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IN-VITRO ANTIFUNGAL ACTIVITY OF LEAF EXTRACTS OF *LEUCAS ASPERA* AND *LEUCAS ZEYLANICA*

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ABSTRACT: The aim of the present study is to probe the antimicrobial activities of methanol leaf extract of *Leucas sp.* Antifungal susceptibilities of certain clinically isolated dermatophytes to methanol extracts of *Leucas aspera* and *Leucas zeylanica* leaves were performed using agar well diffusion method. The result obtained shows that all the extracts expressed remarkable antifungal activity with zone of inhibition ranging from 5 to 10mm. Maximum inhibition zone was recorded with *Penicillium sp.* (10 mm) while minimum inhibition zone of was recorded for *Candida tropicalis* (3 mm). From the study it is evident that *Leucas aspera* and *Leucas zeylanica* possess potential antidermatophytic activity and further study on these plants may lead to explore novel bioactive compounds.

INTRODUCTION: In recent years microorganisms have developed resistance to many commercial antibiotics due to indiscriminate use of antimicrobial drugs and this has created immense clinical problem in the treatment of infectious diseases¹. The increase in resistance of microorganisms encouraged scientists to search for new antimicrobial agents from plants. They are very efficient in treating contagious diseases without extenuating any harmful side effects that are related to pharmaceutically available antimicrobials². Search for novel antifungal agents with wide range of structural classes acting on selectively new targets with fewer side effects has been going on. One approach might be the testing of plants traditionally used for their antifungal activities as potential sources for drug development³.

Various compounds obtained from different parts of plants like roots, shoots and leaves could be used in the form of crude extracts to treat some common infections.

According to folklore medicine, plants from the genus *Leucas* show various therapeutic activities. In this work two species of the genus *Leucas* have been chosen *Leucas aspera* and *Leucas zeylanica*. *Leucas aspera* (Willd.) Link. belongs to the family Lamiaceae is locally known as “Thumbe (English), Dronapushpi, Chitrapatrika (Sanskrit), Thumbai (Tamil) widely distributed throughout South Asia (India, Bangladesh, Nepal), Malaysia and Mauritius⁴. Traditionally, *Leucas aspera* has been reported to be used for coughs, cold, painful swelling, and in chronic skin eruptions⁵.

The leaf extracts are applied to the bites of serpents, poisonous insects, and scorpion sting. The leaves are also used as insecticides and mosquito repellent in rural area⁶. Different types of chemicals such as glucosides, tannins, saponins, sterols, oleic acid, linoleic acid, linolenic acid, palmitic acid, stearic acid, oleanolic acid, ursolic acid and nicotin have already been isolated from

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the leaves, roots, flower and seeds of this plant^{7, 8}. *Leucas zeylanica* (L.) R. Br., commonly known as 'Ceylon slitwort' is being used in the treatment of burning and urination within the frame of traditional medicine, antirheumatic, wild-crafted, coughs, colds, toothaches, abdominal pains and thrombolytic activity⁹. Though adequate evaluation lacks regarding its pharmacological activity¹⁰. The presence of alkaloids, steroids, tannins, flavonoids and glycosides were confirmed during preliminary phytochemical screening of this plant¹¹. Despite of its long history as medicinal values, allelopathic activity of *Leucas sp* were not reported earlier.

In this investigation, we examined the antifungal activity of the *Leucas sp.* against skin pathogens. We suggest the potential of *Leucas sp.* as an antifungal drug against various dermatophytes. In the present work we evaluate the therapeutic potential of methanolic extract of leaf of *Leucas sp.* for its antimicrobial activity against certain clinically isolated dermatophytes.

MATERIALS AND METHODS:

Collection of plant material:

The healthy mature plants of *Leucas aspera* and *Leucas zeylanica* were collected during the month of April/May, from Thuthipattu and Karuvatchi village of Villupuram District, Tamil Nadu. Identification of the plant was authenticated at the Department of Botany, Ramakrishna Mission Vivekananda College, Chennai, Tamil Nadu, India. Herbarium has been deposited in the departmental herbarium.

Extraction of Bioactive compound from plant material: 50g of shade-dried pulverized plant leaves were subjected to extraction in a Soxhlet apparatus using methanol. The extract obtained was filtered using membrane filter paper. The filtrate

was concentrated under vacuum in a rotary evaporator (80°C, 110 RPM) and stored at 4°C until further use².

Fungal strains:

Clinically isolated strains of dermatophytes culture were obtained from Department of Microbiology, Presidency College, Chennai and were used for the antifungal assay.

Antifungal activity by agar well diffusion method:

A sterile swab was used to spread fungal culture evenly over the medium. A well of 10mm diameter was made using a sterile cork borer. Standard drug was prepared by dissolving 1mg of crude extract in 1ml of DMSO (1:1) for both the plants. Each well was impregnated with 20µl of extract in 10mm diameter well. Antifungal assay plates were incubated at 28 ± 2°C for 48 h. The standard well of 10 mm diameter injected with Clotrimazole (20µg/disc) was used as positive control for antifungal activity, and diameter of the zone of inhibition was measured and tabulated.

Statistical analysis:

Data from *in-vitro* antifungal assays (obtained from the fourth day after inoculation) were subjected to an ANOVA analysis, followed by a pair wise comparison of means (Tukey). Statistical analyses were conducted with SAS/STAT software for windows¹².

RESULTS:

Antifungal activity of *Leucas sp.* against Dermatophytes:

In this study, agar well diffusion method was used to determine the susceptibilities of dermatophytes to methanol extracts of both the plants. The antifungal activity of plants extract was given in the **Table 1**.

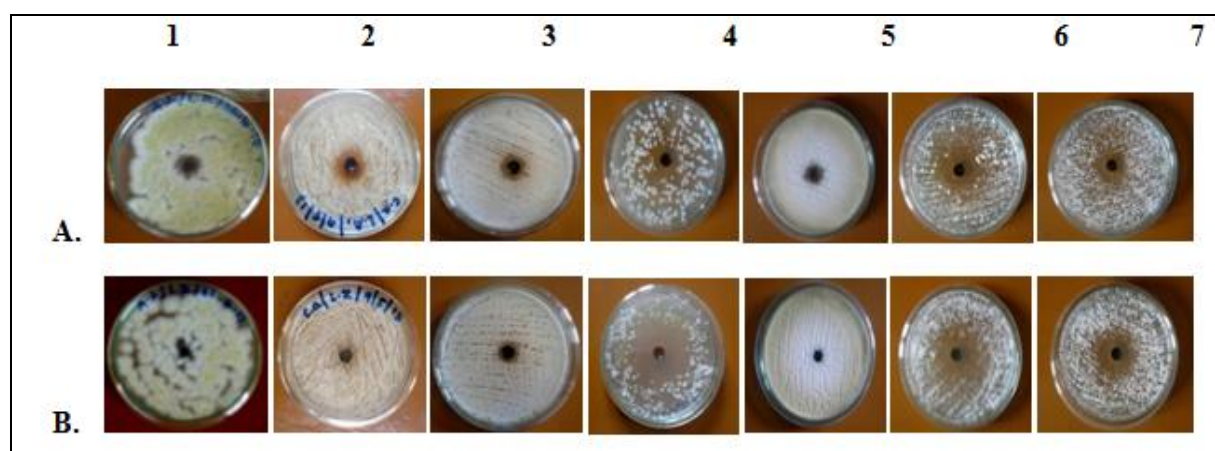
TABLE 1: IN -VITRO ANTIFUNGAL ACTIVITIES OF LEUCAS ASPERA AND LEUCAS ZEYLANICALEAF EXTRACTS

S.no.	Name of fungus	ZONE OF INHIBITION (mm)			
		<i>Leucas aspera</i>	<i>Leucas zeylanica</i>	Control	Methanol
1.	<i>Candida tropicalis</i>	5.0	0.45	10.0	2.0
2.	<i>Candida albicans</i>	3.0	4.0	9.0	–
3.	<i>Trichophyton mentagrophytes</i>	6.0	7.75	10.50	5.0
4.	<i>Microsporium gypsum</i>	5.0	–	10.1	0.40
5.	<i>Microsporium nanum</i>	5.1	–	5.0	–
6.	<i>Aspergillus flavus</i>	5.0	4.0	10.20	–
7.	<i>Epidermophyton floccosum</i>	6.0	–	8.0	3.0
8.	<i>Penicillium sp.</i>	7.5	10.0	10.50	9.0

Data were expressed as means ± SD; P<0.05.

From the case it is evident that all the studied pathogens were found to be susceptible to methanol extract of *Leucas aspera* while only some pathogens were susceptible to *Leucas zeylanica*. Highest activity was recorded against penicillin by the methanol extract of *Leucas zeylanica* (with zone of inhibition of 10mm) which is nearer to control where the control clotrimazole showed an inhibition zone of 10.5mm (**Table 1**) (**Fig.1**). Minimum activity was showed by *Leucas zeylanica* against *Candida tropicalis* but both the plant extracts express reduced activity against the yeast *Candida albicans* recording 3mm & 4mm respectively.

The methanol extract of *Leucas aspera* evaluated for antifungal activity is found to have higher range of zone of inhibition for *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Candida tropicalis*, *Microsporium gypsum*, *Microsporium nanum*, *Aspergillus flavus* and *Penicillium sp.* On the other hand *Trichophyton mentagrophytes*, *Candida albicans*, *Aspergillus flavus* and *Penicillium sp.* were found susceptible to methanol extract of *Leucas zeylanica* where *Microsporium gypsum*, *Microsporium nanum*, *Epidermophyton floccosum* showed resistance to methanol extract of *Leucas zeylanica* (**Fig.1**).



A. Methanol leaf extract of *Leucas aspera*. B. Methanol leaf extract of *Leucas zeylanica*.

FIG.1: IN -VITRO ANTIFUNGAL ACTIVITY OF METHANOLIC LEAF EXTRACT OF LEUCAS SP.

In - vitro fungi:

1. *Aspergillus flavus*, 2. *Candida albicans*, 3. *Candida tropicalis*, 4. *Epidermophyton floccosum*, 5. *Microsporium nanum*, 6. *Penicillium*, 7. *Trichophyton mentagrophytes*

DISCUSSION: Two commonly used medicinal plants *Leucas aspera* and *Leucas zeylanica* were investigated for their biological potentials.

In a previous study, ² showed that the methanol extract of leaf of *Leucas aspera* did not restrain the growth of fungal strains *Candida albicans* and *Aspergillus fumigates*, but in this case study it is apparent to facilitate that the methanol extract of both the selected plants of Lamiaceae family showed a negligible inhibition activity against *Candida albicans* and other species of *Aspergillus* genera, *Aspergillus flavus*. Similarly the study performed by ¹³ showed the antifungal activity of

ethanol and methanol extract of *Spilanthes paniculata* against *Aspergillus niger*, at the same time it is also stated in the above study that ethyl acetate extract of *Leucas aspera* also showed the antifungal effect against *Aspergillus niger*, where as in this experiment methanol extract of leaf of *Leucas aspera* and *Leucas zeylanica* exhibited antifungal activity against *Aspergillus flavus* which is reported for the first time.

In a similar study, ¹⁴ stated that essential oil extracted from the plant *Syzygium aromaticum* when tested for its possible antidermatophyte activity against *Candida tropicalis*, *Candida albicans*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Microsporium gypseum*, *Aspergillus flavus* and the minimum inhibition concentration were determined by a macrodilution method and expressed in $\mu\text{l/ml}$. It is found to possess antidermatophytic properties which

encouraged searching herbal cure for dermatophytosis which in this case was performed with the methanol extract of *Leucas* sp. and the endophytic isolates from *Leucas aspera* against the eight clinical isolates of dermatogens and were found successful leading to new alternate form of cure. The difference of the result may be due to the different solvent used for the extraction and also the fact of using whole plant and part of the plant.

Nevertheless, data obtained from the results are, in general agreement with other authors^{15, 16, 17}. Proposed that different bioactive compounds illustrate various modes of antimicrobial activity. The activity may be due to their hydrophobic action which is responsible for the breakage of lipid bilayer of the cell membrane, leading to losing its permeability and a subsequent leakage of its cell contents¹⁸.

Recent investigations on the antimicrobial actions of some of the bioactive compounds showed rupture of the bacterial and fungal membrane^{19, 20, 21, 22}. Overall reports suggest that this antimicrobial mechanism involves damage of membrane and our results further authenticate that phytochemicals extracts of medicinal plants compromises the structural and functional integrity of cytoplasmic membranes.

Interest in plants with antimicrobial properties increased because of current problems associated with the antibiotics^{23, 24}. Plant extracts showing antimicrobial activity against certain pathogens have been reported by various researchers^{25, 26, 27, 28, 29, 30}.

Given the results discussed above, it becomes evident that methanolic extracts considered for the study is found to possess antifungal activity and can be considered for further investigation with minimal side effects in humans. Analyzing its toxicity, improving its formulations may increase the therapeutic efficacy of these bioactive compounds. Thus optimization of these compounds for clinical applications, to control fungal infections, should be encouraged.

CONCLUSION: The results of this study support the use of *Leucas aspera* and *Leucas zeylanica* in

traditional Indian medicine and show that methanolic leaf extract of these plants can be used as an easily accessible source of natural antifungal agent and can be of assistance in some dermatological problems. This was the first report that *Leucas zeylanica* showed antifungal activity against these dermatophytes. Further studies might aim at the isolation and identification of active substances from the active plant extracts which could also disclose compounds with better therapeutic value.

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