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Canvassing of biological attributes by *In vitro* screening of *Canarium bengalense*

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Various Hoang family remedies known as Dai-Thien-Nuong, utilize *Canarium bengalense* Roxb. (Family: *Burseraceae*) as a major component for the treatment of tumor and liver damage. This study was performed using the crude methanol extract of *C. bengalense* Roxb. bark and its petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble fractions for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. The antioxidant activity was assessed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay. The cytotoxic and thrombolytic activities were determined using vincristine sulphate and *streptokinase* as standards, respectively. Heat and hypotonic solution-induced conditions were induced to determine membrane stabilizing activity. Disc diffusion method was used to determine the antimicrobial activity of the test samples. Among all the test samples, the crude methanol extract showed the highest free radical scavenging activity (IC_{50} = 50.62±0.26 µg/ml). In cytotoxic activity assay, the pet-ether soluble fraction revealed the highest cytotoxic potentials (LC_{50} = 8.96 ± 0.64 µg/ml). The same sample showed 31.08±0.12% of clot lysis in thrombolytic activity assay. In membrane stabilizing activity assay, the dichloromethane soluble fraction showed 60.94 ± 0.31% inhibition of heat induced haemolysis of RBC and this finding was found to be more significant than that of acetyl salicylic acid (42.12±0.38%) used as standard in this assay. Phytocomponents responsible for the observed activities should be isolated for new drug development.

Key words: *Canarium bengalense* Roxb, total phenolic content, cytotoxicity, thrombolytic activity, membrane stabilizing activity.

INTRODUCTION

Many medicines used nowadays are plant derived. Some of these medicines are directly isolated from their natural sources and used as it is. Others are modified using different drug designing techniques to maximize the

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desired effects, improve potency and minimize the side effects. Reports of World Health Organization (WHO) indicate that a huge percentage of global population (about 80%) prefer traditional medicines for their primary health care (Kabir et al., 2015). It is necessary to screen the medicinal plants, isolate and modify the active components and develop new medicines.

The local name of *C. bengalense* Roxb. (Family: Burseraceae) is Dhuna Rata or East Indian Copal. The native origin of the plant is not known but the plant is abundant in Vietnam, China, Laos, Myanmar, Thailand and India-Assam (Wu et al., 2008). Extracts from leaf and root is used in bronchitis, leprosy, jaundice, cough and asthma. Anti-inflammatory, antiseptic and anti-asthmatic activities were demonstrated by leaf and bark extracts (Le et al., 2012). The species has been reported to be useful in skin rashes and snake bite (Sarkar et al., 2017). A new flavone glycoside and six known compounds with cytoprotective properties have been isolated from the stem bark of *C. bengalense.* This might be the reason of the traditional use of this plant in tumor and liver damage (Le et al., 2012).

It is important to investigate the medicinal plants of Bangladesh for prominent biological activities (Sharmin et al., 2017; 2018). *C. bengalense* is abundant in Bangladesh and very few experiments were carried out using the bark of the plant. Therefore, antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activity assays were conducted using the crude methanol extract of *C. bengalense* bark extract as well as its organic and aqueous soluble fractions.

MATERIALS AND METHODS

Plant materials

C. bengalense bark was collected from Mirpur botanical garden, Dhaka, Bangladesh. A voucher specimen (DACB 47558) for this collection has been maintained in Bangladesh National Herbarium for future reference.

Small pieces of the bark were sun dried for several days. Then the bark pieces were oven dried for 24 h for better grinding. High capacity grinding machine was used to powder the dried bark. 500 g powdered material was soaked in 3.0 l of methanol in a clean, ambered color reagent bottle (5.0 l) for 10 days. The bottle was occasionally shaken and stirred. Then the plant material was filtered, and the filtrate was evaporated to dryness using a rotary evaporator at 40°C and 50 r.p.m to give the crude methanol extract. The concentrated methanol extract (5 g) was fractionated by modified Kupchan partition protocol (Van Wagenen et al., 1993) and the petroleum ether (PESF, 0.8 g), carbon tetrachloride (CTCSF, 1.7 g), dichloromethane (DCMSF, 1.3 g) and aqueous (AQSF, 0.7 g) soluble materials were obtained and refrigerated until further use.

Drugs and chemicals

Beacon Pharmaceutical Ltd provided *Streptokinase*. All other drugs, reagents and solvents were obtained from Sigma-Aldrich, Munich, Germany.

Total phenolic content

The method developed by Harbertson and Spayd (2006) was used to determine the total phenolic content of the test samples.

Antioxidant activity

The antioxidant activity of the test samples was determined using BHT and ascorbic acid as reference standards (Brand-Williams et al., 1995).

Brine shrimp lethality bioassay

The cytotoxic potential of the plant samples was determined using single day in vivo assay. The test samples were assayed using *Artemia salina*. Vincristine sulphate was used as the reference standard (Meyer et al., 1982). The lethality of brine shrimp nauplii was used in this assay to determine cytotoxic activity.

Thrombolytic activity

The thrombolytic activity was determined following the method developed by Prasad et al. (2007). *Streptokinase* was used as positive control.

Membrane stabilizing activity

The ability of the extract and fractionates to inhibit heat and hypotonic solution induced haemolysis of human erythrocytes was assessed following the method developed by Omale et al. (2008).

Antimicrobial screening

Disc diffusion method was used to investigate the antimicrobial potential of the crude extract and its aqueous and organic soluble fractionates by observing their ability to generate zone of inhibition (Bayer et al., 1966).

Statistical analysis

Three replicates of each sample were used for statistical analysis and all of the values are expressed as the mean ± standard deviation (SD). The results were evaluated by a two-tailed non-parametric pair t-test. $P < 0.05$ was considered statistically significant.

RESULTS

The present study was undertaken to assess the antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities of the crude methanol extract of *C. bengalense* and its organic and aqueous soluble materials.

Table 1. Total phenolic content, antioxidant and cytotoxic activities of *Canarium bengalense*.

Samples/ Standard	Total phenolic content (mg of GAE/g of dried extract)	Free radical scavenging activity IC_{50} (µg/ml)	Brine shrimp lethality bioassay LC_{50} (µg/ml)
ME	14.31 ± 0.23	50.62 ± 0.26	18.37 ± 0.38
PESF	10.75 ± 0.41	59.42 ± 0.63	8.96 ± 0.64
CTCSF	9.44 ± 0.16	514.65 ± 0.10	25.12 ± 0.61
DCMSF	4.31 ± 0.25	361.35 ± 0.21	27.00 ± 0.22
AQSF	2.75 ± 0.11	266.74 ± 0.46	54.27 ± 0.32
Vincristine sulfate	-	-	0.45 ± 0.04
BHT	-	20.93 ± 0.54	-
Ascorbic acid	-	3.63 ± 0.21	-

ME= Crude methanol extract; PESF= Pet-ether Soluble Fraction; CTCSF= Carbon tetrachloride soluble fraction; DCMSF= Dichloromethane soluble fraction; AQSF= Aqueous soluble fraction; GAE= Gallic acid equivalent; BHT= Butylated hydroxytoluene.

Table 2. Thrombolytic and membrane stabilizing activities of *Canarium bengalense* bark extract and soluble fractions.

Samples/Standard	% of lysis of RBCs	% Inhibition of haemolysis	
		Heat induced	Hypotonic solution induced
ME	20.69 ± 0.28	9.29 ± 0.19	9.76 ± 0.06
PESF	31.08 ± 0.12	46.50 ± 0.48	4.28 ± 0.32
CTCSF	27.58 ± 0.55	33.12 ± 0.11	12.93 ± 0.17
DCMSF	21.96 ± 0.23	60.94 ± 0.31	14.85 ± 0.22
AQSF	16.35 ± 0.21	17.21 ± 0.25	49.17 ± 0.31
Water	3.79 ± 0.55	-	-
Streptokinase	65.01 ± 0.36	-	-
Acetyl salicylic acid	-	42.12 ± 0.38	72.00

ME= Crude methanol extract; PESF= Pet-ether Soluble Fraction; CTCSF= Carbon tetrachloride soluble fraction; DCMSF= Dichloromethane soluble fraction; AQSF= Aqueous soluble fraction.

Different test samples of *C. bengalense* demonstrated presence of phenolic components within the range of 2.75 to 14.31 mg of GAE/g of sample. Among the test samples, the crude methanol extract showed the highest value of phenolic content (14.31 ± 0.23 mg of GAE/g of sample). In free radical scavenging activity assay, the highest free radical scavenging activity was given by the crude methanol extract (IC_{50} = 50.62 ± 0.26 µg/ml) followed by pet-ether soluble fraction (IC_{50} = 59.42 ± 0.63 µg/ml), as compared to ascorbic acid and BHT exhibiting IC_{50} values of 3.63 µg/ml and 20.93 µg/ml, respectively (Table 1).

In case of brine shrimp lethality bioassay, among all the test samples of the bark of *C. Bengalense*, the highest cytotoxic activity was given by the pet-ether soluble fraction (LC_{50} = 8.96 ± 0.64 µg/ml) followed by the crude methanol extract (LC_{50} = 18.37 ± 0.38 µg/ml) as shown in Table 1.

In thromboltic activity assay, the pet-ether soluble and the carbon tetrachloride soluble fractions showed 31.08 ± 0.12% and 27.58 ± 0.55% of clot lysis, respectively as compared to 65.01% clot lysis by the standard *streptokinase* (Table 2).

At concentration 1.0 mg/ml, the test samples of *C. bengalense* protected the haemolysis of RBCs induced by heat and hypotonic solution as compared to the standard acetyl salicylic acid (0.10 mg/ml). The dichloromethane soluble fraction showed 60.94 ± 0.31% inhibition of heat induced haemolysis which is found to be more significant than acetyl salicylic acid (42.12 %). On the other hand, the aqueous soluble fraction inhibited 49.17 ± 0.31 % hypotonic solution-induced haemolysis of RBCs as compared to 72.00% by acetyl salicylic acid (Table 2).

The antimicrobial activity of *C. bengalense* test samples was evaluated against gram positive and gram negative bacteria and the results were compared with standard antibiotic, ciprofloxacin. Among the test samples of *C. bengalense*, only the carbon tetrachloride soluble fraction and dichloromethane soluble fraction revealed antimicrobial activity with zone of inhibition ranging from 7.0 to 9.0 mm. The highest zone of inhibition (9.0 mm)

Table 3. Antimicrobial activity of test samples of *Canarium bengalense*.

Test microorganisms	Diameter of zone of inhibition (mm)		
	CTCSF	DCMSF	Ciprofloxacin (30 µg/disc)
Bacillus cereus	8.0 ± 0.14	7.0 ± 0.31	45.0 ± 2.01
B. megaterium	8.0 ± 0.42	7.0 ± 0.21	42.0 ± 1.17
B. subtilis	7.0 ± 0.33	-	42.0 ± 0.73
Staphylococcus aureus	7.0 ± 0.55	-	42.0 ± 0.23
Sarcina lutea	8.0 ± 0.24	-	42.0 ± 0.56
Escherichia coli	7.0 ± 0.13	8.0 ± 0.45	42.0 ± 0.43
Pseudomonas aeruginosa	7.0 ± 0.32	7.0 ± 0.31	42.0 ± 1.11
Salmonella typhi	9.0 ± 0.51	-	42.0 ± 1.11
S. paratyphi	8.0 ± 0.12	7.0 ± 0.22	47.0 ± 2.33

CTCSF= Carbon tetrachloride soluble fraction; DCMSF= Dichloromethane soluble fraction.

was showed against *Salmonella typhi* by the carbon tetrachloride soluble fraction (Table 3).

DISCUSSION

A few phyto-components like sabinene, caryophyllene and α-humulene have been previously isolated from *C. bengalense* (Thang et al., 2004). Among them sabinene has been reported to possess antioxidant activity (Quiroga et al., 2015). Again, β- caryophyllene from the essential oil of *Aquilaria crassna*, demonstrated significant antioxidant potential (Dahham et al., 2015). Therefore, these phytocomponents might have contributed to the observed antioxidant activity of the species under investigation (Table 1).

In Vietnam, *C. bengalense* has been used as an ingredient for making remedies of cancer and liver damage for a long period of time (Le et al., 2012). β-caryophyllene has been found to show anti-cancer activity against colorectal cancer cells (Dahham et al., 2015). Therefore, this phyto-component might be held responsible for the observed cytotoxic potential of the test samples (Table 1). In case of thrombolytic activity assay, the findings may help in the development of new cardiovascular drugs using the bark of *C. bengalense* (Table 2).

There are lots of similarities between human red blood cell membranes with that of lysosome. Hence, the membrane stabilizing activity can be correlated to anti-inflammatory effect (Mounnissamy et al., 2008). Sabinene has been reported to exhibit strong anti-inflammatory activity by inhibiting nitric oxide production in lipopolysaccharide and interferon gamma-triggered macrophages (Valente et al., 2013). 1% sabinene was observed to inhibit lens protein-induced inflammation in rabbit's eye (Quan-Sheng et al., 1993). Therefore, this phytocomponent might be considered responsible for the observed membrane stabilizing activity.

In support of the observed antimicrobial activity, it can be stated that sabinene, β-caryophyllene and α - humulene have been found to exhibit significant antimicrobial activity (Arunkumar et al., 2014; Dahham et al., 2015; Rahman et al., 2016). Therefore, the presence of these compounds in the species under investigation might be the reason of observed antimicrobial activity (Table 3).

Conclusion

Mankind has been fighting against many diseases such as cancer, heart diseases and neurodegenerative diseases for many years. Some medicines like antibiotics that are found to be useful now may not be found to be effective in future due to development of resistance. Therefore, mankind is continuously searching for better and more potent medicines with fewer side effects. In this investigation, the test samples of *C. bengalense* showed significant cytotoxic and membrane stabilizing potentials. The plant should be further analyzed for the identification of the compounds responsible for the observed activities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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