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COMPARISON OF FUNGICIDAL EFFECT OF PROPOLIS EXTRACTS AND METRONIDAZOLE

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Abstract: *Propolis is a natural substance that has anti-inflammatory (immunomodulatory and microbicidal) properties and has a long history of use in traditional medicine. The aim of this study was to evaluate the antifungal properties of 10% and 30% ethanolic extract of propolis prepared traditionally and of a commercial drug metronidazole, against selected strains of Candida albicans isolated from food samples, reference strain Candida albicans WDCM 00054, reference strain Aspergillus brasilliensis WDCM 00053 and reference strain Saccharomyces cerevisialis WDCM 3058. The agar diffusion method was used to investigate the fungicidal effect of propolis and metronidazole on the growth of selected cultures. The results of the study showed that both tested concentrations of ethanolic extract of propolis originating from the Republic of Srpska acted fungicidally on the Candida albicans isolate as well as on the reference strains Candida albicans WDMC 00054, Aspergillus brasilliensis WDCM 00053 and Saccharomyces cerevisialis WDCM 3058. Metronidazole showed weak antifungal activity.*

Key words: *antifungal activity, propolis, Candida albicans, Aspergillus brasilliensis, Saccharomyces cerevisialis*

Introduction

Propolis is a resinous substance created by the collection of resins from plant buds and tree exudates by bees, enriched with salivary enzymes, pollen and wax. Propolis consists: resin from plants collected by honey bees, wax from bee metabolism, substances that honey bees add when making propolis, and other components such as essential oils, pollen, etc. [1,2,3,4].

Propolis is a significant source of natural chemical compounds with a wide spectrum of pharmacological activities. Many scientific studies have investigated the origin of propolis and the influence of geographical and environmental factors, chemical composition and pharmacological activity [5,6,7,8,9].

Different plant species contain different primary and secondary metabolites (phenolic compounds-flavonoids, phenolic acids, terpenoids and essential oils) that enter the

chemical composition of propolis [3]. Also, it should be noted that the chemical composition of finished products obtained from propolis may depend on the physical and chemical conditions during extraction (solvent, temperature, extraction time, standardization, etc.) [10].

Pobiega et al. [11] compared the antifungal activity of propolis ethanol extracts obtained by different extraction methods. Propolis extraction was performed using 70% ethanol (extraction by shaking, extraction with the help of ultrasound) and the antifungal activity against selected species of bacteria and fungi was evaluated by the disk diffusion method. No differences were observed regarding the qualitative composition of the extracts obtained by any of the methods.

The chemical composition of propolis differs qualitatively and quantitatively in different geographical regions of the world. Also, propolis as a natural mixture shows a better antifungal effect than its individual components [4].

Propolis has antibacterial [12,13,14], antiviral [15,16,17], antifungal [18,19,20], antiparasitic [9,21], anti-inflammatory [18,22], neuroprotective [23,24] and anticancer properties [25,26].

The aim of this reserch is to test the antifungal properties of the ethanol extract of propolis prepared in the traditional way by extraction in 70% and 96% ethanol and commercial drug metronidazole according to two strains of *Candida albicans* isolated from food samples and three reference strains of fungi (*Candida albicans* WDCM 00054, *Aspergilus brasiliensis* WDCM 00053 and *Saccharomyces cerevisialis* WDCM 3058).

Material and methods

A 10% ethanol extract of propolis extracted in the traditional way with 96% ethanol and a 30% ethanol extract of propolis extracted with 70% ethanol were used as test material. In order to obtain the appropriate concentration of propolis extract, crushed propolis was suspended in 100 ml of 70% and 96% ethanol. After 30 days (with daily shaking) at room temperature and in a dark place, the supernatant was centrifuged at 3000 at a temperature of 24ºC.

Preparing discs:

15 µl of each tested concentration of propolis extract was applied to commercial sterile paper discs with a diameter of 6 mm, and the control with 15 µl ethanol preparations without the addition of propolis (control). To compare and evaluate the antifungal effect of propolis extracts we used the antifungal drug metronidazole (10 µl).

Preparation of fungal strains:

Three reference strains (*Candida albicans* WDCM00054, *Aspergillus brasiliensis* WDCM00053, *Saccharomyces cerevisial* WDCM 3058) and two isolates of *Candida albicans* from the isolate collection of the Laboratory for Microbiology of food, feed and water were used in this test. The strains were cultured on Sabouraud dextrose agar for 48-72 hours. They were examined microscopically and subcultured in test

tubes with Sabouraud dextrose broth for 48 hours at 25°C, and then used to prepare test suspensions.

Antifugal activity test:

The antifugal activity of 10% and 30% propolis extract was determined by the disc diffusion method [27]. On the surface of sterile Petri plates with Sabouraud dextrose agar, 100 µL of each strain from broth culture was seeded. Discs with 10% and 30% propolis extract, control discs and discs with metronidazole, were placed on plates with seeded agar and incubated at 25ºC for 42-72 hours. After incubation, the diameter of the clear zone of inhibition around the discs was measured in millimeters (mm). The test was repeated three times and the mean value was calculated. Antifugal activity is expressed as zone of inhibition (mm) ± standard deviation .

The relative antifungal activity, expressed as a percentage, compared to the activity of metronidazole was calculated according to the equation:

$$\mathbf{R\ (\%) = ((Z_n - Z_m)/Z_m) \times 100} \qquad \mathbf{(1)}$$

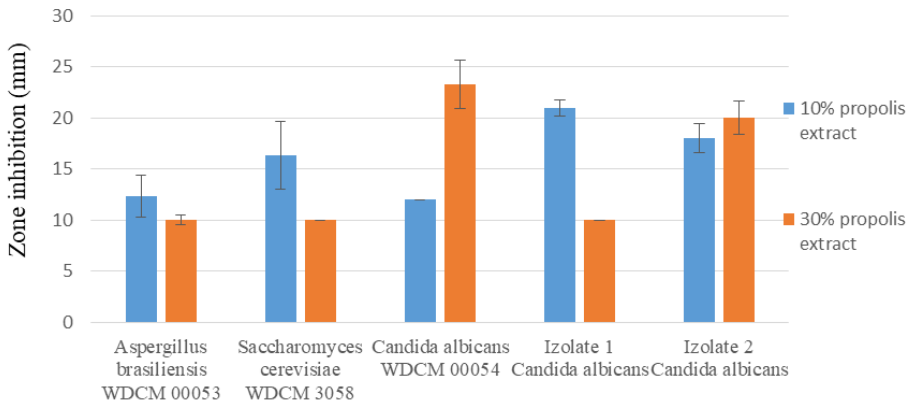
where is

Z_n – mean value of the zone of the tested extract

Z_m – metronidazole zone

Results and discussion

The results of testing different concentrations of propolis on selected fungal strains are shown in graph 1. Control discs with alcohol preparations without propolis did not show an inhibitory zone.



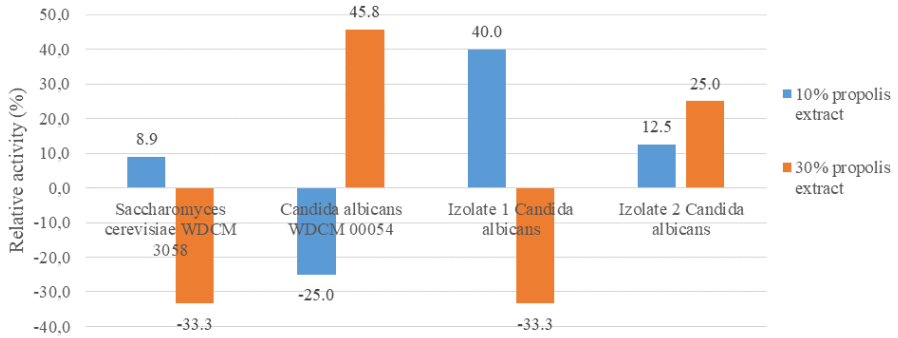
Graph 1. The mean value of activity of 10% and 30% propolis on isolates and reference strains of *Aspergilus brasiliensis, Saccharomyces cerevisiae and Candida albicans*

The test results showed that the ethanol extract of propolis from the territory of the Republic of Srpska has pronounced anti-fungal activity against the tested fungal strains with both tested concentrations. The range of inhibition zones ranged

from 10.00 mm to 21.00 mm for isolates from food and from 10.00 to 23.33 mm for reference strains.

The activity of 10% propolis was more pronounced in strains isolated from food (with an inhibition zone of 21.00 mm and 18.00 mm) compared to the tested reference fungal strains (inhibition zone of 12.00 and 16.33 mm), while 30% propolis had a stronger effect on one isolate *C. albicans* (inhibition zone extent of 20.00 mm) and reference strain *C. albicans* (with inhibition zone extent of 23.33 mm)

The activity of the 10% ethanol extract of propolis was more pronounced with the reference strain *Aspergillus brasilliensis* and *Saccharomyces cerevisiae*, while the antifugal activity of the 30% ethanol extract of propolis was stronger with the reference strain *Candida albicans*.



Graph 2. Relative activity of propolis extracts compared to metronidazole.

From the graph (Graph 2) we can observe that metronidazole did not have a fungicidal effect on one *Candida albicans* from the food isolates.

The results of the study are consistent with the results of other authors who have investigated the fungicidal effect of metronidazole and found that it has a weakly expressed fungicidal effect [28,29].

Metronidazole is one of the main drugs for the treatment of anaerobic bacterial infections, protozoal infections and microaerophilic bacterial infections. It is cytotoxic for facultative anaerobic microorganisms [30]. Although there are studies [28] that indicate a low antifungal activity of metronidazole, our finding is only activity against *Candida albicans*. The absence of activity against *Aspergillus brasiliensis* agrees with other authors [29].

The relative activity of propolis extract compared to metronidazole (Graph 2) shows variations depending on the used strain and propolis extract.

Antifugal activity of propolis on *Candida spp*., *Saccharomyces spp*., *Aspergilus niger*, *Trichophiton spp*. has been known for many decades [31,32], but the mechanism of action of propolis is not fully known, so it is assumed that is connected to the cell wall and plasma membrane [17,19]. A test with *C. albicans* showed that

propolis causes the separation of the fungal cell wall and disruption of division, which leads to dysfunction of the cell wall of the daughter cells [19].

Some studies have shown that the cell membrane of fungi could be a possible target of propolis extract besides inducing cell death [33, 34]. D'Auria et al. [35] have shown that propolis extract can inhibit the activity of extracellular phospholipases, leading to a weakening of the adhesion of fungal cells to the epithelium. Gucva et al. [34] in more recent studies observed that propolis can affect the formation and integrity of the fungal cell wall and can inhibit the morphological transformation of *C. albicans*. It has also been observed in some studies that ethanol extract of propolis causes loss of integrity of the cell wall of *C. albicans* and reduces metabolic activity [36] and that ethanol extract of propolis inhibits the filamentation of *C. albicans* cells, yeast germination, and increases the production of superoxide anion radical [37].

Interesting results were also found for the synergistic action of Polish propolis extract with voriconazole and fluconazole against *C. albicans* [34], as well as the combination of propolis with carnosic acid (diterpene found in *Rosmarinus officinalis* and *Salvia officinalis*) synergistic action, can lead to a drastic reduction in the survival of *C. albicans* cells, leading to a fungicidal effect [40]. The most studied propolis is Brazilian propolis. The results of testing and comparing Brazilian propolis with propolis from Europe, Africa and South America showed that Brazilian propolis has the strongest antifungal effect on the growth of *C. albicans* with an inhibition zone of 15.7 to 18.2 mm [38], Likewise, testing done with propolis originating from Turkey gave good antifungal effects for C. albicans with inhibition zones of 8-12 mm [39].

According to the available data, the inhibition zones varied depending on the chemical composition of propolis and the tested strain of *C. albicans* [11,12,35,36].

The chemical composition of propolis varies qualitatively and quantitatively in different geographical regions of the world and it is believed that the chemical diversity depends on the type of honeybee and the extraction method [4,6].

Conclusion

The two ethanolic extracts of propolis of origin from the Republic of Srpska, obtained by traditional methods, showed good fungicidal activity against the tested strains of *C. albicans*, *A. brasiliensis* and *Saccharomyces cerevisiae*. The commercial drug metronidazole did not act fungicidally against one *Candida albicans* isolate from a food sample and weakly acted against the other tested strains.

Considering that propolis is a natural, inexpensive, non-toxic product with proven fungicidal activity it should be considered as another option in the treatment of fungal infections.

In recent years, researchers have mainly investigated the susceptibility of *Candida albicans* strains to ethanol extracts of various propolis, which often contain different chemical compositions, resulting from the unique flora of the region where bees produce propolis. The wide range of activity against *Candida albicans* leads to the

conclusion that the need for standardization of the chemical composition of propolis extracts should be considered, especially in the case of the same types of propolis.

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POREĐENJE FUNGICIDNOG DEJSTVA EKSTRAKATA PROPOLISA I METRONIZADOLA

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Sažetak: *Propolis je prirodna supstanca koja djeluje protuupalno (imunomodulirajuće i mikrobicidno) i ima dugu istoriju upotrebe u tradicionalnoj medicini. Cilj rada je bio da se ispitaju antifugalna svojstva 10% i 30% etanolnog ekstrakta propolisa pripremljenog na tradicionalan način i komercijalnog lijeka metronidazola prema odabranim sojevima Candida albicans izolovanih iz uzoraka hrane, referentnog soja Candida albicans WDCM 00054, referentnog soja Aspergillus brasilliensis WDCM 00053 i referentnog soja Saccharomyces cerevisialis WDCM 3058. Za ispitivanje fungicidnog dejstva propolisa i metronidazola na rast odabranih*

kultura korištena je agar difuziona metoda. Rezultati rada su pokazali da su obe ispitane koncentracije etanolnog ekstrakta propolisa porijeklom iz Republike Srpske djelovala fungicidno na izolate Candida albicans kao i na referentne sojeve Candida albicans WDMC 00054, Aspergillus brasilliensis WDCM 00053 i Saccharomyces cerevisialis WDCM 3058. Metronidazol je djelovao slabo antifugalno.

Ključne riječi: *antifugalna aktivnost, propolis, Candida albicans, Aspergillus brasilliensis, Saccharomyces cerevisialis*