# SYNERGISTIC PROPERTIES THE COMPLEX OF ESSENTIAL OILS WITH MICROBIAL SURFACTANTS

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According to recent studies of World Health Organization (WHO), almost half of clinical isolates of methicillin-resistant strains of *Klebsiella pneumoniae* and *Staphylococcus aureus*, and of *Escherichia coli* are resistant to 3rd generation cephalosporins, fluoroquinolones and carbapenems [1]. Likewise, the resistance of representatives of the genus *Candida* is increasingly reported against fluconazole (93%), amphotericin B (35%) and echinocandins (7%) [2]. Those pathogens annually cause illnesses of nearly 700 thousand people worldwide, and according to some experts those numbers may reach 10 billion as early as 2050 [3].

Reducing the number of antibiotic-resistant microorganisms can be achieved by using alternative compounds of natural origin, such as bacteriocins, microbial peptides, surfactants (SA) [4, 5] and essential oils (EO) [6-8]. The latter contain aldehydes, alcohols and phenolic compounds and thus are effective antimicrobial agents. That is why EO can be used instead of antibiotics and synthetic compounds in the cosmetic, food and pharmaceutical industries. However, the minimum inhibitory concentrations (MIC) of EO are rather high (400-1600 μg/ml) [6-8], leading to high EO content in the various products. Simultaneously, EO in such concentrations are known to cause severe damage to the central nervous system, and aspiration pneumonia [9]. The concentration of EO can be reduced without affecting their properties if they are used in combination with other biocides.

The present review is aimed to analyze and summarize the published data on the synergic antimicrobial activity of essential oils and other antimicrobial compounds, and on their synergic activity on biofilms.

It should be noted that the information is extremely limited on the synergism of the antimicrobial activity of EO with microbial surfactants. In 2014, Haba et al. [10] found that rhamnolipids synthesized by *Pseudomonas aeruginosa* 47T2 in an emulsion with EO of tea tree, lavender, oregano and cinnamon show an antimicrobial effect against *S. aureus* ATCC 43300 and *C. albicans* ATCC 10231. Thus, emultion of water: rhamnolipids: tea tree EO in ratio (%) 71.8: 2.8: 25.3 inhibited the growth of methicillin-resistant strain *S. aureus* ATCC 43300. The growth inhibition zone was 15.2 mm, while under the effect of essential oil or rhamnolipids separately, it was 11 and 9 mm, respectively. More effective antimicrobial agents were emulsions (%) of water: rhamnolipids: oregano EO (72.2: 11.1: 16.7) and water: rhamnolipids: cinnamon EO (80.9: 1.9: 17.1): the inhibition zones for *C. albicans* ATCC 10231 were 39.3 and 36.0 mm, respectively. Interestingly, rhamnolipids at a concentration of 1.9% (effective concentration of rhamnolids in the composition of an emulsion with cinnamon EO) did not inhibit the growth of *C. albicans* ATCC 10231 at all. The authors note that rhamnolipids are effective emulsifying agents that, by dispersing essential oils, increase their antimicrobial activity.

Our own studies [11] have shown that, with the simultaneous introduction of emulsions based on tea tree oil (12.5 μl/ml) and surfactant (0.43 mg/ml) to the suspension of test cultures of *C. albicans* D-6, *Aspergillus niger* P-3, and *S. aureus* BMS-1 ($10^4$ –$10^5$ cells/ml), the number of living cells after 15 min of exposure was 0.7% to 66% lower than if the microbial suspension were treated with oil preparations without surfactants.

In the following studies, we established a synergism of the antimicrobial activity of tea tree EO and surfactants of *Nocardia vaccinii* IMV B-7405 against *Pseudomonas* sp. MI-2, *S. aureus* BMS-1, *Escherihia coli* IEM-1 and *Bacillus subtilis* BT-2. MIC of essential oil in the test cultures were 625-156 μg/ml, and in the presence of surfactants they decreased by 2 to 260 times. MIC of the mixtures of EO and surfactant were three orders of magnitude lower against *S. aureus* BMS-1 and *B. subtilis* BT-2 than MIC established for essential oil only.

Further experiments showed that surfactants of *N. vaccinii* IMV B-7405 exhibited a synergistic effect when mixed with cinnamon and lemongrass EO. Thus, MIC of EO against *C. albicans* D-6, *C. tropicalis* PE-2 and *C. utilis* BMS-65 were in the range of 312–156 μg/ml, and if EO were added to the surfactant solution, their MIC decreased to 9.7–39 μg/ml.

In addition to antimicrobial activity, essential oils have the ability to degrade biofilms [12–14]. The mechanism of biofilm degradation under the activity of EO is associated with the presence of phenolic terpenoids (thymol, carvacrol) in their composition. The terpenoids can penetrate the polysaccharide matrix and cause antimicrobial action. Due to their hydrophobic nature, EO interact with the bilipid layer of the cytoplasmic membrane, causing it to lose integrity and hence impairing its function [15, 16].

The need of new compounds capable of destroying biofilms is primarily due to the fact that microorganisms in the biofilm have increased resistance to known biocides and rapidly acquire that resistance [15–19].

The available literature on the use of a mixture of essential oils with other antimicrobial compounds for the degradation of biofilms relates mainly to the complex of EO with antibiotics [17, 19, 20] or fluconazole [17].

Our studies have shown that in addition to synergistic antimicrobial action, a mixture of *N. vaccinii* IMV B-7405 surfactants with essential oils of cinnamon and lemongrass was effective for the degradation of yeast biofilms. The highest degree (43-60%) of degradation of *C. albicans* D-6, *C. tropicalis* PE-2 and *C. utilis* BMS-65 biofilms was observed by the activity of microbial surfactants and essential oils of cinnamon and lemongrass at a concentration of 300 μg/ml. The use of a mixture of surfactants and EO in a ratio of 1 : 1 was accompanied by an increase in the degree of biofilms degradation to 70%. In the available literature we found no information about the ability of EO in a mixture with microbial surfactants to increase the degree of destruction of biofilms.

Therefore, our own studies are among the first few to demonstrate the synergistic antimicrobial activity of essential oils with microbial surfactants.

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