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Antifungal activity of *Swietenia mahogany* on *Candida albicans* and *Cryptococcus neoformans*

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The present study was carried out to evaluate the antifungal activities of aqueous and solvent extract of *Swietenia mahogany* which is used as a traditional folk medicine in India for the treatment of different infectious diseases and disorders. The antifungal activities of the extracts were tested against *Cryptococcus neoformans* and *Candida albicans* by disc diffusion assay and broth dilution method to determine minimum inhibitory concentration. Extracts were prepared from aerial part of *S. mahogany* with various organic solvents and the concentrated extracts were reconstituted in dimethyl sulphoxide which was used for anti fungal assay. Among the different extracts tested, methanol revealed distinct anti fungal activity against both tested strains. Minimum inhibitory concentration (MIC) of the methanol extract against *C. neoformans* and *C. albicans* was found to be 200 and 1000 µg/ml, respectively.

Key words: *Cryptococcus neoformans*, *Candida albicans*, *Swietenia mahogany*, antifungal activity, extracts.

INTRODUCTION

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (World Health Organization, 1998). During the past several years, there has been an increasing incidence of bacterial and fungal infections in immunocompromised populations such as during organ transplants, cancer and HIV/AIDS. This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antimicrobial drugs (Giordani et al., 2001) has been the reasons for an extended search for newer drugs to treat opportunistic microbial infections (Fostel and Lartey, 2000). Antifungal therapy offers a unique challenge, primarily because fungi are eukaryotic organisms that share a common line with their mammalian host in terms of replication and metabolism (Polak, 1998), and secondarily because the physically tough cell wall, offering considerable chemical fungus is well protected inside a chemically complex and and steric hindrance to the drug molecule. Although, only few fungi (*Histoplasma*, *Coccidioides*, *Blastomyces* and *Paracoccidioides*) are known to be potentially pathogenic (Rippon, 1982), the commonest occurring infections (oral thrush, oesophageal and vulvo-vaginal thrush, etc.) are caused by normal flora fungi that cause infection when the opportunities arise, e.g. *Candida* spp. and other yeast like fungi. The predisposing factors include excessive use of antibiotics that disturb microbial equilibrium at mucosal surfaces, drug induced immune suppression as in the case of transplant and cancer patients and acquired immunodeficiency as a result of HIV infection. Although, the phenomenon of multi-drug resistance is better recognized and characterized in the case of cancer and bacteria, it is also an emerging problem in antifungal chemotherapy, thus making worse the already difficult situation resulting from an increased number of immunocompromised patients, the appearance of new human pathogenic fungi and the very limited number of available antifungal drugs. Long-term antifungal treatment

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with the commonly used antifungals, such as Amphotericin B has toxic effects; ketoconazole, fluconazole and clotrimazole are limited in their spectrum and efficacy and use may result in strain resistance (Helmerhorst et al., 1999; Wakabayashi et al., 1998). Hence, considerable research is being directed for the screening of lead compounds with a defined mechanism of action that can serve as template for further medicinal chemistry modifications (Fostel and Lartey, 2000).

Cryptococcosis is generally believed to be contracted by inhalation of infectious particles, which might be either spores or desiccated yeast cells (Mokoka et al., 2010; Lemos et al., 2005). In immunodeficient hosts, the infection can be disseminated from the lung to other sites, particularly the central nervous system, although frequent targets also include the skin, eyes and prostate. The most common and devastating consequence of dissemination is Cryptococcal meningitis, which is fatal if not treated. The current treatment for Cryptococcosis is not satisfactory, owing to the toxicity of existing therapies, their limited ability to clear infections completely and the emergence of drug resistant strains (Sagarika and Pratima, 2012) *Swieteniam mahogany* (Meliaceae) is a valuable timber tree native to the new world tropics. Some of the bioactivities reported for extracts from *S. mahogany* include activity against hypertension, diabetes, malaria (Eiichi et al., 1990; Hisao et al., 1990), helminthis, ameobiasis, cough, cancer and HIV (Hattori et al., 2000), inflamatory, mutagenicity (Atilado et al., 1996), astringent, wound healing Kritikar and Basu 1999 and antipyretic (CSIR,1976). *S. mahogany* has been found to contain the following chemical constituents: methyl anglonsate, methyl 6-hydroxyangolensate, 3-o-acetylswietenolide, swietemahonin(A-G), 6-O-acetyleswietenolide, 3-O-tigloyl-6-O-acetylswietenolide, swietenine, swietenine acetate, swietenolide, 3, 6-O, O-diacetylswietenolide, proceranolide, 7-deacetoxy-7-oxogedunolide, 3-O-tigloylswietenolide, swietemahonin (A,E,F) mahonin, secomahonin (Hisao et al., 1990), cyclomahogenol (Chatterjee et al., 1960), swietemahonins G, khayasin T in *S. mahogany* seeds, melianone (triterpene) in *S. mahogany* dried leaves, mahoganin (Hisao et al., 1990), swietemahonolide in *S. mahogany* cotyledons of seeds (Allan et al., 1999).

MATERIALS AND METHODS

Plant material

S. mahogany plantlets were purchased from the nurseries of Theosophical Society, Adayar. Leaves from adult (>20 years) trees were collected. They were cleaned and air dried. A herbarium for these trees was also prepared and verified with samples from the forest department that was identical.

Extraction

S. mahogany dry leaves were powdered, sieved and stored for further work. One hundred grams of dried material of *S. mahogany* was extracted with ethanol, methanol, chloroform, petroleum ether, and water. The extraction was repeated twice. The filtrate was then lyophilized to a powder and stored in a vacuum at 4°C. For the experiments, the powder was dissolved and diluted in distilled water to various concentrations.

Evaluation of anti fungal activity of crude extract

Fungal strain

Candida albicans and *Cryptococcus neoformans:* standard ATCC strains obtained as a gift from clinical laboratory was used for antifungal assays. 37 clinical isolates and standard ATCC strains were used in this study which were obtained from the Department of Dermatology, Sri Ramachandra Medical College and Research Institute.

Fungal inoculum preparation

C. albicans and *C. neoformans* inoculum were prepared according to NCCLS method (M27-A2) (National Committee for Clinical Laboratory Standards, 2002; Arthington et al., 2000). The transmittance was adjusted at 530 nm, which was equivalent to 0.5 Mc Farland standards. This saline suspension contains 1 to 5 x 10^6 CFU/ml.

Anti fungal assay

In order to identify the antifungal activity of total extracts and fractions (1 mg/ml) against *C. neoformans* and *C. albicans*, agar diffusion assay was performed in Brain Heart Infusion (BHI) culture media (pH 6.5). Cultures were grown in MSDA broth for 3 days at 37°C. Fungal cells were obtained by centrifugation at 1500 g at 4°C for 15 min and diluted in buffered saline, pH 7.2 and adjusted to 10^6 cells/ml. In BHI agar plates, 100 µl of fungi (final concentration) were spread and 25 µl of each total extracts and fractions were applied to sterile discs surfaces. The plates were refrigerated for 2 h in order to stop fungal growth and facilitate diffusion of the substances. Plates were then incubated at 37°C for 48 h (Schmourlo et al., 2005).

For MIC evaluation, the fungi (10^6 cells/ml) were incubated in fresh BHI medium in the presence of various concentrations (1 pg/ml to 1 mg/ml) of active total extracts and fractions at 37°C for 48 h. Cell growth was determined daily by visible turbidity and recorded (Newton et al., 2002). The lowest concentration of each active sample that prevented fungal growth was considered as MIC. Amphoterecin B was used as positive control in both methods.

Minimum fungicidal concentration

The minimum fungicidal concentration (MFC) was determined using the method of Rotimi et al. (1988) .The samples in MIC studies which showed no visible growth after incubation were sub-cultured onto Sabouraud's dextrose agar plate using an inoculum of 0.01 ml. The plates were incubated at 25°C for 48 h for yeast and 7 days for filamentous fungi. The MFC was regarded as the lowest concentration of the extracts that prevented the growth of any fungal colony on the solid medium.

Thin layer chromatography

Silica gel alumina (Merck, India) backed plates (8 x 8 cm) were used. Either crude plant extracts (1 mg) or fractions (0.5 mg) (0.01

Table 1. Antifungal activity of *S. mahogany* by disc diffusion assay method at a concentration of 300 µg/disc.

Plant	Fungi	Inhibition zone diameter (mm)				Amphotericin B 20 (mm)
		Aqueous extracts	Solvent extracts			
			Ch	E	M	PE
S. mahogany	*C. albicans*				12±0.5	6±0.2
	C. neoformans	5±1		16±5	22±1.5	14±1

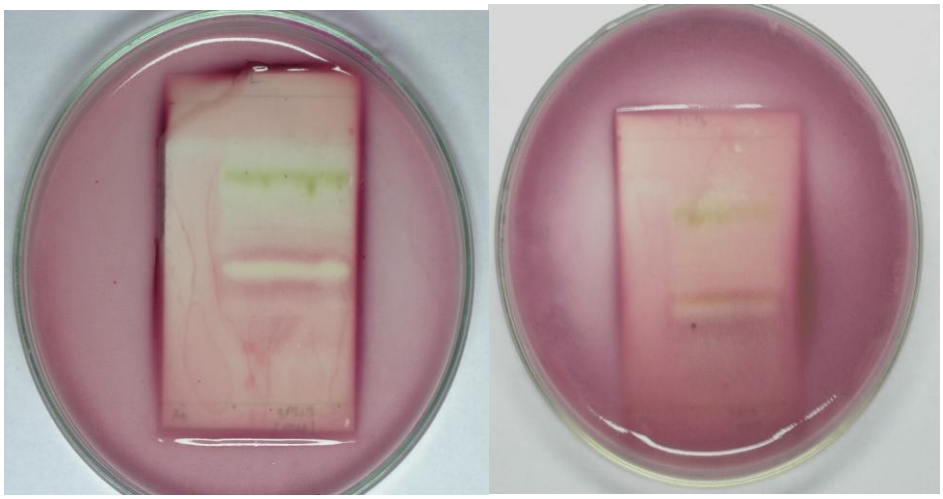


Figure 1. Bioautography pattern of *S. mahogany.*

mg of each sample) were applied to TLC plates. The plates were eluted with Ethyl acetate : chloroform (E: C) 1:2. All TLC plates were run in duplicate and one set was used as the reference chromatogram. The UV active absorbing spots were detected at 254 and 366 nm on the reference chromatogram, which was finally stained with Ceric sulfate.

Bioautography

Chromatograms were placed in 9 x 9 cm sterile Petri dishes with covers and exposed to UV light for 30 min. Overlay media (BHI with phenol red 1%, 10 ml), to which was added 1 ml of the detecting strain at a final concentration of 10^6 cells/ml, was distributed over the developed TLC plates. After solidification of the media, the TLC plates were incubated for 48 h at 37°C. The bioautograms were sprayed with methylthiazolytetrazolium chloride (MTT), 5 mg/ml (w/v) for observation of the inhibition zones (Saxena et al., 1995).

RESULTS AND DISCUSSION

Plants have been rich source of medicines because they produce a host of biomolecules most of which probably involved chemical defenses against predation or infection. These may be a multitude of compounds like glycosides, alkaloids, terpenes, essential oils, steroids, hormones, vitamins, enzymes, plant acids, sugars, starches, fats, waxes, oleoresins, oleogum-resins, resins, balsams etc. Indians have been using several common medicinal plants for their therapeutic use (Sagarika and Pratima, 2012). In the present study, the antifungal activity of the solvent extracts and aqueous extracts of *S. mahogany* were evaluated *in vitro* against *C. albicans* and *C. neoformans*. In disc diffusion assay, methanol extract of *S. mahogany* was found to be most effective against *C. neoformans* by producing an inhibition zone of 22 ± 1.5 mm followed by petroleum ether and ethanol extracts with 14.0 and 16.0 mm (Table 1). Anti fungal activity was not recorded in aqueous and chloroform extracts in the case of *C. albicans,* 17.5 mm of zone of inhibition was recorded in methanol extract.

Minimum inhibitory concentration of 200 µg/ml of *S. mahogany* methanol extract was found to effectively inhibit the growth of *C. neoformans* but comparatively a higher dose of 1000 µg/ml of the extract is required to inhibit the growth of *C. albicans*. Minimum inhibitory concentration of the extracts was found to effectively inhibit the growth of the fungi when introduced into suitable solid medium which confirms the inhibitory concentration as an effective fungicidal activity (Table 2).

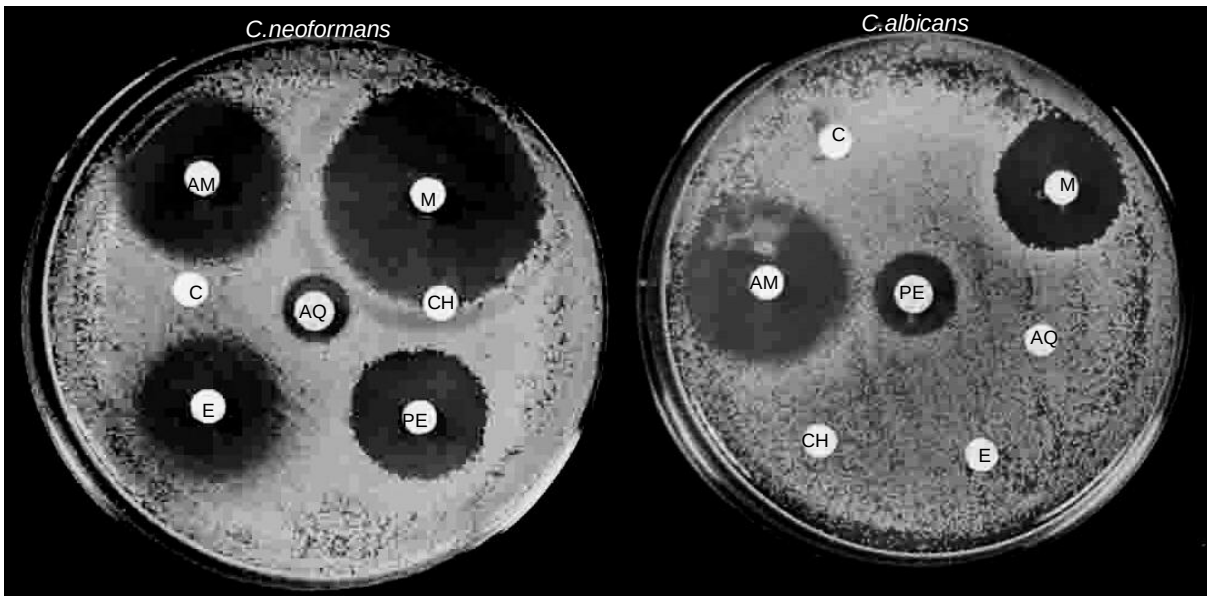
The bioautography of the plant extracts showed antifungal activity against C*. neoformans*, using ethyl acetate : chloroform (1:2) to develop silica gel TLC plates (Figure 1). Clear inhibition zones at an R_f of 0.44 using methanol extract of *C. albicans* and R_f of 0.71 using methanol extract of *S. mahogany* was found against *C. neoformans* (Table 3). The results of the present work indicate that *S. mahogany* possess antifungal properties,

Table 2. Minimum inhibitory concentration of *S. mahogany* extracts against clinical isolates of fungi.

Plant	fungi	Inhibition zone diameter (mm)	Amphotericin B 20 (mm)			
		Aqueous extracts	Solvent extracts			
			Ch	E	M	PE
S. mahogany	*C. albicans*	1800	1500	1300	1000	1300
	C. neoformans	400	300	250	200	250

Table 3. Bioautography profile of *S. mahogany*.

Sample	Solvent	Running Solvent	R_f	Visible light (colour rection)	R_f	UV light (colour reaction)	R_f	Sprayed reaction colour
SM13metha	Methanol	Ethyl acetate:	0.43	Light green	0.43	Light green	0.05	Yellow
nol(leaf		chloroform	0.58	Dark green	0.58	Dark green	0.10	Yellow
powder)		(1:3)	0.60	Light green	0.60	Light green	0.36	Brown
			0.65	Red	0.65	Red	0.40	Brown
							0.50	Brown
							0.63	Dark green
							0.76	Yellow



PE - Petroleum ether, CH – chloroform, E– ethanol, M – methanol,
AQ – aqueous extracts, C– control solvent, AM – Amphotericin B

Figure 2. Zone of inhibition of solvent extracts of *S. mahogany* against *C. neoformans* and *C. albicans.*

which explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve fungal infections, and underline the importance of the ethnobotanical approach for the selection of this plant in the discovery of new bioactive compounds (Figure 2). Further phytochemical research is needed to identify the active principles responsible for the antifungal activity of *S. mahogany*.

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