ORIGINAL ARTICLE

Antimicrobial, cytotoxic and antidiarrhoeal activity of *Fimbristylis aphylla* L.

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This study was conducted to evaluate the antimicrobial, cytotoxic and antidiarrhoeal activity of *Fimbristylis aphylla* L methanol extract. Preliminary phytochemical screenings with the crude extractives demonstrated the presence of alkaloids, glycosides, saponins and reducing sugars. In the disc diffusion antimicrobial sensitivity test, the crude extractive of the whole plant produced moderate to strong antimicrobial activity against the test microorganisms. The zone of inhibition was found within the range of 10.33–15.33 mm. The strongest zone of inhibition was found against *Shigella dysenteriae*. The extractive was found active against only a few number of test pathogens. In the cytotoxicity test by brine shrimp lethality bioassay, the extract exhibited moderate cytotoxic activity by 50 and 90% mortality rates as LC_{50} and LC_{90} values of 5.87 and 9.33 µg/ml respectively. A moderate dose-dependent antidiarrhoeal activity was found by the methanol extract of the plant.

Key words: Antidiarrhoeal, antimicrobial, cytotoxic, *Fimbristylis aphylla*

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were 1 based on the uses of the agents in traditional medicines.[1] Bangladesh is a developing country and it covers a number of people unable to access the modern medical support. Most of the ill-fated people are usually dependent upon the Kabiraj (traditional medicine practitioners) for their health troubles.

Fimbristylis aphylla L. (Family: Cyperaceae) (Bengali: Boro-nirbish Ghass); a herb which is used as food for buffaloes. Traditionally it is used in eczema, burn and diarrhoea in the western area of Bangladesh having no published authentication. The other species of the genus *F. miliacea* L. roots are given in dysentery by a tribe in central India. *F. globulosa* (Retz.) is used as a medicine to treat enlarged spleen in Philippines.[2] The aim of the present study is to evaluate the antimicrobial sensitivity, cytotoxic and antidiarrhoeal activity of crude methanol extract of the targeted plant and to search logical evidence for its folk use and further exploitation.

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MATERIALS AND METHODS

Collection and Identification

The plant *F. aphylla* L. was collected from the potato field of Jessore in the month of December and was identified by the Forest Research Institute (FRI); Chittagong, Bangladesh where voucher specimens have been maintained.

Extraction

The plant under present study, after collection and proper isolation was subjected for shed-drying. Then it was ground into coarse powder and hot extraction[3] was carried out with 97% methanol by the soxhlet apparatus (Quickfit, England). The extraction was carried out about 18 h and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated under reduced pressure at 50°c by using a rotary evaporator (Heidolph, 560-91110-00-0, Germany).

Preliminary Phytochemical Screenings

For preliminary phytochemical screening of the crude ethanol extract was subjected to various tests [Table 1] for determination of chemical nature.[4-6]

Antimicrobial Screening

The antibacterial and antifungal activities of the methanol extract was evaluated by the disc diffusion method[7] against 4 Gram positive and 7 Gram negative

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pathogenic bacteria and 7 fungi [Table 2] using ciprofloxacin (CIPROCIN, 500 mg/Tab., Square Pharmaceuticals Ltd., Bangladesh) and fluconazole (FLUGAL, 50 mg/Cap., Square Pharmaceuticals Ltd.) as standards. The test pathogens were obtained as pure culture from the Faculty of Biology, University of Chittagong, Bangladesh. The antimicrobial activity due to the extractive was expressed by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiments were carried out in triplicate.

Table 1: Presumed chemical groups of the crude extract

Examination	Name of the test	Presumption
Reducing sugar	Fehling's solution test	+
	Benedict's test	+
Steroids	Salkowski test	-
	Libermann–Burchared test	-
Glycosides	Salkowski test	+
	Libermann–Burchared test	+
Tannins	Ferric chloride Test	-
	Potassium dichromate test	-
Alkaloids	Mayer's test	+
	Dragendorff's reagent test	+
	Wagner's reagent test	+
	Hager's reagent test	+
	Tannic acid test	+
Flavonoids	HCl test	-
Saponins	Shake test	+
Gums	Molisch's reagent test	-

(+) – Presence, (-) – Absence

Table 2: Growth inhibition by the methanol extract of *Fimbristylis aphylla*

Test microorganisms	Diameter zone of inhibition (mm)		MIC (µg/ml)
	FAME (50 µg/µl)	STD	
Gram positive bacteria		CFN (50 µg/µl)	
Bacillus subtilis	12.0±0.70[a]	16.0±0.82	31.25
Bacillus megaterium	nd	17.33±1.25	nd
Bacillus cereus	nd	16.67±0.94	nd
Staphylococcus aureus	10.33±1.25[a]	18.67±0.94	125
Gram negative bacteria			
Pseudomonas aeruginosa	nd	18.0±1.63	nd
Escherichia coli	nd	17.67±1.25	nd
Shigella dysenteriae	15.33±1.25[c]	15.67±0.47	15.625
Shigella sonnei	nd	20.67±0.47	nd
Salmonella typhi	nd	15.0±0.82	nd
Vibrio cholerae	nd	19.33±0.47	nd
Salmonella paratyphi	nd	17.33±0.47	nd
Fungi		FCN (50 µg/µl)	
Aspergillus niger	15.0±0.82[b]	16.67±0.47	15.625
Blastomyces dermatitidis	nd	16.0±0.82	nd
Candida albicans	nd	16.67±1.69	nd
Pityrosporum ovale	nd	17.33±0.47	nd
Trichophyton spp.	15.0±0.82b	16.33±1.25	15.625
Microsporum spp.	nd	16.67±0.47	nd
Cryptococcus neoformans	12.33±0.47[a]	16.0±0.82	31.25

[a]P<0.001; [b]P<0.01; [c]P<0.5; nd – Not determined, The diameter of zone of inhibition are expressed as Mean±SEM (n=3); SEM – Standard error of mean; A diameter less than 8 mm was considered inactive; STD – Standard drug; CFN – Cirpofloxacin; FCN – Fluconazole; MIC – Minimum inhibitory concentration; FAME – Methanol extract of *Fimbristylis aphylla*

Minimum Inhibitory Concentration

The Minimum inhibitory concentrations (MICs) of the extract was determined by serial tube dilution technique[8] in nutrient broth medium (HiMedia Laboratories Ltd., India), containing graded concentration of the plant extract and inoculated test micro-organisms. To detect MICs for test bacteria and fungi, ciprofloxacin and fluconazole were taken as standards respectively.

Brine Shrimp Lethality Bioassay

Cytotoxic activity test by brine shrimp lethality bioassay[9] was applied for determination of general toxic property of the plant crude methanol extract. Di-methyl sulfoxide (DMSO) solutions of the samples were applied against *Artemia salina* in a 1-day *exvivo* assay. The plant extract was dissolved in DMSO and solution of varying concentrations (10.0, 5.0, 2.5, 1.25, 0.625, 0.3125 and 0.15625 µg/ml) were obtained by serial dilution. Vincristine sulphate (VINCRISTIN-RICHTER Inj., Powder for reconstitution, 1 mg vial, Gedeon Richter/City Overseas Ltd.) was taken as standard. The experiments were carried out in triplicate.

Antidiarrhoeal Activity

In antidiarrhoeal activity by castor oil induced diarrhoea in mice,[10] young *Swiss-albino* mice (18–25 gm bw) of either sex were divided into control, positive control and two test groups containing five mice in each. Control group received 1% tween-80 (10 ml/kg o.p). The positive control group received loperamide (3 mg/kg o.p.) (IMOTIL, 2mg/Cap., Square Pharmaceuticals Ltd., Bangladesh); test groups received the methanol extract (250 and 500 mg/kg bw) orally. Acute diarrhoea was produced by oral administration of 0.4 ml of castor oil to each mouse. Then the latency period and total diarrheic secretion were counted for 4 h.

Statistical Analysis

Experimentally obtained primary data were manipulated as the source of responses. All experiments were performed in duplicate and replicated at least three times. Data were manipulated as mean±SEM (standard error of mean). Statistical differences between extract activities were determined using ANOVA followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when P<0.5.

RESULTS AND DISCUSSION

In the phytochemical investigation, it was observed that the

methanol extract of *F. aphylla* (FAME) contains alkaloids, glycosides, saponins and reducing sugars.

In the antimicrobial screening, the crude methanol extract of *F. aphylla* showed a short spectrum antimicrobial activity against the test pathogens. The highest zone of inhibition (15.33±1.25 mm) was found against the Gram negative bacteria, *Shigella dysenteriae*. Then followed by 15.0±0.82, 15.0±0.82, 12.33±0.47, 12.0±0.70 and 10.33±1.25 mm against, *Aspergillus niger*, *Trichophyton spp.*, *Cryptococcus neoformans*, *Bacillus subtilis* and *Staphylococcus aureus*. The extract was found to be inactive against *Bacillus megaterium*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella sonnei*, *Salmonella typhi*, *Vibrio cholerae*, *Salmonella paratyphi*, *Blastomyces dermatitidis*, *Candida albicans*, *Pityrosporum ovale* and *Microsporum spp.*

The crude extractive exhibited about a similar activity of standard on the growth inhibition of *Shigella dysenteriae.* Ciprofloxacin and fluconazole were taken as standards for antibacterial and antifungal sensitivity test respectively.

During the MICs test, mild to strong minimum inhibitory concentrations were produced by the extractive. Strong MIC (15.625 µg/ml) was found against *Shigella dysenteriae*, *Aspergillus niger* and *Trichophyton spp.* On the other hand, moderate MIC (62.50 µg/ml) was exhibited against *Bacillus subtilis* and *Cryptococcus neoformans*. A mild MIC was observed in case of the test bacteria, *Staphylococcus aureus*. The other test species were irresponsive to the methanol extract of *F. aphylla* L.

In the cytotoxicity test by brine shrimp lethality bioassay, the extract FAME showed a moderate cytotoxic activity in comparison to the standard, vincristine sulphate. The 50 and 90% mortality concentrations (LC_{50} and LC_{90}) were found to be of 5.87 and 9.33 µg/ml by the crude extract [Table 3].

Again, in the antidiarrhoeal test, the methanol extract of *F. aphylla* L. at the dose of 500 mg/kg reduced the total number of diarrhoeal faeces by 10.0±2.23 and increased the latency period by 6.65±9.29 h in comparison to the standard, loperamide. A half-fold graded activity was observed incase of the 250 mg/kg bw dose by the extractive [Table 4].

Table 3: Brine shrimp lethality bioassay by the extract of *Fimbristylis aphylla*

Sample	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Vincristine sulphate	0.45	0.89
FAME	5.87	9.33

Values LC_{50} and LC_{90} are expressed as µg/ml; FAME – Methanol extract of *Fimbristylis aphylla*

Table 4: Castor induced antidiarrhoeal episode by the extract *Fimbristylis aphylla*

Sample	TLP (hr)	TNF
Loperamide (3 mg/kg bw)	7.25±6.78[a]	6.4±1.151[c]
EEULS (250 mg/kg bw)	4.9±12.43[a]	14.4±2.44[a]
EEULS (500 mg/kg bw)	6.65±9.29[a]	10.0±2.23[a]

[a]$P<0.01$; [b]$P<0.02$; [c]$P<0.05$; TLP – Total latent period (Mean latent period±SEM); TNF – Total number of faeces (Mean defecation±SEM); FAME – Methanol extract of *Fimbristylis aphylla*

CONCLUSION

From the study, it is evident that the crude methanol extract of *Fimbristylis aphylla* L. showed moderate to strong antimicrobial activity. The extractive was also exhibited moderate cytotoxic and antidiarrhoeal activity. Antimicrobial and cytotoxic activity tests were carried out along with the justification of its traditional use in diarrhoea. Further investigation is required to isolate the bioactive principles.

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