



## EVALUATION OF ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACT OF 'ANDROGRAPHIS ECHIOIDES' – AN INVITRO STUDY

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### ABSTRACT

*Andrographis echioides* is a medicinal plant included in Indian Materia Medica as a remedy for fevers, skin infections. This study was conducted to find out the antifungal activity of the ethanolic extract of the whole plant "Andrographis echioides" using standard agar disc diffusion method. Pulverized whole plant of *A.echioides* was subjected to soxhlet extraction using organic solvent of ethanol. The antifungal activity of *A.echioides* extract was done using standard agar disc diffusion method using Sabouraud Dextrose agar (SDA) medium against five strains of fungi. Amphotericin B was the standard positive control and the negative control was DMSO. 20µl of plant extract in varying concentrations were tested & activity was determined by diameter of the zone of inhibition. The ethanolic whole plant extract of *A.echioides* showed antifungal activity against fungal species *candida albicans*, *Aspergillus flavus*, *Penicillium spp.*, *Aspergillus niger*, *Trichophyton* in 1000µg concentration of the sample showing the zone of inhibition of 6,7,7,8,5 mm respectively. The ethanolic extract of *Andrographis echioides* has shown to have significant antifungal activity against *Aspergillus niger*, *Penicillium* and *Aspergillus flavus* species of fungi.

**KEY WORDS:** Andrographisechioides, antifungal activity, agar disc diffusion method.



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## INTRODUCTION

Opportunistic fungal infections due to diabetes, HIV and the wide use of immunosuppressant drugs have become prevalent worldwide. The treatment of the fungal infections is complicated by the rapid emergence of resistant strains and toxicity of the current antifungal drugs.<sup>1,2</sup> The focus of the researchers is now on the development of novel antifungal drugs from newer resources like plants, proteins and peptides. Plants are abundantly found in nature of which 1-10% is used for medicinal purposes in humans.<sup>3,4</sup> Based on the knowledge that plants develop their own defense against fungal pathogens they also appear as an interesting source for antifungal compounds.<sup>5</sup> Moreover plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides and steroids that have antimicrobial properties.<sup>6</sup> These plant extracts and polyherbal preparations are used in traditional medicine for various diseases including fungal infections from time unknown and widely accepted by people throughout the world. The classical example of plant derived drugs is artemisinin derivatives which have proved valuable in antimicrobial therapeutics. According to a study among 109 new antibacterial drugs, approved in the period 1981–2006, 69% originated from natural products, and 21% of antifungal drugs were natural derivatives or compounds mimicking natural products.<sup>7</sup> The highlights of the currently used antifungal drugs like Amphotericin B and Fluconazole are used for systemic deep fungal infections and topical antifungal preparations like Miconazole, Econazole are used for superficial skin infections like dermatophytes. Most of the commonly used antifungal drugs target fungal ergosterol, an important component of fungal cell wall. The main adverse effects of these antifungal agents are hypersensitivity, nephrotoxicity and hepatotoxicity. Since only a few antifungal drug classes are available we are in a dire need of newer drugs with less toxicity. *Andrographis Echioides* is a tropical medicinal plant widely found in south India and Srilanka. The plant is used in alternative medicine for various infections orally and topically. This plant is reported to constitute variety of phytochemicals like flavonoids, phenols, dihydroechioidinin, androechins, coumarins, lignans, essential oil, monoterpenes, carotenoids, saponins, alkaloids and xanthenes.<sup>8-11</sup> There are many reports on the presence of antimicrobial compounds in various plants but there are no published reports on antimicrobial especially antifungal action of *Andrographis echioides*. The aim of this study is to evaluate the in-vitro antifungal activity of *Andrographis Echioides* in five species of common pathogenic fungi.

## MATERIALS

### Plant Identification

The whole plants of *Andrographis Echioides* were collected from Salem, Tamilnadu. The plant authenticity was confirmed by Research scientist from the department of Botany and a specimen voucher (dated. 17.01.2014) was deposited in the pharmacognosy museum of Siddha Institute of Research, Chennai.

### Plant extract

Ethanol is widely accepted solvent for extracting biomolecules like flavonoids and glycosides specifically. Whole plant sample were taken, cut into pieces, air dried and powdered. 25 gram of powdered sample were taken and extracted with 300ml of ethanol in soxhlet apparatus for 12 hours of time. The solvents were condensed using rotary evaporator. The residue was stored for further analysis. A portion of the residue is used for antifungal assay.

### Test Organisms

Test organisms used for susceptibility testing were *Candida Albicans* (MTCC 1637) *Aspergillus Flavus* (MTCC 9167) , *Penicillium spp*( MTCC 8897), *Aspergillus Niger*(MTCC 404) and *Trichophyton spp*(MTCC 8476) purchased from MTCC, Chandigarh. Sabouraud (SAB) agar or broth is a general growth media for fungi. Stock cultures were maintained at 4°C on Sabouraud Dextrose agar Slant. The culture media and the Amphoterin B discs were purchased from hi media labs, Mumbai, India. Dimethylsulfoxide was purchased from sigma labs, Chennai.

### Methodology

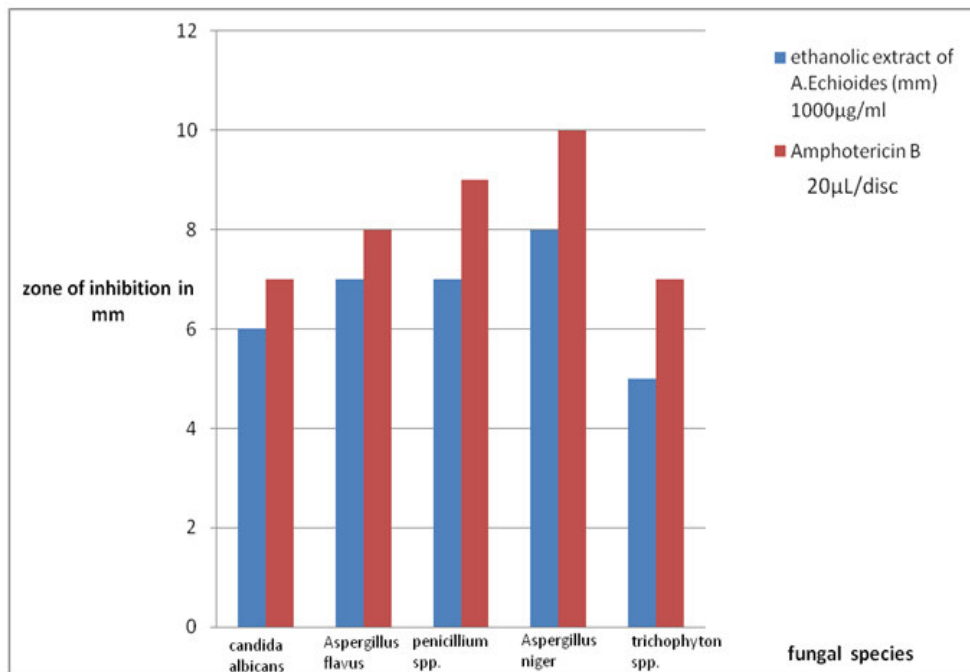
The Assay was performed by agar disc diffusion method.<sup>12</sup> Active cultures for the experiment were prepared by transferring a loop of full of culture from the stock cultures into the test tubes containing Sabouraud Dextrose broth , that were incubated at 48hrs at 37°C. Antifungal activity of the plant extract was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. The disc were placed in SDA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc .The plates were incubated at 37°C for 24 hrs. This procedure was done in triplicate and the mean values were taken as zone of inhibition. The antifungal activity of the plant extract was determined by measuring the diameter of the inhibitory zones using a transparent ruler and compared with those produced by the antibiotic Amphotericin B (20µg/disc) Amphotericin B is used as a positive control and the diameter of zones of inhibition less than 5 mm were considered as insignificant.

## RESULTS

The antifungal activity of ethanolic extract of *Andrographis echioides* was measured by the zone of Inhibition against five common fungal pathogens using agar disc diffusion compared with Amphotericin B (20ug/ml) and DMSO as positive and negative control. The plant extract was prepared in three dilutions 1000µg, 750µg and 500µg and tested against the fungal pathogens. The maximum antifungal activity was shown in 1000 µg concentration of the plant extract. The plant extract showed moderate fungal inhibitory activity in comparison with control drug. The zone inhibition are 8mm for *Aspergillus niger*, 7mm for *Penicillium spp* and *Aspergillus Flavus*, 6mm for *Candida* and 5mm for *Trichophyton spp*. The maximum zone of inhibition of 8mm was shown for *Aspergillus niger* fungal organism against 10mm for Amphotericin B (20µg/disc).

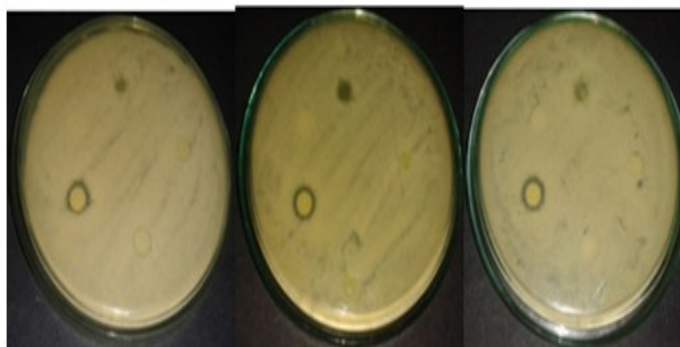
**Table 1**  
**Antifungal activity of ethanolic extract of *A.Echioides* determined by the zone of inhibition using agar diffusion method in various concentrations compared with Amphotericin B as control**

Organisms	MTCC No.	Zone of Inhibition (mm)			Amphotericin B 20µg/disc	DMSO (20µg)
		Concentration(µg/ml)				
		1000	750	500		
<i>Candida albicans</i>	1637	6 mm	3 mm	1 mm	7 mm	-
<i>Aspergillus flavus</i>	9167	7 mm	5mm	2mm	8mm	-
<i>Penicillium spp.</i>	8897	7 mm	6mm	4mm	9mm	-
<i>Aspergillus niger</i>	404	8 mm	6mm	2mm	10mm	-
<i>Trichophyton spp.</i>	8476	5 mm	4mm	1mm	7mm	-

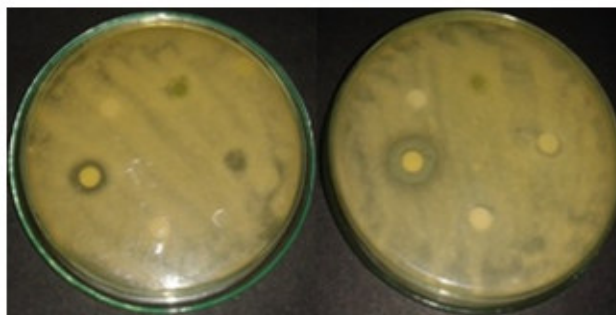


**Graph 1**  
**Antifungal activity of *Andrographis Echioides* Extract (1000µg/ml) in comparison with the control Amphotericin B (20µl/disc) using diameter of zone of inhibition in agar diffusion method.**

Candida albicans      Aspergillus flavus      Trichophyton spp.



**Penicillium spp      Aspergillus niger**



**FIGURE 1**

**Figure showing the zone of inhibition of the *Andrographis Echioides* plant extract in SDA medium**

## DISCUSSION

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the discovery of new lead drug compounds.<sup>13</sup> Ethanolic extract of *Andrographis echioides* in high concentration has shown mild to moderate antifungal activity as measured by the zone of inhibition in the drug impregnated agar disc diffusion method but less than the control drug Amphotericin B. This plant extract showed Maximum zone of inhibition was found to be around 8 mm for *Aspergillus Niger* and 7mm for *Penicillium spp* and *Aspergillus Flavus* strains. Other culture isolates of fungi showed moderate susceptibility with zones of inhibition around 5-6mm with 1000µg concentration of the plant extract. The effects were consistent with replication of tests in various dilutions. So the study results reveal there is a moderate antifungal activity was shown by the ethanolic extract of *Andrographis echioides* when compared with Amphotericin B as a control drug in the assay. Elaiyaraja A et al, reported highest antifungal activity was demonstrated by the ethanolic extracts of *Indoneesiella echioides* (also known as *Andrographis Echioides*) against *Candida Albicans* and *Aspergillus Flavus* fungi, when compared with Nystatin as standard.<sup>14</sup> So our study also showed the moderate activity of *Andrographis echioides* against *Aspergillus Flavus* and additionally we found out this plant extract showed significant antifungal activity against *Aspergillus Niger* and *Penicillus Spp*. Since the major phytoconstituents of *Andrographis Echioides* are flavonoids which are known to be synthesized by plants in response to microbial infection, so it is natural that they are effective antimicrobial substances against a wide range of microorganisms.<sup>15</sup> Chlorine containing

flavonoids like Chlorflavonin, produced by *Aspergillus candidus* have shown antifungal activity.<sup>16</sup> In a study done on the antifungal activity of methanolic and aqueous extracts of *Andrographis paniculata*, a medicinal plant of *Andrographis* family it is proved to have shown antimicrobial activity against bacteria and fungi.<sup>17</sup> Limitations were further studies are needed to isolate and study the active compounds like androechins, andrographolides from *Andrographis echioides* and evaluate the mechanism of action of these phytochemicals against fungi. Purified phytochemicals of this plant if proven for antifungal activity can be potential and adjuvant drugs for mycoses.

## CONCLUSION

*Andrographis echioides* plant extract rich in flavonoids has shown significant antifungal activity against *Aspergillus Niger* than other strains. It has mild to moderate antifungal activity against pathological fungi like *Candida Albicans*, *Aspergillus Flavus*, *Penicillium spp*, and *Trichophyton* but activity was lesser than to the control drug Amphotericin B.

## ACKNOWLEDGEMENT

The authors are thankful to the management of Sri Ramachandra University for supporting the conduction of the study.

## CONFLICTS OF INTEREST

Conflict of interests declared none.

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