



EVALUATION OF PHYTOCHEMICAL AND ANTI-BACTERIAL ACTIVITY OF HOT AND COLD METHANOLIC EXTRACT OF LEAVES AND WHOLE PLANT OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT

In the present study hot and cold methanol extract of leaves and whole plant of *Andrographis Paniculata* were screened separately for their anti-bacterial activity against *Staphylococcus aureus* (MTCC-737) and *Escherichia coli* (MTCC-452) 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 hot methanol extracts of leaves showed the significant anti-bacterial activity against both bacteria while least bacterial activity was recorded with cold methanolic extracts of whole plant of *Andrographis Paniculata*. Phytochemical analysis revealed the presence of flavonoids, alkaloids, phenols, glycoside, tannins and saponins.

Key words: *Andrographis Paniculata*, Methanol extracts, Anti-bacterial activity, Phytochemical.

INTRODUCTION

Andrographis paniculata belongs to the family of Acanthaceae and is popular worldwide with the name of “King of Bitters” in English. It is an annual herbaceous plant which is widely cultivated in Southern Asia, India, China and some parts of Europe.

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

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Pharmacopoeia and is one of the most widely used plants in ayurvedic formulations¹. 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, etc². 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 throat³. *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 purpose⁴.

The plant is also reported effective against diarrhoea⁵ and it is commonly used to prevent and treat common cold⁶. It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia⁷. The plant contains a number of diterpenoids. However, the major bitter constituent is andrographolide, which is diterpene lactone. Diterpenoids and flavonoids are the main chemical constituents of *Andrographis paniculata* which are believed to be responsible for the most biological activities of this plant⁸. Two flavonoids, identified as 5, 7, 2', 3'-tetramethoxyflavone and 5-hydroxy-7, 2', 3'-trimethoxyflavone, as well as several other flavonoids were obtained from the whole plant⁹. The bitter principle andrographolide was isolated in pure form by Goiter¹⁰. Andrographolide is also attributed with some other activities like liver protection¹¹, anticancer activity¹², anti-diabetic activity¹³ and anti-malarial activity¹⁴. The plant extract exhibits anti typhoid, antifungal, antiviral¹⁵ and anti-pyretic¹⁶ activities. It is also reported to possess anti-inflammatory and anti snake venom properties¹⁷. 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.

EXPERIMENTAL

Collection of plant material

Two plants of *Andrographis paniculata* were obtained as a gift from Forest Research Institute (FRI), Dehradun, Uttarakhand, India in the month of June 2009. After one complete life cycle of the plants, fully matured seeds were collected and sown in nursery and the crop was grown as per cultivation practices developed by Pandey and Mandal¹⁸. Whole plant material and only leaves were collected at the same time and dried in shade for seven days followed by grinding and then coarse powder stored in air tight bottles.

Preparation of extracts

Hot methanol extraction

The shade dried coarse powder of the leaves and whole plant material of *Andrographis paniculata* (50 gm each) was packed well in soxhlet apparatus and was subjected with methanol by continuous hot extraction for about 24 hrs. The extracts were filtered through Whatman filter paper and concentrated on a water bath. The final concentrated extracts were stored at -18°C in labeled sterile bottles.

Cold methanol extraction

The shade dried coarse powder of the leaves and whole plant material of *Andrographis paniculata* (50 gm each) was kept in stoppered flask and were macerated with 250 mL of methanol for 24-48 hrs with frequent stirring. Then the extracts were filtered through Whatman filter paper and concentrated under air. Obtained extracts were stored at -18°C in labeled sterile bottles.

The above prepared hot and cold methanol extracts were used for phytochemical and anti-bacterial investigation. The dried extracts were tested for their phyto constituent's by standard methods¹⁹.

Micro-organisms

The hot and cold methanol extracts of leaves and whole plant material of *Andrographis paniculata* were screened against gram-positive (*Staphylococcus aureus* "MTCC-737") and gram-negative (*Escherichia coli* "MTCC-452") bacteria. The test organisms were sub cultured at 37°C for 24 hr and maintained on nutrients agar media. The experimental conditions (temperature and aeration) were maintained constant before the anti-microbial assay was carried out.

Anti-bacterial screening

Agar well-diffusion method²⁰ was used for the anti-bacterial study. The overnight culture grown was used for inoculation. For working stock 1 mL of each bacterial strain was initially inoculated in 100 mL of sterile nutrient broth and incubated for 37° ± 1°C for 24 hr respectively. Then 0.2 mL of the each test organisms from the working stock was seeded into 100 mL sterile nutrient agar medium and cooled to 48°C to 50°C in a sterile Petri dish respectively. When the nutrient agar medium solidifies, four holes of uniform diameter (6 mm) were made using sterilized cork borer. Then, 0.2 mL of each hot, cold methanol

extracts and standard solution were placed in each hole separately. The plates were maintained at room temperature for 2 hr to allow the diffusion of the solution into the medium. All the bacterial plates were incubated at $37^{\circ} \pm 1^{\circ}\text{C}$ for 18 hr and the zone of inhibition was measured. Triplicates were maintained for each sample of the extracts respectively. For each bacterial strains control were maintained where pure solvents were used. Apart from that, the comparative study also conducted with standard antibiotic Norfloxacin and Ciprofloxacin with the test drug *Andrographis paniculata* against *S. aureus* and *E. coli*. All the diameters of inhibition zone were measured in mm.

RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical analysis of methanolic extract revealed the presence of flavonoids, alkaloids, phenols, glycoside, tannins and saponins. Hot methanolic leaves extract was rich in phytochemical constituents (Table1).

Table 1: Phytochemical screening of methanolic extract of *Andrographis paniculata*

Phyto-constituents	Hot methanol extract		Cold methanol extract	
	M ₁	M ₂	M ₁	M ₂
Phenols	+++	++	++	+
Flavonoids	++	+	+	+
Saponins	+	+	+	-
Alkaloids	++	+	+	+
Glycosides	++	++	++	+
Tannins	+++	++	++	++

Abbr: - = Absent, + = Presence, ++ = Moderate, +++ = Maximum

M₁ = Only leaves, M₂ = Whole plant material

The inhibitory facts of *Andrographis paniculata* on the test micro-organisms may be due to the presence of the above phytochemical components. The various phytochemical compounds detected are known to have beneficial importance in medicinal science. Phenols are said to offer resistance to diseases and accounts for most of the anti-oxidant activity in plants²¹. Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities²². Alkaloids have been used to treat diseases like malaria and glycosides serve as

defense mechanisms against many micro-organisms. Saponin protects the plant against microbes and fungi.

Anti-bacterial screening

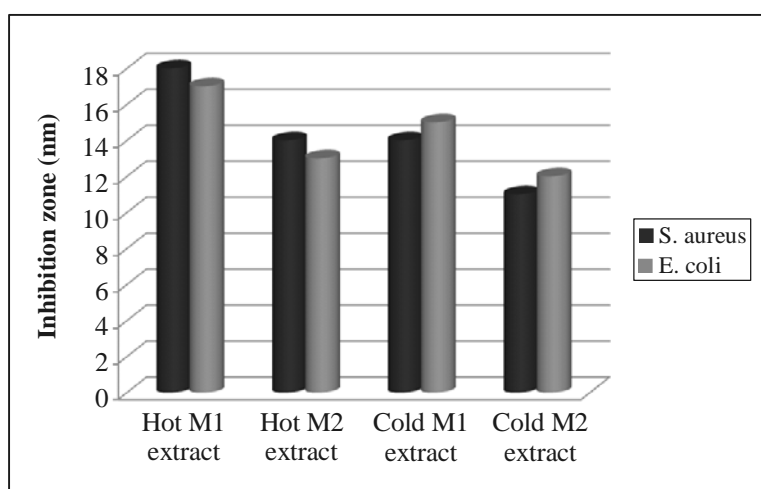
Results of the antibacterial screening of hot and cold methanol extracts of leaves and whole plant material of *Andrographis paniculata* revealed significant antibacterial activity against all tested bacterial strains (Table 2).

Table 2: Evaluation of anti-bacterial activity of methanolic extract of *Andrographis paniculata*

Name of pathogen	Inhibition zone (mm)			
	Hot methanol extract		Cold methanol extract	
	M ₁	M ₂	M ₁	M ₂
<i>S. aureus</i>	18 ± 0.5	14 ± 0.5	14 ± 0.5	11 ± 1.0
<i>E. coli</i>	17 ± 0.5	13 ± 1.0	15 ± 0.5	12 ± 1.0

Values are mean with ± SD (n = 3).

Abbr: M₁ = only leaves, M₂ = whole plant material



Abbr: M₁ = only leaves, M₂ = whole plant material.

Fig. 1: Anti-bacterial effects of hot and cold methanolic extracts of *Andrographis paniculata*

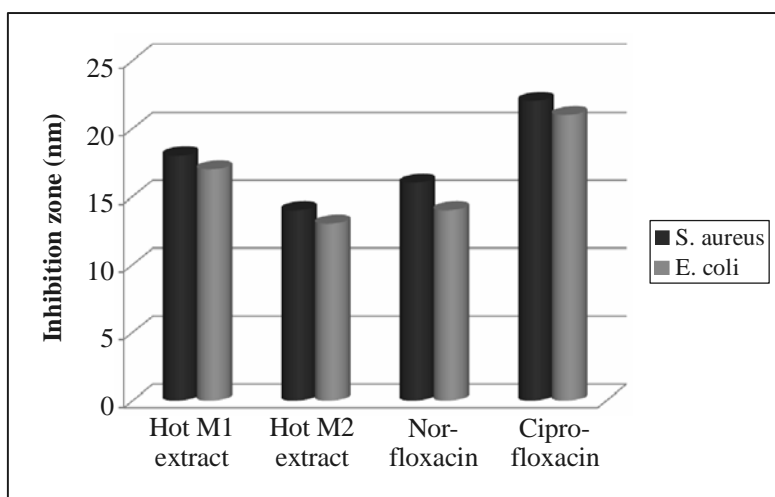
The hot methanol extract of leaves of *Andrographis paniculata* showed more significant activity against all tested bacterial organisms than that of the cold methanol extracts. The maximum antibacterial activity of hot and cold methanol extracts of leaves was exhibited against *Staphylococcus aureus* when compared with standard anti-biotic (Table 3).

Table 3: Comparative anti-bacterial sensitivity of hot methanolic extracts of *Andrographis paniculata* with standard anti-biotic

Name of pathogen	Inhibition zone (mm)			
	Hot methanol extract		Antibiotic	
	M ₁	M ₂	Norfloxacine	Ciprofloxacine
<i>S. aureus</i>	18 ± 0.5	14 ± 0.5	16 ± 0.5	22 ± 0.5
<i>E. coli</i>	17 ± 0.5	13 ± 1.0	14 ± 1.0	21 ± 1.0

Values are mean with ± SD (n=3).

Abbr: M₁ = Only leaves, M₂ = Whole plant material.



Abbr: M₁ = only leaves, M₂ = whole plant material.

Fig. 2: Comparative study of anti-bacterial sensitivity of hot methanolic extracts of leaves of *Andrographis paniculata* with standard anti-biotic

CONCLUSION

Hence the crude extract of *Andrographis paniculata* leaves in hot methanol can be used for further purification and preparation of new anti-microbial for the more resistant type of micro-organism. The above findings recommend the further investigation of *Andrographis paniculata* leaves to evaluate their chemical potential.

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