

Research Article



Phytochemical and *in vitro* Screening of *Adhatoda vesica* L. Extracts against UTI Causing Microorganism

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ABSTRACT

In the present study the antimicrobial activity of *Adhatoda vesica* L. medicinal plants used against UTI causing pathogen. Microbes were isolated from the UTI infected patient and characterised by using microscopic, staining morphological and biochemical method. The present study evaluates the qualitative analysis of phytochemicals and antimicrobial activity of methanolic plant extract of *Adhatoda vesica* L. Acanthaceae plants against some bacteria causing urinary tract Infection (UTI) in humans. Best activity was observed against *B. Subtilis* with maximum zone of inhibition 24.67 ± 0.34 at concentration of 500mg/ml.

Keywords: Phytochemical, Alkaloids, UTI, Antimicrobial activity and *Adhatoda vesica* L.

INTRODUCTION

The urinary tract infection (UTI) begins in the urinary system. It is the most common disease after respiratory infection. The urinary tract consists of the kidneys, bladder, and the urethra¹. There are higher incidence of female UTI (75%) than in male (25%) where multiple factors have probably loaded to the emergence and spread of UTI². According to WHO around 80% of the world's inhabitants depend on traditional medicines for their primary healthcare. Further about 80% of the drugs used in modern medicine are the products of plant origin, their sales exceeding US \$ 65 billion in 2003³.

Multiple drug resistance in human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatments of infectious disease. This situation forced for searching new antimicrobial substance from various sources of novel antimicrobial chemotherapeutic agents^{4,5}. The increasing failure of the chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity⁶. Thus the present study is carried out to study the antibacterial activity of *Adhatoda vesica* against UTI causing bacteria in human.

MATERIALS AND METHODS

Collection and Preparation of Sample

Adhatoda vesica L. family Acanthaceae were collected from areas around Bhopal regions. The collected plant materials were dried in shade at room temperature for 20 days. The thoroughly air dried plant material milled to powder from by electrical blender to about 40-60 mesh size weighted and stored in large plastic bottles.

Solvent Extraction

The plant powder is extracted according to Harborne⁷. The 40-60 mesh size powdered plant material was extracted with soxhlet apparatus using 80 and 50% methanol for 38 hrs. After defatting with petroleum ether for 24 hrs, the extraction was done for 48 hrs duration or till cycles are completed. Almost all the chlorophyll and lipid is deposited on the side of the flask and with skill is was removed. The crude was pipette off skill fully almost completely free of lipid impurities and filtered through whatmann filter paper no.1. Filtrate is then evaporated to 1/10th volume (<40^o), acidified by 2M H₂SO₄ and was extracted with chloroform leaving behind the aqueous acidic layer. This extract is then dried in vacuum evaporator at <40^oC⁸.

Phytochemical Screening

Phytochemical screening of the extracts was carried out according to the methods^{9, 10} for the detection of active components like saponins, tannins, alkaloids, glycosides and etc. The phytochemical analyses were carried out standard procedures.

Test for Alkaloids

To 0.1ml of the crude extract in a test tube, 2-3 drops of Dragendoof's reagent was added. An orange red precipitate with turbidity denoted the presence of alkaloids.

Test for Flavonoids

To 4mg/ml of the extract and a piece of magnesium ribbon was added followed by drop-wise addition of concentrated HCL. A colour change from orange to red indicated the presence of flavones: red to crimson indicated the presence of flavonoids.



Test for Glycosides

10 ml of H₂SO₄ was added to 1ml of the filtrate in separate test tubes and the mixture heated for 15 minutes followed by addition of 10ml of Fehling's solution and boiled. A brick red precipitate indicated presence of glycosides.

Test for Reducing Sugars

To 1ml of extract and fraction in separate test tube, 2.0ml of distilled water were added followed by addition of Fehling's solution (A+B) and the mixtures were warmed at 40^oc. Appearance of brick red precipitate at the bottom of the tube indicated the presence of reducing sugar.

Test for Saponins

Half gram the powdered leaf was dispersed in a test-tube and 5.0 ml of distilled water was added and shaken vigorously. A persistent foam that lasted for about 15 minutes indicated the presence of saponins.

Test for Steroids

2 ml of the extracts were evaporated to dryness in separate test tubes and the residues dissolved in acetic anhydride followed by addition of chloroform. Concentrated sulphuric acid was added by means of a pipette via to side of the test tubes. Formation of brown ring at the interface of the two liquid and violet colours in the supernatant layer denoted the presence of steroids.

Antibacterial Assay

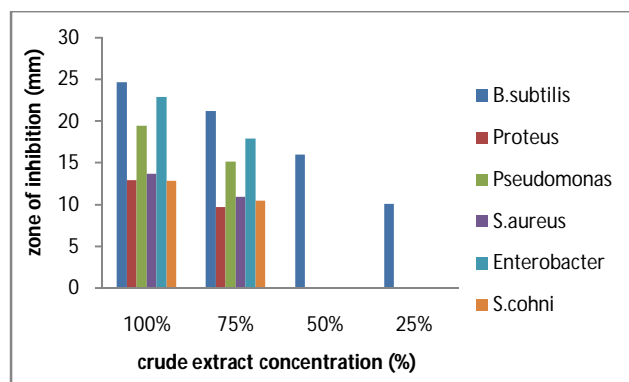
The plate disc diffusion assay was used to determine the growth inhibition of bacteria by plant crude extract¹¹. The bacteria cultures were maintained on nutrient broth. The bacteria were incubated at 37^oc for 24 hrs. Nutrient agar media was prepared and poured in autoclaved Petri dishes. Each bacterial culture was spread on poured plates.

Then the sterilized discs were filled with 50µl of the crude extract. The four different concentrations (50, 100, 250 and 500mg/ml) of plant extract were prepared by dissolving in DMSO (Dimethyl sulphoxide) and were tested for antibacterial activity. The experiments were performed in triplicates.

RESULTS

The antibacterial activity of Methanolic extract of *Adhatoda vesica* was assayed by disc diffusion method against bacteria causing urinary tract infection (UTI) in humans. Graph-1 summarizes the microbial growth inhibition of the methanolic extract. The crude extract showed maximum antibacterial response against *Bacillus subtilis* with maximum zone of inhibition 24.67±0.34 at concentration of 500mg/ml. The extract also showed antibacterial against *Proteus vulgaris*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Enterobacter* and *Staphylococcus choni* with zone of inhibition 12.95±0.12, 19.52±0.39, 13.72±0.17, 22.91±0.16 and 12.92±0.13 respectively at concentration of 500mg/ml. All the six

selected strains of bacteria are inhibited by starting concentration of 50mg/ml i.e. 1mg of the crude extract.



Graph 1: Antimicrobial activity of *Adhatoda vesica* (L.) against UTI Microbes

DISCUSSION

The main advantages of using *in vitro* assays to screen the antibacterial properties of the plant and plant extract include low costs and rapid turnover which allow the screening of plants at large scales¹². The antibacterial activity was expressed at varying degrees and the activity was strain and dose dependent. As flavonoids are synthesized by plants in response to microbial infection it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms.

Generally alkaloids are extremely toxic though they do have a marked therapeutic effect in minute quantities. For this reason alkaloids were not often used in folk medicine and then for external application only. Pure isolated plant alkaloids and their synthetic derivative are used as basic medicinal agents over the world for their analgesic, antispasmodic and bactericidal effects¹³. This antibacterial activity would support the folk therapy of infections whose symptoms might involve bacteria¹⁴. Antibiotics provide the base for treatment of bacterial infections. But, for the possession of high genetic variability bacteria easily develop antibiotic resistance. Thus, there has been a continuing search for new and more potent-antibiotics¹⁵. Methanolic extract from *Adhatoda vesica* plant showed potent antibacterial activity against UTI causing bacteria thus this plant can be used for isolation of bioactive compound especially alkaloids, as lead compound for development of novel antibiotics¹⁶.

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