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Preliminary Phytochemical and Pharmacological Investigations of *Alpinia conchigera* Griff. and *Plumbago indica* L.

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Abstract

Preliminary phytochemical screenings with the crude extractives of *Alpinia conchigera* Griff. and *Plumbago indica* L. demonstrated the presence of alkaloids, steroids, saponins and reducing sugars. The antibacterial and antifungal activities of methanol extracts of *A. conchigera* and *P. indica* have been evaluated against 4 Gram positive and 7 Gram negative pathogenic bacteria and 7 fungi using ciprofloxacin and fluconazole as standards, respectively, where the extract showed varying degrees of antimicrobial activity with zone of inhibition ranging from 15.0 to 27.0 mm. *A. conchigera* demonstrated significant zone of inhibition against *Aspergillus niger, Blastomyces dermatitidis, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Bacillus cereus Pseudomonas aeruginosa, Shigella sonnei, Vibrio cholerae* while *P. indica* revealed strong inhibitory activity against *Candida albicans, Blastomyces dermatitidis, Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Salmonella paratyphi.* The MICs of the both plant extractives were found to be 31.25 µg/ml against *B. cereus, S. paratyphi, V. cholerae, A. niger and B. dermatitidis.* In the brine shrimp lethality bioassay, the LC_{50} and LC_{90} of *A. conchigera* and *P. indica* were found to be 6.1 & 12.2 µg/ml and 5.0 &12.0 µg/ml, respectively. A significant dose dependent antidiarrhoeal, antimotility and analgesic activities were observed during screening in mice.

Key words: *Alpinia conchigera, Plumbago indica,* analgesic, antidiarrhoeal, antimicrobial, antimotility, cytotoxicity, MIC.

Introduction

Many of the plant materials used in traditional medicines are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine (Apulu *et al.*, 1994). Bangladesh is a developing country and it covers a large number of poor people having no access to modern medical support. Most of them are usually dependent upon the traditional practitioners for their health troubles.

Alpinia conchigera Griff. (Family: Zingiberaceae; Bengali: Burkill), a slender herb, about 0.6 to 1.5 m tall, is native to Bangladesh, Cambodia, India and Indonesia and naturalized to Laos, Malaysia, Myanmar, Thailand and Vietnam. Traditionally it is used in gastric pain, diarrhoea and dysentery in the southeast region of Bangladesh (Yusuf *et al*., 2007).

Plumbago indica L. (Family: Plumbaginaceae; Bengali: Agnichita), a perennial evergreen shrub with about 2 to 4 feet in height is traditionally used in skin disease, anaemia, irregular menstruation and leucorrhoea in the southeast area of Bangladesh (Yusuf *et al.,* 2007).

A comprehensive literature search revealed that *A. conchigera* have been studied for chemical constituents (Wong *et al.,* 2005), antimicrobial (Wannissorn *et al.,* 2009), gastroprotective (Pongpiriyadacha *et al*, 2008) and anticancer activities. On the other hand, *P. indica* have been subjected for preliminary phytochemical screening as well as antioxidant and antibacterial activities against few microorganisms. The antifungal activity of *Plumbago* species against *C. gloeosporidodes* has also been documented.

As a part of our continuing effect to study the medicinal plants of Bangladesh (Kaisar *et al.,* 2011; Kabir *et al.,* 2010), we evaluated the phytoconstituents, antimicrobial, antidiarrhoeal, antimotility and analgesic activities of crude methanol extract of *A. conchigera* and *P. indica* and we in here report the results of our preliminary investigation.

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Materials and Methods

Collection and identification: The plants selected for the present work, *A. conchigera* Griff. (Family: Zingiberaceae) and *P. indica* L. (Family: Plumbaginaceae), were collected from Naramuk, Rajasthali of Rangamati district, Bangladesh in October, 2010 and were identified at the Forest Research Institute; Chittagong, Bangladesh where voucher specimens have been maintained.

Extraction: The plant materials were subjected to drying in an oven below 40^0c. Then the crude dried plant was ground into coarse powder and subjected to hot extraction with 97% methanol by using a Soxhlet apparatus. The extraction was carried out about 18 hrs and filtered through a cotton plug followed by Whatman filter paper number # 1. The extract was then concentrated by using rotary evaporator.

Preliminary phytochemical screenings: The crude extracts were subjected to various tests (Table 1) for determination of chemical nature of the phytoconstituents.(Ali, 2009; Evans, 1934; Finer, 1983).

Antimicrobial screening: The antibacterial and antifungal activities of the crude extracts were evaluated by the disc diffusion method (Aboaba *et al.,* 2001) against 4 Gram positive and 7 Gram negative pathogenic bacteria and 7 fungi (Table-2) using ciprofloxacin and fluconazole as standards. The organisms were obtained as pure culture from the Faculty of Biology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was expressed by measuring the diameter of zone of inhibition expressed in mm. The experiments were carried out in triplicate.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of all the extract was determined by the serial dilution technique (Andrews *et al.,* 2001) in nutrient broth medium, containing graded concentration of the plant extracts and inoculated test organisms.

Brine shrimp lethality bioassay: Brine shrimp lethality bioassay (Laughlin, 1988; Lughilin, 1992; Maghrani *et al*., 2005; Persoone, 1980) technique was applied for determination of general toxic property of the plant extractives.

Antidiarrhoeal Activity: This test was carried out by castor oil-induced diarrhoea in mice (Thomas, 2001). Young *Swiss albino* mice, average weight 18-25gm of either sex were employed in the experiment. The animals were divided into control, positive control and two test groups containing five mice in each. Control group received 1% Tween-80 (10 ml/kg p.o). The positive control group received loperamide (3 mg/kg p.o) while the test groups received the methanol extract (250 and 500 mg/kg) 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 hrs.

Antimotility activity: In the *charcoal-meal* defecation method (Hérida and Maria, 2004) the experimental animals (Swiss albino mice) were divided into six groups each containing five mice. After fasting for 180 min the mice were administered with loperamide (*IMOTIL*, 2 mg/cap. Square Pharmaceuticals Ltd., Bangladesh) (5 mg/kg b.w. orally) as positive control, 1% Tween-80 as negative control group and 250 mg/kg b.w. and 500 mg/kg b.w. extracts as the test drugs. After 90 min, 0.3 ml of an aqueous suspension of 5% charcoal was orally administered to each animal. After 60 min the mice had free access to food and the animals were observed at 5 min intervals until faeces with charcoal were eliminated. The maximum time of observation was 450 min. Charcoal was observed on the faeces using normal light when it was easily visible, or using a microscope to help the detection of black spots. The results were based on the time for the charcoal to be eliminated.

Analgesic activity: For acetic acid induced (Whittle, 1964.) analgesic test, the samples, control and diclofenac-Na were given orally by means of a feeding needle to the test, control and positive control groups, respectively. After 30 minutes acute pain was induced by acetic acid solution (0.7%, 15 ml/kg; i.p. to each mouse) and the number of squirms (writhing) due to pain in the abdominal cavity was calculated for 20 minutes after administration of acetic acid.

Statistical analysis: The primary data obtained from the experiments were manipulated as the source of responses. For each of the extracts, three samples were prepared for each of the bioassays. Data were expressed 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

Preliminary phytochemical screenings: The crude extractives when tested with various chemical reagents demonstrated the presence of alkaloids, glycosides, saponins, tannins, steroids and reducing sugars as shown in Table 1.

Antimicrobial screening: The extracts of both plants showed varying degrees of antimicrobial activity (Table 2) with zone of inhibition ranging from 15.0 to 27.0 mm. *A. conchigera* demonstrated significant zone of inhibition against *A. niger, B. dermatitidis, S. aureus, S. typhi, Sh. dysenteriae, B. cereus P. aeruginosa, Sh. sonnei, V. cholerae* while *P. indica* revealed strong inhibitory activity towards *C. albicans, B. dermatitidis, B. cereus, S. aureus, E. coli* and *S. paratyphi.* Ciprofloxacin and fluconazole were taken as standards for antibacterial and antifungal test.

Table 1. Chemical groups present in methanol extracts of *A. conchigera* and *P. indica*

Test for	Name of the test/ Reagent	*A. conchigera*	*P. indica*
Reducing sugar	Fehling's solution test	+	+
	Benedict's test	+	+
Steroids	Salkowski test	+	+
Glycosides	Salkowski test	-	+
	Libermann-burchared test	-	-
Tannins	Ferric chloride	-	-
	Potassium dichromate	-	-
Alkaloids	Mayer's test	+	-
	Dragendorff's reagent	+	+
	Wagner's reagent	+	+
	Hager's reagent	+	+
	Tannic acid	+	+
Saponins	Shaking test for foaming	+	+

+ = presence, - = absence

Table 2. Antimicrobial activity of methanol extracts of *A. conchigera and P. indica* (500 µg /disc) and standard (50 µg/disc)

Test microorganisms	Zone of inhibition (mm)		
	MeOH extract of		Standard
	P. indica	*A. conchigera*	
Gram positive bacteria			Ciprofloxacin
Bacillus cereus	25.0±0.58[b]	23.0 ±0.34[b]	28.0 ±0.34[b]
B. megaterium	17.0 ±0.34[e]	21.0 ±1.17	25.0 ± 0.67[e]
B. subtilis	18.0 ±0.34[a]	20.0 ±0.34[b]	24.0 ±0.34[b]
Staphylococcus aureus	24.0±0.58[b]	24.0 ±1.17	27.5 ±0.34[d]
Gram negative bacteria			
Escherichia coli	23.0 ±0.34[b]	22.0 ± 0.58[e]	28.0 ±0.34[c]
Pseudomonas aeruginosa	21.0 ±0.67[c]	23.0 ±1.21	31.0 ±0.34[e]
Salmonella paratyphi	22.0 ±0.34[d]	20.0 ±0.34[b]	23.5 ±0.34[b]
S. typhi	20.0 ±0.34[d]	23.67±0.89	23.67±0.89
Shigella dysenteriae	21.0 ±0.34[a]	23.5 ± 0.58[e]	32.0 ± 0.34[b]
Sh. sonnei	18.0 ±0.34[a]	23.0 ± 0.34[b]	27.0 ± 0.58[e]
Vibrio cholerae	19.0 ±0.34[d]	19.33±0.34[b]	24.5±0.34[b]
Fungi			Fluconazole
Aspergillus niger	21.0 ±0.34[a]	26.0±0.58	27.0 ±0.34[b]
Blastomyces dermatitidis	25.0 ±0.34[a]	25.0 ±0.34[a]	22.0 ±0.34[a]
Candida albicans	27.0 ±0.34[b]	15.0±0.58	26.0 ±0.58
Cryptococcus neoformans	20.0 ±0.34[e]	15.0 ±0.89	21.0 ±0.34[a]
Microsporum spp.	25.0 ±0.34[e]	20.0 ±0.34[a]	23.0 ±0.34[a]
Pityrosporum ovale	20.0 ±0.34[a]	19.0 ±0.34[a]	22.0 ±0.34[a]
Trichophyton spp.	20.0 ±0.58[d]	24.0±0.58	29.0 ±0.34[a]

Minimum inhibitory concentration (MIC): During the MIC determination, the methanol extract of both of *A. conchigera* and *P. indica* inhibited the growth of test organisms between 31.25-62.50 µg/ml (Table 3). The low MIC values of the extract of *A. conchigera*, against *A. niger, B. cereus, B. dermatitidis, S. typhi, S. sonnei*, and *V. cholerae* that of *P. indica* against *B. cereus, B. dermatitidis, C. albicans, E. coli, S. aureus and S. paratyphi* suggest the presence of strong antimicrobial compounds in the extractives. Here, ciprofloxacin and fluconazole were taken as standard antibacterial and antifungal agent, respectively.

Table 3. Minimum inhibitory concentration (MIC) of methanol extract of *A. conchigera* and *P. indica*

Test organisms	Minimum inhibitory concentration (µg/ml)	
	A. conchigera	*P. indica*
Bacteria		
Bacillus cereus	31.25	31.25
Escherichia coli	62.50	31.25
Staphylococcus aureus	62.50	62.50
Salmonella paratyphi	-	31.25
S. typhi	31.25	62.50
Shigella dysenteriae	-	62.50
Sh. sonnei	31.25	-
Vibrio cholerae	31.25	-
Fungi		
Aspergillus niger	31.25	31.25
Blastomyces dermatitidis	31.25	31.25
Candida albicans	62.50	31.25

(-): MIC>100 µg/ml

Brine shrimp lethality bioassay: During brine shrimp lethality bioassay, the LC_{50} and LC_{90} of methanol extracts of *A. conchigera* and *P. indica* (Table 4) were found to 6.1- & 12.2 - and 5.0- & 12.0 µg/ml, respectively; as compared to 0.44- and 0.82 µg/ml for standard Vincristine sulphate.

Antidiarrhoeal activity: During antidiarrhoeal activity by castor oil induced test in mice, the methanol extract of *A. conchigera* and *P. indica* produced a dose dependent and significant antidiarrhoeal episode (Table 5). The onset of action and the total diarrheic faces were found to be 1.32 hr & 9.0 and 1.24 hr & 8.6 at 500 mg/kg for *A. conchigera* and *P. indica*, respectively. The half dose of the crude extract also prolonged the latency period and reduced the defecation in comparison to the standard loperamide.

Table 4. Brine shrimp lethality bioassay of methanol extract of *A. conchigera* and *P. indica*

Sample	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Vincristine sulphate	0.44	0.82
A. conchigera	6.1	12.2
P. indica	5.0	12.0

Table 5. Antidiarrhoeal episode of methanol extract of *A. conchigera* and *P. indica*

Sample	TLP (hr)	TNF
Loperamide (3 mg/kg)	1.35 ± 6.02^{a}	6.4 ± 1.151^{a}
A. conchigera (250 mg/kg)	1.16 ± 3.88^{b}	13.0 ± 0.79^{b}
A. conchigera (500 mg/kg)	1.32 ± 2.86^{b}	9.0 ± 1.369^{b}
P. indica (250 mg/kg)	0.94 ± 4.64^{b}	12.6 ± 2.10^{b}
P. indica (500 mg/kg)	1.24 ± 5.25^{b}	8.6 ± 1.20^{b}

$^{a}p<0.02$, $^{b}p<0.05$
TLP: Total latent period (Mean latent period± SEM); TNF: Total number of faeces (Mean defecation± SEM);

Antimotility activity: In antimotility activity by charcoal meal defecation, the extract produced significant delay for total excretion of charcoal from the intestinal cavity of the mice (Table 6). The time taken for charcoal meal defecation of *A. conchigera* and *P. indica* at 500 mg/kg b.w. were 370.8 min and 376.2 min, respectively. The half dose of the crude extract also delayed the defecation period in comparison to the standard loperamide.

Table 6. Antimotility activity of methanol extract of *A. conchigera* and *P. indica*

Treatment groups	Time until charcoal defecation (Min)
Loperamide (5 mg/kg)	379.8 ± 9.59
A. conchigera (250 mg/kg)	297.0 ± 15.06
A. conchigera (500 mg/kg)	370.8 ± 6.024
P. indica (250 mg/kg)	302.4 ± 15.11^{b}
P. indica (500 mg/kg)	376.2 ± 5.57^{b}

$^{a}p<0.001$, $^{b}p<0.05$. Total charcoal defecation: (Mean defecation period ± SEM)

Analgesic activity: During acetic acid induced writhing test for the analgesic activity in mice, the pain killer dose 500 mg/kg was found to be significant active in comparison to the standard, diclofenac-Na (Table 7). Total writhing by this dose were 32.6 and 31.4 for A. *conchigera* and *P. indica, respectively;* while the standard drug was 29.2. Both the selected dose for the extract also produced a dose dependent activity.

Table 7. Analgesic activity of methanol extract of *A. conchigera* and *P. indica*

Sample	No. of Writhing
Diclofenac-Na (25 mg/kg)	29.2±4.307[b]
A. conchigera.(250 mg/kg)	38.6±5.392[b]
A. conchigera 500 mg/kg)	32.6±5.76[b]
P. indica. (250 mg/kg)	37.8±4.82[b]
P. indica (500 mg/kg)	31.4±5.03[b]

[a]p<0.001, [b]p<0.05; No. of writhing: (Mean writhing± SEM);

Conclusion

From the study, it is evident that, the methanol extract of *A. conchigera* and *P. indica* showed moderate to strong antimicrobial activity, mild cytotoxicity against brine shrimp nauplii, significant dose dependent antidiarrhoeal, antimotility and analgesic activities. Further investigation is required to isolate the bioactive moieties. Bioactivities demonstrated by the extracts support the traditional uses of the plant in various diseases.

References

Aboaba, O.O., Smith, S.I. and Olude, F.O. 2006. Antibacterial effect of edible plant extracts on *Escherichia coli* 0157:H7. *Pak. J. Nutr*., **5**, 325-327.

Aboaba, O.O. and Efuwape, B.M. 2001. Antibacterial properties of some Nigerian spices. *Bio. Res. Comm.*, **13**, 183-188.

Andrews, J.M. 2001. Determination of minimum inhibitory concentrations. *J. Antimicrobial Chemothe.*, **48**, 5-16.

Apollo, N., Dada, J.O., Odama, L.E. and Galadima, M. 1994. Antibacterial activities of aqueous extracts of some Nigerian medicinal plants. *Nigerian J. Bot.*, **7,** 45-48.

Ali, M. 2009. Text Book of Pharmacognosy, New edn. CBS Publishers and Distributors, New Delhi, India, pp. 96-97, 140 & 283.

Bhal, B.S. and Bhal, A. 1992. A Text Book of Organic Chemistry, 13th edn. S. Chand & Company Ltd. pp. 5-6, 111-12 & 114.

Brunton, L.L. 1996. Agents affecting gastrointestinal water efflux and motility; emesis and anti-emetics; bile acids and pancreatic enzymes, 9th edn. pp. 917-36.

Evans, W.C. 1934. Trease and Evans Pharmacognosy, 15th edn. p. 64.

Finer, I.L.1983. Organic Chemistry, Vol. 2, 5th edn. Longman, Singapore Publisher Ltd. pp. 518, 767.

Ghani, A. 2003. Medicinal Plants of Bangladesh, 2nd edn. *Asiatic society of Bangladesh*, Dhaka, p. 418.

Hérida, R., Nunes, M., Maria, B. and Bastos, L. 2004. Protocol to refine intestinal motility test in mice, **38**, 257–260.

Kaisar, M.A. Rahman, M.S. Rahman, M.Z. Hasan, C.M. and Rashid, M.A. 2011. A review on phytochemicals from some medicinal plants of Bangladesh. *J. Pharm. Nutri. Sci.*, **1**, 87-95.

Kabir, S., Rahman, M.S., Chowdhury, A.M., Hasan C.M. and Rashid, M.A. 2010. An unusual bisnor clerodane diterpenoid from polygonum simiarum. *Nat. Prod. Commun.*, **5,** 1543-1546.

Laughlin, M.J.L. 1988. Brine Shrimp and Crown Gall Tumors: simple Bioassay for the Discovery of Plant Antitumor Agents. Proceedings, NIH Workshop, Bioassays for Discovery of Antitumor and antiviral Agent from Natural Sources. Bethesda, 18-19, 220.

Laties, V. 1987. Control of animal pain and distress in behavioral studies that use food deprivation or aversive stimuli. **191,** 1290–1.

Lughilin, M.J.L. 1992. Bench-top Bioassay for the Discovery of Bioactive Compound in Higher Plants. Brenesic, **29**.

Maghrani, M., Michel, J.B., Eddouks, M. and UFR PNPE, P.B. 2005. 21 Errachidia, Morocco, Hypoglycaemic activity of *Retama raetam* in rats, **19,** 125-8.

Yusuf, M.A. Wahab, M.Y., Chowdhury, J.U. and Begum, J. 2007. Some tribal medicinal plants of Chittagong Hill Tracts, Bangladesh. *Bangladesh J. Plant Taxon.*, **14,** 117-128.

Paech, K. and Tracey, M.V. Modern Method of Plant Analysis, **4**, 373.

Persoone, G. 1980. Proceeding of the International Symposium on Brine Shrimp, *Artemia Salina*, Belgium, Vol. 1-3.

Srivastava, O.P. 1984. Technology for Evaluation of Antimicrobial Properties of Natural Product. In: The use of Pharmacological Tech. for evaluation of natural products, Edited by Dewn, b. N., Srinal, R. C. 75.

Shoba, F.G., Thomas, M. 2001. Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea. *J. Ethnopharmacol.*, **76,** 73-76.

Whittle, B.A. 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesic. *Br. J. Pharmacol. Chemo.*, **22,** 246.