

Full Length Research Paper

Phytochemical and pharmacological screenings of organic crude fractions of *Maesa acuminata*

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The present study is aimed to investigate phytochemical and pharmacological activities of crude organic fractional extracts of *Maesa acuminata* (MA). Preliminary test for phytoconstituents shows the presence of alkaloid, glycoside, steroids, tannins, saponins and reducing sugars. Fractionation was done by ethanol (leaf: ELMA), chloroform (bark: CBMA) and n-hexane (leaf: HLMA). The crude fractions were then subjected for antioxidant, anti-inflammatory and membrane stabilizing, antimicrobial and analgesic activities. In the antioxidant test performed by 1,1-diphenyl-picrylhydrazyl (DPPH) assay, the CBMA and HLMA significantly scavenged DPPH radicals, where HLMA at 100 µg/ml inhibited DPPH by 57.01% and the standard, ascorbic acid (AA) by 95.47%, respectively. In the disc diffusion antimicrobial sensitivity test, all the crude fractions of MA produce zones of inhibition between 8 to 18 mm. ELMA showed prominent antimicrobial activity than the CBMA and HLMA, where it strongly inhibited *Staphylococcus aureus* with the inhibition zones by 18 mm at 500 µg/disc. Minimum inhibitory concentration (MIC) detected with the CBMA, ELMA and HLMA were between 61.25 and 250 µg/ml for the test microorganisms. In the anti-diarrheal activity the HLMA and CBMA in oral dose (p.o.) showed 62.79 and 61.62% of percent inhibition of defection as compared to standard, loperamide (93.00%). Additionally, the fractional extracts of MA also possess anti-inflammatory, membrane stabilization and analgesic activities. In conclusion, *M. acuminata* may be a good source of therapeutic components.

Key words: Antioxidant, antimicrobial, *Maesa acuminata*.

INTRODUCTION

Plants are the backbone of life on earth and an essential resource for the well-being of mankind. One-quarter of all

prescription drugs come directly from plants or their derivatives. Additionally, four out of five people around the world today rely on plants for primary health-care (Loya et al., 2009). Notably, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. In this context, medicinal plants are considered as rich resource of ingredients which can be used in drug development and synthesis. World Health Organization (WHO) enlisted approximately 21,000 important medicinal plants. Besides, these plants are playing a crucial role in the development of human cultures from the beginning of life. About 80% of the populations of many developing countries still use traditional medicines for their health care. On the other hand the resistance to the conventional drugs in a regular basis stimulating the search of natural medicines.

Maesa acuminata DC. (Family: Myrsinaceae) is a tree and its leaf juice is given to the children with symptoms of diarrhoea by the Marma and Chakma in Chittagong hill tracts in Bangladesh. Moreover, its juice is also used in cuts by the Marma (Yusuf et al., 2009). By considering the potentiality of this plant as a source of drugs, a systemic investigation was carried out to screen the phytochemical and pharmacological activities of *M. acuminata*.

MATERIALS AND METHODS

Plant collection and identification

For the investigation, the bark and leaves of *M. acuminata* was collected from the Chittagong district in Bangladesh in the month of June and July. The plant material was identified by the taxonomist (without voucher specimen), from Bangladesh Forest Research Institute Herbarium (BFRIH), Chittagong, Bangladesh.

Extraction and fractionation

Approximately 10 h hot extraction (with 95% ethanol) was carried out by using a Soxhlet extractor (Quickfit, England) after drying (temperature not exceeding 50°C) and pulverized of the plant materials. Extracts were then filtered through a cotton plug followed by Whatman filter paper (no. 1) and finally, concentrated by evaporating the solvent below 75°C. The yield of the crude MA leaves and bark extracts were 4.966 and 5.067%, respectively.

Solvent-solvent partitioning was done using the protocol described by Islam et al. (2014). The extracts were subjected to fractionation using n-hexane (leaf: 2.94 out of 3.724 gm), ethanol (leaf: 0.72 g; bark: 1.68 g), and chloroform (bark: 2.56 out of 4.307 g).

Preliminary screening for phytoconstituents

Findings of the preliminary phytochemical screenings done

according to Sultana et al. (2014) are given in the Table 1.

Screening for antioxidant activity (DPPH scavenging assay)

The antioxidant activities of the crude extracts were determined as per Islam et al. (2016). Stock solutions of the plant extracts were prepared in ethanol with a concentration range from 10 and 100 µg/ml. To the diluted sample (0.3 ml), 2.7 ml of 0.004 % DPPH ethanolic solution was added. Then the contents were mixed properly, allowed to stand at dark for 30 min to complete the reaction and absorbance was taken using a spectrophotometer at 517 nm. A similar concentration of ascorbic acid (AA) served as the positive control, while only 0.3 ml vehicle was added to DPPH solution for negative control (NC). The blank contained no sample. The DPPH radical scavenging potential was calculated using the following equation: % inhibition of DPPH radical = $[(A_{br} - A_{ar})/A_{br}] \times 100$ where, A_{br} is the absorbance of DPPH free radicals before reaction and A_{ar} is the absorbance of DPPH free radicals after reaction.

Screening for *in-vitro* anti-inflammatory and *ex vivo* membrane stabilizing activity (EAL and HRBC assays)

The anti-inflammatory (EAL; *in vitro*) and membrane stabilization (HRBC; *ex vivo*) tests of plant extracts were carried out according to Hossain et al. (2013). In anti-inflammatory test, 1% egg albumin (EAL) was constituted in phosphate buffer saline solution (PBS, pH 7.4), while for membrane stabilization test, fresh human RBC was reconstituted as 10% suspension human red blood cell reconstitution (HRBC) in isosaline (0.9% NaCl, pH 7.4). The assay mixture contains 1 ml phosphate buffer (pH 7.4, 0.15 M), 2 ml hypo-saline (0.36%). 0.5 ml of EAL/HRBC (125 - 500 µg/ml) was mixed with 0.5 ml of test sample. Acetyl salicylic acid (ASA) and distilled water (DW) were taken as positive and negative controls (NC). After incubation at 37°C for 30 min, the reaction mixtures were centrifuged and supernatant was collected for spectrophotometric analysis at 560 nm. Activity was measured by the following equation.

Inhibition (%) = $100 - [(absorbance \text{ of test solution}) \div (absorbance \text{ of control}) \times 100]$

Screening for antimicrobial activity (Disc diffusion assay)

The anti-bacterial action of the crude fractions were screened by the disk diffusion method described in Bauer et al. (1966). All the apparatus used were sterilized by autoclaving at a temperature of 121°C and a pressure of 15 lbs/sq.inch for 15 min. The test was conducted with 500 µg/disc of crude fractions against 10 pathogenic bacteria (Gram positive: *Lactobacillus casei*, *Lactobacillus coryniformis*, *Bacillus cereus*, *Bacillus azotoformans* and *Staphylococcus aureus*; Gram negative: *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae* and *Klebsiella pneumonia*) listed in Table 4.

In addition, anti-fungal activity was done in 7 fungal species (not shown). Microorganisms were collected as pure subculture from Microbiology Laboratory, Department of Pharmacy, BGC Trust University, Chittagong, Bangladesh. Azithromycin (AZ) and fluconazole were taken as standards in this context. The test

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organisms were maintained on nutrient agar slants and were sub-cultured prior to this study. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeters (mm).

Minimum inhibitory concentrations (MIC) determination (Micro-dilution test)

The MIC of the crude fractions were determined by 'micro-dilution technique' (Bauer et al., 1966) in broth medium (Hi Media Laboratories Ltd., India). Briefly, a half-fold dilution from 7.81 to 1000 µg/ml was performed in this occasion. Then the turbidity due to growth of organisms was visually observed by naked eye.

Screening for anti-diarrheal activity (Castor oil-induced diarrheal model)

The anti-diarrheal activity of the crude extracts was determined by the method described in Nur et al. (2015). Briefly, the animals were all screened initially by giving 0.4 ml of castor oil and only those showing diarrhea were selected for the final experiment. Swiss mice (25-30 g of body-weight; 2 months old; both sexes) 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.); test groups received the CBMA and HLMA extracts at a dose of 500 mg/kg (p.o.). Each animal was placed in an individual cage, the floor of which was lined with blotting paper, which was changed every hour. Diarrhea was induced by oral administration of castor oil (0.4 ml) to all mice, 30 min after the above treatments. During the observation period (4 h), the latency period (first diarrheal defecation time) and frequency (number of defecation) were counted manually. Percent inhibition of defecation in mice was calculated by using the following equation:

$$\% \text{ inhibition} = \{(Mo - M) / Mo\} \times 100$$

Where, Mo = mean defecation of control and M = mean defecation of test sample.

Screening for antinoceptive activity (Hot plate test)

The antinoceptive activity of the crude fractions of MA was determined by the method described in Sultana et al. (2015). Diclofenac-Na (DFN) was used as standard. Young Swiss mice of either sex (body weight: 18-24 g) were divided into four groups, 5 animals in each. Group I served as control (DW, p.o.). Group II served as standard and were given DFN (9 mg/kg, i.p.). Group III and IV were treated with CBMA and HLMA at 500 mg/kg (p.o.), respectively. The animals were individually placed on the hot plate maintained at 55±2°C for 15 min after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first (latency). Moreover the number of times jumped and number of times of the paw licked was noted as a pain stimulus for 2 min. The cut off time for the reaction was 15 s. The greater the latency period and the lesser the paw licking or jump response, the more is the positive activity by the test extracts.

Screening for anti-atherothrombotic activity (Clot lysis test in human blood)

The thrombolytic activity of the extract was evaluated by the method

of Prasad et al. (2006) using streptokinase (SK) 1500000 I.U and ethanol as standard and negative controls, respectively. Briefly, blood was collected from 10 healthy volunteers and distributed into pre-weighed (W_1) micro-centrifuge tubes (0.5 ml/tube) and incubated at 37°C for 45 min and then weight (W_2) was taken. The weight of clotted blood (ΔW) was taken by subtracting the pre-weight and the weight of clotted blood containing tube. Then 100 µl test samples were added to the clot containing tubes marked. Each crude sample was tested for three doses (125, 250 and 500 µg). Similarly, 100 µl of streptokinase (15,00,000 U/vial/10ml) and 100 µl of ethanol were added to the controls marked tubes. Then all the tubes were incubated at 37°C for 90 min. After incubation, fluid released was removed carefully without disrupting the clot, and tubes were again weighed for getting the weight variation among the pre-weight and final weight (W_3) that was achieved for clot lyses (thrombolysis).

Statistical analysis

Values are mean ± SD (standard deviation) and percentage forms. The data were analyzed by means of analysis of variance (ANOVA) followed by *t*-Student–Newman–Keuls's as post-hoc test (except antimicrobial) using the GraphPadPrism software (version 6.0) with 95% confidence interval at $p < 0.05$.

RESULTS AND DISCUSSION

Preliminary phytochemical study revealed the presence of alkaloids, glycosides, tannins, flavonoids in HLMA, while glycosides, alkaloids, flavonoids, tannins and reducing sugars in ELMA, and alkaloid, glycoside, steroids and reducing sugar in CBMA, respectively (Table 1).

In the DPPH[•] assay, HLMA at 100 µg/ml showed 57.01% radical scavenging capacity, while CBMA produced 55.77%. At all concentrations both ELMA and CBMA produced lower inhibition than AA. However, there was a dose-response relationship in extracts and AA. The IC₅₀ calculated for HLMA, CBMA and AA are 101.12, 80.27 and 20.01 µg/ml, respectively (Table 2).

In the EAL (*in vitro*) anti-inflammatory test, 500, 250 and 125 µg/ml of CBMA showed mean inhibition of protein denaturation 85.15, 73.05 and 26.17%; whereas, HLMA by 62.25, 47.25 and 18.36% and ELMA by 46.25, 32.25 and 26.17%, respectively. The standard drug, ASA exhibited inhibition of protein denaturation by 96.09, 73.83 and 70.31% with the concentrations of 500, 250 and 125 µg/ml, respectively (Table 3).

Otherwise, in the *ex-vivo* membrane stabilization test, 500 µg/ml of CBMA inhibited hemolysis by 24.47%, while, the HLMA by 43.42%. In this occasion, ELMA (55.25%) was found to be more active than the CBMA and HLMA. At 250 µg/ml ASA produced percent inhibition by 65.20% (Table 3).

ELMA at 500 µg/disc strongly inhibited *S. aureus* (18 mm) followed by *S. typhi* (15 mm), *K. pneumoniae* (14 mm), *L. casei* (14 mm), *V. cholera* (12 mm), *L. coryniformis* (11 mm), *P. aeruginosa* (11 mm), *B.*

Table 1. Phytoconstituents found in the plant extracts.

Phytoconstituent	Alkaloids	Glycosides	Steroids	Tannins	Falvonoids	Saponins	Reducing sugars	Gums
ELMA	+	+	-	+	+	+	-	-
Consequence HLMA	+	+	-	+	+	-	-	-
CBMA	+	+	+	-	-	-	+	-

*+ = Presence; - = Absence; number indicates number of test performed. CBMA: Chloroform bark extract of *M. acuminata*; HLMA: n-hexane leaf extract of *M. acuminata*; ELMA: Ethanol leaf extract of *M. acuminata*.

Table 2. Antioxidant activity of the crude fractions of *M. acuminata* and standard.

Concentration (µg/ml)	Percent inhibition of DPPH radical		
	HLMA	CBMA	AA
100	57.02	55.78	95.47
80	49.69	51.80	94.41
60	42.61	51.06	91.93
40	37.52	49.57	84.09
20	30.68	39.88	62.32
10	23.60	31.18	45.21
IC ₅₀ (µg/ml)	101.12	80.27	20.01

Values are percentage of inhibition of DPPH*. CBMA, chloroform bark extract of *M. acuminata*; HLMA, n-hexane leaf extract of *M. acuminata*; AA, ascorbic acid; IC₅₀, half minimal inhibitory concentration.

Table 3. Anti-inflammatory and membrane stabilizing activities of the crude fractions of *M. acuminata* and controls.

Conc. (µg/ml)	% inhibition of hemolysis				% inhibition of protein denaturation			
	CBMA	ELMA	HLMA	ASA	CBMA	ELMA	HLMA	ASA
125	18.42	20.36	27.63	62.68	26.17	26.17	18.36	70.31
250	22.38	35.25	33.42	65.20	73.05	32.25	47.25	73.83
500	24.47	55.25	43.42	67.65	85.16	46.25	62.25	96.09

Values are percentage inhibition. CBMA, chloroform bark extract of *M. acuminata*; ELMA, ethanol leaf extract of *M. acuminata*; HLMA, n-hexane leaf extract of *M. acuminata*; ASA, acetyl salicylic acid.

azotoformans (10 mm), *E. coli* (9 mm) and *B. cereus* (8 mm). However, the HLMA and CBMA were inactive against the test strains (Table 4). In the MIC test it was depicted that the ELMA inhibited the growth of *S. aureus* significantly at the dose of 61.25 µg/ml then followed by 72.5, 182.5, 250, 160.5, 220.5, 162.5, 125, 72.5 µg/ml against *L. casei*, *L. coryniformis*, *B. cereus*, *B. azotoformans*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, *V. cholerae*. AZ (30 µg/disc) produced zones of inhibition within 12 to 21 mm. Although fluconazole (zones of inhibition between 17 to 26 mm) was active against all the test fungi but, crude fractions were found inactive for all of them (*Candida albicans*, *Pityrosporum ovale*, *Trichophyton sp.*, *Cryptococcus neoformans*, *Aspergillus niger*, *Rhizopus oryzae*, *Aspergillus fumigatus*) (data not shown) (Table 4).

In the castor oil induced diarrhoeal mice, the CLMA and

HLMA at 500 mg/kg (p.o.) showed 61.63 and 62.79% inhibition of defecation compared to standard, loperamide (3 mg/kg; p.o.) with 93% inhibition of defecation. Though, CBMA increased the number of defecation, it significantly ($p < 0.05$) increased the latency (92.20 ± 4.21) as compared to DW (Table 5).

In antinoceptive activity study by hot-plate method, both CLMA and HLMA significantly ($p < 0.05$) increased latency after 30 min of an oral administration of 500 mg/kg. However, CLMA was found better than HLMA, despite of prominent activity of DFN (Table 6).

In the ex-vivo thrombolytic activity test, after addition of 100 µl SK, the clots showed 81.48% clot lysis. On the other hand clots when treated with 100 µl DW (negative control) showed only negligible lysis (2.25%). All the crude fractions produced significant ($p < 0.05$) anti-thrombosis activity when compared to the DW group.

Table 4. Antibacterial activity of crude fractions of *M. acuminata* and standard.

Test microorganisms	Zone of Inhibition (mm)				MICs (µg/ml)
	CBMA (500 µg/disc)	ELMA (500 µg/disc)	HLMA (500 µg/disc)	AZ (30 µg/disc)	
<i>L. casei</i>	NI	14	NI	18	72.5
<i>L. coryniformis</i>	NI	11	NI	12	182.5
<i>B. cereus</i>	NI	7.33	NI	15	250
<i>S. aureus</i>	NI	18	NI	21	61.25
<i>B. azotoformans</i>	NI	10	NI	20	160.5
<i>E. coli</i>	NI	9	NI	16	220.5
<i>P. aeruginosa</i>	NI	11	NI	21	162.5
<i>K. pneumonia</i>	NI	14	NI	15	125
<i>S. typhi</i>	NI	15	NI	17	72.5
<i>V. cholera</i>	NI	12	NI	17	125

CBMA, chloroform bark extract of *M. acuminata*; HLMA, n-hexane leaf extract of *M. acuminata*; ELMA, ethanol leaf extract of *M. acuminata*; AZ, Azithromycin; NI, No. inhibition; Zones of inhibition less than 8 mm were considered as poor and were discarded.

Table 5. Anti-diarrheal activity of crude fractions of *M. acuminata* and controls.

Treatment groups	Dose (kg; p.o.)	Latent time	% inhibition of defecation	No. of faeces
DW	10 ml	4.20 ± 0.84	16.28	14.40 ± 3.36
Loperamide	3 mg	183.40 ± 6.88*	93.00*	4.20 ± 1.92*
CBMA	500 mg	92.20 ± 4.21*	61.63*	6.60 ± 1.52*
HLMA	500 mg	84.20 ± 5.10*	62.79*	6.40 ± 2.07*

*p < 0.05 compared to DW (control); values are expressed as mean ± SD (n=5). CBMA, chloroform bark extract of *M. acuminata*; HLMA, n-hexane leaf extract of *M. acuminata*; DW, distilled water.

Table 6. Antinociceptive activity of the crude fractions of *M. acuminata* and controls in Swiss mice.

Treatment groups	Dose (kg)	Mean latency (s)	
		Initial	After 30 min
Control (1% v/v tween 80)	10 ml	4.50 ± 0.41	6.25 ± 0.20
DFN	9 mg	4.87 ± 0.18	22.05 ± 0.49*
CLMA	500 mg	5.33 ± 0.20	10.75 ± 1.10*
HLMA	500 mg	5.36 ± 0.16	10.58 ± 0.36*

*p < 0.05 compared to the control group; values are mean ± SD (n=5). CBMA, chloroform bark extract of *M. acuminata*; HLMA, n-hexane leaf extract of *M. acuminata*; DFN, diclofenac-Na

However, the activity was prominent to HLMA (45.63 ± 10.32%) followed by CBMA (35.64 ± 3.69%) and ELMA (26.35 ± 8.19%) (Table 7).

Polyphenols, especially the flavonoids are known for their antioxidant and cytoprotective activities (Bhullar and Rupasinghe, 2015). Our data revealed that both ELMA and HLMA contain flavonoids, thus the highest antioxidant activity of HLMA and better anti-inflammatory activity of ELMA and HLMA may be plugged with the flavonoids present in the extracts. However, the CBMA containing alkaloids and glycosides may be link to its

antioxidant as well as membrane stabilization potentials.

Essential oils, especially the diterpenes those have active hydroxyl groups in their structure are capable to kill bacteria (Carvalho et al., 2011). In this study, we found ELMA has promising antibacterial activity. However, HLMA did not show this kind of effect. *Salmonella*, *Shigella* and *E. coli* are most well-known pathogens involve in diarrhea (Nur et al., 2015). However, in this study, both HLMA and CLMA were found inactive against the tested pathogenic strains, including *Salmonella* and *E. coli*, thus other activity mechanism may be involved in

Table 7. Anti-atherothrombosis activities of crude fractions of *M. acuminata* and controls.

Treatments	Concentration/tube containing 500 µl of blood	Percentage of clotlysis
DW	100 µl	2.26±8.23
SK	100 µl	81.48±2.021*
CBMA	500 µg/100 µl	35.64±3.69*
ELMA	500 µg/100 µl	26.35±8.19*
HLMA	500 µg/100 µl	45.63±10.32*

*p <0.05 compared to DW (control); values are mean ± SD; CBMA, chloroform bark extract of *M. acuminata*; ELMA, ethanol leaf extract of *M. acuminata*; HLMA, n-hexane leaf extract of *M. acuminata*; DW, distilled water; SK, streptokinase.

their anti-diarrheal activity. Saponions and flavonoids are evident to impart the neurobiological effect in experimental animals (Musa et al., 2006). The antinoceptive activity of CLMA and HLMA may be an attribute of the presence of such types of phytochemicals. Otherwise, thrombus formation inside the blood vessel is thought to be one of the major consequences for cardiac failure and many other heart diseases (Viles-Gonzalez et al., 2004). Notably, HLMA exhibited better anti-thrombosis activity than the other crude fractions.

CONCLUSION

A plant provides the main ingredients of medicines in traditional systems and is a source of inspiration for several major pharmaceutical drugs. The present work carried out with the ethanol (ELMA), chloroform (CBMA) and n-hexane (HLMA) extracts of *M. acuminata* represents statistically significant (p <0.05) anti-oxidant activity, anti-inflammatory and membrane stabilizing activity, anti-diarrheal, analgesic and anti-atherothrombosis activities in comparison to the respective control groups. Otherwise, the ELMA showed good action in anti-bacterial and anti-fungal activities. The results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants estimated. The obtained results could be helpful for further study prior to isolate and more advanced non-and/or pre-clinical trials with the responsible phytochemicals in *M. acuminata*. Again, we are concluding with a recommendation for further research with this medicinal plant.

Conflict of Interests

The authors have not declared any conflict of interests.

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