

# *In vitro* antibacterial activity in the extracts of *Andrographis paniculata* Burm. F

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**Abstract:** *Andrographis paniculata* Burm.F(Acanthaceae) is a potent medicinal plant in the Indian systems of medicine. Traditionally the leaves are used as/in influenza, bronchitis, gonorrhoea, cholera, fertility and antibacterial, anticancer, antidiabetic, anti-inflammatory and anti snake venom etc. In the present study the aqueous and methanol extracts of the leaves, stem, root and whole plant of *Andrographis paniculata* were studied for their antibacterial activity by Agar-well diffusion method against different gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*) bacteria. It was observed that, methanol extracts of whole plant and leaves showed the significant antibacterial activity against Gram-positive bacteria. No bacterial activity was recorded with aqueous extracts of stem and root.

**Key words:** *Andrographis paniculata*, Agar-well diffusion method, Methanol extract, Antibacterial activity

## Introduction

Infectious diseases are the number one among all causes of death, accounting approximately one-half all deaths throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases<sup>1</sup>. These numbers are still increasing due to development of resistance in microorganisms to the existing first line drugs. Scientists from divergent fields are investigating plants with a new eye for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity<sup>2</sup>. There are several reports on the presence of antimicrobial compounds in various plants<sup>3, 4,5,6,7</sup> but there are only few reports on antimicrobial property on *Andrographis paniculata*<sup>3,4,8,9</sup>.

*Andrographis paniculata* is a herbaceous plant belongs to family Acanthaceae, native to India and Sri Lanka. Mostly the leaves and roots were used for medicinal purposes. *Andrographis paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. The plant extract exhibits antityphoid, antifungal, antifertility and antinematocidal activities. It is also reported to possess antihepatitic, antithrombogenic, anti-inflammatory, anti snake venom, antipyretic and

anticancer properties etc. The primary medicinal component of *Andrographis* is andrographolide, which is a 'diterpene lactone' water soluble substance and is distributed all over the plant body in different proportions. Recent research has thrown light on cultivated of this plant on large because of its high medicinal value. Hence, the present investigation was taken up with an objective to evaluate the antibacterial potential against the microorganisms.

## Materials and Methods

### Collection of plant material:

The different parts of *Andrographis paniculata* Burm.F such as leaf, root and stem were collected from the field grown plants in Botanical garden, Andhra University, Visakhapatnam, Andhra Pradesh and were collected for the following experiments. The plant materials were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in airtight container. The powder obtained was subjected to successive Soxhlet extraction with the organic solvents with increasing order.

### Test microorganisms:

Five bacterial cultures namely *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were used in this investigation. All the cultures were procured from

Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

#### Media and Inoculum preparation:

The media used for antibacterial test were Nutrient Agar/Broth and Muller Hinton agar. All the media were obtained from Himedia Pvt Ltd, Mumbai, India. The test bacterial strains were inoculated into nutrient broth and incubated at 37°C for 24hrs. After the incubation period, the culture tubes were compared with the turbidity (opacity) standard.

#### Antibacterial assay:

Bioassay was carried out by Agar well diffusion method<sup>10, 11, 12</sup>. Fresh bacterial culture of 0.1ml having 10<sup>8</sup> CFU was spread on nutrient agar plate with glass spreader. A well of 6mm diameter was punched off into agar medium with sterile cork borer and filled with 50µl of aqueous and methanol extracts by using micro pipette in each well in aseptic condition. Plates were then kept in a refrigerator to allow pre-diffusion of extract for 30minutes and further incubated in a incubator at 37°C for 24hrs. The antibacterial activity was evaluated by measuring the zone of inhibition. The experiment was done in triplicate and the mean diameter of the inhibition zone was calculated. Antibiotic Tetracycline at a concentration of 30 µg/ml as positive control and 100% DMSO (Dimethyl sulphoxide) as a negative control were used.

#### Results and Discussion

The antibacterial activity of methanol and water extracts of different parts of *Andrographis paniculata* Burm.F were observed using Agar well diffusion method by measuring the diameter of the growth inhibition zone. The results are depicted in Table-1. In total of 8 extracts belonging to different plant parts of *Andrographis paniculata* Burm.F were tested in the present investigation. The aqueous extract of stem and root exhibited no bacterial activity whereas, the methanol extracts of leaf, stem, root and whole plant showed antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) as well as Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*) respectively. In case of whole plant, methanol extracts showed significant and highest antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. Whereas moderate degree of activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*. On other hand water extract of the same showed moderate activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus vulgaris*. No bacterial activity was recorded against *Klebsiella pneumoniae* and *Escherichia coli*. The significant and higher antibacterial activity of the methanol extracts of whole

plant of *Andrographis paniculata* is probably due to the presence of the andrographolids (bicyclic diterpenoid lactone) and other chemical constituents (Table-1).

The leaf extracts of methanol showed a positive significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. While, moderate activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*. Whereas the water extract of the same exhibited moderate degree of activity against *Staphylococcus aureus* and *Bacillus subtilis* but low activity against *Escherichia coli*. No bacterial activity was found against *Klebsiella pneumoniae* and *Proteus vulgaris*. This may be due to the presence of bicyclic diterpenoid lactone (andrographolide), deoxyandrographolide, which possess antibacterial, antifungal, and anti-insect activities. Further, the presence of antibacterial activity in leaf extracts also implies that there would be possibilities of substituting leaves for roots and stem which utilizing this plant species for bacterial related infections. However, use of leaves for medicinal purposes is more sustainable compared to harvesting of plant parts such as stem and roots. Similarly, methanolic extracts of stem and roots exhibited moderate degree of bacterial activity against all the five tested bacteria. The methanolic extract showed considerably more bacterial activity than the water extract. This is interesting in that the traditional method of treating a bacterial infection was by administering a decoction of the plant or a part thereof by boiling it in water, whereas according to our results an organic solvent is better, hence this may be more beneficial. Among of the five bacterial strains investigated *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris* were most resistant. It is also evident that Gram-negative bacteria were more resistant than Gram-positive bacteria (Table-1). The standard drug Tetracycline (30µg/ml) showed high degree of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*.

From the above results it can be concluded that plant extracts have great potential as antibacterial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. *Andrographis paniculata* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hitherto unmet therapeutic needs. However, further investigation on isolation and characterization of the active principle(s) of the plant extracts responsible for the antibacterial activity is necessary and it would give a comprehensive evidence of bioactive potential of medicinal plants.

**Table-1: Antibacterial activity of different parts of *Andrographis paniculata* extracts**

Plant part	Plant extracts	Zone of inhibition (mm)				
		<i>E.coli</i>	<i>K.pneumoniae</i>	<i>P.vulgaris</i>	<i>B.subtilis</i>	<i>S.aureus</i>
Leaves	Aqueous	9	-	-	12	13
	Methanol	17	15	12	21	22
Stem	Aqueous	-	-	-	-	-
	Methanol	15	13	10	18	19
Root	Aqueous	-	-	-	-	-
	Methanol	9	8	7	10	12
Whole plant	Aqueous	-	-	11	12	14
	Methanol	19	16	18	22	23
Tetracycline (30µg/ml)		21	19	20	24	26

Mean of three replicates

- : No inhibition

Concentration used 750mg/ml

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