

## ACHYRANTHES ASPERA LEAF EXTRACTS INHIBITED FUNGAL GROWTH

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**ABSTRACT:** The aim of the study was investigate antifungal activity of the various leaves extracts of *Achyranthes aspera* Linn. The aqueous, ethanol and methanol leaves extracts of *Achyranthes aspera* Linn. (Family: Amaranthaceae) were evaluated for antifungal activity against clinically important fungal spp viz. *Candida albicans* (MTCC 227), *C. tropicalis* (MTCC 750), *C. krusei* (ATCC 6258), *C. kefyr* (ATCC 4235), *C. guilliermondi* (ATCC 6260), *C. glabrata* (ATCC 2001), *Cryptococcus neoformans* (MTCC 1346), *Aspergillus niger* (MTCC 277), *Aspergillus fumigatus* (MTCC 343), *Aspergillus flavus* (MTCC 418), *Rhizopus oryzae* (MTCC 262). The in vitro antifungal activity was performed by agar well diffusion method. The ethanol extract of the leaves of *Achyranthes aspera* Linn revealed an elevated antifungal activity against *C. kefyr*, *Cryptococcus neoformans*, *Aspergillus niger* and *Aspergillus flavus*. The methanol extract of the leaves showed higher antifungal activity against *Cryptococcus neoformans* and *Aspergillus flavus*. Aqueous extract of the leaves did not show activity against tested fungal strains. The results obtained in the present study suggest that the ethanol and methanol extracts of the leaves of *Achyranthes aspera* Linn revealed a significant scope to develop a novel broad spectrum of antifungal herbal formulation.

**KEYWORDS:** antifungal activity, aqueous extract, ethanolic extract, methanol extract, *Achyranthes aspera* Linn.

### INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources<sup>1</sup>. India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species. The plants are potential source of medicines since ancient times. According to World Health Organization, 80% of the populations in the world depend on traditional medical practitioners for their medicinal needs. Yet a scientific

study on plant to determine their antimicrobial material is comparatively new. Numerous surveys on antimicrobial medicinal plants had been made in United States and in many countries throughout the world. Such study had demonstrated the wide occurrences of active compounds in higher plants<sup>2</sup>.

*Achyranthes aspera* Linn. belongs to the family Amaranthaceae. It is an annual, stiff erect herb, and found commonly as a weed throughout India and used by traditional healers for the treatment of fever, dysentery and diabetes<sup>3,4</sup>. Leaf decoction for cardiovascular toxicity has been reported<sup>5</sup>, and the ethanol crude extract showed high larvicidal activity on the tick larvae against *Boophilis microplus*<sup>6</sup>. The root extract is well reputed for its pronounced insect molting hormonal activity<sup>7</sup> and the ethanolic extract of the leaves and stem of the plant inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* bacterial strains<sup>8</sup>.

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Roots are used as astringents to wounds, in abdominal tumor and stomach pain<sup>9</sup>. The benzene extract of the stem bark shows abortifacient activity in the rat<sup>10</sup>. Leaf extracts were reported to possess thyroid-stimulating and antiperoxidative properties<sup>11</sup>. The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan diabetic rabbits<sup>12</sup>. It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids<sup>13</sup>. The water soluble alkaloid achyranthine isolated from *Achyranthes aspera* possess anti-inflammatory activity<sup>13</sup>. The present study was carried out to test the antifungal efficacy of the leaves extract of *Achyranthes aspera* Linn with reference to fungal spp.

## MATERIALS AND METHODS

### Plant material

The plant material of the leaves of *Achyranthes aspera* Linn were freshly collected during January-March 2009 in and around Edhapattu village (Villupuram Dt, Tamilnadu, India) and were cleaned with distilled water and shade dried at room temperature. The plant was authenticated and a voucher specimen (VCV-8) was kept at the Department of Botany, Voorhees College, Vellore, Tamilnadu, India.

### Preparation of leaf extracts

The powdered leaves (135 g) of *Achyranthes aspera* Linn was extracted separately to exhaustion in a Soxhlet apparatus using aqueous, ethanol and methanol solvent systems. All the extracts were filtered through a cotton plug followed by Whatman filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get 4.87g, 3.82 g and 3.02g yield from aqueous, ethanol and methanol fractions respectively. The extracts were preserved in airtight containers and kept at 4-5°C until further use. All the extracts were tested for antifungal activity against the fungal spp.

### Test organisms

The fungal spp used for the test were *Candida albicans* (MTCC 227), *C.tropicalis* (MTCC 750), *C.krusei* (ATCC 6258), *C.kefyr* (ATCC 4235), *C. guilliermondi* (ATCC 6260), *C.glabrata* (ATCC 2001), *Cryptococcus neoformans* (MTCC 1346), *Aspergillus niger* (MTCC 277), *Aspergillus fumigatus* (MTCC 343), *Aspergillus flavus* (MTCC 418) *Rhizopus oryzae*(MTCC 262. All the stock cultures were obtained from Microbial Type Culture Collection (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.

## ANTIFUNGAL ACTIVITY STUDY

### Agar well diffusion method

The extracts obtained from the leaves were used for studying their antifungal activity. A loop full of fungal strain was inoculated in 30 ml of Sabouraud dextrose broth in a conical flask and incubated for 72 hrs to get active strain by using agar well diffusion method<sup>14</sup>. The media was poured into petridishes. After solidification 0.25 ml of test strains were inoculated in the media separately. Care was taken to ensure proper homogenization.

The experiment was performed under strict aseptic conditions. After the medium was solidified, a well was made in the plates with sterile borer (4mm). The extract compound (50 µl) was introduced into the well and plates were incubated at 37°C for 72 hrs. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition. Controls with ketoconazole was kept for all test strains except *C.kefyr* and *Cryptococcus neoformans* for which Itraconazole was used as control and the control activity was deducted from the test and results were recorded.

Data are expressed as means ±SEM statistical analysis was performed with SPSS (8<sup>th</sup> version). Difference on statistical analysis of data were considered significant at P<0.05.

## RESULTS AND DISCUSSION

In the present study the antifungal activity of plant extracts aqueous, ethanol and methanol was evaluated against eleven fungal spp (Table 1, Fig 1). In the first stage, aqueous, ethanol and methanol leaves extracts of *Achyranthes aspera* Linn applied on one isolate of each fungal species. Ethanol leaves extract of *Achyranthes aspera* Linn showed high antifungal activity against *C.kefyr*, *Cryptococcus neoformans*, *Aspergillus niger* and *Aspergillus flavus*. The methanol leaves extracts showed high antifungal activity against *Cryptococcus neoformans* and *Aspergillus flavus* but aqueous leaves extract did not show antifungal activity against tested fungal strains. Whereas ethanol and methanol leaves extracts showed an intermediate activities against *C.albicans*, *C.tropicalis*, *C.krusei*, *C.glabrata*, *C. guilliermondi* *Aspergillus fumigatus* and *Rhizopus oryzae*. The inhibitory activities of all the extracts reported in Table 1 are comparable with standard antimicrobics Ketoconazole (30mg) and Itraconazole (30mg).

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but our studies showed that ethanol, methanol extracts of these plants were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvent<sup>15</sup>. In the present study the ethanol leaves extract revealed higher degree of antifungal activity for *C.kefyr*, *Cryptococcus neoformans*, *Aspergillus niger* and *Aspergillus flavus* when compared with that of other fungal spp tested.

However, the antifungal activity of methanol leaves extract recorded less potent in comparison to ethanol leaves extract. Similar studies elsewhere recorded antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus subtilis*<sup>16</sup>. The antifungal activity of *Achyranthes aspera* Linn against test strains such as *C.albicans* , *C.tropicalis*, *C.krusei* , *C.kefyr* , *C.glabarata* *C.guilliermondi* , *Cryptococcus neoformans*, *A. niger*, *A. fumigates* , *A. flavus* and *Rhizopus oryzae* compared to control can be attributed to the chemical profile of the extracts containing saponins ,alkaloids etc.

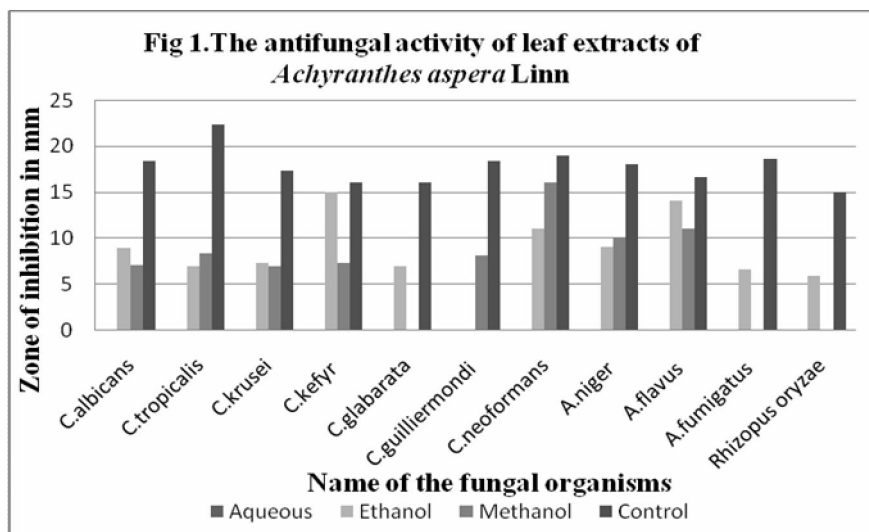
**CONCLUSION**

The results of present study supports the traditional usage of the studied plants and suggests that some of the plant 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. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

**Table 1. The antifungal activity of leaf extracts of *Achyranthes aspera* Linn.**

Microorganisms	Zone of the inhibition in mm				
	AQ	ET	MT	Control 1	Control 2
<i>C.albicans</i>	-	8.96±0.88	7.13±0.80	18.33±0.33	ND
<i>C.tropicalis</i>	-	7.03±0.14	8.33±0.33	22.33±0.32	ND
<i>C.krusei</i>	-	7.33±0.31	7.00±0.01	17.31±0.30	ND
<i>C.kefyr</i>	-	15.00±0.57	7.33±0.57	ND	16.00±0.53
<i>C.glabarata</i>	-	7.00±0.01	-	16.00±0.52	ND
<i>C.guilliermondi</i>	-	-	8.10±0.57	18.33±0.31	ND
<i>Cryptococcus</i>					
<i>Neoformans</i>	-	11.03±0.88	16.03±0.14	ND	19.00±0.57
<i>A.niger</i>	-	9.06±3.78	9.96±0.3	18.03±0.34	ND
<i>A.flavus</i>	-	14.06±0.66	11.10±0.15	16.66±0.32	ND
<i>A.fumigatus</i>	-	6.66±0.33	-	18.66±0.30	ND
<i>Rhizopus oryzae</i>	-	5.96±0.33	-	15.00±0.57	ND

**Key words:** AQ-Aqueous extract,ET-Ethanol extract,MT-Methanol extract, Control 1- Ketoconazole,Control 2- Itraconazole, “-“No activity, ND-Not done.



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