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THE EFFECT OF ESSENTIAL OILS ON THE VIABILITY AND ADHESIVE PROPERTIES OF CLINICAL STRAINS OF *CANDIDA ALBICANS* AND *CANDIDA GLABRATA* OBTAINED FROM PATIENTS WITH PHARYNGOMYCOSIS

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Summary

Aim. To investigate the antifungal and anti-adhesive activities of Monarda fistulosa essential oil and cinnamon essential oil on clinical strains of fungi of the genus *Candida* obtained from patients with pharyngomycosis.
Materials and methods. The object of the study was essential oil of Monarda fistulosa and essential oil of cinnamon, the subject of the study was clinical strains of *Candida albicans* and *Candida glabrata* isolated from patients with pharyngomycosis. Antifungal effect of essential oils was determined by micromethod of serial dilutions with determination of minimum inhibitory concentration and minimum fungicidal concentration according to the recommendations of International Standards Organisation. The viability of micromycetes was determined by crossing the suspension from the test and control wells on Sabouraud dense nutrient medium with subsequent determination of colony-forming units/ml. Adhesion of *Candida* fungi was determined according to the method of Brilis V. I. and co-authors.
Results. Our studies proved the antifungal effect of essential oil of Monarda fistulosa and essential oil of cinnamon on the studied clinical strains of *Candida* fungi. The range of subinhibitory concentrations of essential oils that inhibited the viability and adhesive activity of clinical strains of *C. albicans* and *C. glabrata* was established. Under the conditions of our experiment, the greatest suppressive ability of subinhibitory concentrations affecting the biological properties of *Candida spp*. was demonstrated by essential oil of Monarda fistulosa.
Conclusions. The antifungal effect of essential oils on clinical strains of micromycetes differed in the strength of effect. The maximum expression of antimycotic effect was demonstrated by the essential oil of Monarda fistulosa. Under the conditions of action of subinhibitory samples of essential oil of Monarda fistulosa and cinnamon essential oil, the viability indices of *Candida spp*. were significantly lower than those of the culture control, which was not exposed to the action of essential oils. Clinical strains of *C. albicans* and *C. glabrata* isolated from patients with pharyngomycosis were characterised by medium-adhesive and low-adhesive activity, respectively. Essential oils in subinhibitory concentrations led to inhibition of the adhesive potential of the studied *Candida spp.* isolates, namely, there was a decrease in the adhesive activity of *C. albicans* strains from the category of 'medium-adhesive' to the category of 'non-adhesive' and 'low-adhesive', and of *C. glabrata* isolates – from the category of 'low-adhesive' to the category of 'non-adhesive'.

Keywords: **essential oils, pharyngeal candidiasis, micromycetes, adhesion**

INTRODUCTION

Currently mycotic diseases are gaining social significance and remain one of the most pressing medical problems due to their widespread prevalence. Almost a billion people worldwide suffer from fungal infections, the severity of which varies from asymptomatic and mild to potentially life-threatening conditions [1]. The results of scientific studies indicate an increase in the etiological significance of fungal pathogens in the

development of acute and chronic inflammatory diseases of the upper respiratory tract [2]. The dominant place in the structure of fungal diseases of the ENT organs belongs to pharyngomycoses, the proportion of which has recently reached 39-45 % [3]. Mycotic lesions of these human biotopes are usually opportunistic infections, the leading causative agents of which can be various types of opportunistic microbial agents that are part of the normobiota of mucous membranes and skin or penetrate the macroorganism from the environment. Fungal lesions of the pharynx in the vast majority of cases (97 %) are caused by yeast-like fungi of the genus *Candida*, and mycelial fungi of the genera *Aspergillus, Penicillium* and *Mucor* are etiological factors in only 3 % of cases [4, 5]. The main causative agent of opportunistic candidal infections of the oropharynx is *C. albicans*, which causes the disease in 62 % of cases. Among the non-albicans species, *C. glabrata* (46.4 %), *C. parapsilosis* (24.7 %), *C. tropicalis* (13.9 %), *C. krusei* (5.5 %) are the most common etiological factors of pharyngomycosis [4, 6]. Opportunistic infections caused by *Candida spp.* are difficult to treat, prone to chronicity of the disease, recurrent and persistent course and create the basis for the development of an associative infectious process, under which fungi of the genus *Candida* are able to enhance their pathogenic properties. The increasing influence of fungal microorganisms in the development of opportunistic infections has led to the inclusion of a significant number of antimycotic drugs and their combinations in treatment algorithms and widespread use in clinical practice. The empirical prescription of antifungal agents, their large number, variety of dosage forms, differences in mechanisms of action, and long treatment periods contribute to the formation of multiple resistance of fungal pathogens to these agents [7, 8]. In addition to the growing level of acquired resistance to antifungal drugs, there are a number of other factors that contribute to the lack of pathogen eradication. One of these factors is the ability of microorganisms to exist in biofilms.

According to scientific studies, 60-80 % of infectious pathology in various human biotopes is biofilm-associated, including all major respiratory pathogens capable of forming biofilms [9]. It has been proven that biofilms facilitate the transmission of antimicrobial resistance genes, as well as the expression of virulence factors of microorganisms that make up their composition. Therefore, the phenomenon of film formation should not be underestimated. In this regard, the problem of choosing an antifungal agent for the effective treatment of mycoses of the ENT organs remains topical today and requires new alternative approaches and solutions.

According to modern concepts, biofilms have a complex three-dimensional spatial and metabolic structure, which is represented by a community of microorganisms in an exopolymer matrix. Biofilms are formed by sessile cells, which are characterised by a pronounced ability to adhere and form cellular consortia, as well as a low level of metabolic processes, growth and reproduction parameters, unlike planktonic states [10]. The phenotypic properties of cecal cells provide resistance to cellular and humoral factors of human adaptive immunity and have a 100-1000 times lower sensitivity to antimicrobial agents than planktonic cells. Due to these common characteristics, biofilms potentiate the development of numerous chronic and acute infections, which are mostly refractory to traditional antimicrobial therapies and pose a serious threat to human health. Fungi of the genus *Candida* are one of the most common biofilm-associated pathogens, with strains having a high colonisation potential, which ensures their adaptation to the microbiome of any biotope and avoids interspecific competition [11]. The adhesive capacity of cells plays a key role in the colonisation potential of microbial species, which allows them to attach to living or abiotic surfaces, i.e. to switch from planktonic to attached life. Interaction between microbial adhesions and receptors of sensitive eukaryotic cells is specific and irreversible, which determines the first stage of colonisation of a biotope. *Candida* adhesions are usually cell wall proteins represented by integrin-like proteins, mannan proteins, and carbohydrate components of mannan proteins. *C. albicans* strains are characterized by the presence of specific adhesions, mannan proteins of the germ tube cell wall and hyphal cells, which are directly involved in biofilm formation [11, 12]. The adhesive properties of the pathogen determine its virulence, and their adhesive activity provides the initial link in the pathogenesis of the pathological process of mycotic inflammatory lesions.

In view of the above, we believe that one of the most urgent tasks of modern clinical medicine in the fight against infectious biofilm-associated diseases is the search for drugs that can inhibit the adhesion of pathogenic microorganisms, cause disintegration of biofilms, destroy their structure, and thus block the disease at an early stage of its development, as well as reduce the risk of emergence and spread of drug-resistant strains. To address these problematic issues in the treatment of otorhinolaryngological pathology, including pharyngomycosis, the search for new antimicrobial drugs, the use of combinations of existing antibiotics with drugs with antibiofilm activity and the combined use of drugs with ultrasound, ultraviolet irradiation, hyperbaric oxygenation, etc. are being discussed [13, 14, 15]. However, these therapeutic approaches require special medical equipment, maintenance and may be accompanied by the development of adverse side effects in the macroorganism. Therefore, the use of environmentally friendly biological products based on essential oils that combine anti-inflammatory and regenerative properties with antifungal ones may be a promising direction. The antimicrobial activity of essential oils, their effect on

planktonic and film-forming strains of microorganisms of different taxonomic species, as well as their pathogenic potential, are widely covered in the scientific literature [16, 17]. To date, the effectiveness of essential oils has been proven in medical practice, in particular in the treatment of upper respiratory tract infections when using a combination of essential oils in the form of an aerosol during passive inhalation [18, 19].

AIM

The aim of this study was to investigate the antifungal and antiadhesive activities of Monarda fistulosa essential oil and cinnamon essential oil on clinical strains of fungi of the genus *Candida* obtained from patients with pharyngomycosis.

MATERIALS AND METHODS

The object of the study was the essential oil of Monarda fistulosa (manufacturer: UBA LLC, Houston, USA) and cinnamon essential oil (manufacturer: Aroma Group LLC, Odesa). Essential oils are readily soluble in most organic solvents: ether, chloroform, absolute alcohol, carbon disulfide, hexane, ethyl acetate and acetone. The subject of the study was clinical strains of *Candida albicans* and *Candida glabrata*, which were obtained from clinical material taken from the oropharyngeal mucosa of patients with pharyngomycosis who were treated in the otolaryngology department of the Municipal Enterprise '2nd City Clinical Hospital of Poltava City Council'. The study was performed in accordance with the basic provisions of the World Medical Association's Declaration of Helsinki for the Ethical Principles of Medical Research Involving Human Subjects [20]. The diagnosis of pharyngomycosis was made on the basis of patient complaints, anamnesis, pharyngoscopic signs, and microbiological examination results with the identification of *C. albicans* and *C. glabrata*, as well as the calculation of their population level. The population level of the studied clinical fungal isolates was determined by counting colony-forming units in 1 ml (CFU/ml) and was considered clinically significant at a quantitative level of at least 1.0×10^4 CFU/ml. The biomaterial was collected from the surface of the oropharyngeal mucosa with a sterile swab applicator using a spatula on an empty stomach before treatment. The probe-tampon was introduced into Ames transport medium and delivered to the bacteriological laboratory within 2 hours under isothermal conditions. During mycological examination for identification of *Candida* species, morphological, tinctorial properties, as well as biochemical properties (cleavage of glucose, maltose and galactose to acid and gas) were taken into account. When identifying *C. albicans*, the ability of pathogens to form growth tubes and chlamydospores was taken into account. Final identification of isolated fungal strains was performed using an automatic analyser Vitek 2 compact bioMarieux (France). In accordance with the recommendations of the International Standards Organisation (ISO), the antifungal effect of essential oils was determined by the micro method of serial dilutions in Sabouraud broth with the determination of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) [21]. To obtain the emulsion, we used polysorbate-80 emulsifier, which is capable of ensuring high-quality distribution of the active substance in the solution. To prepare the working solution, essential oils were emulsified in polysorbate-80 in a 1:1 ratio. Then, using Sabouraud broth, a working solution of essential oils was obtained, which was subjected to serial dilutions in the range from 1:1000 to 1:32000 of the active ingredient. The specified range of essential oil dilutions was chosen in accordance with our own research results [22]. As an inoculum, a suspension of micromycetes equivalent to 0.5 according to the McFarland standard was used. The resulting suspension contained $1\text{-}5\times10^6$ CFU/ml. The standardised suspension was diluted 1:10 to prepare a working suspension which contained $1\text{-}5\times10^5$ CFU/ml yeast cells. The minimum inhibitory concentration was determined by the lowest concentration of essential oil solution that inhibited the visible growth of the fungal culture under study. To study the fungicidal effect of essential oils, their minimum fungicidal concentration was determined. To establish the MFC, inoculations were made from each well with no growth on Sabouraud agar sectors in Petri dishes. After 24 hours of incubation under optimal cultivation conditions, the lowest concentration of essential oils in the well was noted, which did not give rise to colony growth. The experimental series contained all components in appropriate proportions. The emulsifier control contained all components except essential oil. Culture controls contained nutrient medium and inoculum of clinical strains. Subinhibitory concentrations of essential oils were used to study the viability (CFU/ml) of clinical strains of *C. albicans and C. glabrata* and their adhesive properties. The subinhibitory samples of Monarda essential oil for *C. albicans* and *C. glabrata* were 0.12-0.03 mg/ml. Subinhibitory samples of cinnamon essential oil for *C. albicans* were 0.26-0.03 mg/ml, and for *C. glabrata* – 0.13-0.03 mg/ml. The viability of the micromycetes was determined by sowing the suspension from the test and control wells by the sector method on dense Sabouraud medium in Petri dishes, followed by determination of CFU/ml. The adhesive process of *Candida* fungi was determined by the method of Brilis V. I. and co-authors using blood erythrocytes of group I(0) Rh+, taking into account the following indicators: average adhesion index (AAI), erythrocyte participation rate (EPR), index of adhesion of microorganisms (IAM) [23]. The following formula was used to calculate the IAM: IAM = (SPA/CFU) x100. According to the methodology of Brillis and co-authors, microorganisms were considered

non-adhesive with an IAM≤1.75, low-adhesive – from 1.76 to 2.50, medium-adhesive – from 2.51 to 4.00, and highly adhesive – with an IAM of 4.00.

Descriptive statistics included calculation of mean values, errors of mean values and testing the normality of distributions using the Shapiro-Wilk method. Comparisons between the main and control groups were performed using Student's t test and the Mann-Whitney method for indicators that did not have a normal distribution. Comparison of related was performed by Student's method for paired indicators and Wilcoxon method for indicators that did not have normal distribution. The limit of statistical significance was considered to be p≤0.05. JASP 0.16.0.0 software was used for data analysis.

RESULTS AND DISCUSSION

Recently, due to the decreasing effectiveness of antifungal therapy of mycotic diseases of various localisations, including oropharynx, more and more attention is paid to phytonying preparations, which, if used rationally, cause minimal side effects, prevent the formation of secondary resistance and have a powerful antimicrobial activity and can act as adjuvants of chemotherapeutic drugs. Our studies proved high antifungal activity of essential oils on the studied clinical strains of fungi of the genus *Candida*, which were isolated from patients with pharyngomycosis. The results of studies of antifungal effect of essential oils are presented in Table 1.

Table 1

Sensitivity of clinical isolates of C. albicans and C. glabrata to essential oils by MIC (M±m) and MFC (M±m)

Microorganisms	Essential oil of Monarda fistulosa		Cinnamon essential oil	
	MIC (mg/ml)	MFC (mg/ml)	MIC (mg/ml)	MFC (mg/ml)
C. albicans (n=8)	0,26±0,08	0,52±0,06	0,45±0,05*	0,45±0,07
C. glabrata (n=5)	0,28±0,10	0,37±0,05	0,31±0,06	0,46±0,09

Note: * – statistical significance of the difference in MIC values of Monarda essential oil to MIC values of cinnamon essential oil, p<0.05.

According to the results of the studies, the minimum inhibitory concentration of Monarda fistulosa essential oil for clinical strains of *C. albicans* was 0.26±0.08 mg/ml, and the minimum fungicidal concentration was 0.52±0.06 mg/ml. For clinical strains of *C. albicans*, the MIC and MFC of cinnamon essential oil were determined at a concentration of 0.45±0,05 mg/ml. Thus, cinnamon essential oil has an exclusively fungicidal effect on fungi of the *C. albicans* species.

The sensitivity of clinical isolates of *C. glabrata* to the action of essential oil of Monarda fistulosa was not significantly different from that of clinical strains of *C. albicans*. However, the MIC of cinnamon essential oil for *C. glabrata* fungi was determined at a concentration of 0.31±0.06 mg/ml, which indicated their higher sensitivity to cinnamon oil (1,7 times) compared to the sensitivity of clinical strains of *C. albicans*. Thus, the results of our studies demonstrated high inhibitory activity of Monarda essential oil against clinical isolates of *C. albicans* and *C. glabrata* and are consistent with the data of other scientists. According to literature sources, essential oils containing phenols (thymol and carvacrol), eugenol and cinnamaldehyde were found to have the most pronounced antimicrobial activity [24]. The main chemical constituents of Monarda essential oil are thymol, carvacrol, terpinenes, carvacrol methyl ester. By chemical composition, cinnamon essential oil is characterised by the presence of the following active components: alpha/beta-pinene, linalool, cymene, sabinene, eugenol, limonene, cinnamaldehyde. So, Monarda essential oil and cinnamon oil by their chemical nature can be classified as a group of essential oils with high antimicrobial activity. The mechanism of their antimicrobial action is related to the lipophilicity of essential oils, which is characterised by the affinity of organic substances to fats and oils. Lipophilicity of essential oils provides increase of permeability of cell membranes of microorganisms and inhibition of membrane enzymes. Damage to cell membrane proteins and degradation of the cell wall have a toxic effect on their structure and function, resulting in the cell membrane losing its ability to perform its functions as a permeability barrier. The consequence of increased permeability of damaged membranes is the loss of cytoplasmic components of the cell, inhibition of hydrogen metabolism, ATP synthesis, mitochondrial destruction, and inhibition of cytoplasmic enzymes. These processes lead to structural and functional changes and end in cell lysis [25, 26].

The next stage in our study was to investigate the effect of sub-inhibitory concentrations of essential oils on the viability of clinical isolates of fungi of the genus *Candida*. Since the use of sub-inhibitory concentrations of essential oils, in our opinion, minimises the risks of toxic and allergic reactions in patients and will allow safe use for a longer period. The results of the study of viability (CFU/ml) of clinical strains of fungi of the genus *C. albicans* and *C. glabrata* are presented in Fig. 1 and Fig. 2

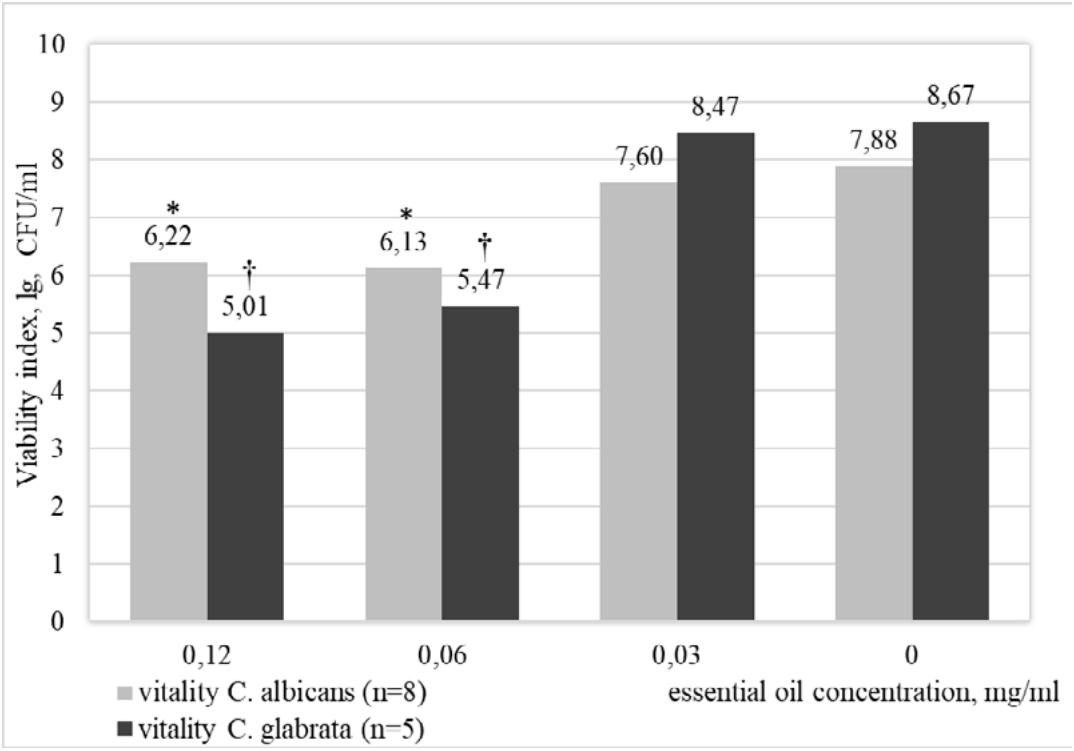


Figure 1. Viability of fungi of *C. albicans* and *C. glabrata* species under the influence of subinhibitory concentrations of Monarda essential oil; * – statistical significance of the difference with the control culture of *C. albicans*; +- statistical significance of the difference with the control culture of *C. glabrata*.

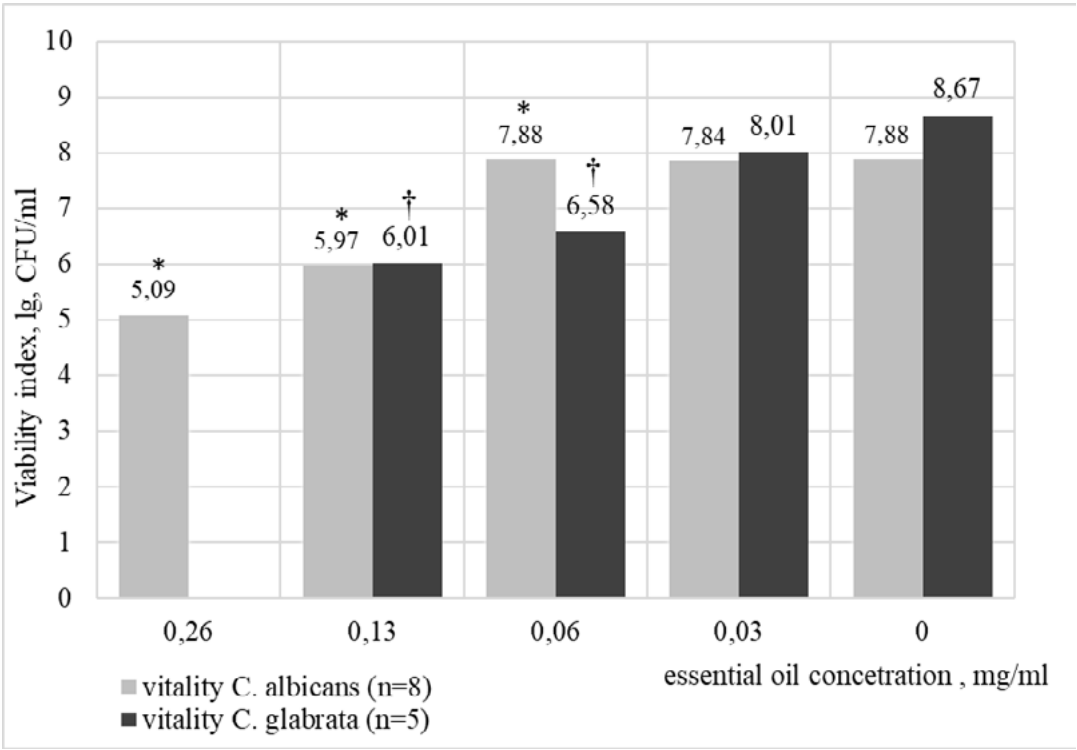


Figure 2. Viability of fungi of *C. albicans* and *C. glabrata* species under the influence of subinhibitory concentrations of cinnamon essential oil; * – statistical significance of the difference with the control culture of *C. albicans;* +- statistical significance of the difference with the control culture of *C. glabrata*.

The analysis of the results (Fig. 1) showed that Monarda essential oil in subinhibitory concentrations of 0.12-0.06 mg/ml inhibited the growth and reproduction of clinical strains of *C. albicans*. The cell viability according to the oil samples was 6.22±0.23 lg CFU/ml and 6.13±0.28 lg CFU/ml, which is 1.3 times lower compared to the control, which was determined at 7.88±0.55 lg CFU/ml. The sample of Monarda essential oil at a subinhibitory concentration of 0.03 mg/ml did not affect or inhibit the viability of microbial cells.

According to the data presented in Figure 1, the viability indicators of clinical isolates of microscopic fungi of the *C. glabrata* species under the influence of subinhibitory concentrations of essential oil of Monarda fistulosa were also determined in the range of 0.12-0.06 mg/ml and amounted to 5.01±0.18 lg CFU/ml and 8.47±0.20 lg CFU/ml, respectively. The obtained viability indices of clinical strains of *C. glabrata* were 43 % and 37 % lower than those of the control culture, which was 8.67±0.18 lg CFU/ml. The level of viability of *C. glabrata* isolates under the influence of Monarda fistulosa essential oil at a concentration of 0.03 mg/ml did not differ from the culture control.

The obtained results, shown in Figure 2, indicate that the effect of subinhibitory concentrations of cinnamon essential oil in the range of 0.26-0.13 mg/ml on clinical strains of *C. albicans* caused inhibition of their viability by 34 % and 25 % compared to the control culture. Samples of essential oil at a concentration of 0.06-0.03 mg/ml did not affect the growth and reproduction of fungal cells and, accordingly, their viability indicators reached the values of the control culture, which was not exposed to cinnamon oil.

Taking into account that the MIC of cinnamon oil for clinical strains of *C. glabrata* was 0.31 mg/ml, the sub-inhibitory concentrations in the range of 0.13-0.03 mg/ml were investigated. Under the action of cinnamon essential oil samples at concentrations of 0.13 and 0.06 mg/ml, inhibition of growth and multiplication of *C. glabrata* micromycete cells was observed 1.5-1.3 times compared to CFU/ml of culture control. Cinnamon oil at a concentration of 0.03 mg/ml did not inhibit the viability of *C. glabrata*.

Thus, the results obtained indicate that even subinhibitory concentrations of essential oils inhibit the viability of clinical strains of *Candida spp.* In our experiment, the highest inhibitory ability of subinhibitory concentrations was demonstrated by the essential oil of Monarda fistulosa. The inhibition of the viability of microorganisms under the influence of subinhibitory samples of essential oils is to some extent consistent with the data of other authors on the effect of the main components of essential oils and their derivatives on the growth and reproduction of bacteria [17, 26]. The most likely factors of disruption of the processes that ensure the vital activity of fungi are a decrease in their adaptive potential, namely, inhibition of cellular respiration, increased permeability of yeast cell membranes, the outflow of trace elements, and disruption of plastic and energy metabolism.

Given the fact that we have established the inhibition of the viability of micromycetes under the influence of subinhibitory samples of essential oils, it was advisable to study the processes of fungal adhesion in this range of essential oil concentrations. After all, adhesive activity is one of the factors of virulence, which ensures the high ability of fungi to colonise and their further reproduction in certain biotopes. It is generally recognised that the adhesion of microorganisms to epithelial cells is a major prerequisite and trigger in the pathogenesis of infectious diseases, so reducing the adhesive potential of microorganisms is a pathogenetically sound approach to combating infectious diseases. For all clinical strains of *C. albicans* and *C. glabrata* that colonised the oropharyngeal mucosa under conditions of infectious and inflammatory process, the manifestation of adhesive properties was established. According to the average number of microorganisms involved in the adhesive process, clinical strains of *C. albicans* were classified as 'medium-adhesive', and clinical strains of *C. glabrata* as 'low-adhesive'. The IAM calculated for them were 3.02±0.09 and 2.35±0.16, respectively. The results of the study of the adhesive properties of clinical isolates of *C. albicans* and *C. glabrata* under the influence of subinhibitory concentrations of essential oils are presented and summarised in Tables 2 and 3.

Table 2

Characterisation of the adhesive properties of clinical strains of *C. albicans* and *C. glabrata* in the presence of subinhibitory concentrations of monarda essential oil by IAM (M±m)

Microorganisms	Control	monarda essential oil (mg/ml)			
	-	0,23	0,12	0,06	0,03
	Adhesion index (IAM)				
C. albicans (n=8)	3,02±0,09	_	1,51±0,10*	1,89±0,08*	2,52±0,11
C. glabrata (n=5)	2,35±0,16	1,32±0,06†	1,40±0,08†	1,96±0,11	2,06±0,08

Note: * – statistical significance of the difference in the IAM of *C. albicans* under the influence of subinhibitory concentrations of essential oils to the IAM of the control culture, $p<0.05$. +- statistical significance of the difference in the IAM of *C. glabrata* under the influence of subinhibitory concentrations of essential oils to the control culture, $p<0.05$.

The analysis of the results of the studies (Table 2) shows that the essential oil of Monarda fistulosa in subinhibitory concentrations of 0.12 mg/ml-0.06 mg/ml reduced the adhesiveness of clinical strains of *C. albicans*. Under the influence of monarda oil at a concentration of 0.12 mg/ml, IAM decreased by 49 % and *C. albicans* fungi lost their adhesive potential and were defined as non-adhesive (Fig. 3).

Under the conditions of exposure to essential oil at a concentration of 0.06 mg/ml, clinical strains acquired low-adhesive characteristics, but reducing the concentration of essential oil to 0.03 mg/ml did not lead to a significant decrease in IAM compared to the control (Fig. 4) and clinical isolates acquired the characteristics of medium-adhesive micromycetes.

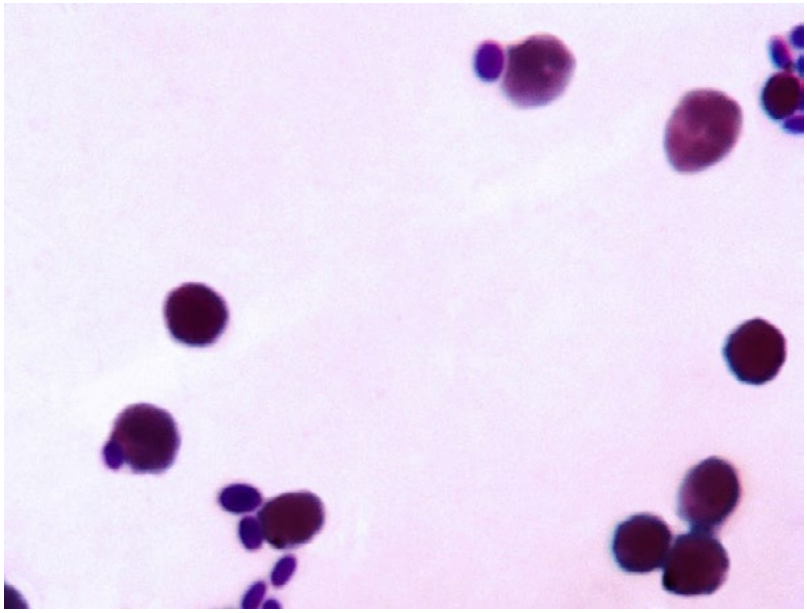


Figure 3. Microscopic picture of adhesion of *C. albicans* strains on erythrocytes according to Brillis method under the influence of subinhibitory concentrations of monarda essential oil.

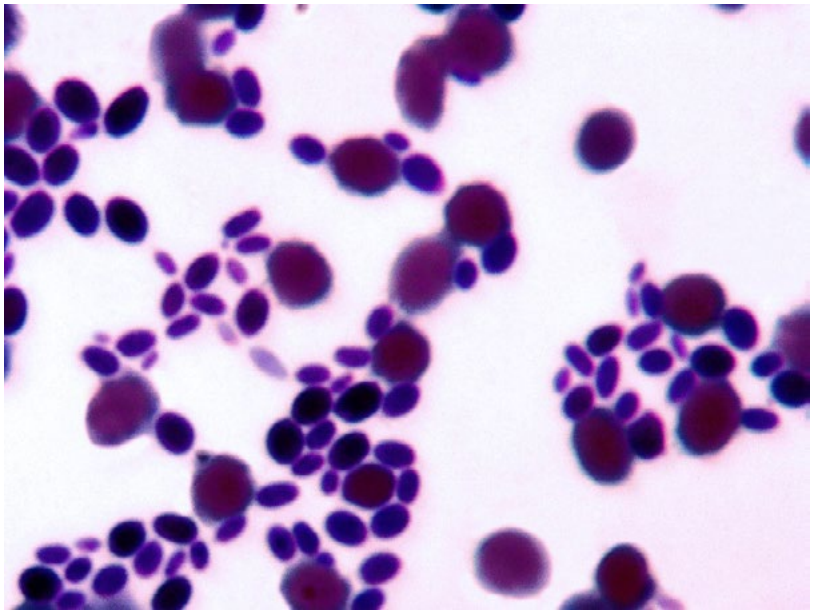


Figure 4. Microscopic pattern of adhesion of *C. albicans* strains on Brillis erythrocytes that were not exposed to essential oils.

In clinical isolates of *C. glabrata* under the influence of subinhibitory concentrations of monarda essential oil in the range of 0.23 mg/ml and 0.12 mg/ml, the inhibition of the adhesive activity of fungi to the category of 'non-adhesive' was found, the IAM values were 1.32±0.06-1.4±0.08, respectively. Under the conditions of exposure of the sample of monarda essential oil at a concentration of 0.06 mg/ml and 0.03 mg/ml to *C. glabrata* fungi, no significant difference in their adhesive activity to the IAM of the control culture was found.

According to the results shown in Table 3, subinhibitory concentrations of cinnamon essential oil at 0.26 mg/ml and 0.13 mg/ml contributed to a significant reduction of *C. albicans* IAM by 50 % and 51 % compared to the control culture and acquired the characteristics of the 'low-adhesive' category. The presence of cinnamon essential oil only at a concentration of 0.13 mg/ml contributed to an effective reduction of *C. glabrata* IAM compared to the control.

Table 3

Characterisation of the adhesive properties of clinical strains of *C. albicans* and *C. glabrata* in the presence of subinhibitory concentrations of cinnamon essential oil by IAM indicators (M±m)

Microorganisms	Control	Cinnamon essential oil (mg/ml)			
	-	0,26	0,13	0,06	0,03
	Adhesion index (IAM)				
C. albicans (n=8)	3,02±0,11	1,52±0,06*	1,53±0,07*	2,89±0,08	2,84±0,13
C. glabrata (n=5)	2,35±0,16	-	1,64±0,08†	1,91±0,10	2,16±0,13

Note: * – statistical significance of the difference in the IAM of *C. albicans* under the influence of subinhibitory concentrations of essential oils to the IAM of the control culture, $p<0.05$. + – statistical significance of the difference in the IAM of *C. glabrata* under the influence of subinhibitory concentrations of essential oils to the control culture, $p<0.05$.

Thus, the pathogens of pharyngomycosis *C. albicans* and *C. glabrata* are characterised by a pronounced adhesive activity, which was effectively reduced in the presence of sublethal concentrations of essential oils. In our opinion, the inhibition of fungal cell attachment under the influence of sublethal concentrations of essential oils can occur already at the stage of nonspecific adhesion due to the inhibition of electrostatic forces, hydrophobic forces, and Van der Waals forces. [10, 27]. The inhibition of the stage of specific adhesion under the influence of subinhibitory essential oils is due to changes in the cell wall and cytoplasmic membrane, which lead to damage to cell membranes and adhesion receptors. Other authors have shown that the inhibition of the adhesive properties of microorganisms by natural compounds (phenols, essential oils), terpenoids, lectins, alkaloids) leads to a decrease in their film-forming potential and, as a result, inhibition of biofilm viability [28]. The ability of essential oils to inhibit the adhesive activity of micromycetes in the process of biofilm formation is clinically relevant, as it leads to the development of a therapeutic strategy aimed at suppressing the viability of biofilms and overcoming the biofilm microorganisms' tolerance to antimicrobial agents.

CONCLUSIONS

1. Essential oil of Monarda fistulosa and essential oil of cinnamon demonstrated high fungistatic and fungicidal effects on clinical strains of *C. albicans* and *C. glabrata* isolated from patients with pharyngomycosis.

2. The antifungal effect of essential oils on clinical strains of micromycetes varied in strength. The maximum severity of the antimycotic effect was demonstrated by the essential oil of Monarda fistulosa. The MIC of monarda essential oil for *C. albicans* was 71 % lower than the MIC of cinnamon essential oil. The MFC of monarda essential oil for *C. glabrata* was 24 % lower than the MIC of cinnamon essential oil.

3. Subinhibitory concentrations of essential oils caused inhibition of the viability of fungal cells of the species *C. albicans* and *C. glabrata*. Under the influence of subinhibitory samples of monarda essential oil, the viability of *C. albicans* was significantly lower than the viability of these microorganisms under the influence of cinnamon essential oil.

4. Clinical strains of *C. albicans* and *C. glabrata* isolated from patients with pharyngomycosis were characterised by medium- and low-adhesive activity, respectively.

5. Essential oils in subinhibitory concentrations led to inhibition of the adhesive potential of the studied isolates of *Candida spp.* Under the influence of essential oils, there was a decrease in the adhesive activity of *C. albicans* strains, which was accompanied by their transition from the category of 'medium-adhesive' to the category of 'non-adhesive' and 'low-adhesive', and *C. glabrata* isolates – from the category of 'low-adhesive' to the category of 'non-adhesive'.

Prospects for further research. Given that yeast-like fungi have acquired or natural resistance to antifungal drugs, our research expands the existing knowledge of the pharmacological properties of essential oils and identifies prospects for further study of the effect of phytonutrient drugs on the pathogenic potential of microbial species.

COMPLIANCE WITH ETHICAL REQUIREMENTS

The study was conducted in accordance with the protocols and procedures required by the Biomedical Research Ethics Committee and complies with the directives of Ukrainian healthcare legislation and the World Medical Association's Declaration of Helsinki for the Ethical Review of Medical Research Involving Human Subjects.

FUNDING AND CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Valentina P. Polianska – conception and design of the study, analysis and interpretation of data, writing the article; Mykhailo I. Bezega – conception and design of the study, analysis and interpretation of data, final approval of the article; Svitlana V. Zachepylo – data collection, data analysis and interpretation, writing the article; Iryna M. Zviagolska – data collection, writing of the article.

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Резюме

ВПЛИВ ЕФІРНИХ ОЛІЙ НА ЖИТТЄЗДАТНІСТЬ ТА АДҐЕЗИВНІ ВЛАСТИВОСТІ КЛІНІЧНИХ ШТАМІВ *CANDIDA ALBICANS* І *CANDIDA GLABRATA*, ОТРИМАНИХ ВІД ПАЦІЄНТІВ З ФАРИНГОМІКОЗОМ

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Мета. Вивчення протигрибкової та протиадгезивної активностей ефірної олії монарди трубчастої та ефірної олії кориці на клінічні штами грибів роду *Candida*, отримані від пацієнтів з фарингомікозом.
Матеріали та методи. Об'єктом дослідження були ефірна олія монарди трубчастої та ефірна олія кориці, предметом дослідження – клінічні штами *Candida albicans* і *Candida glabrata*, виділені від пацієнтів з фарингомікозом. Протигрибкову дію ефірних олій визначали мікрометодом серійних розведень з визначенням мінімальної інгібуючої концентрації та мінімальної фунгіцидної концентрації відповідно до рекомендацій International Standards Organization. Життєздатність мікроміцетів визначали шляхом пересіву суспензії з досліджуваних і контрольних лунок на щільне живильне середовище Сабуро з наступним визначенням колонієутворюючих одиниць/мл. Визначення адгезії грибів роду *Candida* проводили за методом Бриліса В. І. та співавторів.
Результати. Проведені нами дослідження довели протигрибкову дію ефірної олії монарди трубчастої та ефірної олії кориці на досліджувані клінічні штами грибів роду *Candida.* Встановлено діапазон субінгібуючих концентрацій ефірних олій, які пригнічували життєздатність та адгезивну активність клінічних штамів *C. albicans* і *C. glabrata.* В умовах нашого експерименту найбільшу пригнічуючу здатність субінгібуючих концентрацій, що впливають на біологічні властивості *Candida spp.* продемонструвала ефірна олія монарди трубчастої.
Висновки. Протигрибкова дія ефірних олій на клінічні штами мікроміцетів різнилася за силою впливу. Максимальну виразність антимікотичного ефекту продемонструвала ефірна олія монарди трубчастої. В умовах дії субінгібуючих зразків ефірної олії монарди трубчастої та ефірної олії кориці показники життєздатності *Candida spp.* були значно нижчими від показників контролю культури, яка не піддавалася дії ефірних олій. Клінічні штами *C. albicans* і *C. glabrata* виділені від пацієнтів з фарингомікозом, характеризувалися відповідно середньоадгезивною та низькоадгезивною активністю. Ефірні олії в субінгібуючих концентраціях призводили до пригнічення адгезивного потенціалу досліджуваних ізолятів *Candida spp,* а саме відбувалося зниження адгезивної активності штамів *C. albicans* з категорії «середньоадгезивних» в категорію «неадгезивних» і «низькоадгезивних», а ізолятів *C. glabrata* – з категорії «низькоадгезивних» в категорію «неадгезивних».

Ключові слова: **ефірні олії, кандидоз глотки, мікроміцети, адгезія**