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STUDIES ON *IN VITRO* EVALUATION OF ANTFUNGAL ACTIVITIES OF *STERCULIA FOETIDA* L. BARK

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ABSTRACT

The present investigation was attempted in order to evaluate the antifungal potential of successively extracted n-hexane and methanolic extracts of *Sterculia foetida* L. bark. The fungi-toxic efficacy of the extracts was tested against both yeast like fungi [*Candida albicans* (MTCC-3017), *Candida krusei*, *Candida tropicalis*, *Cryptococcus marinus* (MTCC-1029)] as well as mycelial like fungi such as *Aspergillus niger* (MTCC-9933) and *Rhizopus oryzae* employing well diffusion and poison food method respectively. The study revealed that n-hexane extract in high inhibition against *Candida krusei*, whereas *Candida albicans* and *Candida tropicalis* showed no response and *Cryptococcus marinus* responded moderately. The methanolic extract showed high inhibition against *Candida krusei*, whereas *Candida albicans* and *Candida tropicalis* showed no response and moderate effect against *Cryptococcus marinus*. The n-hexane and methanolic extracts of this plant showed better zone of inhibition against *Aspergillus niger* than *Rhizopus oryzae*. Fluconazole and Clotrimazole were taken as reference antifungal.

KEY WORDS: **Agar well diffusion, Poison food method, Antifungal activity, *Sterculia foetida* L. Bark.**

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INTRODUCTION

In the developing countries, synthetic drugs are expensive, inadequate for the treatment of diseases and are also often with adulterations and many side effects.[1] Medicinal plants can be exploited to find out effective alternative to synthetic drugs.[2] The plant kingdom has provided a variety of medicines throughout history and across the globe. The Indian flora offers variety of plants having medicinal properties. There has always been a great interest of scientist on biologically active compounds from natural sources who are working on infectious diseases. Many plant species belonging to different families of angiosperms have been reported to shows antimicrobial activity. Fungal infection has become more frequent because they are ubiquitous in the environment. Presently efforts are being made to study the fungi-toxic potential of a number of medicinal plants. Many workers all over the world carried out studies to ascertain the medicinal value of the phytochemicals for use in pharmacological studies. The phytochemical compounds from medicinal plants mostly serve as lead compounds in drug discovery and design. *Sterculia foetida* L. is a tree belonging to the family Sterculiaceae with digitately lobed leaves (5-7 leaflets), elliptic, margin entire and flowers in axillary panicles. *S. foetida* has vast hidden potential for its medicinal as well as economical importance. The seeds were edible and can be roasted and eaten like chestnuts and it is considered as substitute for Cocao. Seed oil used as local culinary and traditional medicine. Fiber obtained from the bark is used as cord, and the timber yields a gum or glue which is used in bookbinding. *S. foetida* is a source of secondary metabolites and is also well known for its phenolic content, antibacterial[3] and antioxidant[4] activities. The phytochemical constituent of interpolar-methanolic and low polar petroleum ether extract of *S. foetida* indicated presence of alkaloids, phenols, flavonoids and triterpenoids. The seeds of this plant are used as anti-dermatophytic,[5] aperient, diuretic, as insect repellant,[6] laxative, carminative, astringent, anti-inflammatory, antifungal,[7] and central nervous (CNS) depressant.[8] The seeds are also used in treating diseases like itch, rheumatism,[9] skin diseases.[10] The phytoconstituents of seeds like sterculic acid triglycerides, cyclopropenoid fatty acids contains anti-fungal compounds,[11] antibiotic, insecticide, antiviral, hormonal, carcinogenic or antitumor activities.[12] The crude extract of seed acted as insecticide to Asian army worm and as antifeedent to the semi polar, *Achaea janata. S. foetida* oil (3%) in the diet definitely delayed sexual maturity of the female rat and length of consecutive estrous cycles. Oil used in the treatment of nausea and skin diseases. The flavonoid content of *S. foetida* showed anti-oxidant activity.[13] Fruit bodies of *S. foetida* were used to explore novel approaches for biosynthesis of silver nanoparticles and then antibacterial property of synthesized nanoprticles was observed against some gram positive and gram negative bacteria.The aim of the present investigation was to evaluate the *in vitro* antifungal activity of n-hexane and methanolic extracts of bark of *Sterculia foetida* L. against certain fungus.

MATERIALS AND METHODS

1. Collection and identification of plant material

The plant *Sterculia foetida* L. was collected from the "Chandaka reserve forest area" near Bhubaneswar, Odisha, India in the month of April 2014. Identification of the voucher specimen was done by following the Flora of Orissa.[14] The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India. The bark were collected, washed in running tap water, dried under shade and made to coarse powder.

2. Processing of plant material and preparation of extract

The bark were collected, washed in running tap water, dried under shade and ground to form a coarse powder. It has been successively extracted with the solvent n-hexane and methanol by soxhlet apparatus[15] and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

3. Evaluation of the in vitro antifungal activity

3.1. Standard drugs used and preparation of doses for antifungal assay

Fluconazole and Clotrimazole were used as Reference Antifungal (RA). The stock solutions of RA were prepared in 10 % dimethylsulphoxide (DMSO) to give a concentration of 1.56 mg / ml for RA respectively.

3.2. In vitro antifungal assay

The fungal strains were sub-cultured in Sabouraud's Dextrose Agar (SDA HiMedia, Laboratory Ltd, Mumbai) medium. The fungal susceptibility test was determined by measurement of zone of inhibition (ZI) using agar well diffusion assay against some yeast like fungal strains. However, the antifungal activity of the extracts against some mycelial fungi was determined by poison food method and expressed in terms of zone of restricted growth.[15]

3.2.1. Agar well diffusion assay

Agar well diffusion method[15] was followed to determine the zone of inhibition of microbes in Sabouraud's Dextrose Agar (SDA, HiMedia Laboratories Ltd., Mumbai). Plates were swabbed (sterile cotton swabs) with 8 hr old broth culture of fungi. Wells (8 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. Stock solution of plant extracts were prepared at a concentration of 3 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 hrs. Control treatments comprising inoculums without plant extract were set up. The plates were incubated at 28 °C for 48 hrs for fungal pathogens. Triplicates were maintained and the diameter of the zone of inhibition

(mm) was measured and statistical analysis was carried out.

3.2.2. Poison food method

Poison food method [15] was followed to determine the zone of restriction of fungus. It is used for that fungus having high mycelial spreading. For those strains of fungus, the agar plates are prepared by mixing the Sabouraud's Dextrose Agar (SDA, HiMedia Laboratories Ltd., Mumbai) with the plant extract at the concentration of 3 mg/ml. Then a disc of the fungal mycelium was taken out using the sterile cork borer and placed by inverting the disc so that the portion of the fungal disc touches the SDA medium and kept in the centre of the plate. These plates were kept in the room temperature (28 °C) for 2-3 days for measured the zone of restriction.

RESULTS AND DISCUSSION

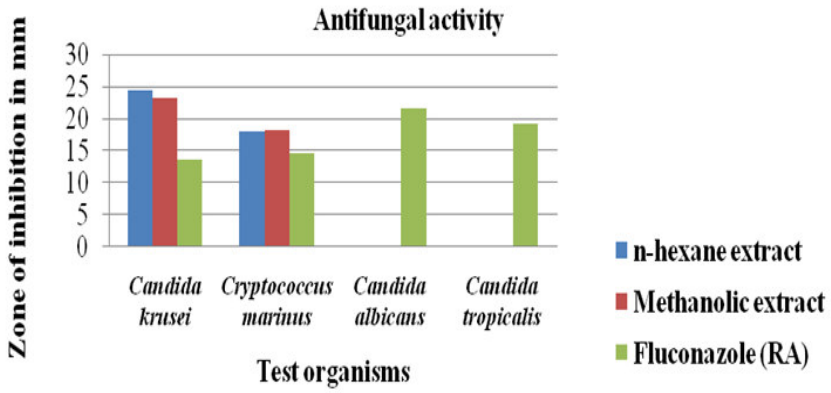
The bark extract of this plant was subjected to antifungal screening against some yeast like as well as mycelial like fungi. The results of antifungal activities were expressed in terms of zone of inhibition and zone of restriction respectively. The result indicated that n-hexane and methanolic extracts of *Sterculia foetida* L. exhibited highest zone of inhibition against *Candida krusei* (24.36 ± 0.32 mm) and (23.3± 0.36 mm) respectively. The extracts showed least activity against *Cryptococcus marinus* (n-Hexane: 18 ± 0.2 mm) & (Methanolic: 18.26 ± 0.20 mm) while did not respond to *Candida albicans* and *Candida tropicalis*. The n-hexane and methanolic extracts of *Sterculia foetida* L. also showed highest zone of restriction against *Aspergillus niger* (n-Hexane: 17.36 ± 0.40 mm) & (Methanolic: 10.2 ± 0.26 mm), least against *Rhizopus oryzae* (n-Hexane: 24.93 ± 0.40 mm) & (Methanolic: 17.86 ± 0.32 mm) respectively. (Table 1 and Graph 1 & 2)

Table 1
In vitro Antifungal activity (Zone of inhibition & Zone of restriction in mm) of different plant extracts of Sterculia foetida L. bark

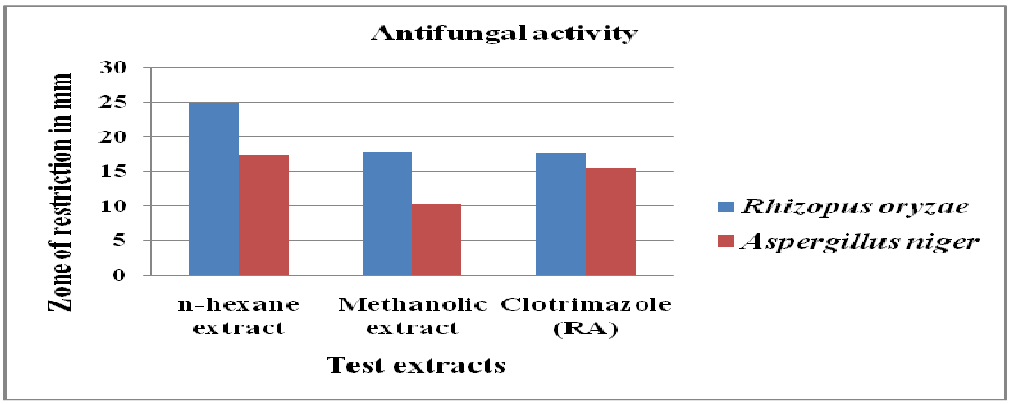
Test organism (Yeast like fungi)	Zone of Inhibition (in mm)		
	n-hexane extract (3 mg/ml)	Methanol extract (3 mg/ml)	Reference Antifungal (RA) Fluconazole (1.56 mg/ml)
Candida krusei	24.36 ± 0.32	23.3 ± 0.36	13.7 ± 1.32
Cryptococcus marinus	18 ± 0.2	18.26 ± 0.20	14.7 ± 0.72
Candida albicans	--	--	21.6 ± 0.5
Candida tropicalis	--	--	19.2 ± 0.2
Rhizopus oryzae	24.93 ± 0.40	17.86 ± 0.32	17.6 ± 0.8
Aspergillus niger	17.36 ± 0.40	10.2 ± 0.26	15.4 ± 0.45

**Results expressed as mean ± S.D. of three determinations, (-) denotes no zone of inhibition*

Graph 1
In vitro antifungal activity (zone of inhibition in mm) of different plant extracts of Sterculia foetida L. bark



Graph 2
In vitro antifungal activity (zone of restriction in mm) of different plant extracts of Sterculia foetida L. bark



CONCLUSION

It was found that *Sterculia foetida* L. has toxic effect against fungal strains. The methanolic extracts of the plant has higher antifungal activity against yeast like and mycelial fungi than n-hexane extract. The fungi-toxic potential of the extract of *Sterculia foetida* against pathogenic fungi under study indicated that further studies were required for the identification of the bioactive molecules for development of antifungal drugs. Further studied are recommended for the isolation of important chemical constituents which may be specific for the antifungal activity.

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