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Study of the antimicrobial and antibiofilm activity of romanian propolis

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ABSTRACT

Although antibiotics have drastically reduced illness and death from infectious diseases, bacteria have exibited a remarkable capacity to quickly become resistant to antibiotics. The increase in antibiotic resistance is thus a global problem, due to the increased incidence of opportunistic infections with bacterial strains, aggravated by producing bacterial biofilms on cell substrates or biomaterials used in medicine. The emergence of multidrug-resistant phenomena caused an increasing concern and sustained researches for finding new antimicrobial agents or new therapeutical strategies for infectious diseases. In recent years, following the general trend of using direct resources provided by nature, bee products too have became an important source for different active factors, essential to promote a range of harmless therapeutic drugs. Among these, propolis is one of the strongest challenges to nutritionists and medical world due to the recognition of its high biological value and both prophylactic and therapeutic effects. The chemical composition of propolis is very complex and varies according to the geographical area and plant origin. The purpose of this study is to demonstrate the antimicrobial and antibiofilm activity of propolis from Romania on some Gram positive and Gram negative clinical bacterial strains. The tested product was 30% propolis tincture (propolis ethanolic extract = PEE) from Romania. PEE antibacterial activity was qualitatively and quantitatively evaluated by two methods: modified agar difussion method and serial two-fold microdilution method. The antibiofilm activity of propolis was determinated by the assay of its inhibitory influence on microbial biofilm formation on inert substratum. The microbial strains used were: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*. The qualitative screening releaved that the PEE exerted an antimicrobial action against all tested bacterial strains, evidenced by the appearance of growth inhibition zones. The quantitative analysis of antimicrobial activity of PEE showed that the product exerted an inhibitory effect up to a concentration of 0.1875mg/ml against *S. aureus*. Also, the results revealed that *S. aureus* biofilm development on inert substratum was inhibited by propolis tincture at a concentration of 0.1875 mg/ml. The inhibitory activity of PEE against biofilm formation of Gram positive bacteria (*S. aureus*) and the positive results of qualitative screening tests on all tested Gram positive and Gram negative bacterial reference strains belonging to clinically significant species proved an antibacterial and antibiofilm effect of the tested samples of Romanian propolis.

Keywords: *propolis, antibiotic resistance, antimicrobial activity, antibiofilm effect.*

1. INTRODUCTION

At present, the quite often used antibiotherapy (more or less necessary) for fighting against microbial infections during the last decades exerted a high selective pressure and conducted to the actual level of antibioresistance and infections very hard to be solved. The increased antibiotic resistance is thus a global problem, due to the increased incidence of opportunistic infections with bacterial strains, aggravated by the capacity of producing biofilms on cellular substrata or on different medical devices. The emergence of multidrug-resistant phenomena caused increasing concern for finding

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new antimicrobial agents and new antiinfectious therapeutical strategies. In recent years, following the general trend of using direct resources provided by nature, bee products too have became an important source for different active factors, essential to promote a range of harmless therapeutic drugs. Among these, propolis is one of the strongest challenges to nutritionists and medical world due to the recognition of its high biological value and both prophylactic and therapeutic effects.

The propolis is a resinous mixture of vegetable and volatile oils collected by bees from buds and exudates of the plants, which are masticated with bee salivary enzymes and mixed with beewax [1]. Bees use this product to seal holes in their honeycombs, to polish internal walls as well as to cover carcasses of intruders who died inside the hive, in order to avoid their decomposition; propolis also protects the bee colony from infectious diseases by its high antiseptic efficacy. So, this natural product have a double origin: vegetal and animal too, and it can be the explanation for its very wide range of biological effects.

Some of the propolis components can be identified in plant sources visited by bees, which can belong to conifers, poplars *(Populus sp.)* and other species: beech *(Fagus sylvatica),* chestnut *(Aesculus hippocastanum);* the bees collect resin from buds, bark and wound exudates of these trees. In Europe, the main sources of propolis include: hazel *(Alnus spp),* birch *(Betula spp),* hazelnuts *(Corylus spp),* oak *(Quercus spp),* poplar *(Populus spp)* and willow *(Salix spp)* [2]. According to the paper of Bankova [3], poplar buds are the main source of propolis in Europe, South America, Western Asia and North Africa.

The chemical composition of propolis is very complex and varies according to geographical area and plant origin [4]. The major components of propolis are resins, wax and flavonoids and minor components are essential oils, tannins, enzymes, vitamins, minerals etc. In propolis there are few pollen grains and varying amounts of impurities, as well as soluble sugars and nitrogenous substances in very small quantities (traces). Pharmacologically active constituents of propolis are found in different fractions soluble in organic solvents such as ethyl alcohol - the main solvent used in the production of extracts for pharmaceutical use [5]. The most important fraction for biological activities is represented by flavonoids (flavones, flavonols and flavanones) and various phenolic and aromatic compounds [6,7].

The studies carried out in well known laboratories of pharmacology and medicine have confirmed the important role of this bee product in prevention and treatment of a wide range of diseases. Because of multiple chemical components, propolis is considered the most valuable bee product, with a wide variety of therapeutic actions: bactericidal, antiseptic, antiparasitic, antiviral, antitoxic, epithelizant, healing, anti-inflammatory, diuretic, analgesic, antitumoral, regenerating and immunostimulating [8]. Native or prepared as extracts, tinctures and different pharmaceutical forms, the propolis is now one of the most important subjects of study and work for apitherapy [2].

Biological effects of propolis are different according to the geographic area, plant origin and also to bacteria species. Thus, this bee product could be used as antibacterial agent in infectious diseases therapy [9].

A lot of studies have been carried out to demonstrate antimicrobial activity of propolis extracts. According to the results of these studies, propolis possesses a broad spectrum against various Gram-positive and Gram-negative bacteria: *Staphylococcus spp., Streptococcus spp., Listeria spp., Bacillus spp., enterobacteria (Klebsiella pneumoniae, Escherichia coli), Pseudomonas aeruginosa, Helicobacter pylori* etc. Some differences concerning the results of the antimicrobial activity testing of propolis extracts are due to: seasonal variation of chemical composition of plants and propolis too, extraction method and various methods for determination of the antimicrobial activity of propolis

extracts. The results obtained are also influenced by the experimental conditions (thickness of agar layer, type of agar, inoculum size) and bacterial strains tested [10].

Many authors compare the chemical composition of ethanol extracts of propolis and its antimicrobial activity. In 2005, Kosalec showed that those samples of propolis where the flavonoid content was higher than 1% exerted an antimicrobial activity against *S. aureus, S. pyogenes, Enterococcus faecalis, B. subtilis, Candida albicans* [11]. Other studies indicate that the components which are involved in European propolis activity are flavonoids, aromatic acids and esters, while in Brazilian propolis are amyrins [5].

The antibacterial action of propolis was investigated by Grange *et al.* using a propolis ethanolic extract (PEE, 70% ethanolic extract) known as "propolis balm"; they applied PEE on bacterial cultures on agar plates, demonstrating its effectiveness in inhibition of the development of *Staphylococcus aureus* strains, including meticillin resistant strains (MRSA) [12].

Associated with the use of some antibiotics, the efficacy and duration of propolis extract action is more pronounced and these organisms do not develop antibiotics resistance. In a study of 2011, Helaly *et al*., demonstrated the antimicrobial and antibiofilm activity of dexapenthanol and propolis extract, alone or in combination with antimicrobial agents on *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains isolated from infected wounds [13].

Bacteria possess specialized surface structures called *adhesins*, able to interact stereospecifically with receptors on the host cell membrane, in a manner analogous with the interaction antigen-antibody or lectin-carbohydrate. Adhesion to the substratum is the initial stage of biofilm formation. In order to form a biofilm the microorganisms must adhere to a cellular or inert substratum, and then they will grow and survive even if the environmental conditions are not so favorable (pH, redox potential, nutrients). The biofilm formation either in natural or industrial systems is now seen as one of the adaptation and survival strategies of microorganisms, the biofilm cells being protected from all stress conditions (including all kind of antimicrobials).

In medical "ecosystems", microbial adhesion leads to biofilms formation on various surfaces, natural (teguments and mucosa, intact or damaged, teeth, endothelial cells) or artificial (different prosthetic materials) and is a precondition to pathogenesis of many bacterial infections, difficult to treat, due to the different behavior of cells in biofilms, respectively phenotypical, behavioural resistance or, more recently called *tolerance* to antimicrobial factors [14,15].

One of the most important consequences of biofilm formation is that the biofilm cells are more resistant to both host defenses and conventional doses of antibiotics and biocides, even the same cells tested by the standard method for M.I.C. determination (with cells in suspension) are susceptible to antimicrobials action.

At the molecular level the study of biofilms showed that they are formed and controlled by complex mechanisms of intercellular signalling. Communication between the constituent cells of a biofilm is achieved by a signalling system known as *quorum sensing (QS)* mechanism considered as a real bacterial “language”, mediated by small signal molecules: peptides (in Gram-positive bacteria) and L- homoserin - lactones (HSL/AHSL) (in Gram-negative species) [16] and AI-2 molecules common to Gram positive and Gram negative bacteria. The signalling molecules allow to bacterial cells the environment monitoring and a modified gene expression and in the same time, the acquirement of a competitive advantage, important for survival and dissemination in natural environments, high competitive, such as in the oral cavity and intestinal tract [15]. QS systems discovery allowed to open new prospects for treatment and prevention of bacterial infections by finding the inhibitors of bacterial QS mechanism, inhibitors which are represented by natural or obtained by chemical synthesis compounds [17,18].

Due to the importance of biofilm formation at medical, ecological, and economic level, in the last ten years an extensive research on this phenomenon has been done. A very attractive alternative to the antibiotherapy and in the same time an ecological strategy for fighting against the biofilm associated infections is the inhibition of intercellular signalling by natural QS inhibitors (QSI) secreted by other organisms, microbial, vegetal and animal too [15]. A lot of natural products and components were tested, especially of vegetal origin, but only relatively a few research works on propolis ability to inhibit biofilm formation have been published. Thus, Hyun Koo *et al*. [19], Duarte *et al*. [20] have shown that propolis inhibits the growth of oral microorganisms and the activity of bacteria-derived glucosyltransferases (GTFs), responsible for glucan synthesis which favors bacterial adhesion and play an essential role in the development of pathogenic dental plaque. Bulman *et al.* [1] studied the composition of propolis and revealed that it contains compounds that inhibit signaling mediated by N-acyl-homoserin-lactone in *Pseudomonas aeruginosa PAO1*.

The purpose of this study is to investigate the antimicrobial and antibiofilm activity of a sample of Romanian propolis on some Gram positive and Gram negative bacterial reference strains belonging to clinically significant species. For this purpose we performed qualitative and quantitative methods to determinate the antimicrobial and antibiofilm activity of propolis. The antibiofilm activity of Romanian propolis has not been yet studied and therefore to determine the antibacterial activity and especially antibiofilm activity of this bee product, is a starting point for future studies to identify biologically active compounds and their targets on microbial biofilm cells.

2. EXPERIMENTAL SECTION

The tested sample of propolis was a 30% *propolis tincture* (30% ethanolic extract of propolis), from *Romanian Institute for Beekeeping Research & Development*. The raw propolis used to obtain propolis tincture was harvested from various regions of Romania; the solvent used for extraction was 96 ° ethanol.

Bacterial reference strains. All tested strains were from Collection of Microorganisms of the Dept. of Microbiology-Immunology, Faculty of Biology, University of Bucharest. Bacterial strains were**:** *Gram positive* bacteria*: Staphylococcus aureus* 13024; *Gram negative* bacteria*: Escherichia coli* 13147, *Klebsiella pneumoniae 13420, Pseudomonas aeruginosa* 13202.

2.1. Quality screening of microbial strains sensitivity to ethanolic extract of propolis was performed with an adapted diffusion method: propolis tincture was spread in *"spot"* on Muller-Hinton medium inoculated with a bacterial suspension. Inoculum was prepared with fresh cultures of bacterial strains cultured on simple agar medium for 24 hours at 37°C with pysiological saline solution. Inoculum was prepared with fresh cultures of bacterial strains cultured on simple nutrient agar medium for 24 hours at 37°C with pysiological saline solution. The spot volume of tested product was 5 µl (in each binary dilution of propolis tincture in ethyl alcohol 96 °: 1/2, 1/4, 1/8, 1/16, 1/32, 1 / 64, 1/128, 1/256, 1/512). After inoculation, the plates were incubated for 24 hours at 37°C. The solvent, ethanol 96%, was tested (as control) to evidence its potential antimicrobial activity. The bactericidal effect of product (bacterial growth inhibition) was measured by the appearance of a zone of inhibition (clear zone) around the spot.

2.2. Quantitative testing of the antimicrobial activity of propolis ethanolic extract (PEE) of was performed by serial two-fold microdilution method in liquid medium (Mueller Hinton) using 96-well microtitrer plates, to determine the MIC (minimum inhibitory concentration). MIC is defined as the lowest concentation of a tested product that inhibit the growth of microbial cells. In a volume of 180 µL of medium were obtained serial dilutions of the stock solution product (0, 3 g / mL). In the

first well were pipetted 180 µL nutrient broth and 20 µL propolis tincture. In the first well were transferred to 100 µL l in the second, the second well were transferred to 100 µL in the third and so on until the last well, which were thrown 100 µL. Microbial suspensions were prepared in sterile physiological water from 24 hours bacterial cultures on nutrient agar. Sterility control wells (glucose broth) and microbial growth controls (inoculated glucose broth) were used. After incubating the plates at 37ºC for 24 hours, the results were analyzed by macroscopic observation and spectrophotometrically measured at A 620 nm. MIC value (µg/mL) for PEE is represented by the PEE concentration from the last well in which no cultural development was observed.

The antibiofilm activity of PEE was determined by measuring the sensitivity of a biofilm developed on inert substratum, by the following steps:

• bacterial cells cultivation in 96-well plates with nutrient broth in the presence of test product (following readings MIC) and incubation at 37C for 24 hours; plates were emptied and washed twice with AFS;

• adhered cells fitting for 5 minutes with 100 µL 80% methanol; methanol solution was removed by inversion;

• adhered cells staining with alkaline crystal violet 1% (100 µL / well) for 15 minutes; staining solution was removed and then the plates were washed under running tap water;

• microbial biofilms formed on plastic plates were resuspended in 33% acetic acid (by bubbling), and the intensity of colored suspensions was assessed by measuring absorption at 490 nm with Appolo LB 911 spectrophotometer.

3. RESULTS SECTION

Determinations have revealed that product show an antimicrobial action against all tested bacterial strains, evidenced by the appearance of growth inhibition zones.

Figure 1: Qualitative screening of antibacterial activity of propolis tincture to Gram positive bacterial strains - *S. aureus*

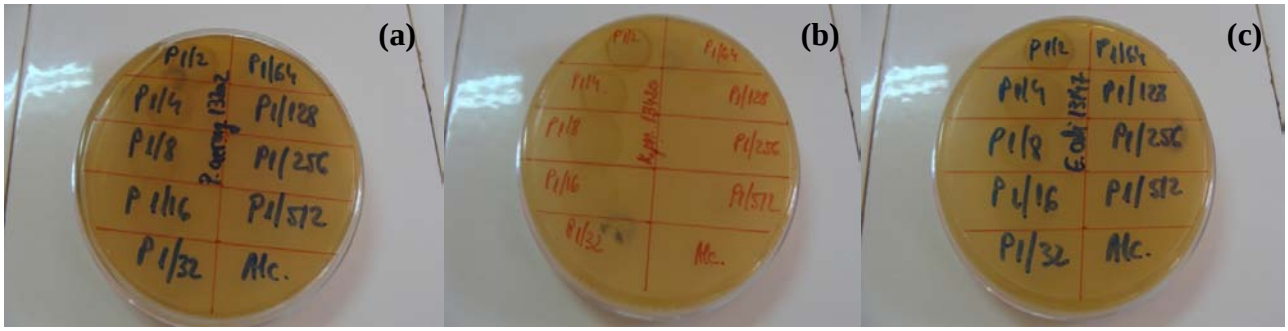

Figure 2: Qualitative *screening* of antimicrobial activity of propolis tincture to Gram-negative bacterial strains: a) *P. aeruginosa 13202, b) K. pneumoniae 13420, c) E.coli 13147.*

The results of qualitative analysis of the antimicrobial activity of propolis tincture have revealed that PEE had an intensive antimicrobial activity against all tested bacterial strains, while 96°C ethylic alcohol (as a control) showed a weak antimicrobial activity (table 1).

Table 1: Qualitative testing of propolis tincture antimicrobial activity

Product	Tested microbial strain			
	E. coli 13147	*P. aeruginosa 13202*	*K. pneumoniae 13420*	*S.aureus 13024*
Propolis tincture(PEE)	++	++	++	++
96° ethilic alcohol	+	+	+	+

Legend : → ”+ + ” intense antimicrobial activity was present
→ ” + ” weak antimicrobial activity was present

For *S.aureus* strain, tests showed a microbial growth inhibition of propolis tincture up to a concentration of 0,1875 mg/mL (M.I.C.) (figure 3). This inhibitory effect was observed by absorbance values of microbial cultures obtained in the presence of propolis tincture that are lower than absorbance values for positive control (microbial growth) and those obtained in the presence of the solvent (96°C ethanol). We have not recorded MIC values of propolis tincture for gram negative microbial strains because the values absorbance of this microbial cultures were higher than the values obtained for positive control (propolis tincture produced turbidity - an opaque solution when was diluted in culture medium and was not possible to determine the minimum inhibitory concentration).

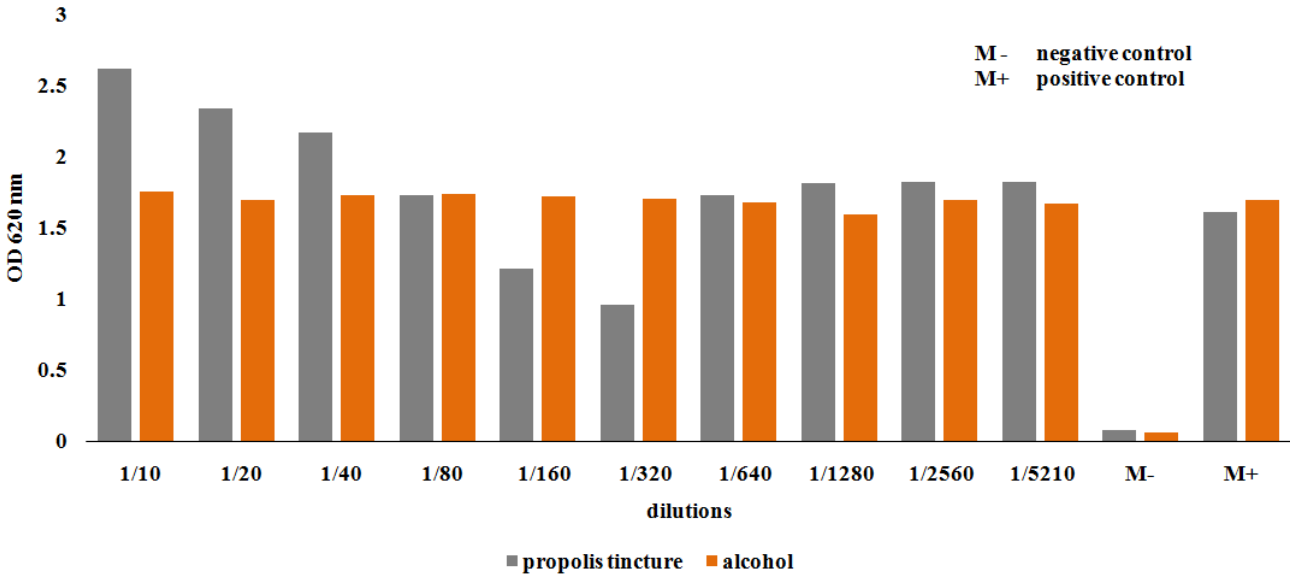

Figure 3: Graphical representation of absorbance values of *S. aureus 13024* cultures recorded at 620 nm and obtained for binary concentration of propolis tincture.

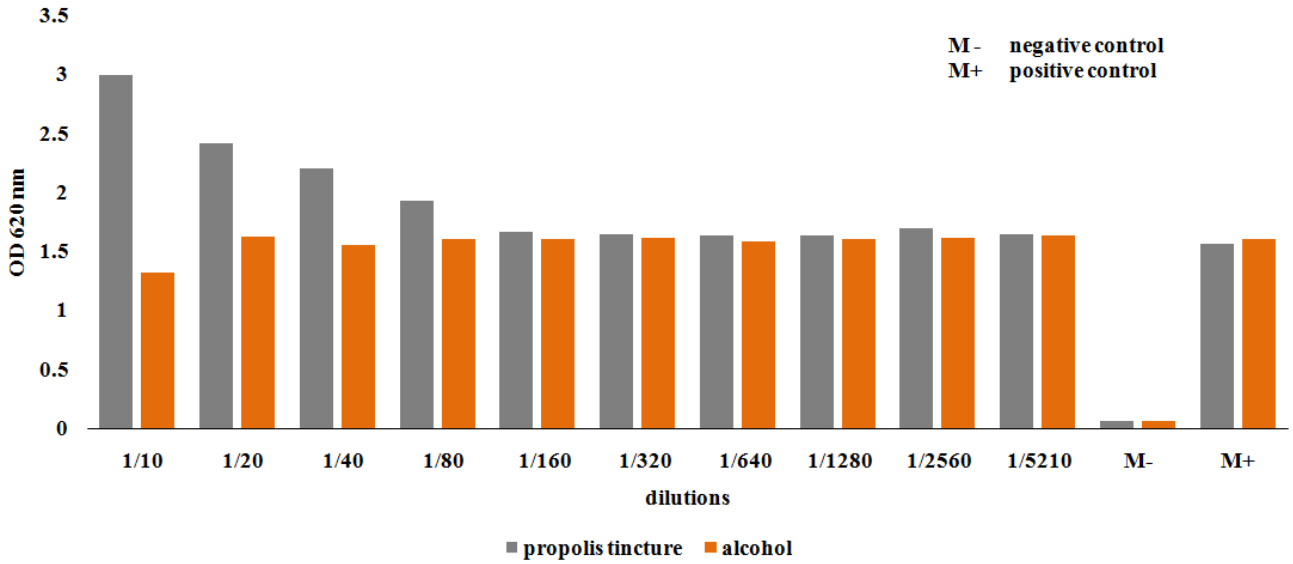

Figure 4: Graphical representation of absorbance values of *E. coli 13147* cultures recorded at 620 nm and obtained for binary concentration of propolis tincture.

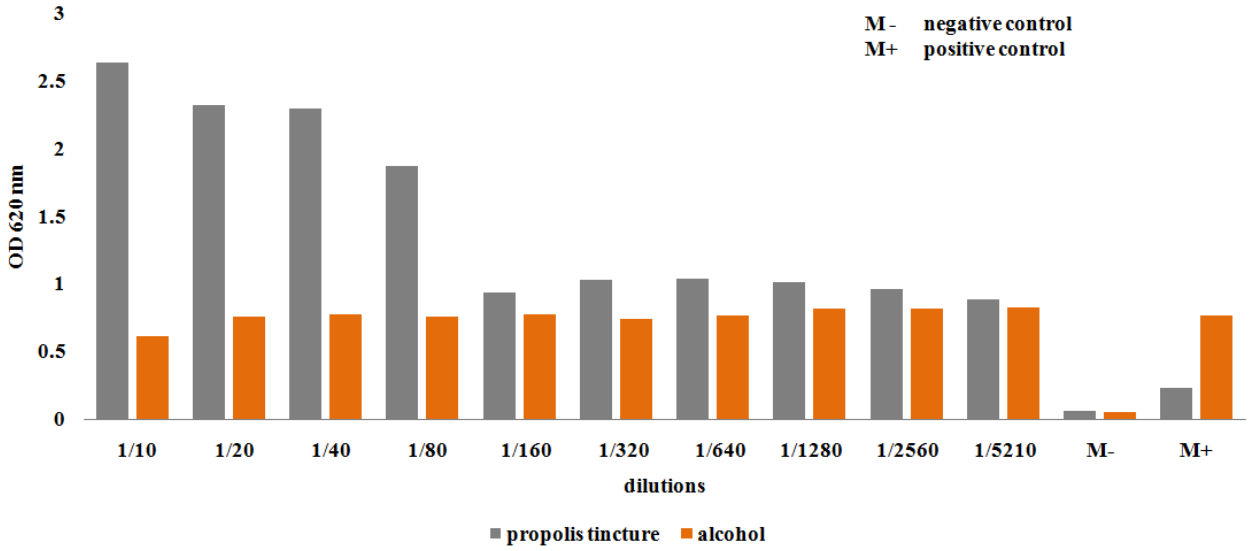

Figure 5: Graphical representation of absorbance values of *P.aeruginosa 13202* cultures recorded at 620 nm and obtained for binary concentration of propolis tincture.

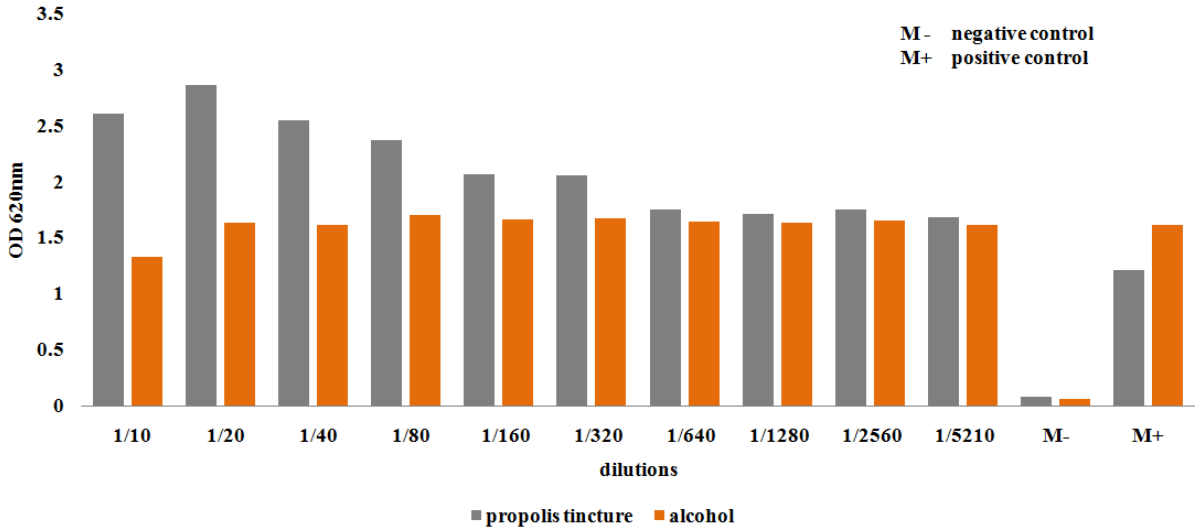

Figure 6: Graphical representation of absorbance values of *K. pneumoniae 13420* cultures recorded at 620 nm and obtained for binary concentration of propolis tincture.

Performed tests revealed that *S. aureus 13024* biofilm development on inert substrate was inhibited by propolis tincture to a concentration of 0,1875 mg/mL. The antibiofilm activity of propolis tincture was recorded only for gram-positive microbial strain (*S. aureus 13024*).

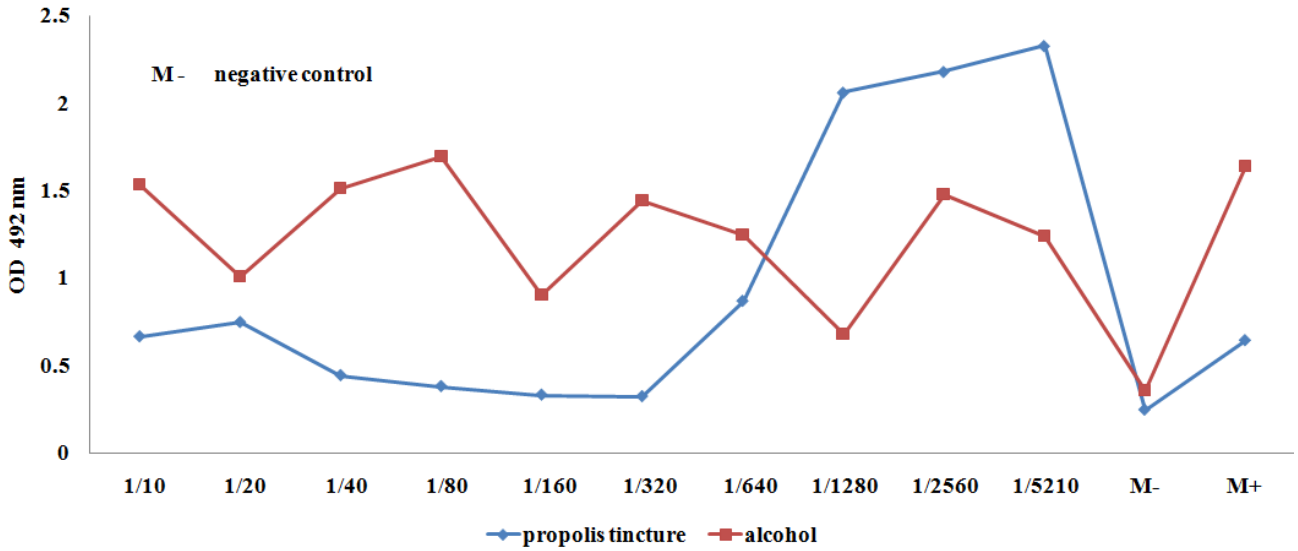

Figure 7: Graphical representation of microbial biofilm development degree on inert substrate, in the presence of various dilutions of propolis tincture on *S. aureus* 13024 strain

Qualitative determination of the antimicrobial activity revealed that propolis tincture had an inhibitor effect against all tested Gram-positive and Gram-negative bacterial strains. These results were in agreement with those of Helaly *et al.* [13] who noticed that PEE had an antimicrobial activity against *Staphilococcus aureus* and *Pseudomonas aeruginosa*. But our results vere not in accordance with the observations of other authors [12, 21]. For instance, Grange and Davey [12] observed that propolis partially inhibited growth of *Pseudomonas aeruginosa* and *Escherichia coli* and did not demonstrate any antibacterial activity against *Klebsiella pneumoniae*. Similar results obtained Katircioglu and Mercan [21]: propolis did not inhibited growth of *K. pneumoniae*, but it had a strong inhibitory effect against *E. coli*.

Most studies conducted to determine antimicrobial activity of propolis have shown that propolis exerts a stronger antibacterial action against Gram-positive bacteria than against Gram negative bacteria. Also, many authors correlated the antibacterial activity of propolis with its chemical composition, so they found that caffeic acids, flavonoids and phenolic esters are important for biological effects of propolis [5,21]. For this reason, propolis from various geographical areas and with different chemical composition exhibit different activities against Gram-positive and Gram-negative bacteria [5]. MIC value (0.1875 mg/mL) obtained in our study shown that propolis tincture exerted a strong inhibitory activity on the growth of *Staphylococcus aureus* 13024. This MIC value was much lower than MIC values obtained by Helaly *et al.* [13] in their study, respectively 2500 µg/mL for *Staphylococcus* sp. isolates N31 and N25 and 1250 µg/mL for *Staphylococcus* isolate N6. Other researchers obtained MIC values for *Staphylococcus sp*. higher then MIC value determined in from our study: Schazzocchio et al.(2005) recorded MIC values for 63 strains of *S. epidermidis* and 42 strains of *Staphylococcus spp.*: 1.25 mg/mL and 2.5 mg/mL. Because we did not recorded MIC values of propolis tincture for Gram-negative bacteria (propolis tincture produced turbidity), we intend to modify microdilution method (different medium, inoculum size). The results of the present study on the influence of propolis tincture on biofilm development of Gram positive microbial strain (*Staphylococcus aureus* 13024) on inert substratum revealed that ethanolic extract of propolis exerted an inhibitory effect of bacterial cell adhesion, antimicrobial activity was observed up to a

concentration of 0.1875 mg/mL. The used solvent (96° ethanol) also inhibited biofilm development but in a lesser extent than the propolis tincture. These results show a positive correlation between propolis tincture concentration and decrease of bacterial cell adhesion. Moreover, Helaly *et al.* [13] reported that PEE inhibited *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms formation.

4. CONCLUSIONS

The positive results of qualitative *screening* tests both on Gram positive and Gram negative clinically significant species and the inhibitory activity of propolis tincture against biofilm formation in Gram positive bacteria (*Staphylococcus aureus*) prove an antimicrobial and antibiofilm effect of Romanian propolis. These results suggest the Romanian propolis as subject for next studies in determination of its inhibitory potential against microbial biofilms and the clinical use of this product in infectious diseases therapy. The next step in our researches is to identify biologically active compounds from chemical composition of Romanian propolis and to establish the inhibition mechanism on biofilm formation.

5. REFERENCES

[1] Bulman Z., Le P., Hudson A.O., Savka M.A., A novel property of propolis (bee glue): Anti-pathogenic activity by inhibition of N-acyl-homoserine lactone mediated signaling in bacteria, *Journal of Ethnopharmacology*, 138, 788-797, **2011**.

[2] Mateescu C., *Apiterapia sau cum să folosim produsele stupului pentru sănătate*, Ed. Fiat Lux, Bucureşti, **2008**.

[3] Bankova V.S., De Castro S.L., Marcucci M.C., Propolis: recent advances in chemistry and plant origin. *Apidologie*, **31,** 3-15, **2000**.

[4] Popova M., Silici S., Kaftanoglu O., Bankova V., Antibacterial activity ofTurkish propolis and qualitative and quantitative chemical composition, *Phytomedicine*, 12**,** 221-228, **2005**.

[5] Mateescu C., Dumitru I.F**.,** *Propolisul şi extractele de propolis. Noutăţi în biotehnologie*. Editura Ilex, Bucureşti, **2001**.

[6] Salomao K., Dantas A.P., Borba C.M., Campos L.C., Machado D.G., Aquino Neto F.R., de Castro S.L., Chemical composition and microbicidal activity of extracts from Brazilian and Bulgarian propolis. *Letters in Applied Microbiology***,** 38, 87–92, **2004**.

[7] Lotfy M., Biological activity of bee propolis in health and disease. *Asian Pacific Journal of Cancer Prevention*, **7,** 22-31, **2006**.

[8] Marcucci M.C., Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie*, 26, 83-99, **1995**.

[9] Najmadeen H.H., Kakamand F.K., Antimicrobial activity of propolis collected in different regions of Sulaimani province-Kurdistan region/Iraq, *Journal of Duhok University*, 12**,** 233-239, **2009**.

[10] Da Silva Ribeiro de Rezende G.P., Pimenta F.C., Ribeiro de Rezende Sucasas da Costa L., Antimicrobial activity of two brazilian commercial propolis extracts. *Brazilian Journal Oral Science*, 5, 967-970, **2006**.

[11] Kosalec I., Pepeljnjak S., Bakmaz M., Vladimir-Knezevic S., Flavonoid analysis and antimicrobial activity of commercially available propolis products, *Acta Pharmaceutica*, 55, 423-430, **2005**.

[12] Grange J.M., Davey R.W., Antibacterial properties of propolis (bee glue), *Journal of the Royal Society of Medicine*; 83, 159-160, **1990**.

[13] Helaly G.F., Abd El-Aziz A.A., Sonbol F.I., El-Banna T.E., Louise N.L. Dexpanthenol and propolis extract in combination with local antibiotics for treatment of *Staphylococcal* and *Pseudomonal* wound infections, *Archives of Clinical Microbiology*, 2, 1-15, **2011**.

[14] Lazar V., Chifiriuc M.C., Medical significance and new therapeutical strategies for biofilm associated infections, *Roumanian Archives of Microbiology and Immunol*ogy, 69, 3, 121-127, **2010**.

[15] Lazar V., Quorum sensing in biofilms - How to destroy the bacterial citadels or their cohesion/power?, *Anaerobe*, 17, 1-6, **2011**.

[16] Davey M.E., O'Toole G.A., Microbial biofilms: from ecology to molecular genetics, *Microbiology and Molecular Biology Reviews*, 64, 847-867, **2000**.
[17] Hentzer M., Givskov M., Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections, *Journal of Clinical Investigation*, 112, 1300-1307, **2003**.
[18] Høiby N., Ciofu O., Johansen H.K., Song Z., Moser C., Jensen P.O., Molin S., Givskov M., Tolker-Nielsen
T., Bjarnsholt T., The clinical impact of bacterial biofilms, *International Journal of Oral Science*, 3, 55-65, **2011**.
[19] Koo H., Rosalen P.L., Cury J.A., Park Y.A., Bowen W.H., Effects of compounds found in propolis on *Streptococcus mutans* growth and on glucosyltransferase activity, *Antimicrobial Agents and Chemotherapy*, 46, 1302-1309, **2002**.
[20] Duarte S., Koo H., Bowen W. H., Hayacibara M. F., Cury J. A., Ikegaki M., Rosalen P. L., Effect of a novel type of propolis and its chemical fractions on glucosyltransferases and on growth and adherence of *mutans streptococci, Biological & Pharmaceutical Bulletin*, 26, 4, 527—531, **2003**.
[21] Katircioglu H., Mercan N., Antimicrobial activity and chemical compositions of Turkish propolis from different regions, *African Journal of Biotechnology*, 5, 1151-1153, **2006**.