

Antimicrobial Properties of Various Extracts of *Andrographis neesiana* Wight - An Endemic Medicinal Species from India

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Abstract : In the present study deals with the antimicrobial properties of the *Andrographis neesiana* were tested against five human bacterial pathogens by using five various solvent namely, ethanol, methanol, acetone, chloroform and petroleum ether. The maximum antimicrobial activity was noted in petroleum ether extracts of stem showed activity against *Escherichia coli* (16mm) and the minimum was recorded in ethanol stem extracts against *Staphylococcus aureus* (2mm), *Klebsiella pneumonia* (2mm) and *Proteus vulgaris* (3 mm). The maximum antimicrobial activity recorded in chloroform leaves extracts against *Klebsiella pneumonia* (12mm). The minimum activity was noted in acetone leaves extracts against *Staphylococcus aureus* (2mm) and *Pseudomonas aeruginosa* (3mm). The stem extract showed more inhibitory effect than the leaf extracts. The results of our antimicrobial assay revealed that plant extracts showed inhibitory activity against the tested organisms.

Key words: *Andrographis neesiana*, Antimicrobial activity, Medicinal plant, Plant extracts, Agar diffusion method.

Introduction

Microorganisms and medicinal plants are rich sources of secondary metabolites which are potential sources of useful drugs and other useful bioactive products¹. Medicinal plants are an important medicinal aid for various diseases. Scientific experiments on the antimicrobial properties of plant components were first documented in the 19th century². In India from ancient times, different parts of medicinal plants have been used to cure specific diseases. Today, there is widespread interest in drugs derived from plants. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms³.

Microorganisms are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. Plants are used as medicines since time immemorial. India has rich

heritage of using medicinal plants in traditional medicines such as Siddha, Ayurveda and Unani besides folklore practices. Antibacterial properties of various plants parts like leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades⁴. Antibiotic principles are distributed widely among angiospermic plants. A variety of compounds are accumulated in plant parts accounting for their constitutive antimicrobial activities⁵.

Antimicrobial drugs are used in medicinal practices for treating food - borne diseases⁶. Use of medicinal plant extract which are rich in antibacterial compounds could be an alternate way to eliminate these bacteria from palatable items^{7,8,9} which has already been proved *in vitro* by Kaushik and Dhiman¹⁰ and Kaushik¹¹. Over the last 25 years a large number

of plant species have been evaluated for their antimicrobial activity. So in the present study is an attempt to evaluate the antimicrobial activities of the leaves and stem of *Andrographis neesiana* against selected pathogenic bacteria. Many workers throughout the world doing investigation on potential applications on medicinal plants like *Nerium oleander*¹², *Adhatoda vasica* and *Plumbago zeylanica* and many more. Surprisingly it was found effective against many pathogenic microorganisms. Hence, they can be used for the treatment and cure the various diseases.

Species of *Andrographis* Wallich ex Nees (Acanthaceae) are used in the Indian systems of medicine namely Siddha, Ayurvedha and Unani¹³. The genus exhibits antipyretic properties¹⁴. This genus consists of 40 species distributed in Tropical Asia¹⁵. About 21 species are distributed in India¹⁶ and all of them available in Tamilnadu¹⁷. Among the 21 species 18 species are reported to be endemic to India¹⁸. *Andrographis neesiana* Wight (Acanthaceae) is an endemic medicinal herb¹⁹ found in wild in Kotagiri of Nilgiri District, Tamil Nadu. The Nilgiris, the so called blue mountains, is one of the richest hill district with its plant diversity, culture and ethnic groups of Tamil Nadu in Southern Peninsular India. It lies between 11°12' and 11°43' N and 76°14' to 77°1'E.

Since there is no earlier report on antimicrobial activity of leaves and stem of *Andrographis neesiana* against these five microorganisms, an attempt was made to evaluate the antimicrobial activity of ethanol, methanol, acetone, chloroform and petroleum ether extract of the plant by agar diffusion method using *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* as test microorganism. So in the present study is an attempt to evaluate the antimicrobial activities of the leaves and stem of *Andrographis neesiana* against selected pathogenic bacteria.

Material and Methods

Collection of Plant material

Andrographis neesiana Wight was collected in October month 2009 from Kottagiri (Western Ghats) of Nilgiri district of Tamilnadu. The plant were taxonomically identified and confirmed with the Flora of Tamilnadu and voucher specimen (No.17/8.10.09CA) deposited in Department of Botany, Government Arts College (Autonomous), Salem for the future reference.

Preparation of plant extracts

The fresh plant samples (leaves and stem) collected were washed individually under running water to remove soil particles and other dirt. The leaves were air dried in the laboratory at room temperature ($30 \pm 2^\circ\text{C}$) for 15 days. While the stem samples were dried at 60°C for 2 days in an oven. The dried leaves, stem samples were ground well into a fine powder in a mixer grinder. The powder was stored in air sealed polythen bags at room temperature before extraction.

The method of Alade and Irobi¹⁹ was adopted for preparation of plant extracts. A fixed weight (20 gm) of powdered plant material was soaked separately in 50 ml of distilled water acetone and petroleum ether for 72 hours. Each mixture was stirred at 24 hours interval using a sterile glass rod. At the end of extraction each extract was passed through Whatman No.1 filter paper (Whatman, England), the filtrate obtained was concentrated in vacuum using rotator evaporator. Then the extracts were used for antimicrobial activity.

Bacterial strains used for Assay

Staphylococcus aureus, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Microbial strains were obtained from the Calicut Medical College, Kerala, India.

Antimicrobial assay

The media and the test bacterial cultures were poured into dishes (Muller -Hinton agar media). The test strain (0.2ml) was inoculum size (108 cells/ml) when the temperature reached $40-42^\circ\text{C}$. Care was taken to ensure proper homogenization. The plant extracts were tested for antimicrobial activity in the agar well diffusion assay²⁰ against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Agar well diffusion method:

The antimicrobial activity was tested against (Methanol, Ethanol, Acetone, Chloroform and Petroleum ether) leaves and stem of *Andrographis neesiana*. The inoculation of microorganism was prepared from bacterial culture²¹. About 15-20 ml of Muller - Hinton agar medium was poured in the sterilized petridish and allows solidifying. One drop of bacterial strains was spread over the medium by a rod. Wells of 6mm in diameter and about 2cm apart punctured in the culture medium using sterile cork borers. About 100 ml of plant extracts was added to the wells. Plates were incubated in air at 37°C for 24 hours. Antimicrobial activities were evaluated by measuring inhibition zone diameters.

Results and Discussion

The results antimicrobial activity are given in the Table 1 and 2. The in vitro antimicrobial activity of ethanol, methanol, acetone, chloroform and petroleum ether extracts of *Andrographis neesiana* leaf showed in Table 1. The antimicrobial activity of methanol, ethanol, acetone, chloroform and petroleum ether extracts of stem showed in Table 2.

Antimicrobial activity of the leaf extracts

The investigation made on chloroform extracts maximum activity against *Klebsiella pneumoniae* (12 mm) and *Proteus vulgaris* (7mm) and the minimum activity against *Escherichia coli* (5mm), *Staphylococcus aureus* (5mm) and *Pseudomonas aeruginosa* (4mm). The extracts using petroleum ether showed maximum activity against *Staphylococcus aureus* (8mm) and *Pseudomonas aeruginosa* (8mm) and the minimum activity against pathogen like

Proteus vulgaris (4mm) and *Escherichia coli* (4mm). Where as no activity pathogen like *Klebsiella pneumoniae*. Methanol extract pointed out maximum activity against pathogens *Staphylococcus aureus* (9mm) and *Klebsiella pneumoniae* (8mm) and the minimum activity against *Proteus vulgaris* (5mm), *Escherichia coli* (4mm) and *Pseudomonas aeruginosa* (4mm). The ethanol extract observed maximum activity against *Klebsiella pneumoniae* (7mm) and *Pseudomonas aeruginosa* (6mm). Showed the minimum activity against pathogen like *Proteus vulgaris* (4mm) and *Escherichia coli* (4mm). Showed no activity against pathogen like *Staphylococcus aureus*. Observation made from acetone extract showed a maximum activity against *Proteus vulgaris* (8mm) and *Escherichia coli* (6mm) and the minimum activity against *Staphylococcus aureus* (2mm). It has no activity against pathogen like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Table 1: Shows the antimicrobial activity of the leaf extracts of *Andrographis neesiana* against human pathogens.

<u>Solvents used zone of inhibition mm in diameter</u>						
S.No.	Microorganisms	Ethanol	Methanol	Acetone	Chloroform	Petroleum ether
1.	<i>Staphylococcus aureus</i>	NA	9	2	5	8
2.	<i>Proteus vulgaris</i>	4	5	8	7	4
3.	<i>Escherichia coli</i>	4	4	6	5	4
4.	<i>Pseudomonas aeruginosa</i>	6	4	NA	4	8
5.	<i>Klebsiella pneumoniae</i>	7	8	NA	12	NA

NA - No Activity

Table 2: Shows the antimicrobial activity of the stem extracts of *Andrographis neesiana* against human pathogens.

<u>Solvents used zone of inhibition mm in diameter</u>						
S.No.	Microorganisms	Ethanol	Methanol	Acetone	Chloroform	Petroleum ether
1.	<i>Staphylococcus aureus</i>	NA	3	4	11	6
2.	<i>Proteus vulgaris</i>	3	NA	6	NA	5
3.	<i>Escherichia coli</i>	10	NA	8	4	16
4.	<i>Pseudomonas aeruginosa</i>	5	6	11	8	5
5.	<i>Klebsiella pneumoniae</i>	NA	7	9	6	5

NA - No Activity

Antimicrobial activity of the stem extracts

The petroleum ether extract observed the maximum activity against pathogen like *Escherichia coli* (16 mm) and *Staphylococcus aureus* (6mm) and the minimum activity against *Proteus vulgaris* (5mm), *Pseudomonas aeruginosa* (5mm) and *Klebsiella pneumoniae*(5mm). Chloroform extract obtained maximum activity against *Staphylococcus aureus* (11mm) and *Pseudomonas aeruginosa* (8mm) minimum activity against *Klebsiella pneumonia* (6mm) and *Escherichia coli* (4mm). Showed no activity against pathogen like *Proteus vulgaris*. The extract obtained using acetone showed a maximum activity against pathogen like *Pseudomonas aeruginosa* (11mm), *Klebsiella pneumoniae* (9mm) and *Escherichia coli* (8mm) and the minimum activity against *Proteus vulgaris* (6mm) and *Staphylococcus aureus* (4mm). The extraction by way of methanol observed maximum activity against *Klebsiella pneumoniae* (7mm) and *Pseudomonas aeruginosa* (3mm). Where as no activity pathogen like *Proteus vulgaris* and *Escherichia coli*. Ethanol extract obtained maximum activity against *Escherichia coli* (10mm) and *Pseudomonas aeruginosa* (5mm) and the minimum activity against *Proteus vulgaris* (3mm). Where it has no activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Suresh *et al.*,²² reported the better antimicrobial activity of ethanol extract obtained from *Rauvolfia tetraphylla* showed maximum activity against *Alcaligenes faecalis*, *Escherichia coli*, *Enterobacter aerogenes* and various fungi tested *Aspergillus niger* and *Penicillium spp* were found to be

more sensitive to crude extract when compared to others. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents such as tannins²³, flavanoids²⁴, terpenoids²⁵, Phenolics and Polyphenois²⁶ are effective antimicrobial substances against a wide range of microorganisms.

The findings of the present investigation clearly indicate that the antimicrobial activity vary with the plant material used. These results suggest that the leaf and stem extracts possess compounds with antimicrobial activity. These plants could serve as useful sources for new antimicrobial agents. Apart from this study, there are no reports of antimicrobial studies on *A. neesiana*. Present investigation is teh first experimental demonstration of any biological activity as well as antimicrobial activity of *A. neesiana*. It is also first report of antimicrobial potential of leaf and stem of *A. neesiana*. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

Conclusion

In conclusion, the ethanol, methoanol, acetone, chloroform and petroleum ether extracts of *Andrographis neesiana* leaf and stem possess significant inhibitory effect against the tested pathogens. The results of the study support the folklore claim of this plant.

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