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## Phytochemical screening and antimicrobial activity of ethanolic extract of *Andrographis paniculata*

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### Abstract

Medicines of plant origin have been used to combat human diseases since time immemorial even before the history of their documentation. *Andrographis paniculata* belongs to the Acanthaceae family and is popularly known as king of bitters in English. The leaf extracts of *A. Paniculata* were analysed for the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside. Antibiogram was done by disc diffusion method using plant extracts. The antimicrobial potential of ethanolic extract of *Andrographis Paniculata* was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The ethanolic extract of leaves exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* and antifungal activity against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*.

**Keywords:** *Andrographis paniculata*, Phytochemicals, Antibacterial activity, Antibiogram

### 1. Introduction

Medicines of plant origin have been used to combat human diseases since time immemorial even before the history of their documentation. Medicines are regarded actually the first awareness about plants after food and shelter. As far as India is concerned documentation of knowledge about the healing virtues of plants can be traced back to the vedic period (1000-5000 B.C). The Rigvedha and Atharvedha are sacred monumental works of India which enlightened Medicinal values of plants [1]. According to world health organization plant derived drugs constitute the mainstay of nearly 80% of the population for their Primary Health Care. Presently plant based drugs are being used worldwide. These are either used directly extracted from plants or modified through further synthesis (cox and Ballick, 1994). These specific chemicals belong to plant derived compounds called phytochemicals such as the metabolites of primary and secondary metabolites. Primary metabolites are high volume chemicals of low value. Secondary metabolites are biosynthetically derived from primary metabolites in lower volume and have higher value compounds. The secondary metabolites are much of interest because of their antimicrobial. Antibiotic, insecticidal and hormonal properties [2].

*Andrographis paniculata* belongs to the Acanthaceae family and is popularly known as king of bitters in English. *Andrographis paniculata* grows erect to a height of 30– 110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance shaped leaves have hairless blades mea ruing up to 8 centimeters long by 2.5 wide. The small flowers are borne in spreading racemes. The fruit is a capsule around 2 centimeters long and a few millimeters wide. It contains many yellow brown seeds. It is also known as *Bhui-neem*, meaning “neem of the ground”, since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree. It is an annual herbaceous plant which is commonly cultivated in Southern Asia, in China, and in some parts of South East Asia. *A. paniculata* has antimicrobial [3-5] and anti-snake-bite activities [6]. Diterpenoids and flavonoids are the main antimicrobial constituents of *A. paniculata* [7]. It is used to overcome *sannipata* type of fever, difficulty in breathing, hemopathy burning sensation, cough, skin diseases, fever, ulcer and worms. It is also useful in acidity and liver complaints [8].

The present study was aimed to investigate the phytochemical constituents and antibacterial effects of *A. paniculata* against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* specie of bacteria and antifungal effects of *A. Paniculata* against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* specie of fungi.

## 2. Materials and Methods

### Collection of plant material

The fresh leaves of the plant *Andrographis Paniculata* were collected during the month of October 2015 from the area of Trichy. The fresh leaves of that plant were taxonomically identified from the Rabinet Herbarium (RHT) of St. Joseph College, Trichy.

### Extraction of plant material

Healthy plant leaves were collected, washed thoroughly in tap water and dried in room temperature for 30 days. The dried leaves were powdered and 25 g leaf powder soaked separately in 225 ml of ethanol for 3 days. The extracts were filtered through whatman No.1 filter paper. Similar process was repeated twice with fresh solvent and the filtrate was collected together. The extract was stored at the refrigerator for further studies.

### Screening of phytochemical components

The leaf extracts of *A.Paniculata* were analysed for the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside according to the common phytochemical methods described by Harborne (1998).

### Microorganisms

Pure culture of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* specie of bacteria and *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* 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.

### Antimicrobial assay

Antibiogram was done by disc diffusion method [8, 9] using plant extracts. Petri plates were prepared by pouring 30 ml of

NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mins. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* was spread on potato dextrose agar 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 plate. The plates were incubated at 37 °C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

### 3. Results and discussion

Phytochemical screening of the ethanolic extracts of *A.Paniculata* revealed the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside (Table 1). Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, Alkaloids protect against chronic disease. Saponins protect against hypercholesterolemia and antibiotic properties [10]. Steroids and triterpenoids show the analgesic properties. The Steroids and saponins were responsible for central nervous system activities [11], flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [12].

**Table 1:** The analysis of phytochemicals in the ethanolic extracts of *Andrographis paniculata*

S. No.	Phytochemical Tests	Ethanol
1.	Alkaloids ( Mayer's reagent)	+
2.	Carbohydrates & Glycosides ( molisch's test)	+
3.	Sugar (Benedict's reagent)	+
4.	Saponin ( Foam test)	+
5.	Protein ( Millon's test)	+
6.	Phytosterol (Liebermann Burchard's test)	+
7.	Phenolic compounds and tannin ( Ferric chloride test)	+
8.	Flavonoid (Alkaline reagent test)	+
9.	Glycoside (Legal's test)	+

(+) – Present, (-) – Absent

The antimicrobial potential of ethanolic extract of *Andrographis Paniculata* was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale. The ethanolic extract of leaves exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*

and antifungal activity against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. (Table 2 and 3). Among these three bacteria, ethanolic extract has exhibit more antimicrobial activity against *Escherichia coli* than other two bacteria and also exhibit more fungal activity against *Candida albicans* than other two fungai.

**Table 2:** Antibacterial activity of ethanolic extracts of *Andrographis paniculata*

Microorganisms	50 µl	100 µl	150 µl	Standard (Chloramphenicol for bacteria)	Control (solvent)
<i>Escherichia coli</i> (mm)	2.74±0.19	3.87±0.27	5.67±0.39	9.57±0.65	0
<i>Staphylococcus aureus</i> (mm)	1.49±0.10	1.92±0.13	3.14±0.21	8.76±0.61	0
<i>Bacillus subtilis</i> (mm)	1.25±0.08	1.83±0.12	3.46±0.24	8.94±0.62	0

Values were expressed as Mean ± SD

**Table 3:** Antifungal activity of ethanolic extracts of *Andrographis paniculata*

Microorganisms	50 $\mu$ l	100 $\mu$ l	150 $\mu$ l	Standard (fluconazole for fungal)	Control (solvent)
<i>Candida albicans</i> (mm)	1.21 $\pm$ 0.08	2.34 $\pm$ 0.16	3.86 $\pm$ 0.27	8.74 $\pm$ 0.61	0
<i>Aspergillus niger</i> (mm)	1.84 $\pm$ 0.12	2.68 $\pm$ 0.18	3.67 $\pm$ 0.25	9.46 $\pm$ 0.65	0
<i>Aspergillus flavus</i> (mm)	1.18 $\pm$ 0.07	2.17 $\pm$ 0.15	3.21 $\pm$ 0.22	8.64 $\pm$ 0.60	0

Values were expressed as Mean $\pm$ SD

#### 4. Conclusion

*Andrographis paniculata* extract have great potential as antimicrobial compounds against bacteria and fungi. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. Based on the results we concluded that ethanolic extract of *Andrographis paniculata* has exhibit antimicrobial property. Plant tissues contain secondary metabolites with antibacterial and antifungal activities and thus are sources of natural bioactive molecules to control pathogens that cause disease in humans.

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