

## Antimicrobial activity of *Andrographis paniculata* stem extracts.

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### Abstract

The present study describes the phytochemical profile and antimicrobial activity of *Andrographis paniculata*. For the present investigation, samples of *Andrographis paniculata* extracts, obtained by extraction in chloroform were used for their antimicrobial activity. The antibacterial activities were assessed by measuring the diameter of the inhibition zones, MIC and MBC values. Antimicrobial activity of stem extract of *Andrographis paniculata* was studied using solvent chloroform against bacterial strains like gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and five strains of fungi which are *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*. The antimicrobial activity was determined by disc diffusion method. The chloroform extract were found to be highly active against *Staphylococcus aureus* and *Escherichia coli*.

**Keywords:** *Andrographis paniculata*, Antimicrobial activity

### 1. Introduction

Infectious diseases are one of the significant causes of mortality and morbidity in developing countries. The prevalence of MRSA (Methicillin Resistant *Staphylococcus Aureus*) in nosocomial infections has been on the continuous rise and its prevalence has increased from 14.3% in 1987 to 60% in 2006<sup>[1]</sup>. Recently, carbapenem resistant Gram negative bacterial superbugs have been reported from patients admitted in hospitals of India and Pakistan creating a major global health problem<sup>[2]</sup>. Resistance to available therapeutic agents and the limited development of new agents are threatening to worsen the burden of infections and cancers that are already the leading cause of morbidity and mortality<sup>[3]</sup>. To overcome the problem, knowledge about production of allelochemicals by the plants it has created a interest in use of plants.

Higher plants, as sources of medicinal compounds, have continued to play an important role in the maintenance of human health since antiquity, especially in developing countries. Historically different herbal preparations have been used for the treatment of various types of illness in Indian medicine (Ayurvedic) system<sup>[4]</sup>. Although, this approach accepts the emergency use of modern drugs, but recommends the use of traditional herbal combinations and extracts to improve health, as well as to prevent microbial infections<sup>[5]</sup>. Presently, 50% of all modern drugs are also of plant origin<sup>[6]</sup>. Therefore, the present investigation has been carried out to evaluate the specific antimicrobial potential of *Andrographis paniculata* against resistant clinical pathogens. The plant was selected from Indian system of medicine and it was already known by traditional information that is reduce microbial infections.

## **2. Materials and Methods**

### **2.1. Collection of the plant material**

The fresh stem of the plant *Andrographis Paniculata* were collected during the month of October 2016 from the area of Trichy. The fresh stem of that plant were taxonomically authenticated from the Rabinet Herbarium (RHT) of St. Joseph College, Tiruchirappalli, Tamilnadu .

### **2.2. Test organisms**

Pure culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Bacillus subtilis* specie of bacteria and *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* specie of fungi were procured from the Department of Microbiology of Thanjavur Medical College, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

### **2.3. Preparation of extract from stem**

The freshly collected stems were washed with distilled water and air-dried at 40°C and powdered. About 50g of dried powder was extracted with solvent using chloroform by cold percolation method. The mixture was kept undisturbed at room temperature for 72 hrs in a sterile flask covered with aluminium foil to avoid evaporation and subjected to filtration through

sterilized Whatman no.1 filter paper. The extract was concentrated to dryness in rotary pressure evaporator at 40° C and stored until use.

#### **2.4.Preparation of discs**

The crude extract was dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 50µl (50µg) 100µl (100µg) and 150µl (150µg). Standard solution was *Chloromphenical* for bacteria and *fluconazole* (25mg/ml distilled water- 30µl) for fungi used to compare the test solution. Whatman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter and sterilized in hot air oven. After sterilization, the discs were loaded with different concentrations of prepared plant extract solutions and it was kept under refrigeration for 24 hrs. Above discs were dispensed onto the surface of the inoculated agar plates. Each disc was pressed down firmly to ensure completely contact with the agar surface. Then the plates were incubated at 5°C for 1 hr to permitted good diffusion and transferred in to incubator at 37°C for 24 hrs. After completion of 24hrs, the plates were inverted and placed in an incubator set to respective temperature for 24 hrs.

#### **2.5. Antimicrobial assay**

Antibiogram was done by disc diffusion method<sup>[7]</sup> using plant extracts. Petri plates were prepared by pouring 30 ml of Nutrient agar(NA) medium for bacteria and Potato Dextrose Agar (PDA) medium for fungi. The test organisms were inoculated on solidified agar plates with the help of micropipette and spreaded and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria and fungi from a broth culture. A sterile cotton swab was dipped into a standardized bacterial and fungi test suspension and used to evenly inoculated the entire surface of the NA plates and PDA plates. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Pseudomonas aeruginosa* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans*, *candida tropicalis*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* was spread on PDA for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plates for each bacteria and fungi. The plates were incubated at 37 °C for 24 hrs

for the bacteria and at room temperature ( $30\pm 1$ ) for 24-48 hrs. for yeasts strains. Each sample was tested in triplicate.

### 3. Results and Discussion

The chloroform extract of *Andrographis paniculata* was tested for zone of inhibition and MIC against Gram – Positive bacteria: *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 102) and *Bacillus subtilis* (MTCC 441) and Gram – negative bacteria: *Escherichia coli* (MTCC 119), *Pseudomonas aeruginosa* (MTCC 741). The results of these bacterial bioassays were given in Table 1. This antibacterial assay revealed that out of the five different bacteria, the chloroform stem extract of *Andrographis paniculata* posses highest antibacterial activity against *Escherichia coli*,. Even though the significant antibacterial activity was observed the other three bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacillus subtilis*. The extract was not found more active against *Pseudomonas aeruginosa*. The MIC value of the active extract against the strains showing promising results of antibacterial potential of the *Andrographis paniculata*.

The chloroform extract of *Andrographis paniculata* was tested for zone of inhibition and MIC against fungal strains containing *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*. This assay revealed that the chloroform stem extract of *Andrographis paniculata* exhibited the highest antifungal activity against *Candida tropicalis*. The moderate antifungal activity was observed against *Candida albicans*. The lowest antifungal activity were encountered in the remaining three fungai as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. The results of these fungal bioassays were given in Table 2. This assay showed that *Andrographis paniculata* had strong antifungal potential also.

**3.1. Table 1: antibacterial assay**

Microorganisms	50 µl	100 µl	150 µl	Standard	Control
<i>Escherichia coli</i> (mm)	3.60±0.25	6.50±0.45	9.70±0.67	12.30±0.86	0
<i>Staphylococcus aureus</i> (mm)	2.30±0.16	5.10±0.35	7.50±0.52	10.70±0.74	0
<i>Streptococcus pyogenes</i> (mm)	2.10±0.14	5.00±0.54	7.20±0.50	10.50±0.73	0
<i>Bacillus subtilis</i> (mm)	1.80±0.12	4.70±0.32	6.80±0.47	10.20±0.71	0
<i>Pseudomonas aeruginosa</i> (mm)	1.50±0.10	4.40±0.30	6.70±0.46	9.90±0.69	0

Values were expressed as Mean ± SD

Bacterial standard : *Chloromphenical*

**3.2. Table 2: antifungal assay**

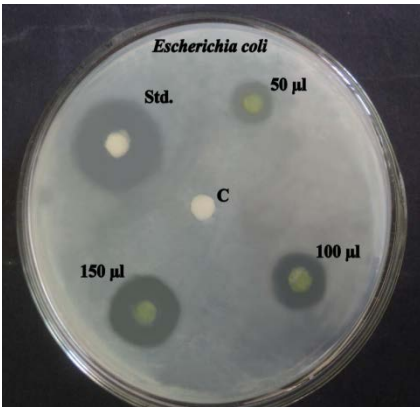
Microorganisms	50 µl	100 µl	150 µl	Standard	Control
<i>Candida albicans</i> (mm)	1.20±0.08	2.50 ±0.17	4.70 ±0.32	7.90 ±0.55	0
<i>Candida tropicalis</i> (mm)	1.10±0.07	2.70 ±0.18	5.00 ±0.35	8.10 ±0.56	0
<i>Aspergillus niger</i> (mm)	0.70 ±0.04	2.30 ±0.16	3.60 ±0.25	7.60 ±0.53	0
<i>Aspergillus flavus</i> (mm)	0.40±0.04	2.10 ±0.14	3.50 ±0.24	7.40 ±0.52	0
<i>Aspergillus fumigatus</i> (mm)	0.10±0.01	1.80 ±0.12	3.20 ±0.22	7.30 ±0.51	0

Values were expressed as Mean ± SD

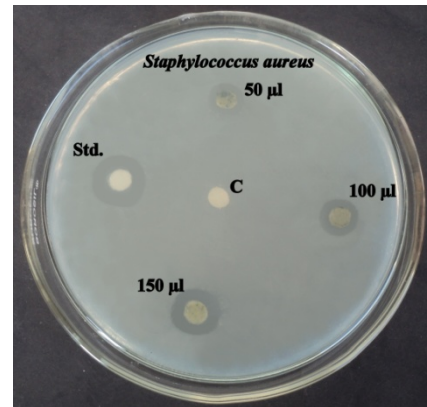
Fungal standard : *Fluconazole*

### 3.3. Antibacterial assay

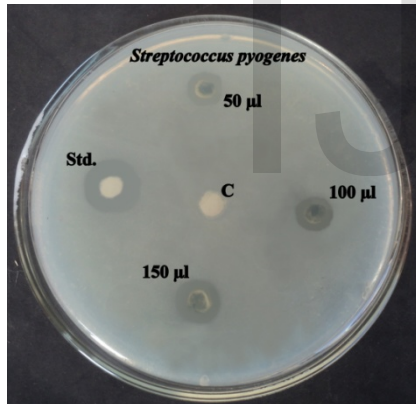
#### *Escherichia coli*



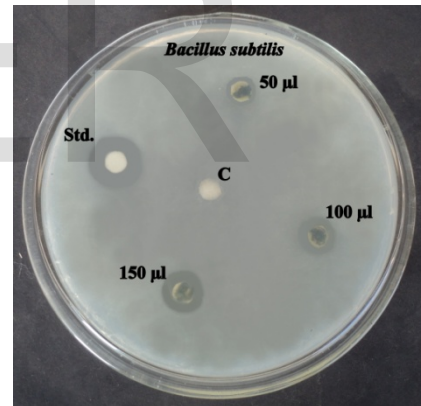
#### *Staphylococcus aureus*



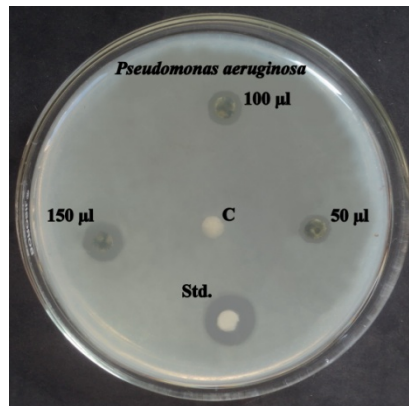
#### *Streptococcus pyogenes*



#### *Bacillus subtilis*



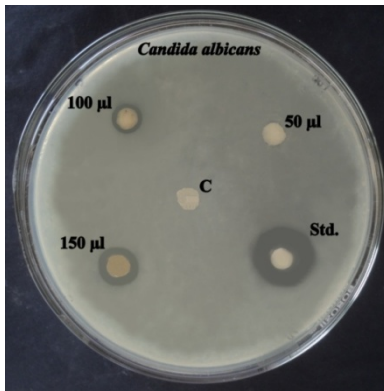
#### *Pseudomonas aeruginosa*



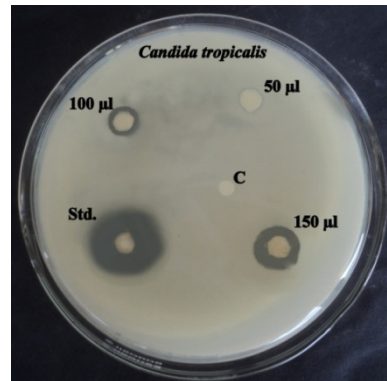


### 3.4. Antifungal assay

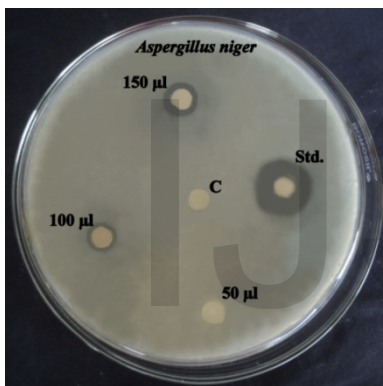
*Candida albicans*



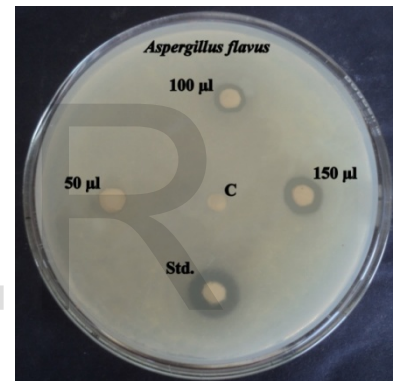
*Candida tropicalis*



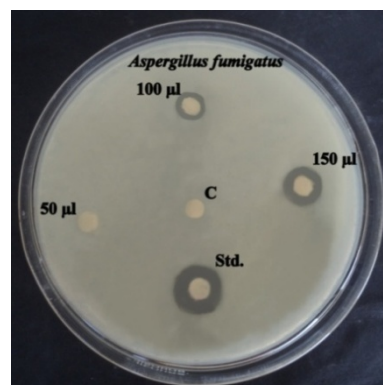
*Aspergillus niger*



*Aspergillus flavus*



*Aspergillus fumigatus*



#### 4. Conclusion

The threat of bioterrorism with multi-drug resistant pathogens emphasized the need for continued development of new antibiotics. Currently, the ongoing battle against bacteria and fungi prevails certainty of evolving resistance. Plants may be an important source of potentially useful structures for the development of new chemotherapeutic agents<sup>[8]</sup>. The first step towards this goal is the *in vitro* analysis of plant extracts for their biological activity. The present study, stem extract of *Andrographis paniculata* have tested against clinical pathogens. The number of bacterial and fungal strains was determined in accordance with their cell wall structure. Based on this analysis, out of the five bacterial and five fungal pathogens against chloroform stem extract of *Andrographis paniculata* exhibited the highest antibacterial activity against *E.coli* and highest antifungal activity against *Candida tropicalis*. The present study opens a new era in correlating the Ayurveda and Siddha with modern microbiology. The promising result obtained in this study may lead to the development of a potential antibiotic from the stem extract of *Andrographis paniculata* against bacteria and fungi. Further it also encourages the young researchers to test other medicinal plants for their biological activities.

#### 5. References

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