Antimicrobial, Antioxidant and Cytotoxic Activities of *Glochidion multiloculare* (Roxb. ex Willd.) Müll. Arg. (Euphorbiaceae)

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ABSTRACT: The methanol extract (ME) of the powdered bark of *Glochidion multiloculare* and its six vacuum liquid chromatographic (VLC) fractions (F_{a-f}) were investigated for antimicrobial, cytotoxic and antioxidant activities. Only fractions F_c and F_d showed mild antimicrobial activity. Significant free radical (DPPH) scavenging activity was found in F_f (IC_{50} value = 9.27±0.117 µg/ml). The total phenolic content was measured involving Folin-Ciocalteu reagent and it was the highest in fraction F_e (187.00±1.74 mg of GAE/gm of sample). Cytotoxicity (LC_{50}) by brine shrimp lethality bioassay was found to be significant for F_b (0.023±0.001 µg/ml), F_c (0.3±0.01 µg/ml) and F_d (0.117±0.0015 µg/ml).

Keywords: *Glochidion*, antimicrobial, brine shrimp lethality bioassay, total phenolic content, free radical scavenging.

INTRODUCTION

Glochidion was regarded as a genus of the family Euphorbiaceae. But molecular phylogenetic studies have shown that *Phyllanthus* is paraphyletic over *Glochidion*. A recent revision of the family Phyllanthaceae has subsumed *Glochidion* into *Phyllanthus.*[1] *Glochidion multiloculare* (Roxb. ex Willd.) Müll. Arg., Phyllanthaceae (synonym: *Phyllanthus multilocularis*) is an evergreen shrub or small tree which is found in Bhutan, India, Myanmar, Nepal and Bangladesh.

Biological investigations of *Phyllanthus* species revealed that many members of the genus possess anti-tumor promoting ability,[2-4] antiviral activity against hepatitis B virus,[5,6] anti-angiogenic,[2] lipid lowering activity,[7] antidiabetic,[8-10] antiherpetic activity,[11,12] anti-HIV,[13-15] antiplasmodial[16] and other activities. However, no biological studies of *G. multiloculare* have been found in literature to date.

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Several secondary metabolites were isolated from different *Glochidion* species, including tannins,[17] glycosides,[18] lignans,[19] terpenoids.[20] Previous investigations of *Glochidion multiloculare* revealed glochidiol, glochilocudiol, glochidone, lupeol, dimedone etc.[21,22]

The present work was an endeavor to screen the methanolic extract (ME) of the barks of *G. multiloculare* and its chromatographic fractions for probable antibacterial, cytotoxic and antioxidant activities and we, herein, report the results of our preliminary studies.

MATERIALS AND METHODS

Plant material. *Glochidion multiloculare* (Roxb. ex Willd.) Müll. Arg., (Euphorbiaceae) was collected from Rajendrapur, Gazipur, Bangladesh in the month of August, 2009 and was identified in Bangladesh National Herbarium, Dhaka, Bangladesh. A voucher specimen (DACB Accession No. 34391) of the plant has been deposited in the herbarium.

Production of extracts. The powdered bark (700 g) of *G. multiloculare* was soaked in methanol (3 L) for 15 days. Part of the residue (10 g) obtained

from methanol extract was subjected to vacuum liquid chromatography using *n*-hexane, *n*-hexane–EtOAc, EtOAc, EtOAc-MeOH, MeOH, MeOH-H_2O and H_2O in order of increasing polarities. As a result, 15 fractions (each 100 ml) were obtained and on the basis of TLC behavior same were combined to yield test samples F_a (1-3), F_b (4-6), F_c (7-8), F_d (9-10), F_e (11-12) and F_f (13-15).

***In vitro* antimicrobial activity.** The samples were tested for antimicrobial activity by the disc diffusion method.[23] The screening was done against 13 strains of bacteria. The results thus obtained were compared with standard antibiotic, kanamycin (30 µg/disc).

Cytotoxicity by brine shrimp lethality bioassay. In brine shrimp lethality bioassay[24] dimethyl sulfoxide (DMSO) was used as a solvent and negative control while vincristine sulfate (VS) served as the positive control. For cytotoxicity screening, DMSO solutions of the test samples were applied against *Artemia salina* in a 1-day *in vivo* assay. For the experiment, 4 mg of each of the test samples was dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ ml) were obtained by serial dilution technique.

Total phenolics analysis. Total phenolics of the samples were measured by Folin-Ciocalteu reagent.[25] To 0.5 ml of sample solution (0.25 mg/ml) in water, 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of sodium carbonate (7.5 % w/v) solution were added. After 20 minutes of incubation at room temperature the absorbance was measured at 760 nm using UV-visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the known concentrations of standard gallic acid (0-100 µg/ml). The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent)/gm of the sample.

Free radical scavenging activity. The free radical scavenging activity (antioxidant capacity) of the test samples was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH).[26] Here, 2.0 ml of a methanol solution of the sample (test sample/ standard) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm by UV spectrophotometer by using methanol as blank. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\ \%) = (1 - A_{sample}/A_{blank}) \times 100$$

Where A_{sample} is the absorbance of the sample and A_{blank} is the absorbance of the control (containing all reagents except the test material). Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with inhibition percentage against sample/standard concentration.

Statistical analysis. Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD (n=3). Probability (P) value of 0.05 or less ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

***In vitro* antimicrobial activity.** *In vitro* antibacterial activity of the test samples were investigated against five gram positive bacteria namely, *Bacillus cereus, B. megaterium, B. subtilis, Sarcina lutea, Staphylococcus aureus* and eight gram negative bacteria namely, *Escherichia coli, Pseudomonas aeruginosa, Salmonella* Paratyphi*, Salmonella* Typhi*, Shigella boydii, Sh. dysenteriae, Vibrio mimicus,* and *V. parahaemolyticus.* Test samples F_c and F_d showed weak antimicrobial activity but other samples were found to be inactive. The zone of inhibition of F_c and F_d were found to be 8-9 mm and 7-9 mm, respectively, at 400 µg/disc against all test organisms. The zone of inhibition of samples was compared with the zone of inhibition of kanamycin (30 µg/disc) which showed 30-32 mm of zone of inhibition against all test organisms.

Cytotoxicity by brine shrimp lethality bioassay. In the cytotoxicity screening the LC_{50} values obtained from the best-fit line slope were found to be significant (in comparison with VS, 0.423 µg/ml) for F_b (0.023±0.001 µg/ml), F_c

(0.3±0.01 µg/ml), F_d (0.117±0.0015 µg/ml) and F_e (8.17±0.036 µg/ml). The LC_{50} values for the other samples were found to be somewhat significant (9.04-85.92 µg/ml) in comparison to positive control.

Antioxidant activity. Total phenolic content of *G. multiloculare* extractives was found to be the highest in F_e (187.00±1.74 mg of GAE/gm of sample) and the lowest in F_b (18.82±0.36 mg of GAE/gm of sample) (Table 1). In addition, *G. multiloculare* extractives were subjected to free radical scavenging activity using DPPH by using ascorbic acid (ASA) and tert-butyl-1-hydroxytoluene (BHT) as reference standards (Table 1). Free radical scavenging activity was found to be significant in F_f (IC_{50} value is 9.27±0.117 µg/ml).

Table 1. Total phenolic content and free radical scavenging activity of *G. multiloculare* extractives

Sample	Total phenolic content (mg of GAE/gm of sample)	Free radical scavenging activity (IC_{50} in µg/ml)
BHT	-	20.45±0.17
ASA	-	2.9±0.04
ME	100.87±1.88	16.46±0.32
F_a	19.82±0.26	Value too high *i.e.* no activity
F_b	18.82±0.36	Value too high *i.e.* no activity
F_c	23.87±0.35	17.82±0.106
F_d	186.45±2.57	33.92±0.175
F_e	187.00±1.74	23.72±0.11
F_f	176.22±3.6	9.27±0.117

The average values of three replicates are presented as mean ± S.D. (Standard Deviation).

Therefore, it can be concluded that, in the preliminary studies, some of the test samples obtained from the bark of *G. multiloculare* revealed mild antibacterial activity while significant antioxidant activity as well as strong cytotoxicity.

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