

**POTENTIAL ANTIMICROBIAL ACTIVITY OF ACHYRANTHES BIDENTATA METHANOL EXTRACT AGAINST BOTH GRAM (+) VE AND GRAM (-) VE BACTERIA**

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**Abstract**

In vitro antibacterial activity of methanol extract of whole plant (leave, stem, flower & root) of *Achyranthes bidentata* were found to be the most effective against the bacterial strains *Bacillus cereus*, *Salmonella typhi*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*. The maximum zone of inhibition of *achyranthesbidentata* extract was 10 mm against *Bacillus cereus* at the dose of 100 µg/disc. A qualitative phytochemical analysis was performed for the detection of Reducing Sugars, Carbohydrate, Saponins, Alkaloids, Flavonoids, Tannins, Gums, Steroid and Glycosides. The photochemical of flavonoids, tannins and alkaloids show antimicrobial activity and saponin may be attributed to anti-infecting agent where flavonoids showed antimicrobial activity by complexing with cell wall and also binds to adhesions. The present study is done to evaluate the antibacterial efficacy of *achyranthesbidentata* against several pathogenic bacterial strains which could be further exploited for isolation and characterization of the novel phytochemicals in the treatment of infectious disease especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents.

**Keywords:** Antibacterial activity, zone of inhibition, phytochemical analysis, drug-resistant.

## Introduction

There are thousands of plant species on earth which are endowed with an enormous wealth of medicinal remedies from nature.<sup>[1]</sup> Medicinal plants are rich sources of bioactive compounds and thus serve as important raw material for drug production. It has now been established that the plants synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils etc. that may possess a great potential for biological activity and can be a curative agent in therapeutic purposes.<sup>[2]</sup> Antimicrobial susceptibility testing can be used for drug discovery, development and prediction of therapeutic outcome. After the revolution in the "golden era", when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered but now-a-days, antibiotic resistance is an increasingly serious threat to global public health. Today the situation for bacterial pathogens is very different, and the need for potent new antimicrobial agents has probably never been greater.<sup>[3]</sup>

Currently, its impact is noticeable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health. For this reason, discovery of new antibiotics is an exclusively important objective. Medicinal plants are still one of the major sources of new drug molecules today.<sup>[3]</sup>

In vitro antibacterial activity of plants can be detected by observing the growth response of various microorganisms to those plant extracts

## Methods

### *Collection of samples*

The whole plant of *Achyranthes bidentata* was collected from West Medda, Brahmanbaria, Bangladesh, and was identified by the Bangladesh National Herbarium, Mirpur, Dhaka.

### *Preparation of crude extract*

### *Drying and Grinding*

The collected plant parts were separated from undesirable materials or plants or plant parts and air dried for three weeks. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

### *Cold Extraction (Methanol extraction)*

The leaves (120 gm), stem (120 gm), root (120 gm) were extracted by cold extraction method. 360 gm grinded powder was soaked in 2000 ml of 95% methanol in a glass container for fifteen days accompanying regular shaking and stirring. After fifteen days the extract was separated from the plant debris by filtration by a piece of clean, white cotton cloth and finally by cotton.

### *Filtration of the solvent*

After fifteen days the extract was separated from the plant debris by filtration by a piece of clean, white cotton cloth and finally by cotton. The filtrate (methanol extract) was taken into a beaker. Then this filtrate was taken into rotary evaporator to evaporate methanol.

### *Evaporation*

Then the opening of beaker was wrapped by a sheet of aluminum foil to which perforation was done for evaporation of the rest of the methanol & was kept in dry & cool place for several days. Then table fan was used until dried. The concentrate was designated as crude extract of methanol.

## Antibacterial activity assay

### *Preparation of standard culture inoculum of test organism*

Three isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent with MacFarland standard (0.5%) as recommended by WHO.<sup>[4]</sup>

### *Preparation of test sample*

For the preparation of the test solution, 5 mg of methanol extract were accurately measured by the electronic balance and taken into a test tube. Then small amount (2.5 ml) of methanol was added by a calibrated pipette and triturated in unidirectional manner using a vortex mixer and the concentration of the solution became 2 µg/µl.

#### *Application of discs*

Three types of discs were used for antibacterial screening:

- a) Sample discs
- b) Blank discs
- c) Standard discs

Each agar plates were divided into six portions i.e. two for samples (30 µg/disc, 50 µg/disc, 100 µg/disc, 150 µg/disc, 200 µg/disc and 250 µg/disc), one for standard and one for blank.

#### *Sample disc application*

Two blank discs were placed into respected portions on agar plates with the help of a sterile forceps to assure complete contact with medium surface. The spatial arrangement of the discs was such that the discs were no closer than 15 mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. 15µl, 25 µl, 50 µl, 75 µl, 100 µl, 125 µl of the test sample from 2 µg/µl solutions were applied on the respective discs with the help of a micropipette in an aseptic condition under the laminar air flow to get per disc concentration 30 µg, 50 µg, 100 µg, 150 µg, 200 µg, 250 µg respectively.

#### **Identification tests for active compounds**

The tests were done to find the presence of the active chemical constituents such as Reducing Sugars, Carbohydrate, Saponins, Alkaloids, Flavonoids, Tannins, Gums, Steroid, Glycosides by the following procedure.

#### *Tests for reducing sugar*

0.5 ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. A red color precipitate of cuprous oxide indicates the presence of a reducing sugar.

#### *Test for carbohydrates / gums*

5 ml solution of the extract was taken and then Molish's reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicates the presence of gums and carbohydrate.

#### *Test for saponins*

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A layer of foam above the solution indicates the presence of saponins.

#### *Test for alkaloids*

2 ml solution of the extract and 5 ml of dilute hydrochloric acid (1%) were taken in a test tube. Then 1 ml of Mayer's reagent was added. A white or creamy white color precipitate indicates the presence of alkaloids.

#### *Test for flavonoids:*

0.2 gm extract was dissolved in dilute sodium hydroxide and then neutralized with dilute hydrochloric acid. Formation of yellow color and disappearance of color indicate the presence of flavonoid.

#### *Tests for tannins*

5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added. Greenish black precipitate indicates the presence of tannins.

#### *Tests for steroid*

1 ml sulfuric acid was taken in a small amount of an alcoholic extract. Reddish brown color and acid layer showed green fluorescence which indicates the presence of steroid.

### Test for glycosides

A small amount of an alcoholic extract was taken in 1ml of water. A few drops of aqueous NaOH were added. A yellow color indicates the presence of glycosides.

### Results

The phytochemical tests, carried out on leaves & aerial root extract revealed the presence of Alkaloids, Flavonoids, Tannins, Gums, Steroids which may lead to the development of drug formulation after investigation of further pharmacological and other activity.

The crude extract of methanolic *achyranthes bidentata* showed observable antibacterial activity against the bacterial strains *Bacillus cereus* (BC), *Salmonella typhi* (ST), *Streptococcus pyogenes* (SP), *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA). The maximum zone of inhibition of *achyranthes bidentata* extract was 10 mm against *Bacillus cereus* at the dose of 100 µg/disc and which could be comparable to kanamycin. Further investigation is required to identify the active compounds and to determine the mechanism of action of the compounds present in the plant

### Discussion

There are thousands of plant species on earth which are endowed with an enormous wealth of medicinal remedies from nature. Natural products and their derivatives represent more than 50% of all the drugs in modern therapeutics but still there is an enormous possibility to isolate and identify a new compound from natural source having a potential pharmacological activity.[1]

In this present study, the freshly prepared extract of whole plant of *achyranthes bidentata* was subjected to preliminary phytochemical screening for various constituents. The qualitative phytochemical investigation of methanolic extract of *achyranthes bidentata* revealed the presence of alkaloids, flavonoids, tannins, gums, steroid (Table-1) which may lead to the development of new drug formulation. The percentage yield of methanol

extract *achyranthes bidentata* was found to be 1.45% w/w.

Tannins may protect plants from invasion of pathogenic microorganism due to their antimicrobial and antifungal properties.<sup>[5][6][8]</sup> The photochemical of flavonoids, tannins and alkaloids show antimicrobial activity.<sup>[9]</sup> Saponin may be attributed to anti-infecting agent (Saxena et al., 2012; Halilu et al., 2012). Flavonoids showed antimicrobial activity by complexing with cell wall and also binds to adhesions.<sup>[9]</sup> The present study is done to evaluate the antibacterial efficacy of *achyranthes bidentata* against several pathogenic bacterial strains such as *bacillus cereus*, *salmonella typhi*, *streptococcus pyogenes*, *escherichia coli* and *pseudomonas aeruginosa*. The values of zone of inhibition were measured and compared with the positive control kanamycin. The crude extract of *achyranthes bidentata* extract showed observable antibacterial activity against all the bacterial strains. The maximum zone of inhibition of *achyranthes bidentata* extract was 10 mm against *bacillus cereus* at the dose of 100 µg/disc and which could be comparable to kanamycin. So methanol extract of *achyranthes bidentata* whole plant showed dose depended antibacterial activity and effective against both gram-positive and gram-negative bacteria.

Therefore, further investigation is required to identify the active compounds and to determine the mechanism of action of the compounds present in the plant extract.

### Acknowledgement

The authors acknowledge their profound gratitude to the Daffodil International University (DIU) for providing the facilities for research work. I heartily thanks Dr. Md. Anwar Hossain, Department of Microbiology University of Dhaka, for providing the pure cultures of bacterial strain.

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**Table 1:** Phytochemical qualitative evaluation of bidanteta extract

Test for	<i>Achyranthes bidandeta</i> whole plant extract
Reducing Sugars	-
Carbohydrate	-
Saponins	-
Alkaloids	+++
Flavonoids	+++
Tannins	++
Gums	+
Steroid	+
Glycosides	-

**Figure 1:** Antibacterial activity of methanolic extract of *Achyranthes bidentata* whole plant at different doses in comparison of kanamycin (Std in red color). The bacterial strains, *Bacillus cereus* (BC), *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Salmonella typhi* (ST), and *Streptococcus pyogenes* (SP) were evaluated and found kanamycin potential activity, significantly.

