

Original Research Article

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Evaluation of Antifungal Activity of *Amaranthus spinosus* L. (Amaranthaceae)

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ABSTRACT

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The plants have investigated in the search for new substances against microorganisms resistant to current pesticides and antibiotics. The various organic extracts of *A.spinosa* were evaluated for *invitro* antifungal activity against some yeasts including two yeasts viz. *Candida albicans* SRTCC (3971) and *Saccharomyces cerevisia* SRTCC (1781) and three fungi viz. *Aspergillus niger* SRTCC (1073), *Fusarium oxysporium* SRTCC (611) and *Aspergillus flavus* SRTCC (1072). The *in vitro* antifungal activity was evaluated at different concentrations (1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml) by agar disc diffusion method. *A.niger* were the most susceptible fungal strain while *F.oxysporium* was the most resistant one. The results were compared with the standard antifungal. Through this research was showed the presence of antifungal constituents in extracts of *Amaranthus spinosus* L., revealing its potential against these pathogenic strains tested.

Introduction

Traditional and folklore medicines play important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. The subcontinent is rich in medicinal plants and is one of the richest countries in the world as regards genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Krishnaraju *et*

al., 2005). Several plants have been used in folklore medicine (Premanathan *et al.*, 2000). The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents (McNeil *et al.*, 2001).

The plant *Amaranthus spinosus* Linn. (Amaranthaceae) (Kirtikar *et al.*, 1999) is an annual herb found in throughout India and also many tropical countries. Traditionally the plant is used to treat various diseases.

Although the leaves part are used as a laxative and an applied as an emollient poultice to abscesses, boils and burns and reported as antimalarial (Hilou *et al.*, 2006) antioxidant (Amin *et al.*, 2006) and antihepatotoxic actions. (Yang *et al.*, 1987) The presence of spinoside, new coumaroyl flavone glycoside, (Azhar-ul-Haq *et al.*, 2004) lectins, (Singh *et al.*, 1993) betacyanins and phenolic compounds has been reported. (Stintzing *et al.*, 2004) Pharmacognostic studies have not been reported for the leaves of this plant. *Candida albicans*, the agent of candidiasis, is an increasingly important disease that has a worldwide distribution due to the fact that it is a frequent opportunistic pathogen in AIDS patients (De Pavia *et al.*, 2003). It is a common commensal of the gastrointestinal and urogenital tracts of human (Black, 1996) and is also the cause of Candiasis in women (Demarch *et al.*, 1995). There are alarming reports of opportunistic fungal infections. The infections caused by opportunistic fungi are included under new spectrum of fungal pathogens. Such fungi were earlier reported from various plants as pathogens (Singh, 2001). But now they are known to cause disease in human being.

Material and Methods

Plant material

Amaranthus spinosous (VTJ 30) was collected from various districts of Marathwada. The plants were collected and identified by using standard flora (Naik, 1979) and also authenticated by Botanical Survey of India, Pune. The collected plant materials (leaves, seeds) were separated and washed several times with distilled water to remove foreign material then it is air dried for 24 h. Thereafter, they were dried in oven at 25⁰C for 7 days. The dried leaves and seeds were powdered using mechanical grinder and stored in schott bottles until use.

Extraction of plant material

The 10g leaf powder of *A. spinosous* was extracted using Soxhlet apparatus for 6 to 8 hours with the solvents of increasing polarity viz. petroleum ether(60-80⁰C)>ethanol (60⁰C)> methanol (65⁰C)>distilled water (100⁰C) etc. Extracts were filtered, evaporated and weighted. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) for further use of plant extracts.

Fungal Cultures and growth media

The two yeasts viz. *Candida albicans* SRTCC (3971) and *Saccharomyces cerevisia* SRTCC (1781) and three fungi viz. *Aspergillus niger* SRTCC (1073), *Fusarium oxysporium* SRTCC (611) and *Aspergillus flavus* SRTCC (1072) were used as test organisms. The cultures were purchased from School of Life Sciences, Nanded. The yeast & fungi cultures were maintained on nutrient agar and Potato Dextrose Agar slants at 30⁰C respectively. Standard antifungal agent Amphotericin B was used and dissolved in sterile distilled water and prepared as a stock of 1 mg/ml concentration.

Antifungal assay

Paper Disc Method

The yeast cultures were recovered by growing from stock cultures in Potato Dextrose Broth for 24 h at 37⁰C. The moulds were grown on Potato Dextrose Agar at 28⁰C for 4 to 7 days until sporulation. Sterile distilled water containing the fungal spores (10⁶ spores/ml) were poured over the Potato Dextrose Agar (PDA) base plates. The 50 µl extract of each were used to prepare different concentrations (1000 µg/ml, 500 µg/ml, 250

µg/ml, 100 µg/ml) then they are transferred on each of three sterile discs having diameter 9 mm (Whatman No. 1). The 50 µl of solvent served as the negative control, and 5 µg/ml Amphotericin B was used as a positive control. Each plant extracts and control were tested in triplicate. The plant extracts and solvent extracts impregnated discs were dried in sterile Petridishes and incubated at 30°C. Antifungal activities were recorded as the width (mm). (NCCLS 1997)

Results and Discussion

Antifungal assay of *A. spinosus*

Petroleum ether extract of 500µg/ml and 100µg/ml had maximum activity against *C. albicans* (17.50mm), *A. niger* (17.50mm). *F. oxysporium* (15.50mm) exhibited considerable activity at 1000 µg/ml concentration (Table No.1). The 500µg/ml concentration of ethanolic extract had maximum zone of inhibition against *A.niger* (16.50mm) which was followed by *A. flavus* at 100µg/ml (15.25mm) (Table No.2).

Methanolic extract of 100µg/ml concentration showed maximum antifungal activity with 16.50 mm inhibitory zone against *A. flavus*, whereas *A. niger* with 16.50mm zone of inhibition at 500µg/ml concentration had moderate activity (Table No.3). Distilled water extract of 100 µg/ml of *C. albicans* exhibited 16.50mm inhibitory zone whereas *A. niger* at 1000µg/ml showed 16.3mm inhibitory zone (Table No.4).

It is also important to note that susceptibility of the pathogens was different with solvent extract and aqueous extract. This indicates the presence of more than one active principle in these plants. Plants are a rich reservoir of antimicrobials. It is observed that a single plant is known to contain several active principles, which are compounds of biological significance. The findings of Bruna Carminate (2012) supports the antifungal efficacy of *Amaranthus viridis* against *Colletotrichum musae* causing anthracnose of banana and against *Fusarium solani* f. *piperis* responsible for fusariosis in black pepper.

Table.1 Screening of Antifungal activity of Petroleum ether extracts of *A. spinosus*

Organism	Zone of inhibition in mm				Standard	control	
	Conc.µg/ml	100	250	500			1000
<i>C .albicans</i>		11.50±0.19	12.00±0.19	17.50±0.19	13.50±0.19	13.3±0.40	-----
<i>S .cerevisia</i>		14.20±1.38	15.20±0.00	14.75±0.25	12.13±0.44	18.6±3.00	15.6±0.57
<i>A .niger</i>		17.50±0.19	12.13±0.44	12.70±0.00	-----	17.33±1.15	-----
<i>F. oxysporium</i>		14.13±0.23	12.25±0.00	12.70±0.00	15.50±0.19	20.6±1.15	-----
<i>A .flavus</i>		14.00±0.46	10.25±0.19	09.50±0.00	11.50±0.00	24.6±1.15	-----

Table.2 Screening of Antifungal activity of Ethanol extracts of *A. spinosus*

Organism	Zone of inhibition in mm				Standard	Control
	100	250	500	1000		
Conc. µg/ml	100	250	500	1000	Amphicillin	
<i>C. albicans</i>	14.20±1.38	11.50±0.00	14.63±0.18	12.6±0.57	19.1±0.20	-----
<i>S. cerevisia</i>	11.50±0.19	12.3±0.57	14.20±1.38	12.00±0.19	10.90±0.00	13.6±0.30
<i>A. niger</i>	10.25±0.19	13.3±0.57	16.50±0.19	10.90±0.00	20.5±0.30	16.6±1.15
<i>F. oxysporium</i>	13.70±0.19	13.6±0.57	12.6±0.57	12.13±0.44	19.1±0.20	-----
<i>A. flavus</i>	15.25±0.00	11.6±0.57	13.6±0.57	11.50±0.19	20.5±0.30	-----

Table.3 Screening of Antifungal activity of Methanol extracts of *A. spinosus*

Organism	Zone of inhibition in mm				Standard	Control
	100	250	500	1000		
Conc. µg/ml	100	250	500	1000	Amphicillin	
<i>C. albicans</i>	12.70±0.00	16.25±0.25	10.3±0.57	14.20±1.38	16.6±2.30	10.3±0.57
<i>S. cerevisia</i>	16.50±0.19	13.0±0.00	9.6±1.52	12.00±0.19	20.0±0.00	-----
<i>A. niger</i>	14.20±1.38	9.3±0.57	16.6±2.30	11.50±0.19	17.3±1.13	12.0±0.00
<i>F. oxysporium</i>	10.25±0.19	10.6±0.57	17.3±1.13	14.13±0.23	23.3±1.15	-----
<i>A. flavus</i>	17.50±0.19	9.0±0.00	9.6±1.52	10.6±0.57	19.3±1.15	9.6±0.57

Table.4 Screening of Antifungal activity of distilled water extract of *A. spinosus*

Organism	Zone of inhibition in mm				Standard	control
	100	250	500	1000		
Conc. µg/ml	100	250	500	1000	Amphicillin	
<i>C. albicans</i>	16.50±0.19	12.3±0.57	11.50±0.19	11.3±3.21	20.6±1.15	-----
<i>S. cerevisia</i>	11.25±0.00	10.6±0.57	16.00±0.27	11.6±0.57	15.3±1.10	15.3±1.10
<i>A. niger</i>	17.5±0.50	13.0±1.00	10.25±0.19	16.3±1.52	22.3±1.50	-----
<i>F. oxysporium</i>	09.25±0.25	9.6±0.57	13.70±0.19	12.13±0.44	21.0±1.00	11.3±0.57
<i>A. flavus</i>	11.3±3.21	14.75±0.25	15.25±0.00	11.3±1.15	14.3±1.50	-----

In addition to this antifungal efficacy of *A.spinosus*, *A.tricolor*, *A.aspera*, *A.viridis* and *A.hybridus* extracts were tested against plant pathogenic fungi causing diseases to various crop plants like *Fusarium oxysporium f.var.vasinfetum* strains, *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Aspergillus flavus*. The petroleum ether, ethanol, methanol, distilled water extracts were used in the present study of opposite polarity. This helps in determining the activity of compounds of different polarities. Biological activities in man and animals. The active components usually interfere with growth and metabolism of microorganisms in a negative manner.

In conclusion, methanolic extract of *A.spinosus* of 100 and 500µg/ml concentration shows maximum inhibitory zone of 17.50 mm against *A.niger* and *C.albicans* which is more in comparison to standard antimicrobial agent i.e. Amphotericin. Through this research was showed the presence of antifungal constituents in extracts of *Amaranthus spinosus* L., revealing its potential antimicrobial effect against these pathogenic strains tested.

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