



ANTIMICROBIAL POTENTIAL AND PHYTOCHEMICAL SCREENING OF *ANDROGRAPHIS AFFINIS* NEES - AN ENDEMIC MEDICINAL PLANT FROM INDIA

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ABSTRACT

The present study designed for antimicrobial potential and phytochemical screening of various extracts of leaf and stem of *Andrographis affinis* Nees belongs to the family Acanthaceae. *Andrographis affinis* Nees is an endemic medicinal plant. The has been used in the folklore system of medicine for the treatment of snake bite and fever. Only one biological active compounds have been isolated from the plant. However, its phytochemical screening and antimicrobial activity has not been investigated still now. Hence it was considered to evaluate the qualitative phytochemical analysis and antimicrobial activity. The antimicrobial activity of various extracts (ethanol, methanol, acetone, chloroform and petroleum ether) of *Andrographis affinis* was studied against five different microorganisms by disc diffusion method. Among different solvent extracts studied, acetone extract showed higher antimicrobial activity followed by petroleum ether, ethanol, chloroform and methanol. Phytochemical investigation of ethanol, acetone, methanol, chloroform and petroleum ether extracts revealed the presence of flavonoids, gums and mucilages triterpenoids, steroids and sterols, and glycosides have been reported here in this herb for the first time. The main aim of the present investigation is to study the antimicrobial activity and phytochemical standards of leaves and stem of *Andrographis affinis*. The study suggest further research regarding the pharmacological investigations of this plant.

Keywords: Medicinal plant, *Andrographis affinis*, Phytochemical analysis, Antimicrobial activity.

INTRODUCTION

Medicinal plants are an important therapeutic aid for various disease. In India, from ancient times, different parts of medicinal plants have been used to cure specific diseases. India is known for its rich diversity of medicinal plants and hence called botanical garden of the world¹. Microorganisms and medicinal plants are rich sources of secondary metabolites which are potential sources of useful drugs and other useful bioactive products². A large number of these medicinal plants are used in several formulations for the treatment of various diseases caused by microbes. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. The increasing failure chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial injections agents have lead to the screening of several medicinal plants for their potential antimicrobial activity³. Herbs have been used as sources of food and medicinal purposes for centuries and knowledge have been passed on from generation to generation⁴. Several workers throughout the world have carried out antimicrobial studies on some medicinal plants including *Betula pendula*⁵ and *Ageratum houstonianum*⁶.

Plants are widely in the traditional medicine and their curative potentials are well documented⁷. Medicinal plants are an important source for the therapeutic remedies of various diseases. Plants are used medicinally in different countries and are a source of many potent and powerful drugs⁸. The inhabitants of an area utilize plant wealth for medicine, food and many other purposes. Many of the food and medicinal plants contain a variety of chemical substances such as alkaloids, tannins, flavonoids, steroids, glycosides, saponins, oxalates, etc. Thus phytochemical screening of such plants is an important aspect for the scientific verification of folklore claim with regard to the utility of plants.

Andrographis affinis Nees is a herb species belonging to the family Acanthaceae. This plant is used for snake-bite and fever^{9,10,11}. *Andrographis affinis* is an endemic plant to India¹² found in wild in the Shevaroy Hills of the Eastern Ghats, is situated in the Salem District of Tamilnadu, lying in between 11°45' to 11°55' N and 78°10' to 78°20'E. Yercaud is a popular hill station on this range and connected by road from Salem town. Vijaya Bhasker Reddy *et al.*,¹³ reported the New 2-Oxygenated Flavonoids from the whole plant extract A literature survey shows that no antimicrobial activity and phytochemical work done so far. Therefore, an attempt has been made to study the phytochemical screening and antimicrobial activity on the leaf and stem of *Andrographis affinis* Nees.

MATERIALS AND METHODS

Collection of plant material

The plant were collected from Shevaroy Hills (Eastern Ghats) of Salem District, Tamilnadu, The plant were taxonomically identified and confirmed with the Flora of Tamilnadu and voucher specimen (No. 21/15.07.2010 CA) deposited in Department of Botany, Government Arts College (Autonomous), Salem, Tamilnadu, India for future reference.

Preparation of extracts

The fresh plant samples (leaf and stem) collected were washed individually under running tap water to remove soil particles and other dirt. The leaf was air dried in the laboratory at room temperature (30± 2°C) for 15 days. While the stem samples were dried at 60°C for 2 days in an oven. The dried leaves and stem samples were ground well into a fine powder in a mixer grinder. The powder was stored in air sealed polythene bags at room temperature before extraction. The method of Alade and Irobi¹⁴ was adopted for preparation of plant extracts. A fixed weight (25g) of powdered plant material was soaked separately in 250 ml of acetone, methanol, chloroform, ethanol and petroleum ether for 72 h. Each mixture was stirred at 24h 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 stained was concentrated in vacuum using rotator evaporator. Then the extracts were used for antimicrobial activity.

Collection and maintenance of microbial culture

The bacterial strains were collected from the Calicut Medical College, Kerala, India and freeze preserved in nutrient agar slants. The bacterial strain namely *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were inoculated in a nutrient broth at 37°C for 24 hour in incubator. The 36g of Muller -Hinton agar (Himedia) was mixed with double distilled water and then sterilized in autoclave at 15 lbs pressure for 15 min. The sterilized media was poured into petridishes; the solidified plates were bored with 6 mm diameter cork borers. The plates with wells were used for the antimicrobial activities. The various extracts were tested against the *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* for antimicrobial assay. Wells of equal size were cut and the ampicillin trihydrate was added into it for positive

control; respective solvents acting as a negative control. The plates were incubated at 37°C overnight.

Antimicrobial activity

The antimicrobial activity was tested against (methanol, ethanol, acetone, chloroform and petroleum ether) leaves and stem of *Andrographis affinis*. The inoculation of microorganism was prepared from bacterial culture¹⁵. About 15-20ml of Muller - Hinton agar medium was poured in the sterilized petridishes and allows solidifying. One drop of bacterial strains was spread over the medium by a rod. Wells of 6 mm in diameter and about 2 cm apart punctured in the culture medium using sterile cork borers. About 100 ml of plant extract was added to the wells. Plates were incubated in air at 37°C for 24h. Antimicrobial activities were evaluated by measuring inhibition zone diameters.

Preliminary phytochemical screening

The preliminary phytochemical studies were carried out by the standard methods described by Horborne¹⁶ and Kokate *et al.*,¹⁷ with some modifications. The leaves and stem extracts was assayed for the presence of flavonoids, gums and mucilages, triterpenoids, steroids and sterols and glycosides.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of preliminary phytochemical are presented in Table 1. Preliminary phytochemical test for ethanol, methanol, acetone, chloroform and petroleum ether extracts of the drug carried out. The phytochemical analysis of the various extracts from the leaf and

stem sample of *Andrographis affinis* showed the flavonoids, gums and mucilages and triterpenoids, showed the positive result in all the extracts. Alkaloids, carbohydrates and protein and amino acids were absent in all the extracts. Steroids and sterols were absent in acetone (leaf) extract but other extracts exhibited positive result. Glycosides were present only in ethanol, acetone and chloroform, extracts, methanol and petroleum ether extracts showed negative result.

Antimicrobial activity

The results of antimicrobial activity are given in the Table 2. The *in vitro* antimicrobial activities of acetone, methanol, ethanol, chloroform and petroleum ether extracts of *Andrographis affinis* leaf and stem showed in Table 2. The results reveal that all the extracts are powerful antimicrobials against all the microorganisms studied. The antimicrobial activity was screened from the zone of inhibition. Among different solvent extracts studied, acetone extract showed higher degree of inhibition followed by petroleum ether, ethanol, chloroform and methanol. The methanol extract show minimum inhibitory effect compare to the all the other extracts. The diameter of inhibition zones standard ampicillin trihydrate. Highest *Pneumonia* in acetone leaf extract, followed by *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*. Similar types of results were also noticed in all the other extracts.

Several phytoconstituents such as flavonoids¹⁸, terpenoids¹⁹, tannins²⁰ phenolics and polyphenolics²¹ 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 materials used.

Table 1: Preliminary phytochemical screening of the leaf and stem of *Andrographis affinis*

Plant parts	Extract	Constituents							
		Flavonoids	Alkaloids	Gums and mucilages	Carbo hydrates	Triter penoids	Protein and Amino acids	Steroids and Sterols	Glycosides
Leaf	Ethanol	+	-	+	-	+	-	+	+
	Methanol	+	-	+	-	+	-	+	-
	Acetone	+	-	+	-	+	-	-	+
	Chloroform	+	-	+	-	+	-	+	+
	Petroleum ether	+	-	+	-	+	-	+	-
Stem	Ethanol	+	-	+	-	+	-	+	+
	Methanol	+	-	+	-	+	-	+	-
	Acetone	+	-	+	-	+	-	+	+
	Chloroform	+	-	+	-	+	-	+	+
	Petroleum ether	+	-	+	-	+	-	+	-

+ indicates presence of active constituents; - indicates absence of active constituents.

Table 2: Antimicrobial activity of leaf and stem extracts of *Andrographis affinis*

Plant parts	Extract	Diameter of zone of inhibition (in mm)				
		<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
Leaf	Ampicillin trihydrate (Standard)	11	10	NA	8	NA
	Acetone	7	8	8	12	NA
	Methanol	2	3	4	2	5
	Ethanol	3	NA	NA	NA	5
	Petroleum ether	NA	11	5	5	NA
	Chloroform	5	NA	7	NA	7
Stem	Acetone	7	6	8	6	5
	Methanol	3	NA	3	NA	NA
	Ethanol	4	4	11	9	NA
	Petroleum ether	NA	5	NA	NA	4
	Chloroform	11	4	4	5	NA

NA - No activity

CONCLUSION

In conclusion the present investigation the plant contains potential antimicrobial components that may be of use for development of pharmaceutical for the therapy of infections. The ethanol, acetone, methanol, chloroform and petroleum ether extracts of *Andrographis affinis* leaf and stem possess significant inhibitory effect against the tested pathogens. The results of the study support the folklore claim of this plant. The study is also aimed at extensive investigation, isolation and purification of active constituents with broad spectrum of antimicrobial activity. Further studies are needed to isolate and characterized the bioactive principles to develop new antimicrobial drugs.

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