

Prophylactic effect of *Andrographis paniculata* extracts against fungal species

G.Rajalakshmi^{1*}, D.Aruna¹, B. Bhuvaneswari¹, R.S. Venkatesan¹, A.Natarajan², K. Jegatheesan³

¹Research Department of Biochemistry Adhiparasakthi College of Arts and Science, Kalavai- PIN- 632506, Vellore District, Tamilnadu, India.

²PG & Research Department of Zoology, C. Abdul Hakeem College, Melvisharam-632 509. Tamil Nadu, India.

³Director, VI Institute of Technology, Chengalpet Taluk, Kanchipuram District- 603108, Tamilnadu, India.

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ABSTRACT

Andrographis paniculata (Acanthaceae) has been used to treat hepatoprotective, antidiarrhoeal, anti-inflammatory, antimalarial, and antihypertensive. The aim of the study was to investigate the antifungal activity of different part of extracts of *A. paniculata*. The antifungal activity of extracts of *A. paniculata* was evaluated by Agar well diffusion method against five selected fungal species. Stem extracts of *A. paniculata* showed high antifungal activity against *A.oryzae*, *Penicillium sp* and *C.albicans*. The root extracts showed high antifungal activity against *A. niger*, *A. flavus*, *C.albicans*, *Penicillium sp* and *A.oryzae* and also leaf extracts showed high antifungal activity against *Penicillium sp* and *A. flavus* but did not show antifungal activity against *C.albicans*, *A. niger*, *A.oryzae*. The results obtained in the present study suggest that *A. paniculata* plant can be used in treating various diseases caused by the test organisms.

INTRODUCTION

Medicinal plants are widely used for treatment of diseases all over the world. According to WHO report about 80% of the world populations are taking interest in indigenous medicinal plants remedies. Herbal medicines have usually been used in the form of fruit and vegetables, drugs or their extract for the treatment of the diseases and for maintenance health (Sahito *et al.*, 2003). Skin disease, diarrhea, diabetes, malaria, respiratory infection, fungal and bacterial infection are the common health problem in rural areas. In under developing countries numerous medicinal plants are used traditionally which are remedial against these disease (Pinn, 2000). Fungal diseases cause significant loss in many important vegetable crops and plants. Many fungi are harmful as they are pathogens of plants, animals and human

* Corresponding Author

Adhiparasakthi College of Arts and Science, Kalavai,
Vellore District, Tamilnadu, India; Tel: +91-8870690296

beings or produce metabolites that are toxic to plants and animals (Richard *et al.*, 1993; Bowers and Locke, 2000). Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to nutritional value, organoleptic characteristics, and limited shelf life (Agrios, 2004). In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. *Andrographis paniculata* Nees (Acanthaceae), commonly known as *Nilavembu* is an annual herb. It is found in wild throughout the plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Maharashtra, Orissa and Uttar Pradesh. Some recent researcher reported on these plant having medicinal properties like antidiarrhoeal, anti-inflammatory, choleric, antimalarial, antihypertensive, antipyretic, antithrombotic, antidote and hepatoprotective.

The bioactive compound andrographolide has been reported to be effective in the treatment of upper respiratory tract infection (Gupta *et al.*, 1990; Yin and Guo, 1993; Chturvedi *et al.*, 1983; Chang and But, 1986; Hancke *et al.*, 1995; Melchior *et al.*, 1997; Melchior *et al.*, 2004; Thamilikui *et al.*, 1991; Poolsup *et al.*, 2004; Coon and Ernst, 2004).

MATERIALS AND METHODS

Plant Material

Andrographis paniculata was collected from Adhiparasakthi Agriculture and Horticulture College, G.B. Nagar, Kalavai, Vellore District, Tamilnadu, India. Professor V. Durai authenticated the sample. Voucher specimen of the same was maintained in Adhiparasakthi Agriculture and Horticulture College.

Plant extract preparation

The collected plant materials were chopped into small pieces separately, shade-dried, at room temperature for 15 days (Mukherjee, 2002). Then coarsely powdered using pulverizer. The coarse powders were subjected to successive extraction with organic solvent such as ethanol by Soxhlet apparatus. The extracts were collected and distilled on a water bath at atmospheric pressure and the last trace of solvents was removed in vacuum. The resulted extracts were used for antibacterial studies (Rammohan Subramanian *et al.*, 2008).

Test Organisms

The test organisms used were *Candida albicans* (MTCC 10231), *Aspergillus niger* (MTCC 16404), *Aspergillus oryzae* (MTCC 42149), *Aspergillus flavus* (MTCC 9643), *Penicillium species* (MTCC 9480). All the stock cultures were obtained from Microbial Type Culture (IMTECH, India).

Culture Media and Inoculums Preparation

Sabouraud dextrose agar /broth (Himedia, India.) were used as the media for the culturing of fungal strains. Loops full of all the fungal cultures were inoculated in the Sabouraud dextrose broth (SDB) at 37°C for 72 hrs.

Reference drug

Fluconazole was purchased from Biochem pharmaceutical industries Ltd., Mumbai. 30mg dissolved in 1ml with distilled water.

Antifungal activity study

Antifungal activities of the *A. paniculata* extracts *viz.*

ethanol were determined, using the agar well diffusion assay method (Perez *et al.*, 1990). Approximately 20 ml of molten and cooled media (SDA) was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The fungal test organisms were grown in Sabouraud dextrose broth for 24 h. A 100 ml Sabouraud dextrose broth culture of each fungal organism (1×10⁵cfu/ml) was used to prepare fungal lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Five wells were prepared in the agar plates. The wells were labeled as A,B,C,D,E 'A' well was loaded with 20 µl of solvent, 'B' well was loaded with 20 µl of leaf extracts, 'C' well loaded with 20 µl of stem extracts, 'D' well loaded with 20 µl of root extracts and 'E' well loaded with 20 µl of positive control drug fluconazole. The plates containing the fungal and plant extracts were incubated at 37°C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells (Cheesbrough, 2000). The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

RESULTS

The present study the antifungal activity of seed extracts *viz.* leaf, stem and root were evaluated against five fungal sp (Table 1). In the first stage, leaf, stem and root plant extracts of *Andrographis paniculata* applied on one isolate of each fungal species. Stem extract of *Andrographis paniculata* showed significant antifungal activity against *A. oryzae*, *Penicillium sp* and *C.albicans*. The root extracts was recorded significant antifungal activity against *A. niger*, *A.flavus*, *Penicillium sp*, *C.albicans* and *A.oryzae* on the other hand the leaf extracts revealed significant antifungal effect on *Penicillium sp* and *A.flavus* but did not show same on *C.albicans*, *A.niger*, *A. oryzae*. The inhibitory activities of all the three extracts of the plant reported in the present study were compared with standard drug.

DISCUSSION

Antifungal activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers (Mau *et al.*, 2001). Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. In the present study three extract *viz.* leaf, stem and root were used for extraction. The antifungal activity was significantly recorded in stem and root extracts. This might be due to the better solubility of the active

Table. 1: Antifungal activity of different extract of *Andrographis paniculata*.

S.No	Samples	Zone of inhibition in mm				
		<i>C.albicans</i>	<i>A. niger</i>	<i>A.oryzae</i>	<i>A. flavus</i>	<i>Penicillium sp</i>
1	Control	03	0	02	03	05
2	Leaf	08	07	09	15	17
3	Stem	12	09	14	08	13
4	Root	16	18	15	17	16
5	Fluconazole	19	21	19	21	20

components in the organic solvents (De Boer *et al.*, 2005). The results obtained in the present study, wherein the leaf extracts revealed antifungal effect on only *Penicillium sp* and *A.flavus* (Figure 1), when compared to stem (*A. oryzae*, *Penicillium sp* and *C.albicans*) root (*A.niger*, *A.flavus*, *C.albicans* and *Penicillium sp*) plant extracts this would supplement the above observation. The root extract revealed higher degree of antifungal activity for five fungal spp (*C.albicans*, *A.niger*, *A.flavus*, *A. oryzae* and *Penicillium sp*) out of five tested. However stem extract's antifungal efficacy was on *A. oryzae*, *Penicillium sp* and *C.albicans* revealing that it is less potent compared with that of root extract. The therapeutic value of medicinal plants lies in the various chemical constituents presents it. The bioactivity of plant extracts was attributed to phytochemical constituents.

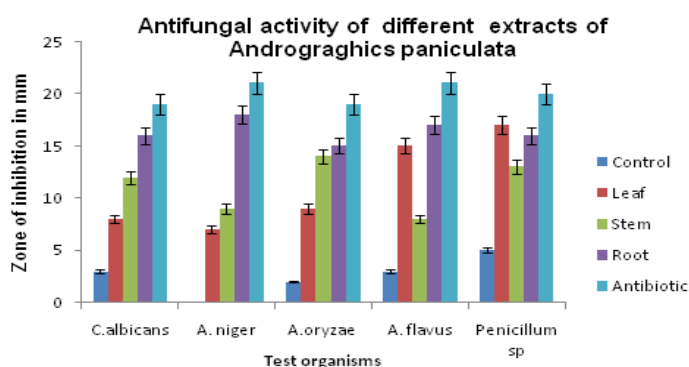


Fig. 1: Antifungal activity of different extract of *Andrographis paniculata*

CONCLUSION

Traditional medicinal plants *Andrographis paniculata* plant extract (root) revealed higher degree of antifungal potency against five fungal species tested. Further studies are required to isolate, identify and elucidate the structure of the bioactive compound.

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