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IN-VITRO ANTIDERMATOPHYTIC ACTIVITY OF LOW POLAR PETROLEUM ETHER AND INTER POLAR METHANOLIC SEED EXTRACTS OF *STERCULIA FOETIDA* L.

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ABSTRACT

The antidermatophytic activity of interpolar methanolic and low polar petroleum ether soxhlet extracts of *Sterculia foetida* seed was evaluated by agar well diffusion method, The maximum activity was observed in interpolar methanolic extract against *C .albicans* followed by *E. coli, B. Subtilis, P. aeruginosa, S. aureus, T. rubrum, M.gypseum, T.mentagrophytes and T.tonsurans.* When compared to low polar petroleum ether extract. The Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), minimum bactericidal concentration (MBC) were determined using broth dilution method. The preliminary phytochemical tests for secondary metabolites were carried out in both the extracts but the results indicated presence of alkaloids, phenols, flavonoids and triterpenoids in the interpolar extract. This study provides a basis for the isolation and purification of anti-dermatophytic compound(s) from the seeds of *S. foetida.*

KEY WORDS: Antidermatophytic activity, interpolar methanolic seeds extract, *Sterculia foetida,* MIC, MFC, MBC.

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1. INTRODUCTION

Medicinal plants are natural sources of compounds that can be used against many diseases today[1]. Since a variety of plants grow in every conceivable place, having access to them would require only previous knowledge of their location and certain unique characteristics, such as a plant's habit of growth. As such, plants can be obtained easily. This aspect would be vital in discovering medicinal plants with high biological activity, low toxicity and which are acquired at a low price[2]. Various studies have been done which utilized plants in investigating possible antimicrobial drugs and in discovering the different medicinal properties of plants, although, these studies are not enough to cover the world's biodiversity and the traditional use of medicinal plants. WHO estimated that 80% of the people of the world living in developing countries rely on medicinal plants for primary health care needs[3]. The high cost of acquiring synthetic drugs, their inadequate supplies, the side effects associated with their uses, and the belief that plants hold cure to many disease conditions have led to a reawakening of interest in the utilization of plants and plant products in recent years. There is a need to intensify research into medicinal flora especially those claimed to have beneficial effects in serious disorders [4]. Agents to prevent growth of fungi are important in medicine. Fungi that infect the skin, nails, and hair, generally called ''ringworm'' or ''tinea,'' are classified as dermatophytes. The three important genera that are closely related botanically are *Microsporum, Trichophyton*, and *Epidermophyton*. *Microsporum* is commonly causing ringworm of the scalp and may also causing ringworm in other parts of the body whereas the *Trichophyton* causes ringworm of the scalp, beard, and other areas of the skin and nails. Epidermophyton is largely responsible for ringworm of the skin, hand and feet and appears as interlacing threads in the skin, but does not invade the hair[5]. Candida spp. have been reported to be commensal fungi commonly found in the gastrointestinal tract, mouth, and vagina[6]. *Sterculia foetida* Linn. belongs to the family Sterculiaceae, thirty three species were distinguished on the basis of seeds structure. This was originated from East Africa, North Australia and Asia. Commonly called Java olives in English and Jangli badam or Pinari in Hindi is a large evergreen tree found usually in the western and southern parts of India[7]. It is a tall stately tree, deciduous in the cold season and produces more or less whorled, horizontal branches. The follicles are boat shaped woody bright red when ripe. The seeds are black, ellipsoid, 2 cm long, 10 - 15 in each follicle, not winged[8]. The seeds of this plant are used as aperient, diuretic and as insect repellant[9] (Chopra et al., 1992). Laxative, carminative, astringent, anti-inflammatory and central nervous (CNS) depressant activity [10], antifungal[11]. The seeds of this plant are roasted and eaten like chestnuts, and also used in treating diseases like itch, skin diseases [12], rheumatism [13]. The phytoconstituents from seeds like sterculic acid triglycerides [14], cyclopropenoid fatty acids contains anti-fungal compounds [15], insecticide, antibiotic, antiviral, hormonal, carcinogenic or antitumor activities[16]. The authors have documented ethno-antidermatophytic plants from Hyderabad Karnataka region, however, there was no previous report on in vitro antidermatophytic activity of interpolar methanolic seed extract. Therefore the present work was carried out.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The seeds of *Sterculia foetida* were collected in fresh bags from different places of Gulbarga University, identified with the help of Flora of Gulbarga district [17]. The voucher specimen (HGUG-997) deposited in herbarium centre, department of Botany, Gulbarga University, Karnataka, India. The collected plant materials were initially rinsed with distilled water to remove soil and other contaminants and dried on paper towel in laboratory at 37 ± 2°C for week.

2.2 Extraction of plant material by soxhlet apparatus

The seed materials after drying were ground in a grinding machine in the laboratory. 25g of shade dried powder was weighed and extracted successively with petroleum ether (low polar) and 98% methanol (inter polar) in soxhlet extractor for 48h. The extracts were concentrated under reduced pressure and preserved in refrigerator in airtight bottles for further use.

2.3 Test microorganisms

Five fungal cultures strains, *Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum gypseum, Candida albicans* and five bacterial strains, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* obtained from M.R. medical college, Gulbarga, Karnataka, India were used in the present study. The bacterial strains were grown in NB (Himedia, M002) at 37ºC and maintained on nutrient agar slants at 4ºC, fungal cultures were grown in PDB broth at 28 ºC and maintained on PDA slants at 4ºC.

2.4 Agar-well diffusion method[18]

The assay was conducted by agar well diffusion method. About 15 to 20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using 5 days old culture strains. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations of serially diluted extract (0.62, 1.25, 2.5, 5, 10, 20 and 40mg/ml) was added to the 20µl to each wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37^{o}C. After incubation for 48h, the plates were observed for zone of inhibition. Diameter zone of inhibition was measured and expressed in millimetres. Dimethyl formamide (DMF) was used as a negative control. The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37^{o}C for 18h.

2.5 Minimum Inhibitory Concentration[19]

One ml of sterile liquid Sabouraud medium was added to 11 sterile capped tubes, 1 ml of each solvent extracts suspension was added to tube 1. The contents were mixed and 1 ml was transferred to tube 2. This serial dilution was repeated through to tube nine and 1 ml was discarded from tube 9. Fifty µl of inoculum was added to tubes 1-10 and the contents were mixed. Medium control (no inoculum and no drug) and inoculum control (no drug) tubes were prepared. The final concentrations of each plant solvent extracts ranged from 05mg/ml to 0.02 mg/ml. The tubes were incubated at 30^{o} C for 96 h. The fungal growth in each tube was evaluated visually depending up on the turbidity in the tubes. MIC was defined as the drug concentration at which the turbidity of the medium was the same as the medium control. Ten µl aliquot of cell suspension from the tube without observed growth of dermatophytes was inoculated on to Sabouraud dextrose agar. Minimum fungicidal concentration (MFC), Minimum bactericidal concentration (MBC) of test compound were determined as the lowest concentration of the agent at which no colonies were seen after 4 days at 30^{o}C. Triplicate sets were maintained for each experiment.

2.6 Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference p ~ 0.05 was considered to denote a statistically significance All data were presented as mean values ± standard deviation (SD).

3. RESULTS

The preliminary phytochemical analysis of the low polar petroleum ether and interpolar methanolic seeds extracts of *Sterculia foetida* by adopting standard methods [20]. The results were represented in table-2 which reveals the

presence of various phytochemicals such as alkaloids, flavonoids, phenols, triterpenoids, steroids and saponins. The interpolar extract shows strong positive response to flavonoids, phenols, Triterpenoids and steroids. Whereas the low polar extract showed positive response to alkaloids and saponins. The antidermatophytic investigation of five fungal and five bacterial strains were tested to determine the effect of two different polar solvents seeds extracts of *S. foetida.* The interpolar methanolic extract exhibited an effective antidermatophytic activity as compared with petroleum ether extract. The maximum antidermatophytic activity was observed against *C. albicans* (22.00±0.00 mm) followed by *T. rubrum* (14.66±1.15 mm), *M. gypseum* (12.33±1.15), *T.* mentagrophytes(11.00±0.00), and *T. tonsurans* (10.33±0.57). Whereas, maximum antibacterial activity of 21.00±0.00 mm inhibition was observed in *E. coli* followed by *B. subtilis* (18.00±00 mm), *P. aeruginosa* (16.33±0.57 mm) and *S. aureus* (15.66±0.57). The zone of inhibition was found to be concentration dependent.

The negative control (DMF) was not shown inhibition against all the tested fungal and bacterial strains. Ketoconazole used as a positive control at concentration of 2 mg/ml showed 24.00±0.00 to 26.33±1.15 mm in antifungal activity, whereas the streptomycin sulphate standard antibacterial drug showed 26.00±0.00 to 29.33±0.57 mm inhibition zone. The MIC value 0.15 mg^{-1} recorded against *Ec* followed by 0.31 mg^{-1} was recorded against *Ca, Sa, Bs* and 0.62 mg^{-1} showed *Tr, Tm, Mg* and *Pa.* Whereas 1.25 mg^{-1} MIC showed by *Tt* (Table:4). Similarly, the MFC value against *Tt, Ca* was 0.6 mg^{-1}. While 0.3 mg^{-1} MBC value recorded against *Sa, Ec.* (Figure 1, 2).

Table 1
Botanical identification, folk indication of Sterculia foetida seed.

Botanical name	Voucher specimen (Registration number)	Family	Common name	Part used	Therapeutic indication
Sterculia foetida L.	HGUG-997	Sterculiaceae	Bhootale	Seed	Laxative, carminative, astringent, anti-inflammatory, (CNS) depressant activity [9] Antifungal [10] Ringworm [11] Aperient, diuretic [27]

Table 2
Preliminary phytochemical screening for secondary metabolites of Sterculia foetida seed extracts.

	Inference	
Phytochemical	PE	ME
Alkaloids	+	+
Phenol	-	+
Flavonoids	-	+
Tannins	-	-
Triterpenoids	-	+
Steroids	-	+
Saponins	+	-
Glycosides	-	-

PE: Petroleum ether extract, ME- Interpolar methanolic extract + present, - absent.

Table 3
Antidermatophytic activity of petroleum ether and interpolar methanolic seed extracts of Sterculia foetida

Botanical name of the medicinal plant & Part used	Concentrations mg/ml	Test organisms & inhibition of zones in mm								
		Fungal strains					Bacterial strains			
		T. rubrum	*T.tonsurans*	*T.mentagrophytes*	*M.gypseum*	*C .albicans*	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*E. coli*
S. foetida L. (Seed)										
A	40	05.00±0.00	04.66±0.57	05.66±0.57	04.00±00	07.33±1.15	05.33±1.15	06.33±1.15	07.66±0.57	06.66±0.57
	20	NA	NA	NA	NA	05.00±1.00	04.66±0.57	04.66±1.15	05.33±1.15	05.00±0.00
	10	NA	NA	NA	NA	NA	NA	NA	NA	NA
	5	NA	NA	NA	NA	NA	NA	NA	NA	NA
	2.5	NA	NA	NA	NA	NA	NA	NA	NA	NA
	1.25	NA	NA	NA	NA	NA	NA	NA	NA	NA
	0.62	NA	NA	NA	NA	NA	NA	NA	NA	NA
B	40	14.66±1.15	10.33±0.57	11.00±0.00	12.33±1.15	22.00±0.00	15.66±0.57	16.33±0.57	18.00±00	21.00±0.00
	20	12.00±0.00	09.66±0.57	09.66±1.15	10.00±0.00	13.33±1.15	13.66±1.52	14.00±0.00	15.33±0.57	20.66±0.57
	10	09.00±0.00	08.33±1.15	07.66±0.57	08.66±1.15	12.33±0.57	10.00±0.00	13.66±1.15	14.66±1.52	17.33±1.15
	5	08.66±1.52	07.33±0.57	06.00±0.00	08.00±0.00	11.66±1.52	09.66±0.57	11.00±0.00	13.00±0.00	14.66±0.57
	2.5	07.66±0.57	05.33±1.15	05.00±0.00	06.33±0.57	10.33±1.15	07.66±1.15	10.66±1.15	10.33±1.15	12.66±1.15
	1.25	06.00±0.00	NA	NA	05.00±1.00	09.00±0.00	06.33±0.57	08.66±0.57	09.66±1.52	10.00±0.00
	0.62	NA	NA	NA	NA	06.33±0.57	05.00±0.00	06.66±1.15	07.33±0.57	08.00±0.00
K	02	24.00±0.57	26.33±1.15	26.00±1.00	24.66±1.52	24.00±0.00	-	-	-	-
S	02	-	-	-	-	-	29.33±0.57	28.33±1.15	26.00±0.00	26.33±1.15

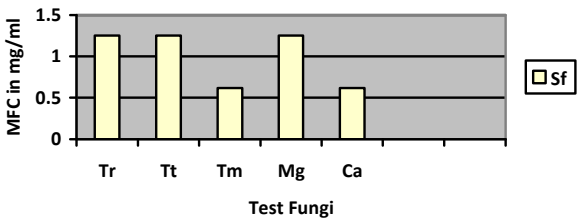
A- Petroleum ether extract, B- 98% methanolic extract, Tr - Trichophyton rubrum, Tt – Trichophyton tonsurans, Tm- Trichophyton mentagrophytes, Mg- Microsporium gypseum, Ca - Candida albicans, Sa- Staphylococcus aureus, P a- Psudomonas aeruginosa, Bs- Bacillus subtilis, Ec- Escherichia coli, NA-Not Active, K-Ketoconazole, S-Streptomycin Sulphate.

Table 4
Minimum Inhibitory Concentrations against test strains of S. foetida seed interpolar extract.

Botanical name of the medicinal plant crude extracts	Test organisms & Minimum Inhibitory Concentrations in mg/ml								
	Fungal strains				Bacterial strains				
	Tr	*Tt*	*Tm*	*M g*	*Ca*	*Sa*	*Pa*	*Bs*	*Ec*
S. foetida L.	0.62≤	1.25≤	0.62≤	0.62≤	0.31≤	0.31≤	0.62≤	0.31≤	0.15≤

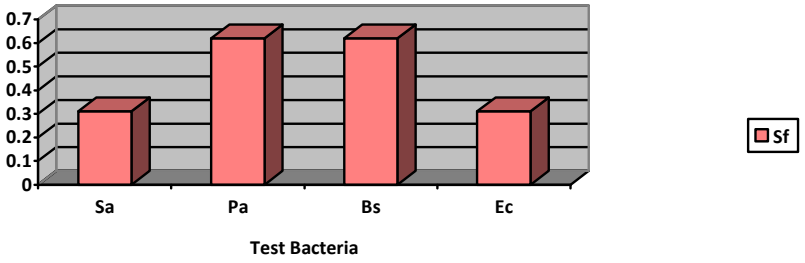
Tr - Trichophyton rubrum, Tt – Trichophyton tonsurans, Tm- Trichophyton mentagrophytes, Mg- Microsporium gypseum, Ca - Candida albicans, Sa- Staphylococcus aureus, P a- Psudomonas aeruginosa, Bs- Bacillus subtilis, Ec- Escherichia coli

Figure 1
Minimum Fungicidal Concentration (mg/ml) of S. foetida seed interpolar extract.



Sf- Sterculia foetida Tr - Trichophyton rubrum, Mg- Microsporum gypseum, Ca - Candida albicans, Tt -Trichophyton tonsurans, Tm -Trichophyton mentagrophytes.

Figure 2
Minimum Bactericidal Concentration (mg/ml) of S. foetida seed interpolar extract.



Sf- Sterculia foetida . Sa- Staphylococcus aureus, Bs- Bacillus subtilis, Ec- Escherichia coli, Pa- Pseudomonas aeruginosa.

4. DISCUSSION

The preliminary phytochemical results of the present report reveals the presence of phytochemicals such as alkaloids, flavonoids, phenols, triterpenoids, steroids and saponins from low and inter polar extracts. The previous report of the same region reveals the other solvent alcoholic extracts indicated high concentration of flavonoids, alkaloids, along with other constituents like glycosides, saponins and steroids[8]. In the present report interpolar methanolic seed extract shows effective antidermatophytic activity, the previous reports supported due to the seeds contains 65–78% of cyclopropene fatty acids and are associated with a large spectrum of biological properties ranging from insecticidal, antifungal, antibiotic, antiviral, herbicidal, hormonal, carcinogenic or antitumoral activities, enzyme, and gluconeogenesis inhibitions to neurochemical activity[21, 22]. Wide arrays of unusual fatty acids are found in seed oil[23]. These fatty acids contain a highly strained propene ring in their carbon chains and are associated with several biological properties[24]. The insecticidal activities of the cyclopropene fatty acid from *S. foetida* seeds against three major stored product pests, namely, *Sitophilus oryzae* L., *Callosobruchus chinensis* L., and *Tribolium castaneum* H., through contact and fumigation bioassay[25]. In the present study the maximum antibacterial activity 21.00±0.00, 15.66±0.57 mm inhibition was observed against *E. coli* and *S. aureus* respectively, whereas in previous report the ethanolic leaf extract of *S. foetida* showed weak inhibition zones 1.44 and 1.38 mm of showed against *E. coli* and *S. aureus* respectively[26]. The potent herbicidal constituent of *S. foetida* seeds was characterized as a cyclopropene fatty acid (2n-octyl*cyclo*prop-1-enyl-octanoic acid) against the 4-Indian major weeds: *Calotropis gigantea* (R.Br.), *Parthenium hysterophorus* (L.), *Datura metel* (L.) *and Tridax procumbens* (L.) were assessed[27]. In the present report, seeds of *S. foetida* interpolar extract (98% methanolic) showed effective activity against all the test strains, whereas in previous report the effective activity observed in ethyl acetate extracts of different plants[28]. The phytoconstituents of the seed extract, such as phenols, triterpenoids and flavonoids may play a major role in the antidermatophytic process observed in this study, however, further phytochemical studies are needed to isolate the active compound(s) responsible for this pharmacological activity.

5. CONCLUSION

The antidermatophytic activity of interpolar methanolic seed extract of *S. foetida* may be attributed to the phytochemical constituents like flavonoids, phenols, triterpenoids and steroids present in crude extract. The purified components may have even more potency with respect to inhibition of dermatophytes. The work carried out was a basic approach to find out the antidermatophytic activity in *S. foetida*. Further study on the purification of

individual groups of bioactive interpolar-compound(s) can reveal the exact potential of the plant to inhibit skin pathogenic microbes.

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