THE IN VITRO ANTI-YEAST ACTIVITY OF SOME ESSENTIAL OILS

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SUMMARY: The in vitro anti-fungal activity of twelve commercially available essential oils were tested against five pathogenic species of yeasts (Candida albicans, C. stellatoidea, C. tropicalis, Torulopsis candida and T. versitilis) using the paper disc plate method. Trosyd (tioconazole) was taken as standard inhibitor. From the investigated oils, five exhibited various degrees of inhibition to all test yeasts; these were cinnamon, clove, horseradish, marjoram and peppermint. Cinnamon is the most effective oil in front of all pathogenic yeasts studied and C. tropicalis has shown the greatest degree of sensitivity to this oil. On the other hand, cacao oil exhibited weak suppressive effect against C. stellatoidea only.

Key Words : Essential oils, pathogenic yeasts, anti-yeast activity.

INTRODUCTION

It has been known since ancient times that essential oils of certain plants have preservative qualities (5). The use of oils such as cinnamon, clove and cassia by ancient Egyptians in the processes of mummification of their dead is well documented. Several investigations have been directed towards the antimicrobial activity of essential oils (3,8,11,12,15,18-20,23). In North America, pathogenic yeasts are the second most common cause of vaginitis (21). In United Kingdom, vaginal candidosis is the third commonest condition diagnosed in departments of genitourinary medicine (4). In Assiut (Upper Egypt), vaginal candidosis is responsible for nearly half the cases of vaginitis (2). Therefore the aim of the present investigation was to find a suitable anti-fungal agent, capable of inhibiting pathogenic yeasts which cause vaginitis.

MATERIALS AND METHODS

The following twelve commercially essential oils collected from spices and aromatics shops in Assiut (Egypt) have been studied: Anise (*Pimpinella anisum*), black cumin (*Nigella sativa*), cacao (*Theobroma cacao*), cinnamon (*Cinnamomum verum*), clove (*Syzygium aromaticum*), fenugreek (*Trigonella foenum-gracenum*), garlic (*Allium sativum*), horseradish (*Raphanus sativus*), marjoram (*Majorana hortensis*), onion (*Allium cepa*), peppermint (*Mentha piperita*), and tamarind (Tamarindus indica). These oils extracted from seeds except, cinnamon from bark, clove from flower buds, and marjoram and peppermint from leaves.

Organisms

Cultures of five species of yeasts namely; *Candida albicans, C. stellatoidea, C. tropicalis, Torulopsis candida* and *T. versitilis* isolated from 225 women with abnormal vaginal discharge and/or pruritis, burning and dysparuenia (2) were maintained on Sabouraud's dextrose agar and used *in vitro* studies reported in this paper.

Sensitivity test

The anti-fungal effect of essential oils was examined using the paper disc plate method. Twenty ml of sterilized Sabouraud's dextrose agar medium were poured into a 15 cm diameter Petri dish. After its hardening 1ml of spore suspension of each test organism was distributed evenly over the surface. Three paper discs (Whatman No. 3; 3 mm diameter) fully saturated individually by the tested oil were placed on the seeded agar et equal distances. The plates were incubated for

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2-3 days at 37±1°C. The diameter of the inhibition zone around the disc was measured and the mean of the replicates is given. Results were compared with 1% lotion of tioconazole (Trosyd drug, manufactured by Pfizer Egypt). It is a member of the imidazole class of compounds which is used as a fungicide in the treatment of dermatophytic diseases.

RESULTS

The results in Figure 1 show the anti-fungal properties of twelve investigated essential oils in their commercial forms against five pathogenic yeast species comparable with tioconazole.

All tested yeasts were affected by tioconazole. The greatest inhibitory effect was recorded with *Torulopsis candida* (36mm; inhibition zone) followed by T. versitilis (25 mm), *Candida stellatoidea* (20 mm), *C. tropicalis* (19 mm) and *C. albicans* (14 mm).

Candida albicans was affected by six (out of twelve) of the investigated oils. The marked inhibition zone (42.5 mm) was observed by cinnamon followed by clove (30 mm), horseradish (28.5 mm), marjoram (25 mm), peppermint (23.5 mm) and anise (17.5 mm). These oils showed better anti-yeast activity than trosyd (14 mm). On the other hand, the remaining six essential oils did not cause any inhibition zone.

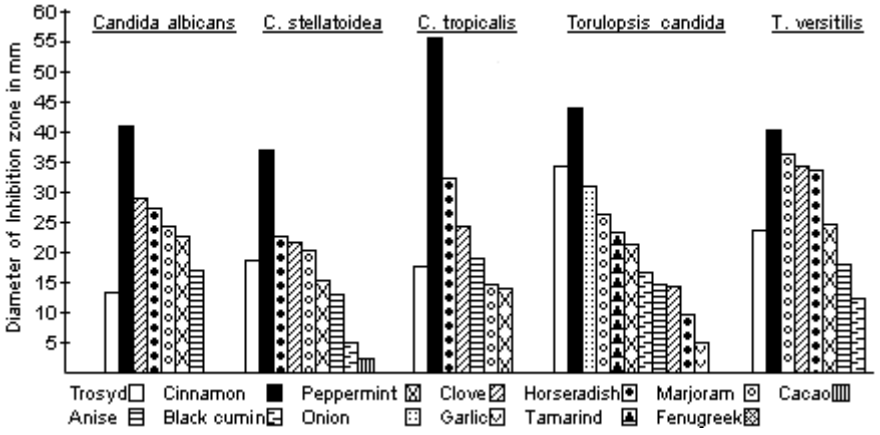
Eight oils affected the growth of *C. stellatoidea* from which cinnamon, horseradish, clove and marjoram exerted higher inhibitory effects comparable with trosyd. However, peppermint, anise, black cumin and cacao showed less anti-yeast activity against *C. stellatoidea.* The highest inhibition zone was observed in *C. tropicalis* culture plate with cinnamon. The growth of *C. tropicalis* was also inhibited by horseradish, clove, anise, marjoram and peppermint, respectively. The remaining oils did not cause any inhibitory effect against *C. tropicalis*.

Torulopsis candida was affected by 10 out of 12 oils tested from which cinnamon oil only showed better anti-yeast activity than trosyd, whereas in case of *T. versitilis,* it is growth was inhibited more than trosyd by each of cinnamon, marjoram, clove, horseradish and peppermint. The inhibition zone ranged from 42 mm with cinnamon to 25 mm with peppermint.

DISCUSSION

The inhibitory effects of twelve essential oils in their commercial forms against five species of pathogenic yeasts were studied. Cinnamon is the most effective oil in front of all the yeast species studied and its effect was more pronounced than trosyd. *Candida tropicalis* has shown the greatest degree of sensitivity to this oil. Also, five types of oils namely: anise, clove, horseradish, marjoram and peppermint inhibited the growth of all tested yeast species. The black cumin was of moderate inhibitory effects. It inhibits three (out of five) species; these were *C. stellatoidea, T. candida* and *T. versitilis.* On the other hand, garlic, onion and tamarind exhibited weak suppressive effect where they affected two out of five species. Cacao oil affected only *C. stellatoidea;* the minimum inhibition zone (2.5 mm) was observed in *C. stellatoidea* with this oil. In accordance with our results, cinnamon was recorded as strong inhibitory agent against several pathogenic fungi (17).

Figure 1: Effect of some essential oils on the growth of five species of yeast comparable with trosyd (tioconazole).



(14) and (22) reported that cinnamon contains 0.5-1.0% volatile oil which consists mainly of cinnamaldehyde (65-75%), eugenol (4-10%) and these compounds are the major anti-fungal substances of cinnamon oil (5).

Peppermint oil contains 50-78% free methanol, 5-20% combined in various esters, L-limonen, menthone, cineol and phellandrene (7) and these constituents may be responsible for the inhibition of different fungal species (6). (19) provided evidence that garlic extract has inhibitory and cidal properties to yeast-like fungi including Candida, *Cryptococcus, Rhodotorula, Torulopsis* and *Trichosporon*. The depressive effect against several pathogenic fungi of cinnamon was reported by (17); of peppermint by (13) and (16) of anise by (23); of clove and peppermint by (9); of *Eugenia uniflora* by (3); of marjoram by (8); of thyme, cinnamon and peppermint by (2); of eucalyptus by (18); of palmarosa and aniseed by (11); of *Capillipedium foetidum* by (12) and of *Marjorana hortensis* and *Anismeles indica* by (24).

According to the explanation of (10) it appears that there is a relationship between the chemical structure of the most abundant compounds in the essential oils and the anti-microbial activity.

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