

Ex-Vivo anti-inflammatory and antimicrobial activities of the leaves of *Bauhinia acuminata*

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Abstract: In present study, the methanol extracts and all fractions of leaves of *Bauhinia acuminata* were subjected to various biological screening such as membrane stabilizing activity and antimicrobial screening. This investigation suggested that, leaves of *Bauhinia acuminata* were highly effective in the membrane *stabilizing* activity as the extractives prevented the lysis of erythrocyte by hypotonic solution and heat induced. In hypotonic solution induced condition the highest membrane *stabilizing* activity was exhibited by the Pet ether soluble fraction (PESF-418.26%) followed by carbon tetra chloride soluble fraction (CTCSF-418.26%). Significant amount of membrane *stabilizing* activity were also present in methanol soluble fraction (MESF-13.46%) and aqueous soluble fraction (AQSF-167.30%). On the other hand, heat induced condition the maximum membrane *stabilizing* activity was exhibited by the carbon tetra chloride soluble fraction (CTCSF-853.3%) followed by Methanol soluble fraction (MESF-423.88%). Significant amount of membrane *stabilizing* activity were also present in Pet ether soluble fraction (PESF-81.77%) and aqueous soluble fraction (AQSF-40.46%). In this study, the antimicrobial activity, among all extractives of leaves of *Bauhinia acuminata* was found to be negligible.

Keywords: *Bauhinia Acuminata*, Anti-Inflammatory, Antimicrobial Activity

1. Introduction

Bauhinia acuminata belonging to the family; Fabaceae, an evergreen large shrub, grows in disturbed areas of Southeast Asia such as Indonesia, Malaysia or the Philippines [1]. In Bangladesh, it grows in hilly forests of Sylhet and Chittagong. It grows 2 to 3 meters tall. Leaves with petioles 1.5-4 cm long; broadly ovate or sub orbicular, blades ovate, divided about 1/3 their length, membranous, densely puberulent abaxially, glabrous adaxially, apex of lobes acute, base cordate to rounded. They are apical cleft up to 5 cm deep with the 6 to 15 centimeters long and broad. The flowers are 8 to 12 centimeters in diameter with fragrant, with five white petals, ten yellow-tipped stamens and a green stigma [2]. The species occurs in deciduous forests and scrub. Several chemical compounds including palmitic acid, three phthalic acid esters, phthalic acid, gallic acid, ursolic acid were identified from the leaves of *B. acuminata* [3]. In this study, we report the

anti-inflammatory activity and the antimicrobial activity of the methanol leaf extract of this plant.

As a part of our continuous search on medicinal plants of Bangladesh, the organic soluble materials of leaves of *Bauhinia acuminata* were evaluated for anti-inflammatory and antimicrobial activities.

2. Materials and Methods

2.1. Plant Material

The leaves of *B. acuminata* were collected from Mirpur Botanical Garden, Dhaka, Bangladesh, in the month of November 2011. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. 38305). The fruit were picked and washed with water to remove all unwanted plant materials and sand, air dried under light exposure (27°C-30°C for 7 days),

pulverized in a mill and stored in an airtight container for further study.

2.2. Preparation of Extract

The air dried and powdered fruit (500 gm) of *B. acuminata* was macerated in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. All the extracts were concentrated with a rotary evaporator at low temperature (40-45 °C) with reduced pressure. The concentrated methanolic extract (ME) was fractionated by modified Kupchan partitioning method [4] and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

2.3. Drugs and Chemicals

The drugs such as Ciprofloxacin and nutrient agar medium (Difco) were collected from Oponin Pharmaceuticals Ltd, Dhaka, Bangladesh as gift sample. Chemicals were obtained from Merck limited.

2.4. Membrane Stabilizing Activity

The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced hemolysis of mice erythrocyte by the method developed by [5, 6] as well as describe by [7]. In hypotonic solution-induced method, the test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) is mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extracts (1.0 mg/mL) or acetyl salicylic acid (0.1 g/mL). 0.5 mL of RBCs mixed with hypotonic-buffered saline alone for prepared control sample. The mixture sample was incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g as well as taken the absorbance of the supernatant at 540 nm. The percentage inhibition of either membrane stabilization or haemolysis was determined using the following equation $-\% \text{ inhibition of haemolysis} = 100 \times (\text{OD1}-\text{OD2}/\text{OD1})$

Where, OD1=optical density of hypotonic - buffered saline solution alone (control) and OD2= optical density of test sample in hypotonic solution.

In heat-induced haemolysis, isotonic buffer containing aliquots (5 ml) of the different extracts were put into two duplicate sets of centrifuge tubes. Two tubes were prepared with same amount of vehicle and another tube as control. Erythrocyte suspension (30 μ L) was added to each tube and mixed gently by inversion. One pair was maintained at (0-5) °C in an ice bath while the other pair of the tubes was incubated at 54 °C for 20 min in a water bath. The vehicle and Erythrocyte suspension containing mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was determined at 540 nm. The acceleration of hemolysis or percentage inhibition tests was calculated according to the equation:

$$\% \text{ Inhibition of hemolysis} = 100 \times [1 - (\text{OD2} - \text{OD1} / \text{OD3} - \text{OD1})]$$

Where, OD1= optical density of unheated test sample, OD2= optical density of heated test sample and OD3=optical density of heated control sample.

2.5. Antimicrobial Activity

The antimicrobial test was performed by the disc diffusion method [8] against eleven bacteria and three fungi (Table-1) collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The fungal and bacterial suspensions were used to inoculate Petri plates (90 mm) and wells (6 mm) were filled with the extracts (400 μ g/disc). Standard disc of Ciprofloxacin (30 μ g/disc) and blank discs (impregnated with solvents followed by evaporation) were used as negative control as well as positive, respectively. The plates were incubated at 37°C for 24 hours for microbial growth. The antimicrobial activity of the extract was determined by measuring the diameter of zone of inhibition (mm) at the end of the incubation period.

2.6. Statistical Analysis

The results were expressed as the mean \pm standard deviation (SD). Statistical significance of the mean mortality at each concentration was analyzed using one-way analysis of variance (ANOVA) and compared using Duncan's multiple range tests. Values of $p \leq 0.05$ were taken to be statistically significant.

3. Results and Discussion

The different methanol extracts of leaves of *B. acuminata* at concentration 2.0 mg/mL significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution whereas acetyl salicylic acid (0.10 mg/mL) used as standard (Table-1).

It is clear evident that the extractives of leaves of *B. acuminata* were highly effective in the membrane stabilizing activity as the extractives prevented the lysis of erythrocyte induced by hypotonic solution and heat.

In hypotonic solution induced condition the highest membrane stabilizing activity was exhibited by the Pet ether soluble fraction (PESF-418.26%) followed by carbon tetra chlororide soluble fraction (CTCSF-418.26%). Significant amount of membrane stabilizing activity were also present in methanol soluble fraction (MESF-13.46%) and aqueous soluble fraction (AQSF-167.30%).

On the other hand, heat induced condition the maximum membrane stabilizing activity was exhibited by the carbon tetra chlororide soluble fraction (CTCSF-853.3%) followed by Methanol soluble fraction (MESF-423.88%). Significant amount of membrane stabilizing activity were also present in Pet ether soluble fraction (PESF-81.77%) and aqueous soluble fraction (AQSF-40.46%).

The effect of synthetic and herbal anti-inflammatory agents on the stabilization of erythrocyte membrane exposed to hypotonic solution has been studied extensively. The

erythrocyte membrane resembles to lysosomal membrane, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [9].

The results showed that the extracts were potent on human erythrocyte adequately protecting it against hypotonic induced lysis. The activity was compared with standard anti-inflammatory drug (Acetyl Salicylic Acid). It has been reported that flavonoids exert profound stabilizing effects on lysosomes both in vitro and in vivo experimental animals while tannin and saponins have the ability to bind cations and other biomolecules, and are able to stabilize erythrocyte membrane.

The present investigation suggests that the membrane stabilizing activity of leaves of *Bauhinia acuminata* plays a significant role in its anti-inflammatory activity may be due to its high flavonoids and tannin content.

Prevention of leakage of serum proteins and fluids into the tissues during a period of increased permeability caused by inflammatory mediators by membrane stabilizes results [4]. Phytochemicals screening demonstrates that the plant extract contains flavonoids which have been reported to possess potent anti-inflammatory property [5, 6]. The anti-inflammatory activity is may be inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid [7].

The Methanol extract of leaves of *B. acuminata* (MESF) and different partitionates i.e. Pet Ether (PESF), chloroform (CSF), carbon tetrachloride (CTCSF) and aqueous (AQSF) soluble partitionate of the methanol extract of leaves of *B. acuminata* were subjected to antimicrobial screening with a concentration of 400 µg/disc in every case. The results are given in the Table -2.

In this study, the antimicrobial activity, among all extractives of leaves of *Bauhinia acuminata* was found to be negligible.

The Plant produces several secondary metabolites which are includes like phenolics, flavonoids, flavones, flavonols, coumarins, tannins, lectins, polypeptides, alkaloids and other compounds which support the plant defense against the microorganisms [8].

4. Conclusion

In the present study, demonstrate significant anti-inflammatory activity. The plant may be an economical, safe and easily available source of natural agents used in inflammation and anti-microbial. Future study would be conducted for identification of lead compounds and purification of the active principles of the plant responsible for the observed biological effects.

Table 1. Effect of different extractives of leaves of *Bauhinia acuminata* on hypotonic solution-induced haemolysis of erythrocyte membrane.

Sample code	Concentration	Absorbance	% inhibition of haemolysis
Hypotonic medium	50 mM	3.225	--
MESF	2 mg/mL	0.118	13.46
PESF	2 mg/mL	0.539	418.26
CTCSF	2 mg/mL	0.289	177.88
AQSF	2 mg/mL	0.278	167.30
Acetyl salicylic acid	0.10 mg/mL	0.906	71.9

Table 2. Antimicrobial activity of test samples of *Bauhinia acuminata*

Test m.o	Diameter of zone of inhibition (mm)					
	MESF	PESF	CTCSF	CSF	AQSF	Ciprofloxacin
Gram positive bacteria						
Bacillus cereus	-	-	-	-	-	15
Bacillus megaterium	-	-	-	-	-	15
Bacillus subtilis	-	-	-	-	-	17
Staphylococcus aureus	-	-	-	-	-	21
Sarcina lutea	-	-	-	-	-	17
Gram negative bacteria						
Escherichia coli	-	-	-	-	-	22
Pseudomonas aeruginosa	-	2	3	4	-	17
Salmonella paratyphi	-	3	2	-	3	15
Salmonella typhi	-	3	1	6	-	20
Shigella boydii	-	-	-	-	-	17
Shigella dysenteriae	-	-	-	3	-	21
Vibrio mimicus	-	-	-	-	-	16
Vibrio parahemolyticus	-	-	-	-	5	15
Fungi						
Candida albicans	-	-	-	-	-	20
Aspergillus niger	-	-	-	-	-	17
Sacharomyces cerevaceae	-	-	-	-	1	19

Abbreviations

ME= Methanolic extract; PESF= Pet-ether soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF =Chloroform soluble fraction; AQSF =Aqueous soluble fraction of the methanolic extract of *Bauhinia acuminata*

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