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COMBINED ACTION OF ANTIMYCOTICS AND ESSENTIAL OILS ON THE REFERENCE CANDIDA STRAIN IN VITRO

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Introduction. The frequency of fungal infections has increased in recent years as a result of an increase in the number of immunocompromised patients due to HIV infection, cancer chemotherapy, and organ or bone marrow transplants. *Candida* infections are very common in these people, including oral, vaginal, and systemic candidiasis [1]. Polyenes and azoles are considered the drugs of choice in the treatment of these fungal infections, but the toxicity associated with the use of amphotericin B and the failure of azole therapy due to internal resistance of *Candida spp.* are the serious problem [2]. Given the limitations of currently available antimycotics in terms of toxicity, potency, cost, and emerging resistance, the search for new alternative strategies, such as the use of drug combinations, is warranted [3]. These combinations may include herbal products and, especially, essential oils (EOs), which have long been known in ethnomedicine as effective and safe remedies for candidiasis [4]. Because natural products are less expensive and considered safer, they should be studied for synergistic interactions with the drugs of choice for treating *Candida* infections, which could lead to more economical and safer formulations. As ingredients for compositions with nystatin, amphotericin B and fluconazole, tea tree, eucalyptus and cloves EOs, which are among the most popular at the pharmaceutical market, can be useful.

The aim of the study is to investigate the combined effect of traditional antifungal drugs (antibiotics and azoles) and EOs on the susceptibility of the reference strain of *C. albicans*.

Main part. Determination of the susceptibility of the reference strain *C. albicans* ATCC 10231 was carried out by the disk diffusion method [5]. It were used standard discs with nystatin (100 U), amphotericin B (100 U), and fluconazole (10 mg) manufactured by Himedia Laboratories Pvt. Limited, India. EOs of cloves (*Eugenia caryophyllata*), tea tree (*Melaleuca alternifolia*) and eucalyptus (*Eucalyptus globulus*) (Green Pharm Cosmetic, Ukraine) in the amount of 10 µl were applied to empty sterile discs or to standard discs with antifungal drugs immediately before they were placed on the surface of Mueller-Hinton agar, modified to determine the susceptibility to antimycotics (Himedia Laboratories Pvt. Limited, India), in Petri dishes with a test culture of microorganisms. The results were recorded after 24 hours of incubation at 35°C. The susceptibility of microorganisms was assessed by the growth inhibition zones more than 10 mm [6]. If the zone of inhibition exceeded 25 mm, the microorganism was considered highly susceptible; from 16 to 25 mm – moderately susceptible; from 10 to 16 mm – low susceptible. The determination was repeated 5 times followed by statistical processing using the Newman-Keuls test and Statistica for Windows 6.0 software.

It was shown that diameters of the growth inhibition zones of *C. albicans* ATCC 10231 around the disks with nystatin averaged 23 mm, with amphotericin B – 16 mm, with fluconazole – 22 mm, which corresponded to the reference susceptibility values for this etalon strain. *C. albicans* ATCC 10231 showed high susceptibility to the clove EO (growth inhibition zones averaging 29 mm), low susceptibility to the tea tree EO (growth inhibition zones averaging 12.6 mm), and was insusceptible to the eucalyptus EO (growth inhibition zone 8.3 mm). Nystatin. amphotericin B, or fluconazole combining with the clove EO increased growth inhibition zones of the test culture by 12 mm, 4 mm and 20 mm compared with these antimycotics themselves ($p<0.05$), which obviously reflects an increase in *Candida* susceptibility. The combined use of amphotericin B and fluconazole with the tea tree EO also led to an increase in the susceptibility of *C. albicans* ATCC 10231, as evidenced by an increase in the diameter of growth inhibition zones by 5 mm and 8 mm, respectively ($p<0.05$) compared with the control. At the same time, the combination of nystatin with the tea tree EO caused a decrease in growth inhibition zones compared to those around discs with nystatin, which, however, was not significant. The eucalyptus EO increased the susceptibility of the used test culture of fungi to fluconazole ($p<0.05$) compared to the same parameter for the drug itself and did not change it with respect to amphotericin B. The addition of the eucalyptus EO to nystatin was characterized by a decrease in susceptibility of the microorganisms test culture to this agent, which was confirmed a decrease in growth inhibition zones by an average of 4 mm ($p<0.05$).

Conclusions. Thus, not all combinations of traditional antimycotics, which are known treatments for candidiasis, with cloves, tea tree, and eucalyptus EOs showed an increase in antifungal activity against the

reference strain *C. albicans* ATCC 10231. The observed differences (intensification of the effect, its weakening or absence), apparently, can be due to the different ratio of the prooxidant properties of antifungal drugs as a component of their mechanism of action and the antioxidant activity of EOs. The most consistently synergistic effect was observed when the cloves EO had been added to the antimycotics, which concerned both polyene antibiotics and the representative of azoles. The revealed features of the combined action of traditional antimycotics with EOs require further study in order to substantiate a purposeful differentiated approach to the creation of pharmaceutical compositions containing these ingredients.

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