

IN VITRO SENSITIVITY STUDY OF ANTIFUNGAL DRUGS AND PLANT EXTRACTS AGAINST MYCOTIC MASTITIS CASES

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ABSTRACT

A comparative study was made to find out the in vitro antifungal susceptibility of fungal isolates to commercially available antifungal drugs and plant extracts. The sensitivity was studied by disc diffusion method. Out of the three essential oils, cinnamon leaf at 1 in 10 dilution showed better in vitro antifungal activity to all the yeast and yeast like fungal organism compared to Amphotericin.B, Fluconazole and Griseofulvin. *Geotrichum candidum* was found to be more amenable to cinnamon leaf oil than all the commercial antifungal agents except Nystatin.

KEYWORDS: Fungal Mastitis, Antifungal drugs.

INTRODUCTION

The various agents which cause clinical and subclinical mastitis may be hazardous to human health. Previously bacteria were considered to be the most causative agents of mastitis but now a days fungus, especially yeast and yeast like fungal organisms are also being recognized as primary agents for this condition. Frazier (1967) reported that essential oils of certain spices possess antimicrobial activities. The present study was undertaken to compare in vitro efficacy of different commercially available antifungal drugs and plant extracts against fungi isolated from bovine mastitis cases.

MATERIALS AND METHODS

Twenty species of yeasts and yeast like fungi viz. *Candida tropicalis* (7 isolates), *Candida parapsilosis* (1 isolate) *Candida guilliermondii* (1 isolate) *Geotrichum candidum* (4 isolates), *Trichosporon cutaneum* (3 isolates), *Saccharomyces cerevisiae* (1 isolate), *Rhodotorula rubra* (1 isolate) and *Torulopsis Sp.* (1 isolate) and mould isolates namely *Penicillium Sp.* (2 isolates), *Sepedonium Sp.* (1 isolate), *Aspergillus ochraceous* group (1 isolate), *Cladosporium carrionii* (1 isolate) and *Trichophyton verrucosum* (1 isolate) isolated from mastitic milk were used for sensitivity trials. Eight drugs namely Amphotericin- B (100 units), Clotrimazole (10µg), Fluconazole (10µg), Nystatin (100 units), Griseofulvin (250µg, 125µg & 50µg), Itraconazole (0.3125µg, 0.0156µg & 0.0078µg), Ketoconazole (100µg, 80µg & 60µg) and Pimaricin (5µg, 2.5µg & 1.25µg) were used in this study. The drug sensitivity was studied by disc diffusion method as adopted by Bauer et al. (1966).

Aqueous extracts of cinnamon leaf (*Cinnamomum zeylanicum*), lemon grass (*Cymbopogon citrates*), clove (*Syzygium aromaticum*), and methanol extracts of Cassia alata were used in this study. Discs of 1:10 and 1:20 dilutions of plant extracts were prepared by standard method as adopted by Bauer et al. (1966).

The yeast suspension and mould suspension was adsorbed onto a sterile cotton swab, uniformly inoculated on yeast nitrogen base agar plates and allowed to dry for 10 minutes. The different antifungal discs were then placed on the medium suitably spaced with the help of sterile forceps. The yeast-inoculated plates were incubated at 37°C for 24 to 48 hours and the filamentous fungi inoculated plates were incubated at room temperature for 5 days. Inhibitory zones were read visually

and measured with the help of a scale. The inhibitory zone of 15 mm and above was marked as highly sensitive, 10-15mm as moderately sensitive, 5-10 mm as less sensitive and below 5 mm was declared as resistant as adopted by Shah et al. (1986).

RESULTS AND DISCUSSION

The antifungal susceptibility of yeast and yeast like fungi showed better *in vitro* antifungal activity to Cinnamon leaf oil at 1 : 10 dilution compared to Amphotericin B, Fluconazole and Griseofulvin. Cinnamon leaf oil at 1 : 10 dilution could produce more diameter zone of inhibition for the yeast and yeast like fungi except *C. parapsilosis* and *R. rubra* when compared with Pimaricin. *Geotrichum candidum* was found to be more amenable to cinnamon leaf oil than all the commercial antifungal agents except nystatin. Comparative evaluation of the efficacy of Cinnamon leaf oil with common antifungal agents revealed that Cinnamon leaf oil was more powerful than Amphotericin B, Clotrimazole, Nystatin and Itraconazole to inhibit *R. rubra*. It was found to be more effective in suppressing the growth of fungal organism than Fluconazole and Griseofulvin.

Lemon grass oil at 1 : 10 dilution found to be more effective than Amphotericin B, Fluconazole and Pimaricin in combating *G. candidum*. In the suppression of *R. rubra* this oil was found superior to Amphotericin B and Clotrimazole. Even though at 1 : 10 dilution it has good suppressive action on *G. candidum* and *R. rubra* some antifungal agents were superior to this oil. Lemon grass oil at 1:10 and 1:20 dilutions revealed no antifungal activity against mould.

Cinnamon leaf oil at 1:10 dilution showed more *in vitro* antifungal activity to all the mould isolates compared to Amphotericin B, Fluconazole, Griseofulvin, Ketoconazole and Nystatin. Cinnamon leaf oil at 1:10 dilution could produce inhibition to *Penicillium sp.* when compared with pimaricin and showed inhibition to *A. ochraceous* group when compared to Itraconazole. It showed inhibition to *C. carrionii* when compared to Clotrimazole,

Clove oil at 1:10 dilution was able to suppress the growth of *Sepedonium Sp.* and *C. carrionii*. It was found to be more effective in suppressing the growth of mould than Fluconazole, Griseofulvin and Nystatin. Aqueous and methanol extract of Cassia alata revealed no antifungal property even though Fuzellier et al. (1982) had reported the antifungal property of the aqueous extract from Cassia alata leaves.

The results accrued out of the present study points to the possibility of employing cinnamon leaf oil at 1 : 10 dilution than the commonly available antifungal agents to combat fungal mastitis.

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