

## MICROBIOLOGICAL ASPECTS OF CHLOROPHYLLIPT EXTRACT USED FOR PREVENTION OF CANDIDA POSTOPERATIVE COMPLICATIONS

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### ABSTRACT

The purpose of this research paper was to investigate the chlorophyllipt extract fungicidal action against *Candida* spp. fungi.

We studied the reference strain *C. albicans*, clinical strains of *C. albicans* and *C. glabrata*, isolated from patients of surgical units. The sensitivity assessment was performed on the derived strains for chlorophyllipt and ethanol using double serial dilutions according to the standard procedure.

According to the results, the reference and clinical strains of *Candida* were sensitive to the action of the chlorophyllipt extract. The control strain of *C. albicans* was found to be the most sensitive at the time, as the antifungal effect of the extract studied against the clinical strains did not differ significantly from each other. In turn, it was found that ethanol had a weaker fungicidal action.

The results of the study prove the antifungal effect of the chlorophyllipt extract against *Candida* spp. isolated from patients of surgical units.

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### 1. Introduction

Postoperative infectious complications are one of the most preventive infections associated with a medical service and are a significant problem for healthcare systems around the world, from the position of incidence and morbidity [1, 2].

The etiology of infectious complications in general surgery has not changed in the past 30 years. Gram-positive microorganisms such as coagulase-negative staphylococci, *Enterococcus* spp., along with gram-negative pathogens of *Escherichia*, *Enterobacter*, *Klebsiella*, *Pseudomonas* genera are its basis. Previously, it was believed that yeast-like fungi (*Candida* spp. mainly) cause approximately 3% of postoperative infectious complications [3, 4]. However, their role in developing diseases in severe surgical patients has grown substantially in recent years. Thus, representatives of the *Candida* genus are in sixth place among main hospital pathogens and fourth among nosocomial bacteremias. Until recently, in the majority of countries, the dominant species among them was *C. albicans* causing two-thirds of all cases of invasive candidiasis. Simultaneously, changes are occurring in an epidemiology of the candidiasis infection with a progressive shift towards non-*albicans* species such as *C. glabrata*, *C. krusei* [5 - 7].

This is associated with an increase of using azoles (fluconazole, itraconazole, clotrimazole, etc.), which are showing high fungistatic action against *C. albicans* in prevention and treatment of postoperative fungal complications. At the same time, non-*albicans* species *C. glabrata* and *C. krusei* are demonstrating considerable resistance to these drugs that promote their uncontrolled growth in surgical inpatients [6, 8].

In this regard, an increase of scientific interest related to the search for new alternative drugs that possess antifungal properties not only against *C. albicans* but other representatives of this species to an equal extent is natural. This will help to improve an efficiency of candidiasis prevention and treatment in severe surgical, burn, and intensive care inpatients [9].

Drugs based on plant extracts, with little to no side effects and show good antimicrobial activity, are considered as perspective [10 - 13].

The purpose of this study is to analyze an antifungal action of the chlorophyllipt extract on *Candida* spp. fungi.

### 2. Material and methods

For the study, we used the standard strain of *C. albicans* (American Type Culture Collection (ATCC) 10231) received from the Public Institution "Institute of Epidemiology and Infectious Diseases L.V. Gromashevsky

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National Academy of Medical Sciences of Ukraine” (Kyiv), 5 clinical strains of *C. albicans* and 5 clinical strains of *C. glabrata*, which were isolated from inpatients of thoracic and vascular surgery departments and were identified in the bacteriological laboratory of M.V. Sklifosovsky Poltava Regional Clinical Hospital. We tested an alcohol solution of chlorophyllipt, which contained a chlorophyllipt thick extract as the active substance and 96% ethanol (Halychpharm, JSC, Lviv, Ukraine, № UA/4551/02/01 from 31.10.2016) as an excipient, for determination of the antifungal action. The sensitivity assessment of the derived strains to the chlorophyllipt extract was performed using double serial dilutions according to the standard procedure approved by the Order № 167 of the Ministry of Public Health of Ukraine on “On Approval of Training Guidance “Assessment of the sensitivity of microorganisms to antibiotics” [14].

The tests were conducted in a 0.5 cm<sup>3</sup> volume of each dilution of the extract with a final concentration of the microbial culture 10<sup>6</sup> CFU/cm<sup>3</sup>. 1.0 cm<sup>3</sup> of the Sabouraud broth was transferred into each of the 10 vials considering “positive” and “negative” controls. A serial dilution series in the Sabouraud broth with 1.0ml of the alcohol solution of chlorophyllipt was prepared, which contained 10.0mg of a dry matter of the chlorophyllipt thick extract in recalculation on 100%. For inoculation we used a microbial suspension equivalent to 0.5 by McFarland Equivalence Standards diluted 1/100 in saline, and then the concentration of microorganisms in this suspension was 5×10<sup>5</sup> CFU/cm<sup>3</sup>. 0.5 cm<sup>3</sup> of the suspension was added to each vial with the chlorophyllipt extract and into one vial with the Sabouraud broth without the testing drug as a negative control. The vials with dilutions were incubated in a normal atmosphere at the temperature 37 °C for 48 hours. Determination of the minimal inhibiting concentration of the testing solution was difficult because of the appearance of opacities. Therefore, we determined the minimum fungicidal concentrations (MFCs) of the extract by placing microorganisms from 10 vials on sectors of Petri dishes containing solid nutrient medium Sabouraud, and then incubated at 37°C for an additional 48 hours. The MFC was considered as the maximal dilution of the chlorophyllipt extract when growth of microorganisms was absent.

An action of 96% ethanol (Private Enterprise "Kilaff", Sumy, Ukraine), which is a part of the test solution, against *Candida spp.* was estimated as a control.

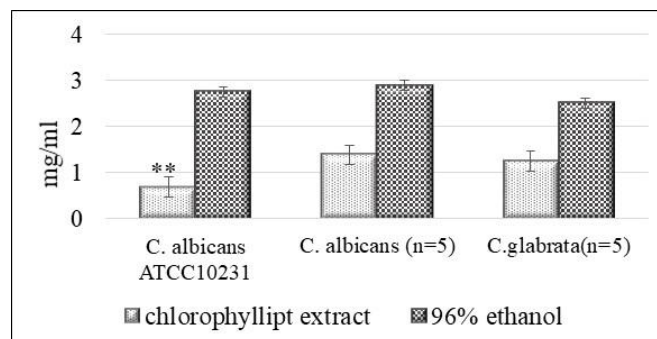
The statistical analysis of data was conducted with a standard package of programs Microsoft Excel 2013, Statistica 6.0., where arithmetical mean (M), standard error of the mean (m) were calculated. The Student t-test was used to evaluate the degree of reliability of differences between groups. The results were considered statistically significant at  $p < 0.05$ .

### 3. Results

The standard strain of *C. albicans* was the most sensitive to the chlorophyllipt extract and the MFC against it was 0.688±0.25 mg/ml (Figure 1). However, there was no significant difference in the antifungal action of the test extract against clinical strains of *C. albicans* and *C. glabrata*. The MFCs of the chlorophyllipt extract against them were 1.38±0.45 mg/ml, 1.25±0.5 mg/ml respectively.

It was also established that 96% ethanol had a weaker fungicidal action against the examined strains of yeast-like fungi.

Moreover, it should be noted that its MFC was within the same limits both against the standard and clinical strains of *Candida spp.* Thus, the MFC of 96% ethanol against the standard strain of *C. albicans* was 2.75±0.9 mg/ml, the clinical strains of *C. albicans* – 2.88±1.7 mg/ml, and the clinical isolates of *C. glabrata* – 2.5±1.0 mg/ml. The MFC of 96% ethanol was 4.0 times higher than this figure of the chlorophyllipt extract for the standard strain, and 2.0 times higher than of the clinical isolates.



**Figure 1 - Susceptibility of *Candida spp.* to the alcohol extract of chlorophyllipt and 96% ethanol, M+m (\* - in recalculation on the active substance; \*\* - reliability of results differences of the MFC of the chlorophyllipt extract comparatively to the MFC of 96% ethanol,  $p < 0.05$ ).**

### 4. Discussion

Elaboration and implementation of drugs based on plant extracts into medical practice obtain significant relevance in an environment of a rapid development of resistance to known antibiotics in microorganisms. An antibacterial action of the chlorophyllipt extract against resistant to antibiotics strains of *Staphylococcus spp.*, *Pseudomonas spp.*, *Listeria spp.* has been extensively researched. This explains its successful use in a treatment of infectious processes associated with these pathogens [15 -17]. At the same time, there isn't much information about its antifungal activity. A number of authors point to an ability of the extract to oppress vital activity of *Fusarium graminearum*, *Fusarium asiaticum*, *Fusarium redolens f.sp. dianthus*, *Fusarium verticillioides*, *Fusarium oxysporum f.sp. lentis*, *Sclerotinia sclerotiorum*, *Aspergillus flavus*, *Aspergillus tubingensis*, *Botrytis cinerea* and *Cladosporium cladosporioides*. However, according to the literature, the antifungal spectrum of the chlorophyllipt extract is limited to hyphal fungi, while its influence on yeast forms is insufficiently described [18, 19].

The results of the research submitted in this article prove antifungal action of the chlorophyllipt extract against *Candida spp.* isolated from surgical inpatients. It is quite natural that clinical isolates of this species were less sensitive to have an impact on the tested extract in comparison to the standard strain. A reliable distinction of the MFCs between the chlorophyllipt extract and the 96% ethanol, which is the excipient of the extract, against fungi of the *Candida* genus indicates the direct fungicidal action of the active substance of the studied drug.

## 5. Conclusions

The alcohol extract of chlorophyllipt has the antifungal action against yeast-like *Candida spp.* fungi, reliably oppressing vital activity both of standard and clinical representatives of this species. Moreover, the testing extract demonstrates an equally high antifungal activity against *C. albicans* and *C. glabrata* that reduces a risk of postoperative candidiasis caused by non-albicans species and *C. albicans*. This allows us to look at the possibility of expanding the indications of the alcohol extract of chlorophyllipt usage in treatment and prevention of postoperative candidiasis in severe inpatients.

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