Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

A COMPARATIVE STUDY: DIFFERENTIAL ANTIMYCOSES ACTIVITY OF CRUDE LEAF EXTRACTS OF *CALOTROPIS SPP*.

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Received: 26 Mar 2012, Revised and Accepted: 28 May 2012

ABSTRACT

The differential antimycoses activities of chloroform, methanol and ethyl acetate extracts of *Calotropis spp* were evaluated against dermatophytes and *Aspergillus flavus* using agar-well diffusion assay. *Calotropis gigantea* producing purple colour flower (P), *Calotropis gigantea* producing white colour flower (W) and *Calotropis procera* (R) were taken as samples for the activity. White flowered *C. gigantean* (W) showed good activity in comparison with other samples. Chloroform extracts showed highest activity, methanol extracts showed moderate activity and ethyl acetate extract showed least activity.

Keywords: *Calotropis spp*, Leaf, Agar well diffusion, Differential antimycoses.

INTRODUCTION

Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents[1]. The most serious and widest range of fungal infections are found in tropical developing countries[2]. Dermatophytes and *Aspergillus spp* are among this group of pathogens. Skin, hair, nail and subcutaneous tissues in human and animal are subjected to infections by dermatophytes[3,4]. Dermatophytic infections are caused by 40 species of fungi which are grouped into three genera viz., *Trichophyton, Microsporum* and *Epidermophyton*[5]. *Aspergillus flavus* cause pulmonary aspergillosis, aspergilloma, pneumonia, invasive stomatitis[6-9]. Aflatoxins produced by *A. flavus* are toxic and carcinogenic[10]. They cause severe aflatoxicosis leading to hemorrhagic necrosis of the liver, bile duct proliferation, edema and lethargy[11].

Medicinal plants are gifts of nature to cure number of diseases among human beings[12,13]. The traditional medicine related to treatment of both human and animal mycoses with plant-derived preparations is considered a valuable knowledge for the discovery of new antimicrobial drugs[14]. A lot of work have been under taken with reference to anti dermatophytic and anti *Aspergillus flavus* activities of plants[15-28]. *Calotropis is* one of such important traditional medicinal plants reported to be used in distinct ways to treat infections caused by dermatophytes[29-33] and *Aspergillus* flavus[34-41].

Calotropis has two species viz., *Calotropis gigantea* and *Calotropis procera*. The species of *C. gigantea* has flowers ranging from purple to white colour. In traditional medications the local healers prefer white flowered *C. gigantea* over purple flowered *C. gigantea*. For this reason these two C. *gigantea* plants were considered as two different samples. Further work was carried out considering purple flowered *C. gigantea* (P), *C. gigantea* white flowered (W) and *C. procera* (R). The present work was thus designed to determine the differential antimycoses activity of crude leaf extracts of these three samples in various solvents.

MATERIALS AND METHODS

Collection of plant material

The leaf material was collected from Gulbarga University, Gulbarga campus. The material was air dried at room temperature for 10 to 15 days, powdered and stored in airtight container for further use.

Preparation of extracts

Powdered plant materials (100g) were extracted successively with 500 ml of ethyl acetate, methanol and chloroform in a soxhlet apparatus. The extraction was stopped when the solvent was drained colourless. The solvent was completely removed and extracted with successive solvents. These extracts were air dried and later serially diluted in dimethyl sulphoxide (DMSO) to yield required concentrations for antimycoses activity.

Test microorganisms

A total of five fungi were used in the screening viz., *Aspergillus flavus, Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophyte, Epidermophyton floccosum.* The Microorganisms were obtained from laboratory, Department of Botany, Gulbarga University, Gulbarga.

Morphology of flowers of samples P, W and R

Antimicrobial activity test

About 15 to 20 ml of Sabaraud's dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using 5 days old culture strain. 1 ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 6 mm diameter were punctured in the culture medium. Required concentrations of serially diluted extracts (50, 100, 150, 200 and 250mg/ml) were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37^{0}C. After incubation for 48 h, the plates were observed for zone of inhibition surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters. Dimethyl sulphoxide (DMSO) was used as a negative control.

RESULT

The data pertaining to antimycoses potential of various extracts are tabulated in Table 1, 2 *and 3.*

Table 1: Antimycoses activity of chloroform extracts of leaves of *Calotropis spp*

Sample	Conc. of extract (mg/ml)	Zone of inhibition *T.r T. t T. m E. f A .f*				
P	50	-	-	-	-	-
	100	-	-	-	-	-
	150	-	3	-	-	-
W	50	-	-	1	1	1
	100	4	-	3	4	2
	150	-	-	-	-	-
R	50	-	2	1	2	1
	100	-	-	1	0	0
	150	-	3	-	1	1

Table 2: Antimycoses activity of methanol extracts of leaves of *Calotropis spp*

Sample	Conc. of extract (mg/ml)	Zone of inhibition *T.r T. t T. m E. f A .f*				
P	50	-	-	-	-	-
	100	2.5	-	-	-	-
	150	3.5	-	-	-	-
W	50	5	-	-	1	-
	100	3	-	-	7	-
	150	5	-	-	-	-
R	50	-	-	-	-	-
	100	-	-	-	-	-
	150	-	-	-	-	-

Table 3: Antimycoses activity of ethyl acetate extracts of leaves of *Calotropis spp*

Sample	Conc. of extract (mg/ml)	Zone of inhibition *T.r T. t T. m E. f A .f*				
P	150	-	-	-	-	-
	200	-	-	-	-	-
	250	-	-	2	-	-
W	150	-	-	-	-	-
	200	-	-	-	-	-
	250	5	-	1	5	-
R	150	-	-	4	6	5
	200	-	-	6	10	8
	250	-	-	11	11	10

*T.*r- Trichophyton *rubrum, T.t- Trichophyton tonsurans, T.m- Trichophyton mentagrophyte,*

E.f- Epidermophyton floccosum,. A.f- Aspergillus flavus, - - no zone of inhibition.

P- purple flowered *C. gigantea,* W- white flowered *C. gigantea,* R- *C. procera*.

DISCUSSION

All three samples showed differential antimycoses activity. The chloroform extracts showed highest activity, methanol extracts showed moderate activity and ethyl acetate extracts showed lowest acitvity. All fungi responded to chloroform extracts. Methanolic extract was active against *T. rubrum and E. floccosum*. Ethyl acetate extract showed antimycoses activity against *T. rubrum, T. mentagrophytes, E. floccosum* and *A .flavus*. Among tested fungi *T. tonsurans* showed more resistivity and was found to be susceptible to chloroform extracts of purple flowered C. gigantea and C. procera.

Chloroform and methanol extracts showed activity at concentration of 50mg/ml whereas ethyl acetate showed activity at high concentration of 150mg/ml. Chloroform and methanol extracts of both samples of *C. gigantea* showed better activity than *C. procera* extract. The ethyl acetate extracts of C. procera was better compared to both samples of *C. gigantea*. Among samples of *C. gigantea*, white flowered *C. gigantea* showed better activity than purple flowered *C. gigantea*.

C. gigantea showed good antimycoses activity in comparison with *C. procera.* As per information from traditional practitioners, it was found that white flowered *C. gigantea* showed better activity than purple flowered *C. gigantea*.

ACKNOWLEDGEMENT

The first author is thankful to the Chairman, Department of Botany, Gulbarga University, Gulbarga, for providing laboratory facilities and also to University Grants Commission for providing Rajiv Gandhi National Fellowship. Authors are thankful to traditional healers for providing information regarding the plant.

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