

Antimicrobial Activity of the Chloroform Extracts of the Root and the Stem of *Andrographis paniculata* Nees

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In vitro antibacterial and antifungal activity of the chloroform extracts of the root and the stem of *Andrographis paniculata* at different concentrations were screened against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria *Escherichia coli*, *Proteus vulgaris* and fungi *Aspergillus niger*, *Penicillium chrysogenum* by cup plate method. The extracts were found to inhibit the growth of all the bacteria and fungal organisms tested. The effect produced by the extracts were comparable with the standard antibacterial agent, benzyl penicillin and with the standard antifungal agent, fluconazole and were found to be active against all the organisms tested.

Keywords: *Andrographis paniculata*, stem, root, chloroform extracts, antibacterial activity, antifungal activity

INTRODUCTION

Andrographis paniculata Nees is an herbaceous plant, commonly known as “king of bitters” or Kalmegh belongs to the family Acanthaceae. Mostly the leaves and roots have been traditionally used over centuries for different medicinal purposes in Asia and Europe as a folklore remedy for a wide spectrum of ailments or as an herbal supplement for health promotion. It has been used to treat disorders of gastro-intestinal tract and upper respiratory infections, herpes, sore throat and a variety of other chronic and infectious diseases. In traditional Chinese Medicine (TCM) *Andrographis* is considered as the herb possessing an important “Cold Property” used to treat the heat of the body in fevers, and to dispel toxins from the body (Deng, 1978). In Scandinavian countries, it is commonly used to prevent and treat common colds. It is rated very high in therapeutic action in curing liver disorders and common cough and cold in humans.

The Indian Pharmacopoeia mention that it is a predominant constituent of at least 26 Ayurvedic formulations (Zhang, 2004; Mishra et al., 2007). Extensive research has revealed that *Andrographis paniculata* has a broad range of pharmacological effects such as anti-inflammatory (Sheeza et al., 2006), antidiarrhoeal (Gupta et al., 1990), antibacterial, antiviral (Wiart et al., 2005),

antimalarial (Misra et al., 1992; Rahman et al., 1999), filaricidal, hepatoprotective (Trivedi and Rawal, 2001; Visen et al., 2007), cardiovascular (Zhang and Tan, 1997; Tan and Zhang, 2004), anticancer (Kumar et al., 2004; Rajagopal et al., 2003; Cheung et al., 2005; Li et al., 2007; Sukardiman et al., 2007; Zhon et al., 2006), male reproductive toxicity (Akbarsha and Murugaian, 2000), cytotoxicity (Nanduri et al., 2004), immunostimulatory (Calabrese et al., 2000; Basak et al., 1999; Iruretagoyena et al., 2005) and antifertility (Akbarsha et al., 1990) activities. In view of its wide variety of above biological activities and in continuation of our studies (Radhika et al., 2008) the efficacy of the *A. paniculata* stem and root chloroform extracts were screened for their antibacterial activity against gram positive bacteria *Staphylococcus aureus* (NCIM 5021), *Bacillus subtilis* (NCIM 2439), gram negative bacteria *Escherichia coli* (NCIM 2067), *Proteus vulgaris* (NCIM 2027) and antifungal activity against *Aspergillus niger* (NCIM 1055), *Penicillium chrysogenum* (NCIM 722) by cup plate method (Kavanagh, 1963).

MATERIALS AND METHODS

Plant material

Andrographis paniculata (Acanthaceae) (10 kg) was collected from the Sri Venkateswara University gardens and Mamundur forest, Mallimadugu village, Tirupati (rural), Chittoor District, Andhra

Table 1.Antibacterial activity of the chloroform extracts of the stem and the root of *Andrographis paniculata*

Test organisms	Inhibition zone diameter (mm)				
	Chloroform extract of the stem		Chloroform extract of the root		Standard (Benzyl penicillin)
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	100 µg/ml (DMSO)
<i>Staphylococcus aureus</i>	10	12	9	11	17
<i>Bacillus subtilis</i>	11	12	9	11	18
<i>Escherichia coli</i>	12	13	10	11	16
<i>Proteus vulgaris</i>	12	13	11	12	15

Table 2.Antifungal activity of the Chloroform extracts of the stem and the root of *Andrographis paniculata*

Test organisms	Inhibition zone diameter (mm)				
	Chloroform extract of the stem		Chloroform extract of the root		Standard (fluconazole)
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	100 µg/ml (DMSO)
<i>Aspergillus niger</i>					
<i>Penicillium chrysogenum</i>	10	12	10	12	15
	10	12	10	11	16

Pradesh, India. The parts of this plant such as the roots, stem and leaves have been dried separately in shade and powdered. The identification of the plant, *Andrographis paniculata* was done by Dr. K Madhava Chetty, Dept. of Botany, Sri Venkateswara University, Tirupati, India. A voucher specimen (No. 0054/AP, dt. 13-07-2006) has been deposited in the Department of Botany, Andhra University, Visakhapatnam, India.

Extraction

The powdered form of the root (400 g) and the stem (700 g) of *Andrographis paniculata* were subjected to step wise extraction using *n*-hexane, chloroform and methanol exhaustively by successive cold and hot extraction processes. These extracts were concentrated to dryness *in vacuo*. The chloroform extract of the stem (4 g) and the root (3 g) were tested for antimicrobial activity.

Antibacterial activity

The cultures of *Staphylococcus aureus* (NCIM 5021), *Bacillus subtilis* (NCIM 2439), *Escherichia coli* (NCIM 2067) and *Proteus vulgaris* (NCIM 2027) grown overnight at 37°C were used for testing the antibacterial activity. Nutrient agar medium (Himedia, India) was dissolved in water (2.8% w/v) and pH adjusted to 7.0. This was distributed in 25 ml quantities in 100 ml conical flasks and were sterilized in an autoclave at 121 °C (15 lbs/sq.in) for 20 minutes. The bacterial culture was added aseptically to the agar medium maintained at 45°C, mixed well and poured immediately in sterilized petri plates. After hardening, four cups of 8 mm diameter each were cut into agar. 50 µL of test solutions of various concentrations (50 µg/ml, 100 µg/ml) and standard benzyl penicillin (100 µg/ml) were placed in these cups. Solvent alone in the fourth cup was kept in control. The plates were incubated at 37°C and observations recorded after 24-72 h. Each experiment was carried out in triplicate and the mean diameter of inhibition zone was recorded. The inhibition zones produced by the test solutions were compared with

the inhibition zone produced by pure benzyl penicillin used as the standard. The results are presented in Table 1.

Antifungal activity

Antifungal activity of the stem and the root chloroform extracts were tested against *A. niger* (NCIM 1055) and *P. chrysogenum* (NCIM 722) using the diffusion plate method (Kavanagh, 1963). In this 0.1 mL of fungal spore suspension (grown for 3 days on 10 mL of nutrient dextrose agar) was thoroughly mixed with 25 ml of melted Potato dextrose agar (Himedia, India) and was poured into sterilized petri plates. When the agar solidified, 4 cups of 8 mm diameter were made on each of the seeded plates. These cups were filled with 50 µL of the test samples of various concentrations (50µg/ml and 100µg/ml) and standard fluconazole (100 µg/ml). Solvent alone in the fourth cup was kept in control. The petri plates were incubated at 28°C for 2-4 days. All these experiments were carried out in triplicate. All the culture plates were examined from 24 h onwards and the results are tabulated (Table 2). The inhibition zones produced by the test samples were compared with the inhibition zone produced by fluconazole used as the standard.

RESULTS AND DISCUSSION

The chloroform extracts of the stem and the root of *Andrographis paniculata* showed considerable antibacterial and antifungal activities. The chloroform extract of the stem (100 µg/ml) showed the significant antibacterial activity against all the tested organisms compared with the standard benzyl penicillin, but the chloroform extract of the root (100 µg/ml) showed moderate activity against the organisms tested with the standard benzyl penicillin. These extracts also showed moderate antifungal activity against the tested organisms compared with the standard fluconazole.

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