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Phytochemical and Pharmacological Evaluation of Fruits of *Sonneratia apetala*

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I. 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. *S. apetala* popularly known as mangrove apple plant belonging to the Lythraceae family and it grows as tree or shrub, distributed through out saline area. The mangrove plant 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 parts of the plant contain a large number of terpenoid, steroid, alkaloid & polysaccharide (Jamale & Joshi, 1998). The antimicrobial activity of the extract of *S. apetala,* on the various test microorganisms, including clinical multiple antibiotic resistant bacteria and phytopathogens were investigated (The Ayurvedic Pharmacopoeia of India.1998 & John, 2007). The seeds contain polyphenols which may be responsible for antibacterial activity and antioxidant activity (Hossain, Basar, Rokeya, Sultana & Rahman, 2012).

Fig.1 : Fruits of *Sonneratia apetala.*

Folk Medicinal Healers in Bagerhat district of Bangladesh uses *S. apetala* as Anti-inflammatory and to treat gastrointestinal disorders (including dysentery, diarrhoea, indigestion, colic, acidity, bloating, lack of appetite, stomachache) (Mollik, Hossan, Rahman,, Rownak & Mohammed Rahmatullah, 2010). There is a number of traditional uses and medicinal benefits that is why this plant part was selected for pharmacological investigations.

II. Materials and Methods

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.

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b) Cold Extraction

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%.

c) Test Animals and Parasites

Young Swiss-albino mice aged 4-5 weeks, average weight 28-35gm were used for the experiment. The mice were purchased from Department of Pharmacy, Jahangirnagar University, Dhaka. They were housed in standard environmental conditions at animal house of Pharmacy Discipline 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. During investigation of anthelmintic property of extract, *Haemonchus contortus* (Nematode), was considered and isolated because it has similarity with parasites living in human body.

d) Phytochemical Screening Methods

For alkaloid analysis the extracts was treated with 1% HCl, boiled and filtered. Dragendorff's was used to indicate the presence of alkaloids (Harborne, 1998) (Trease & Evans 1989), Libermann Burchard and Salkowski tests for the presence steroids, foam test for the presence of saponins, Benedict's and Fehling's test for the presence of carbohydrates, test for the presence of glycoside. 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicates the presence of flavonoids. 20gm extract was dissolved in dilute sodium hydroxide and then neutralized with dilute hydrochloric acid. Formation of yellow color and disappearance of color indicate the presence of flavonoid (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. H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of 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 of hind legs) for 5 min.

Fig. 2 : Test animal Swiss-albino mice.

f) Evaluation of Antidiarrhoeal Activity

The test was performed by castor oil induced diarrhoea in young Swiss-albino mice (Reiner, R. (1982)). The animals are divided into control, positive control and six test groups containing five mice in each group. Control group receive vehicle (1% Tween-80 in water) at a dose of 10 ml/kg body weight orally. The positive control group receive loperamide (IMOTIL 2 mg/Cap., Square Pharmaceuticals Ltd., Bangladesh) at the dose of 3 mg/kg orally; test groups received the crude extracts at the doses of 250 and 500 mg/kg body weight orally. Each animal was placed in individual cage, the floor of which was lined with blotting paper which was changed every hour. Diarrhoea is induced by oral administration of 0.3 ml castor oil to each mouse, 30 min after the above treatments. During the observation period (4hr), the latency periods and the number of diarrheic faeces excrete by the animals is recorded. A numerical score based on stool consistency is assigned as follows: normal stool =1/2 and watery stool = 1.

g) Evaluation of Anthelmintic Activity

Live parasites *Paramphistomum cervi* (Trematoda) and *Haemonchus contortus* (Nematode) were collected from freshly slaughtered cattle at local abattoirs and identified by Dr Md. Royhan Gofur, Lecturer, Department of Animal Husbandry and Vaterinary Science, Rajshahi University, Rajshahi. During investigation of anthelmintic property of extract, *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 Na_2HPO_4 and 0.27 g KH_2PO_4 in 1L of distilled water at 37±1 ^oC. 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^oC) or subjected to external stimuli. Anthelmintic activity was expressed as the time required for paralysis and death of parasites as compared to control.

h) Screening for Cytotoxic Activity

The cytotoxicity assay was performed on brine shrimp nauplii using the method described by Mayer, (1982). Simple zoological organism *Artemia salina* was used as convenient monitor for the screening. DMSO (Dimethyl sulfoxide) solutions of the fractions were applied to *A. salina* in a one-day in vivo assay. For the 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 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 percent of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50} and LC_{90}) 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.

III. Results and Discussion

a) Phytochemical Screening

The ethanolic extract of *S. apetala* 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.

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 2). The active principle responsible for this analgesic activity may be acidic compounds, terpenoids, reducing sugar, gums, xanthoprotein, flavonoids and tannins (Ahmadiani, Hosseiny, Semnanian, Javan, Saeedi, Kamalinejad & Saremi. 2000; Rajnarayana, Sripal, Chaluvadi. 2001; Choi, Lee, Park. 2005).

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 3). % Inhibition of defecation at doses 62.5mg/kg, 125mg/kg, 250 and 500 mg/kg b. wt. was 74.19, 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, antidysenteric 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.

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, 441.5, 276, 255, 209 seconds and death time found at 615, 438, 354, 222 seconds respectively. Standard albendazole also showed paralysis time 334, 273 seconds and death time at 379, 338 seconds for concentration of 10 and 15 mg/ml respectively. From the above result, it is clear that *S. apetala* has significant

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 polymerization by binding to β-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. 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 *S. apetala* has many *in vitro* and *in vivo* biological 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.

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Table 1 : Phytochemical Investigation of Ethanolic Extract of *S. apetala* Fruits

Phytochemical Group	Result
Reducing sugars	+
Tannins	+
Flavonoids	+
Saponins	-
Gums	-
Steroids	+
Alkaloids	+
Glycosides	+
Proteins	-
Terpenoids	+
Acidic compounds	+

Table 2 : Effect of *S. apetala* on Acetic Acid Induced Writhing of Mice

Group	Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Control	1% tween 80 solution 10ml/kg, p.o.	26±2.003 (100)	---
Standard	Diclofenac Na 25 mg/kg p.o.	4.6±0.65** (17.69)	82.31
Test Group-I	*S. apetala* extract 250 mg/kg p.o.	13.9±1.825** (53.46)	46.54
Test Group-II	*S. apetala* extract 500 mg/kg p.o.	7.9±2.25** (30.38)	69.62

Note: 1. Values are expressed as mean± SEM (Standard Error Mean);
*2. ** indicates P>0.001;*
3. one way ANOVA followed by Dunnet's test as compared to control;
4. n=Number of mice; p.o.: per oral.

Table 3 : Effect of *S. apetala* on Castor oil Induced Dirrhoea in Mice

Group	Dose(/kg-bw)	Mean Latent Period in min ± SEM	Mean Number of Stool in 4hr ± SEM
Control	1% tween-80 in distilled water	36.2±3.54	24.8±1.68
Standard	Loperamide	191.6±3.52	1.2±0.18
Test Group I	*S. apetala* extract 62.5mg p.o.	63.2±3.08	6.4±1.08
Test Group II	*S. apetala* extract 125mg p.o.	77.2±2.82	4.4±0.46
Test Group III	*S. apetala* extract 250mg p.o.	112.6±3.64	3±0.05
Test Group IV	*S. apetala* extract 500mg p.o.	183.4±10.06	1.4±0.36

Note: 1. Values are expressed as mean±SEM(Standard Error Mean);
*2. ** indicates P>0.001;*
3. one way ANOVA followed by Dunnet's test as compared to control;
4. n=Number of mice five; p.o.: per ora.

Table 4 : Result of Anthelmintic Activity Test of *S. apetala*

Group	Concentration (mg/ml)	Time in seconds	
		Paralysis	Death
Control	0.2% Tween-80 in water	---	---
Standard Group I	Albendazole 10	334.17±5.45	379.17±6.17
Standard Group II	Albendazole 15	273.33±3.26	338.67±2.42
Test Group I	*S. apetala* extract 25	441.5±7.58	615.67±4.45
Test Group II	*S. apetala* extract 50	276.67±7.78	438±9.70
Test Group III	*S. apetala* extract 100	255±3.76	354.83±7.42
Test Group IV	*S. apetala* extract 200	209±5.11	222.16±4.12

Note: 1. Values are expressed as mean±SEM(Standard Error Mean);
*2. ** indicates P>0.001;*
3. one way ANOVA followed by Dunnet's test as compared to control;
4. n=Number of mice;

Table 5 : Graphical Representation of Brine Shrimp Lethality Bioassay and Both LC_{50} and LC_{90} for the *S. apetala* by Ldp Line Software