Antioxidant, Thrombolytic and Cytotoxic Activities of *Picrasma javanica*

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ABSTRACT: The methanol extract of leaf of *Picrasma javanica* as well as its *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screening for antioxidant and thrombolytic activities and brine shrimp lethality. The antioxidant potential was evaluated by DPPH, Folin-Ciocalteau reagent and phosphomolybdenum total antioxidant assays using butylatedhydroxytolune (BHT) and ascorbic acid as standards. All fractions showed moderate to strong antioxidant activity, of which the chloroform and aqueous soluble fractions demonstrated the highest activity with the IC_{50} value of 14.59 ± 0.73 µg/ml and 18.6 ± 0.15 µg/ml, respectively. The total phenolic content of the chloroform and aqueous soluble fractions was 10.4 ± 0.64 and 5.15 ± 0.22 mg of GAE/g of extractive, respectively. Thus, a positive correlation was found between the total phenolic content and total antioxidant activity of *P. javanica*. The general toxicity was determined by brine shrimp lethality bioassay where the crude extract (LC_{50} 1.04 ± 0.31 µg/ml) and its *n*-hexane (LC_{50} 1.28 ± 0.45µg/ml) soluble partitionate demonstrated the presence of considerable bioactive principles. Mild to moderate thrombolytic activity was discerned by the methanol extract of leaf of *P. javanica* and its different fractions. During assay for thrombolytic activity, the carbon tetrachloride soluble materials revealed 34.165±1.57 % of clot lysis while standard streptokinase and water, used as positive and negative controls, demonstrated 66.77% and 3.791% lysis of clot, respectively.

Key words: *Picrasma javanica*, antioxidant, DPPH, total phenolic, thrombolytic and cytotoxicity.

INTRODUCTION

Picrasma javanica Blume. (Synonyms: *Picrasma nepalensis* A.W. Bennet and *Picrasma philippinensis* Elmer., Bengali name: Nilghanta) belonging to the family *Simarubaceae*, grows in Java Island at 150-1400 m altitude. The plant occurs from the North-Eastern India throughout South East Asia to the Solomon's islands. In Bangladesh, it can be found in forests of Chittagong, Chittagong Hill Tracts and Cox's Bazar. Traditionally, the bark of the plant is used as a febrifuge where it is known to be a substitute for quinine.[1] Previous studies with *P. javanica* revealed anti-malarial and anti-cancer activities.[2] As part of our ongoing investigations on medicinal plants of Bangladesh[3,4] the methanol extract of leaves of *P. javanica* and growing in Bangladesh as well as its organic and aqueous soluble fractions were studied for the antioxidant potential in terms of total phenolic content and free radical scavenging property, thrombolytic activity in addition to general toxicity by brine shrimp lethality bioassay for the first time.

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MATERIALS AND METHODS

Collection of plant materials and extraction. The leaves of *P. javanica* were collected in mid 2011 from Khagrachori and a voucher specimen (DUSH - 10771) has been deposited in Dhaka University Salar Khan Herbarium for future reference.

The collected plant materials were chopped, dried and powdered and about 500 gm of the powdered material was soaked in 1.5 liter of methanol at room

temperature for 7 days. The extract was filtered by using Whatman filter paper number 1 and concentrated with a rotary evaporator. An aliquot of the concentrated methanol extract was fractionated by the modified Kupchan method[5] and the resultant partitionates were evaporated to dryness with a rotary evaporator to yield hexane (HXSF, 1500 mg), carbon tetrachloride (CTCSF, 950 mg), chloroform (CSF, 800 mg) and aqueous (AQSF, 500 mg) soluble materials. The residues were then stored in a refrigerator until further use.

Total phenolic content. The total phenolic content of the extractives were determined with Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd.[6] Sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau reagent and 2.0 ml of sodium carbonate (7.5%, w/v) in water were added and incubated for 15 min at 45°C. The absorbance of all samples was measured at 765 nm with a visible spectrophotometer. The phenolic contents were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of dry extract.

DPPH free radical scavenging assay. Following the method developed by Brand-Williams *et al*.[7] the antioxidant activity of the methanol extract and its sub-fractions was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Then, 2.0 ml of the different concentrations (500 µg/ml to 0.977 µg/ml) of the test samples were mixed with 3.0 ml of DPPH solution (20 µg/ml) in methanol. After 30 minutes of reaction period at room temperature in dark, the absorbance was measured at 517 nm. Then the IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, developed by plotting concentration of the samples versus percentage inhibition of free radicals. Here, synthetic antioxidants, butylatedhydroxytoluene (BHT) and L-ascorbic acid were used as positive controls.

Phosphomolybdenum antioxidant assay. The total antioxidant capacity of the extract was evaluated by the phosphomolybdenum assay method[8], the details of which has been published previously. In brief, the extract (2 mg/ml, 0.3 ml) was allowed to mix with 3.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the reaction mixture was incubated at 95°C for 90 minutes. After cooling at room temperature, the absorbance of the solution was measured at 695 nm by using a visible spectrophotometer against reagent blank.

Brine shrimp lethality bioassay. Brine shrimp bioassay method indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anti-cancer, anti-viral and pesticidal properties.[9] Test samples of different concentrations (400 µg/ml to 0.781 µg/ml) were prepared in dimethylsulfoxide (DMSO). Ten brine shrimp nauplii were taken in vials containing 5 ml of simulated sea water. Then test samples were added to the pre-marked vials with micropipette and after 24 hours, the number of the survivors was counted and the LC_{50} was calculated from the regression equation, obtained from the logarithm of sample concentration versus percentage mortality of the shrimp nauplii.

Thrombolytic activity. The thombolytic activity of all extractives was evaluated by the method developed by Prasad *et al*.[10] using streptokinase (SK) as positive control. Aliquots (5 ml) of venous blood were drawn from healthy volunteers that were distributed in five different pre-weighed sterile micro-centrifuge tubes (500 µl/tube) and incubated at 37°C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone).

To each micro-centrifuge tube containing pre-weighed clot, 100 µl aqueous solution of different partitionates and the crude extract was added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes. All tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluid was

removed and tubes were again weighed to observe the difference in weight after clot disruption. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt of released clot /clot wt) × 100

Table1. Total phenolic content, free radical scavenging activity, total antioxidant capacity and cytotoxicity of *P. javanica.*

Sample	Total phenolic content (mg of GAE/gm of dried extract)	Free radical scavenging activity IC_{50} (µg/ml)	Total antioxidant capacity (mg of ascorbic acid/100 g of extract)	Brine shrimp lethality bioassay LC_{50} (µg/ml)
Vincristine sulfate	-	-	-	0.451
BHT	-	27.5 ± 0.54	-	-
Ascorbic acid	-	5.8 ± 0.21	-	-
ME	5.09 ± 0.22	41.53 ± 0.21	1.128 ± 0.61	1.04 ± 0.31
HXSF	5.09 ± 0.56	71.2 ± 0.45	1.073 ± 0.52	1.28 ± 0.45
CTCSF	0.833 ± 0.45	133.6 ± 0.84	0.89 ± 0.25	2.00 ± 0.32
CSF	10.4 ± 0.64	14.59 ± 0.73	1.465 ± 0.45	2.30 ± 0.51
AQSF	5.15 ± 0.22	18.6 ± 0.15	1.399 ± 0.15	3.37 ± 0.82

The average values of three calculations are presented as mean ± S.D. ; BHT= Butylatedhydroxytolune; ME= Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction.

RESULTS AND DISCUSSION

The crude methanol extract of *P. javanica* as well as different Kupchan partitionates derived from it were subjected to assays for total phenolic content, free radical scavenging activity, preliminary cytotoxicity and thrombolytic activity. The total phenolic content in the samples were found in the range of 0.833 to 10.4 mg of GAE/g of sample. The highest amount of phenolic compounds (10.4 mg of GAE/g of extractive) was found in the chloroform soluble fraction (Table 1).

In the DPPH free radical scavenging assay, the chloroform soluble fraction revealed maximum free radical scavenging activity (IC_{50} = 14.59±0.73 µg/ml) when compared to ascorbic acid (IC_{50} = 5.8±0.21 µg/ml). This prominent free radical scavenging may be correlated to its high phenolic content (10.4±0.64 mg of GAE/gm of sample) or due to synergistic activity of various chemical entities present in the extractive. A positive correlation was seen between total phenolic content and total antioxidant activity of *P. javanica.*

In the brine shrimp lethality bioassay, the lowest LC_{50} (1.04±0.31 µg/ml) value was obtained with the crude extract whereas Vincristine sulphate exhibited an LC_{50} value of 0.451 µg/ml (Table 1). This suggested the presence of potent bioactive components in the crude extract.

In order to identify the drugs with the ability to promote lysis of blood clot from natural resources, the extractives of *P. javanica* were assessed for thrombolytic activity and the results are presented in Table 2. Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots of human blood and subsequent incubation for 90 minutes at 37°C, showed 66.77% lysis of clot. On the other hand, distilled water when treated as negative control, showed negligible lysis of clot (3.793%). The mean difference in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study, all the samples showed mild to moderate activity where the carbon tetrachloride and hexane soluble fractions exhibited 34.165±1.57 and 33.17±0.81% clot lysis, respectively (Table 2).

It is clearly evident from the above findings that the leaves of *P. javanica* have potent antioxidant and cytotoxic properties. The plant also exhibited moderate thrombolytic activity. Therefore, it is a good candidate for further systematic chemical and biological studies to isolate the active principles.

Table 2. Thrombolytic activity of test samples of *P. javanica.*

Sample code	Sample description	% of lysis
SK	Streptokinase	66.77 ± 1.08
ME	Methanol extract	29.583 ± 0.23
HXSF	Pet ether soluble fraction	33.17 ± 0.81
CTCSF	Carbon tetrachloride soluble fraction	34.165 ± 1.57
CSF	Chloroform soluble fraction	10.94 ± 1.66
AQSF	Aqueous soluble fraction of methanol extract	30.196 ± 1.79
Water		3.791 ± 0.55

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