

**EVALUATION OF ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT
OF LEAVES AND STEMS OF
SOLANUM SISYMBRIIFOLIUM LAM.**

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Summary

Invasive fungal infections are significant causes of morbidity and mortality, particularly in immunocompromised patients. *In-vitro* antifungal activity of methanolic extract of leaves (MELS) and stems (MESS) of *Solanum sisymbriifolium* Lam. was assessed against *Aspergillus niger*, *A.Flavus*, *A.xylinium* and *Candida albicans* by agar well diffusion method using Fluconazole (100 µg/ml) as a positive control. The stem extract was shown large zone of inhibition against all three species of *Aspergillus* and also active against *Candida albicans* compare to stem extract. The MESS was found active against all three species of *Aspergillus* and also active against *Candida albicans*. MESS has significant antifungal activity compare to MELS.

Keywords: *Solanum sisymbriifolium*, Antifungal activity, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus xylinium* and *Candida albicans*.

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Introduction

Higher incidence of fungal infection caused by various species of fungi has been reported, hence, research on bioactive substances that may lead to the discovery of new compounds is required. There has been a dramatic increase in the use of antifungal agents for the treatment of both systemic and localized fungal infection but the expanded use of antifungal agents has accelerated the development of resistance to antifungal drugs followed by frequent therapeutic failures and increasing mortality rate^[1]. The most important and dreadful species of fungi includes *Aspergillus niger*, *A.flavus*, *A.xylinium* and *Candida albicans*. Despite advances in antifungal therapies, many problems related to drug resistance and toxicity remains to be solved, for example, the toxicity of Amphotercin and development of clinically resistance strains of various fungal species on continuous use of Azoles.

This situation highlights the need for advent of safe, novel and effective antifungal agents. The main objective of the present study is to explore the antifungal potential of *Solanum sisymbriifolium* Lam. It is commonly known as Red Buffalo bur belongs to Solanaceace family. It is a native of South America. In India it was found in Central, East, North and South^[2-6]. The Leaves pinnately lobed with yellow spines, alternate; Flowers pale blue to white, 11/4 in. in diameter; The Berry globose, 2.5 cm in diameter, yellowish-red, included in the accrescent calyx which has a prickly tube, with the apex open; Stem branched, woody at base, villose-pubescent with long viscid hairs and armed all over with bright yellow prickles.

In traditional medicine it is used in the treatment of respiratory diseases, as anti-inflammatory drug. The drug is of high interest as it has been mentioned as possible treatment of female infertility and for promoting conception in females^[7,8]. Roots are used in the treatment of hypertensive diseases in Paraguay^[12], as diuretic, analgesic, contraceptive, antisypilitic and hepatoprotective in Argentine^[9]. Aerial parts are used in Argentine to treat diarrhea, infections of respiratory and urinary tracts^[10] and Flowers are used as analgesic in India^[11]. Leaves are as febrifuge in Peru and as diuretic in Brazil^[12,13]. The plant is also used as emenagogue and for fertility regulation^[14,15]. The hypotensive effect of the root extract and its components shown in both normo- and hypotensive rats^[16,17]. Alkaloid solasodine from leaves^[18], alkaloids from roots^[19-21], Lignans and Steroids from fruits^[21,24] are already reported. The present study aims at evaluation of leaf and stem extract for its antifungal activity.

Materials and Methods

Collection and identification of Plant material.

Collection of stem and leaf part of *Solanum sisymbriifolium* were done from the wild sources near by Saurashtra university campus during August/September, 2007 and identification and authentication were done by Dr. Reddy, Department of Bioscience, S. P. University, V. V. Nagar, Gujarat. The voucher specimen no. Herbarium/2007-08/03 of the collected sample was deposited in the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat for future reference.

Preparation of methanolic extract

About 500 gm of dried powder of both leaf and stem were obtained from fresh leaves and stem weighing about 3 and 4 kg, respectively. They were powder and extracted with 80 % methanol using the soxhlet apparatus (soxhlet F) for about 48 hours. After extraction, the methanolic extracts filtered through Whatmann filter paper No.1. The filtrate were dried in the vacuum distillation and then in dessicator.

Antifungal activity

Cup-plate method was used for screening the antifungal activity of methanolic extract of leaf (MELS) and stem (MESS) of *Solanum sisymbriifolium*^[25,26]. A commercial sample of fluconazol^[27] was used as a standard and Potato-Dextrose Agar Media was used as culture medium.

The petri dishes filled to depth of 4.5 mm with potato dextrose agar medium and were placed on a level surface so as to ensure that the level of the medium was of uniform thickness. The petri dishes were sterilized at 160-170 °C for 1 hour before use.

Small sterile borer of uniform size having an internal diameter of 6-8 mm and made up of stainless stell was placed at 10cm height. Six holes were made in the medium with the sterile borer. Two holes for MELS, MESS each, one hole for positive control (Fluconazol) and one for solvent control dimethyle sulfoxide (DMSO). Solutions of the standard and the extract being examined were prepared in five different concentrations (10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml) using sterile DMSO as a solvent. For experiment 50 ul of the solution of fluconazol, DMSO, MELS and MESS were filled with the help of micro pipette. The zones of the inhibition were measured in diameter (CM) produced around the hole after incubation at 37 °C for 24 hours. The experiment was done in triplicates. The results were expressed in diameter of inhibition zones \pm S.E.M.

Result and Discussion

The results of present study are shown in Table 1. The zone of inhibition of MESS with five different concentrations were found to be comparable with that of standard drug in case of *A.niger*, *A.flavus*, *A.xylinium* and *C.albicans*, reflecting the potency of extract against these pathogens, whereas diameter of zone of inhibition of MELS with five different concentrations were found to be far less then standard drug. In case of *A.niger*, when the concentration of the 40 and 50 mg/ml of MESS were used, the diameter of zone of inhibition, was more compare to other concentrations used, but in the cases of *A.xylinium*, *A.flavus* and *C.albicans*, it was observed that increase in concentration inevitably increases the diameter of zone of inhibition.

The use of methanol as a solvent for exctraction and subsequent good antifungal activity exhibited by the MESS extract.

Table 1: The concentration of zone of inhibition values at different concentrations of 80% of methanolic extract of leaf and stem of *Solanum sisymbriifolium* Lam. with Fluconazol as standard drug

Name of the organism	Concentration (mg/ml)	Zone of inhibition (cm) with MELS	Zone of inhibition (cm) with MESS	Zone of inhibition (cm) with Fluconazol
<i>Aspergillus niger</i>	10	1.13	1.87	2.01
	20	1.21	1.83	2.0
	30	1.26	1.78	2.2
	40	1.39	1.89	2.0
	50	1.433 ± 0.88	1.9 ± 0.50	2.0 ± 0.33
<i>Aspergillus flavus</i>	10	1.10	1.5	1.63
	20	1.19	1.76	2.01
	30	1.25	1.75	2.2
	40	1.40	1.81	2.1
	50	1.71	1.92	2.1
<i>Aspergillus xylinium</i>	10	1.02	1.48	1.63
	20	1.31	1.74	1.93
	30	1.39	1.80	2.01
	40	1.42	1.84	2.2
	50	1.55	1.89	2.0
<i>Candida albicans</i>	10	1.00	1.75	2.2
	20	1.21	1.71	2.01
	30	1.30	1.83	2.01
	40	1.36	1.90	2.0
	50	1.43	1.94 ± 0.50	2.1

MESS: Methanolic extract of Stem of *Solanum sisymbriifolium*

MELS: Methanolic extract of leaves of *Solanum sisymbriifolium*

Conclusion

Almost all the antifungal agents, in-use shown toxic side effects^[28] and relatively expensive. In search of efficacious, less toxic and economical antifungal agent this study was performed and it was found that 80% methanolic extract of stem of *S. sisymbriifolium* Lam. (MESS) possesses good antifungal activity and MELS possesses less antifungal activity when compared with Fluconazol. The future prospective includes isolation and characterization of active constituents responsible for anti-inflammatory activity of the plant.

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