standard drug. Extract at concentrations of 25, 50, 100 and 200 mg/ml showed paralysis at, 441. anthelmintic activity in dose dependent manner which was comparable with standard anthelmintic drug. This also supports its traditional use as anthelmintic.

Albendazole inhibits parasitic microtubule polymerrization by binding to ?-tubulin (Goodman LS., Gilman A. 11th Edn). As *S. apetala* showed anthelmintic activity compared to albendazole, may be their pathway is same.

e) Screening for Cytotoxic Activity

In brine shrimp lethality bioassay, the crude extract of *S. apetala* fruit showed lethality indicating the biological activity of the extract. The percent of mortality vs log concentration was plotted and a best fit line was obtained using LDP line probit analysis software.

Through the software LC 50 and LC 90 of the test sample were found to be 61µg/ml and 616 µg/ml. As it contains many tanins and other polyphenols, its cytotoxic effect may be correlated some polyphenols perturb the membrane structure (Hossain 2002;Aoshima, 2005).

IV.

12 Conclusion

The fruit of *S. apetala* is extensively consumed in coastal areas of Bangladesh without any known toxicity in humans. Based on our present observations activity. It may be concluded that this mangrove fruit merits further exploration both chemically and biologically to identify the functional principle(s) and mechanism of action.

13 *S. apetala* has many in vitro and in vivo biological

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Figure 1:



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Figure 2: Fig. 1 :



2

Figure 3: Fig. 2 :

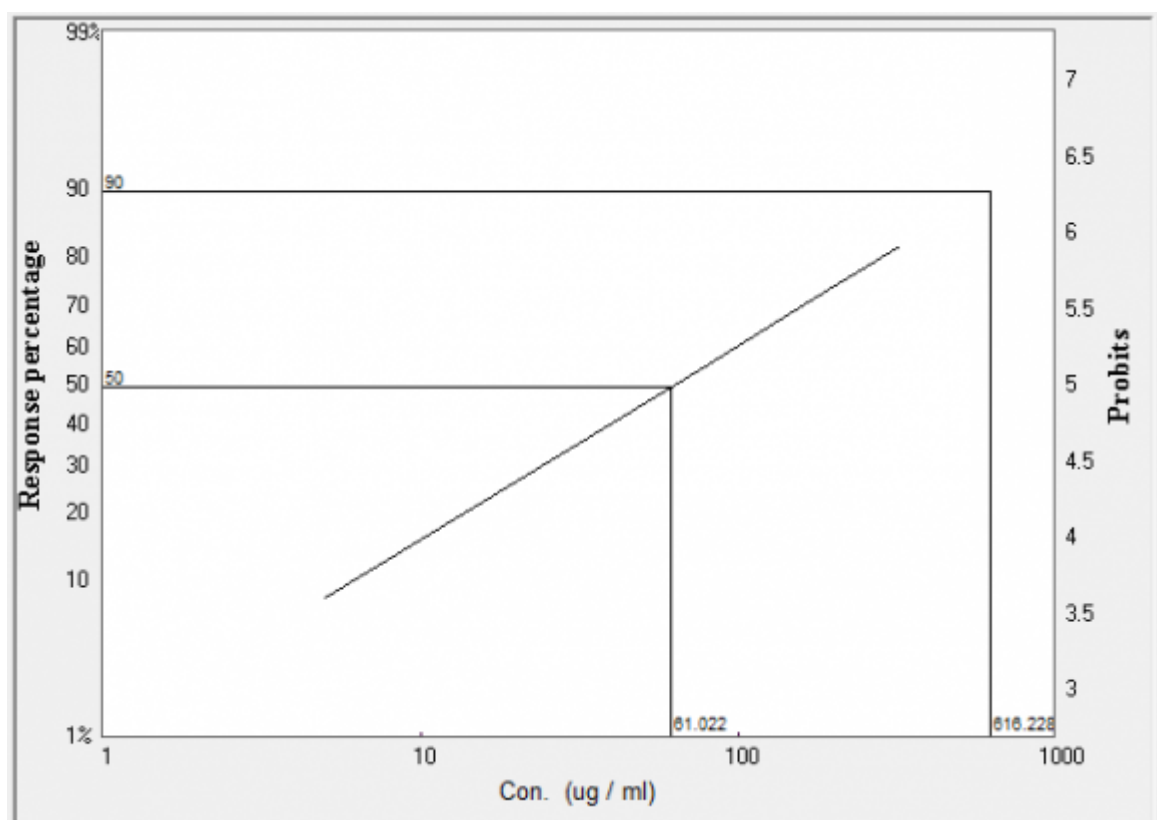


Figure 4:

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[Graphical Representation of Brine Shrimp Lethality Bioassay and Both LC 50 and LC 90 for the *S. apetala* by Ldp Line Software] *Graphical Representation of Brine Shrimp Lethality Bioassay and Both LC 50 and LC 90 for the *S. apetala* by Ldp Line Software*, 5.

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- 190 Mean.
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- 195 [per ora. Table 4 : Result of Anthelmintic Activity Test of S. apetala] *per ora. Table 4 : Result of Anthelmintic*
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