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Research Article

ANTIFUNGAL POTENTIAL OF DIFFERENT EXTRACTS OF *SYZYGIUM AROMATICUM* AGAINST *MICROSPORUM GYPSEUM*

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ABSTRACT

Present research was carried out to evaluate the effectiveness of different extracts of *Syzygium aromaticum* in the treatment of multifarious dermal infections caused by *Microsporum gypseum*. The present work was conducted to find out the antifungal potential of various extracts of dried powder of buds of *Syzygium aromaticum*by means of paper disc diffusion method, with petroleum ether, ethyl acetate, ethanol, and aqueous solvents in 25 μml, 50 μml and 100 μml concentrations against *Microsporum gypseum*. Clotrimazole, a synthetic antifungal drug was used as a standard. The present study revealed that *Syzygium aromaticum* is a potent antifungal agent against *Microsporum gypseum*. The ethanol extract of *Syzygium aromaticum* using 100 μml concentrations depicted the highest zone of inhibition of 19.690+ 0.86 mm and 45.790 % of mycelial inhibition against a tested pathogen.

Keywords: *Syzygium aromaticum,* antifungal, *Microsporum gypseum.*

INTRODUCTION

Dermatophytoses is a general skin disorder commonly found in both human and animals around the globe. The most common pathogens responsible for dermal infections are in the genera of *Epidermophyton, Trichophyton* and *Microsporum*[1]. The major proven symptoms are generally mild, but the implication of the disease is its high cost, ability to get transferred from animals to human and time-consuming treatment. Anti-dermatophytic drugs include several chemical groups and the important drugs are griseofulvin, terbinafine and azoles drugs such as ketoconazole and itraconazole[2]. Although they are beneficial and effective, simultaneously their unwanted adverse effects such as drug toxicity and drug resistance are of great concern. As long-term therapy is required for the treatment of dermatophytoses, the toxic effects of antifungal drugs cannot be avoided. For instance, griseofulvin is responsible for hepatotoxicity and gastrointestinal irritation on the other hand ketoconazole can restrain adrenal steroid synthesis[3]. Chronic infection and re-occurrence of skin infections are also a common nature of dermatophytoses and as a consequence repeated treatment is required which results into the development of drug resistance. In addition, antifungal activities of azoles agents, griseofulvin and terbinafine are fungistatic which could contribute to the emergence of drug resistance[4]. These adverse effects are issue of concern and are responsible for development of novel antifungal agents of herbal origin. In order to overcome the ill effects and resistance caused due to synthetic drugs, the World Health Organization has motivated many researchers to exploit natural products for their great therapeutic potential[5]. A huge variety of herbal antifungal agents derived from traditional medicinal plants are existing for the treatment of dermatophytoses[6]. In the present scenario, medicinal plants and their phytoconstituents are gaining attention owing to the fact that herbal drugs are lesser in cost, easily accessible and with fewer or no side effects[7]. Clove (*Syzygium aromaticum*) is one of the most valuable spices that have been used from centuries as food preservative and for many medicinal purposes. Cloves are considered to be inhabitant of Indonesia, but these days are cultivated around the globe[8]. The essential oils contain a variety of volatile molecules such as terpenes, terpenoids and phenol derived from many aromatic and aliphatic compounds, which might have bactericidal, antiviral and fungicidal consequences[9]. Eugenol is the main volatile compound of extracted oil from clove bud (*S. aromaticum* L.) that is used in traditional medicine, as a bactericide, fungicide[10,11] anesthetic and other infections[12]. During antifungal studies of *Syzygium aromaticum*, the hexane extract, ethyl acetate extract and methanol extracts and clove oil, the n-hexane extract showed the highest antifungal activity, followed by ethyl acetate extract, clove oil and methanol extract with 90%, 78%, 72% and 29% inhibition respectively against *Phytophthora palmivora* at 10 mg/ml[13].

MATERIAL AND METHODS

Preparation of Extract

Syzygium aromaticum dried flower buds were purchased from the local market for the preparation of the extract. Herbarium sheet is submitted with the Pharmacognosy department Chandigarh college of Pharmacy, Landran Mohali with voucher no. CCP/TFG/078. The dried flower buds of clove were grounded to form a powder with the help of a mechanical grinder. Clove buds' powder (500 g) was macerated successively at room temperature for 24 hours with petroleum ether, ethyl acetate, ethanol and aqueous solvents and tested against *Microsporum gypseum*. Each extract was evaporated using a rotary evaporator at 50°C

respectively. The prepared extract was weighed and stored in airtight sample bottles. The filtered extracts were tested against *Microsporum gypseum* at three different concentrations viz. 25 μml, 50 μml and 100 μml.

Procedure and Procurement of strain

The antifungal potential of an extract of dried flower buds of *Syzygium aromaticum* was evaluated by the Paper disc diffusion method. The test organisms used were the dermatophyte strains of *Microsporum gypseum*, which was procured from IMTECH, Chandigarh with MTCC No. 2829. Sabouraud Dextrose agar was used as a culture media according to the manufacturer's direction. The dermatophyte cultures were aseptically inoculated on Sabouraud agar plate and subjected to incubation at 28°C for approximately 5 days[14].

Antifungal activity

In this research, Paper disc diffusion method was employed, and some amount of Sabouraud Dextrose agar was dispersed in Petri dishes, which were allowed to solidify. A micropipette was employed to introduce 0.1 ml. Spores on agar medium and was spread with the help of glass rod spreader under aseptic conditions. Sterilized discs (5 mm, Whatman No. 1 filter paper) were prepared by soaking in different concentrations of the extracts, i.e., 25 μml, 50 μml and 100 μml for approximately 5- 6 hour. After this duration, discs were removed and then allowed to dry. To evaluate the antifungal potential of dried flower buds of *Syzygium aromaticum* extracts, various discs impregnated with different concentrations of the dried flower buds of *Syzygium aromaticum* extracts were positioned on the fungal spore or mycelium with the help of sterilized forceps. The Petri dishes incubated at 28 ºC for 72 hours. The antifungal potential was determined by measuring the zone of inhibition (ZOI) around the discs and percentage inhibition after the period of incubation[14].

Data analysis

Data from antifungal screening was analyzed with the help of simple statistics from Microsoft Excel and recorded in appropriate tables as a mean $\pm$ standard deviation of the mean.

RESULT AND DISCUSSION

Antifungal potential of extracts of dried flower buds of *Syzygium aromaticum* which were tested against fungal strain *Microsporum gypseum* is depicted in Table 1. The Pet ether extract of dried flower buds of *Syzygium aromaticum* showed 7.45 mm ZOI at 25 μml concentration. 50 μml concentrations were moderately effective with 12.10 mm zone of inhibition. At 100 μml, the zone of inhibition was observed to be as 14.36 mm. The ethyl acetate extract showed a 9.49 mm inhibition zone at 25 μml concentration. 50 μml concentrations were effective with 11.99 mm inhibition zone. 16.89 mm inhibition zone was observed at 100 μml. The ethanol extract showed a 12.54 mm inhibition zone at 25 μml concentration. 50 μml concentrations were moderately effective with 15.82 mm inhibition zone. At 100 μml, the inhibition zone was observed to be as 19.69 mm. While it's aqueous extract showed a 4.12 mm inhibition zone at 25 μml concentration. 50 μml concentrations were effective with a 9.85 mm inhibition zone. 11.15 mm inhibition zone was observed at 100 μml concentration. The antifungal potential was determined by comparing the activity of extracts with the Clotrimazole, in which the zone of inhibition was 43 mm. Percentage inhibition was also calculated, which was 45.79% with 100 μml ethanol extract depicted in Table 2.

Table 1: Mean Zone of Inhibition in different solvents (mm) of the *Syzygium aromaticum*

Crude Drug	Conc.	Pet Ether	Ethyl Acetate	Ethanol	Aqueous	Clotrimazole
Syzygium aromaticum	25 μml	7.455 $\pm$ 0.85	9.486 $\pm$ 0.86	12.542 $\pm$ 0.65	4.122 $\pm$ 0.96	43.000 $\pm$ 0.20
Syzygium aromaticum	50 μml	12.100 $\pm$ 0.65	11.990 $\pm$ 0.75	15.820 $\pm$ 0.86	9.850 $\pm$ 0.74	43.000 $\pm$ 0.20
Syzygium aromaticum	100 μml	14.360 $\pm$ 0.65	16.890 $\pm$ 0.75	19.690 $\pm$ 0.86	11.150 $\pm$ 0.89	43.000 $\pm$ 0.20

Table 2: Percentage inhibition (%) of various extracts of the *Syzygium aromaticum*

Crude Drug	Conc.	Pet Ether	Ethyl Acetate	Ethanol	Aqueous	Clotrimazole
Syzygium aromaticum	25 μml	17.325%	2.046%	29.162%	9.581%	100%
Syzygium aromaticum	50 μml	24.464%	27.206%	36.197%	23.004%	100%
Syzygium aromaticum	100 μml	33.395%	39.279%	45.790%	25.930%	100%

CONCLUSION

The present research provides evidence about the antifungal potential of crude dried flower buds of *Syzygium aromaticum* against *Microsporum gypseum*. The antifungal potential is different depending on the polarity of the solvent utilized in the extraction process. From the study, it can be depicted that ethanol extract of clove are promising as compared to other solvents. The level of activity was comparable to those previously shown to be effective in treating dermatophytes in several species. The major active substances in these oils were eugenol and its derivatives. Cloves are traditional and aromatic drug which is exploited for many beneficial and therapeutic purposes. Clove extract was successfully effective in suppressing the growth of *Microsporum gypseum in vitro*. Ethanol extract of clove could be promising as a source of herbal antifungal compound for *in vivo* applications.

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