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Original Research Article

Pharmacological Investigation of Selected Medicinal Plants of Bangladesh

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Abstract

Purpose: *To pharmacologically investigate the methanol and petroleum ether extracts of the plant leaves of Manilkara zapota (MZME and MZPE, respectively), Abroma augusta (AAME and AAPE, respectively) and Vitex negundo (VNME and VNPE, respectively).*
Methods: *Analgesic and anti-diarrheal activities were assessed by acetic acid-induced writhing and castor oil-induced diarrhea in mice, respectively, while CNS depressant activity was evaluated using hole-cross and open-field method by observing the decrease in exploratory behavior and spontaneous motor activity in mice, respectively.*
Results: *All the extracts exhibited good analgesic activity at a dose of 200 mg/kg with the following rank order of activity: MZME > MZPE > VNME > VNPE > AAME > AAPE. Analgesic activity was insignificant at 100 mg/kg dose except for VNPE (67.81 % inhibition). The extracts produced significant reduction in diarrheal episodes in mice at a dose of 400 mg/kg MZPE (highest protection: 80.3 %, $p < 0.05$) and VNME (lowest protection: 38.6 %, $p < 0.001$). The extracts demonstrated CNS depressant activity in a dose-dependent manner ($p < 0.05$ compared to the standard except for AAME and AAPE which showed insignificant activity).*
Conclusion: *The results indicate that the traditional use of the investigated plants appears to be justified; however, further studies are required to unravel the underlying mechanisms of action.*

Keywords: *Manilkara zapota, Abroma augusta, Vitex negundo, Analgesic, Central nervous system depressant, Anti-diarrhoeal*

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INTRODUCTION

Among the south Asian countries, Bangladesh has a rich and prestigious heritage of herbal medicines. Out of 500 species of medicinal plants about 250 species are used for the preparation of traditional medicines in Bangladesh. But majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compounds [1].

Manilkara zapota (L) is an evergreen, glabrous tree under the family *Sapotaceae*. It is cultivated throughout the Indian subcontinent. In Bangladesh the local name is Sofeda. The leaves of this plant are used to treat cough, cold, and diarrhoea [2]. *Abroma augusta* (L) is a plant

under family of *Sterculiaceae* and Ulat kambal is the trade name of this plant. Leaves are used to treat uterine disorders, diabetes, rheumatic pain of joints, and headache with sinusitis [3]. Leaves and stem are demulcent and an infusion of fresh leaves and stem in cold water is very efficacious in gonorrhea [4]. *Vitex negundo* (L) is a plant under family of *Verbenaceae* which is locally known as nishinda in Bangladesh. The leaves and the roots of this plant are important as drugs. Anti-inflammatory and analgesic properties of mature fresh leaves have been reported [5].

In our laboratory we previously investigated the leaves of these plants for antioxidant & antimicrobial potential which were collected from a different region of Bangladesh [6]. The present study was undertaken to further explore these plants as a part of our endeavor to look for medicinal properties in local floristic resources.

EXPERIMENTAL

Collection and identification of plant material

The investigated plants, namely, *Manilkara zapota, Abroma augusta* and *Vitex negundo* were collected from Magura, Bhola, Dhaka, Bangladesh in February 2011 and Dr MA Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh authenticated the plants and their voucher specimens (nos. 35493, 35510 and 35401 respectively) were deposied in Bangladesh National Herbarium, Mirpur, Dhaka for future reference.

Chemicals and drugs

Tween-80 (as suspending agent), DMSO (as suspending & solubilizing agent) were purchased from BDH Chemicals Ltd and Merck, Germany; Diazepam and Sterile normal saline solution (0.9 % NaCl) were collected from Incepta Pharmaceuticals Ltd., and Beximco Infusion Ltd. Bangladesh.

Drying and pulverization

The fresh leaves of the plants were first washed with water to remove adhering dirt and then cut into small pieces, dried in the laboratory for 7 days. After complete drying, the entire portions were pulverized into a coarse powder with the help of a grinding machine and were stored in an airtight container for further use. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Cold extraction

About 180 gm of *Manilkara zapota*, 150 gm of *Abroma augusta* Linn, 500 gm of *Vitex negundo* Linn powdered materials were placed in separate glass containers and soaked in 700 ml of 95 % methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK).

Extraction with methanol

The concentrated methanol extract was made slurry with water. The slurry was taken in a separating funnel and methanol (50 ml) was added to the aqueous solution and the mass was shaken vigorously in a separating funnel. Then the funnel was allowed to stand for few minutes for the complete separation of the layers. The organic (lower layer) layer was collected. The process was repeated two times.

Extraction with petroleum ether

After methanol extraction, petroleum ether (70 ml) was added to the methanolic aqueous solution and the mass was shaken vigorously in a separating funnel. Then the funnel was allowed to stand for few minutes for the complete separation of the layers. The organic (upper layer) layer was collected. The process was repeated two times.

The filtrate (methanol and petroleum ether extract) obtained was evaporated at 50 ºC under reduced pressure using vacuum pump rotary evaporator (STUART RF3022C, UK). It rendered a gummy concentrate of reddish black color. The extract was transferred to a closed container for further use and protection.

Experimental animal

Young Swiss-albino mice of either sex, aged 4 - 5 weeks, average weight 25 - 30 g were used for the experiment. The mice were purchased from the animal research branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental conditions (24.0 ± 0 °C 55 - 65 % relative humidity and 12 h light/12 h dark cycle) for one week for acclimation, and fed on ICDDR, B-formulated rodent food and water *ad libitum*. All protocols were approved by the institutional animal ethical committee (Southeast University Animal Ethics committee, Southeast

University, Banani, Dhaka (approval ref no. SEU-01) and followed international guidelines for handling of animals for the investigation of experimental pain [7].

Evaluation of analgesic potential

The analgesic activity of the extracts was evaluated using acetic acid induced writhing method in mice [8,9]. As a positive control, any standard NSAID drug, indomethacin was used. Thirty six experimental animals were randomly selected and divided into six groups denoted as group-I, group-II, group-III, group-IV, group-V and group-VI consisting of 6 mice in each group. Each group received a particular treatment i.e. control, standard and the dose of the extracts of the plant respectively. Prior to any treatment, each mouse was weighed properly and the dose of the test sample and control materials was adjusted accordingly. The plant extracts were administered orally in two different doses (100 and 200 mg/kg body weight) to Swiss albino mice after an overnight fast. Each mouse in all the groups was observed carefully to count the number of writhing.

Evaluation of CNS depressant effect

CNS depressant activity was evaluated using hole cross and open field method.

Hole cross test

The method was adopted as described by [10]. A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The number of passage of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the crude extracts at doses of 250 and 500 mg/kg body weight of mice.

Open field test

This experiment was carried out as described by [11]. The animals were divided into control and test groups containing 6 mice each. The test group received extract at the doses of 250 and 500 mg/kg body weight orally whereas the control group received vehicle (1 % Tween 80 in water). The floor of an open field of half square meter was divided into a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90 and 120 min after oral administration of the crude extracts at doses of 250 and 500 mg/kg body weight of mice.

Evaluation of anti-diarrhoeal activity

The anti-diarrheal activity was performed by the method developed by [12]. Animals were divided into six groups of six animals in each group. Group I received 1 ml castor oil and served as control. Groups II received castor oil and standard drug, (Loperamide, 3 mg/kg) and served as standard. Group V-VI received methanolic extracts (200 and 400 mg/kg respectively). Diarrhea was induced in all the overnight fasted animals by administering 1 ml of castor oil orally. The test extracts and the standard drug were administered one hour prior to the treatment of castor oil. Each mouse was housed separately and observed for diarrheal episode, for a period of 3 h. During that period, number and weight of diarrheal feces were taken and noted at every half an hour. The mean diarrheal episodes and percent protection was calculated. The anti-diarrheal activity was determined in terms of percentage protection. The data of stool weight were expressed as mean ± SEM.

Statistical analysis

Data of all experiments were reported as mean ± SEM. Statistical significance testing of the values obtained were performed by one-way analysis of variance (ANOVA) and the group means were evaluated using Dunnett's multiple comparisons for analgesic screening tests and Tukey's multiple comparisons for neuropharmacological tests using SPSS program (SPSS 16.0, USA). The results were further analyzed using Student's t-test for castor oil-induced diarrhoea. In all cases, the data obtained were compared with the vehicle control group. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Analgesic activity

Table 1 shows the result for the writhing tests for methanol and pet ether extracts of the three plants. Compared with the standard, the effects of analgesic activity of petroleum ether and methanolic extracts at the dose of 100 mg/kg showed insignificant writhing in mice except VNPE and VNME. At the dose of 200 mg/kg all the extracts showed significant writhing and sequence of percent inhibition is MZME > MZPE > VNME > VNPE > AAPE > AAME. Among the

extracts, the MZME was the most potent and AAME was least potent.

CNS depressant activity

Hole cross

Both petroleum ether and methanolic extracts of *Manilkara zapota* and *Vitex negundo* showed a noticeable decrease of exploratory behavior in the test animals from its initial value at 0 to 120 min (Table 2). The results were dose-dependent and significant at $p < 0.05$ level, At 30 min, VNME (500 mg/ kg) and VNPE (250 mg/ kg) are significant compared to standard. At 60 min, all test samples are significant compared to standard. At 90 minutes, both MZME and MZPE are significant compared to control but not standard at the dose of 250 and 500 mg/kg. In case of *Abroma augusta,* both extracts insignificantly decreased the locomotor activity.

Open field

The petroleum ether and methanol extracts of *Manilkara zapota* and *Vitex negundo* at various dose levels both decreased locomotor and exploratory activity of experimental animal. In *Manilkara zapota,* at 0 min pet ether 250 mg/ kg is significant ($p < 0.05$) compared to standard. Otherwise all data are insignificant compared to control and standard (Table 3). In case of *Abroma augusta*, the result indicates that the both extracts insignificantly decreased the locomotor activity.

Table 1: Effect of methanol and petroleum ether extracts of *Manilkara zapota, Abroma augusta,* and *Vitex negundo* on acetic acid induced writhing in mice

Treatment	Dose (mg/ kg, p.o)	No. of writhing	% Inhibition
Control	0.5 ml/mouse	39.25 ± 8.02	0
Standard control	10 ml/ kg	18.50± 7.64*	52.87
MZPE	100	15.75 ±9.98	59.87
	200	2.25 ±0.87**	94.27
MZME	100	25.25 ± 9.11	35.67
	200	1.25 ± 1.09**	96.82
AAPE	100	14.50 ± 4.01	17.14
	200	6.75 ± 3.10*	61.43
AAME	100	12.25 ± 3.81	30.00
	200	7.12 ± 3.32*	59.30
VNPE	100	4.62 ± 1.85*	67.81
	200	5.87 ± 2.50*	74.66
VNME	100	3.00 ± 2.05**	81.16
	200	3.25 ± 0.99**	82.62

*Values are presented as mean ± SEM (n = 6); *p < 0.05, **p < 0.001 compared to control (Dunnett's test)*

Table 2: Effects of petroleum ether and methanolic extracts of *Manilkara zapota, Abroma augusta,* and *Vitex negundo* in mice on hole cross test

Groups	Dose (mg/kg, p.o)	Mean movements on open field before and after drug administration				
		0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in water	7.25±2.08	5.00 ± 1.25	2.75± 1.85	2.75± 1.09	1.50± 1.00
Diazepam	1	9.75±3.18	7.75 ± 2.23	6.50± 2.77	3.25± 0.87	2.00± 1.33
MZPE	250	3.75±2.89	0.75± 0.55*	0.50± 0.33	0.25±0.29*	0
	500	7.50±5.22	2.75 ± 2.02	0.75± 0.55	0*	0
MZME	250	4.00±2.16	4.00 ± 2.11	0*	0.25±0.29*	0
	500	5.25±4.09	1.50 ±1.00	1.25± 1.09	0*	0
AAME	250	8.00±4.03	2.75 ± 1.28	2.50± 0.75	2.00± 1.25	0.50± 0.58
	500	3.75±0.55	2.25 ± 0.73	0.75± 0.55	2.25± 0.99	1.00± 1.16
AAPE	250	6.50±1.73	3.25 ± 1.28	0.75± 0.55	0.50± 0.33	0
	500	1.50±1.11	4.50 ± 2.13	1.25± 0.87	1.25± 0.55	1.00± 0.82
VNME	250	3.25±1.72	3.25 ± 1.59	0.25±0.29*	0.50±0.58*	0.25 ± 0.29
	500	4.00±3.27	1.25± 0.87*	1.00± 0.82*	0.25±0.29*	0.25 ± 0.29
VNPE	250	3.50±2.24	1.25± 0.87*	0.75± 0.87*	0.75±0.55	0.50 ± 0.33
	500	6.25±4.20	2.25± 1.52	1.00± 0.47*	0.25±0.29*	0.25 ± 0.29

*Values are presented as mean±SEM (n=6); *: p<0.05; **:p<0.01 in ANOVA and post hoc Tukey's test*

Table 3: Effects of petroleum ether and methanolic extracts of *Manilkara zapota, Abroma augusta,* and *Vitex negundo* in mice on open field test

Group	Dose (mg/kg) (p.o)	Mean movements on open field before and after drug administration				
		0 min	*30 min*	*60 min*	*90 min*	*120 min*
Control	1%Tween 80 in water	115.00±45.56	50.00±15.61	45.25±27.11	35.25±22.84	32.25±22.71
Diazepam	1	209.50±12.71	98.00±16.91	32.00±22.33	14.75±17.03	0
MZPE	250	42.50± 23.88*	34.50±15.84	24.25 ± 6.35	0	0
	500	138.25±66.23	33.00±14.86	30.00±24.50	10.00±11.55	9.00±8.25
MZME	250	148.00±22.50	18.75±12.68*	0.25±0.29*	0	0
	500	232.50±38.38	98.50±36.48	26.25±17.50	12.50±14.43	3.75± 4.33
AAME	250	132. 00±6.13	101.75±14.27	69.25±12.42	26.00±12.76	5.00±5.77
	500	54.75±10.18	10.25±1.09	4.00±2.11	0.75±0.55	0
AAPE	250	178.75±16.62	100.75±7.92	64.75±4.72	20.50±9.10	3.50±2.52
	500	62.75±31.07	36.50±19.91	12.50±5.93	5.00±5.77	3.00±3.46
VNME	250	153.25±25.75	21.25±10.10	1.25±1.44	0.25 ± 0.29	0.25±0.29
	500	220.00±46.13	108.00±44.58	29.75±16.68	11.50±11.79	3.50 ± 4.04
VNPE	250	47.50±25.12*	33.25±13.61	25.00± .99	0.50±0.58	0
	500	139.25±70.12	38.00±16.19	31.25±25.86	10.50±11.34	14.25±12.27

*Values are presented as mean±SEM (n=6); *p<0.05; **p<0.01 in ANOVA and post hoc Tukey's test*

Table 4: Effects of petroleum ether and methanol extracts of *Manilkara zapota, Abroma augusta* and *Vitex negundo* on castor oil-induced diarrhea in mice

Treatment	Dose (mg/ kg)	Number of wet stools	Total number of stools	Total weight of stools	Protection (%)
Castor oil	10 ml/kg	2.75 ± 0.87	9.50 ± 1.80	2.44 ± 0.35	0
Loperamide	1	3.25 ± 1.52	8.75 ± 0.87	0.79 ± 0.12**	67.69
MZME	200	4.50 ± 3.14	8.50 ± 2.56	0.92±0.27**	43.59
	400	5.50 ± 1.53	9.50 ± 1.80	1.38± 0.22*	62.26
MZPE	200	4.75±2.37	9.25±2.38	0.48±0.11**	78.15
	400	2.75±1.85	7.00±2.05	0.53±0.25**	80.31
AAME	200	2.50 ± 1.53	8.50 ± 2.13	1.30 ± 0.40*	46.67
	400	0.25 ± 0.23	4.25 ± 1.97	0.93 ± 0.59*	62.05
AAPE	200	1.50 ± 1.00	4.00 ± 0.82	0.72 ± 0.28**	70.67
	400	2.00 ± 2.31	5.25 ± 3.54	0.57 ± 0.31**	76.82
VNME	200	3.25 ± 2.33	7.00 ±1.25	0.86±0.22**	61.74
	400	4.75 ± 2.47	9.75± 2.56	1.38 ± 0.22*	38.61
VNPE	200	3.25±0.73	8.75±2.37	0.45±0.10**	79.98
	400	1.75±0.73	6.00±1.41	0.51±0.24**	77.18

*Values are presented as Mean±SEM (n=6); *p<0.05; **p<0.01 in unpaired Student's t- test*

Antidiarrhoeal activity

Petroleum ether and methanol extracts showed statistically significant (p < 0.05) reduction of diarrheal episode in mice (Table 4). Both extracts administered at the dose of 200 and 400 mg/kg and showed 78.2 % and 80.3 % protection respectively for MZPE where the MZME showed respectively 43.6 and 62.3 % protection. The frequency of stooling as well as fresh weight also decreased significantly and the result was found significant. For both doses of *Abroma augusta* and *Vitex negundo* extracts, the protection was 46.7% and 62.1% for AAME and 70.7% and 76.8% for AAPE, respectively. The VNPE at two doses showed 80.0% and 77.2% protection where methanolic extracts of *Vitex negundo* showed 61.74 and 38.6 % protection, respectively.

DISCUSSION

Acetic acid writhing is a model of pain involved mainly in the peripheral mechanism [13]. This model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [8]. In our experiment, both MZME and MZPE showed that the plant extracts has good analgesic activity. Acetic acid is supposed to release prostaglandins E2 and F2α in the peritoneal fluid that excite pain nerve endings [14]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing

capillary permeability [15]. It has been suggested that these endogenous mediators of pain are sensitive to non-steroidal anti-inflammatory drugs and opioids [16].

Both doses of extracts of *V. negundo* showed significant reduction in writhing induced by the acetic acid after oral administration in a dose dependent manner. At all dose levels, VNME was exhibited higher analgesic activity than VNPE. Our observations support previous results for the analgesic activity of *V. negundo* leaf extract (500 and 1000 mg/Kg) using acetic acid induced writhing test in mice for assessing peripheral analgesic effect [17].

The results of *Manilkara zapota* caused a general inhibition of neuronal activity in the CNS. In the hole cross test the extracts reduced the exploration capacity significantly ($p < 0.05$) which might be considered to be an index of depressors on CNS. A reduction in exploratory behavior with the extract is in conformity with similar actions produced by other CNS depressant drugs [18]. But it is difficult to separate the hole cross test from the open field test by which decreased the locomotor activity was measured. The results obtained demonstrate that the leaf extract has effective CNS depressant potential through diminishing exploratory activity but its action is not promising via locomotor reduction pathway. So it is assumed that the extracts may contain some compounds which decrease the exploratory as well as locomotor activity.

To the best of our knowledge, this is the first report of the *Abroma augusta* plant extracts on CNS depressant activity. The obtained results showed a decrease in exploratory conduct as well as reduction of spontaneous motility by the methanolic and petroleum ether extracts in a dose-dependent manner. However, the alteration in the general behavior patterns and decreased effects on motor coordination found by the extracts were insignificant compared to vehicle control. However, the general inhibition of neuronal activity in the CNS caused by AAME and AAPE might be considered to be the depressive on CNS.

Both the methanol and pet ether extracts of *Vitex negundo* exhibited reduction of both exploratory and spontaneous locomotor activity at doses of 250 and 500 mg/kg. The data obtained in our investigation demonstrate that the extracts are not promising for eliciting such activities at threshold level in both hole cross and open field tests. Previously, Gupta *et al* [11] showed that the methanol extracts of *Vitex negundo* significant exhibited CNS depressant activity in a dose-dependent manner.

It is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [19]. Therefore, the decreased frequency of stooling and fecal parameters observed with the *Manilkara zapota* and *Abroma augusta* extracts are indications of antidiarrhoeal potential. In this study, the 200 and 400 mg/kg body weight of the extracts of *Manilkara zapota* showed the best antidiarrhoeal activities. The data presented in our investigation suggest that the antidiarrhoeal activity of *Vitex negundo* is not promising.

CONCLUSION

On the basis of the results obtained, there is some justification for the traditional use of the investigated plants in ethnomedical practice. However, further studies are required to identify the exact bioactive compounds responsible for the pharmacological effects observed as well as fully elucidate the underlying mechanisms of action.

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