

***In vitro* inhibitory activity of *Achyranthes aspera* L. seed against some test bacteria**

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Abstract: The inhibitory activity of seed extracts of *Achyranthes aspera*, a widely used folk medicinal plant in Bangladesh, was examined using methanol, acetone, ethyl acetate and petroleum spirit as solvents against five test bacteria by disc diffusion method. Methanol extract was found to reveal significant inhibitory activity against the pathogenic *B. subtilis*, *E. coli* and *K. pneumoniae*. Only the methanol and ethyl acetate extracts were effective against all bacteria and the best activity was found against *B. subtilis* in terms of zone of clearance. The best minimum inhibitory concentration value was found by methanol extract against *B. subtilis*. The present study suggests that the methanol extract of seed of this plant could be a possible source of obtaining new and effective herbal medicines to treat infections; hence, it justified the ethnic use.

Keywords: *Achyranthes Aspera*, Inhibitory Activity, Medicinal Plant

1. Introduction

Medicinal plants have been playing significant roles since ancient traditional systems of medication in many countries. They are rich source of bioactive compounds and thus serve as important raw materials for drug production [1]. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [2] due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [3], has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages [4]. This situation forced scientists to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [5]. The antimicrobial activities of plant extracts may reside in a variety of different secondary metabolites, including aldehyde and phenolic compounds [6]. A number of scientific investigations have highlighted the importance and the contribution of so many plant families namely Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae, Amaranthaceae etc. used as medicinal plants [1,7]. Hence the sensitivity study of bacterial strains to the plant

Achyranthes aspera was evaluated.

A. aspera Linn. belongs to the family Amaranthaceae, is an annual, stiff erect herb, and found commonly as a weed throughout Bangladesh. The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative, ecboic, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anticoagulant, diuretic and anti-tumor [8-9]. It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles and snake bites [10]. This plant is astringent, digestive, diuretic, laxative, purgative and stomachic. The juice of the plant is used in the treatment of boils, diarrhea, dysentery, hemorrhoids, rheumatic pains, itches and skin eruptions [11]. It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids. Flavonoids have shown to prevent or slows the development of some cancers [12] and mostly act as an anti-oxidant and anti-inflammatory agents. Saponins have long been known to have strong biological activity. The water soluble alkaloid achyranthine isolated from *A. aspera* possess anti-inflammatory activity [13]. Many *in vitro* antimicrobial studies of different extracts of roots, leaves and stems of *A. aspera* has been carried out but none of them used seed.

The present study was carried out to test the antibacterial

efficacy of the seed extract of *A. aspera*.

2. Materials and Methods

2.1. Plant Material

Healthy, disease free, mature Apang (*A. aspera*) plant was collected directly from nature (university region of Kushtia, Bangladesh) on September, 2010. The herb was then botanically identified and characterized by a taxonomist. After cleaning the waste materials with water, the plant was air dried. The seeds were then collected and the name of the plant, time, place, and date of collection were recorded.

2.2. Extract Preparation

Collected *A. aspera* seeds were cleaned with deionized water and dried in shade and pulverized into fine powdered substance by a grinding machine. Each 10 gm of powdered seed was weighted with the electric balance and transferred into five separate 100 ml conical flasks. Then each 40 ml of methanol, ethyl acetate, acetone and petroleum spirit was added in the flasks respectively. The conical flasks were closed by foil paper and placed on a shaker at 37°C temperature for 24 h. The crude extracts were then filtered by passing the extracts through Whatmann No. 1 filter paper (UK) and concentrated under vacuum at 40°C by using a rotary evaporator. The residual extracts were stored in refrigerator at 4°C in small and sterile plastic bottles.

2.3. Test Bacteria

Antibacterial activity of *A. aspera* seed extracts was investigated against three gram-negative (*Xanthomonas campestris*, *Escherichia coli* and *Klebsiella pneumoniae*) and two gram positive (*Bacillus subtilis* and *Sarcina lutea*) bacterial isolates, obtained from the Microbiology Laboratory, Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh. The test microorganisms were cultured on nutrient agar (Oxoid, UK) at 37°C for 24 h. The cultures were sub-cultured regularly (every 30 days) and stored at 4°C.

2.4. Inoculum Preparation

Ten (10) ml of distilled water containing screw capped tube was added with pure colony of freshly cultured bacteria followed by vortexing. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be within in 10^7 ml⁻¹ to 10^8 ml⁻¹ and then plated out as inoculums [14].

2.5. Antimicrobial Bioassay

The *in vitro* antimicrobial activities of the test samples were carried out by disc diffusion method [15-16]. Dried and sterilized filter paper discs (6 mm diameter) were impregnated with test substances (extracts) dissolved in solvents and the residual solvents were completely

evaporated. Discs containing the test materials were placed on nutrient agar medium uniformly seeded with the test bacteria. Standard disc of nalidixic acid (30µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4°C) for 24 h to allow maximum diffusion of test samples and then incubated at 37°C for 24 h to allow maximum growth of the bacteria. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter. Minimum inhibitory concentration (MIC) values of the extracts showing significant results (methanol and ethyl acetate extracts) were determined in the present study following the serial dilution technique [17].

2.6. Statistical Evolution

The experiments were repeated three times and the results were determined as an average value. Readings were made visually. The MIC endpoint was considered as the lowest drug concentration of antibacterial agent inhibiting the total growth.

3. Results and Discussion

Higher zone of inhibition against the bacterial pathogens exhibited by the extracts is of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections, especially as they frequently develop resistance to known antibiotics [10]. Among all tested extracts in the present study, methanol and ethyl acetate extracts were found to be most sensitive than corresponding organic extracts (Table 1). Methanol extract revealed comparatively better results found to have maximum zone of inhibition against *B. subtilis* (12 mm) and it was significantly susceptible against *K. pneumoniae* (11 mm). Earlier study reported similar result revealed with methanol extract [18]. The alcoholic extracts have been reported to have wound healing and antioxidant activities [19]. The lowest MIC value was 256 µgml⁻¹ against *B. subtilis* (Table 2) showed by methanol extract. Methanolic leaf extract of *A. aspera* reported as potent inhibitor of Gram positive *Staphylococcus aureus* with a minimal inhibitory concentration of 5000 µlml⁻¹ [11]. Our study possessed relatively good results. The acetone extract was most effective against *B. subtilis* (11 mm) whereas inactive against *E. coli*. Ethyl acetate extract was found to be most active against both *B. subtilis* and *E. coli* (9 mm). The petroleum spirit extract showed highest activity against *E. coli* (9 mm) but inactive against *K. pneumoniae*. It has been found to support these findings likely [20]. Other studies on antimicrobial screening of different parts of *A. aspera* revealed its poor antibacterial activity and broad spectrum antifungal activity [21-22]. Negative controls (disc containing only solvents) showed no zone against any test

bacteria. Thus no solvent process any antibacterial activity. Positive control (nalidixic acid) produce greater zone of inhibitions against all the test bacteria (Table 1).

Table 1. Antibacterial activity of different extract of *A. aspera* seed

Test Bacteria	Diameter of Zone of Inhibition (mm)				
	Methanol	Acetone	Ethyl acetate	Petroleum sprit	Nalidixic acid (30 µg)
<i>B. subtilis</i>	12	11	9	7.5	27
<i>S. lutea</i>	7	8	7	7	24
<i>X. campestris</i>	8.5	7	7	7	27
<i>E. coli</i>	10.5	0	9	9	28
<i>K. pneumoniae</i>	11	7.5	8	0	29

Table 2. MIC values of methanol and ethyl acetate extracts of *A. aspera* seed

Test Bacteria	Methanol extract (µgml ⁻¹)														Ethyl acetate extract (µgml ⁻¹)													
	4096	2048	1024	512	256	128	64	32	16	8	4	2	0	4096	2048	1024	512	256	128	64	32	16	8	4	2	0		
<i>B. subtilis</i>	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+		
<i>S. lutea</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+		
<i>X. campestris</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+		
<i>E. coli</i>	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+		
<i>K. pneumoniae</i>	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+		

‘+’= No zone formation; ‘-’= Formation of inhibition zone

4. Conclusion

The seed extracts of *A. aspera* seed were found to be effective against test bacteria, three of which are pathogenic for human. This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity, to characterize the active compounds and to elucidate the exact mechanism of action by which they exert their antibacterial effects.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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