



## EVALUATION OF ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF 'ANDROGRAPHIS ECHIOIDES' – AN *IN VITRO* STUDY

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### ABSTRACT

**Background and Objectives:** *Andrographis echioides* is a unique medicinal plant included in Indian material medical as a remedy for fevers, malaria, respiratory infections and various ailments. This study is conducted to evaluate the antibacterial activity of the ethanolic extract of the whole plant "*Andrographis echioides*" using standard disc diffusion method. Whole plant of *A.echioides* was subjected to soxhlet extraction using organic solvent of ethanol. The antibacterial activity of *A.echioides* extract was done using standard disc diffusion method using Muller Hinton agar (MHA) against ten strains of bacteria (both two gram positive and eight gram negative strains). Ampicillin is the standard positive control and Negative control was DMSO. 20µl of varying concentrations (1000 µg, 750µg, 500 µg, and 250µg) of the extract sample were tested. The antibacterial effect was determined by measuring the diameter of the zone of inhibition. The ethanolic whole plant extract showed the maximum antibacterial activity against gram negative bacterial strains like *Salmonella typhi* (20mm), *klebsiella pneumonia* (14mm) and *Vibrio parahaemolyticus* (15mm) as measured by the zone of inhibition produced by the plant extract when compared with the control drug Ampicillin. The antibacterial activity of this plant extract was least significant against gram positive bacteria like *Bacillus subtilis* (9mm) and *Staphylococcus aureus* (9mm). There was moderate inhibitory activity on *Aeromonas hydrophilia* (10mm), *E.coli* (10mm). In our study the ethanolic extract of *Andrographis echioides* has shown to have good antibacterial activity against gram negative bacteria in high concentration. In comparison to the positive control drug Ampicillin the antibacterial activity of this plant extract is not very significant against gram positive bacteria.

**Key words:** *Andrographis echioides*, Antibacterial effect, Zone of inhibition.

### INTRODUCTION

In the last century we saw the discovery of wonder drugs called antibiotics from microorganisms. There is also a constant need for newer antimicrobial agents because of their rampant overuse and misuse leading to emergence of drug resistance (Nascimento *et al.*, 2000).

Plants are one of the alternative sources of a large number of drugs and phytochemicals used to treat infections in allopathy, ayurvedha and siddha. Plant derived drug research if proved safe and effective through proper methodology have more likely chance of being accepted by the physicians and people than new chemical entities. *Andrographis Echioides* (Indoneesiella echioides) is an erect, annual medicinal plant of Acanthaceae family. It is widely found in Tamilnadu, Kerala and Srilanka. This plant has been listed in Indian Materia Medica as remedy for many illnesses particularly, to reduce fever, Malaria, respiratory infections, liver

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diseases and goiter (Munuswamy Hemalatha *et al.*, 2013). The whole plant extract is applied topically over fungal infections, to control hair fall, snake bite, cuts and wounds (Anonymous, 1990).

Medicinal properties of this plant are more or less similar to those of *Andrographis paniculata* a widely studied plant for wide range of pharmacological activities (Kokate, 1994). In a study done to analyze the phytochemistry of *Andrographis echinoides*, the major constituents of the plant were flavonoids rather than the crystalline bitter principle analogous to diterpenoids as in other *Andrographis* species (Pandi Kumar *et al.*, 2007). Disc diffusion method is one of the reliable, commonly used in vitro screening methods to evaluate the antibacterial activity of various drugs and phytochemicals (Bauer *et al.*, 1996).

As *Andrographis Echinoides* is commonly used and unique in treating wide range of diseases and infections in traditional medicine, it is important for us to prove the untapped antibacterial activity of this plant scientifically. The aim of this study is to evaluate the antibacterial activity of the ethanolic extract of the whole plant of '*Andrographis echinoides*' against selected common disease causing gram positive and gram negative microorganisms using the in vitro standard disc diffusion method.

#### Plant Materials:

The whole plants of *Andrographis Echinoides* were collected from West Tambaram, Chennai in December 2013. The plant identity was confirmed by Prof. Sasikala, Research officer and botanist and a specimen voucher (dated. 17.01.2014) was deposited in the pharmacognosy museum of Siddha Institute of Research, Chennai.

#### Extraction:

Whole plant sample were taken, cut into pieces, air dried and powdered. 25 gram of powdered sample were taken and extracted with 300ml of ethanol in soxhlet apparatus for 12 hours of time. The crude extract was filtered and the solvents were further condensed using rotary evaporator. The crude extract was stored at room temperature in airtight container for further analysis. A portion of the extract is used for antibacterial evaluation.

#### Bacterial culture & Preparation of inoculum:

The bacterial strains used in the study are clinical isolates purchased from MTCC, Chandigarh. The isolates were identified by a standard method (Cowan and Steel, 1993). Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loopful of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 24hrs at 37°C.

#### Bacterial susceptibility testing:

In vitro Antibacterial activity of extract was determined against two specimens of gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and eight gram negative bacteria specimens (*E. coli*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Salmonella*, *Aeromonas*, *Klebsiella*, *Proteus*) by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The discs were placed in MHA plates and 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc. The plates were incubated at 37°C for 24 hrs. Pure DMSO was taken as a negative control. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition. The diameter of the zone of inhibition produced by each agent were measured with a transparent ruler in millimeter and compared with those produced by the commercial antibiotic Ampicillin (20µl/disc) served as a positive control.

#### RESULTS

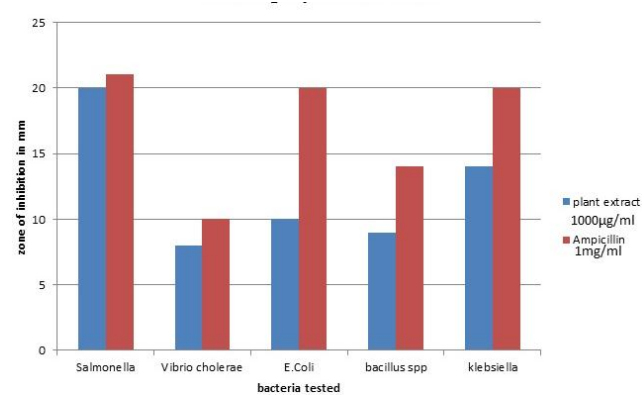
The ethanolic whole plant extract showed the maximum antibacterial activity against gram negative bacterial strains like *Salmonella typhi* (20mm), *Klebsiella pneumoniae* (14mm) and *Vibrio parahaemolyticus* (15mm) as measured by the zone of inhibition produced by the plant extract when compared with the control drug Ampicillin. The antibacterial activity of this plant extract was least significant against gram positive bacteria like *Bacillus subtilis* (9mm) and *Staphylococcus aureus* (9mm). There was moderate inhibitory activity on *Aeromonas hydrophilia* (10mm), *E. coli* (10mm).

**Table 1. Zone of inhibition of bacterial colonies by different concentrations of *Andrographis Echinoides* using disc diffusion method**

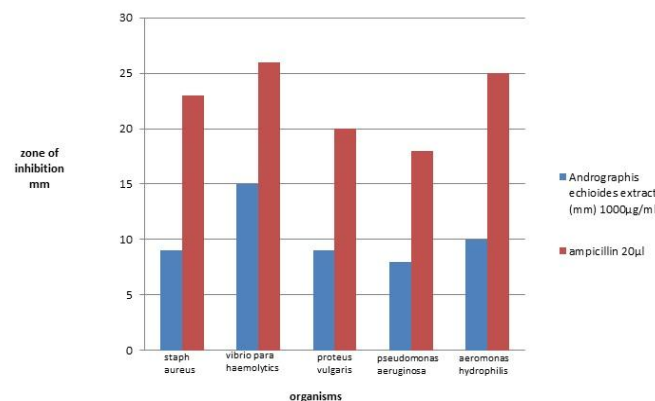
ORGANISMS	MTCC No.	Zone of Inhibition (mm)			Antibiotic (1mg/ml)	DMSO (20µl)
		Concentration(µg/ml)				
		1000	750	500		
E.coli	9537	10mm	8mm	5 mm	20mm	-
Vibrio cholera	3906	8mm	5 mm	3 mm	10 mm	-
Staphylococcus aureus	7443	9mm	6mm	4 mm	23mm	-
Pseudomonas aeruginosa	3542	8mm	6mm	4 mm	18mm	-
Bacillus subtilis	2057	9mm	6 mm	4 mm	14 mm	-

Vibrio parahaemolyticus	451	15mm	9mm	6 mm	26mm	-
Salmonella typhi	3216	20 mm	10 mm	8 mm	21mm	-
Aeromonas hydrophilia	1739	10 mm	7 mm	5 mm	25 mm	-
Klebsiella pneumoniae	4032	14mm	12 mm	8 mm	20 mm	-
Proteus vulgaris	7299	9 mm	6 mm	4 mm	20 mm	-

**Figure 1. Graph showing the highest antibacterial activity of ethanolic extract of *Andrographis echiodes* extract**



**Figure 2. Graph showing moderate to least antibacterial activity of ethanolic extract of *andrographis echiodes* in bacteria**



**Salmonella typhi**



**Aeromonas hydrophilia**



**Klebsiella pneumoniae**



**Vibrio Parahaemolyticus**



**Proteus vulgaris**



**E. coli**



## DISCUSSION

The presence of flavonoids, tannins, terpenoids, gums, cardiac glycosides and phytosteroids in *Andrographis echiodes* have proven antioxidant, cytoprotective and anti-inflammatory properties (Xie *et al.*, 1993). These phytochemicals have free radical scavenging and prevent lipid peroxidation of cell membranes (Cuvelier *et al.*, 1992). A study on analgesic,

anti-inflammatory and antipyretic effect of *Andrographis echiodes* have reported its efficacy is similar to paracetamol and phenylbutazone and the activity is attributed to the presence of flavonoids (Basu *et al.*, 2009). Studies have proven the *Andrographis* species have nitric oxide inhibition activity so that this plant can be valuable source for anti-inflammatory drugs (Shen *et*

*al.*, 2013). A study done by Qadrie *et al.*, have also shown *Andrographis echinoides* possess good hepatoprotective activity with antioxidant effect and it may be due to the presence of flavonoids (Qadrie *et al.*, 2011). Ganesan *et al.* have reported in their research about the antibacterial activity of hydroalcoholic extract of *Andrographis Echinoides* against both gram positive bacteria like corynebacterium, staphylococcus and gram negative bacteria like salmonella typhi, klebsiella etc (Kanchana *et al.*, 2014).

In our study, the ethanolic extract of *Andrographis echinoides* has shown various degrees of inhibitory activity on all the bacterial strains used. The plant extract as found to have a highest inhibitory activity against gram negative bacteria in high concentration of 1000 µg/ml and moderate inhibitory activity with 500 µg/ml. The maximum antibacterial activity as shown in Fig 1 was seen against gram negative bacterial strains like *Salmonella typhi*, *klebsiella pneumoniae* and *Vibrio parahaemolyticus* as measured by the zone of inhibition produced by the plant extract when compared with the control drug Ampicillin. The antibacterial activity of this plant extract was least significant against gram positive bacteria and had minimal inhibitory activity against bacillus subtilis and staphylococcus aureus as shown in Table 1. There was moderate inhibitory activity on *Aeromonas hydrophilia*, *E.coli* and *Proteus vulgaris*. The presence of Flavanoids, tannins and phytosteroids in the

ethanolic extract of our preparation can be responsible for the antibacterial action against gram negative organisms. A lot of factors like the binding capacity, chelation of iron and proteins of the bacterial cell membranes are suggested antibacterial mechanisms of the phytochemicals like tannins (Premkumar *et al.*, 2010). Further studies are needed evaluate the exact mechanism of action and safety profile of *Andrographis echinoides* plant. The results of this study can be tested further with phytochemicals extracted in pure form from this plant and tested further against gram negative infections especially urinary tract infection, gastroenteritis using animal models.

## CONCLUSION

The ethanolic extract of *Andrographis echinoides* which is commonly used in fevers and infections in traditional medicine was found to have antibacterial activity against both gram positive and gram negative bacteria and has a significant antibacterial against *Salmonella typhi*, *Vibrio parahemolyticus* and *klebsiella pneumonia* and least antibacterial activity against gram positive bacteria like *bacillus subtilis* and *staphylococcus aureus*.

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## CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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