

SHORT COMMUNICATION

Antifungal activity of curry leaf (*Murraya koenigii*) extract and an imidazole fungicide on two dermatophyte taxa

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Abstract

Antifungal efficacy of the ethanolic extract of *Murraya koenigii* was evaluated against two dermatophyte taxa, namely, *Trichophyton mentagrophytes* and *Microsporum gypseum*. The therapeutic value of the ethanolic *Murraya koenigii* extract was assessed against an imidazole fungicide. The extract equally inhibited vegetative growth like that of the fungicide, both the fungicide and the extract exerted significant effect on the hyphal morphology. The effect of extract is almost equal that of the fungicide on conidiation and germination. The total lipid content and ergosterol content of the treated mycelium dwindled in both the dermatophyte treated with the extract. The crude leaf extract inhibited both lipid and sterol synthesis. The crude extract inhibited lipase secretion compared to the fungicide.

Keywords: *Murraya koenigii*, *Trichophyton mentagrophytes* and *Microsporum gypseum*, fungicide.

Introduction

Infectious diseases pose a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (World Health Organization, 1998). During the past several years, there has been an increasing incidence of fungal infections due to growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the question of resistance to antibiotics and with the toxicity caused by prolonged treatment with several antifungal drugs (Giordani *et al.*, 2001) has prompted an extended search for newer drugs to combat opportunistic fungal infections. Dermatophytoses (dermatomycosis, tinea, ringworm) are the most common forms of fungal infections encountered in most countries (Ribbon, 1988). The diseases are caused by keratinophilic fungi called dermatophytes. The dermatophytes can cause diseases of the skin, hair and nails. Therefore, these fungi have become a public health problem. In the Indian subcontinent, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicine.

According to the national health experts, (2000) around 1500 drugs are extracted from various plants. In an effort to find alternative remedies for the various maladies caused by infectious fungi, researchers are on an endless search. Many plant-part extracts have proved to be promising to this effect. For instance, the methanolic extract and total alkaloids of the aerial parts of *Glaucium oxylobum* exhibited good activity against *M. gypseum*,

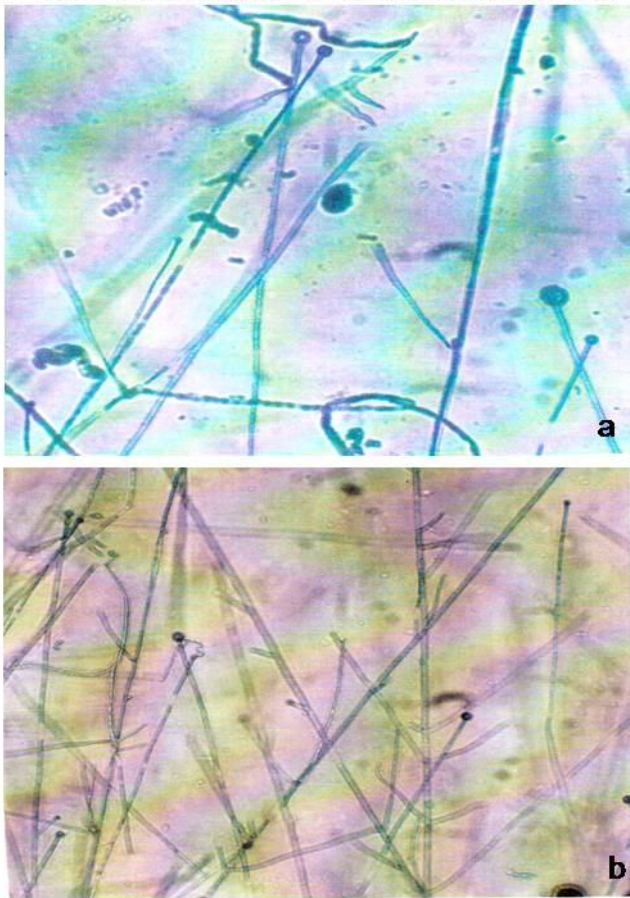
M. canis, *T. mentagrophytes* and *E. floccosum* (Morteza-Semmani *et al.*, 2003). *Syzygium jambolanum* seed water and methanolic extracts proved to be antimycotic against *T. rubrum*, *T. mentagrophytes* and *M. gypseum* besides yeast and other filamentous fungi (Chandrasekaran and Venkatesalu, 2004). Methanolic extracts of *Pistia stratiotes* were found to be active against *T. rubrum*, *T. mentagrophytes*, *E. floccosum*, *M. gypseum* and *M. nanum* (Premkumar and Shyamsundar, 2005).

The aim of this study is to evaluate the antifungal efficacy of the ethanolic extract of *Murraya koenigii* (Fig. 1) against two dermatophyte taxa, namely, *Trichophyton mentagrophytes* and *Microsporum gypseum*. The therapeutic value of the ethanolic *Murraya koenigii* extract has been assessed against an imidazole fungicide.

Fig. 1. *Murraya Koenigii*.



Fig. 2. Hyphal branches of *T. mentagrophytes* (320 X).



a. Curry leaf extract; b. Imidazole

Materials and methods

Preparation of ethanolic *Murraya koenigii* leaf extract
 The fresh and healthy *Murraya koenigii* leaves (500 g) were extracted with 1.5 L ethanol (95%) for 24 h. This suspension was filtered and the residue was re suspended in an equal volume of 95% ethanol for 48 h and was filtered again. The two filtrates were pooled, and the solvents were evaporated in a rotavapour at 40-50°C under reduced pressure. The resultant semisolid substance was used for the study.

Test microorganisms

The cultures of *Trichophyton mentagrophytes* and *Microsporum gypseum* obtained from the culture collection, Department of Medical Microbiology, PGIBMS, University of Madras were used in the study.

Antifungal activity of the extract

SDA medium was amended with the extract to yield the final concentrations of 50, 100, 150, 200 and 250 µg/mL, an unamended aliquot serving as the control. The petri dishes containing the medium were inoculated with the test organism and the same were incubated for 10 d at 30°C. Growth was assessed in terms of the colony diameter as well as dry weight in mg. The degree of sporulation was also assessed after inducing the same by incubating the plates under the white fluorescent light

under the light regimes, 12:12 darkness and light. Spore germination potential under the influence of the extract was also assessed in terms of percentage inhibition in germination. Suitable controls were maintained for these assessments. In another set of petriplates an imidazole fungicide was amended in place of the extract to yield the final concentrations of 10-50 µg/mL.

Estimation of lipid and ergosterol

Lipid and ergosterol levels of the treated samples were compared with the untreated samples as per Folch *et al.* (1957) method. The contents were expressed as µg g⁻¹ dry weight of the mycelium. Extracellular lipase activity was assessed in the treated samples by the method of Sierra (1957) by growing the cultures on Tween-20 medium and by measuring the extent of the lytic zone in the culture plates.

Results and discussion

The antifungal efficacy of the curry leaf extract (ethanolic) in relation to an ergosterol biosynthesis inhibiting fungicide, imidazole has been evaluated against two well-known dermatophyte, *T. mentagrophytes* and *M. gypseum*. The effect of the extract equaled that of the fungicide as far as the vegetative growth was concerned (Fig. 2 and 3). However, both the fungicide and the extract exerted significant effect on the hyphal morphology. The branching potential increased; numerous short slender branches of hyphae with swollen tips were observed (Fig. 4). This is an usual effect of any antifungal compound. The effect of the extract is almost equal that of the fungicide on conidiation and germination as well (Fig. 5 and 6).

Fig.3. Effect of the extract and fungicide on radial growth of dermatophytes.

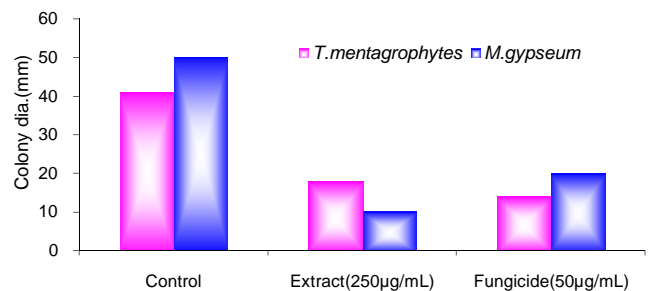
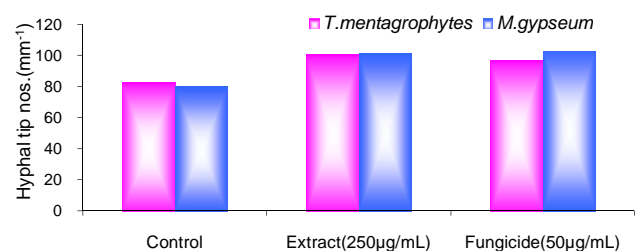
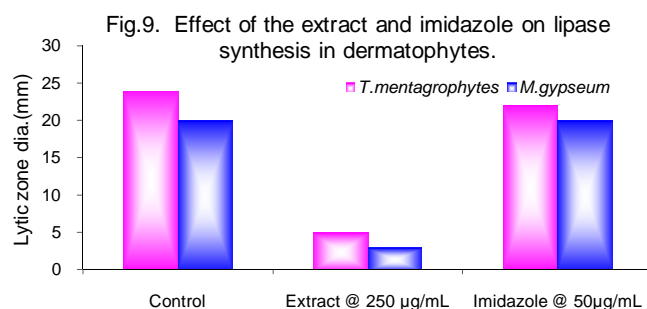
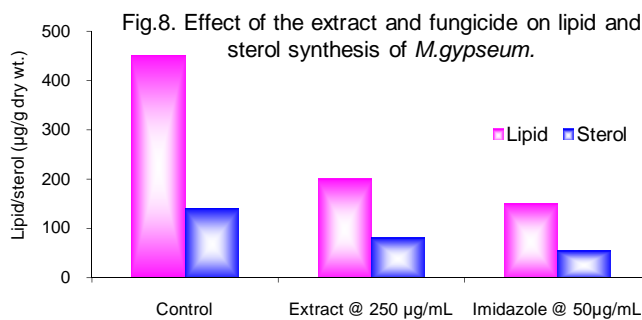
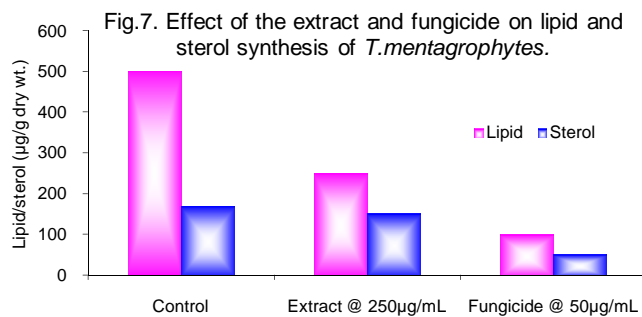
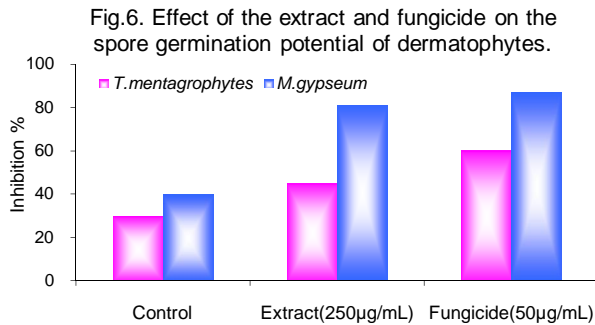
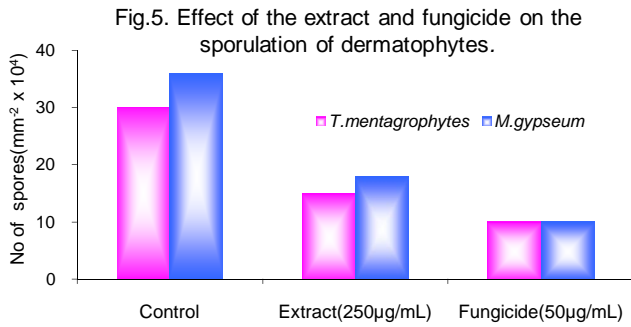


Fig.4. Effect of the extract and fungicide on branching potential of hyphae of dermatophytes.





The total lipid content and ergosterol content of the treated mycelium dwindled in both the dermatophyte. It is natural with imidazole because it is an ergosterol biosynthesis inhibitor. Interestingly, a crude leaf extract has considerably inhibited both lipid and sterol synthesis (Fig. 7 and 8). Perhaps a refined extract would be much more efficacious and would perhaps replace a synthetic compound like imidazole. An other interesting finding is that of the effect of the extract on lipase secretion. It is surprising that the crude extract inhibited lipase secretion but not the fungicide (Fig. 9). From this it is suggestive that, the extract contains some substance which is a potent lipase inhibitor. Lipases are important for the dermatophytes since the fungi need to gain entry in to the host tissue, whose epidermis is rich in lipid substances.

Conclusion

To conclude, this study evaluated the antifungal efficacy of the ethanolic extract of *Murraya koenigii* against two dermatophyte taxa, namely, *Trichophyton mentagrophytes* and *Microsporum gypseum* and revealed the therapeutic value of the ethanolic *Murraya koenigii* extract. Further investigations should be carried out to isolate and evaluate the antifungal compound in the extract to replace synthetic fungicides.

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