Preliminary Phytochemical Screenings and Pharmacological Activities of Three Medicinal Plants of Bangladesh

Mohammed Ibrahim[1], Md. Ruhul Kuddus[1], Md. Aslam Hossain[1], Muhammad Abdullah Al-Mansur[2] and Mohammad A. Rashid[1]

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka Dhaka-1000, Bangladesh
[2]Institute of National Analytical Research and Services (INARS), BCSIR, Dhaka-1205, Bangladesh

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ABSTRACT: The present study was designed to evaluate the preliminary phytochemical screening and antimicrobial, antidiarrheal, anti-inflammatory, analgesic, antipyretic, anxiolytic, thrombolytic and membrane stabilizing properties of the methanolic extract of three medicinal plants *Perilla ocymoides* L., *Murraya koenigii* (Linn.) Spreng., *Baliospermum montanum* (Wild.) Muell growing in Bangladesh. In antimicrobial test, maximum zone of inhibition was found against *Salmonella* typhi (18.0 mm) and *Escherichia coli* (17.0 mm) by *B. montanum* extract. In the castor oil-induced antidiarrheal assay, the methanol extract of *M. koenigii* showed maximum 50% inhibition of defecation. During *in-vitro* anti-inflammatory test, the methanol extract of *B. montanum* at 500 µg/ml, b.w. revealed 39.62% inhibition of protein denaturation. Due to analgesia, the *M. koenigii* extract showed 53.29% inhibition of acetic acid-induced writhing reflex in experimental mice. Antipyretic effect of *P. ocymoides*, *M. Koenigii* and *B. montanum* extractives was assessed by Brewer's yeast-induced pyrexia in mice. The *B. montanum* extract possesses significant anxiolytic effect that was evidenced by both hole cross test and open field test in mice. In thrombolytic assay, the highest activity (57.81%) was observed by *B. montanum* extract. Results of the preliminary phytochemical screenings demonstrated the presence of alkaloids, glycosides, flavonoids, reducing sugars, gums etc.

Key words: *Perilla ocymoides*, *Murraya koenigii*, *Baliospermum montanum*, antimicrobial, antidiarrheal, anti-inflammatory, analgesic, antipyretic, anxiolytic, thrombolytic, membrane stabilizing.

INTRODUCTION

Bangladesh is a prominent source of numerous medicinal plants among the South Asian countries. About 250 species of medicinal plants are used for the preparation of traditional medicines which is the half of total species of plants grown in Bangladesh.[1] But the uses of most of these plants have no scientific basis. The majority of these plants have not yet undergone extensive chemical, pharmacological, and toxicological studies to identify their bioactive compound(s). Therefore, evaluation of medicinal plants with important biological activity is an essential task to discover new lead compounds from natural sources.[2,3]

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

Perilla ocymoides L. (Bengali name: Ban tulsi; Family: Lamiaceae) is an annual herb of the mint family which is an edible plant and native to East Asia. The entire plant is rich in vitamins and minerals.[4] It has antiasthmatic, antidote, antimicrobial, antipyretic, antiseptic, anti-allergic properties.[5,6] The seeds of *Perilla* species contain different polyphenols or flavones. The essential oil of the plant is used as flavouring agent in food industry.

Murraya koenigii (Linn.) Spreng. (Family: Rutaceae), commonly termed as curry leaves, is basically known for its smell and medicinal property. This plant has been reported to show antidiabetic and immunomodulatory effects.[7,8] The aromatic leaves used in cooking curry are reported to possess antioxidant, antidiabetic, anti-inflammatory, hepatoprotective and hypolipidemic activities.[9-12]

The carbazole alkaloids isolated from leaves are suggested to have stimulating effects on the central nervous system.[13]

Baliospermum montanum (Wild.) Muell. is a Bangladeshi medicinal plant which belongs to Euphorbiaceae family. The roots, leaves and seeds of this plant are used medicinally for different activities. Root is used in abdominal pain, constipation, piles, calculus, helminthiasis and scabies. The leaves are used in treating asthma and seeds are used in snakebite. Recently, the ethanolic extract of leaves of this plant has shown antimicrobial activity against test microorganisms. The plant contains phorbol esters, 12-deoxyphorbol and 12-deoxy-16-hydroxyphorbol which are reported to exhibit antilukaemic and cytotoxic activities.[14,15]

As part of our continuing studies on medicinal plants of Bangladesh,[16,17] we conducted phytochemical- and pharmacological-screenings of methanol extract of three Bangladeshi medicinal plants *i.e., Perilla ocymoides, Murraya koenigii, Baliospermum montanum* as well as to find out the logical evidence for their folk uses.

MATERIALS AND METHODS

Plant materials. The seeds of *Perilla ocymoides,* leaves of both *Murraya koenigii* and *Baliospermum montanum* were collected from Naramuk, Rajasthali of Rangamati district, Bangladesh in June 2010 and were identified at the Forest Research Institute, Chittagong, Bangladesh, where voucher specimens have been maintained for future reference.

Drying and grinding. The collected plant materials were washed with running tap water and then were dried at a temperature not exceeding 40ºC in an oven. The dry materials were ground to a coarse powder with the help of a grinding machine and kept in an airtight container until extraction was commenced.

Hot extraction. The powdered materials (150 g each) were extracted with 750 ml of methanol (99.98%) in a Soxhlet apparatus (Quickfit, England). The extracts were separately concentrated with a rotary evaporator (Heidolph, Germany) under reduced temperature and pressure to provide gummy residues.

Chemicals. All chemicals and solvents used in this study were of analytical grade and purchased from Merck, Germany. Standard drugs such as loperamide, acetyl salicylic 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 of 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[18] of temperature (27.0 ± 1.0°C), relative humidity (55-65%) and 12 hr light/12 hr dark cycle. The animals are fed with icddr,b formulated diet and water *ad libitum.*

Preliminary phytochemical investigations. For preliminary phytochemical investigation, the crude methanol extracts of *P. ocymoides, M. koenigii* and *B. montanum* were subjected to various tests (Table 1) to determine the chemical nature of the extracts.[19-21]

Test for antimicrobial activity. The preliminary antimicrobial activity of *P. ocymoidies, M. koenigii* and *B. montanum* extractives were determined by the disc diffusion method[22] 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 (30 µg) discs were used as references.

Test for anti-diarrheal activity. The *P. ocymoides, M. koenigii* and *B. montanum* extractives were subjected to assay for anti-diarrheal activity which was carried out by castor oil-induced diarrhea in mice.[23] For this purpose, the animals were divided into negative control, positive control and three test groups containing five mice in each. The negative control group mice received 1% Tween-80 (10 ml/kg, p.o). The positive control group received loperamide (3 mg/kg b.w., p.o.), while the test groups were

administered with each of the methanol extract (500 mg/kg, b.w.) orally. Acute diarrhea was induced 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 using standard protocol.[23]

Test for anti-inflammatory activity. To determine the anti-inflammatory activity of the *P. ocymoides, M. koenigii* and *B. montanum* extractives, 15 clean centrifuge tubes (three for standard acetyl salicylic acid, three for negative control methanol and three for each crude extract) were used. Then, 1.0 ml of 5% egg albumin solution was added to all test tubes. Later on, 1.0 ml of acetyl salicylic acid (0.1 mg), 1.0 ml of Tween-80 and 1.0 ml of crude extract (500 µg/ml of Tween-80) were added to the positive- and negative-controls and test groups, respectively. All the reaction mixtures were adjusted to pH 5.6±0.2 by 1N HCl, heated, cooled and finally, after filtration, the absorbance was measured spectrophotometrically at 660 nm.[24]

Test for analgesic activity. The analgesic activity of the *P. ocymoides*, *M. koenigii* and *B. montanum* extractives was evaluated using acetic acid-induced writhing method in mice.[25] Experimental animals were divided into negative control, positive control and three test groups containing five mice in each. The mice of each group received a particular treatment *i.e.* negative control, standard drug and one dose of each extract. Test samples (500 mg/kg, b.w. of the plant extract), 1% Tween-80 and diclofenac-Na were given orally by means of feeding needle. An interval of thirty minutes was given to ensure proper absorption of the administered substances. Then, the writhing inducing chemical acetic acid solution (0.7%, 15 ml/kg b.w.) was administered intraperitoneally to all mice. After an interval of 5 mins, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 5 mins.

Test for antipyretic activity. The antipyretic activity of the *P. ocymoides*, *M. koenigii* and *B. montanum* extractives was evaluated on Swiss Albino mice (25-30 g) of either sex. The animals were divided into five groups, each group containing five mice. The normal body temperature of each mouse was recorded using digital thermometer and then pyrexia was induced in all mice by injecting 20% aqueous suspension of Brewer's yeast (10 ml/kg b.w., s.c.).[26] All groups were fasted overnight but free access to drinking water was provided. After 24 h, rectal temperature of each mouse was recorded again. The induction of pyrexia was confirmed by rise in temperature of more than 32.9 °F, while animals showing less than 32.9 °F rise 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 received 500 mg/kg b.w. of the plant extract, respectively. Rectal temperature was recorded periodically after 1, 2 and 3 hrs of drugs administration.

Test for anxiolytic activity

Treatment schedule. The anxiolytic activity of the plant extractives was examined by using the hole board test and open field test. The animals were divided in to five groups, with each group consisting of three mice. First group received normal saline, second group received diazepam (1 mg/kg b.w.) and the remaining groups received plant extract (500 mg/kg b.w.).

Hole cross test. The hole board is a white painted wooden board (30 cm×20 cm×14 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the base of box. The test groups received crude extract at the dose of 500 mg/kg b.w. orally whereas the negative- and positive- control group mice received saline and diazepam (2 mg/kg, i.p.), respectively. The number of passages of a mouse through the hole from one chamber to the other was counted for a period of 30 min after 30 min of oral administration of the test drug.[27]

Open field test. The open field test is employed to observe general motor activity, exploratory behavior and measure anxiety. The open field area was made of plain wood and consisted of a square area (45 cm × 45 cm × 20 cm). The floor had a

square sheet of wood (45 cm ×45 cm) with the surface divided into sixteen small squares. Experimental animals were divided into five groups of 3 mice and treated similarly as described in the hole cross test. About 30 min after treatment, mice of both the control and treated groups were placed individually in the center of the open field and behavioral activities were video recorded for 30 min. Subsequently, hand operated counters and stopwatches were used to score the following behavioral parameters for a period of 30 min: (1) the number of entries and time spent in the centre, (2) periphery and corners of the field, (3) the number of crossings (number of square floor units entered) as a measure of distance travelled, (4) rearing (number of times the animal stood on hind legs) and (5) assisted rearing (forepaws touching the walls of the apparatus).[27]

Test for thrombolytic activity. The thrombolytic activity of the *P. ocymoides, M. koenigii* and *B. montanum* extractives was evaluated following the method developed by Prasad *et al.*[28]using streptokinase as standard.

Test for membrane stabilizing activity. The membrane stabilizing activity of all extracts was assessed by hypotonic solution-induced erythrocyte hemolysis designed by Shinde *et al.*[29]using acetyl salicylic acid as standard.

Statistical analysis. Results are expressed as the mean ± SEM. Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group, where $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical tests. The phytochemical screenings of *P. ocymoides, M. koenigii,* and *B. montanum* extractives revealed the occurrence of various bioactive secondary metabolites such as alkaloids, glycosides, flavonoids, reducing sugars, gums etc. (Table 1). All these extracts contained alkaloids, flavonoids and reducing sugars while steroids and tannins were present only in *M. koenigii* extract and *P. ocymoides* extract, respectively. The presence of biologically important phytochemicals in the *P. ocymoidies, M. koenigii* and *B. montanum* extractives, is believed to contribute to their medicinal values, and therefore, point to potential sources for useful drugs.

Antimicrobial activity. *P. ocymoides, M. koenigii,* and *B. montanum* extracts were screened for antibacterial and antifungal activities by employing the disc diffusion method.[22] The activity was recorded as diameter of zone of inhibition using the crude extract at the concentration of 500 µg/disc. During screening for antibacterial activity, all the plant extracts exhibited mild to strong antibacterial activity (zone of inhibition = 9.0-18.0 mm) against both Gram positive and Gram negative bacteria (Table 2). Among all test organisms, *S.* Typhi and *E. coli* were found to be most sensitive to *B. montanum* extracts. On the other hand, the extracts of both *P. ocymoides* and *M. koenigii* showed moderate antifungal (zone of inhibition = 9.0-16.0 mm activity while *B. montanum* did not show any activity against the fungal species (Table 2) used in the screening.

Antidiarrheal activity. In castor oil-induced diarrhea, crude extract of *P. ocymoides, M. koenigii* and *B. montanum* at 500 mg/kg b.w. revealed different degrees of anti-diarrheal activity as evident by the reduction of defecation (Table 3). In general, the imbalance between the absorptive and secretory mechanisms in the intestinal tract leads to induce diarrhea. Ricilonic acid, an active component of castor oil, stimulates the peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. In this study, the plant extracts exhibited prominent anti-diarrheal activity, which may be attributed to the phytochemical constituents such as flavonoids, tannins, saponins etc.[30]

***In vitro* anti-inflammatory activity**. In the present study for *in vitro* anti-inflammatory properties, the crude methanol extract of *B. montanum* and *M. koenigii* at 500 µg/ml showed 39.62% and 32.08% inhibition of protein

denaturation, respectively whereas standard ASA exhibited 62.26% (Table 4). On the other hand, the ability of *P. ocymoides* extract was found to be mild in inhibiting heat-induced protein denaturation.

Table 1. Chemical analysis for phytoconstituents in crude methanol extract of *P. ocymoides, M. koenigii* and *B. montanum*.

Plant extract	Chemical groups							
	Alkaloids	Glycosides	Steroids	Tannins	Flavonoids	Saponins	Gums	Amides
P. ocymoides	+	-	-	+	+	-	+	-
M. koenigii	+	+	+	-	+	-	-	-
B. montanum	+	+	-	-	+	-	+	-

Table 2. Antimicrobial activity of methanol extract of *P. ocymoides, M. koenigii* and *B. montanum*.

Microorganisms	Diameter of zone of inhibition (mm)			
	POME (500 µg/disc)	MKME (500 µg/disc)	BMME (500 µg/disc)	Ciprofloxacin (30 µg/disc)
Gram positive bacteria				
Bacillus cereus	15.0±0.60	13.0±1.03	14.0+0.59	22.0±0.09
B. megaterium	11.0±0.36	15.0±0.62	16.0+0.15	25.0±1.53
B. subtilis	12.0±1.45	-	11.0+0.28	21.0±0.42
Staphylococcus aureus	12.0±1.61	12.0±1.57	-	28.0±0.65
Gram negative bacteria				
Escherichia coli	-	14.0±0.47	17.0+0.75	23.0±1.05
Pseudomonas aeruginosa	14.0±.57	12.0±0.58	9.0+0.09	26.0±0.56
Salmonella paratyphi	14.0±0.80	9.0±1.25	14.0+1.25	23.0±1.25
S. typhi	15.0±1.58	17.0±1.03	18.0+0.15	28.0±0.76
Shigella dysenteriae	12.0±0.57	12.0±0.57	-	25.0±0.83
Sh. sonnei	13.0±0.47	16.0±1.63	13.0+0.33	22.0±0.59
Vibrio cholerae	15.0±1.70	15.0±1.08	10.0+0.57	23.0±1.34
Fungi				**Fluconazole** (30 µg/disc)
Aspergillus niger	12.0 ± 0.57	14.0 ± 0.64	-	26.0 ± 0.12
Blastomyces dermatitidis	15.0 ± 1.88	11.0 ± 1.45	-	22.0 ± 0.13
Candida albicans	10.0 ± 0.49	11.0 ± 0.76	-	22.0 ± 0.57
Cryptococcus neoformans	12.0 ± 0.76	13.0±0.35	-	25.0 ± 1.22
Microsporum spp.	11.0 ± 0.55	13.0 ± 0.34	-	24.0 ± 0.55
Pityrosporum ovale	12.0 ± 2.08	09.0 ± 1.15	-	26.0 ± 1.12
Trichophyton spp.	10.0 ± 1.09	16.0 ± 0.67	-	23.0 ± 0.99

Values are expressed as mean ± SEM, Zones of inhibition < 8 mm were discarded. POME = Methanolic extract of *Perilla ocymoides*, MKME = Methanolic extract of *Murraya koenigii*, BMME =Methanolic extract of *Baliospermum montanum*

Table 3. Antidiarrheal activity of *P. ocymoides, M. koenigii* and *B. montanum* extractives in mice.

Test groups	% Inhibition of defecation	TNF (240min)*
Negative control	0	98.00±1.35
Loperamide (3mg/kg, b.w.)	65.31	34.00±1.34
POME (500 mg/kg, b.w.)	37.76	61.00±1.14
MKME (500 mg/kg, b.w.)	50.00	49.00±1.43
BMME (500 mg/kg, b.w.)	39.80	59.00±1.64

*****TNF = Total number of faeces (MD×5 ± SEM)

Table 4. *In vitro* anti-inflammatory activity of the *P. ocymoides, M. koenigii* and *B. montanum* extractives.

Test groups	Total inhibition of protein denaturation
Negative control	0±0.001
Standard ASA (0.1 mg/ml)	62.26±0.001
POME (500 µg/ml)	18.87±0.001
MKME (500 µg/ml)	32.08±0.008
BMME (500 µg/ml)	39.62±0.001

Table 5. Analgesic activity of *P. ocymoides, M. koenigii* and *B. montanum* extracts on acetic acid-induced writhing in mice.

Clinical groups	Total writhing	% Writhing	% Protection	t- test (p-values)
Control (1% Tween-80)	170.0±2.35	100	0	-
Diclofenac-Na (25 mg/kg, b.w.)	51.0±0.82	30	70	9.58 (p<0.0003)
POME (500 mg/kg, b.w.)	110.0±0.50	64.71	35.29	6.84 (p<0.004)
MKME(500 mg/kg, b.w.)	76.0±1.53	44.71	53.29	6.84 (p<0.001)
BMME(500 mg/kg, b.w.)	155.0±2.37	91.18	8.82	0.899 (p<0.05)

Table 6. Antipyretic effect of *P. ocymoides, M. koenigii* and *B. montanum* extractives on yeast-induced pyrexia in mice.

Drug	Rectal temperature in ºF at time (hr) after administration of drug			
	0 hr	1 hr	2 hrs	3hrs
Control [DW, 10 ml/kg]	101.23 ± 0.71	101.41 ± 0.36	101.74 ± 0.58	101.32 ± 0.77
Paracetamol (150 mg/kg, b.w.)	102.38± 0.57	99.47± 0.82	97.56± 0.73	96.49± 0.85
POME (500 mg/kg, b.w.)	101.52± 0.19	100.43± 0.88	99.14± 0.32	97.85± 0.59
MKME (500 mg/kg, b.w.)	102.56± 0.37	101.63± 0.41	101.05± 0.18	98.74± 0.22
BMME (500 mg/kg, b.w.)	101.37± 0.43	101.13± 0.59	101.22± 0.17	99.98± 0.32

Analgesic activity. The analgesic effect of *P. ocymoides, M. koenigii* and *B. montanum* extractives on the acetic acid-induced abdominal constrictions in mice has been presented in table 5. The results show that the plant extract (500 mg/kg b.w.), and the reference drug diclofenac-Na (25 mg/kg b.w.) significantly reduced abdominal writhing in mice.

Antipyretic activity. The methanolic crude extract of *P. ocymoides, M. koenigii* and *B. montanum* markedly diminished yeast-induced hyperthermia in mice. The inhibition remained significant up to 3 hour of administration as shown in table 6. About three hour of drug administration, the maximum antipyretic effect was observed by *P. ocymoides* extractive *i.e.,* 97.85ºF while the antipyretic effect of paracetamol (150 mg/kg) was 96.49 ºF. The antipyretic effects of the extracts were compared with that of standard paracetamol (Table 6).

Table 7. Anxiolytic activity of *P. ocymoides, M. koenigii* and *B. montanum* extracts in mice.

Clinical groups	Hole cross test		Open field test	
	Number of hole crossed	% Inhibition of hole cross	Number of square crossed	% Inhibition of square crossed
Normal saline	33.99±0.41	0	240.99 ± 2.95	0
Diazepam (1.0mg/kg)	8.01±0.41	76.52	36.0 ± 2.12	85.06
POME(400mg/kg)	32.01±1.08	6.50	198.0 ± 3.08	17.84
MKME(400mg/kg)	27.00±0.71	20.62	219.0 ± 1.87	9.12
BMME(400mg/kg)	21.99±1.08	35.34	152.01 ± 3.19	36.92

Anxiolytic activity. During the evaluation of anxiolytic activity by hole cross test, the plant extracts at 500 mg/kg, b.w. showed significant increase in the number of line crossing as compared to control animals as shown (Table 7). In the open field test, administration of plant extract in mice demonstrated substantial increase in number of squares crossed as compared to the control (Table 7). Medicinal plants are a good source to find new remedies for anxiety disorders. Mechanism of anxiolytic action of plants might be due to the interaction with some of the natural endogenous mediators in the body.[31]There could also be a linkage in the interaction of the plant extract with serotonergic pathway.[32] Thus the present study revealed that the plant extracts specially *B. montanum* extract possesses significant anxiolytic effect.

Thrombolytic activity. The purpose of this experiment was to identify the fibrinolytic drugs from natural sources. These drugs are used for the treatment of myocardial infarction to dissolve the thrombin in occluded coronary arteries and thereby helpto restore normal blood supply to ischemic myocardium.[33] As shown in table 8, streptokinase (SK), a positive control (30,000I.U.) showed 69.23% lysis of clot after incubation with clot of human blood at 37°C for 90 minutes. In this study, the highest thrombolytic activity (57.81%) was observed by *B. montanum* extract (Table 8).

Table 8. Thrombolytic effect (%clot lysis) and membrane stabilizing effect of *P. ocymoides, M. koenigii* and *B. montanum* extract.

Samples	% Clot lysis	% Inhibition of hemolysis
SK	69.23	--
ASA	--	61.33±0.004
POME	3.44	14.67±0.001
MKME	29.16	36.00±0.003
BMME	57.81	10.67±0.001

In this study, the stabilization of RBC membrane by the methanolic crude extract of *P. ocymoides, M. koenigii* and *B. montanum* was found to be moderate (Table 8). Previously, it has been reported that certain bioactive secondary metabolites isolated from plant

sources were capable of stabilizing the RBC membrane and this may be indicative of their ability to exert anti- inflammatory activity.[34]

CONCLUSIONS

It can be concluded that the methanol extracts of *P. ocymoides*, *M. koenigii* and *B. montanum* exhibited moderate antidiarrheal, antipyretic, analgesic and anxiolytic activities in animal models. These properties strongly support the ethno-pharmacological uses of these plants in Bangladesh. However, further phytochemical and pharmacological investigations of the active compounds from these plants should be conducted for extensive traditional uses and potential therapeutic applications.

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