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Short Communication

Antibacterial Activity of Different Organic Extracts of *Achyranthes Aspera* and *Cassia Alata*

M. T. Alam, M. M. Karim, and Shakila N. Khan[1]

Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh

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Abstract

Extracts in organic solvents (namely methanol, ethanol, ethyl acetate and chloroform) of two medicinal plants - *Achyranthes aspera* and *Cassia alata* were evaluated for their antibacterial activities against *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi* and *Staphylococcus aureus*. These were carried out by taking the organic extracts of both the leaf and stem parts of the plants at a concentration of 5 mg/ml and their activities were recorded by estimating zones of inhibition as produced by disc-diffusion method on Mueller-Hinton agar media. While neither the leaf nor stem parts of *A. aspera* in any organic extractions showed antibacterial activity, the methanolic extracts of both the leaf and stem parts of *C. alata* exhibited antibacterial activity, but only to *B. subtilis* and *S. typhi*, and the corresponding MIC values of the leaf extracts were estimated as 1.25 and 1.5 mg/ml respectively. However, the ethanolic extracts of both the stem and leaf parts were found equally effective only to *S. aureus* (MIC= 1.25 mg/ml). The corresponding MBC values are reported.

Keywords: *Achyranthes aspera*; *Cassia alata;* Antibacterial.

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1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources [1]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The use of plant extract for medical treatments is enjoying great popularity since 1990s when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance [2].

[1] *Corresponding author*: shakilamanjur@yahoo.com

The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds [3]. Naturally occurring combinations of these compounds can be synergistic and often results in crude extracts having greater antimicrobial activity than the purified individual constituents [4]. In this work, we have selected two plants, namely *Achyranthes aspera* (family Amaranthaceae; local name, *apang*) and *Cassia alata* (family Caesalpinaceae; local name, *Dadmardan*). *A. aspera* is distributed throughout the tropical and subtropical regions, including the Indian sub-continent, Africa, Australia and America [5]. *C. alata*, commonly known as Candlebrush, is a tropical shrub having yellow flowers and large leaves whose juice is used as a cure for ringworm and poisonous bites [6]. Both of them are commonly used in Bangladesh as herbal medicine. This study was conducted to address their antimicrobial activities against some pathogenic bacteria causing acute diarrhea, food poisoning and other diseases, when extracted their leaf and stem parts in different organic solvents.

2. Materials and Methods

2.1. *Bacteria and growth conditions*

Five bacterial species were employed as test organisms which include *E. coli* (ATCC 25922), *Salmonella typhi* (ATCC 27785), *Staphylococcus aureus* (ATCC 103207), *Bacillus subtilis* (ATCC 6623) and *Vibrio cholerae* (ATCC 9458). The bacteria were maintained in Mueller-Hinton Agar (MH). Inocula were prepared by adding an overnight culture of the organism in MH broth to obtain an OD_{600} 0.1. The cells were allowed to grow until they obtain the McFarland standard 0.5 (approximately 10^8 CFU/ml). The suspension were then diluted 1:100 in MH broth to obtain 10^6 CFU/ml.

2.2. *Test plants and their extraction*

Cassia alata and *Achyranthes aspera* were collected from the Chittagong region of Bangladesh for the study. Both the leaf and stem parts of the plants were separated, washed with sterile water, dried at room temperature and then ground to powder using a grinder. 10 g of the powder is mixed with 40 ml of chloroform in a 250-ml conical flask and was kept at 25ºC for 12 h. The suspension was filtered through a Whatman no. 4 filter paper and the filtrate was evaporated by vacuum dryer at 40ºC overnight to get the chloroform extract. After chloroform extraction, a part of the solid residue was dried at 40ºC overnight to remove residual chloroform. The solid powder was resuspended in 40 ml ethyl acetate and kept at 25ºC for 12 h. Ethyl acetate extract was recovered following the same procedure as stated for chloroform extract. Similarly, methanol and ethanol extracts were prepared by applying the same procedure. Finally, the extracted powder was resuspended in the respective organic solvents at a concentration of 100 mg/ml before it was tested for the antibacterial activity.

2.3. *Determination of antibacterial activity*

Sterile discs (Oxoid) were soaked separately with 50 µl of each of the organic extract prepared in chloroform, ethyl acetate, methanol and ethanol solvents, at a concentration of 100 mg/ml and then dried. These discs were placed on Mueller-Hinton agar plates, previously swabbed with the target bacterial isolate at a concentration of 10^6 CFU/ml. In one disc, the respective organic solvent was added as negative control to determine possible inhibitory activity of the solvent. This preparation was incubated for a period of 24 h at 30°C. Antibacterial activity was defined as the diameter (mm) of the clear inhibitory zone formed around the discs.

The MIC of the extract was determined by tube dilution techniques in Mueller-Hinton broth (Merck) according to NCCLS [7]. The range of concentration used was 156.25 to 5000 µg/ml. The four last vials of each bacterium with no growth from the MIC procedure were streaked onto nutrient agar (NA) plates. The plates were then incubated at 37°C for 24 h. The lowest concentration that killed 100% of the inoculum bacteria (no growth on plate) was recorded as Minimum Bactericidal Concentrations (MBC).

3. Results

The results of the antimicrobial determinations for all the organic extracts of the leaf and stem parts of *Cassia alata* and *Achyranthes aspera* against the five bacterial species are investigated in a disc-diffusion assay. Fig. 1 illustrates a representative plate showing the antibacterial activity of ethanolic extracts from *A. aspera* and *C. alata* that produced zones of inhibition against *Staphylococcus aureus*.

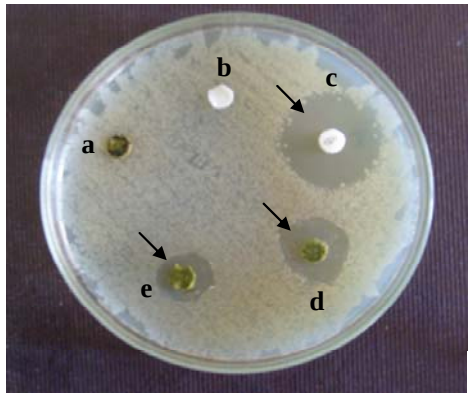

Fig. 1. Antibacterial activity. Petridish containing 50 µl each of ethanolic extracts of the (a) leaf part of *A. aspera*, and leaf (d) and stem (e) parts of *C. alata* are placed in discs in a media previously swabbed with *S. aureus* culture. Zones of inhibition are shown in arrows as produced after overnight incubation. As positive and negative controls, chloramphenicol (c) and the ethanol solvent (b) absorbed in empty disc were used respectively.

While neither the leaf nor stem parts of *A. aspera* in any organic extractions displayed no or low antibacterial activity, the methanolic extracts of both the leaf and stem parts of *C. alata* exhibited antibacterial activity, but only to *B. subtilis* and *S. typhi*. However, the ethanolic extracts of both the stem and leaf parts were found equally effective only to *S. aureus*. Virtually no inhibition of bacterial growth was observed for fraction extracted in ethyl acetate. *E. coli* and *V. cholerae* were found resistant to the tested plants (Table 1).

Table 1. Antibacterial assay of plant extracts by disc-diffusion method.

Target organism	Medicinal plant	Zone of inhibition (mm) as produced in different organic solvent extracts of the plants			
		Chloroform	Methanol	Ethanol	Et-acetate
Escherichia coli	*C. alata* (leaf)	0	0	0	0
	C. alata (stem)	0	0	0	0
	A. aspera (leaf)	0	0	0	0
	A. aspera (stem)	0	0	0	0
Bacillus subtilis	*C. alata* (leaf)	17	22	0	0
	C. alata (stem)	13	16	0	0
	A. aspera (leaf)	0	0	0	0
	A. aspera (stem)	0	0	0	0
Vibrio cholerae	*C. alata* (leaf)	0	0	0	0
	C. alata (stem)	0	0	0	0
	A. aspera (leaf)	0	0	0	0
	A. aspera (stem)	0	0	0	0
Salmonella typhi	*C. alata* (leaf)	0	15	0	0
	C. alata (stem)	0	13	0	0
	A. aspera (leaf)	0	0	0	0
	A. aspera (stem)	0	0	0	0
Staphylococcus aureus	*C. alata* (leaf)	0	0	16	0
	C. alata (stem)	0	0	15	0
	A. aspera (leaf)	0	0	0	0
	A. aspera (stem)	0	0	0	0

The MIC and MBC of the organic extracts of *C. alata* that showed antibacterial activity were determined by macro-dilution method and the results are summarized in Fig. 2. Organic extracts from leaf part of *C. alata* appeared to be more potent than that of the stem part. The methanolic extract from the leaves produced significant activity against *B. subtilis* (MIC= 1250 µg/ml), while the activity was moderate against *S. typhi* (MIC= 1500 µg/ml). Comparable activity of ethanolic extract was observed to inhibit *S. aureus* growth (Fig. 2).

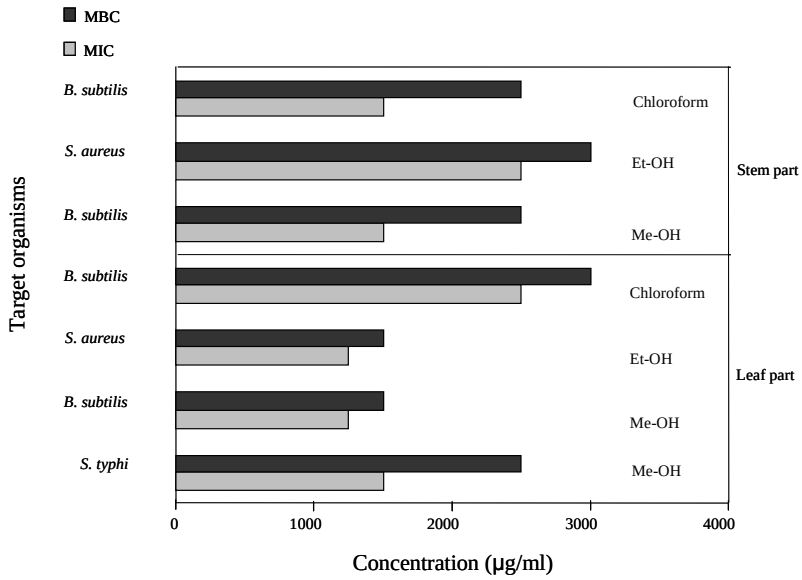

Fig. 2. MIC and MBC of organic extracts of *C. alata.*

4. Discussion

Plants and plant products have been used extensively throughout history to treat medical problem. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal remedies has risen in the developed countries in the last decades. In this connection, plants continue to be a rich source of therapeutic agents. The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industry was possible, because of the large number of phytochemical and biological studies all over the world. Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents.

In this study, two plants, named *Achyranthes aspera* and *Cassia alata* were used which are easily available in Bangladesh and other tropical countries. We did not find any antibacterial activity of the organic extracts of *A. aspera*, although it is popularly used in herbal practices. However, the aqueous extract of the plant was not tested in this study, and therefore demands to be investigated that might yield any antibacterial effect.

The organic extracts of *C. alata* exhibit significant antimicrobial activity against *B. subtilis*, *S. typhi* and *S. aureus*; with no activity observed against *E. coli* and *V. cholerae*. Extracts from leaf part of *C. alata* appeared to be more potent than that of the stem part, and the methanolic extract produced consistent level of inhibition of bacterial growth. The leaves of *Senna alata* also demonstrated significant antibacterial and antifungal activities when extracted with methanol [8]. It is interesting to note that the leaf extract of *C. alata* could be used against salmonellae. Since *Salmonella* is a frequent candidate causing food-borne illnesses in addition to the typhoid and paratyphoid infection, the therapeutic value of this plant could be an effective remedy. Further, it was revealed that the extract has the ability to inhibit the growth of *Staphylococcus aureus*, another food-poisoning organism. Hence it would be interesting to investigate the potentiality of this plant for possible application in foods to increase shelf life or promote safety.

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