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PRELIMINARY PHYTOCHEMICAL, ANTI-BACTERIAL, ANALGESIC, ANTI-DIARRHOEAL AND CYTOTOXIC ACTIVITY OF METHANOLIC EXTRACT OF POLYALTHIA *SUBEROSA* LEAVES

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ABSTRACT

The MeOH extract of leaves of *Polyalthia suberosa* Roxb. (Annonaceae) was screened for its antibacterial, analgesic, Antidiarrhoeal and cytotoxic activities. The extract showed moderate anti-bacterial activity against *Vibrio cholerae, Sheigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus saprophyticus.* It also produced significant (P<0.01) writhing inhibition in acetic acid induced writhing in mice at dose of 250 and 500 mg/kg, which was comparable to the standard drug diclofenac sodium. Moreover, when tested for its antidiarrhoeal effects on castor oil induced diarrhea in mice, it decreased the frequency of defecation and increased mean latent period significantly (P<0.01) at the dose of 500 mg/kg comparable to the standard drug loperamide. The extract also exhibited high level of cytotoxicity in brine shrimp lethality bioassay (LC_{50}: 30 µg/ml). The overall results tend to suggest the antibacterial, analgesic, antidiarrhoeal and cytotoxic activities of the extract.

INTRODUCTION: *Polyalthia suberosa* Roxb. (Synonym: *Uvaria suberosa* Roxb.)(Family: Annonaceae), locally known as Jam Debdaru, ham jam, is a short small tree widely distributed in Bangladesh, West Indies, Philippine, India, Sri-Lanka, Malaysia and Myanmar [1]. Fruits are used to stop diarrhea. Fruits and flowers are used to relieve pulmonary complaints. Leaves are used as a remedy for coughs, colds and diarrhea. It is also used in flatulence and as Anti-HIV agent.

Bark is regarded as a febrifuge and is said to halt diarrhea and dysentery. It is powerful astringent and also used as analgesic and laxative. Seed have a diuretic action and is a sedative and soporific. The latex is used in the tropics as a crude filling for tooth cavities [2]. A literature survey of this plant retrieves only little information regarding the nature of its chemical constituents and pharmacological activity.

Chemical characterization of the stem revealed the presence of two new 2-substituted furans, 1-(2-furyl) pentacosa-16,18-diyne and 23-(2-furyl) tricosa-5,7-diynoic acid [3], an azaanthracene alkaloid, 1-aza-9,10-dimethoxy- 4- methyl- 2- oxo- 1, 2-dihydroanthracene (kalasinamide) [4] and anti-AIDS agent, 9 Suberosol [5]. However, no biological activity has been reported.

The aim of the present study was to investigate the antibacterial, analgesic, antidiarrhoeal and cytotoxic activities of the crude MeOH extract of *Polyalthia suberosa (P. suberosa).*

MATERIALS AND METHODS

Plant material collection and extraction: The plant was collected from Satkhira, Bangladesh in May 2006 and was identified in the National Herbarium of

Bangladesh (Accession no.: 31305). About 400 g of pulverized leaves were taken in a clean, flat-bottomed glass container and soaked in 1300 ml of 80% methanol. 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 followed by filtration through whatmann filter paper. The filtrate thus obtained was concentrated under ceiling fan followed by vacuum desiccation (% yield: 5.5).

Tests for different Chemical groups: The crude MeOH extract was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins [6]. In each test 10% (w/v) solution of the extract in methanol was taken unless otherwise mentioned in individual test.

Animals: Young Swiss-albino mice of either sex, weighing 20-25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) were used for analgesic and antidiarrhoeal activity tests. The animals were kept at animal house of Pharmacy Discipline, Khulna University, for adaptation under standard environmental condition and fed with standard diets (ICDDR, B formulated).

Microorganisms: Both gram positive and gram-negative bacterial strains were taken for antibacterial test. The bacterial strains used for the investigation are listed in Table 2.

Drugs: Diclofenac Na and Loperamide.

Antibacterial Activity: Antibacterial activity of the leaf extract was tested by disc diffusion method [7, 8]. Sample impregnated discs, standard antibiotic discs and negative control discs were placed gently on the seeded agar plates with the help of sterile forceps to assure complete contact with medium surface. The plates were then inverted and kept in refrigeration for about 2 h at 4°C to allow the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37°C for 24 h. After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in terms of millimeter with a slide calipers.

Analgesic Activity: Analgesic activity of the MeOH extract of *P. suberosa* was tested using the model of acetic acid induced writhing in mice [9, 10]. The experimental animals were randomly divided into four groups, each consisting of five animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 solution in water; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg; group III and group IV were test groups and were treated with MeOH extracts at the doses of 250 and 500 mg/kg respectively. Control, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7 %) injection. Then after an interval of 5 min, the number of writhes (squirms) was counted for 15 min.

Antidiarrhoeal Activity: Antidiarrhoeal activity of *P. suberosa* was tested by using the model of castor oil induced diarrhea in mice [11]. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Group-I was kept as control and received only distilled water containing 1% Tween-80; group-II was treated as 'positive control' and received standard antimotility drug Loperamide at a dose of 50 mg/ kg; group III was 'test group' and was treated with MeOH extract at dose of 500 mg/kg.

Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in 4 h study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (4 h). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones.

Cytotoxic Activity: Cytotoxicity of the extract was tested by using brine shrimp lethality bioassay [12]. Test solution (MeOH extract in DMSO) of different concentrations as 5, 15, 30, 60, 120, 240 and 400 µg/ml was applied to the test tubes containing hatched

brine shrimp naupli in sea water followed by counting the survived naupli after 24 h.

Statistical Analysis: Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

RESULTS:

Preliminary Phytochemical Analysis: Results of different chemical tests on the methanol extract of leaves of *P. suberosa* showed the presence of alkaloids, tannins, steroids, glycosides and saponins (**Table 1**).

TABLE 1: RESULTS OF DIFFERENT CHEMICAL GROUP TESTS OF *P. SUBEROSA*

Plant Extract	Alkaloids	Reducing Sugars	Tannins	Gums	Flavonoids	Saponins	Steroids	Glycosides
MeOH extract of *P. suberosa*	+	-	+	-	-	+	+	+

+: Positive result; -: Negative result

Antibacterial Activity: Table 2 showed the antibacterial activity of *P. suberosa* relative to that of the standard drug kanamycin. It showed moderate antibacterial activity against *Vibrio cholerae, Sheigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus saprophyticus* where the zone of inhibition was 13, 11, 12, 15, and 10 mm respectively while mild activity against *Shigella dysenteriae, Plesiomonas, Hafinia* and *Streptococcus pyogenes* where the zone of inhibition was 6-8 mm.

TABLE 2: *IN VITRO* ANTIBACTERIAL ACTIVITY OF MEOH EXTRACT OF *P. SUBEROSA*

Bacterial strains	Diameter of zone of inhibition in mm	
	MeOH extract (500 µg/disc)	Kanamycin (30 µg/disc)
Gram positive		
Staphylococcus aureus	12.7	24.9
Staphylococcus epidermis	15.14	25.82
Streptococcus saprophyticus	10.0	27.40
Streptococcus pyogenes	8.1	26.0
Gram negative		
Vibrio cholerae	13.0	26.68
Shigella dysenteriae	7.08	24.60
Plesiomonas	6.2	29.1
Sheigella sonnei	11.0	31.0
Hafinia	7.8	15.32

Analgesic Activity: Table 3 showed the effect of the MeOH extract of *P. suberosa* on acetic acid-induced writhing model in mice. The extract produced about 38.60 % and 59.07% ($P<0.01$) writhing inhibition at doses of 250 and 500 mg/kg respectively, which was comparable to the standard drug diclofenac sodium where the inhibition was about 77.78 % ($P<0.001$) at the dose of 25 mg/kg.

TABLE 3: EFFECT OF *P. SUBEROSA* ON ACETIC ACID INDUCED WRITHING IN MICE

Animal Group/Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 solution in water, p.o.	34.2 ± 3.22 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	7.6 ± 2.926** (22.22)	77.78
Test group-1 MeOH extract 250 mg/kg, p.o.	21 ± 2.168* (61.40)	38.60
Test group-II MeOH extract 500 mg/kg, p.o.	14 ± 3.86* (40.93)	59.07

Values are expressed as mean ± SEM (n=5); **: $P<0.001$; *: $P<0.01$; vs. control

Antidiarrhoeal Activity: Table 4 showed the effect of *P. suberosa* on castor oil induced diarrhea in mice. The extract caused an increase in latent period (0.60 hr) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg as compared to the standard antidiarrhoeal agent loperamide where the mean latent period was 0.93 min (**Table 4a**). The extract also decreased the frequency of defecation at the dose of 500 mg/kg where the mean numbers of stool at the 1st, 2nd, 3rd, and 4th hour of study were 7.4, 8, 6.6 and 3.2 respectively which was comparable to the standard drug loperamide where the mean numbers of stool were 7, 8.4, 6.2 and 3.4 respectively (**Table 4b**).

TABLE 4A: EFFECT OF *P. SUBEROSA* ON CASTOR OIL INDUCED DIARRHOEA IN MICE (LATENT PERIOD)

Animal Group/Treatment	Dose (p.o.)	Latent period (h)
Control (1% tween-80)	10 ml/kg	0.399 ± 0.039
Positive control Loperamide	50 mg/kg	0.929 ± 0.025**
Test group MeOH extract	500 mg/kg	0.602 ± 0.019*

Values are expressed as mean ± SEM (n=5); **: $P<0.001$; *: $P<0.01$; vs. control

TABLE 4B: EFFECT OF *P. SUBEROSA* ON CASTOR OIL INDUCED DIARRHOEA IN MICE (NUMBER OF STOOLS)

Animal Group/Treatment	Dose (p.o.)	Period of study (hr)	Total number of stool
Control (1% tween-80 solution in water.)	10 ml/kg	1	13.2 ± 0.583
		2	14.4 ± 0.678
		3	9.0 ± 0.447
		4	6.6 ± 1.077
Positive control (Loperamide)	50 mg/kg	1	7.0 ± 1.00*** 8.4 ± 0.748***
		2	6.2 ± 2.154
		3	3.4 ± 1.777*
		4	
Test group (MeOH extract)	500 mg/kg	1	7.4 ± 1.077**
		2	8.0 ± 1.00**
		3	6.6 ± 1.288
		4	3.2 ± 1.068*

Values are expressed as mean ± SEM (n=5); ***: *P*<0.001; **: *P*<0.01; *: *P*<0.02; vs. control

Cytotoxic Activity (Brine Shrimp Lethality Bio-Assay): In this bioassay, the extract showed lethality against the brine shrimp nauplii. The extract showed different mortality rate at different concentrations. The plot of percent mortality versus log concentration on the graph paper produced an approximate linear correlation between them. From the graph, the concentrations at which 50 and 90% mortality occurred were obtained by extrapolation (LC_{50}: 30 µg/ml; LC_{90}: 400 µg/ml) (**Table 5**).

TABLE 5: BRINE SHRIMP LETHALITY BIOASSAY OF THE MEOH EXTRACT OF *P. SUBEROSA*

Test sample	Conc. (µg/ml)	Log (Conc.)	Avg. no. of alive shrimp	% mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
MeOH extract	5	0.6989	8	20	30	400
	15	1.176	6	40		
	30	1.477	5	50		
	100	1.778	4	60		
	120	2.079	3	70		
Me. extract of *C. odollam*	240	2.380	2.5	75	10	39.81
MeOH extract	400	2.602	1	90	03	32

DISCUSSION: *In-vitro* antibacterial activity of *Polyalthia suberosa* was determined by disc diffusion method. Disc diffusion method is widely acceptable for the preliminary screening of antimicrobial activity. It is essentially a qualitative or semi qualitative test indicating the sensitivity or resistance of micro-organisms to the test materials [13]. On the basis of the result of disc diffusion method, it can be concluded that the MeOH extract of *P. suberosa* might possess antibacterial activity.

Analgesic activity of the MeOH extract of *P. suberosa* leaves was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [14]. Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intra-peritoneal administration of acetic acid [15]. On the basis of the result of acetic acid induced writhing test, it can be concluded that the MeOH extract of *P. suberosa* might possess analgesic activity.

Antidiarrhoeal activity of the MeOH extract of *P. suberosa* was tested using the model by castor oil induced diarrhea in mice. Castor oil, which is used to induce diarrhea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine.

The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface [16]. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase [17] or release prostaglandin [18].

On the basis of the result of castor oil induced diarrhoea, it can be concluded that the MeOH extract of *P. suberosa* might possess a significant antidiarrhoeal activity.

The cytotoxic activity of extract was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc.[19]. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

In conclusion, it can be suggested that the crude extract of leaves of *Polyalthia suberosa* may possess antibacterial, analgesic, antidiarrhoeal and cytotoxic effects, which correlate well with the traditional use of the plant. The activity of the leaf extract may be due to the presence of alkaloid and/or saponins present in it. Further researches, however, are essential to find out the active principles responsible for these activities.

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