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Preliminary Biological Investigations of *Lophopetalum fimbriatum* and *Calophyllum inophyllum*

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Abstract

The crude methanol extracts of the leaf of *Lophopetalum fimbriatum* (non Wight) F. Vill. and *Calophyllum inophyllum* L. as well as their pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions were evaluated for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. The antioxidant potential was evaluated by DPPH and Folin-Ciocalteau reagent using butylated hydroxytolune (BHT) and ascorbic acid as standards, respectively. Among the extractives of *L. fimbriatum* and *C. inophyllum*, the chloroform soluble fraction and methanolic crude extract demonstrated the highest free radical scavenging activity (IC_{50} = 175.57 ± 0.02 µg/ml and IC_{50} = 1.0 ± 0.22 µg/ml) which could be correlated with their total phenol contents 82.15 ± 0.89 and 32.19 ± 0.81 mg of GAE /g of extractives, respectively. In the brine shrimp lethality bioassay, the carbon tetrachloride soluble fractions of *L. fimbriatum* (LC_{50} = 0.515 ± 0.03 µg/ml) and *C. inophyllum* (LC_{50} = 0.77 ± 0.18 µg/ml) revealed general toxicity. During assay for thrombolytic activity, the carbon tetrachloride soluble materials of *L. fimbriatum* and the chloroform soluble fraction of leaf of *C. inophyllum* revealed clot lysis by 8.89 ± 1.410% and 27.84 ± 0.94%, while the standard streptokinase and water, used as positive and negative controls, demonstrated 66.77% and 3.79% clot lysis, respectively. In hypotonic solution and heat induced conditions, the crude methanol extracts of *L. fimbriatum* and *C. inophyllum* inhibited haemolysis of human erythrocyte by 68.14 ± 2.05% & 40.00 ± 1.6% and 57.67 ± 0.26% and 28.12 ± 0.38%, respectively. Here, acetyl salicylic acid (0.1 mg/ml) was used as reference showing 72.79% and 42.12% haemolysis of RBCs in hypotonic solution and heat induced conditions, respectively. The antimicrobial activity was assessed by the disc diffusion method and the chloroform soluble fraction of *L. fimbriatum* demonstrated 16.0 mm zone of inhibition against *Sarcina lutea*. Different extractives of *C. inophyllum* inhibited microbial growth with zone of inhibition ranging from 10.0 mm to 22.0 mm. Among the different extractives of *C. inophyllum, the p*et-ether and carbon tetrarchloride soluble fractions demonstrated 22.0 mm zone of inhibition against *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa*, respectively.

Key words: *Lophopetalum fimbriatum* (non Wight) F. Vill., *Calophyllum inophyllum* L., antioxidant, DPPH, cytotoxicity, thrombolytic, membrane stabilizing, zone of inhibition.

Introduction

Lophopetalum fimbriatum (non Wight) F. Vil. (Synonyms: *Lophopetalum javanicum* (Zoll.) Turcz., *Lophopetalum intermedium* Ridl., Bengali name: Raktan) is a small tree belonging to the family Celastraceae. The plant is distributed in Thailand, Peninsular Malaysia, Sumatra, Java, Borneo, Philippines, Celebes, Moluccas and New Guinea. The bark is used as a constituent of dart poison (www.asianplant.net).

Calophyllum inophyllum L. (Synonyms: *Calophyllum blumei* Wight., *Balsamaria inophyllum* Lour., Bengali name: Punnag) commonly called Alexandrian laurel, is an evergreen tree belonging to Calophyllaceae family. It is native from East Africa, Southern coast of India, Malaysia and Australia. In Bangladesh, the plant is distributed in coastal forests of the country, especially Noakhali, Bhola, Sandwip and Patuakhali. Bark is astringent. Pounded bark is used topically in orchitis and internal haemorrhages while its juice is used as purgative. Decoction of the bark is employed as a lotion for indolent ulcers. The gum resin is considered emetic, purgative, vulnerary, resolvent and anodyne and is applied to ulcers and wounds. Leaves are applied to sore eyes. Seed oil is applied externally in rheumatism and it is a reputed antipsoric. Seed oil is also

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used in gonorrhoea, gleet and scabies (Medicinal Plants Database of Bangladesh).

As part of our ongoing investigations on medicinal plants of Bangladesh (Kaisar *et al.*, 2011 and Sharmin *et al.*, 2012), the crude methanol extracts of leaves of *L. fimbriatum* and *C. inophyllum* growing in Bangladesh as well as their organic and aqueous soluble fractions were studied for the antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities for the first time and we, here in, report the results of our preliminary investigations.

Materials and Methods

Plant materials: The leaves of *L. fimbriatum* and *C. inophyllum* were collected from Mirpur Botanical garden, Dhaka in November 2011. Voucher specimens DACB-24336 and DACB-37785 for *L. fimbriatum* and *C. inophyllum*, respectively have been maintained in Bangladesh National Herbarium, Dhaka Bangladesh for future references.

The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (500g each) of both the plants were separately soaked in 2.0 liters of methanol at room temperature for 7 days. The extracts were filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature (40-45ºC) and pressure. An aliquot (5g) of each of the concentrated methanol extracts was fractionated by the modified Kupchan partitioning protocol (VanWagenen *et al.*, 1993) and the resultant partitionates were evaporated to dryness with rotary evaporator to yield pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble materials (Table 1). 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 Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006).

DPPH free radical scavenging assay: Following the method developed by Brand-Williams *et al.* (1995), the antioxidant activity of the test samples was assessed by determining 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.

Table 1. Kupchan partionates of *L. fimbriatum* and *C. inophyllum.*

Crude extract/ Fractions	*L. fimbriatum* (g)	*C. inophyllum* (g)
Me	5.0	5.0
PESF	0.5	0.5
CTCSF	1.5	1.0
CSF	0.5	1.5
AQSF	1.5	1.5

ME= Methanolic crude extract; PESF= Pet-ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction.

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 one day *in vivo* assay (Meyer *et al*., 1982). Vincristine sulphate was used as positive control.

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

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

Antimicrobial screening: Antimicrobial activity was determined by disc diffusion method (Bauer *et al.*, 1966).

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

Results and Discussion

The aim of the study was to evaluate the crude methanol extracts of *L. fimbriatum* and *C. inophyllum* as well as their pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions for antioxidant potential in terms of total phenolic content and free radical scavenging property as well as cytotoxic, thrombolytic, membrane stabilizing and antimicrobial potential.

In DPPH free radical scavenging assay, all the test samples of *L. fimbriatum* demonstrated mild free radical scavenging potential with IC_{50} values ranging from 175.57 µg/ml to 619.0 µg/ml. The highest free radical scavenging activity was demonstrated by the chloroform soluble fraction (IC_{50}= 175.57 ± 0.02 µg/ml) which can be

correlated to its phenolic content (82.15 ± 0.89 mg of GAE / g of extractives). Highly significant free radical scavenging potentials were demonstrated by all the test samples of *C. inophyllum* with IC_{50} values ranging from 1.0 µg/ml to 13.0 µg/ml. The highest free radical scavenging activity was revealed by the crude methanol extract (IC_{50}= 1.0 ± 0.22 µg/ml) which could be correlated to its phenolic content 32.19 ± 0.81 mg of GAE/g of extractives (Table 2).

All the test samples of *L. fimbriatum* and *C. inophyllum* displayed significant cytotoxic potential against *A. salina.* The carbon tetrachloride soluble fractions of *L. fimbriatum* and *C. inophyllum* exhibited the highest cytotoxic activity with LC_{50} values 0.515 ± 0.03 µg/ml and 0.77 ± 0.18 µg/ml, respectively as compared to 0.451 µg/ml for Vincristine sulphate (Table 2).

The extractives of *L. fimbriatum* demonstrated weak thrombolytic activity. The carbon tetrachloride soluble fraction showed 8.89 ± 1.410% clot lysis as compared to 66.77% clot lysis exhibited by the standard streptokinase. The extractives of *C. inophyllum* demonstrated mild to moderate thrombolytic activity. The chloroform soluble fraction and the crude methanol extract showed 27.84 ± 0.94 % and 27.38 ± 1.03 % clot lysis, respectively (Table 3).

At concentration 1.0 mg/ml, *L. fimbriatum* extractives possess significant membrane stabilizing activity as compared to the standard acetyl salicylic acid (0.10 mg/ml). The crude methanol extract inhibited 68.14 ± 2.05 % and 40.00 ± 1.60 % hypotonic solution and heat induced haemolysis of RBCs. The extractives of *C. inophyllum* significantly protected the haemolysis of RBC induced by hypotonic solution and heat. The methanolic crude extract and the carbon tetrachloride soluble fraction inhibited 57.67 ± 0.26% & 28.12 ± 0.38% and 48.29 ± 0.01% and 14.43 ± 0.71% hypotonic solution and heat induced haemolysis of RBC as compared to 72.79% and 42.12% by acetyl salicylic acid, respectively (Table 4).

L. fimbriatum and *C. inophyllum* test samples were screened against five gram positive and eight gram negative bacteria and one fungus their microbial growth inhibitory potentials. The crude methanol extract and the chloroform soluble fraction of *L. fimbriatum* demonstrated zone of inhibition ranging from 8.0 to 16.0 mm. The highest zone of inhibition (16 mm) was shown by the chloroform soluble fraction against *Sarcina lutea*. The crude methanol extract and its chloroform soluble fraction exhibited 15 mm zone of inhibition against *S. lutea* and *Shigella boydii*, respectively (Table 5). On the other hand, the test samples of *C. inophyllum* displayed zone of inhibition ranging from 10.0 to 22.0 mm. The highest zone of inhibition (22.0 mm) was observed by the pet-ether and carbon tetrarchloride soluble fractions against *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa*, respectively. The chloroform soluble fraction also showed strong zone of inhibition (21.0 mm) against *Sh. boydii* and *V. parahaemolyticus* (Table 6). Standard antibiotic, Ciprofloxacin was involved as the reference standard in this assay.

Table 2. The total phenolic content, free radical scavenging and cytotoxic activities of *L. fimbriatum* and *C. inophyllum.*

Plants	Samples/ Standards	Total phenolic content (mg of GAE/g of extract)	DPPH Free radical scavenging activity (IC_{50} µg/ml)	Cytotoxic activity (LC_{50} µg/ml)
L. fimbriatum	ME	80.10 ± 0.74	249.55 ± 0.57	0.731 ± 0.22
	PESF	11.89 ± 0.95	619.0 ± 0.77	0.856 ± 0.68
	CTCSF	15.88 ± 0.83	562.69 ± 0.38	0.515 ± 0.03
	CSF	82.15 ± 0.89	175.57 ± 0.02	6.79 ± 1.01
	AQSF	28.10 ± 0.73	423.10 ± 0.18	73.18 ± 0.37
C. inophyllum	ME	32.19 ± 0.81	1.0 ± 0.22	1.93 ± 0.77
	PESF	11.45 ± 1.24	3.95 ± 0.34	1.12 ± 0.41
	CTCSF	18.24 ± 0.47	1.71 ± 0.59	0.77 ± 0.18
	CSF	12.31 ± 0.39	3.0 ± 0.11	7.97 ± 0.69
	AQSF	9.60 ± 0.24	13.0 ± 1.21	1.83 ± 0.51
Standards	VS	-	-	0.451
	BHT	-	27.5 ± 0.54	-
	Ascorbic acid	-	5.8 ± 0.21	-

BHT= Butylated hydroxytolune; VS= Vincristine sulfate; ME= Methanolic crude extract; PESF= Pet-ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction

Table 3. Thrombolytic activity of *L. fimbriatum* and *C. inophyllum* extractives.

Sample	% of lysis of RBC	
	L. fimbriatum	*C. inophyllum*
ME	4.64 ± 1.200	27.38 ± 1.03
PESF	1.945 ± 0.818	23.96 ± 0.23
CTCSF	8.89 ± 1.410	24.23 ± 0.48
CSF	2.963 ± 0.352	27.84 ± 0.94
AQSF	1.00 ± 0.906	14.08 ± 0.55
Water	3.79 ± 0.55	
SK	66.77 ± 1.08	

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction; SK =Streptokinase

Table 4. Effect of different extractives of leaf of *L. fimbriatum* and *C. inophyllum* on hypotonic solution- and heat-induced haemolysis of erythrocyte membrane.

Sample	% Inhibition of haemolysis			
	L. fimbriatum		*C. inophyllum*	
	Hypnotic solution induced	Heat induced	Hypnotic solution induced	Heat induced
ME	68.14 ± 2.05	40.00 ± 1.60	57.67 ± 0.26	28.12 ± 0.38
PESF	14.08 ± 2.06	19.00 ± 0.71	42.14 ± 0.33	13.55 ± 0.82
CTCSF	18.36 ± 1.63	30.40 ± 1.43	48.29 ± 0.01	14.43 ± 0.71
CSF	66.33 ± 0.88	32.46 ± 1.63	38.6 ± 0.64	4.23 ± 0.37
AQSF	1.43 ± 0.80	16.05 ± 0.76	30.61 ± 0.49	16.05 ± 0.76
ASA	72.79 ± 0.47	42.12 ± 0.23	72.79 ± 0.47	42.12 ± 0.23

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction; ASA= Acetyl salicylic acid

Table 5. Antimicrobial activity of *L. fimbriatum.*

Test microorganisms	Diameter of zone of inhibition (mm)		
	ME	CSF	Ciprofloxacin (30µg/disc)
Bacillus cereus	8.0 ± 1.65	9.0 ± 1.53	45.0 ± 2.01
B. megaterium	14.0 ± 1.53	15.0 ± 1.53	42.0 ± 1.17
B. subtilis	10.0 ± 2.5	12.0 ± 3.06	42.0 ± 0.73
Staphylococcus aureus	11.0 ± 12.55	13.0 ± 2.08	-
Sarcina lutea	15.0 ± 2.35	16.0 ± 1.52	42.0 ± 0.56
Escherichia coli	13.0 ± 2.55	14.0 ± 1.52	42.0 ± 0.43
Pseudomonas aeruginosa	11.0 ± 1.65	12.0 ± 2.52	42.0 ± 1.11
Salmonella Typhi	-	-	45.0 ± 0.73
S. Paratyphi	11.0 ± 3.55	13.0 ± 2.52	47.0 ± 2.33
Shigella boydii	14.0 ± 2.55	15.0 ± 1.53	34.0 ± 0.58
Sh. dysenteriae	11.0 ± 3.25	12.0 ± 2.08	42.0 ± 0.22
Vibrio mimicus	10.0 ± 1.75	11.0 ± 2.51	40.0 ± 0.45
V. parahaemolyticus	9.0 ± 2.75	11.0 ± 3.05	35.0 ± 0.44
Saccharomyces cerevisiae	9.0 ± 2.25	10.0 ± 1.52	38.0 ± 0.49

ME = Methanol extract; CSF = Chloroform soluble fraction

Table 6. Antimicrobial activity of *C. inophyllum.*

Test Microorganisms	Diameter of zone of inhibition (mm)				
	ME	PESF	CTCSF	CSF	Ciprofloxacin (30µg/disc)
Bacillus cereus	14.0 ± 0.13	16.0 ± 0.26	16.0 ± 0.08	14.0 ± 0.36	45.0 ± 2.01
B. megaterium	14.0 ± 0.34	16.0 ± 0.71	14.0 ± 0.38	16.0 ± 0.61	42.0 ± 1.17
B. subtilis	14.0 ± 0.44	18.0 ± 0.43	18.0 ± 0.19	16.0 ± 0.43	42.0 ± 0.73
Sarcina lutea	14.0 ± 0.81	14.0 ± 0.54	14.0 ± 0.47	13.0 ± 0.38	42.0 ± 0.56
Escherichia coli	14.0 ± 0.06	18.0 ± 0.44	16.0 ± 0.29	14.0 ± 0.84	42.0 ± 0.43
Pseudomonas aeruginosa	16.0 ± 0.12	14.0 ± 0.61	22.0 ± 0.19	14.0 ± 0.11	42.0 ± 1.11
Salmonella Typhi	14.0 ± 0.28	18.0 ± 0.17	20.0 ± 0.77	14.0 ± 0.24	45.0 ± 0.73
S. Paratyphi	12.0 ± 0.60	-	-	-	47.0 ± 2.33
Shigella boydii	12.0 ± 0.33	14.0 ± 0.69	16.0 ± 0.90	21.0 ± 0.03	34.0 ± 0.58
Sh. dysenteriae	14.0 ± 0.48	14.0 ± 0.78	16.0 ± 1.03	16.0 ± 0.44	42.0 ± 0.22
Vibrio mimicus	10.0 ± 0.02	16.0 ± 0.13	18.0 ± 0.53	16.0 ± 0.38	40.0 ± 0.45
V. parahaemolyticus	12.0 ± 0.57	22.0 ± 0.33	16.0 ± 0.76	21.0 ± 0.55	35.0 ± 0.44
Sacharomyces cerevasiae	14.0 ± 0.38	16.0 ± 0.52	14.0 ± 0.61	16.0 ± 0.74	38.0 ± 0.49

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction

It is clearly evident from the above findings that the test samples of *L. fimbriatum* have significant cytotoxic and membrane stabilizing potentials. The plant also exhibited mild antioxidant and antimicrobial activities while the test samples of *C. inophyllum* have strong antioxidant, cytotoxic, membrane stabilizing and antimicrobial potentials. The carbon tetrachloride soluble fraction of *C. inophyllum* exhibited 22.0 mm zone of inhibition against *Pseudomonas aeruginosa*, a bacteria whose resistance is well documented. The plant also demonstrated mild to moderate thrombolytic activity.

Therefore, both the plants are good candidates for further systematic, chemical and biological studies to isolate the active principles.

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