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ANTI-DIARRHEAL, ANTIMICROBIAL AND MEMBRANE STABILIZING ACTIVITY OF *SARCOCHLAMYS PULCHERRIMA* GAUDICH

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ABSTRACT

In this study, the methanol extract of leaf of *Sarcochlamys pulcherrima* Gaudich (SPME) was investigated for anti-diarrheal, antimicrobial and membrane stabilizing activities. Test for anti-diarrheal activity was carried out by castor oil-induced diarrhea in mice. During antimicrobial assay test by agar disc diffusion method, the plant extract showed strong activity against *Bacillus megaterium* (zone of inhibition = 22.0 mm) *and Candida albicans* (zone of inhibition = 17.0 mm). The MICs of the sample were 250 µg/ml against *Bacillus cereus, B. subtilis, Escherichia coli, Aspergillus niger* and *Microsporum* sp. In membrane stabilizing activity test, the crude methanol extract at 500 µg/ml inhibited the heat-induced haemolysis of RBCs by 47.77% whereas the standard acetyl salicylic acid (ASA) demonstrated 81.72% inhibition of haemolysis. Preliminary phytochemical screening revealed that the crude extract contains reducing sugar, glycosides, alkaloids, flavonoids and saponins.

Introduction

In developing countries, diarrhea is a major cause of infant mortality and morbidity.[1] Despite the availability of wide spectrum of approaches for diarrheal management, a vast majority of the people in the developing countries rely on herbal drugs for the management of diarrhea. WHO has encouraged studies for treatment and prevention of diarrheal diseases using traditional medical practices.[2] Besides diarrheal diseases, infections caused by bacteria, fungi, viruses and parasites are also a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance.[3] In Ayurveda, the use of herbal extracts and nutritional supplements is well documented for treatment of inflammatory diseases in the Indian subcontinent for 5000 years.[4] The increasing failure of chemotherapeutics and development of antibiotic resistance exhibited by pathogenic microorganisms have emphasized the need for screening of several medicinal plants for their potential antimicrobial activity.[5,6]

Sarcochlamys pulcherrima Gaudich (Family- Urticaceae) is an evergreen shrub or small tree growing from 2-6 m tall. In Bangladesh, this plant is widely distributed in Forests of Chittagong, Chittagong Hill Tracts, Sylhet and Mymensingh. The Chakma tribe in Chittagong Hill Tracts use the leaf paste for the treatment of boils and blisters on the lips.[7,8] Boiled leaf is taken as vegetable by Khumi community.[9] Significant literature survey revealed that found only a little research work has been performed with this plant to evaluate its medicinal value and pharmacologically active constituents. As part of our ongoing research with medicinal plant of Bangladesh[10,11] the present study has been undertaken for the first time to evaluate the anti-diarrheal, antimicrobial and membrane stabilizing activities of different Kupchan partitionates of *S. pulcherrima* leaf as well as to find out the rationale behind the folk uses of this plant.

MATERIALS AND METHOD

Collection of plant materials

Dried leaves of *S. pulcherrima* were collected from Chittagong Hill Tracts, Bangladesh in June 2012 and were identified at Forest Research Institute, Chittagong, Bangladesh, where a voucher specimen has been maintained for future reference.

Drying and grinding

After collection, the dried leaves were washed with running tap water. These clean leaves were dried at a temperature not exceeding 50 ºC. The dry materials were ground to a coarse powder with the help of a grinder and kept in airtight container and stored in a cool and dark place until extraction was commenced.

Hot extraction by Soxhlet extractor

Exactly 140 gm of powdered leaf was extracted with 750 ml of methanol (99.98%) with a Soxhlet apparatus (Quickfit, England). The extract was concentrated with a rotary evaporator (Heidolph, Germany) under reduced temperature and pressure to provide a gummy residue (yield 18.70%).

Chemicals

All the chemicals and solvents used in this study were of analytical grade and purchased from Merck, Germany. Standard drugs such as loperamide, ciprofloxacin, fluconazole and acetyl salicylic acid were obtained from Square Pharmaceuticals Ltd.

Experimental animals

For the experiment *Swiss albino* mice of either sex, 6-7 weeks of age, weighing between 25-30 g, were collected from the Animal Resources Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR,B). Animals were maintained under standard environmental conditions temperature: (27.0 ± 1.0 °C), relative humidity: 55-65% and 12 h light/12 hr dark cycle and had free access to feed and water *ad*

libitum. Appropriate measures were taken to minimize the pain or discomfort of animals and the animals were acclimatized to laboratory condition for one week prior to experiments and details of animal care should be provided. All protocols for animal experiment were approved by the institutional animal ethical committee.[12]

Preliminary phytochemical investigation

For preliminary phytochemical investigation, the crude methanol extract of *S. pulcherrima* was subjected to various tests to determine the chemical nature of the extractive.[13,14] The presence of alkaloid content was determined by performing Mayer's test; white precipitate (ppt) indicated the presence of alkaloids. The formation of intense yellow coloration upon the addition of few drops of sodium hydroxide and the subsequent loss in color upon the addition of dilute acetic acid indicated the presence of flavonoids. The existence of glycoside in the sample was identified by performing Salkowski's test as well as Libermann-burchard's test; Orange-reddish color at the junction of 2 layers confirms the presence of glycosides. The presence of both the reducing sugar and gums were confirmed by Fehling's test and Molisch's reagent, respectively.

Anti-diarrheal activity

Anti-diarrheal activity of crude methanol extract of *S. pulcherrima* was carried out by castor oil-induced diarrhea in mice.[1] Young *Swiss albino* mice, average weight of 20-25g were employed in the experiment. The animals were divided into control, positive control and two test groups containing seven mice in each. Control group received 1% Tween-80 (10 ml/kg, p.o). The positive control group received loperamide (25 mg/kg, p.o.) while the test groups received the methanol extract (200 and 200 mg/kg) orally. Acute diarrhea was produced by oral administration of 0.4 ml of castor oil to each mouse. Then the latency period and diarrheic secretion were counted for 4 hours.

Antimicrobial activity

The preliminary antimicrobial activity of the extractives was determined at 500 µg/disc by the disc diffusion method[15] against a number of Gram positive and Gram negative bacteria and fungi (Table-2). The bacterial and fungal strains used in this experiment were collected from the Microbiology Lab., Chittagong University, Chittagong, Bangladesh. Here, standard Ciprofloxacin (30 µg) and fluconazole (50 µg) disc were used as reference.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of SPME was determined by the serial dilution technique[16] in nutrient broth medium, containing graded concentration of the plant extract and inoculated test organisms.

Membrane stabilizing activity

For this experiment, three clean centrifuge tubes were taken for positive control (acetyl salicylic acid), three for negative control (99.8% ethanol) and six for crude ethanol extract and 1.0 ml of 10% RBC suspension was added to each tube. Then 1.0 ml ethanol and 1.0 ml acetyl salicylic acid were added to the negative control and positive control tubes respectively. On the other hand, for the test group, 1.0 ml of ethanol extract (1000 mg/kg) was mixed. The pH (7.4±0.2) of the reaction mixtures was adjusted by phosphate buffer. The tubes were then incubated in water bath and after cooling these were centrifuged at 2500 rpm for 5 min. After filtration the absorbance of the supernatants were taken at 556 nm. The total inhibition of haemolysis was then calculated (Table 3) by determining the % inhibition of haemolysis.[17]

STATISTICAL ANALYSIS

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 vehicle group. p values <0.05 were considered to be statistically significant compared with the control.

RESULTS AND DISCUSSION

Antidiarrhoeal activity

In the castor oil-induced diarrhea, the extract of the leaf of *S. pulcherrima* (SPME) produced a marked anti-diarrheal effect in the mice, as shown in (Table-1). At doses of 200 and 400 mg/kg b.w, the extract significantly decreased ($p<0.0001$) the total number of feces which was produced upon the administration of castor oil (40.0% at 200 mg/kg b.w, 56.36% at 400 mg/kg b.w) as compared to that in the control group. Both the doses were shown to reduce the total number of feces in the test groups as compared to that in the control.

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, which is accompanied by an excess loss of fluid in the faeces. In some types of diarrhoea, the secretory component predominates, while other types of diarrhoea are characterized by hyper motility. Castor oil causes diarrhoea due to its active metabolite, ricilonic acid[18] which stimulates the peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action stimulates the release of endogenous prostaglandins.[19] In this study, the crude methanol extract of *S. pulcherrima* exhibited a significant anti-diarrheal activity.

Antimicrobial activity

In this screening, the extract showed varying degree of antimicrobial activity against the test organisms (zone of inhibition = 9.33-22.0 mm) (Table-2). The methanol extract showed strong activity against *Bacillus megaterium*, *Escherichia coli, Salmonella* Typhi, *B. subtilis, Shigella dysenteriae* with zone of inhibition of 22.0, 19.0, 18.33, 16.33, 14.0 mm, respectively as compared to the reference standard ciprofloxacin (Table-2). The highest zone of inhibition (22.0 mm) was produced by *S. pulcherrima* against *B. megaterium*. In the antifungal sensitivity test, the plant extract also produced a mild to moderate inhibition of fungal growth used in the screening (Table-2). The plant extract (500 µg/disc) exhibited significant activity against *Candida albicans* (zone of inhibition = 17.0 mm).

During the MIC determination, the methanol extract inhibited the growth of test organisms between 125.0-250.0 µg/ml (Table-2). It inhibited the growth of *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Aspergillus niger* and *Microsporum* sp. at 250 µg/ml and *Bacillus megaterium*, *Staphylococcus aureus, Salmonella* Typhi, *Vibrio cholerae, Trichophyton* sp. and *Candida albicans* at 125µg/ml.

Membrane stabilizing potential

The red blood cell stability test is based on the result that a number of non-steroidal anti-inflammatory agents inhibit heat-induced rupture of erythrocytes, most probably by stabilizing the membrane of the cells. The erythrocyte membrane may be considered as a model of the lysosomal membrane. Certain herbal preparations have been found to capable of stabilizing the red blood cell membrane and this may be indicative of their ability to exert anti-inflammatory activity.[20]

In this experiment, the extract (SPME) at 500 µg/ml inhibited the heat-induced haemolysis of RBCs by 47.77% as compared to 81.72% exhibited by standard acetyl salicylic acid (Table-3). Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is thought that the plant may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.[21]

Phytochemical screening

In the preliminary screening for chemical constituents, the crude extract revealed the presence of reducing sugar, glycosides, alkaloids, flavonoids and saponins.

Conclusion:

Currently there has been an increased interest worldwide to identify secondary metabolites from plant sources which are pharmacologically potent and have small or no side effects for use in protective medicine. The present study demonstrates that the crude extract of *S. pulcherrima* also exhibited good anti-diarrheal, antimicrobial and membrane stabilizing activities. Nevertheless, the isolation of pure secondary metabolites from the plant will help us further in understanding the mechanism of these activities and identification of lead compounds of clinical utility.

Table 1: Effect of SPME on castor oil-induced diarrhea in mice

Test groups	% Inhibition of defecation	TNF (240 min)
Control	0	55.0±1.76
Loperamide (25 mg/kg)	70.91	16.0±1.04
SPME (400 mg/kg)	56.36	24.0±1.0
SPME (200 mg/kg)	40.0	33.0±1.0

TNF = Total number of feces; Values are mean ± SEM (n=7); **p<0.0001 by Dunnett's T test for values between the sample and vehicle treated group.

Table 2: Antimicrobial activity of SPME against test organisms

Microorganisms	Zone of inhibition (MZI±SD) mm		MIC (µg/µl)
	SPME (500 µg/µl)	Standard	
Gram positive bacteria		**Ciprofloxacin (30 µg/µl)**	
Bacillus cereus	11.0±0.57[a]	28.0±1.0	250
B. megaterium	22.0±1.51[b]	30.0±1.0	125
B. subtilis	16.33±1.51[c]	28.67±1.52	250
Staphylococcus aureus	9.33±0.57[d]	30.0±1.0	125
Gram negative bacteria			
Escherichia coli	19.0±0.57[c]	25.0±1.0	250
Salmonella Typhi	18.33±0.57[e]	33.17±0.71	125
Shigella dysenteriae	14.0±0.71[b]	25.33±0.82	NF
Vibrio cholerae	12.67±1.15[a]	31.0±1.0	125
Fungi		**Fluconazole (50 µg/µl)**	
Aspergillus niger	12.33±0.57[a]	34.0±1.0	250
Candida albicans	17.0±1.0	33.0±0.5	125
Microsporum sp.	11.0±1.0[c]	32.33±2.0	250
Trichophyton sp.	8.33±0.5[a]	35.0±1.0	125

[a]p<0.0001, [b]p<0.004 [c]p<0.001, [d]p<0.002, [e]p<0.000001; MZI: Mean zone of inhibition (mm); zone of inhibition under 8 mm were considered as less active and were discarded; MIC = Minimum Inhibitory Concentration; NF= Not Found.

Table 3: *In-vitro* membrane stabilization activity of test sample and controls.

Test groups	Total inhibition of haemolysis
Control (methanol)	00.0±0.0018
Standard (ASA, 0.1 mg/ml)	81.72±0.002[a]
SPME (500 µg/ml)	47.77±0.002[b]

ASA= Acetyl salicylic acid; Total inhibition of haemolysis = %IMHLs±SEM , [a]p<0.0001 and [b]p<0.2.

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