ANTIFUNGAL ACTIVITY OF BENZENE AND ACETONE EXTRACT OF *Leucas lavandulifolia* (SMITH) LINN AGAINST DERMATOPHYTES

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Abstract

*Leucas lavandulifolia* (smith) Linn (Family: Lamiaceae) commonly known as *Thumbai* is distributed throughout India from the Himalayas down to Ceylon. It is reported to have prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities. Traditionally crushed leaves of *Leucas lavandulifolia* is used externally for dermatitis. In this study antifungal activity of the plant was investigated by using dermatophytes to justify the traditional claims that this plant is effective in killing fungus with a concurrent healing from various infectious diseases. Successive Benzene and acetone extract of aerial part of this plant showed good antifungal activity against *Trichophyton rubrum* and *Micosporum fulvum* by Disc diffusion method.

**Keywords:** *Leucas lavandulifolia*, antifungal, dermatophyes

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INTRODUCTION

*Leucas lavandulifolia* (smith) Linn (Family: Lamiaceae) commonly known as *Thumbai* is distributed throughout India from the Himalayas down to Ceylon. The different systems of medicine are practiced in India, which includes Ayurveda, Siddha, Unani, Amchi and local health traditions by using a large number of plants for the treatment of human diseases. A large portion of the world population especially those who are living in the developing countries depends mainly on the traditional system of medicine for getting relief from a variety of diseases. Several hundreds of plants are used medicinally as herbal preparations in the indigenous system of medicine in different countries [1,2]. Plant have been extensively used by rural people of Mithila region (Bihar) in human and cattle ailments, such as cough, cold, fever, loss of appetite, skin diseases, headache, snake bite and scorpion sting [3]. The aerial parts have a strong characteristic odour and are used as sedative, laxative, anthelmintic, inflammation, jaundice, dyspepsia, vermiituge stomachic, scabies, psoriasis, dermatosis, migraine, glaucoma, asthma, anthelmintic, urinary discharge, fever and paralysis [4-7]. Researchers have reported the different biological actions of *L. lavandulifolia* in various test models. *L. lavandulifolia* aerial parts, flowers, whole plant have been found to exhibit hepatoprotective, hypoglycemic, antipyretic, antidiarrhoel, antitussive, wound healing and psychopharmacological, antimicrobial properties [8]. According to the World Health Organization (WHO) report, as many as 80% of the world’s population depends on traditional medicine for their primary health care needs [9] and a major part of the traditional therapies involve the use of plant extracts as their constituents. India is one of the World’s top 12 mega diversity countries with 10 biogeography regions and over 40 sites which are known for their high endemism and genetic diversity. India has more than one fourth (8000) of the world’s known medicinal plant species (30,000), of which 90% are found in forests [10]. Plant species still serves as the rich source of many novel biologically active compounds as very few plants species have been thoroughly investigated for their medicinal properties [11]. Due to the increase in the number of immune compromised individuals, fungal infections have increased in the last two decades. Among them, opportunistic systemic mycoses are associated with high mortality rates [12]. This is essential for systemic mycoses that are typically in immune compromised patients as toxicities are induced by commercial antifungal drugs. The side effects are often observed in these patients because of the dosage and prolonged therapy. Herbal healers suggest that their medicines are cheaper and more efficient than commercial ones [13].
many drugs for the treatment of fungal diseases; however, there are a limited number of efficacious antifungal drugs [14]. They possess a series of limitations such as undesirable side effects and low sensitivity against these fungal infections [15]. Hence, new antifungal agents still require improvement to be effective against opportunistic infections. This study was designed to investigate the effect of the antifungal activity of *Leucas lavandulifolia* plant by using the disc diffusion method.

**MATERIALS AND METHOD**

**COLLECTION OF PLANT MATERIAL**

Aerial parts of *Leucas lavandulifolia* were collected from Azara, Hatkhowapara of Guwahati (Assam) and properly washed in tap water and rinsed in distilled water. Then the plant was dried in an oven at a temperature of 35 – 40°C for 3 days and pulverized using a mortar and pestle, to obtain a powdered form. The powdered form is stored in air tight glass containers, protected from sunlight until required for analysis.

**AUTHENTICATION OF PLANT MATERIAL**

The plant *Leucas lavandulifolia* has been authenticated by the HOD, Department of Botany, Gauhati University, Assam and voucher specimen has been submitted (Acc. No. 181140)

**EXTRACTION OF PLANT MATERIAL**

The dried, coarsely powdered plant material was successively extracted by cold maceration for seven days with benzene and acetone at room temperature. Then the extracts were filtered and concentrated with a rotary evaporator at low temperature (40-50°C) and reduced pressure and subsequently defatted to get the dried petroleum ether, benzene and acetone extracts.

**PHYTOCHEMICAL ANALYSIS**

Phytochemical analysis of extract for qualitative detection of Alkaloids, Carbohydrates, Glycosides, Saponins, Phytosterols, Phenols, Tanins, Flavanoids, Proteins and Amino acids was performed by the extracts [16].

**FUNGAL STRAIN**

The test organisms used for the assay are *Trichophyton rubrum* & *Microsporum fulvum* were collected from Institute of Science and Technology, Guwahati, Assam.

**ANTIFUNGAL ACTIVITY BY DISC DIFFUSION METHOD**

A suspension of *Microsporum fulvum* and *Trichophyton fulvum* was added to the sterile sabouraud media at 45°C and the mixture was transferred to sterile petri dishes and allowed to
solidify. Sterile 5.0 mm diameter blank discs were impregnated with a test (1mg/ml and 2mg/ml of each extracts), standard (Miconazole nitrate, 1mg/ml) and control substances and were placed in petridishes containing a suitable agar medium seeded with the test organism (*Trichophyton rubrum* & *Microsporum fulvum*) using a sterile loop [17]. The plates were made to stand for one hr at room temperature as a period of preincubation diffusion to minimize the effect of variation in time between the applications of the different solutions. All the plates were incubated at 37°C for 24-48 hours. The zones of growth inhibition around the disc were measured after 48 hours. The antifungal activity of the extracts was determined by measuring the sizes of inhibition zones (diameter of the zone) on the agar surface around the disc.

**RESULTS AND DISCUSSION**

The phytochemical screening facilitates quantitative estimation and qualitative separation of pharmacologically active chemical compounds [18]. The result of phytochemical screening has revealed the presence of Alkaloids, glycosides, tannins, saponins and phytosterols in the extracts of *Leucas lavandulifolia*(table 1). These phytoconstituents in the two extracts may be responsible for the therapeutic activities of *Leucas lavandulifolia*.

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>BE</th>
<th>AE</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protiens</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Amino acids</td>
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BE: Benzene Extract; AE: Acetone Extract

The results of antifungal activity of the benzene extract and acetone extracts are presented in Table 2. Benzene extract of *Leucas lavandulifolia* showed a higher zone of inhibition ie 14.7 mm than acetone extract, produced 11.8 mm zone of inhibition against *Trichophyton rubrum* where standard showed 17.5 mm zone of inhibition. Again benzene extract was more active against *Microsporum fulvum* than acetone extract. The control doesn’t show any antifungal zone against

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any of the pathogens used. Both the extract has demonstrated considerable antifungal activity over *Leucas lavandulifolia* and *Microsporum fulvum* by producing adequate zone of inhibition.

**Table 2: Antifungal activity of benzene and acetone extracts obtained from aerial parts of *Leucas lavandulifolia***

<table>
<thead>
<tr>
<th>Extract/ Standard</th>
<th>Zone of Inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td><em>Microsporum fulvum</em></td>
</tr>
<tr>
<td>Benzene (1 mg/ml)</td>
<td>10.2±0.26</td>
</tr>
<tr>
<td>Benzene (2 mg/ml)</td>
<td>13.2±0.15</td>
</tr>
<tr>
<td>Acetone (1 mg/ml)</td>
<td>9.7±0.61</td>
</tr>
<tr>
<td>Acetone (2 mg/ml)</td>
<td>12.4±0.36</td>
</tr>
<tr>
<td>Miconazole nitrate (1 mg/ml)</td>
<td>15.3±0.44</td>
</tr>
</tbody>
</table>

Values were performed in triplicates and represented as mean ± SD

Onychomycosis is a common nail infection caused by dermatophytes or by nondermatophytic molds. *Trichophyton rubrum* is known to be the most common causative agent of dermatophyte nail infections in humans [19]. Onychomycosis is difficult to cure due to its high probability of recurrence and prolonged antifungal agent treatment time. As a result of the side effects observed in commercially available antifungal agents, the development of a novel antifungal agent with less profound adverse effects will be necessary for the treatment of dermatophyte infection of nails [20].

*Leucas aspera* and *Leucas zeylanica* is reported to have significant antifungal and antibacterial activity [21]. Likewise, the other species of this genus, *Leucas lavandulifolia*, is a popular and useful ethno medicinal plant which has been traditionally used for the treatment of diseases along with dermatitis [22]. Therefore, the antifungal activity of the extract of this plant is done against dermatophytes.

Banso et al. [23] reported that the antifungal substances contained in the extracts were fungistatic at lower concentrations, while becoming fungicidal at higher concentrations of the extracts. Our results indicate that the zone of inhibition of both the extracts evaluated were less in lower concentration (1%) and at higher concentration (2%) zone of inhibition is comparatively high. In *Leucas lavandulifolia*, the benzene extract possesses more fungicidal action, as indicated by the high zone of inhibition for both *Microsporum fulvum* and *Trichophyton rubrum*. 

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CONCLUSION

The findings of the present study demonstrated the potential of phytochemicals from aerial parts of *Leucas lavandulifolia*, a natural source, in the pathway of developing a novel antifungal agent able to treat different fungal infections. Among the two solvent tested, benzene extract recorded a significant activity compared to acetone extract. This extract could be promising as a source of natural eco-friendly phyto-fungicidal compounds for *in vivo* applications. Confirmations of the efficacy *in vivo* of these extracts against *Trichophyton* and *Microsporum* are needed.

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REFERENCE


