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Original Research Article

Antifungal activity of ethanolic extract of *Sphaeranthus indicus* Linn. against Dermatophytes and Candida species

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ABSTRACT

Background: *Sphaeranthus indicus* as an entire plant or the specific parts like roots, leaves, flowers are used for treating helminthiasis, jaundice, diabetes, dyspepsia, fever, cough, hernia, gastritis, hemorrhoids, migraine, leprosy and skin diseases. The objective of this study was to evaluate the antifungal activity of ethanolic extract of the whole plant *Sphaeranthus indicus* Linn.

Methods: The antifungal activity of ethanolic extract of the whole plant *Sphaeranthus indicus* Linn was evaluated by incorporating the extract in Sabourauds dextrose agar. This property was studied in vitro using the ethanolic extract on *Candida albicans*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* by using Clotrimazole as standard drug.

Results: This study revealed that the ethanolic extract of *Sphaeranthus indicus* is having good antifungal activity against Dermatophytes and Candida species.

Conclusions: This study demonstrates the promising antifungal action of ethanolic extract of whole plant of *Sphaeranthus indicus* Linn. This feature can be exploited in the development of a newer antifungal agent from plant.

Keywords: *Sphaeranthus indicus* Linn, *Candida albicans*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, antifungal action

INTRODUCTION

Infections induced by pathogenic fungi are increasingly recognized as an emerging threat to public health.^{1,2} The epidemiological data point out that the prevalence and incidence of fungal infection is a major public health issue. The increase in occurrence of fungal infections during recent years is due to a growth in the immune compromised population, such as organ transplant recipients and cancer and HIV patients.³ Antifungal agents are being widely used and this had resulted in the rapid development of resistance to these class of drugs. Certain commensal fungi, such as *Candida* species, cause infections when their human hosts become immune

compromised.⁴ These problems are also associated with resistance to antibiotics and toxicity during prolonged treatment with several antifungal drugs.⁵ In several researches, extracts of various plants have been reported to exhibit antifungal properties under *in vitro* laboratory trials.⁶⁻¹⁰ It has been pointed out that there is a relationship between the antifungal activity of the extracts and its bioactive compounds.¹¹ *Sphaeranthus indicus* Linn is a herb commonly known as Mundi, 30 cm or 1 foot high with spreading branches, found all over the Indian plains, especially in hills, as weed in the rice-fields. All parts of the plant are used as medicine.¹² This study was done to evaluate the antifungal properties of ethanolic extract of whole plant *S. indicus*.

METHODS

Plant collection

Sphaeranthicus indicus as a whole plant was collected locally in January from Thiruvananthapuram district, Kerala, India and it was authenticated by the pharmacognosy unit in ayurveda research institute, Poojappura, located in Thiruvananthapuram, Kerala, India. It was cleaned with distilled water. They were cut into small pieces, shade dried and ground to coarse powder form in an electric blender.

Preparation of ethanolic extract

Petroleum ether was used for pre extraction in order to defatten the material. After that soxhlet extraction was done using 90% ethanol. In the next step, the extract was distilled and then dried up in a previously weighed beaker for getting to get rid of the solvent. The filtration was done using Whatman No.1 filter paper. For further use they were stored at 4°C. The ethanolic extract was prepared in three serial concentrations so that the effective concentration was 5 mg/ml, 10 mg/ml and 15 mg/ml, based on a previous study.¹³

Antifungal strains

Candida albicans, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* were the antifungal strains obtained from the microbiology department of Government medical college, located in Thiruvananthapuram, Kerala, India

In vitro antifungal activity

The ethanolic extract of whole plant of *S. indicus* was incorporated in sabourauds dextrose agar (SDA) and *in vitro* antifungal activity was studied in *C. albicans*, *M. gypseum*, *T. mentagrophytes* and *E. floccosum*. The SDA medium was sterilised by autoclaving at 121°C for 15 minutes. To 5 ml of molten medium in test tubes, the extract was added in serial concentrations so that the concentration was 5 mg, 10 mg and 15 mg/ml of the medium and allowed to slant. Clotrimazole 100 mcg/ml was used as standard and dimethyl formamide as vehicle. The media were allowed to cool to room temperature. A bit of mycelial growth was inoculated on to the medium and it was incubated at room temperature. The rate of growth of fungi was noted at the end of 4 weeks.

RESULTS

The antifungal activity was done in test tubes for greater ease of performance, reproducibility and convenience. The rate of growth of fungi could not be measured quantitatively or in terms of zone of inhibition. Hence at

the end of 4 weeks as shown in the figures the experimental observation of absence of growth, or presence of growth was recorded.

As evident from (Figure 1 to 4) and as summarized in (Table 1), the study revealed that the ethanolic extract of *S. indicus* showed inhibition of growth of Dermatophytes and *Candida* strains in the three doses tested at the end of four weeks. As shown in figures, there was no growth of *C. albicans*, *M. gypseum*, *E. floccosuma* and *T. mentagrophytes* in the test tubes with serial dilutions of extract 5 mg/ml, 10 mg/ml and 15 mg/ml as compared to clotrimazole 100 mcg/ml which showed scanty growth. Test tubes containing dimethyl formamide (DMF), the vehicle and sabourauds dextrose agar (SDA), the culture media which acted as controls showed profound growth of the fungi at the end of 4 weeks.

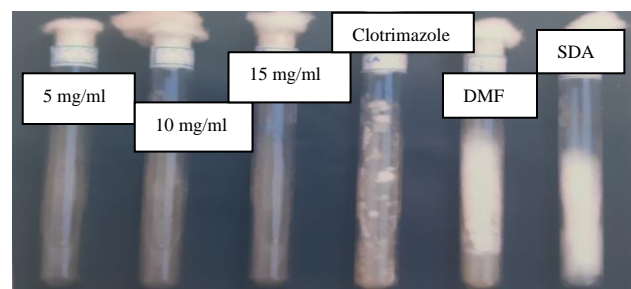


Figure 1: Effect of *S. indicus* on the growth of *C. albicans* in SDA medium compared with clotrimazole.

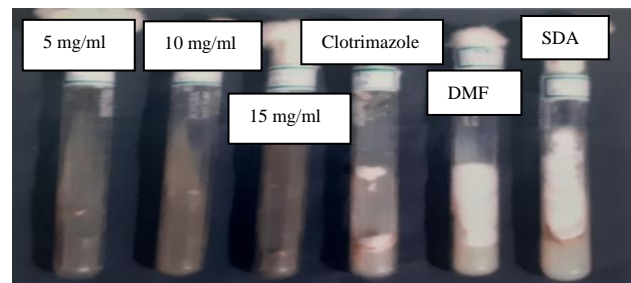


Figure 2: Effect of *S. indicus* on the growth of *M. gypseum* in SDA medium compared with clotrimazole.

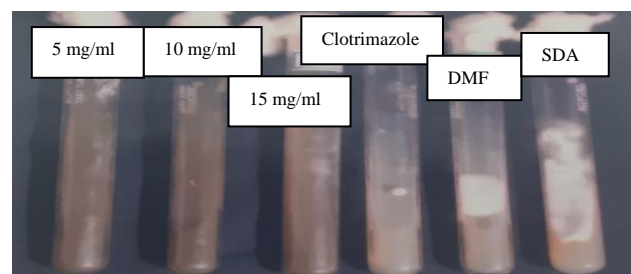
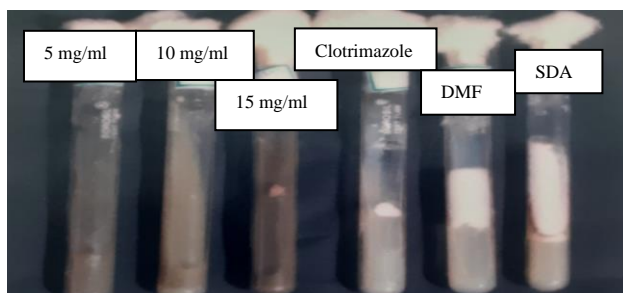


Figure 3: Effect of *S. indicus* on the growth of *E. floccosum* in SDA medium compared with clotrimazole.

Table 1: Effect of *S. indicus* on the growth of fungus in SDA medium compared with clotrimazole.

Fungal strain	Extract 5 mg/ml	Extract 10 mg/ml	Extract 15 mg/ml	Clotrimazole 100 mcg/ml	Dimethyl Formamide	Control
<i>C. albicans</i>	-	-	-	+	+++	+++
<i>M. gypseum</i>	-	-	-	+	+++	+++
<i>E. floccosum</i>	-	-	-	+	+++	+++
<i>T. mentagrophytes</i>	-	-	-	+	+++	+++

**Figure 4: Effect of *S. indicus* on the growth of *T. mentagrophytes* in SDA medium compared with clotrimazole.**

DISCUSSION

This study can be used to justify the use of *S. indicus* in traditional practice as a therapeutic agent as it exhibited significant antifungal properties causing inhibition of growth *Candida* and *Dermatophytes*. The antifungal properties may be due to tannins and saponins.

Hexane extract of *S. indicus* flowers possessed antifungal activity against *C. albicans*. The flower extract showed minimum inhibitory concentration (MIC) at 0.15 mg/ml and aerial parts showed MIC at 1.25 mg/ml against *C. albicans* and it is the first report on antifungal activity of *S. indicus* per the available literature.¹² The antifungal activity against *T. mentagrophytes* and *E. floccosum* was reported in thiophene compound isolated from *Tagetes patula* (Asteraceae).¹⁴ Antifungal activity was also reported for the ethanol extract of underground parts of *Leuzea carthamoides* (Asteraceae) and *Centaurea hermanni* (Asteraceae) against *C. albicans*, *A. fumigatus*.^{15,16}

Juice of *Garcinia mangostana* contains polyphenolic compound such as flavonoid and tannin.¹⁷ It also contains α -mangostin compounds which are proven to have potential antifungal and antibacterial activity.¹⁸ Xanthone is the major compound of *G. mangostana* showed high-antifungal activity (against *C. albicans* and *Aspergillus niger*).¹⁹ In a study evaluating the anticandidal activity of pomegranate peel extracts (PPE) and application of PPE aerosol as sanitizer agent against exhibited potent anticandidal activity against *C. albicans* strains compared with standard fungicides in both susceptibility techniques used. Methanol, ethanol and water extracts were the most effective for inhibiting *C. albicans* growth. PPE aerosol

was an efficient method for complete sanitizing of semi-closed places against *C. albicans* growth.²⁰

Limitations

Since the study was a quantitative one and done in test tube we could not measure the zone of inhibition and hence statistical evaluation could not done. More studies need to be conducted with disc diffusion method for the tested fungal species. We did not identify the active principle. However, to obtain a novel drug chemical nature of active principle should be isolated and standardized and tested in more species of fungi.

CONCLUSION

S. indicus whole plant ethanolic extract can be used as a potential antifungal agent against *Dermatophytes* and *Candida* species. As whole plant was used, various phytochemicals could probably have contributed to antifungal activity. There is paucity of scientific data about the mechanism of action of the extracts. So further studies should be done to find out the molecular actions as well as pre clinical studies done to establish antifungal action of *S. indicus* extracts. The results of this study give a scientific proof for the traditional use of *S. indicus* against common fungal pathogens. This study reveals that the ethanolic extracts can be used as a potential lead to discover newer antifungal agents.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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