

## Original Article

# A study of antifungal activity of ethanolic extracts of *Leucas longifolia* L. against fungal pathogens

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## ABSTRACT

*Leucas longifolia* Benth. (Lamiaceae) is common plant in India is considered carminative, stimulant and emmenagogue. The present study was designed to evaluate the antifungal activity of ethanolic (leaf and root) extracts of *Leucas longifolia* against pathogenic strains of *Aspergillus niger*, *Fusarium oxysporum*, *Candida albicans*, *Trichophyton* sp., and *Alternaria alternata*. The results were compared with standard antibiotics and findings support the traditional use of the plant in the treatment of different infections for the medicinal use. In comparison with the antibiotic, these extracts show no side effects.

**KEYWORDS:** Antifungal Activity, *Leucas longifolia*, Minimum Inhibitory Concentration.

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## INTRODUCTION

All microorganisms have the ability to adapt from the environment. Similarly the fungal pathogens, opportunistic in nature and cause a large number of diseases in healthy and immune-compromised individuals.

The fungal infections are also increasing year by year, especially the candida infection, due to overuse of antibiotics and upcoming immune-compromised state in the human population. Many of the currently used antimicrobials are associated with adverse effects such as toxicity, hypersensitivity, immune suppression and tissue residues posing public health hazard. Therefore further, new alternate remedies for treatment of microbial diseases are required and the people are returning to their traditional

therapies, which possess minimum side effects and naturally heal the infection.

The plants are rich source of bioactive compounds and have the potential to act against deadly pathogens. Plant based antimicrobials may become the base for the development of new antibiotics, because of increasing side effects and complications of chemically synthesized antibiotics. *Leucas longifolia* Benth. (Lamiaceae) is commonly called 'Barumbi or Dudhani' in India. It is perineal herb found in in wasted lands and roadsides. It is considered as carminative and stimulant [1, 2].

Many of (Lamiaceae) species of *Leucas* shows antidiabetic, analgesic, antipyretic, anti-inflammatory, antioxidant, CNS depressant and wound healing activity [3-9].

Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practiced in India. Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture. The term comes from the Sanskrit root Au (life) and Veda (knowledge). As the name implies it is not only the science of treatment of the ill but covers the whole gamut of happy human life involving the physical, metaphysical and the spiritual aspects. Ayurveda recognizes that besides a balance of body elements one has to have an enlightened state of consciousness, sense organs and mind if one has to be perfectly healthy.

In the present study, the selection of this plant for evaluation was based on its traditional usages. A little work has been done on the antimicrobial activity of this medicinal plant, it needs further study for verification of its activity against number of pathogens. The ethanol extracts obtained from the leaves, roots and the combined formulation of wild *Leucaslongifolia*, have been investigated for their antifungal activities.

## MATERIALS AND METHODS

### Plant material

The plant material was collected from wild *Leucaslongifolia*, *Lantana camara* in and around Loni August, 2013. Voucher specimens of the plant were deposited in the Department of Botany, University of Rajasthan, Jaipur, India.

### Preparation of extracts

The plant parts (leaf and rootstock) were air dried. The dry powdered plant material (20 g), either of the leaf or the root, was soaked in the 50% ethanol until complete saturation of the plant material. The extract was filtered using Whatman filter paper no. 1, and the filtrate solvent was evaporated under vacuum using a rotary evaporator at 55°C. The resulting dried extract was stored in labeled sterile screw-capped bottles at -20°C. The extract (in the form of sticky black substances) amounting to around 5 g was dissolved in 1 ml of DMSO (dimethyl sulfoxide) before testing. The combination of

plant extracts, leaf and root, (1:1 ratio) was also used to test *Aspergillusniger*, *Fusarium-oxysporum*, *Candida albicans*, *Trichophyton* sp., *Alternariaalternata* which were used as the test fungi were obtained from Department of Microbiology, University of Rajasthan, Jaipur, India and maintained pure cultures on Sabouraud's Dextrose Agar (SDA) plates and slants.

### Minimum inhibitory concentration (MIC) determination

MICs were performed by the visual broth macro dilution method [10]. Fungal suspensions were diluted added to Medium without bicarbonate (buffered to pH 7.0 with 0.165 M morpho line propane sulfonic acid) broth supplemented with glutamine, to a concentration of approximately  $0.5 \times 10^5$  CFU/ml (verified by colony counts in SDA). A twofold serial dilution of 1ml each of extract was added to 1.8 mL of the Medium. The concentrations were in the range, 0.100 – 1000 mg/ml. Controls used were medium without antifungal agents were used in the test. The results for the extracts were compared with a standard, Ketoconazole. The tubes were then incubated at 35°C for 24 - 48h. MIC was defined as the lowest concentration that did not yield visual growth. All experiments were performed in triplicate.

## RESULTS

The MICs values of the extracts are presented in Table 1. The MIC results for the ethanol extract of the leaf, root and the combination of both, ranged from 6.25 - 25, 12.5 - 25 and 3.12-12.5 mg/ml, respectively and showed that activity of the extracts varied from one fungal strain to another. The extract combination (both leaf and root) exhibited stronger antifungal activity than the individual extract. *Candida albicans*, *Cryptococcus neoformans* and *Botrytis cinerea* with MIC of 3.12 mg/ml were more susceptible to the extract combination than other fungi, followed by *Candida guilliermondii* and *Geotrichumcandidum* with MIC of 6.25 mg/ml. The MIC for the other fungi was 12.5 mg/ml. However, the extracts were less active than the standard antifungal agent, like ketoconazole.

## DISCUSSION

Fungi used in this study were chosen primarily on the basis of their importance as pathogens of humans and plants. *Botrytis cinerea* is a fungus that affects many plant species, although its most notable hosts may be wine Grapes. According to findings from the National Nosocomial Infection Surveillance System (NNIS), 61% of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* sp. [11].

*Candida albicans*, while naturally occurring in the intestinal flora, can cause oral thrush and systemic infections. *Cryptococcus neoformans* causes cryptococcosis, an opportunistic infection of the lungs especially in AIDS patients. Ethanol was found to be the best solvent for extracting antimicrobial substances in a previous study [12, 13].

The previous studies shown that, the essential oil of *S. multicaulis*, *S. kronenburgii* and *S. verticillata* were effective against *Candida albicans* [14]. The essential oil of *S. lachnocalyx* were investigated for antimicrobial activity against fungal cultures [15]. The bioassays showed significant inhibition against fungi with Minimum inhibitory concentration in range of 5-10 mg/ml [16-19]. The results of antifungal effects obtained in this study are similar to those reported in the above studies.

Table 1: Minimum inhibitory concentration of the ethanolic extract of leaf and root.

Fungal Pathogen	Minimum inhibitory concentration (MIC)			
	Leaf extract (5g/ml)	Root extract (5g/ml)	Leaf and root extract (1:1 ratio) (5g/ml)	Ketoconazole (5g/ml)
<i>Candida albicans</i>	7.25	13.5	4.12	1.55
<i>Fusarium oxysporum</i>	26	13.5	4.12	1.75
<i>Alternaria alternata</i>	12.5	25	4.12	1.25
<i>Aspergillus niger</i>	25	25	12.5	1.25
<i>Trichophyton</i> sp.	25	12.5	6.25	1.25

## CONCLUSION

The results obtained in this work are in agreement with recent studies regarding antimicrobial activities of *Leucas longifolia* Benth. This plant could be of importance in the search of new natural sources of bioactive compounds and possess no side effects.

## REFERENCES

- [1]. Davis PH. Flora of Turkey and East Egean Island, Vol.7. Edinburg, Edinburg University Press, 1982; pp. 947.
- [2]. Newall CA, Anderson LA. Herbal medicines. A guide for healthcare professionals. Pharmaceutical Press, London, 1996; p. 231.
- [3]. Kelen M, Tepe B. Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. *Bioresour Technol* 2008; 99(10): 4096-4104.
- [4]. Fiore G, Nencini C, Cavallo F, Capasso A, Bader A, Giorgi G, Micheli L. In vitro antiproliferative effect of six *Salvia* species on human tumor cell lines. *Phytother Res* 2006; 20(8): 701-703.
- [5]. Wu BW, Pan TL, Leu YL, Chang YK, Tai PJ, Lin KH, Horng JT. Antiviral effects of *Salvia miltiorrhiza* (danshen) against enterovirus 71. *Am J Chinese Med* 2007; 35(1): 153-168.
- [6]. Damjanovic Vratnica B, Dakov T, Sukovic D, Damjanovic J. Chemical composition and antimicrobial activity of essential oil of wildgrowing *Salvia officinalis* L. from Montenegro. *J Essential Oil Bearing Plants* 2008; 11(1): 79-89.
- [7]. Tzakou O, Pitarokili D, Chinou IB, Harvala C. Composition and antimicrobial activity of the essential oil of *Salvia ringens*. *Planta Med* 2001; 67(1): 81-83.
- [8]. Haznedaroglu MZ, Karabay NU, Zeybek U. Antibacterial activity of *Salvia tomentosa* essential oil. *Fitoterapia* 2001; 72(7): 829-831.
- [9]. Singh H, Gulati IB. Tribological behavior of some hydrocarbon compounds and their blends. *Wear* 1990; 139(2): 425-437.
- [10]. Dzoyem JP, Tangmouo JG, Lontsi D, Etoa FX, Lohoue PJ. In vitro antifungal activity of extract and plumbagin from the stem bark of *Diospyros crassiflora* Hiern (Ebenaceae). *Phytother Res* 2007; 21: 671-674.
- [11]. Walsh JJ. Invasive fungal infections: problems and challenges for developing new antifungal compounds. In: Sutcliffe JA, Georgapadakov NH, eds., *Merging Targets in Antibacterial and Antifungal Chemotherapy*. New York, Chapman and Hall, 1992; 349-373.
- [12]. Jonathan SG, Fasidi IO. Antimicrobial activities of two Nigerian edible macrofungi, *Lycoperdon pusillum* and *L. giganteum*. *Afr J Biomed Res* 2003; 6: 85-90.
- [13]. Baser KHC. Essential oils of Labiatae from Turkey – Recent Results. *Lamiales Newsletter* 1994; 3:6-11.
- [14]. Jirovetz L, Wleek K, Buchbauer G, Gochev V, Girova T, Stoyanova A, Schmidt E, Geissler M. Antifungal activities of essential oils of *Salvia lavandulifolia*, *Salvia officinalis* and *Salvia sclarea* against various pathogenic *Candida* species. *J Essential Oil Bearing Plants* 2007; 10 (5): 430-439.
- [15]. Altun M, Unal M, Kocagoz T, Goren AC. Essential oil compositions and antimicrobial activity of

- Salvia species. J Essential Oil Bearing Plants 2007; 10 (3): 251-258.
- [16]. Salehi P, Sonboli A, Fathi F. Essential oil composition and antimicrobial activity of *Salvia lachnocalyx* Hedge from Iran. Int J Essent Oil Ther 2007; 1: 45-48.
- [17]. Tiwari K, Chittora M. Assessment of genetic diversity and distribution of endophytic fungal communities of *Alternariasolani* isolates associated with the dominant Karanja plants in Sanganer Region of Rajasthan. Springer Plus 2013; (2): 13.
- [18]. Tiwari K, Lodha P. Isolation, frequency distribution and diversity of novel endophytic fungal communities of *Fusarium* species in *Rhusmysorensis* L. from Sanganer region of Rajasthan. Elixir Bio Technology 68 2014; 21983-21986.
- [19]. Isolation, frequency distribution and diversity of novel fungal endophytes in *Securinegaleucopyrus* L. from Sanganer region of Rajasthan Int. J. Int sci. Inn. Tech. Sec. B, Dec. 2012, Vol. 1(5) 40-43.

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