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SCREENING OF BIOLOGICAL ACTIVITIES OF *ACACIA FARNESIANA* (GUYABABLA), A MEDICINAL PLANT OF BANGLADESH

Tasnuva Sharmin[1,*] and Syeda Nazia Mahazabin[2]

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

***Corresponding Author: Tasnuva Sharmin**
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

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ABSTRACT
The crude methanol extract of bark of *Acacia farnesiana* (L.) Willd. as well as its hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screening for total phenolic content, and cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activity assays. Among the extractives of *A. farnesiana*, the hexane soluble fraction showed the highest **amount** of phenolic content (28.05±0.75 mg of GAE/ g of extractives). In brine shrimp lethality bioassay, the aqueous soluble fraction of crude methanol extract of *A. farnesiana* revealed the presence of the highest amount of cytotoxic principles (LC_{50}= 0.59±0.32 µg/ml) as compared to 0.45 µg/ml for **v**incristine sulphate. This fraction also showed the highest ability to promote lysis of human blood clot (45.36±0.26%) as compared to 66.77% clot lysis by standard streptokinase while evaluating the thrombolytic activity of *A. farnesiana* extractives. At concentration 1.0 mg/ml, the hexane soluble fraction inhibited 63.30±0.33% and 26.39±0.72% of haemolysis of RBCs induced by hypotonic solution and heat as compared to 71.90% and 42.12% by acetyl salicylic acid, respectively. Among the *A. farnesiana* extractives, only the carbon tetrachloride soluble fraction revealed antimicrobial activity against the test organisms with zone of inhibition ranging from 9.0 to 20.0 mm.

KEYWORDS: *Acacia farnesiana* (L.) Willd., total phenolic content, cytotoxicity, thrombolytic activity, membrane stabilizing activity, zone of inhibition.

INTRODUCTION
Acacia farnesiana L. (Willd.) (Synonym: *Acacia indica*, *Acacia minuta*, *Vachellia farnesiana* etc; Bengali names: Guyababla, Belati Babul) commonly known as needle bush and sweet acacia; is a small, branching, deciduous tree or shrub belonging to Leguminoseae family. Its native range is uncertain but the plant is mainly abundant in the tropical and subtropical regions.[1] The bark and the flowers are the parts of the tree mostly used in traditional medicine. Flowers are used to relieve stomachache. *A. farnesiana* pods are used to treat sore throat and conjunctivitis.[2] The bark of this plant is used as astringent and demulcent.[3,4] *A. farnesiana* has been used in Colombia to treat malaria and the extract from the bark and leaf has shown some efficacy against the malarial pathogen *Plasmodium falciparum* in animal models.[5] Crushed and boiled bark and leaf are inhaled for the treatment of malaria.[6] Leaf powder is reported to acts as a healing agent.[2,7] *A. farnesiana* root is used as antispasmodic, aphrodisiac, astringent, demulcent, febrifuge and stimulant and in diarrhea and rheumatism.[8] The gummy root is chewed to ease sore throat pain.[7,8] An active fraction from aqueous extract of *A. farnesiana* showed promising anti-diabetic activity at a dose of 25 mg/kg.[9] The ethanol extract showed considerable anti-inflammatory activity in both carrageenan induced paw oedema for acute inflammation and cotton pellet induced granulation for chronic inflammatory models.[10] Lectin-like agglutin has been isolated from the seeds of *A. farnesiana* and has been found to possess anti-inflammatory activity.[11,12] Different protein fractions isolated from the seeds were reported to exhibit significant analgesic and anti-inflammatory activities.[13] Compounds like alkaloids, saponin, carotenoids, flavonoids and terpenoides have been isolated from pods and seeds.[3,14,15] Four new diterpenes namely acasiane A, acasiane B, farnesirane A and farnesirane B, along with three known diterpenes, two triterpenes and eight flavonoids were isolated from the root. Some of the isolated compounds showed promising cytotoxicity towards human cancer cell lines and antioxidant and moderate anti-inflammatory activities.[16,17]

As part of our ongoing investigations on medicinal plants of Bangladesh[18,19], the crude methanol extract of bark of *A. farnesiana* growing in Bangladesh as well as its organic and aqueous soluble fractions were studied for

total phenolic content, 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 bark of *A. farnesiana* was collected from Dhaka, Bangladesh, in May 2015. A voucher specimen for this collection has been maintained in Dhaka University Salar Khan Herbarium for future reference.

The sun dried and powdered plant materials (800 g) were macerated in 2.0 L methanol for 7 days. The extract was filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. 5 g of the concentrated methanol extract was fractionated by modified Kupchan[20] partition protocol and the resultant partitionates were evaporated to dryness with rotary evaporator to yield hexane (HXSF, 1.0 g), carbon tetrachloride (CTCSF, 1.5 g), chloroform (CSF, 1.5 g) and aqueous (AQSF, 0.5 g) soluble materials. The residues were then stored in the refrigerator until further use.

Total phenolic content

The total phenolic content of the extractives was determined with Folin-Ciocalteau reagent using the method developed by Harbertson and Spayd (2006).[21]

Brine shrimp lethality bioassay

This method was applied for the determination of toxic properties of the dimethylsulfoxide (DMSO) solutions of the extractives against *Artemia salina* in a single day in vivo assay.[22] Vincristine sulphate was used as the standard.

Thrombolytic activity

The thrombolytic activity of the extractives was evaluated by using streptokinase as positive control.[23]

Membrane stabilizing activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale *et al*. (2008).[24]

Antimicrobial screening

Antimicrobial activity was determined by disc diffusion method.[25]

Statistical analysis

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

Table 1: Total phenolic content and cytotoxic activity of *A. farnesiana*.

Samples/ Standard	Total phenolic content (mg of GAE/ g of dried extract)	Brine shrimp lethality bioassay LC_{50} (µg/ml)
ME	10.10±0.61	2.06±0.11
HXSF	28.05±0.75	3.81±0.61
CTCSF	16.83±0.66	3.56±0.04
CSF	12.16±0.57	23.81±1.07
AQSF	10.02±0.46	0.59±0.32
Vincristine sulfate	-	0.45±0.04

ME= Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction.

Table 2: Thrombolytic activity of *A. farnesiana*.

Samples/ Standard	% of lysis of RBC
ME	13.83±0.53
HXSF	14.67±0.47
CTCSF	38.21±0.43
CSF	43.55±0.14
AQSF	45.36±0.26
Water	3.79±0.55
Streptokinase	66.77±0.36

ME = Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction.

Table 3: Effect of different extractives of *A. farnesiana* on heat and hypotonic solution-induced haemolysis of erythrocyte membrane.

Samples/ Standard	% Inhibition of haemolysis	
	Heat induced	Hypotonic solution induced
Hypotonic medium	-	-
ME	18.50±0.39	24.69±0.41
HXSF	26.39±0.72	63.30±0.33
CTCSF	17.04±0.62	30.38±0.59
CSF	20.01±0.51	28.49±0.36
AQSF	18.97±0.44	27.22±0.39
Acetyl salicylic acid.	42.12±-0.38	71.90±0.78

ME = Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction.

Table 4: Antimicrobial activity of *A. farnesiana.*

Test microorganisms	Diameter of zone of inhibition (mm)	
	CTCSF	Ciprofloxacin
Bacillus cereus	-	45.0±2.01
B. megaterium	20.0±1.12	42.0±1.17
B. subtilis	-	42.0±0.73
Staphylococcus aureus	10.0±1.05	42.0±0.23
Sarcina lutea	-	42.0±0.56
Escherichia coli	-	42.0±0.43
Pseudomonas aeruginosa	10.0±0.39	42.0±1.11
S. typhi	13.0±0.58	47.0±2.33
Salmonella paratyphi	-	45.0±0.73
Shigella boydii	10.0±0.56	34.0±0.58
S. dysenteriae	-	42.0±0.22
Vibrio mimicus	13.0±0.22	40.0±0.45
V. parahaemolyticus	9.0±0.32	35.0±0.44
Sacharomyces cerevacae	10.0±0.17	38.0±0.11

CTCSF = Carbon tetrachloride soluble fraction.

RESULTS AND DISCUSSION

The present study was undertaken to assess the total phenolic content, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities of different organic and aqueous soluble materials of *A. farnesiana*.

Different extractives of *A. farnesiana* demonstrated the presence of phenolic components within the range of 10.02 to 28.05 mg of GAE/g of extractives. Among the test samples, the hexane soluble fraction showed the highest value of phenolic content (28.05±0.75 mg of GAE / g of extractives). This finding suggests the extract may possess some antioxidant activities as observed from assays on the *A. farnesiana* pod and leaf extract[26,27] (Table 1).

In case of brine shrimp lethality bioassay, all the fractions demonstrated significant cytotoxic potential against *A. salina* with LC_{50} values ranging from 0.59 to 23.81 µg/ml. The aqueous soluble fraction revealed the highest cytotoxic activity with an LC_{50} value 0.59±0.32 µg/ml as compared to 0.45 µg/ml for Vincristine sulphate. Quercetin deoxyhexoside has been reported as a phytoconsituent of the leaf extract that might have contributed to the cytotoxic potential.[27,28] (Table 1).

The extractives of *A. farnesiana* demonstrated significant thrombolytic activity. The aqueous and chloroform soluble fractions showed 45.36±0.26% and 43.55±0.14% of clot lysis as compared to 66.77% clot lysis by standard streptokinase, respectively. This might be a new observation in the field of cardiovascular diseases.[29] New thrombolytic agents can be developed the bark of the plant. (Table 2).

At concentration 1.0 mg/ml, the extractives of *A. farnesiana* protected the haemolysis of RBCs induced by hypotonic solution and heat as compared to the standard acetyl salicylic acid (0.10 mg/ml). The hexane soluble fraction inhibited 63.30±0.33% and 26.39±0.72% of haemolysis of RBCs induced by hypotonic solution and heat as compared to 71.90% and 42.12% by acetyl salicylic acid, respectively. As the membrane of human red blood cell resembles lysosomal membrane, the membrane stabilizing activity can be correlated to anti-inflammatory effect of the plant extract.[30] This membrane stabilizing activity can be attributed to anti-inflammatory phytoconstituents isolated from the seeds[11,12,13] (Table 3).

The antimicrobial activity of *A. farnesiana* test samples was evaluated against five gram positive and eight gram

negative bacteria and one fungus and the results were compared with standard antibiotic, ciprofloxacin. Among the test samples of *A. farnesiana*, only the carbon tetrachloride soluble fraction revealed antimicrobial activity with zone of inhibition ranging from 9.0 to 20.0 mm. The highest zone of inhibition (20.0 mm) was showed against *Bacillus. megaterium* (Table 4).

CONCLUSION

It is clearly evident from the above findings that the test samples of *A. farnesiana* possess significant cytotoxic, thrombolytic and membrane stabilizing activities. Therefore, the plant is a good candidate for further systematic, chemical and biological studies to isolate the active principles.

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