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Antimicrobial activity and Phytochemical analysis of A*ndrographis alata* Nees from Southern India

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Abstract: *Andrographis alata* Nees is an important medicinal plant. The current study was to investigate the antibacterial, antifungal activity and phytochemical analysis of the various leaf and stem extracts of *A.alata*. Methanol, ethanol, chloroform, acetone and petroleum ether extracts of shade dried plant leaf and stem of *A.alata* were tested for antibacterial, antifungal and phytochemical screening. The antibacterial activity of different extracts (methanol, acetone, ethanol, chloroform and petroleum ether) of leaves and stem of *A.alata* using the standard disc diffusion assay against five strains of antibacterial species, viz., *Escherichia coli, Proteus vulgaris, Staphyllococcus aureus, Pseudomonas aerusinosa* and *Klebsiella pneumoniae*. Among various solvent extracts studied petroleum ether leaf extract showed a highest antibacterial assay followed by chloroform, ethanol, acetone and methanol. A phytochemical screening was conducted on the leaf and stem extracts using standard qualitative methods that revealed the presence of saponins, flavonoids, triterpenoids, gums and mucilages, glycosides, steroids, tannins and phenolic compounds. The chloroform leaf extract of *A.alata* showed most active against *Alternaria alternata*. The ethanol leaf extracts showed high antifungal activity against *Aspergillus niger*. The methanol leaf extracts showed significant activity against *Aspergillus flavus*. The petroleum ether leaf extracts showed high against *Fusarium solani*. The acetone leaf extracts showed highest antifungal activity against *Penicillium pinophiium*. The present study justifies the claimed uses of this herb in the traditional system of medication to treat various sickness. This is the first report wherein antibacterial, antifungal and phytochemical analysis from *A.alata* leaf and stem.

Key words : *Andrographis alata*, Medicinal plant, Antibacterial, Antifungal, Phytochemical, Agar well diffusion method.

INTRODUCTION

Medicinal plants are an important source for the therapeutic remedies of various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century[1]. Natural antimicrobials have been often derived from plants, microorganisms or animal tissues[2]. India is known for its rich diversity of medicinal world [3]. Nearly 70 percent of the world population is dependant on the traditional medicines for primary health care. The knowledge of medicinal plants has been accumulated during the course of many centuries based on different medicinal systems such as Ayurvedha, Unani and Siddha. In India it is reported that traditional healers used 2500 plant species and that 100 species of plants served as regular sources of medicine [4].

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as fungi, bacteria or protozoan's as well as destroying viruses [5]. Antimicrobial drugs either kill microbes or prevent the growth of 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 [6]. There are many reports on the presence of antimicrobial compounds in various plants [7, 8] but there are no reports on antimicrobial potential on *Andrographis alata.* Phytochemical from medicinal plants showing antimicrobial properties have the potential of filling this need, because their structure are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ[9]. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity[10]. Screening active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various ailments, including cancer[11].

Andrographis alata Nees is popularly known as Periyanangai (Tamil), belonging to the family of Acanthaceae is one of the most famous medicinal plant of Tamilnadu. Species of *Andrographis* Wallich ex Nees (Acanthaceae) are used in the Indian systems of medicines such as Ayurvedha, Homeopathy, Naturopathy, Amchi, Modern, Siddha and Unani. The genus exhibits antipyretic properties[12]. This genus consists of 40 species distributed in Tropical Asia[13]. Among these, 24 species have been found to be distributed mainly in the hilly areas of the districts of Tamilnadu, India[14]. *Andrographis alata* is a medicinal herb found in wild in Shevaroy Hills of Salem district, Tamilnadu (11°45' and 11°55' N and 78°11' to 78°20'E) upto 1100 m. There is so far no report available on antibacterial and antifungal properties. *Andrographis alata* has been shown to possess antipyretic, anti-inflammatory, antivenom activity and snake bite [15,16,17,18,19]. A new flavone 2'-glucoside, A flavone glucoside and Acylated 5,7,2',6'-oxygenated flavone glycosides were isolated from the whole plant[20,21,22]. The present investigation attempts to bring out the hitherto unearthed antibacterial, phytochemical and antifungal potentials of the leaves and stem extracts of *Andrographis alata* against some selected microorganisms.

MATERIAL AND METHODS

Plant material extraction

Leaf and Stem of *Andrographis alata* was collected from the Shevaroy Hills, Salem district of Tamilnadu. This plant was identified and confirmed with the authentic. A voucher specimen was deposited (No. CA/25/2010) in the Department of Botany, Government Arts College (Autonomous), Salem for the future reference. Fresh leaves and stem were washed thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender. All parts were extracted with acetone, ethanol, methanol, petroleum ether and chloroform using soxhlet apparatus. After removal of solvents under reduced pressure, extracts were stored at -20°C until use. Then the extracts were used for antibacterial, antifungal and phytochemical activity.

Antibacterial assay

Antibacterial activity of all extracts from *Andrographis alata* were checked against *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The fungal strains used were *Penicillium pinophilum, Alternalia alternata, Aspergillus niger, Aspergillus flavus* and *Fusarium solani.* All the cultures were obtained in pure form from the Biomedical Engineering Research Foundation, Salem, Tamilnadu, India. Antibacterial assay was carried out by Agar well diffusion method [23,24]. Fresh microbial culture of 0.1ml having 10^8 CFU was spread on nutrient agar plate with glass spreader. A well of 6 mm diameter was punched off into agar medium with sterile cork borer and filled with 50 µg of ethanol, methanol, acetone, chloroform and petroleum ether extracts by using micropipette in each well in aseptic condition. The petriplates were then kept in a refrigerator to allow pre-diffusion of extract for 30 minutes and further incubated in a incubator at 37°C for 24 h. The antibacterial screening 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 ciprofloxacin at a concentration of 25 µg/ml as positive control and 100% dimethylsulfoxide (DMSO) as a negative control were used.

Phytochemical analysis

The ethanol, acetone, methanol, petroleum ether and chloroform extracts of *Andrographis alata* were screened for the presence of secondary metabolites using the procedures of Harborne [25] and Kokate *et al.,*[26]. The leaf and stem extracts was assayed for the presence of glycosides, flavonoids, gums and mucilages, steroids, triterpenoids, tannins, saponins and phenolic compounds.

Antifungal activity

The method of Bauer *et al.,*[27] was adopted for the study. Antifungal activities of leaf and stem of *Andrographis alata* was proved in a radical growth inhibition activity. A fungal plug was placed in the center of the Potato Dextrose Agar plate. Extracts of

25 mg/ml concentrate was pipetted into the wells. The petriplates were incubated in the dark at 23°C for 48 h. Antifungal properties was observed as a cresent shaped zone of inhibition at the mycelial form. The effect of fungal growth was expressed qualitatively. Comparison of antifungal activity of various extracts was done with standard antifungal fluconazole at a concentration of 25 µg as a positive control. The diameters of zone of inhibition surrounding each of the well were recorded.

Statistical analysis

Agar well diffusion activity was performed in three replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analysed and expressed as Mean ± Standard Deviation.

Table 1: Antibacterial activity of leaves and stem extracts of *Andrographis alata* Nees

Plant part	Plant Extracts	Zone of inhibition (mm)				
		Escherichia coli	*Proteus vulgaris*	*Staphylococcus aurues*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Leaves	Acetone	10.24 ± 0.07	8.15 ± 0.18	13.10 ± 0.22	9.0 ± 0.12	9.11 ± 0.50
	Methanol	9.11 ± 0.16	10.28 ± 0.50	-	11.0 ± 0.15	10.33 ± 0.44
	Ethanol	11.62 ± 0.14	12.13 ± 0.44	15.41 ± 0.18	10.03 ± 0.18	12.65 ± 0.63
	Petroleum ether	10.08 ± 0.06	11.0 ± 0.15	18.11 ± 0.05	-	-
	Chloroform	17.05 ± 0.04	13.81 ± 0.07	13.24 ± 0.33	10.05 ± 0.70	10.51 ± 0.28
Stem	Acetone	8.13 ± 0.07	9.12 ± 0.20	10.30 ± 0.41	8.15 ± 0.10	10.32 ± 0.04
	Methanol	10.61 ± 0.04	8.41 ± 0.11	9.11 ± 0.03	8.60 ± 0.22	9.37 ± 0.05
	Ethanol	-	10.33 ± 0.60	11.30 ± 0.09	10.19 ± 0.81	8.40 ± 0.02
	Petroleum ether	8.20 ± 0.08	-	10.03 ± 0.08	-	11.21 ± 0.15
	Chloroform	9.42±0.15	10.50±0.70	-	8.43±0.17	8.30±0.21
	Ciprofloxacin (25µg/m1)	24.0 ± 0.12	26.0 ± 0.08	23.0 ± 0.19	26.0 ± 0.05	27.0 ± 0.40

Data given are mean of three replicates ± Standard error.
:- No inhibition
Concentration used 50µg/m1

Table 2: Antifungal activity of leaves and stem extracts of *Andrographis alata*

Microorganisms	**Methanol extract**		**Ethanol extract**		**Chloroform extract**		**Acetone extract**		**Petroleum ether extract**		**Fluconazole (25µg)**
	Leaf	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	
Alternaria alternata	7.51± 0.13	8.20± 0.50	6.20± 0.40	0 ± 00	9.45± 0.11	6.10± 0.71	6.61± 0.37	5.70± 0.03	5.33± 0.49	0.00	15±0.10
Fusarium solani	7.41± 0.22	7.04± 0.14	0.00	6.41± 0.31	8.11± 0.40	7.33± 0.10	0.00	6.70± 0.34	7.11± 0.66	6.10± 0.28	14±0.17
Aspergillus flavus	8.15± 0.15	8.20± 0.40	7.90± 0.15	7.0± 0.10	8.0± 0.30	7.10± 0.05	6.11± 0.31	0.00	6.9± 0.70	6.0± 0.68	17±0.20
Aspergillus niger	6.44± 0.30	6.11± 0.11	9.10± 0.20	7.20± 0.41	8.70± 0.25	6.0± 0.44	7.14± 0.63	6.90± 0.38	7.0± 0.50	6.33± 0.19	15±0.36
Penicillium pinophilum	0.00	6.80± 0.57	7.0± 0.35	0±00	7.10± 0.60	0.00	7.39± 0.71	6.13± 0.25	6.41± 0.05	5.46± 0.07	16±0.05

Table 3: Qualitative analysis of the phytochemicals in the leaf and stem of *Andrographis alata* Nees.

Phyto chemicals	Methanol extract		Ethanol extract		Chloroform extract		Acetone extract		Petroleum ether extract	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Saponins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Triterpenoids	+	+	+	+	+	+	+	+	+	+
Alkaloids	–	–	–	–	–	–	–	–	–	–
Glycosides	–	+	+	–	+	–	+	–	–	+
Gums and Mucilages	+	+	+	+	+	+	+	+	+	+
Steroids	–	–	+	+	–	–	–	+	–	–
Carbohydrates	–	–	–	–	–	–	–	–	–	–
Protein and Amino acids	–	–	–	–	–	–	–	–	–	–
Tannins	+	+	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+	+	+	+

(+) - Positive
(–) - Negative

Table 4: Phytochemicals composition percentage of *Andrographis alata* Nees.

Phyto chemicals	Methanol extract		Ethanol extract		Chloroform extract		Acetone extract		Petroleum ether extract	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Saponins (%)	3.41± 0.12	1.70± 0.20	3.51± 0.33	1.90± 0.07	2.90± 0.27	1.55± 0.46	3.10± 0.15	1.82± 0.6	3.45± 0.19	1.86± 0.20
Flavonoids (%)	14.30± 0.14	7.10± 0.30	12.70± 0.04	6.40± 0.13	18.43± 0.60	8.11± 0.18	13.60± 0.37	7.20± 0.07	15.40± 0.88	7.08± 0.12
Triterpenoids (%)	0.24± 0.6	0.10± 0.07	0.19± 0.18	0.15± 0.22	0.30± 0.36	0.12± 0.22	0.22± 0.40	0.14± 0.31	0.26± 0.16	0.13± 0.50
Alkaloids (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycosides (%)	0.00	0.36± 0.18	0.45± 0.30	0.00	0.24± 0.11	0.00	0.20± 0.26	0.00	0.00	0.21± 0.30
Gums and Mucilages (%)	13.40± 0.19	6.90± 0.11	10.81± 0.35	8.20± 0.10	14.17± 0.66	8.50± 0.22	12.71± 0.47	6.80± 0.09	11.65± 0.15	8.16± 0.30
Steroids (%)	0.00	0.00	0.51± 0.24	0.29± 0.08	0.00	0.00		0.34± 0.19	0.00	0.00
Carbohydrates (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tannins (%)	6.20± 0.27	2.13± 0.16	5.40± 0.28	1.75± 0.40	7.10± 0.55	2.40± 0.1	5.11± 0.65	2.06± 0.33	6.15± 0.48	1.95± 0.36
Phenolic compounds (%)	0.88± 0.17	0.09± 0.30	0.45± 0.25	0.22± 0.11	0.65± 0.42	0.28± 0.15	0.41± 0.34	0.20± 0.10	0.91± 0.23	0.39± 0.65

RESULTS AND DISCUSSION

The determinations from the present study showed that the five leaf and stem extracts (methanol, ethanol, acetone, petroleum ether and chloroform) of *Andrographis alata*, revealed antibacterial properties against all the five human pathogens tested. As noticed from Table 1, All the extracts exhibited broad spectrum of activity. When the five extracts were compared with each other and with that of standard antibiotic ciprofloxacin, the chloroform leaf extract observed to have highest potential compared to that of the acetone, methanol, ethanol and petroleum ether extracts.

The investigation made on chloroform extract highest activity against *Escherichia coli* (17.05 mm) *Proteus vulgaris* (13.81mm) and *Staphylococcus aureus* (13.24 mm) and least inhibition zone was observed against *Klebsiella pneumonia* (8.30 mm) and *Psudomonas aeruginosa* (10.43 mm). Where as no activity pathogen like *Staphylococcus aureus* in chloroform stem extract. The extracts using petroleum ether showed highest inhibition zone observed against *Staphylococcus aureus* (18.11 mm) and *Klebsiella pneumonia* (11.21 mm) and the minimal activity against *Escherichina coli* (8.20 mm). It has no activity against pathogen like *Pseudomonas aeruginosa, Proteus vulgaris* (stem extract) and *Klebsiella pneumoniae* (leaf extract). Acetone extract pointed out maximum activity against *Staphylococcus aureus* (13.10 mm) and *Klebsiella pneumoniae* (10.32 mm). Showed that least activity against *Escherichia coli* (8.13 mm) and *Proteus vulgaris* (8.15 mm). The extract obtained using methanol showed a highest activity against pathogen like *Pseudomonas aeruginosa* (11.0 mm) and *Escherichia coli* (10.61 mm). Observed no activity against pathogen like *Staphylococcus aureus* (leaf extract. The ethanol extract antimicrobial activity results showed diameter of inhibition zones ranging from (8.40 to 15.41 mm), with the highest zone of inhibition shown towards *Staphylococcus aureus* (15.41 mm). Least inhibition zone was observed against *Klebsiella pneumoniae* (8.40 mm). Where it has no activity against *Escherichia coli* in stem extract.

There is no previous report on evaluation of this plant concerning its antibacterial activity. Although, the antibacterial effect of another species of this family (Acanthaceae) has been reported [28, 29]. Table No.2 displayed the antifungal activity of leaves and stem extracts of *A.alata*. The results of minimum inhibitory concentrations (MIC) study proved the antifungal activity of extracts against the tested strains of microorganisms. Antifungal activity denoted that the tested fungal strains are most susceptible to chloroform extract. Chloroform extract antifungal results showed the diameter of inhibition zones ranging from 6.0 to 9.45 mm with the highest inhibition zone observed against *Alternaria alternata* (9.45 mm). Minimal inhibition zone was noticed against *Aspergillus flavus* (6.0 mm). It has no activity against *Penicillium pinophium*. Observation made from methanol extract showed a highest activity against *Aspergillus flavus* (8.15 mm), *Alternaria alternata* (7.51 mm) and *Fusarium solani* (7.41 mm) and the minimum activity against *Aspergillus niger* (6.44mm). Where as no activity against *Penicillium pinophilum*. Petroleum ether extract antifungal results observed the diameter of inhibition zone noticed against *Fusarium solani* (7.11 mm). Least inhibition zone was showed against *Penicillium pinoophilum* (5.46 mm. Showed no activity against *Alternaria alternata*. The ethanol extract observed highest activity against *Alternaria alternata* (9.20 mm) and the minimum activity against *Penicillium pinophilum* (7.0 mm). Where it has no action against *Fusarium solani*. The extract obtained using acetone showed a highest activity against *Penicillium pinophilum* (7.39 mm) minimal inhibition zone was observed against *Alternaria alternata* (5.70 mm). Where it has no activity against *Fusarium solani* and *Aspergillus flavus*.

The present study was to investigate the leaf and stem samples revealed the presence of medicinally bioactive constituents. The phytochemical analysis of *A.alata* investigated are presented in Tables 3 and 4. Qualitative phytochemical test for methanol, ethanol, chloroform, acetone and petroleum ether extracts of the drug carried out. The phytochemical analysis of the different extracts from the leaf and stem sample of *A. alata* revealed the presence of phytochemicals such as saponins, flavonoids, triterpenoids, glycosides, gums and mucilages, tannins, phenolic compounds and steroids. The presence of these phytoconstituents suggests that the plant might be of medicinal importance and pharmaceutical industrial. The phytoconstituents like carbohydrates, alkaloids, protein and amino acids were absence in leaf and stem sample of *A. alata* (Table 3).

Table 4 shows quantitative estimation of the percentage phytochemicals of *A.alata*. *A.alata* contained the highest percentage yield of flavonoids (18.43%) in chloroform leaf extract. The content of gums and mucilages was found highest (14.17%) in chloroform leaf extract. *A.alata* contained the lowest yield of phenolic compounds (0.09%) but the highest percentage yield of tannin (7.10%). Triterpenoids were obtained in the plant but the yields recorded were minimal (0.30 - 0.10%). Saponin high yield of 3.45% and lowest yield was found in 1.55%. Steroids were obtained in the plant but the yields recorded (0.51 -

0.29%). The content of glycosides was found in *A.alata* (0.45 - 0.20%). The phytochemical research and quantitative estimation of the percentage yields of chemical constituents of the plant studied that the leaves and stem were rich in flavonoids, gums and mucilages saponins and tannins. The were known to show medicinal activity as well as exhibiting physiological properties [30]. Saponin has the activity of precipitating and colligating red blood cells. Saponin also foams in aqueous solutions, hemolytic properties and bitterness, flavonoid on the other hand, are effective water soluble anti-oxidants activity with prevent oxidative cell damage, have potent anti-cancer properties. The phytochemicals are known to have antimicrobial screening [31]. The phytochemical screening revealed the presence of the saponins, flavonoids, tannins, steroids, triterpenoids, phenolic compounds, gums and mucilages respectively. It can be suggested that *A. alata* are not only interesting source of medicinal properties but also potential source of phytoconstituents.

It is concluded the present study the plant contains potentional antibacterial and antifungal components that may be of useful for evolution of pharmaceutical for the therapy of ailments. The acetone, chloroform, petroleum ether, methanol and ethanol extracts of *A.alata* leaf and stem possess significant inhibitory effect against the tested organisms. The results of the investigation support the traditional claimed of this plant. Apart from this investigation, there are no reports of antibacterial, antifungal and phytoconstitutents studies of *A.alata*. The current study is the first experimental demonstration of any biological properties as well as antibacterial and antifungal activity of *A.alata*. Further studies are going on these plant in order to isolate, identify, characterized and elucidate the structure of the bioactive principles to develop new antibacterial and antifungal medications.

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