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Biological activities of Bonsupari (*Caryota urens* L.) fruits

Tasnuva Sharmin[1*], Md Shahidur Rahman[2], Sirajis Salekin[1] and Kamrun Nahar[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[2]Department of Chemistry, Faculty of Science, University of Dhaka, Dhaka-1000, Bangladesh.
[3]Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh.

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In Asian subcontinent, *Caryota urens* L. plant is well known. Its leaf has antioxidant and antimicrobial properties. The work aims to analyze the composition of the plant's fruit extract and its aqueous and organic soluble fractions for its cytotoxic, thrombolytic, antioxidant, membrane stabilizing and antimicrobial properties. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay, *phosphomolybdenum* total antioxidant assay and total phenolic content were used to evaluate the antioxidant activity. Brine shrimp lethality bioassay was used to determine the cytotoxic property. In thrombolytic activity assay, streptokinase was the standard. The samples were exposed to membrane stabilizing activity assay under heat and hypotonic solution-induced conditions. The samples' antimicrobial potential was evaluated with Disc diffusion assay. The crude methanol extract showed the highest free radical scavenging activity (IC_{50} = 62.74±0.16 µg/mL) that can correlate with its total phenolic content of 106.88±0.19 mg of GAE / g of sample. The crude methanol extract showed the highest cytotoxic potential (LC_{50} = 0.59±0.34 µg/mL). The carbon tetrachloride soluble materials revealed 20.48±0.44% of clot lysis in the assay for thrombolytic activity. The crude methanol extract prevented haemolysis of human erythrocytes in hypotonic solution-induced condition by 64.17±0.26%.

Key words: *Caryota urens* L., free radical scavenging activity, brine shrimp lethality, thrombolysis, membrane stabilization.

INTRODUCTION

Caryota urens L. (English name: Fishtail palm, Bengali name: Bonsupari) belongs to Arecaceae family. The plant is a native of India, Myanmar and Sri lanka (Uddin et al., 2015). Traditionally root is used to treat tooth ailments. Flower is useful in gastric ulcer and migraine. Bark is used to treat rheumatic swellings and snake bite (Charles et al., 2011; Uddin et al., 2015). Recent scientific investigations report that *C. urens* sap is nutritionally rich

*Corresponding author. E-mail: tasnuva.phr.du@gmail.com. Tel:+880-1720212675.

and contains mixture of simple sugars such as sucrose, glucose and fructose (Somasiri et al., 2008). Flavonoids have been isolated from the methanol extract of the fruits (Srivastav et al., 2015). The plant has been reported to possess significant antioxidant, anti-diabetic and anti-microbial activities in the last few years (Charles and Ramani, 2011; Ranasinghe et al., 2012; Krishnamoorthy et al., 2013; Azam et al., 2016; Wimalasiri et al., 2016; Sujitha and Kripa, 2018).

In investigating Bangladesh medicinal plants (Sharmin et al., 2017-2018), the crude methanol extract of *C. urens* fruits in Bangladesh including its organic and aqueous soluble fractions was for the first time evaluated for the antioxidant potential based on total phenolic content, phosphomolybdenum total antioxidant activity free radical scavenging activity, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial properties.

MATERIALS AND METHODS

Plant materials

Fruits of *C. urens* were obtained from Mirpur, Dhaka, Bangladesh. A voucher specimen (DACB-39528) is maintained in Bangladesh National Herbarium, Dhaka, Bangladesh for use in future.

The fruits (800 g) were dried under the sun and ground into powder. It was then macerated in 2.5 L of methanol for one week. It was filtered via fresh cotton bed and with Whatman filter paper number 1. It was concentrated with a rotary evaporator at low temperature and pressure. A modified version of Kupchan partition protocol (VanWagenen et al., 1993) was used to fractionate an aliquot (5 g) of the concentrated methanol extract; the partitionates obtained from there were evaporated to dryness with rotary evaporator. This yielded hexane soluble fraction (HXSF, 1.8 g), carbon tetrachloride soluble fraction CTCSF, 2.0 g), chloroform soluble fraction (CSF, 0.2 g) and aqueous soluble fraction (AQSF, 0.5 g). The residues were kept in a refrigerator for use later.

Total phenolic content

Folin-Ciocalteau reagent was used to determine the total phenolic content by Harbertson and Spayd (2006)'s method developed.

DPPH free radical scavenging assay

Brand-Williams et al. (1995)'s developed method was used to assess the capacity of the study samples to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The positive controls were Butylated hydroxytoluene (BHT) and ascorbic acid.

Phosphomolybdenum antioxidant assay

Phosphomolybdenum antioxidant assay method (Prieto et al., 1999) was used to evaluate the total antioxidant activity of the extract.

Brine shrimp lethality bioassay

This was done to determine the overall harmful activities of the dimethyl sulfoxide (DMSO) solutions of plant samples against *Artemia salina* in one day *in vivo* assay (Meyer et al., 1982). The positive control was Vincristine sulphate .

Thrombolytic activity

Prasad et al. (2006)'s method was used to evaluate the thrombolytic property. The positive control was streptokinase.

Membrane stabilizing activity

Omale and Okafor (2008)'s method was used to assess the membrane stabilizing property of the samples by analyzing their capacity to prevent hypotonic solution and heat-induced haemolysis of human erythrocytes.

Antimicrobial screening

Disc diffusion method was used to determine antimicrobial property (Bayer et al., 1966).

Statistical analysis

Three replicates of each sample were used for statistical analysis for all bioassays; the values are given as mean ± standard deviation (SD). A two-tailed Student's t-test was used to evaluate the results.

RESULTS

This research was done to analyze *C. urens* fruit extract for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities.

The crude methanol extract of *C. urens* fruits have a high content of phenolic principles (106.88±0.19 mg of GAE/g of sample). The extract's total phenolic content and the free radical scavenging activity correlate positively (IC_{50}= 62.74±0.16 µg/m) (Table 1).

All the fractions had significant cytotoxic potential against *A. salina* in brine shrimp lethality bioassay. The crude methanol extract showed the highest cytotoxic activity with LC_{50} value of 0.59±0.34 µg/mL in comparison to 0.451 µg/mL for Vincristine sulphate (Table 1).

C. urens extract had mild thrombolytic activity. The carbon tetrachloride soluble fraction was 20.48% of clot lysis in contrast to 66.77% clot lysis by streptokinase used as standard (Table 2).

At 1.0 mg/mL, *C. urens samples* protected the haemolysis of RBC caused by hypotonic solution and heat compared to the standard acetyl salicylic acid (0.10 mg/mL). The crude methanol extract prevented 64.17±0.26% of haemolysis of RBCs induced by hypotonic solution in contrast to 71.9% by acetyl salicylic acid (Table 2).

The antimicrobial activity of *C. urens* samples was analyzed against five gram positive and eight gram negative bacteria. The results were compared with

Table 1. Total phenolic content, phosphomolybdenum total antioxidant capacity, free radical scavenging and cytotoxic activities of *C. urens.*

Samples/ standards	Total phenolic content (mg of GAE/ g 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)
ME	106.88±0.19	62.74±0.16	4.0±0.20	0.59±0.34
HXSF	1.45±0.25	387.74±0.46	1.12±0.27	0.71±0.53
CTCSF	7.48±0.18	120.24±0.09	4.0±0.38	3.11±0.12
AQSF	41.42±0.41	-	3.61±0.21	3.51±0.11
Ascorbic acid	-	5.8±0.21	-	-
BHT	-	27.5±0.54	-	-
Vincristine sulfate	-	-	-	0.451±0.04

Table 2. Thrombolytic and membrane stabilizing activities of *C. urens.*

Samples/standards	% of lysis of RBC	% Inhibition of haemolysis	
		Heat-induced	Hypotonic solution-induced
ME	2.40±0.23	19.01±0.43	64.17±0.26
HXSF	7.70±0.18	6.70±0.14	63.28±0.49
CTCSF	20.48±0.44	10.00±0.27	60.91±0.54
Water	3.79±0.21	-	-
Streptokinase	66.77±0.36	-	-
Hypotonic medium	-	-	-
Acetyl salicylic acid	-	42.12±-0.38	71.9±0.78

ME = Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction.

Table 3. Antimicrobial activity of *C. urens.*

Parameter	Diameter of zone of inhibition (mm)			
	ME	CTCSF	CSF	Ciprofloxacin
Bacillus cereus	12.0±0.38	-	-	45.0±2.01
B. megaterium	-	-	-	42.0±1.17
B. subtilis	-	-	-	42.0±0.73
Staphylococcus aureus	-	-	-	42.0±0.23
Sarcina lutea	-	-	8.0±0.12	42.0±0.56
Escherichia coli	-	-	-	42.0±0.43
Pseudomonas aeruginosa	-	-	-	42.0±1.11
Salmonella typhi	-	-	-	45.0±0.73
S. paratyphi	-	8.0±0.15	8.0±0.24	47.0±2.33
Shigella boydii	-	-	-	34.0±0.58
S. dysenteriae	-	13.0±0.39	-	42.0±0.22
Vibrio mimicus	8.0±0.22	8.0±0.18	8.0±0.41	40.0±0.45
V. parahaemolyticus	-	-	-	35.0±0.44

ME = Methanolic crude extract; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction.

ciprofloxacin, standard antibiotic. Among all the samples, the largest zone of inhibition (13.0 mm) was displayed by the carbon tetrachloride soluble fractions against *Shigella dysenteriae* (Table 3).

DISCUSSION

The high phenolic content of *C. urens* extract might contribute to its antioxidant potentials. Lupeol and ursolic acid have been isolated from the leaf of this plant (Muhaisen, 2013). Both compounds possess antioxidant potentials (Santiago et al., 2014; Tchimene et al., 2016). The antioxidant potential of *C. urens* extract might be due to the presence of these two compounds. Lupeol has been also found to be a potent cytotoxic component (Moriarity et al., 1998). Therefore, this compound might be responsible for the observed cytotoxic activity.

C. urens extract showed membrane stabilizing property. Leakage of serum proteins and fluid into the tissue causes inflammation. Membrane stabilizing property can prevent induction of inflammation (Chaitanya et al., 2011). Ursolic acid possesses anti-inflammatory property (Checker et al., 2012; Wang et al., 2018). The presence of this compound in *C. urens* might contribute to the observed membrane stabilizing activity.

Conclusion

People have the common belief that nature is good. This belief has contributed to the increased popularity of traditional medicines. But consuming plant-based medicines might cause unwanted side effects because such medicines contain a large number of compounds with different activities. This study has revealed that *C. urens* fruit extract possesses significant antioxidant and membrane stabilizing potentials. Therefore, it is important to identify the compounds responsible for the observed activities from the fruit extract. Thus, the plant is a good candidate for further systematic, chemical and biological studies to isolate the active principles.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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