

Phytochemical screenings, thrombolytic activity and antimicrobial properties of the bark extracts of *Averrhoa bilimbi*

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ABSTRACT

In this present study, the bark extracts of *Averrhoa bilimbi* were subjected to the thrombolytic activities were assessed by using human erythrocyte and the results were compared with standard streptokinase (SK). On the other hand, bark extracts of *A. bilimbi* revealed moderate antibacterial activity against some microorganisms used in the screening. Preliminary phytochemical investigation suggested the presence of flavonoids, saponins and alkaloids.

INTRODUCTION

Averrhoa bilimbi (Fam. oxalidaceae) locally known as bilimba, is long-lived tree and reaches 5-10 m in height, has a short trunk soon dividing into a number of upright branches. Leaves mainly clustered at the branch tips, are alternate, imparipinnate; 30-60 cm long, with 11-37 alternate or sub opposite leaflets, ovate obovate, with rounded base and pointed tip; downy; medium-green on the upper surface, pale on the underside; 2-10 cm long, 1.2-1.25 cm wide. Fruit ellipsoid, obovoid or nearly cylindrical, faintly 5-sided, 4-10 cm long; capped by a thin, star-shaped calyx at the stem-end and tipped with 5 hair like floral remnants at the apex. Crispy when unripe, the fruit turns from bright green to yellowish-green, ivory or nearly white when ripe and falls to the ground. The outer skin is glossy, very thin, soft and tender, and the flesh green, jelly-like, juicy and extremely acid (Orwa *et al.*, 2009). The generic name is after Averrhoes (1126-98), the widely known Arab philosopher (Orwa *et al.*, 2009).

It is native in Indonesia, Malaysia and as exotic in Argentina, Australia, Brazil, Colombia, Cuba, Ecuador, Guyana, India, Jamaica, Myanmar, Philippines, Puerto Rico, Singapore, Sri Lanka, Surinam, Tanzania, Thailand, Trinidad and Tobago, US, Venezuela (Orwa *et al.*, 2009).

In the Philippines, the leaves are applied as a paste or poulticed on itches, swellings of mumps and rheumatism, and on skin eruptions. Elsewhere, they are applied on bites of poisonous creatures. Malaysians take the leaves fresh or fermented as a treatment for venereal disease. A leaf infusion is a remedy for coughs and is taken after childbirth as a tonic. A leaf decoction is taken to relieve rectal inflammation. A flower infusion is said to be effective against coughs and thrush. In Java, the fruits combined with pepper are eaten to cause sweating when people are feeling "under the weather". A paste of pickled bilimbis is smeared all over the body to hasten recovery after a fever. The fruit conserve is administered as a treatment for coughs, beri-beri and biliousness. Syrup prepared from the fruit is taken as a cure for fever and inflammation and to stop rectal bleeding and alleviate internal hemorrhoids (Orwa *et al.*, 2009).

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As a part of our continuing studies on medicinal plants of Bangladesh (Hossain *et al.*, 2012; Shahriar *et al.*, 2012(a); Shahriar *et al.*, 2012(b); Shahriar *et al.*, 2012(c); Shahriar and Kabir, 2011; Shahriar, 2010), the organic soluble materials of the bark extracts of *Averrhoa bilimbi* were evaluated for phytochemical screenings, anti-thrombolytic activity, antimicrobial activities for the first time.

MATERIALS AND METHODS

Plant materials

The barks of *Averrhoa bilimbi* were collected from Savar, Dhaka, Bangladesh, in November 2010. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no 35410). The sun dried and powdered plant parts (500 gm) of *Averrhoa bilimbi* was successively extracted in a soxhlet extractor at elevated temperature using 200 ml of distilled methanol (40-60)°C which was followed by n-hexane, carbon tetrachloride and chloroform. All extracts were filtered individually through filter paper and poured on petri dishes to evaporate the liquid solvents from the extract to get dry extracts. The dry crude extracts were weighed and stored in air-tight container with necessary markings for identification and kept in refrigerator (0-4)°C for future investigation.

Preliminary phytochemical screening

One gram of the methanol extract was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents (Harbone, 1998; Kokate, 2001).

Streptokinase (SK)

Commercially available lyophilized Altephase (Streptokinase) vial (Beacon pharmaceutical Ltd.) of 15, 00,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U.) was used for *in vitro* thrombolysis.

Blood sample

Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Thrombolytic activity

The thrombolytic activity of all extracts was evaluated by the method developed by Dagainawala (2006) and slightly modified by Kawsar *et al.* (2011) using streptokinase (SK) as the standard.

Antimicrobial activity

The antimicrobial screening, which is the first stage of antimicrobial drug discovery, was performed by the disc diffusion method (Ayafor, 1972) against some gram positive and gram negative bacteria and also against fungi (Table 2) collected as pure

cultures from the department of microbiology, University of Dhaka, Bangladesh. Standard disc of Ciprofloxacin (5 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm (Bauer *et al.*, 1966)

RESULTS AND DISCUSSION

Thrombolytic activity

As a part of discovery of cardio-protective drugs from natural sources the extractives of *Averrhoa bilimbi* were assessed for thrombolytic activity and the results are presented in Table 1. Addition of 100µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed 92.81% lysis of clot. At the same time, distilled water was treated as negative control which exhibited negligible lysis of clot (1.32%). In this study, the chloroform soluble fraction (CSF) exhibited highest thrombolytic activity (8.13%).

Table 1: Thrombolytic activity of different fractions of *Averrhoa bilimbi*.

Sample	Thrombolytic Activity (% of lysis)
SK	92.81%
Water	1.32%
MF	4.11%
HSF	7.81%
CCSF	2.78%
CSF	8.13%

SK= Streptokinase, MF= methanolic fraction, HSF= n-hexane soluble fraction, CCSF= carbon tetra chloride soluble fraction and CSF= chloroform soluble fraction of the bark extracts of *A. bilimbi*.

Antimicrobial activity

The crude extract and its different partitionates when subjected to antimicrobial screening at 400 µg/disc revealed antimicrobial activity against the tested microorganisms having the zone of inhibition ranging from 8 to 15 mm (Table 2).

Table 2: Antimicrobial activity of *Averrhoa bilimbi*.

	HSF	CCSF	CSF	MF	Ciprofloxacin
Gram positive bacteria					
<i>Bacillus cereus</i>	8	8	-	8	44
<i>Bacillus megaterium</i>	8	8	8	8	44
<i>Bacillus subtilis</i>	10	8	8	10	44
<i>Staphylococcus aureus</i>	10	8	6	9	43
<i>Sarcina lutea</i>	9	8	-	9	43
Gram negative bacteria					
<i>Escherichia coli</i>	10	8	-	9	44
<i>Pseudomonas aeruginosa</i>	9	8	8	9	44
<i>Salmonella paratyphi</i>	9	8	13	9	43
<i>Salmonella typhi</i>	9	8	-	9	43
<i>Shigella boydii</i>	9	8	8	9	44
<i>Shigella dysenteriae</i>	9	8	15	9	44
<i>Vibrio mimicus</i>	10	8	-	10	43
<i>Vibrio parahemolyticus</i>	9	8	-	9	43
Fungi					
<i>Candida albicans</i>	9	8	8	8	43
<i>Aspergillus niger</i>	-	8	14	8	43
<i>Sacharomyces cerevacae</i>	9	8	6	9	43

MF= methanolic fraction, HSF= n-hexane soluble fraction, CCSF= carbon tetra chloride soluble fraction and CSF= chloroform soluble fraction of the bark extracts of *A. bilimbi*.

Preliminary phytochemical screening

In preliminary phytochemical screening, the methanol extract of *Averrhoa bilimbi* demonstrated the presence of alkaloids, saponins and flavonoid (Table 3).

Table. 3: Analysis of phytochemical in the methanol extract of *Averrhoa bilimbi*.

Phytochemicals	Result
Alkaloids	+
Saponins	+
Flavonoid	+

+ = Presence.

CONCLUSION

It can be concluded that the extracts of the *Averrhoa bilimbi* can be used to design different antimicrobial agents as well as thrombolytic agent due to its moderate antimicrobial activity. Further work is needed to isolate the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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