

In Vitro Antioxidant, Cytotoxic, Thrombolytic, Antimicrobial and Membrane Stabilizing Activities of *Murraya paniculata*

Tahmina Akter Mita¹, Mahbubul Hoque Shihan¹, Mehreen Rahman¹, Tasnuva Sharmin¹, Mahfuza Maleque¹, Mohammad Razi-Ul-Hasan Alvi² and Sharmin Reza Chowdhury^{1*}

¹Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh.

² Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka-1000, Bangladesh.

*Correspondence to: Sharmin Reza Chowdhury, Assistant Professor, Department of Pharmacy, State University of Bangladesh. Email: sharminreza10@yahoo.com.

ABSTRACT

The methanolic extract of leaves of *Murraya paniculata* and its petroleum ether, carbon tetrachloride, chloroform, and aqueous soluble partitionates were subjected to total phenolic content determination, free radical scavenging activity determination (DPPH assay), cytotoxic, thrombolytic, antimicrobial and membrane stabilizing activity assays. The antioxidant potential was determined in terms of total phenolic content and free radical scavenging activity assay. The highest free radical scavenging potential was demonstrated by the carbon tetrachloride soluble fraction ($IC_{50} = 72.51 \pm 0.69 \mu\text{g/ml}$) which could be correlated to its phenolic content ($52.99 \pm 0.32 \text{ mg of GAE / gm of extractives}$). In brine shrimp lethality bioassay, the pet ether soluble extractives and the crude methanol extract revealed the highest cytotoxic activity having LC_{50} values $0.471 \pm 0.72 \mu\text{g/ml}$ and $0.773 \pm 0.19 \mu\text{g/ml}$, respectively. In thrombolytic activity study, the crude methanolic extract exhibited mild clot lysis activity ($27.94 \pm 0.23 \%$) compared to the standard streptokinase (66.77%). In antimicrobial investigation by disc diffusion method, none of

the extractives of *M. paniculata* exhibited any zone of inhibition. The *M. paniculata* extractives revealed mild to moderate membrane stabilizing activity of which the aqueous soluble partitionate inhibited 30.20 ± 0.75 % and 60.83 ± 1.45 % haemolysis of RBCs under heat and hypotonic solution induced conditions, respectively.

Key words: *Murraya paniculata*, total phenolic content, free radical scavenging activity, brine shrimp lethality bioassay, thrombolysis, zone of inhibition, membrane stabilization.

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INTRODUCTION

Murraya paniculata (L.) Jack commonly called orange jessamine, is a tropical, evergreen plant bearing small, white, scented flowers, which is grown as an ornamental tree or hedge. *Murraya* is closely related to Citrus and bears small orange to red fruit. *Murraya paniculata* belongs to Rutaceae family (commonly known as the rue or citrus family). *M. paniculata* is a native to South and Southeast Asia, China and Australasia. It is naturalized in southern USA.¹ The crude ethanolic extract of leaves of *M. paniculata* has antidiarrhoeal, antinociceptive² and anti-inflammatory³ activities.

As a part of our continuing investigation of medicinal plants of Bangladesh^{4, 5}, the crude methanol extract of *M. paniculata* and its aqueous and organic soluble fractions were

studied for the antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic, thrombolytic, antimicrobial and membrane stabilizing activities for the first time and we, here in, report the results of our preliminary investigations.

MATERIAL AND METHODS

Collection and extraction of plant material

The leaves of *Murraya paniculata* were collected from Khulna. The collected plant materials were cleaned, sun dried for several days and then oven dried for 24 hours at considerably low temperature (not more than 40°C) for better grinding. The dried barks and seeds were then grounded to a coarse powder. The powdered plant material (250 gm) was taken in a cleaned, ambered color reagent bottle and soaked in 2.0 liters methanol at room temperature for 7 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a fresh cotton plug and finally with a Whatman number 1 filter paper and concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 gm) of the concentrated methanol extract was fractionated by modified Kupchan partition protocol⁶ and the resultant partitionates were evaporated to dryness with rotary evaporator to yield pet-ether (PESF, 3.0 gm), carbon tetrachloride (CTCSF, 3.5 gm), chloroform (CSF, 1.5 gm) and aqueous (AQSF, 1.0 gm) soluble materials. The residues were then stored in a refrigerator until further use.

Total phenolic content

The total phenolic content of the extractives was determined with Folin-Ciocalteu reagent by using the method developed by Harbertson and Spayd (2006).⁷

DPPH free radical scavenging assay

Following the method developed by Brand-Williams *et al.* (1995)⁸, the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

Brine shrimp lethality bioassay

This technique was applied for the determination of general toxic properties of the DMSO solutions of plant extractives against *Artemia salina* in a single day *in vivo* assay.⁹ Vincristine sulphate was used as positive control.

Thrombolytic activity

The thrombolytic activity was evaluated by the method developed by Prasad *et al.* (2006)¹⁰ by using streptokinase (SK) as positive control.

Antimicrobial screening

Antimicrobial activity was determined by disc diffusion method developed by Bayer *et al.* (1966).¹¹

Membrane stabilizing activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale *et al.* (2008).¹²

Statistical analysis:

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean \pm SD.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the total phenolic content, free radical scavenging, cytotoxic, thrombolytic, antimicrobial and membrane stabilizing activity of different organic and aqueous soluble partitionates of the crude methanol extract of *M. paniculata*.

The total phenolic contents of the extractives were found between 0.2 to 52.99 mg of GAE/g of sample. The highest total phenolic content was demonstrated by the carbon tetrachloride soluble fraction 52.99 \pm 0.32 mg of GAE/g of sample respectively (Table 1).

In the free radical scavenging (DPPH) assay, the IC₅₀ values of the test fractions were found to be within the range of 72.51 to 250.08 μ g/ml where the highest free radical scavenging activity was demonstrated by the carbon tetrachloride soluble fraction (IC₅₀= 72.51 \pm 0.69 μ g/ml) compared to the standard butylatd hydroxytoluene (BHT) (IC₅₀ = 27.5 μ g/ml) and ascorbic acid (ASA) (IC₅₀ = 5.25 μ g/ml) (Table 1).

In brine shrimp lethality bioassay, the LC₅₀ values of the test fractions were found within the range of 0.471 to 5.4009 µg/ml where the lowest LC₅₀ value (0.471±0.72µg/ml) was revealed by the pet-ether soluble fraction and the highest LC₅₀ value (5.4009±1.91µg/ml) was demonstrated by the carbon tetrachloride fraction whereas the standard Vincristine sulphate showed an LC₅₀ value of 0.451 µg/ml (Table 2).

In thrombolytic activity assay, addition of 100µl streptokinase as positive control (30,000 I.U.) to the clots and subsequent incubation for 90 minutes at 37°C, showed 66.77% lysis of clot. On the other hand, distilled water treated as negative control exhibited a negligible percentage of lysis of clot (2.89%). The mean difference in clot lysis percentage between positive and negative control was found statistically very significant. In this study, the crude methanol extract of *M. paniculata* exhibited highest thrombolytic activity (27.94±0.23%) (Table 3).

In antimicrobial screening by disc diffusion method, the none of the test samples of *M. paniculata* exhibited any zone of inhibition (Table 4).

At concentration 1.0 mg/ml, the extractives of *M. paniculata* protected the haemolysis of RBC induced by heat and hypotonic solution as compared to the standard acetyl salicylic acid (0.10 mg/mL). The aqueous soluble fraction inhibited 30.20±0.75 % and 60.83±1.45 % haemolysis of RBCs induced by heat and hypotonic solution as compared to 42.12 % and 84.444% by acetyl salicylic acid, respectively (Table 5).

CONCLUSION

From the above results it may be concluded that, the leaves of *M. paniculata* has highly significant cytotoxic and membrane stabilizing activities which warrant bioactivity guided

isolation of the active compounds and further investigation. The *M. paniculata* extractives also possess mild to moderate antioxidant and thrombolytic activities. The present findings from the preliminary toxicity studies of the extractives of *M. paniculata* (L) support the insecticidal uses of the plant by the indigenous people.

Table 1. Total phenolic content and free radical scavenging activity of different extractives of *M. paniculata*

Sample code	Total phenolic content (mg of GAE/g of dried extract)	Free radical scavenging activity (IC ₅₀ µg/ml)
ME	0.71±2.01	140.23 ±1.29
PESF	12.13±0.39	96.53±1.07
CTCSF	52.99±0.32	72.51±0.69
CSF	0.2±1.51	250.08 ± 1.2
AQSF	0.463±1.12	180.65 ±3.67
BHT	-	27.5±0.54
Ascorbic acid	-	5.25±0.21

ME= Methanol extract; PESF= Pet ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction and AQSF= Aqueous soluble fraction; BHT= Butylated hydroxytoluene

Table 2. LC₅₀ values in brine shrimp lethality bioassay of different fractions of *M. paniculata*

Samples	LC ₅₀ (µg/ml)
ME	0.773±0.19
PESF	0.471±0.72
CTCSF	5.4009±1.91
AQSF	2.0533±0.81
VS	0.451±0.08

ME= Methanol extract; PESF= Pet ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction, AQSF= Aqueous soluble fraction and VS= Vincristine sulfate

Table 3. Thrombolytic activity of different partitionates of *M. paniculata*

Sample Code	% of lysis
ME	27.94±0.23
PESF	15.17±0.81
CTCSF	13.09±1.57
CSF	10.94±1.66
AQSF	10.36±1.79
SK	66.77±1.08

ME= Methanol extract; PESF= Pet ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction and AQSF= Aqueous soluble fraction, SK= Steptokinase

Table 4. Antimicrobial activity of *M. paniculata* extractives

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	PESF	CTCSF	CSF	AQSF	Ciprofloxacin
<i>Bacillus sereus</i>	-	-	-	-	-	45±2.01
<i>B. megaterium</i>	-	-	-	-	-	42±1.17
<i>B. subtilis</i>	-	-	-	-	-	42±0.73
<i>Staphylococcus aureus</i>	-	-	-	-	-	42±0.23
<i>Sarcina lutea</i>	-	-	-	-	-	42±0.56
<i>Escherichia coli</i>	-	-	-	-	-	42±0.43
<i>Pseudomonas aureus</i>	-	-	-	-	-	42±1.11
<i>Salmonella typhi</i>	-	-	-	-	-	45±0.73
<i>Salmonella paratyphi</i>	-	-	-	-	-	47±2.33
<i>Shigella boydii</i>	-	-	-	-	-	34±0.58
<i>S. dysenteriae</i>	-	-	-	-	-	42±0.22
<i>Vibrio mimicus</i>	-	-	-	-	-	40±0.45
<i>V. parahemolyticus</i>	-	-	-	-	-	35±0.44

Methanol extract; PESF= Pet ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction and AQSF= Aqueous soluble fraction

Table 5: Effect of extractives of *M. paniculata* on heat and hypotonic solution induced hemolysis of erythrocyte membrane.

Treatment	Concentration (mg/mL)	Hemolysis inhibition (%)	
		Heat induced	Hypotonic solution induced
ME	2.0	30.00±0.66	53.22±0.81
PESF	2.0	18.35±0.35	51.65±1.11
CTCSF	2.0	27.86±0.62	53.364±0.31
AQSF	2.0	30.20±0.75	60.83±1.45
Acetyl salicylic acid	0.1	42.12±1.02	84.444±0.88

Methanol extract; PESF= Pet ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction and AQSF= Aqueous soluble fraction

Fig 1: Free radical scavenging activity of different partitionates of *M. paniculata*.

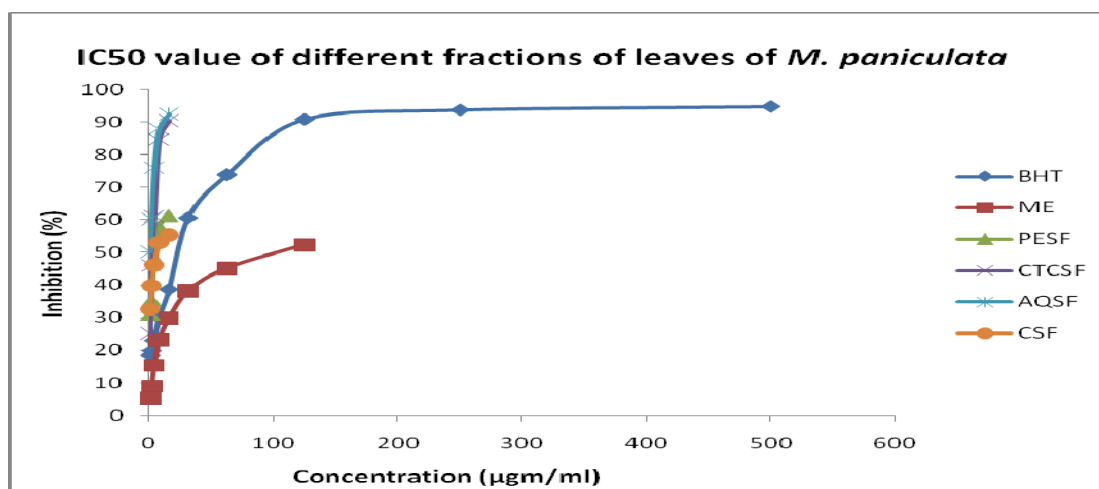


Fig 2: LC50 values in brine shrimp lethality bioassay of different fractions of *M. paniculata*.

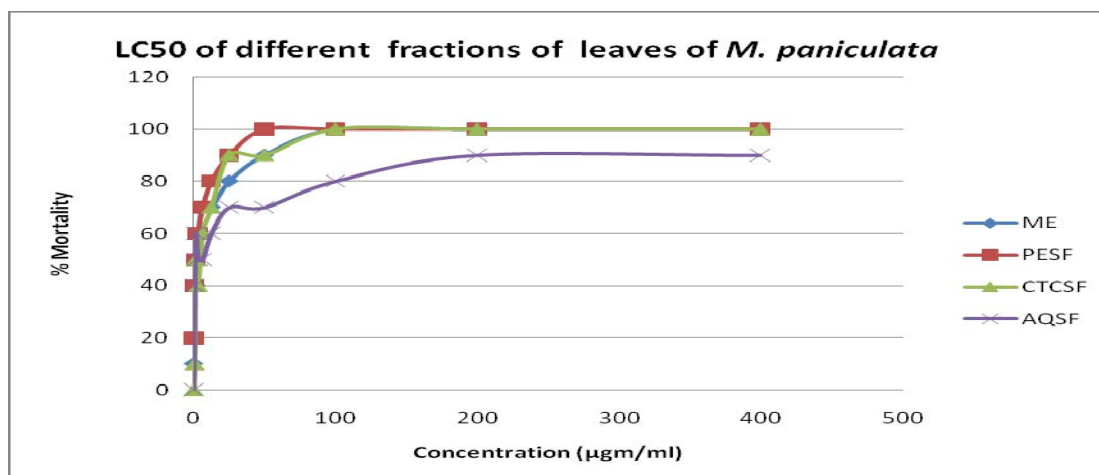
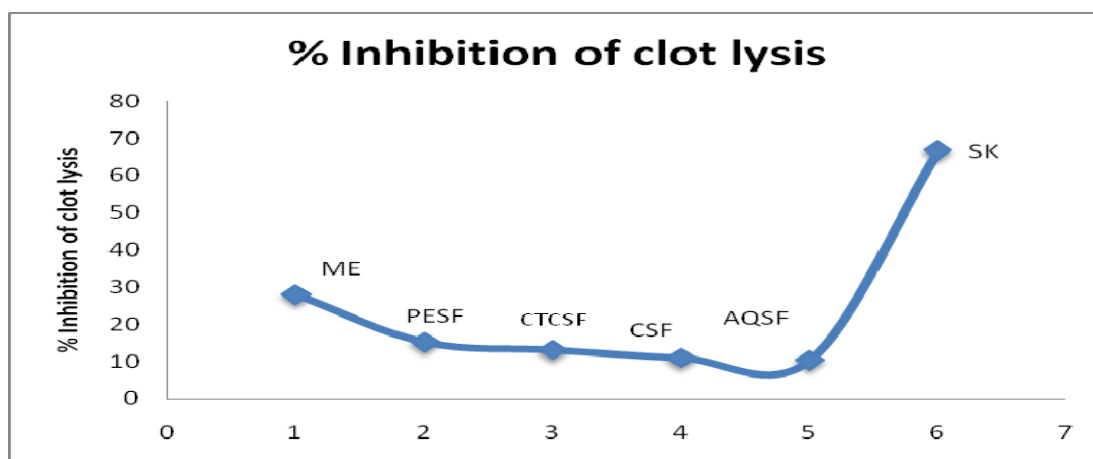
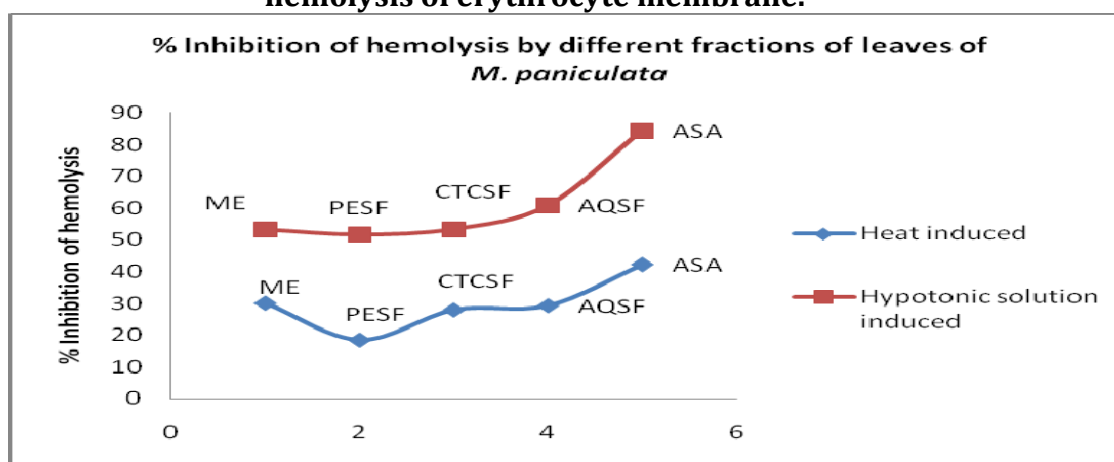


Fig 3: Thrombolytic activity of different partitionates of *M. paniculata* and standard.**Fig 4: Effect of extractives of *M. paniculata* on heat and hypotonic solution induced hemolysis of erythrocyte membrane.**

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