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

Mohammed Ibrahim[1], Md. Aslam Hossain[1], Md. Shafiullah Shajib[2] and Mohammad A. Rashid[1]

[1]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Pharmacy, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka-1217, Bangladesh

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ABSTRACT: The present study was conducted to evaluate the phytochemical constituents, anti-inflammatory, antipyretic, thrombolytic and CNS modulatory activities of the methanol, chloroform and *n*-hexane extracts of *Plumbago indica* L. and *Alpinia conchigera* Griff. The plants are used in the treatment of inflammations and fever in traditional systems of medicine in Bangladesh. Qualitative tests revealed that the crude extracts of the plants contain reducing sugars, steroids, alkaloids and flavonoids. The anti-inflammatory activity was evaluated by means of protein denaturation and membrane stabilization assay. The methanol extract of *P. indica* and *A. conchigera* demonstrated inhibition of protein denaturation by 34.55 and 29.55% while their *n*-hexane soluble extract exhibited 46.87 and 37.21% inhibition of lysis of erythrocyte membrane, respectively which were considerable as compared to the standard drug acetyl salicylic acid. The methanol extract of *P. indica* and *A. conchigera* prominently reduced Brewer's yeast-induced pyrexia which were close to the standard drug, paracetamol. A considerable thrombolytic activity was exhibited by the chloroform soluble extract of *A. conchigera* (48.27%) compared to the reference drug, streptokinase (72.13%) in red blood cell clot lysis test. The chloroform soluble materials of *P. indica* extract demonstrated noticeable reduction of CNS depression activity (63.52 % inhibition of locomotion), compared to reference drug diazepam (80.69 %) in open field experiment. The study justifies the medicinal applications of *P. indica* and *A. conchigera* in traditional systems and reveals the bioactivity of the plants which could be suitable for isolation and identification of the bioactive compounds.

Key words: *Alpiniaconchigera*, *Plumbago indica*, anti-inflammatory, antipyretic, thrombolytic, CNS depressant.

INTRODUCTION

Natural products are the valuable source of structurally diverse compounds, which possess therapeutic potential for treatment of human diseases. Many of the clinically used therapeutic agents are of natural origin, used either in the naturally occurring forms or as the derivatives or analogs after structural optimization. Among the natural resources, plant has been widely studied which include the discovery and development of anticancer, antifungal, antibiotics, antioxidants, immunostimulants, anti-inflammatory agents and others bio-molecules for managements of various life-threatening diseases as well as to improve human health.[1]

Plumbago indica L.is a flowering plant of Plumbaginaceae family. The plant is a less branched climbing herb with oblong leaves and bright red flowers. *Alpinia conchigera* Griff. (Zingiberaceae) is a rhizomatous herb with 90-120 cm high slender leafy stem, oblong leaves, 15-30 cm long, small flowers and pea size fruits. They are found in the southern areas in Bangladesh.[2] *P. indica* is used in skin disease, anemia, irregular menstruation, leucorrhoea and inflammations in the traditional systems of medicine.[3] Its root is implicated in fever and act as narcotic.[4] *A. conchigera* is used to relief gastric pain, diarrhea, dysentery and stop bleeding.

Correspondence to: Mohammad A. Rashid
Tel.: +88-02-9661900-73, Extn.8137; Fax: +88-02-9667222
E-mail: rashidma@du.ac.bd.

Rhizome of the plant is used in fever and skin rashes.[3,5]

As part of our continuing studies on medicinal plants of Bangladesh,[6-8] we conducted phytochemical and pharmacological screenings of methanol extract of two Bangladeshi medicinal plants *i.e., P. indica*. and *A. conchigera*, as well as to find out the scientific evidence for their folk uses.

MATERIALS AND METHODS

Collection and extraction of plant material. *P. indica* and *A. conchigera* were collected from Rajasthali, Bangladesh during October, 2010 and identified by the authentic taxonomist of Forest Research Institute, Chittagong, Bangladesh. Voucher specimens have been deposited to the Institute for future reference. The plants were collected in fresh condition during the season of maturity. They were cut into small pieces, sun-dried and then dried in an oven at 40-45°C. The dried plant materials were made into coarse powder following grinding and stored in an airtight container. The powdered material was extracted separately using 97% methanol by a Soxhlet apparatus. The process was repeated and allowed to continue until complete extraction was achieved. The obtained solution was filtered using Whatman filter paper No. 1. The extracts were then concentrated by using rotary evaporator. The methanol, chloroform and *n*-hexane soluble materials of the extracts were obtained from their aqueous solution (10% water in methanol) following the Kupchan method as described by Van Wagenen *et al*. (1993).[9] The extractives were used for screening of bioactivities.

Chemicals and drugs. The solvents and reagents used in this study were of analytical grade and purchased from Merck, Germany. Standard drugs such as loperamide, acetylsalicylic acid (ASA), diclofenac-Na, paracetamol, and diazepam were obtained from Square Pharmaceuticals Ltd. as gift samples.

Experimental animals. Swiss Albino mice (25-30 g) of either sex, 6-7 weeks age, were collected from the Animal Resources Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (icddr,b). The mice were maintained under standard laboratory conditions, temperature (27.0 ± 1.0°C), relative humidity (55-65%) and 12 hr light/12 hr dark cycle. The animals well fed with icddr,b formulated diet and water *ad libitum.* Animals had been treated and dealt according to the guidelines of the Swiss Academy of Clinical Sciences and Swiss Academy of Sciences. Animals were euthanized according to the principles for the Euthanasia of Animals: 2013 edition. All experiments were accomplished by considering the ethical requirements laid down in the declaration of Helsinki 2013.

Preliminary phytochemical screenings. The crude methanol extract of *P. indica* and *A. conchigera* were subjected to qualitative phytochemical tests to determine the chemical nature of the extracts, following the previously described procedures.[10-12]

***In vitro* anti-inflammatory activity.**

Protein denaturation assay. To determine the anti-inflammatory effect of the *P. indica* and *A. conchigera* extractives protein denaturation assay was performed according to the method described by Ullah *et al*. (2014).[13] Fifteen clean centrifuge tubes (three for standard acetyl salicylic acid, three for negative control methanol and three for each crude extract) were used. Then, 0.2 ml of 5% egg albumin solution and 2.8 ml phosphate buffer (pH 6.4 ±0.2) were added to all test tubes. Later on, 2.0 ml of acetyl salicylic acid (0.1 mg), Tween-80 or crude extract (500 µg/ml of Tween-80) was added as positive- and negative controls and test group, respectively. The reaction mixtures were incubated at 37 °C in a BOD incubator for 15 min. Then the mixtures were heated for 5 min at 70°C. They were filtrated followed by cooling and the absorbance of the filtrate was measured spectrophotometrically at 660 nm.

Membrane stabilizing activity. The membrane stabilizing activity of all extracts was assessed against hypotonic solution-induced erythrocyte

hemolysis as described by Shinde *et al*. (1999)[14], where acetylsalicylic acid was used as standard.

Antipyretic activity. The antipyretic activity of the *P. indica* and *A. conchigera* extractives was evaluated on Swiss albino mice (25-30 g) of either sex according to the method described by Naveed *et al.* (2012).[15] The animals were divided into eight groups, each group containing five mice. The temperature of body of each mouse was recorded before test using a digital thermometer. Then pyrexia was induced in the mice by injecting 20% aqueous suspension of Brewer's yeast (10 ml/kg b.w., s.c.). All groups were fasted overnight but free access to drinking water was provided. After 24h, rectal temperature of each mouse was recorded again. The induction of pyrexia was confirmed by rise in temperature of more than 32.9°C. Animals showing less than 32.9°C of temperature were excluded from the experiment. Group-I received saline (10 ml/kg, b.w.) as a negative control, group-II received paracetamol (150 mg/kg, b.w.) as standard drug while the test groups (Group III-VIII) received 500 mg/kg b.w. of the plants extract. Rectal temperature was recorded periodically after 1, 2 and 3 hrs of drugs administration.

Thrombolytic activity. The thrombolytic activity of the methanol extract of *P. indica* and *A. conchigera* was evaluated following the method developed by Miah *et al.* (2018)[16] using streptokinase as standard.

Open field test. The effect of the plant extracts on central nervous system (CNS) was examined by using open field test as described by Shajib *et al*. (2015).[17] The animals were divided into five groups, with each group consisting of five mice. Control group received normal saline (10 ml/kg b.w, p.o), positive control group received diazepam (1 mg/kg b.w., i.p.) and the test group received *P. indica* or *A. conchigera* extract (500 mg/kg b.w., i.p.). After 30 min of treatment of normal saline, diazepam or the plant extract, mice were transferred individually in the centre of the open field. Then, crossing of open field squares within 5 min was recorded.[18,19]

Data analysis. Results are expressed as the mean ± SEM. The data calculation was performed using the programme Microsoft Excel 2016, Microsoft Corporation, USA.

RESULTS AND DISCUSSION

Egg albumin denaturation assay is a suitable method for the determination of anti-inflammatory effect of drugs.[13] The anti-inflammatory activity of the plant extractives was measured by the means of egg albumin denaturation assay i.e. protein denaturation assay. Protein denaturation evokes release of Type III hyper-sensetive reaction related antigens which are associated with the pathogenesis of diseases such as glomerulo-nephritis and serum sickness.[18] It has been evidenced that NSAID drugs exhibit their effect by inhibition of protein denaturation.[19] In the present study, *n*-hexane soluble material of both *P. indica* and *A. conchigera* demonstrated considerable inhibition of protein denaturation by 34.55 and 29.55%, respectively. However, standard drug acetylsalicylic acid inhibited 58.18% of the protein denaturation which was higher than any of the plant extractives (Table 1). The result suggests that the plant extracts have diminishing effect on denatured protein-induced antigens as well as anti-inflammatory effect like NSAID drugs.

Table 2 shows that the methanol extract of the plants revealed prominent inhibition of erythrocyte red blood cell (RBC) hemolysis. The effect of *P. indica* (46.87%) was higher than *A. conchigera* (37.21 %). However, the maximum inhibitory effect was 45.00 % which was shown by standard drug, acetylsalicylic acid. The chloroform extract of *P. indica* also demonstrated considerable inhibition of RBC hemolysis (32.50%). RBC membrane is considered alike cell membrane.[20] The rupture of cell membrane facilitates the release of content of lysosome (e.g. hydrolytic enzymes) which are associated to inflammatory process.[21] The result (Table 2) suggest that the plant extracts could have anti-inflammatory potential via membrane stabilization mechanism.

Table 1. Effect of *P. indica* and *A. conchigera* extractives in protein denaturation assay.

Test sample	% Inhibition of protein denaturation
ASA	58.18 ± 0.001
MEPI	20.00 ± 0.002
CEPI	28.30 ± 0.002
HEPI	34.55 ± 0.002
MEAC	8.17 ± 0.0004
CEAC	23.08 ± 0.003
HEAC	29.55 ± 0.001

Data are presented as mean ± SEM (n = 3). MEPI = methanol extract of *P. indica*, CEPI = chloroform extract of *P. indica*, HEPI = *n*-hexane extract of *P. indica*, MEAC = methanol extract of*A. conchigera*, CEAC = chloroform extract of *A. conchigera*, HEAC = *n*-hexane extract of*A. conchigera*. ASA = acetylsalicylic acid.

Table 2. Effect of *P. indica* and *A. conchigera* extractives on RBC membrane lysis.

Test sample	%Inhibition of hemolysis
ASA	75.00 ± 0.002
MEPI	46.87 ± 0.007
CEPI	32.50 ± 0.002
HEPI	20.88 ± 0.004
MEAC	37.21 ± 0.001
CEAC	27.40 ± 0.003
HEAC	22.06 ± 0.003

Data are presented as mean ± SEM (n = 3). MEPI = methanol extract of *P. indica*, CEPI = chloroform extract of *P. indica*, HEPI = *n*-hexane extract of *P. indica*, MEAC = methanol extract of*A. conchigera*, CEAC = chloroform extract of *A. conchigera*, HEAC = *n*-hexane extract of *A. conchigera*. ASA = acetylsalicylic acid.

Brewer's yeast-induced pyrexia is a convenient experiment for the assessment of antipyretic potentiality of synthetic drugs as well as plant products.[15] Subcutaneous injection of the yeast could evoke the production of prostaglandin and increases body temperature which is known as pathogenic fever.[22] Antipyretic drug e.g. paracetamol exerts its effect by the inhibition of prostaglandin production.[23] The result demonstrates that methanol extract of *P. indica* noticeably reduced the yeast-induced hyperthermiain mice over the experimental period which was comparable to standard drug paracetamol (Table 3). The effect of chloroform and *n*-hexane extract of *A. conchigera* was also considerable from second hours to end of the experiments. The maximum antipyretic effect was observed for methanol extract of *A. conchigera* by 35.77ºC, while the highest effect for paracetamol was 36.08ºC at 3rd h. The effect of the extractives indicates that they could have potential of inhibition of prostaglandin synthesis as well as prominent antipyretic effect.

Thrombosis occur due to the clot formation of blood as well as interruption of blood supply in the blood vessel which may result in severe diseases e.g. myocardial infarction, venous embolism.[24,25] Antithrombotic drugs (e.g. reteplase, streptokinase, urokinase) help to cleave or dissolve the clotsoccluded in the blood vessel and thereby help to restore normal blood flow.[26] As shown in table 4

Table 3. Antipyretic effect of *P. indica* and *A. conchigera* extractives on yeast-induced pyrexia in mice.

Treatment	Dose	Rectal temperature (ºC)			
		0 hr	1 hr	2 hrs	3 hrs
Normal saline (control group)	10 ml/kg b.w.	39.21 ± 0.28	38.97 ± 0.61	39.34 ± 0.47	38.27 ± 0.91
Paracetamol	150 mg/kg b.w.	38.80 ± 0.31	37.03 ± 0.74	36.29 ± 0.94	36.08 ± 0.87
MEPI	500 mg/kg b.w	38.85 ± 0.73	37.63 ± 0.35	36.92 ± 0.45	36.30 ± 0.68
CEPI	500 mg/kg b.w	38.68 ± 0.69	38.59 ± 0.74	38.18 ± 0.64	36.51 ± 0.55
HEPI	500 mg/kg b.w	38.65 ± 0.81	38.61 ± 1.16	38.81 ± 0.49	38.29 ± 0.91
MEAC	500 mg/kg b.w	39.42 ± 1.04	38.13 ± 0.77	37.52 ± 0.41	35.77 ± 0.57
CEAC	500 mg/kg b.w	38.73 ± 0.35	38.19 ± 0.80	37.50 ± 1.05	36.83 ± 0.29
HEAC	500 mg/kg b.w	38.67 ± 1.03	38.53 ± 0.56	38.39 ± 0.98	38.01 ± 1.35

Data are presented as mean ± SEM (n = 5). MEPI = methanol extract of *P. indica*, CEPI = chloroform extract of *P. indica*, HEPI = *n*-hexane extract of *P. indica*, MEAC = methanol extract of *A. conchigera*, CEAC = chloroform extract of *A. conchigera*, HEAC = *n*-hexane extract of *A. conchigera*.

the chloroform extract of *A. conchigera* produced considerable blood clot lysis by 48.27%. The standard drug, streptokinase showed maximum lysis of clot (72.13%), compared to the extractives of the plants. *P. indica* exhibited highest 27.56% of clot lysis by its chloroform soluble extract. The result indicates that the plant extracts could possesses potential substances responsible for thrombolytic activity.

Table 4. Thrombolytic (blood clot lysis) activity of *P. indica* and *A. conchigera* extractives.

Test sample	Clot lysis (%)
Streptokinase	72.13 ± 0.07
MEPI	16.00 ± 0.04
CEPI	27.56 ± 1.23
HEPI	06.89 ± 0.09
MEAC	30.76 ± 1.04
CEAC	48.27 ± 0.02
HEAC	09.52 ± 0.05

Data are presented as mean ± SEM (n = 3). MEPI = methanol extract of *P. indica*, CEPI = chloroform extract of *P. indica*, HEPI = *n*-hexane extract of *P. indica*, MEAC = methanol extract of *A. conchigera*, CEAC = chloroform extract of *A. conchigera*, HEAC = *n*-hexane extract of *A. conchigera*.

Pharmacological studies evidenced that, CNS acting drugs such as narcotic analgesics (e.g. morphine, codeine) anxiolytics (diazepam, clonazepam) cause significant reduction of locomotion i.e. depression of animals in open field experiment.[27,28] Table 5 demonstrated that, intraperitoneal administration of chloroform soluble extract of *P. indica* remarkably reduced the number of open field square crossing (84.99 ± 2.94) by 63.52 % compared to control group. Standard drug exhibited maximum reduction of square crossing as (45.00 ± 1.41) by 80.69%. Intraperiton ealingestion of methanol extract produced highest reduction (123.99 ± 5.67, 44.65%) of square crossing for *A. conchigera*. Thus, it could be suggested that the plant extractives possess CNS depressant activity. In addition, the effect of *P. indica* complies with its medicinal use as narcotic in folk medicine.

The phytochemical screenings of methanol extract *of P. indica* and *A. conchigera* revealed the occurrence of secondary metabolites including reducing sugars, steroids, alkaloids and flavonoids. The assay showed that, amide was present only in *P. indica* extract and saponins and gums were present in

Table 5. Effect of *P. indica* and *A. conchigera* extractives in open field test.

Treatment	Dose	Number of square crossed	% Inhibition of square crossed
Normal saline (control group)	10 ml/kg, p.o.	233.01±4.97	-
Diazepam	1 mg/kg, i.p.	45.00 ± 1.41	80.69
MEPI	500 mg/kg, i.p	141.00 ± 2.83	39.49
CEPI	500 mg/kg, i.p	84.99 ± 2.94	63.52
HEPI	500 mg/kg, i.p	174.99 ± 2.94	24.90
MEAC	500 mg/kg, i.p	123.99 ± 5.67	44.65
CEAC	500 mg/kg, i.p	159.00 ± 1.87	29.02
HEAC	500 mg/kg, i.p	200.01 ± 3.19	10.71

Data are presented as mean ± SEM(n = 5). MEPI = methanol extract of *P. indica*, CEPI = chloroform extract of *P. indica*, HEPI = *n*-hexane extract of *P. indica*, MEAC = methanol extract of *A. conchigera*, CEAC = chloroform extract of *A. conchigera*, HEAC = *n*-hexane extract of *A. conchigera*.

Table 6. Phytochemical analysis of methanol extract of *P. indica*. and *A. conchigera*.

Chemical group	MEPI	MEAC
Alkaloids	+	+
Glycosides	-	-
Steroids	+	+
Tannins	-	-
Flavonoids	+	+
Saponins	-	+
Reducing sugars	+	+
Gums	-	+
Amides	+	-

MEPI = methanol extract of *P. indica*, MEAC = methanol extract of *A. conchigera*
+ = presence, - = absence.

A. conchigera extract. Both plant extract did not exhibit the presence of glycosides and tannins (Table 6). It has been reported that plant derived alkaloids, flavonoids, steroids exert remarkable anti-inflammatory and CNS modulatory effects.[29-32] Therefore, the presence of such phytochemicals in the investigated plants could be responsible for their anti-inflammatory as well as CNS activities.

CONCLUSION

The results of the study revealed substantial pharmacological activities of *P. indica.* and *A. conchigera.* The methanol and *n*-hexane soluble extracts of *P. indica* and *A. conchigera* possess noticeable anti-inflammatory activity. The methanol extracts of both plants possess considerable antipyretic activity. The chloroform soluble materials of *A. conchigera* exhibited prominent thrombolytic while *P. indica* showed CNS depressant activities. The results of the present investigation rationalize the ethnomedicinal uses of these plants in the treatment of inflammation and fever. This preliminary research outcomes will provide well insight for further phytochemical and pharmacological investigations to identify the bioactive compounds from these plants.

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