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Antifungal activity of *Solanum melongena* L, *Lawsonia inermis* L. and *Justicia gendarussa* B. against Dermatophytes.

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Abstract: *In vitro* antifungal property of the chloroform, methanol and aqueous extracts of *Solanum melongena* L., *Lawsonia inermis* L. and *Justicia gendarussa* B. were evaluated against four common dermatophytic species, viz. *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum* and *Microsporum fulvum* adopting Agar cup diffusion technique. Minimum inhibitory concentrations (MIC) for the extracts of the three plants were estimated by the broth dilution method and values found ranging between 3.12 -12.5 mg/ml and above. The results demonstrate the antidermatophytic nature of the selected plant extracts of which the chloroform extract of *S. melongena* was found to be the most potent.
Key Words: Agar cup diffusion, Minimum inhibitory concentrations, Antidermatophytic.

Introduction

Dermatophytes are fungi capable of invading keratinized regions such as skin, hair, and nails of human beings and animals, causing diseases known as dermatophytoses or dermatomycoses (1). Dermatomycoses due to *Trichophyton mentagrophytes, T. rubrum, Microsporum gypseum* and *Epidermophyton floccosum* occur commonly worldwide and these are one of the most common skin infections in the North East Region of India. Unlike other superficial fungal infections, the incidence of dermatophytoses, commonly called ringworm or tinea, has increased considerably due to several reasons (2-3) and the situation has worsen with the increase in number of immune-compromised hosts (4). Although several synthetic antimycotic drugs are available in the market, at present the use of these drugs has minimized because of a number of factors which includes low potency, poor solubility, development of resistant strains, drug toxicity and side effects, like gastrointestinal disturbances, cutaneous reaction, hepatotoxicity, leucopenia etc (5-9). The poor availability of efficient non toxic antifungals and increasing number of treatment failures have motivated current searches for therapeutic alternatives to include the testing of Medicinal Plant and essential oils as potential antimicrobial agents (10-12). Most of such plants are known to contain large amounts of phenolic monoterpenes and other compounds, which have significant antifungal, antibacterial and antiviral properties (12-14).

Medicinal plants have been used in traditional treatment of skin diseases worldwide (15). In India, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced, such as Ayurveda, Unani and Siddha (16). The rich availability and easy access to these medicinal plant resources have made them almost inevitable in the healthcare practices specially for those residing in the hilly and remote areas. The people of the North East part of India have an age old rich ethno-medical tradition and most of the people living in the interior areas depend on the herbal remedies based on Medicinal plants (17). They use many plants and plant preparations to cure various skin infections and other diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of lower side effects. Unlike the synthetic drugs medicinal plants have the ability to control antibiotic resistant microorganisms (18).

***Solanum melongena* Linn. (Solanaceae)** well known as brinjal, is widely distributed in all parts of India. Roots of which are used as antiasthmatic and general stimulant, juice is employed for otitis, applied to ulcers of the nose. Leaves are used in the treatment of bronchitis, asthma and dysuria, also given in liver complaints and they stimulate the inter hepatic metabolism of cholesterol. The fruit of *Solanum melongena* is a high valued vegetable all over the world because of its taste and higher percentage of Vitamin B_2 (19). ***Lawsonia inermis* Linn. (Lythraceae)** is a small shrub frequently cultivated in India, Persia, and along the African coast of the Mediterranean Sea. Powdered leaves of this plant, in the form of a paste, are used both as a cosmetic dye and as a remedy for boils, wounds, and some mycotic infections in certain countries of South East Asia . ***Justicia gendarussa* Burm. (Acanthaceae)** is a shade loving, quick growing, evergreen scented shrub found through out India and also in all Asian countries like Malaysia, Indonesia, Sri-Lanka etc. The plant is used in traditional medicinal practice for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases, fever, hemiplegia, headache, earache, muscle pain, respiratory disorders, and digestive trouble (19). On the basis of the information of the rural and tribal people of the region, we have taken up the work to evaluate the efficacy of these plants against one of the common skin diseases of the region, Dermatophytosis.

Materials and Methods

Collection and identification of Plant Materials

The leaves and young shoots of all the three plants were collected from Kamrup district, (25.43° and 26.51° North Latitude and between 90.36° & 92.12° East Longitude) of Assam, India. The plant materials were authenticated by a taxonomist from the Gauhati University Assam (India). Herbarium voucher specimens (No. IASST/MEP/H No. 10, 24 & 35) were prepared and deposited in the Life Sciences Division of IASST, Guwahati, Assam, India for future reference.

Collection of Fungal strains

Fungal strains were procured from Institute of Microbial Technology (IMTECH),Chandigarh-160036 (India). The organisms tested were *Trichophyton rubrum* (MTCC 8477), *Trichophyton mentagrophytes* (MTCC 8476), *Microsporum gypseum* (MTCC 8469) and *Microsporum fulvum* (MTCC 8478). The procured samples were sub cultured and maintained in Sabouraud Dextrose Agar (HIMEDIA) slants at 4^{O}C.

Plant extracts preparation

Freshly collected plant materials were washed twice with distilled water to remove the sand and dirt. Plant materials were then dried under shade in a well ventilated room and dried parts of the plants were finely powdered in a mixture grinder. The powdered materials were exhaustively extracted with chloroform, methanol (Merck) and water (double distilled) separately. In brief, about 100 gms of the dried powdered plant materials were dissolved in 1000 ml of solvent for 48 hours for chloroform and methanol extract and repeated for two times. The filtrate is then concentrated under reduced pressure using a rotary evaporator (Buchi R-124) at low temperature (< 40°C). Finally vacuum desiccators were used to completely remove the solvent. For the aqueous extract, 100 gms of powdered plant materials were heated in 1000 ml water for one hour in a water bath at 40^{0}C, filtered and finally lyophilized to dryness. The extracted samples were kept in refrigerator at 4^{O}C for future use (20).

In vitro assay

Agar cup diffusion

The antifungal activity of the test extracts was determined by employing agar cup diffusion technique (21). The concentration of the micro-organism was about $1.5x10^{8}$ cfu/ml. In brief, with a sterilized spreader, 0.2 ml of broth inoculum was swabbed evenly on the surface of the Petri plates containing solidified Sabouraud Dextrose Agar (HIMEDIA). Well of 6 mm diameter was made in the center of the agar plate with a sterile cork borer. The well was then filled

with the respective test extracts (0.3ml) at different concentration and allowed to diffuse at room temperature for an hour. A control set was maintained with DMSO. Clotrimazole was used as a reference standard. The plates were then incubated at 28 ± 2°C for 5 days to 2 weeks depending on the growth rate of the test pathogens. The experiment was replicated thrice and the average results were recorded. The antifungal activities of the extracts were determined by measuring the diameter of the inhibition zone around the well that was filled with the extract.

Minimum Inhibitory Concentration

Determination of the Minimum Inhibitory Concentration (MIC) of all the extract was carried out by broth dilution assay (22). Two-fold serial dilutions of the extracts were prepared in Sabouraud Dextrose broth to give concentrations ranging from 100-1.56mg/ml. 0.2 ml of the micro-organisms suspension was inoculated into the different concentrations of the extract in test tubes. The tubes were incubated at 28 ± 2^{O} C for 4-5 days for *T. mentagrophytes, M. gypseum, M. fulvum* and 10-14 days for *T. rubrum*. The concentration of the extract which exhibited no visible growth of the fungus was considered as the MIC.

Results and Discussion:

The results of the inhibition zone shown by chloroform, methanol and aqueous extracts of the three plants at different concentration are presented in Table 1. At the highest concentration of 100 mg/ml, the maximum inhibition zone for *S. melongena* (42mm against *T. mentagrophytes*) and *J. gendarussa* (22mm against *T. mentagrophytes* and *M. fulvum*) were exhibited by the chloroform extract, while that of *L. inermis* (26 mm against *M. fulvum*) was exhibited by the aqueous extract. Minimum inhibitory concentrations of the extracts against all the tested dermatophytes are given in Table 3. The Chloroform extract of *S. melongena* was found to be the most active extract with its higher inhibition zone diameter and lower MIC values (Table 3) as compared to the other two tested plants. MIC values for *L. inermis* and *J. gendarussa* was found greater than 6.25 mg/ml against all the tested dermatophyte samples. The values of the three plants were compared with that of the reference antifungal drug Clotrimazole and DMSO (Table 2).

Table 1: Antifungal activity of *S. melongena*, *L. inermis* and *J. gendarussa* against dermatophytes.

Plants	Fungi	Concentration (mg/ml)			Concentration (mg/ml)			Concentration (mg/ml)		
		100	50	25	100	50	25	100	50	25
S. melongena		Inhibition zone (mm) Chloroform Extract			Inhibition zone (mm) Methanol Extract			Inhibition zone (mm) Water Extract		
	T. mentagrophyte	42	35	24	26	18	11	17	-	-
	T. rubrum	41	30	21	28	18	-	13	-	-
	M. gypseum	34	24	13	23	13	-	21	-	-
	M. fulvum	35	26.	14	29	21	-	23	-	-
L. inermis	*T. mentagrophyte*	13	-	-	15	8	-	23	12	-
	T. rubrum	10	-	-	13	-	-	20	10	-
	M. gypseum	12	-	-	14	-	-	19	8	-
	M. fulvum	14	8	-	17	10	-	26	14	9
J. gendarussa	*T. mentagrophyte*	22	9	-	15	7.	-	8	-	-
	T. rubrum	21	-	-	12	-	-	9	-	-
	M. gypseum	19	11	-	20	12	-	15	8	-
	M. fulvum	22	13	8	21	13	-	10	-	-

Values are mean of three replicates.

Table 2: Inhibition zone for Clotrimazole and DMSO.

Control	*T. mentagrophytes*	*T. rubrum*	*M. gypseum*	*M. fulvum*
Clotrimazole (500µg/ml.)	19	22	24	20
DMSO	0	0	0	0

Table 3: Minimum inhibitory concentration of the plant extracts.

Plant Type	Extract Type	Minimum inhibitory concentrations (mg/ml)			
		T. mentagrophytes	*T. rubrum*	*M. gypseum*	*M. fulvum*
S. melongena	Chloroform	3.12-6.25	3.12-6.25	3.12-6.25	3.12-6.25
	Methanol	6.25-12.5	6.25-12.5	6.25-12.5	6.25-12.5
	Water	>12.5	>12.5	>12.5	>12.5
L. inermis	Chloroform	>12.5	>12.5	>12.5	>12.5
	Methanol	>12.5	>12.5	>12.5	>12.5
	Water	6.25-12.5	>12.5	>12.5	6.25-12.5
J. gendarussa	Chloroform	>12.5	>12.5	>12.5	>12.5
	Methanol	>12.5	>12.5	>12.5	>12.5
	Water	>12.5	>12.5	>12.5	>12.5

Values are mean of three replicates.

The treatment of mycoses has lagged behind bacterial chemotherapy and fewer antifungal than antibacterial substances are available. Therefore, a search for new antifungal drugs is extremely necessary. Antimicrobial properties of plant extracts are now recognized and have been reported by many researchers (14, 23). It is important to investigate scientifically plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compounds (24). The three plants studied in the present investigation are in wide use in traditional medicines either singly or in combinations with other plants. Traditional ethnobotanical use of these plants against a number of diseases suggests that there is no undesirable effect to human. The results of the present study illustrate the antifungal nature of the three plants against some common skin pathogens of humans and animals. The chloroform extract of *S. melongena* was found to be the best of all the extracts in activity as known from its MIC value. The activity of different solvent extracts for *S. melongena* and *J. gendarussa* in term of inhibition zone diameter in decreasing order can be stated as Chloroform > Methanol > Water, while that for *L. inermis,* the activity was in the order of Water > Methanol > Chloroform.

Antidermatophytic properties of *L. alba* on five strains each of *Tinea rubrum* and *Tinea mentagrophytes* have been studied by Natarajan *et al*. 2000 (25). Das *et al*, 2010 (26) reported promising antifungal activity of *S. melongena* against some human pathogenic dermatophytes. Our results indicate the presence of antifungal agents in the tested plants which were found affective in inhibiting the growth of both *Trichophyton* and *Microsporum* species. The present study reveals a scientific base of the traditional use of *S. melongena, L. inermis* and *J. gendarussa* by traditional practitioners in Assam.

Conclusion

The results of the present investigation indicated that *S. melongena, L. inermis* and *J. gendarussa* are antifungal plant, with broad spectrum of activity. However future studies on the chemical characteristics of extracts and active components are necessary for each plant and antimicrobial property, since only crude extracts have been used in this study.

References:

1. Macura A.B., *In vitro* susceptibility of dermatophytes to antifungal drugs: A comparison of two methods. Int. J. Dermatol, 1993, 32, 533-536.
2. Weitzman I., Summerbell R.C., The dermatophytes, Clin Microbiol Rev, 1995, 8, 240–59.
3. Jessup C.J., Warner J, Isham N et al. Antifungal susceptibility testing of dermatophytes: establishing a medium for inducing conidial growth and evaluation of susceptibility of clinical isolates, J Clin Microbiol, 2000, 38, 341–4.
4. Pinto E, Pina-Vaz C, Salgueiro L et al. Antifungal activity of the essential oils of Thymus pulegioides on Candida, Aspergillus and dermatophyte species. J Med Microbiol 2006, 55, 1367-73.
5. Agrawal A., Srivastava S., Srivastava J. N., Srivasava, M.M., Evaluation Of Inhibitory Effect Of The Plant *Phyllanthus Amarus* Against Dermatophytic Fungi *Microsporum Gypseum,* Biomedical And Environmental Sciences, 2004, 17, 359-365.
6. Del Aguila R., Gei F.M, Robles M., Perera-Ramirez A., Male O., Once-weekly oral doses of fluconazole 150 mg in the treatment of tinea pedis, Clin Exp Dermatol, 199217, 402-6.
7. Torok I., Stehlich G., Double blind comparative examination of Ketokonazole 1% cream and Clotrimazole 2% ointment in superficial dermatomycoses, Ther Hung 1993, 41, 60-63.
8. Lopez-Gomez S., Del Palacio A., Van Gutsem J., Soledad Cuetara M., Igalesias L, Rodriguez-Noriega A., Itraconazole versus Griseofulvin in the treatment of tinea capitis: a double blind randomised study in children, Int J Dermatol 1994, 33, 743-7.
9. Gupta A.K., Del Rosso J.Q., Lynde C.W, Brown G.H., Shear N.H., Hepatitis associated with terbinafine therapy: three case reports and a review of the literature,. Clin Exp Dermatol 1998, 23, 64-67.
10. Mondello F., De Bernardis F., Girolamo A et al., In vitro and in vivo activity of tea tree oil against azole-susceptible and -resistant human pathogenic yeasts, J Antimicrob Chemother, 2003, 51: 1223–9.
11. Hammer K.A., Carson C.F., Riley T.V., In vitro activity of Melaleuca alternifolia (tea tree) oil against dermatophytes and other filamentous fungi, J Antimicrob Chemother, 2002, 50, 195–9.
12. Pina-Vaz C., Gon¸calves Rodrigues A., Pinto E et al., Antifungal activity of Thymus oils and their major compounds, J Eur Acad Dermatol Venereol, 2004, 18, 73-8.
13. Dorman H.J., Deans S.G., Antimicrobial agents from plants: antibacterial activity of plant volatile oils, J Appl Microbiol, 2000, 88, 308–16.
14. Cowan M.M., Plant products as antimicrobial agents, Clin Microbiol Rev, 1999, 12, 564–82.
15. Adeleye I.A., Ogunniyi A.A., Omonigbehin E. A., Antimicrobial activity of some local herbs on common skin pathogens, Bioscience Research Communications 15 (3), 231-236.
16. Sukhder., Ethanotherapeutics and modern drug development, The Potential Aurvedha.Curr.Sci, 1997, 73, 909-928.
17. Kotoky J., Das P.N., Medicinal Plants used for liver diseases in some parts of Kamrup district of Assam, a North Eastern State of India, Fitoterapia, 2008, 79(5), 384-7.
18. Rawat R.B.S., Uniyal R.C., National Medicinal plants Board committed for overall development of the sector. Agro Bios, Med. Plants, 2003, 1**,** 12 –16.
19. The useful plant of India. Publications and information directorate. Council of Scientific & industrial research. New Delhi. 1992, 306, 320, 581.
20. Duraipandiyan V., Ayyanar M., Ignacimuthu S, Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India, BMC Complementary and Alternative Medicine, 2006, 6,35.
21. Motiejunaite O., Peciulyte D., Fungicidal properties of *Pinus sylvestris* L for improvement of air quality, Medicina (Kaunas) 2004, 40 (8), 787-793.
22. Akinpelu DA, Kolawole DO (2004). Phytochemical and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum.). Sci. Focus J., 7: 64-70.
23. Shariff Z.U., Modern Herbal Therapy for common Ailments, Nature Pharmacy Series (Volume 1) Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, 2001, 9-88.
24. Hammer K. A., Carson C.F., Riley T.V., Antimicrobial activity of essential oils and other

plant extracts, J. of Appl. Microbiol, 1999, 86, 985 - 90.

25. Natarajan V., Mahendraraja S., Menon T., Antidermatophytic activities of Lawsonia alba, Biomed, 2000, 20(4), 243-245.
26. Das J., Lahon J. P, Srivastava R.B., *Solanum melongena*: A potential source of antifungal agent, Ind. J. Microbiol, 2010. 1-8.

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