Antibacterial Potency Screening of the Crude Extracts of *Adenanthera pavonina* L.

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Abstract The antibacterial potency screening of the crude extracts of leaves, seed and stem wood of *Adenanthera pavonina* L. were tested against 15 Gram-positive and Gram-negative bacteria along with a standard antibiotic, Ciprofloxacin. The *in vitro* screening for antimicrobial potency was carried out by following the agar diffusion and micro broth dilution techniques. Among the fifteen bacterial strains tested the chloroform fraction of the seed extract showed promising antibacterial efficacy against *Salmonella-β-haemolyticus*, *Serratia dysenteriae* and *Escherichia coli*. The susceptibility order of extracts were seed>leaf>stem. The MIC values of the chloroform seed extract were determined 256 μg/ml against *B. cereus*; 128 μg/ml against *S. β-haemolyticus*, *S. dysenteriae* and 64 μg/ml against *Klebsiella* sp. The MIC values of the chloroform extracts of stem wood were 128 μg/ml *S. β-haemolyticus*; 64 μg/ml against *B. megaterium*, *S. sonnei* and 32 μg/ml against *S. typhi*.

Keywords Adenanthera Pavonina, Extract, Antibacterial Screening, Disc Diffusion Assay, Zone of Inhibition, MIC

1. Introduction

The plants are the natural chemical factories that synthesize unmemorable compounds. The plants-derived compounds have been utilized by the human kind from time immemorial in different sectors of life, including public health and pest management. The test plant is native to Bangladesh and is easily available. They are well known for their medicinal values.

Adenanthera pavonina L. is a deciduous, erect, unarmed tree 6-15 m high, distributed up to native to India, China, Malaysia and Africa. In Bangladesh it is occasionally grown under cultivation reported by Zarnowski et al. [1]. It is a monogenic plant in family Fabaceae and commonly known as Rakta chandan, Ranjana, Rakta kambal, Bead tree, Coral wood tree, Red sandalwood, etc. Traditionally, different parts

of *A. pavonina* have been used for the treatment of different human disorders like chronic rheumatism, diarrhea, dysentery, antiseptic paste, pulmonary affections, chronic ophthalmia and reported to have properties such as antioxidant, antibacterial by Jayasinghe et al. [2]. There have also been reports on cytotoxicity of crude extract determined using the brine shrimp assay observed by Ajaiyeoba et al. [3]. The plants also contain alkaloid, octacosanol, saponin, stigmastrol, HCN-glycoside, lignoceric acid and 2,4-di-OH-benzoic acid. Here we report a comparative potency of extracts obtained in different solvents from leaves, seed and stem wood of *A. pavonina* to inhibit Gram-positive and Gram-negative bacteria due to their random use in customary and traditional medicine to cure common ailments such as rheumatism, diarrhea, chronic ophthalmia etc.

2. Materials and Methods

Plant collection and identification: The plant specimen was collected from Botanical Garden, Rajshahi University Campus. Identification of voucher specimens was confirmed at the Taxonomical Section, Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extract: The leaves, seeds and stem woods were cut into small pieces, dried in shade, stored in cotton bags and then finely powdered (100 g) separately with the help of a grinder. Each ground material was soaked in 500 mL petroleum ether, acetone, chloroform and methanol (BDH, Poole, England) separately for 24-72 h and filtered (Whatman No. 1). The filtered one was then allowed to vaporize in rotary evaporator until completely dried and kept in a refrigerator at -20°C with proper labeling. Then dried extract (100 mg and 50 mg), for further study, was weighed and dissolved in 10 mL of respective solvents for dilution.

Antibacterial screening: Inhibitory activity of the extracts was performed by following the agar well diffusion method of Perez et al. [4]. Fifteen pathogenic bacteria (six gram-positive and nine gram-negative) were selected for the antibacterial test and were cultured at the Molecular Laboratory, Institute of Biological Sciences, Rajshahi

amount (2.048 mg), and then broth medium (2 ml) was added to each of the vials and agitated well to make sample solution whose concentration become 1024 µg/ml. The standard antibiotic Ciprofloxacin solution 512 µg/ml was used for comparison.

3. Results and Discussion

The antibacterial activity of *A. pavonina* extracts collected in petroleum ether, acetone, chloroform and methanol of leaves, seed and stem wood were tested against 15 bacteria. A correlation was found between the antibacterial activity observed by agar disc diffusion assay and MIC determination.

The highest average zone of inhibition was found 25 mm and 22 mm against: *S. β -haemolyticus* by chloroform fraction of seed extract and stem wood extract and also against methanol fraction of same bacteria (Table 2) when compared to the ciprofloxacin 33 mm in diameter against *Proteus* sp. of seed extract and *S. typhi* of stem wood extracts. Then moderate zone of inhibition were recorded respectively against gram positive and gram negative bacterial strains in both type of extraction either chloroform and methanol by seed, leaf and stem wood presented in Tables (1-3). The highest average zone of inhibition was found 24 mm against *S. boydii* by pet. Ether and acetone fraction of leaf extracts compared to the ciprofloxacin 33 mm in diameter against *S. typhi* and *Proteus* sp. of seed extract and leaf extracts respectively (Tables 1-2). Remaining of the lowest average zone inhibition activity was 6 mm against *S. dysenteriae* of leaf extract of chloroform fraction whereas the comparative study of ciprofloxacin showed; 26 mm in diameter against gram positive bacteria *S. aureus* and 28 mm in diameter against gram negative bacteria *B. cereus*, *Klebsiella* sp.

(MIC): The serial dilution technique was followed using nutrient broth medium of Reiner [5] to determine the MIC values of the chloroform and methanol extracts against *B. cerus*, *B. Megaterium*, *S. aureus*, *S. sonnei* and *S. dysenteriae*. The selected extracts were taken into different vials in a fixed

[illegible]

Table 2. Antibacterial activity of the seed extracts of *A. pavonina* and the standard Ciprofloxacin.

[illegible]

Table 3.Antibacterial activity of the stem wood extracts of *A. pavonina* and the standard Ciprofloxacin.

[illegible]

Table 4. Minimum inhibitory concentrations of the chloroform extract of seed and stem wood of *A. pavonina* against pathogenic bacteria.

Test tube No.	Nutrient broth or potato dextrose broth medium added (ml)	Diluted solution of chloroform extract from root (µg/ml)	Inoculums added (µl)	Seed extracts				Stem wood extracts			
				<i>S.-β-haemolyticus</i>	<i>S. dysenteriae</i>	<i>Klebsiella</i> sp.	<i>B. cereus</i>	<i>S.-β-haemolyticus</i>	<i>S. sonnei</i>	<i>S. typhi</i>	<i>B. megaterium</i>
1	1	512	10	-	-	-	-	-	-	-	-
2	1	256	10	-	-	-	-	-	-	-	-
3	1	128	10	-	-	-	+	+	-	-	-
4	1	64	10	+	-	+	+	+	+	-	+
5	1	32	10	+	+	+	+	+	+	+	+
6	1	16	10	+	+	+	+	+	+	+	+
7	1	8	10	+	+	+	+	+	+	+	+
8	1	4	10	+	+	+	+	+	+	+	+
9	1	2	10	+	+	+	+	+	+	+	+
10	1	1	10	+	+	+	+	+	+	+	+
Cm	1	0	0	-	-	-	-	-	-	-	-
Cs	1	1024	0	-	-	-	-	-	-	-	-
Ci	1	0	10	+	+	+	+	+	+	+	+
Results of MIC values (µg/ml)				128	128	64	256	128	64	32	64

Minimum inhibitory concentrations (MICs) against test bacteria

Only chloroform extracts of the seed and the stem wood were subjected to evaluate the minimum inhibition zones just depending on the intensity of activity (Table 4). The MIC value of the chloroform extract of the seed was 256 µg/ml against *B. cereus*, and 128 µg/ml against *S.-β-haemolyticus*, *S. dysenteriae* and 64 µg/ml against *Klebsiella* sp. The MIC values of the chloroform extract of seed were 128 µg/ml against *S.-β-haemolyticus*, *S. dysenteriae*; 64 µg/ml against *Klebsiella* sp. and 256 µg/ml against *B. cereus*. The MIC values of the chloroform extract of stem wood were 128 µg/ml against *S.-β-haemolyticus*; 64 µg/ml against *B. megaterium*, *S. sonnei* and 32 µg/ml against *S. typhi*.

The chloroform and methanol extracts of seed and seed coats of *A. pavonina* especially at 200 µg/disc were very effective. When concentration of the extract was increased, the zone of inhibition was found to be increased. The MIC results indicated that the methanolic extract of the seed has the property of inhibiting bacterial growth even at low concentrations (64-128 µg/disc). This probably explains the use of the extract of this plant in traditional medicines against a number of infections. So more comprehensive works are very much to be solicited for their effective use, especially in medicine and agriculture.

The antibacterial study of *A. pavonina* extracts demonstrates that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests

that they represent an economic and safe alternative to treat infectious diseases, Girish and Satish [6]; Toama et al. [7]. These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of the plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources, Li et al. [8], Eruteya and Odunfa [9]. Our previous studies have demonstrated that the reliable antibacterial potentiality of *Solanum torvum* and *Clerodendrum infortunatum*, Bari et al. [10], Waliullah et al. [11].

The present data on the antibacterial activity of the experimental plant are supported by a number of recent works. Kannur et al. [12] reported that the seed coat extracts of *C. bonduca* was more effective in controlling the inflammation. Cerqueira et al. [13] extracted, purified and characterized galactomannans from non-traditional sources. Four non-traditional galactomannans were isolated from the seeds of *A. pavonina*, *C. pulcherrima*, *Gleditsia triacanthos* and *Sophora japonica*. All the galactomannans from those plants in view of their importance, eg. in the demanding area of food industry. The finding also supports the antibacterial activity against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus* and inhibitory effect on glucoamylase of ethanolic extracts isolated at different temperatures from seeds of *S. cumini* investigated *in vitro*, Gangadhar A. Meshram et al. [14]. Thus, we believe that *A. pavonina* will be a possible source for new antibacterial substances against important pathogens of medically and veterinary importance.

The findings evidently appear promising antibacterial

properties of *A. pavonina* against antagonistic pathogens. Leaf possess quite potent activity than root and stem specially root extract > leaf extract > stem extract. This study serves as basis for further research to lead compounds to be isolated so that may be as a template for the implications of these results for bioactivity and drug discovery potential of herbal products are discussed.

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