International Journal of Sciences & Applied Research

www.ijsar.in

# Evaluation of phytochemical and anti-microbial activity of *Andrographis paniculata* Nees

S. Muthulakshmi*

The Standard Fireworks Rajaratnam College for Women, Sivakasi. Tamil Nadu, India.

**Corresponding author:** *S. Muthulakshmi, The Standard Fireworks Rajaratnam College for Women, Sivakasi. Tamil Nadu, India.

**Abstract**

In the present study aqueous extract of leaves of *Andrographis Paniculata* were screened separately for their antimicrobial activity against *Staphylococcus* and *E.coli* using Agar well-diffusion method. The susceptibility of the micro organisms to the extracts was compared with each other and with selected standard anti-biotic. It was observed that µg /ml aqueous extracts of leaves showed the significant antimicrobial activity against both microorganism while least microbial activity was recorded with µg /ml aqueous extracts of *Andrographis Paniculata*. Phytochemical analysis revealed the presence of flavonoids, alkaloids, phenols, glycoside, tannins and saponins.

**Keywords:** *Andrographis Paniculata*, Aqueous extracts, Antimicrobial activity, Phytochemical

## Introduction

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice.

Andrographis paniculata belongs to the family of Acanthaceae and is popular worldwide with the name of "King of Bitters" in English. The leaves and roots have traditionally been used over the centuries in Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for health promotion. Andrographis paniculata (Kalmegh) has an important place in the Indian Pharmacopoeia and is one of the most widely used plants in ayurvedic formulations (Puri, *et al*., 1996). The whole plant has variety of therapeutic values. It has immunosuppressive properties and is useful in treatment of wounds, ulcers, leprosy, sore throat, and hypertension, etc Chopra, *et al*., 1959. Panchang (stem, leaves, flowers, root and seeds) of the plant is being used in various formulation of Indian system of medicine for the treatment of fever, malaria and sore throat Jain, 1991.

Andrographis paniculata has been used in the treatment of some skin infections in India by folkloric medicine practitioners. It is considered beneficial to the skin and is used both internally and externally for this purpose Saxena, *et al*., 1998. Andrographolide is also attributed with some other activities like liver protection Saraswat, *et al*., 1995, anticancer activity Kumar, *et al*., 2004, anti-diabetic avtivity Lal *et al*., 1986 and anti-malarial activity Rahman, *et al*., 1999. The plant extract exhibits anti typhoid, antifungal, antiviral Chang, *et al*., 199 1n and anti-pyretic Madav, *et al*., 1995 activities. It is also reported to possess anti-inflammatory and anti snake venom properties Samy, et al., 2006. Recent research has thrown light on cultivation of this plant on large scale because of its high medicinal value. Hence, the present investigation was taken up with an objective to evaluate the antibacterial potential against the microorganisms.

## Materials and methods

### Collection of Plant

The saplings of *Andrographis Paniculata* were collected from Srivilliputtur of Virudhunagar District. The shoot of *Andrographis Paniculata* was collected after 45days *i.e*., during the flowering stage and shade dried at room temperature. The dried materials were converted into coarse powder and fine powder. The coarse powder was used for the preliminary phytochemical screening and antimicrobial activities.

### Extraction Procedure

Shade dried leaves of *Solanum nigrum* were packed in 2 lit. soxhlet extractor for continuous extraction. The powdered crude drug was macerated in methanol and the extraction was maintained for 72 hours. Then the extracted components were concentrated by distillation of the solvent and were evaporated to dryness on a water bath. The final products were dried in a desiccator containing anhydrous calcium chloride, for further analyses of preliminary phytochemical screening and antimicrobial activities.

### Antimicrobial Activities

### Sterilization

It is the complete destruction or removal of all living micro organisms from the object being sterilized. Glasswares were sterilized in the hot air oven at a temperature of 160°C for 2 hours. Pressure cookers and autoclaves were used for making sterilization of glasswares and culture media.

### Culture media Preparation

The microbes were isolated and cultivated by using culture media of different types. The following culture media were prepared for isolation and characterization of bacteria.

### Micro organisms Used

Gram positive bacteria: *Staphylococcus aureus*

Gram negative bacteria*: Escherichia coli*

### Antibiotics Used

Cloxacillin - 1 μg/disc

Gentamycin - 0.8 μg/disc

Muller Hinton agar was used for testing the susceptibility of micro organisms to antimicrobial agent using the disc diffusion technique described in NCCLS approved standard.

| Muller Hinton agar medium | - g / L |
|---|---|
| Beef, Hot infusion form | - 300 |
| Caesin acid hydrolysate | - 17.5 |
| Starch | - 1.5 |
| Agar | - 17.0 |

Final pH (at 25°C) 7.4 $\pm$ 0.2

About 38 g of Muller Hinton agar medium was suspended in 1000 ml distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Procedure**

The nephlometric standard according to Mac Farland's standard after sub culturing in peptone water for 4 hours were used for all tests. One milligram of crude drug was dissolved in 1ml distilled water and used in concentration of 1 in 2, 1 in 4 and 1 in 8 dilutions. The discs were prepared from sterile filter paper, 6 mm in diameter and were immersed in the herbal extract from 15 to 30 minutes after which they were placed in pour plate culture of pathogens on Muller Hinton agar medium. The discs were placed on the inoculated plate within 15-30 minutes. The minimal inhibition concentration values were studied for the micro organisms and tabulated. The results were recorded after 24 hrs and 48 hrs.

**Results**

A qualitative screening of the phytochemicals in the acetone extract did not show alkaloids, carbohydrates, proteins, phenolic compounds, gums, mucilage and flavonoids in the leaf samples of *Andrographis paniculata*. Benzene and chloroform extracts did not record the presence of carbohydrates, phytosterols, phenolic compounds, proteins, amino acids, gums, mucilage, flavones and flavonoids. Water, chloroform and methanol extracts showed the presence of alkaloids. Petroleum ether and benzene extracts are exhibited the presence of fixed oils & fats. Phytosterols were found in petroleum ether, acetone and methanol extracts. Methanol and water extracts showed the presence of saponins, flavonones and flavonoids.

**Table 1(a): Phytochemical Screening.**

| Phytochemical Screening | Extract | | | | | |
|---|---|---|---|---|---|---|
| | Petroleum ether | Benzene | Chloroform | Acetone | Methanol | Water |
| Alkaloids | | | | | | |
| Mayer's reagent | - | - | + | - | + | + |
| Dragendroff's reagent | - | - | + | - | + | + |
| Hager's reagent | - | - | + | - | + | + |
| Wagner's reagent | - | - | + | - | + | + |
| Carbohydrates and Glycosides | | | | | | |
| Molisch's reagent | - | - | - | - | + | + |
| Fehling Solution | - | - | - | - | + | + |
| Barfoed's reagent | - | - | - | - | + | + |
| Libermann's Burchard test | - | - | - | - | + | + |
| Borntrager's test | - | - | - | - | + | + |
| Proteins and Amino Acids | | | | | | |
| Millon's test | - | - | - | - | + | + |
| Biuret test | - | - | - | - | + | + |
| Ninhydrin reagent | - | - | - | - | + | + |

+ → Positive result; ⁻ → Negative result

**Table 1(b): Phytochemical Screening.**

| Phytochemical Screening | Extract | | | | | |
|---|---|---|---|---|---|---|
| | Petroleum ether | Benzene | Chloroform | Acetone | Methanol | Water |
| Tannins – Phenolic Compounds | | | | | | |
| Ferric choride solution | - | - | - | - | + | + |
| Lead acetate solution | - | - | - | - | + | + |
| Gelatin solution test | - | - | - | + | + | - |
| Aqueous bromine | - | - | - | - | + | + |
| Phytosterols | | | | | | |
| Libermann's test | + | - | - | + | + | - |
| Libermann's Burchard | + | - | - | + | + | - |
| Fixed oils and fats | | | | | | |
| Spot test | + | + | - | - | - | - |
| Saponification | + | + | - | - | - | - |
| Saponins | | | | | | |
| Foam test | - | - | - | - | + | + |
| Haemolysis test | - | - | - | - | + | + |
| Flavones and Flavonoids | | | | | | |
| Aq. NaOH | - | - | - | - | + | + |
| Conc. $H_2SO_4$ | - | - | - | - | + | + |
| Mg. and Hcl. | - | - | - | - | + | + |
| Gum and Mucilage | | | | | | |
| Alcoholic | - | - | - | - | - | + |
| Molisch's test | - | - | - | - | - | + |

+ → Positive result; ⁻ → Negative result

**Table 2: Antimicrobial Activity of Aqueous Extract of *Andrographis paniculata* Leaves.**

| Samples | Concentration in µg/ml | | | | Cloxacillin | Gentamycin |
|---|---|---|---|---|---|---|
| | 1µg/ml | 0.5µg/ml | 0.25 µg/ml | 0.125 µg/ml | 1 µg/disc | 0.8 µg/disc |
| | Zone Diameter inhibition in mm | | | | | |
| H-Apr. | | | | | | |
| *Staphylococcus* | 18 mm | 15 mm | 12 mm | 11 mm | 30mm | 30mm |
| *E.coli* | 16 mm | 15 mm | 12 mm | 10 mm | | |

The antibacterial activity of the crude aqueous extract of *Andrographis Paniculata* leaf showed inhibition zone. By tube dilution method, diameter of inhibition zone of 1µg/ml concentration revealed 18 mm in *Staphylococcus aureus* and 16mm in *E. coli.* Whereas in other samples inhibition zone remained the same in bacterial strains. But in Cloxacillin, Gentamycin standards antibiotics exhibited 30 mm as the inhibition zone, whereas in 0.5µg/ml dilution less difference was observed with a range of 1mm diameter zone when compared to 1µg /ml concentration. There was a gradual decrease in the diameter of inhibition zone as the dilution increased. At 0.125µg/ml

concentration of *A. paniculata* aqueous extract of leaves, the diameter of inhibition zone is 10mm. Among the dilution 1µg/ml concentration showed better antibacterial effect when compared to others. After 24 hours of incubation there was no increase in the diameter of inhibition zone.

**Discussion**

Aqueous leaf extract with phenol was toxic to micro organisms. Similar results have been reported in Giriram kumar *et al*. (2006) in aqueous extract of leaf and stem of *Santalum album*. The aqueous extract of *Andrographis paniculata* revealed a variation in the inhibition zone depending on the concentration of the drug. Under 1µgm/ml concentration showed maximum inhibitory zone of 18 mm whereas in 0.125µgm/ml concentration it showed 10mm whereas after 24 hours there was no change in the inhibition zone .This was in confirmity with the study of Bhavani *et al*. (2006).The results of antibacterial activity of the aqueous extracts obtained from the leaves *Andrographis paniculata* showed maximum inhibitory effect in 1µg/ml concentration.

**Conclusion**

The results of the present study also support the medicinal usage of *Andrographis paniculata* extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

**References**

A . Nostro, M.P Germanò, V. D'angelo, A. Marino, Cannatelli MA (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 30: 379-384.

A. Puri, R. Saxena, R. P. Saxena, K. C. Saxena, V. Srivastava and J. S. Tandon, Immunostimulant Agents from Andrographis Paniculata, J. Nat. Prod., 56(7), 995-999 (1996).

B. Saraswat, P. K. S. Visen, G. K. Patnaik and B. N. Dhawan, Effect of and Rographolide Against Galactosamine Induced Hepatotoxicity. Fitoterapia, 66, 415- 420 (1995).

B.Ravinder Reddy, Asif Ansari and I. Shivkumar, 2003. Herbal medicine: Need for Stricter regulations. Express pharma pulse, 22nd May. pp: 6.

C.K. Kokate, 1991. In : Practical Pharmacognosy, Vallabh Prakashan. New Delhi, pp: 108-127.

J. D. Hooker, Flora of British India, Vol. IV, L. Reeve & Co. Ltd. Ashford, Kent (1885).

J. Lal, H. C. Tripathi and S. K. Tandon, Antidiabetic Activity of Andrographolide Indian J. Pharmacol., 18, 58-61 (1986).

J.Bhavani, M.Bhuvaneswari, Parameswari, S., Prathiba, G.A., Janardhanan, V., Reddy,C.U. and N. Deepa, 2006. Antibacterial activity of various extracts of *Rhinacanthus nasutus.* L. Kuttz. *Indian Drugs*, 43 (12): 1004-1007.

M. Giriram Kumar, A. Indra, , R. Jeyaraj and D. Loganatham, 2006. Antimicrobial activity of aqueous extract of leaf and stem extract of *Santalum album*. *Anc. Sci. life,* 25 (3,4): 2-9.

M.P,N. Singh and J. Ahmed. 2000. A note on herbal medicinal plants. *J.Env. and pollution,* 7 (4):331-332.

N. N. Rahman, T. Furuta, S. Kojima, K. Takane and M. A. Mohd, Antimalarial Activity of Extracts of Malaysian Medicinal Plants, J. Ethnopharmacol., 64, 249-254 (1999).

R. A. Kumar, K. Sridevi, N. V. Kumar, S. Nanduri and S. Rajagopal, Anticancer and Immunostimulatory Compounds from Andrographis Paniculata, J. Ethnopharma., 92(2-3),291-295 (2004).

R. N. Chopra, S. L. Nayar and I. C. Chopra, Glossary of Indian Medicinal Plants. 1st

Ed. Publication and Information, New Delhi (1956).

R. P. Samy, M. M. Thwin, P. Gopalakrishnakone and S. Ignacimuthu, Ethnobotanical Survey of Folk Plants for the Treatment of Snake Bites in Southern Part of Tamilnadu, India, J. Ethnopharmacol., 115, 302-312 (2008).

R. S. Chang, L. Ding, C. G. O. Hen, Q. C. Pan, K. L Zhao and K. M. Snith Dehydrographolide Succinic Acid Monoester as an Inhibitor Against the Human Immunodeficiency Virus, Proceedings of Society of Experimental Biology and Medicine,197, 59-66 (1991).

S. K. Jain, Dictionary of Indian Folk Medicine and Ethnobotany- A Reference Manual ofMan-Plant Relationships, Ethnic Groups and Ethno Botanists in India. Deep Publication, New Delhi, India (1991).

S. Madav, H. C. Tripathi, S. K. Tandan and S. Mishra, Analgesic, Antipyretic and Antiulcerogenic Effect of Andrographolide, India J. Pharmaceut. Sci., 57, 121-125 (1995).

S. Saxena, D. C. Jain, R. S. Bhakuni and R. P. Sharma, Chemistry and Pharmacology of Andrographis Species, Indian Drugs, 35, 458-467 (1998).