

NAUČNI RAD – Original Paper

**ANTIFUNGAL ACTIVITY OF PROPOLIS ORIGINATED FROM
BOSNIA AND HERZEGOVINA**

**ANTIFUGALNO DJELOVANJE BOSANSKO-HERCEGOVAČKOG
PROPOLISA**

Babić Senka, Pašić Š., Dukić Behija, Čoralić Agnesa

Abstract - Propolis is a resinous substance made by the bees that collect resin from the buds of plants and exudates of trees, enriching it with the salivary enzymes, pollen and wax. Beside of biological activities, propolis also has an antifungal activity. *Candida albicans* is a commensal yeast that may cause candidiasis in humans and animals, too. *Ascospaera apis* is a heterothalic fungus and represents an agent of chalkbrood larval disease (cystic mycosis) of honeybees, *Apis mellifera*. Propolis from Bosnia and Herzegovina has shown an inhibitory effect on *C. albicans* and *A. apis*. 30% concentration of ethanol extracts of propolis has shown the strongest average inhibition zone with the disc diffusion method, with inhibition diameter of 7.56 mm for *C. albicans* and 14.5 mm for *A. apis*, as well as 8 mm for the reference strain of *C. albicans* ATCC 10231 and 12,2 mm for the reference strain of *A. apis* MUCL 30764.

Key word: propolis, antifungal activity, *Candida albicans*, *Ascospaera apis*

Mr. sc. Senka Babić, DVM, viši asistent; Prof. dr. sc. Šemso Pašić, DVM, vanredni profesor, Katedra za mikrobiologiju s imunologijom i zarazne bolesti životinja sa epizootologijom; dr. sc. Behija Dukić, DVM, docent, Katedra za uzgoj i bolesti riba, pčela i divljači; mr. sc. Agnesa Čoralić, DVM, viši asistent, Katedra za unutrašnje bolesti, svi Veterinarski fakultet Univerziteta u Sarajevu.

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Senka Babić, DVM, MSc, Senior Assistant; Šemso Pašić, DVM, PhD, Associate Professor, Department of Microbiology and Immunology; Behija Dukić, DVM, PhD, Assistant Professor, Department of Breeding and Diseases of Fish, Bees and Game; Agnesa Čoralić, DVM, MSc, Senior Assistant, Department of Internal Diseases, Veterinary Faculty, University of Sarajevo.

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Kratak sadržaj - Propolis je smolasta substancija nastala sakupljanjem smola sa pupoljaka biljaka i eksudata drveća od strane pčela obogaćena pljuvačnim enzimima, polenom i voskom. Pored bioloških djelovanja propolis posjeduje i antifungalno djelovanje. *Candida albicans* je komensalni kvasac, koji, s druge strane, može da bude i uzročnik kandidijaze ljudi i životinja. *Ascospaera apis* je heterotalični fungus i predstavlja uzročnika krečnog legla (cistične mikoze) larvi medonosnih pčela, *Apis mellifera*. Propolis porijeklom iz Bosne i Hercegovine pokazao je inhibitorno djelovanje na *C. albicans* i *A. apis*. Najjaču prosječnu zonu inhibicije sa disk difuzionom metodom pokazali su etanolni ekstrakti propolisa u 30%-tnoj koncentraciji gdje je dijametar inhibicije iznosio 7,56 mm za *C. albicans* i 14,5 mm za *A. apis*, te 8 mm za referentni soj *C. albicans* ATCC 10231 i za referentni soj *A. apis* MUCL 30764 12,2 mm.

Ključne riječi: propolis, antifungalno djelovanje, *Candida albicans*, *Ascospaera apis*

Introduction

Propolis is a resinous natural product made by the honey bees who collect resin from the buds of plants and exudates of trees. Bees chew the collected resinous substance adding salivary enzymes, pollen and wax (2, 6). The activity of propolis is diverse and correlates with its chemical composition, i.e. quantity and quality of substances in it. Chemical composition of propolis is variable and depends on flora around the hive as well as geographic region and climate (22, 23).

Some of the main propolis components having biologic properties are: flavonoids, aromatic acids, phenolic compounds, esters and diterpenic acids (6, 19, 24). Mechanisms of propolis activity are not fully known. A possible mechanism of propolis activity would be through the inhibition of the synthesis of proteins and function of the membrane. Flavonoide quercetin is responsible for such kind of activity (14, 26). Yeasts are less sensitive to propolis than bacteria, and more sensitive than mold (7). Mechanism of propolis activity to yeasts is probably related to the cell wall and plasma membrane (13).

Candida spp. are polymorphic eucaryotic organisms (17), classified in *Saccharomycetaceae* family (1). *Candida albicans* is commensal and opportunistic pathogen that causes the whole spectrum of candidiasis forms, from the mild ones to ones that are life-threatening (17, 19). *C. albicans* as an opportunist is a part of normal gastrointestinal, vaginal and oral flora. On the other hand, *C. albicans* is a major pathogen and it causes many manifest diseases, ranging from superficial to systemic mycosis and chronic mucocutaneous diseases (1).

Ascospaera apis is heterothallic fungus causing cystic mycosis (ascosporosis), or chalkbrood of the honey bee larvae *Apis mellifera* L (4). *A. apis* is classified in order

Acosphaerales. Chalkbrood is a fungal infectious disease of larvae of many kinds of social and solitary bees caused by some species of *Ascosphaera* (25).

According to available data, all to-date tested propolis samples showed in different concentrations inhibitory effect to *C. albicans*. Diameters of inhibitory zone are variable depending on propolis chemical composition and tested strain of *C. albicans* (5, 12). On the other hand, literature data are very poor concerning the effects of propolis to the *A. apis*. According to available data, just Samšić et al. (20) and Sahinler and Kurt (18) tested its effect to *A. Apis*, and proved that propolis showed inhibitory activity to *A. apis*.

The aim of this study was to investigate the antifungal activity of propolis obtained from different regions of Bosnia and Herzegovina against *C. albicans* and *A. apis*.

Material and methods

For this study, the following were used: ethanolic extracts of propolis (EEP), nine isolates and one referent strain of *C. albicans* (ATCC 10231), and ten isolates and one referent strain of *A. apis* (MUCL 30764).

The samples of propolis from different regions of Bosnia and Herzegovina in two concentrations, 5 and 30%, were tested.

In order to obtain appropriate concentration of EEP, a suitable amount of grounded propolis was suspended in 100 ml of 96% ethanol. After 30 days of extraction at room temperature in the dark place and with daily mixing, supernatant was centrifugated at 3000 rpm at temperature of 24 °C (modif. of the method according to Krell) (10).

Sterile filter paper discs of 6 mm were impregnated with 10 µl of each of the tested concentrations of EEP, and the control discs with 96% ethanol. Then, the discs were dried at 37°C for 24 hours (modif. of the method according to Silici and al.) (24).

Own isolates and reference strain of *C. albicans* were suspended in saline solution. Inoculum density was adjusted to 0.5 McFarland's (21). Plates with Sabouraud dextrose agar (SDA) were homogeneously inoculated with 100 µl of appropriate inoculum (15), and were kept at room temperature for 2 hours in order for the inoculum to be absorbed into the medium. The two discs with tested concentrations and one control disc were put on each plate, and incubated for 24 hours at 37 °C.

Inoculum density of *A. apis* was set to 4 McFarland's (3). Plates with SDA with supplemented oligoelements were prepared as for *C. albicans*. On the plates placed were two discs previously impregnated with 5 and 30% of EEP and one control disc, and incubated at 30 °C. Evaluation of the results was done after 24, 48 and 72 hours. Diameters of the zone inhibition of growth were measured in millimeters (20).

Results

Tables 1 and 2 present the results of the average activity of 5 and 30% concentrations of EEP of all tested samples of propolis.

Table 1: Average inhibitory activity of EEP to isolates and referent strain of *C. albicans* ATCC 10231

Tabela 1: Srednja vrijednost djelovanja EEP-a na izolate i referentni soj *C. albicans* ATCC 10231

Tested yeast	Mean value of inhibitory zone for the tested fungus (mm)	
	Tested concentrations of EEP	
	5%	30%
Isolates of <i>C. albicans</i>	6,44	7,56
<i>C. albicans</i> ATCC 10231	6,5	8

Table 2: Average inhibitory activity of EEP to isolates and referent strain of *A. apis* MUCL 30764

Tabela 2: Srednja vrijednost djelovanja EEP-a na izolate i referentni soj *A. apis* MUCL 30764

Tested mold	Mean value of inhibitory zone for the tested fungus (mm)	
	Tested concentrations of EEP	
	5%	30%
Isolates <i>A. apis</i>	10,7	14,5
<i>A. apis</i> MUCL 30764	9,6	12,2

Tested samples of propolis originated from Bosnia and Herzegovina showed the strongest average inhibition zone in 30% concentration for *C. albicans* (Table 1) and *A. apis* (Table 2).

Discussion

Antifungal activity of propolis to *Candida* spp., *Saccharomyces* spp., *Aspergillus niger*, *Trichophyton* spp is known for many decades. (9, 11, 16).

As mechanism of propolis action is not fully known, it is assumed, as already mentioned, that it is associated with cell wall and plasma membrane (13, 14, 26). Research with *C. albicans* showed that propolis induced detachment of fungal cell wall and disruption of division, which leads to disfunction of the cell wall of the daughter cells (13).

The strength of propolis inhibitory effect depends on representation of individual components in the sample, and from chemical composition. As highlighted by Burdock (2) and Kujumgijev et al. (12), propolis as natural mixture shows better effect than its individually separated components.

Due to different origin, and thus the chemical composition of propolis, the results of its inhibitory effect to *C. albicans* were different. Kujumgijev et al. (12) investigated the inhibitory effect of Brazilian propolis and propolis originated from

Europa, Africa and South America. Based on their results, they concluded that Brazilian propolis had the strongest inhibited growth of *C. albicans* with inhibitory diameter from 15.7 to 18.2 mm. On the other hand, testing that was done with Turkish propolis showed that inhibitory diameter for *C. albicans* was 8-12 mm (8).

Results of our research showed that propolis originated from Bosnia and Herzegovina had inhibitory activity to *C. albicans* in both tested concentrations, with minimal differences of diameter of the zone of inhibition between isolates and referent strain (Table 1).

Data are poor regarding the activity of propolis to *A. apis*. Samšić et al. (20) tested the inhibitory activity of 15% of the EEP, and they concluded that the more prominent inhibitory effect of propolis to growth of *A. apis* was after 24-hour incubation period, compared to the first hour of incubation period. Sahinler and Kurt (18) added 5% EEP in various concentrations into medium for growth of *A. apis* (1.56-50 ppm), and proved that propolis showed inhibitory activity to *A. apis*.

According to our results, propolis originated from different regions of Bosnia and Herzegovina has inhibitory effect to own isolates and referent strain *A. apis* (table 2).

Conclusion

Propolis originated from different regions of Bosnia and Herzegovina shows inhibitory activity to *Candida albicans* and *Ascosphaera apis*.

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