

Phytochemical Potential and Antimicrobial Activity of Andrographispaniculata

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Abstract:

The Herbal medicine today ensures safety in contrast to the synthetic preparations. Herbs the Nature's Physician, have been reported as an important source of medicine for years and years. Using of herbs for curing diseases dated back to prehistory and people of all continents have this old tradition. Recently, wide research proposals highlight the property of medico potential from phytalsources. My herb of interest is also the above said, ofcourse Andrographispaniculata (Acanthaceae) is a medicinal plant used for the treatment of various ailments, which has been documented in history of all civilizations. The present study is to learn the phytochemical properties and the antimicrobial activity of the above using disc diffusion method

Keywords: Antimicrobial activity, Herb, Phytochemical, Medico potential.

I. Introduction

Medicinal herbs have a long history of use worldwide. Though the process of preparation of synthetic drugs is remarkable, around one to the fourth of Medicinal derivatives are phytoproducts are derived directly from plants (Newman et al.). The World Health Organization says that the phytoremedy is the novel practice to be practiced and also it suggest basic procedures for validation of drugs from plant origin (Anushiaet al.) and also it delivers that about 80% of people still depend on traditional remedies such as herbs for their medicine (TripathiandTripathi). Also there are evidences that herbs can act as antibiotic resistant inhibitors. Eventually combinations of herbs and different antibiotics might affect the inhibitory effect of these antibiotics (Aiyegoroet al., 2009).Ofcourse one to two of current pharmaceuticals are derived from plants. Among the few advantages of using antimicrobial compounds of medicinal plants include fewer side effects, better patient tolerance and relatively less expensive. All these data highlights made me to choose Andrographispaniculata(Acanthaceae) commonly known as "King of Bitters". To describe the plant is an annual herb, branched, erect, and growing up to 1 meter in height. The leaves and the stems of the plant are used to extract the active phytochemicals. Traditional plant remedies might offer a natural key to unlock Medical complications. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate diseases.

II. Materials & Methods

Collection and Preparation of Plant Material

Herb Andrographispaniculata plant parts viz leaves and the nodal stem were collected from my native Alwarkurichi, Tirnelveli, Tamilnadu India and were confirmed by Dr. S. John Britto, The RapinatHerarium, ST. Joseph's college(A), Tiruchirappalli and available literature. The plant parts were thoroughly washed with water and sterilized



Fig-1 Andrographispaniculata

Preparation of Fresh Extract: The plant parts (leaf, stem and nodal branches) were washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. 100gm of dried powder was first defatted with petroleum ether and then extracted with methanol. The solvent was evaporated to dryness and the dried crude extract was stored in airtight bottle at 4°C.

Preparation of Dry Extract: 100g of each plant part (leaf, stem and nodal branches) were washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles and extracted with 100ml of methanol. The extract was stored at 4°C in a airtight bottle and subjected to further analysis.

Phytochemical Screening: Phytochemical analysis of methanol extract of *Andrographis paniculata*, was carried out qualitatively to test for the presence of carbohydrates, proteins, tannins, phenols, flavonoids, cardiac glycosides, phytosterols, saponins and alkaloids (Harborne, et al.,).

Test for Carbohydrates

Molisch's test: Two drops of Molisch's reagent was added to 2 - 5 ml of the plant extract in a test tube to which 1 ml of concentrated sulphuric acid was allowed to flow down the side of the test tube. Appearance of a violet ring at the junction of two layers indicates the presence of Carbohydrates.

Test for proteins

Biuret test: To 2 ml of the plant extract, 2 ml of 10% sodium hydroxide was added and mixed well. To this, 2 drops of 0.1% copper sulphate solution was added. Formation of violet or pink colour indicates the presence of Proteins.

Test for Phenols

FC reagent method: A known volume of the plant extract was made up to 3 ml with distilled water to which 0.5 ml of freshly prepared FC reagent was added. After 3 – 5 min, 2 ml of 20% sodium carbonate was added. Appearance of blue colour indicates the presence of Phenols.

Test for Flavonoids

Ferric chloride test: To the known volume of plant extract, neutral ferric chloride solution was added. Appearance of blackish red colour indicates the presence of Flavonoids.

Test for Tannins

Ferric chloride test: The plant extract was treated with alcoholic ferric chloride solution. Formation of blue colour indicates the presence of Tannins.

Test for Terpenoids

Liebermann-Burchard test: About 10 mg of the plant crude extract was dissolved in 1 ml of chloroform and 1 ml of acetic anhydride. Then 2ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of reddish violet colour ring at the junction of two layers indicates the presence of Terpenoids.

Test for Steroids

Salkowski test: A known volume of the plant extract was dissolved in chloroform and few drops of concentrated sulphuric acid was added and mixed well. Formation of red colour in chloroform layer suggests the presence of Steroids.

Test for Cardiac Glycosides

Keller Kiliani test: A known volume of the plant extract was treated with 1 ml of ferric chloride reagent (mixture of 1 volume of 5% ferric chloride and 99 volumes of glacial acetic acid). To this, few drops of concentrated sulphuric acid was added. Appearance of greenish blue colour indicates the presence of Cardiac Glycosides

Test for Alkaloids

Wagners test: To a known volume of extract, iodine-potassium solution was added. Appearance of reddish brown precipitate indicates the presence of Alkaloids.

Test for Saponins

Frothing test: The crude dry extract was vigorously shaken with distilled water and was allowed to stand for 10 min. Stable froth indicates the presence of Saponins

Antimicrobial screening: The antimicrobial activity was carried out by disc diffusion method (Bauer et al., 1966). Bacterial cultures were used to lawn nutrient agar plates evenly using a sterile swab. The plates were dried for 15 min and sterile discs (5 mm in diameter, Whatman No.1) impregnated with 10 µl (1 mg/ml) of the plant extracts were placed on the nutrient agar surface. 10 µl of the respective solvent served as the negative

control. Streptomycin standard antibiotic disc served as the positive control (10µg/disc). The plates were then incubated at 37°C for 18-24 h. After overnight incubation the plates were examined for the zone of inhibition

Statistical analysis: The experimental results are expressed as mean ± standard deviation (SD) of triplicate measurements. The data was subjected to One Way Analysis of Variance(ANOVA) and the significance of differences between the sample means was calculated by Turkeys test. Data was considered statistically significant at P value ≤ 0.05.

Evaluation of Antibacterial Activity

Inoculum Preparation: Overnight broth culture (in nutrient broth- HiMedia) of the test bacteria was made and the turbidity was compared with McFarland Nephelometer.

Test Organisms: The test organisms such as Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiellapneumoniae, Enterobacterfaecalis and Pseudomonas aeruginosawere used for the bioassay. These strains were isolated from clinical samples collected from The Department of Communicable Diseases, Government Royapettah Hospitals, Royapettah, Chennai, Tamilnadu, The organisms were characterized by biochemical tests.

III. Results & Discussion

Phytochemical Screening: Investigations on the phytochemical screening of methanolic extracts of Andrographispaniculata’s leaf extracts consist various types of pharmacologically active compounds. The commonly identified components of pharmacological importance plant extracts includes Phenols, Flavonoids and Cardiac glycosides. In addition, Tannins and Terpenoids were present in Andrographispaniculata and carbohydrates and proteins were present in all the three plant extracts. Alkaloids were found to be absent in the plant which is clearly represented in Table 1

Table 1: Phytochemical constituents of ethanol leaf extracts of Andrographispaniculata

Phytochemical Constituents	Extract of Andrographispaniculata
Phenol	+
Flavanoids	+
Tannins	+
Terphenoids	+
Alkaloids	-
Polysterols	-
Cardiac glycosides	+
Saponins	-
Carbohydrates	+
Proteins	+

+ Represents presence of the phytoconstituent; - represents absence of the phytoconstituent

Fresh Extract: The results of the agar disc diffusion assay for the fresh extract are presented in Table 2. The fresh methanol extract of both leaf and nodal stem exhibited the maximum inhibitory activity against the G +ve organisms. The G –ve bacterial sensitivity studies revealed the following result: the leaves extract of Andrographispaniculata showed maximum inhibitory activity against Salmonella typhi and Enterobacterfaecalis which are causative organisms for typhoid (enteric fever) and other diseases, The nodal stem extract showed activity against Klebsiellapneumoniae. The extracts of nodal stem, did not have any bactericidal activity to the G +ve organisms. Salmonella typhi showed maximum inhibitory activity to leaf and nodal stem whereas Klebsiella pneumonia showed activity only to the leaves extract

Table 2: Effect of fresh methanol and aqueous extracts of Andrographispaniculata on Gram +ve and Gram -ve bacteria.

Sl No.	Name of the pathogen	Inhibitory action of leaf extract		Inhibitory action of nodal extract	
		Methanol	Water	Methanol	Water
1.	Staphylococcus aureus	15	12	10	NS
2.	Klebsiella pneumonia	10	13	12	NS
3.	Salmonella typhi	12	10	10	10
4.	Escherichia coli	10	12	NS	NS
5.	Enterobacterfaecalis	12	11	10	NS
6.	Pseudomonas aeruginosa	10	12	NS	NS
7.	Bacillus subtilis	20	18	13	NS

NS – Not Significant (values are mean of replicates)

Crude Extract: The zone of inhibition for the dry aqueous and methanol extracts on G +ve and G –ve organisms are given in Table 3. The leaf extract showed maximum activity to Staphylococcus aureus and Pseudomonas aeruginosa and moderate activity towards Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli and Enterobacter faecalis. All the other extracts showed insignificant activity towards G +ve and G negative organisms. While comparing the fresh water and methanol extracts, fresh methanol extract showed maximum inhibitory activity towards G +ve and G –ve organisms.

Also while comparing fresh & dry leaf extracts, showed significant effect in dried form is represented in Table 4. This showed that the active compounds present in the plant materials are not soluble in water when the plant parts are dried it easily dissolves and shows significant zone of inhibition.

Table 3: Effect of crude extracts of methanol & water of Andrographis paniculata on Gram +ve and Gram -ve bacteria.

Sl No.	Name of the pathogen	Inhibitory action of leaf extract		Inhibitory action of nodal extract	
		Methanol	Water	Methanol	Water
1.	Staphylococcus aureus	15	7	NS	R
2.	Klebsiella pneumonia	10	5	NS	R
3.	Salmonella typhi	10	8	NS	R
4.	Escherichia coli	12	R	NS	R
5.	Enterobacter faecalis	10	R	NS	NS
6.	Pseudomonas aeruginosa	12	8	NS	R
7.	Bacillus subtilis	10	5	NS	R

R- Resistant NS – Not Significant (values are mean of replicates)

Table 4: Antibacterial effect of Leaf extract of Andrographis paniculata compared with other commercial antibiotics

Sl No.	Name of the pathogen	Inhibitory Zone			
		Fresh leaf	Dry leaf	Kanamycin	Ampicilin
1.	Staphylococcus aureus	5	13	20	18
2.	Klebsiella pneumonia	10	15	18	15
3.	Salmonella typhi	9	12	15	13
4.	Escherichia coli	13	16	15	16
5.	Enterobacter faecalis	10	17	17	15
6.	Pseudomonas aeruginosa	8	R	NS	NS
7.	Bacillus subtilis	15	20	18	13

R- Resistant NS – Not Significant (values are mean of replicates)

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Reference

- [1]. Coon, J.T., And Ernest, E. 2004. Andrographis paniculata in The Treatment Of Upper Respiratory Tract Infections: A Systematic Review Of Safety And Efficacy. Planta. Med., [70]: [293-298].
- [2]. Eban, R.U.B., Madunagu, B.E., And Ekpe, E.D. 2005. Microbiological Exploitation Of Cardiac Glycosides And Alkaloids From Garcinia kola, Borrelia, Kolanitida, Citrus Aurantifolia. J. Appl. Bacteriol., [71]: [398-401].
- [3]. Hugo, W.B., And Russell, A.D. 2003. Pharmaceutical Microbiology; 6th Edn. Blackwell Science Publishers, Oxford, United Kingdom. Pp. [91-129].
- [4]. Martin, K.W., And Ernst, E. 2003. Herbal Medicine For The Treatment Of Bacterial Infections-A Review Of Controlled Clinical Trials. J. Antimicrobial Chemotherapy, [51]: [241-246].
- [5]. Prescott, H., And Klein, J.O. 2002. Microbiology 6th Ed. Macgraw Hill Publishers, USA. Pp. [808-823].
- [6]. Rajani, M., Shrivastava, N., And Ravishankara, M. N. 2000. A Rapid Method For Isolation Of Andrographolide From Andrographis paniculata nees (Kalmegh). Pharmaceut. Biol., [38]: [204-209].
- [7]. Srivastava, A., Misra, H., And Verma, R.K., And Gupta, M.M. 2004. Chemical Finger Printing Of Andrographis paniculata using HPLC, HPTLC And Densitometry. Phytochem. Anal., [15]: [280-287]S