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Anti-Pathogenic Effect of Propolis Extracts from Different Romanian Regions on *Staphylococcus* Sp. Clinical Strains

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Abstract

Propolis, a natural product elaborated by bees, determines a wide variety of therapeutic actions because of its bioactive compounds. Our researches were focused on evaluating the anti-pathogenic effect of eight ethanolic propolis extracts (EEP) from different Romanian regions on 11 Staphylococcus sp. clinical strains. The content of flavons, flavonols and polyphenols was investigated by spectrophotometric methods. The following parameters were investigated for the biological evaluation: i) the microbial biofilms development on inert the substratum assessed by the microtiter method; ii) the microbial adherence to the eukaryotic cells (adapted Cravioto's method); iii) the expression of soluble enzymatic factors involved in the microbial virulence (cultuvation on specific substrates). The results showed that all studied EEP exhibited an antibiofilm activity. A correlation between the increased polyphenol content (EEP Arad - 17.62% and EEP Cluj - 21.54%) and the lowest value of minimum biofilm eradication concentration (0.2343 mg / ml) was noticed. EEP Arad, EEP Bihor and EEP Dolj exhibited a strong inhibitory effect on Staphylococcus strains adherence to the cellular substratum and induced changes in the adherence pattern. EEP inhibited the secretion of soluble enzymatic factors of tested Staphylococcus strains. These results demonstrate that the tested EEP could be used for the design of new anti-pathogenic agents for preventing microbial colonization and dissemination of infections.

Keywords: *propolis, microbial adherence, virulence factors, microbial biofilm, Staphylococcus aureus*

1. Introduction

The propolis, so called "bee glue", is a natural product with double origin: vegetal and animal, too. So, the bees elaborate this product by adding glandular secretions and wax to resinous substances collected from different plant species. In addition to its mechanical role (sealing holes and crackes, reduceing entrance of the hive, repairing combs), propolis has an aseptic role in the hive microclimate, being a true "chemical weapon" against microorganisms (Bankova [1]; Fokt et al. [2]).

Among the products of the hive, propolis has a special place because of multiple chemical components generating a wide variety of therapeutic actions.

The chemical composition of propolis varies greatly according to the botanical and geographical origin (Bankova [3]), the season of collection and is represented by: 50%resins and vegetable balsams, 30% wax, 10% essential and aromatic oils, 5 % pollen, and 5% traces of other substances (Ozan et al.[4]; Nieva Moreno et al. [5],Miguel and Antunes [6]). The main constituents of propolis are phenolic compounds (flavonoids, phenolic acids and their esters),

aromatic acids and their esters, aliphatic acids and their esters, terpenoids, bioactive compounds responsible for the biological effects of propolis (Kujumgiev et al. [7]). Since ancient time propolis has been used in folk medicine, being one of the most effective natural remedies. Pharmacologically active compounds of propolis are present in varying amounts depending on the propolis sample, the mode of dosage and the nature of extraction method (Ugur şi Arslan [8]). The ratio of organic compounds in propolis is very important for the biological effects (Fokt et al. [2]). The flavonoid compounds are considered to be responsible for its main therapeutic actions. The researches on the antibacterial activity of propolis and its extracts have revealed that they were active against a broad spectrum of Gram-positive bacteria, but exhibited a lower activity or were inactive against Gram-negative bacteria. One of the main opportunistic and nosocomial pathogens is *Staphylococcus aureus,* causing skin, gastrointestinal tract, genital tract, urinary tract infections, hard to treat due to its increasing resistance to antimicrobials and specific and nonspecific host defense mechanisms (Lee et al. [9]).. Moreover, *S.aureus* could develop biofilms both on living tissue and the inert surfaces of medical devices and implants. The pathogenesis of *S. aureus* is facilitated by multiple virulence factors which are implicated in cellular adherence and in invasion and dissemination of infections. The cell surface factors, termed the microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) are involved in the initiation and colonization process (Foster and Hook [10], Speziale et al. [11]). Also, the virulence factors secreted during different stage of infections play an important role in the virulence of *S. aureus*. Enzymatic factors such as lipases, nucleases, proteases, exotoxins may help *S. aureus* to avoid the immune system, to destroy the host tissue and thus facilitate the bacterial spreading (Lowy [12], Dinges et al. [13], Foster [14], Gordon and Lowy [15]). Coagulase-negative staphylococci (CoNS) are opportunistic pathogens that colonize the skin and mucosa normally and can cause serious infections in immunocompromised hosts. *S. haemolyticus, S. sciuri* and *S. warneri* are CoNS species could produce clinically significant infections difficult to treat because of antibiotic resistance and their ability to form biofilms associated with the insertion of medical devices (de Allori et al. [16]). Skin lesions favor the development of acute or chronic skin infections. The process of wound healing is more difficult due to the presence of chronic wounds associated biofilms. The increased incidence of infections caused by bacteria of the genus *Staphylococcus* and their resistance to conventional antibiotic treatment require finding new effective solutions to prevent and to stop the progress of these infections. In recent years there have been extensive studies on the chemical composition and biological effects of natural product, including propolis in order to introduce them in the treatment of various diseases. The purpose of this work was to evaluate the anti-pathogenic effect of eight ethanolic propolis extracts (EEP) from different Romanian regions on 11 *S. aureus* clinical strains.

2. Materials and Methods

Preparation of ethanolic extracts of propolis

We used in our study raw propolis collected from eight Romanian regions. Propolis samples were initially kept in a freezer at -18 ° C and subsequently grounded to obtain a fine powder. In order to obtain the ethanol extract of propolis, there were added 70 ml of 96% ethanol over 30 g of propolis powder. The obtained mixture was kept for 7 days at room temperature while stirring occasionally. The obtained solution was then filtered through filter paper with a pore size of 0.5 mm. (Tagliacollo and Orsi [17]). The extraction was repeated twice, washed with 96% ethanol; the obtained filtrates were combined and the total volume was adjusted to 100 ml with the same extraction solvent. The concentration of the ethanolic

extract obtained was 30%. Ethanolic extracts of propolis were stored in brown tightly closed bottles at room temperature.

Bacterial strains

For testing antipathogenic activity of propolis eight *Staphylococcus aureus* strains, one *Staphylococcus haemolyticus* strain, one *Staphylococcus sciuri* strain and one *Staphylococcus warneri* strain were used. These bacterial strains were isolated from wound secretions found in skin infections.

Assessment of flavanols and flavonols in propolis by the spectrophotometric method

To quantify flavonoids there were used ethanolic extracts of propolis (concentration 30%) obtained from propolis samples of the 8 Romanian counties (Dâmbovița, Calarasi, Dolj Mehedinti, Mures, Arad, Bihor and Cluj). Propolis ethanolic extracts were diluted with ethanol 96% v / v to obtain solutions with a final concentration of 1%. These solutions were then used to determine the content of flavones and flavonols from propolis. The content of flavones and flavonols were determined by the method of aluminum chloride which consist in the development of a color reaction due to the formation of a complex between the aluminum ion Al (III) and the carbonyl and hydroxyl groups of flavonoids (Gomez-Caravaca et al. [18]). In a 25 ml flask were mixed 1 ml of ethanolic extract of propolis concentration 1%), 1 ml of 5% $AlCl_3$ and 10 ml of methanol. The volume was then adjusted to 25 ml with methanol. The resulting solution was kept for 30 minutes at room temperature and the absorbance at 425 nm was measured using a UV-VIS spectrophotometer Shimadzu. For preparing the blank solution the sample was replaced with the same volume of methanol (Popova et al. [19]). The quercetin (3,3-, 4-, 5,7-pentahydroxyflavondihydrate) (Merck) was used as a reference standard for calibration. Calibration was achieved by a series of dilutions from the stock standard solution of quercetin in methanol (0.8 mg / ml), obtaining five standard solutions with concentrations ranging from 0.1- 0.8 mg / ml.

Determination of total polyphenols from propolis by Folin-Ciocalteu method

The method used to determine the total polyphenol content of propolis of the eight ethanolic extracts diluted with 96% ethanol for obtaining working solutions with a concentration of 1% was Folin-Ciocalteu colorimetric method. In a 50 ml flask containing 15 ml of distilled water was added 1 ml of working solution (concentration 1%), 4 ml of Folin-Ciocalteu reagent and 6 ml of 20% sodium carbonate (w/v). The final volume was adjusted to 50 ml with distilled water. The solution was kept for 2 hours in the dark. After 2 hours the absorbance was measured at 760 nm. Blank solution was prepared by replacing the sample with an equal volume of distilled water. (Popova et al. [19]). Gallic acid was used as the standard reference for the calibration curve. There were prepared five standard solutions by dilutions of the gallic acid stock standard solution (1.2mg / ml). The concentrations of these standard solutions were ranged from 0.3 to 1.2 mg / ml.

Antibiofilm assay

In 96-well plates with nutrient broth (TSB Tryptic Soy Broth) there were cultivated bacterial suspensions of 0.5 McFarland (20 µl) density obtained from fresh bacterial cultures. Also, different concentration of EEP (0.058 - 30 mg/ml) were added to the inoculated plates and incubated at 37°C for 24 hours. After incubation, the plates were gently washed twice and the adhered cells were fixed with cold methanol 80% (100µl) for 5 minutes. After this period

methanol solution was removed and microbial biofilm was stained with 0.1% violet crystal solution for 15 minutes. The adhered biomass was resuspended in 33% acetic acid and optical density was assessed by measuring the absorbance at 490 nm using an Appolo LB 911 spectrophotometer.

Microbial adherence to eukaryotic cells

Bacterial adherence to the cellular substrate and the EEP influence on microbial adherence capacity was performed by the adapted Cravioto's method (Cravioto et al. [20]). Cell lines used in this method have been HEp-2 (Human Epithelioma) and HeLa (cervical carcinoma). The cells were cultured in Eagle's MEM (EMEM = Eagle Minimum Essential Medium) (Gibco BRL), with the addition of 10% bovine fetal serum, without antibiotics, in tissue culture plates to reach a 80% cell monolayer confluence. Then, the plates with cells were incubated in 5% CO_2 humid atmosphere at 37 ° C for 24 hours. Microbial strains of 0.5 McFarland density obtained from 24-hour cultures grown on TSB (Tryptase Soy Broth) in the presence of subinhibitory concentrations of EEP were centrifuged at 10,000 rpm for 10 min and the obtained pellets were washed 3 times with PBS (phosphate buffered saline) and resuspended in PBS. After removing the growth medium and washing (3 times) with PBS there was added 1 ml of the microbial suspension to the cell monolayer. Then the tissue culture plates were incubated at 37 ° C for 2 hours. After incubation, the infected cellular monolayer was washed with PBS (3 times), fixed in cold methanol (5 minutes) and then stained with 20% Giemsa solution (20 minutes). The stain was removed with water and plates were dried at room temperature overnight, and then examined by light microscopy (magnification, x 2500) and photographed with a Canon camera adapted to Zeiss microscope. Microscopic examination allowed establishing the adherence pattern (localized, diffuse or aggregative adherence) and the adherence index expressed as the ratio between of the number of adhered bacteria cells and 100 eukaryotic cells counted on the microscopic field.

Expression of solubl enzymatic factors

To evaluate the effect of EEP on the expression of soluble enzymatic factors involved in the virulence of tested microbial strains, eight enzymatic assays were carried out: production of hemolysins, pore-forming toxins (lecithinase, lipase), proteases (caseinase, gelatinase), DN-ase, amylase and esculinase. For this purpose, bacterial strains cultures of 24 hours, grown on nutrient medium (TSB) in the presence of subinhibitory concentrations of EEP were spotted (10 µl) on media containing specific substrates necessary to emphasize the production of different soluble virulence factors. The expression of virulence factors was compared with that of the positive control (bacterial strain cultivated in the absence of propolis extract).

For the detection of hemolysins production the bacterial strains were spotted on 5% sheep blood agar medium. The inoculated plates were incubated at 37°C for 24 hours and then the appearance of a clear zone around colonies indicated a complete hemolysis for β-hemolytic strains and the appearance of a green or pink zone (partial hemolysis) characteristic for α-hemolytic strains. Lipase production was detected after spotting the bacterial strains on 1% Tween 80 (monooleate of sorbitol) agar medium and incubation at 37 ° C for 48 hours. The appearance of opaque areas (precipitation) in the surrounding of growth area was considered positive reaction. Precipitation opaque zone is given by the formation of insoluble crystals of calcium oleate. The study of lecithinases expression was performed by spotting the bacterial strains on 2.5% yolk agar medium and incubation for 24 hours at 37°C. Lecithinase production was indicated by a clear zone around the culture spot. The production of proteases

(enzymes which hydrolyze extracellular proteins to peptides and amino acids) has been investigated using 15% casein agar medium and respectively 3% gelatin agar medium. The strains were inoculated on medium and incubated at 37°C for 24 hours. An opaque (precipitation) zone surrounding the growth area was registered as positive reaction for casein activity and a transparent area around the colonies indicated gelatinase production. In order to study the production of DN-ase, bacterial strains were spotted on DNA supplemented agar with blue toluidin and incubated at 37 ° C for 24 hours. After incubation, the presence of a pink zone around the spot area showed DN-ase activity. Detection of amylase activity was assessed by spotting the strains on agar medium supplemented with 1% starch. After incubation at 37 ° C for 24 hours, a clear zone surrounding the growth area indicated presence of amylase. Hydrolysis is revealed by flooding the plate with Lugol solution (appearance of a yellow ring around the spot of culture, while the medium is blue). Esculinase production was determined on esculin and iron citrate medium. Esculinase (β-galactosidase) hydrolyzes esculin to esculetol and glucose. The strains were spotted on medium and after incubation at 37 ° C for 24 hours, esculetol released under the action of esculinase is combined with salts of iron in the medium (1% ammonium ferric citrate) and esculetin form an iron esculetin black precipitate (medium colored in black).

3. Results and discussions

Determination of the content of flavones and flavonols

As a result of the beneficial biological effects of phenolic compounds and more and more frequent use of propolis for therapeutic purposes, numerous studies were undertaken to determine the content of bioactive compounds. The results of these studies showed a high variability in the concentration of phenolic compounds of propolis correlated with its botanical origin. During this study, the determination of phenolic compounds was achieved by rapid spectrophotometric methods, which have the advantage of being simple, but of acceptable accuracy and good repeatability. These methods quantify the total active compounds, providing more information than measuring the concentration of individual compounds. Based on the calibration curve of quercetin there were calculated the concentrations of flavones and flavonols (mg/g) contained by EEP which were reported as a percentage from the weight of propolis. Flavones and flavonols content values for analyzed samples ranged from 1.94% (Cluj) and 6.07% (Calarasi), with an average of 3.73%. The average content of flavones and flavonols was superior to that obtained by Kosalec et al [21], i.e. 2.2%, respectively 0.14–0.41% in Croatian propolis tinctures (Kosalec et. al. [22]), Woisky and Salatino [23]: 0.77-2.69 % and Silva et al. [24]: 0.15-0.65% for Brazilian propolis, Chang et al. [25]: 6% for Chinese propolis and 3% for Taiwanese propolis. Our results are similar to those of Popova [19] and Bankova [1] who reported an average of flavones and flavonols content of 8 ± 4% for European propolis.

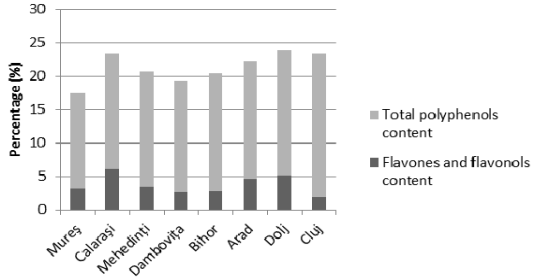

Fig. 1. Flavones and flavonols content and polyphenols content of tested Romanian EEP

Determination of total polyphenols

The calibration curve of gallic acid used as a reference standard allowed calculation of the concentration of the polyphenols from tested EEP. The total polyphenol content was expressed as a percentage from the weight of propolis. The minimum value of total polyphenol content was recorded for EEP Mureş, i.e. 14.34%, and the maximum value of 21.54% was obtained for the sample of Cluj. For EEP Arad and Bihor, total polyphenol content values were identical: 17.62%, and for EEP Calarasi and EEP Mehedinti these values were almost similar: 17.39% and 17.36%. Bankova investigated a large number of propolis sample from Europa and found an average for total phenolics of 28 ± 9% (Bankova [1]), but the results cannot be compared, due to the use of a different standard.

Influence of EEP on the development of microbial biofilm on inert substratum.

Bacterial biofilm consists of bacterial cells adhering closely to substratum and surrounded by a polysaccharide molecular matrix. Biofilm development occurs both on living tissues and inert surfaces of implants and medical devices, causing serious medical problems as urogenital tract, gastrointestinal tract, respiratory tract, eyes infections. Also, it is believed that about 60% of nosocomial infections are caused by microbial biofilms (Ilynia et al. [26]). An essential step in the pathogenesis of various infections associated with implants and medical devices is the ability of *S. aureus* to adhere to the surface of biomaterials through different mechanisms of adhesion. The use of natural products could be a solution for blocking of microbial biofilm formation early stage on prosthetic devices. Intercellular communication within the biofilms is achieved through a system called quorum sensing and response, mediated by small signaling molecules called autoinducers (bacterial pheromones). An ecological strategy to fight recurrent infections to antibiotic treatment and host defense mechanisms is the inhibition of signaling by QS inhibitors secreted by other organisms: microbial, vegetal and animal (Lazar [27]). Many studies have been carried out to determine the inhibitory potential of natural products on quorum sensing mechanisms, but the propolis ability to inhibit microbial biofilm formation has been less investigated. The results of research conducted by Koo et al. [28], Duarte et al. [29] on oral microorganisms have shown the ability of propolis to inhibit the bacterial glucosyltransferases (GTFs) activity involved in the formation of pathogenic dental plaque. Also, Kouidhi et. al demonstrated that Tunisian propolis had a strong antibiofilm activity, inhibiting biofilm formation of oral streptococci (Kouidhi et al. [30]). Bulman et al. [31] found that certain compounds from propolis were responsible for the inhibition of acyl homoserin lactones (AHL) QS signaling systems. In the study conducted in 2013, Stan et al. [32] have demonstrated that the Romanian EEP had an inhibitory effect only on Gram-positive strains (*S. aureus* ATCC 13024) biofilm developed on inert substratum. The results indicated a positive correlation between the concentration of propolis tincture and the inhibition of bacterial cell adhesion. However, in another study, Helaly et al. [33] have reported that EEP not only inhibits the biofilm formation of *S. aureus* but also those developed by *P. aeruginosa*, alone or in combination with other antimicrobial agents (dexapentanol). Regarding the influence of ethanol extracts of propolis on the microbial biofilm development, our results indicated that the tested products have inhibited the adhesion to the inert substratum of all studied microbial strains at concentrations ranging from 0.2343 mg / ml to 15 mg / ml (fig. 2).

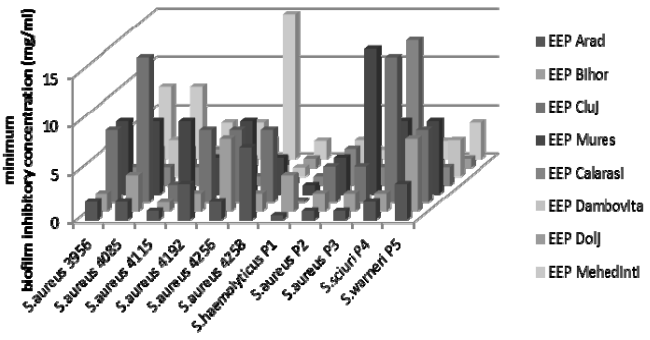

Fig. 2 Distribution of MBEC (minimum biofilm eradication concentration) recorded for propolis extracts from different regions of Romania on tested bacterial strains

Evaluation of EEP influence on microbial adherence to eukaryotic cells

The bacterial adherence to the eukaryotic cells was also influenced by the studied EEPs.

It was noted that many of the tested strains showed a mixed localized-aggregative adherence pattern. Optical microscopy images reveal the inhibitory effect of EEP on the adherence of *Staphylococcus haemolyticus* P1 strain to eukaryotic cells, as well as changing adherence pattern (fig. 3) from localized aggregative to a diffuse one.

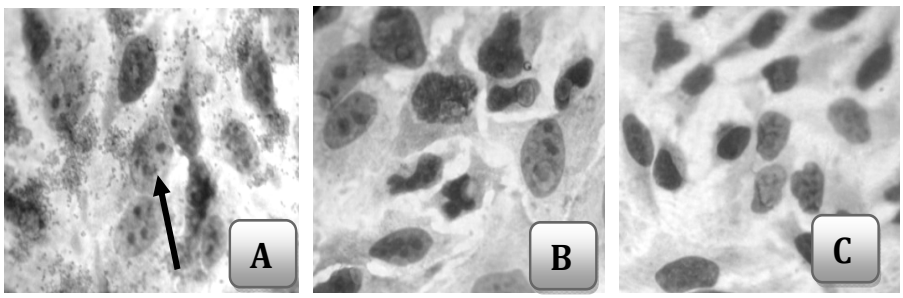

Fig. 3 A. HeLa cells infected with *Staphylococcus haemolyticus* P1 with localized-aggregative adherence pattern; **B.** reducing bacterial adherence to the HeLa cells in the presence of EEP; **C.** HeLa cells - control (Giemsa stain, × 2500).

Table 1. Inhibition of bacterial adherence to cellular substratum

Ethanolic extracts of propolis	% inhibited microbial strains		
	Adherence index < 20	**Adherence index 20 - 30**	**Adherence index > 30**
Arad	72,73	27,27	-
Bihor	36,37	63,63	-
Cluj	-	36,37	63,63
Mureș	-	45,45	54,55
Călărași	45,45	36,37	18,18
Dâmbovița	9,1	63,63	27,27
Dolj	81,81	18,19	-
Mehedinți	9,1	90,9	-

The average adherence index of *Staphylococcus* sp. strains to the HeLa cells ranged from 17.2 % (EEP Dolj) to 38.2 % (EEP Cluj). Three of the eight ethanolic extracts of propolis (EEP Arad, EEP Bihor and EEP Dolj) had a significant inhibitory activity on tested microbial strains adherence to eukaryotic cells. The intensity of the EEP inhibitory effect was quantified depending on the reduction percentage of the adherence index of *Staphylococcus* strains (A.I. below 20, A.I. : 20 - 30, A.I. under 30) (table 1). The lower capacity of tested bacterial strains to adhere to eukaryotic cells in presence of EEP revealed the ability of these extracts to inhibited adhesins involved in the attachment to host cells which mediates the initiation of the infectious process.

Evaluation of the expression of virulence factors of microbial strains tested in the presence of ethanolic extracts of propolis

An important target of infections therapy is to reduce microbial virulence factors. It has been shown that EEP could inhibit the expression of coagulase and lipase in S*taphylococcus sp*. (Scazzocchio et al. [34]). In the present study, all tested EEP had various inhibitory effects on the soluble virulence factors production, depending on the tested bacterial strain. Eight soluble enzymatic virulence factors were tested: hemolysins, lipase, lecithinase, amylase, gelatinase, caseinase, DN-ase and esculinase. None of tested *Staphylococcus* strains expressed gelatinase and amylase. DN-ase was produced by only one bacterial strain (*Staphylococcus haemolyticus* P1).

Table 2. Effect of Romanian EEP on the soluble virulence factors expression of bacterial strains: *S.aureus* 3956 (1), *S.aureus* 4085 (2), *S.aureus* 4115 (3), *S.aureus* 4192 (4), *S.aureus* 4258 (5), *S. haemolyticus* P1(6), *S.aureus* P2 (7), *S.sciuri* P4 (8), *S.warneri* P5 (9).

Bacterial strains	EEP Arad	EEP Bihor	EEP Cluj	EEP Mures	EEP Calarasi	EEP Dambovita	EEP Dolj	EEP Mehedinti
	Lecithinase							
1	-	-	-	-	-	-	-	+
2	+	-	+	-	-	-	-	-
3	-	-	+	-	-	-	-	-
4	-	-	+	-	-	-	-	-
5	-	-	+	-	-	-	-	-
8	+	+	-	-	-	-	-	-
	Lipase							
1	-	-	-	-	-	-	-	+
2	+	-	+	-	-	-	-	-
3	-	-	+	-	-	-	-	-
6	++	++	++	++	++	-	-	-
7	-	-	+	-	+	+	-	+
8	+	-	-	-	-	-	+	-
9	-	-	-	+	-	-	-	-
	Caseinase							
1	-	-	-	-	-	-	-	+
3	-	-	+	-	-	-	-	-
7	-	-	-	-	++	-	-	-
8	++	++	-	-	+	-	+	-
9	-	-	-	+	-	-	-	-
	DN-ase							
6	-	++	-	++	-	-	+	++
	Haemolysins							
1	-	-	-	-	-	-	-	+
2	-	-	+	+	-	-	-	-
3	-	-	+	-	-	-	-	-
4	-	-	+	-	-	-	-	-
5	-	-	+	-	-	-	-	-
9	-	+	-	-	-	-	-	-
	Esculinase							
9	-	+	-	-	-	+	-	-

Legend: „+ " partial inhibition, „++" complete inhibition

In table 2 there is presented the influence of tested EEP on the expression of soluble virulence factors. We observed that EEP Bihor inhibited partially or totally the expression of all soluble virulence factors. Also, EEP Mehedinti and Cluj were efficient, decreasing the virulence factors production. In the case of *S. aureus* P3 strain production of four virulence factors (lecithinase, lipase, caseinase and hemolysin) was not inhibited by any of the eight tested propolis extracts. Our results indicated that some of EEP repressed the expression of soluble virulence factors implicated in bacterial dissemination from invasive infections: lipase determines pore formation in host membrane cell, caseinase contributes to cell damage and invasion and DN-ase is responsible for lesions in host cell and bacteria spreading.

Conclusions

Our results showed that all studied propolis ethanolic extracts affected the ability of various microbial strains to form biofilms on inert substrates, with MBEC values ranging from 0.2343 mg / ml to 15 mg / ml. The antimicrobial activity of the tested propolis extracts varied depending on the geographical area and the chemical composition of propolis. The propolis ethanolic extracts with an increased polyphenols content increased (EEP Arad - 17.62% and EEP Cluj - 21.54%) showed an enhanced inhibitory activity on the microbial biofilms development with the lowest MBEC values (0.2343 mg / ml). Ethanolic extracts of propolis inhibited completly or partially the secretion of soluble enzymatic factors involved in the virulence of the tested *Staphylococcus* strains, except for one *S. aureus* strain. The most frequent inhibited soluble virulence factors were: lipases, caseinases and DN-ases. Also, there was a change in the adherence to the cellular substratum index and pattern of tested bacterial strains in the presence of EEP. In this respect it was found that three of the eight propolis extracts (EEP Arad, EEP Bihor and EEP Dolj) were the most effective with a strong inhibitory effect on *Staphylococcus* sp. strains adherence to the cellular substrate. The other extracts of propolis have induced a decreased adherence index. The Romanian propolis extracts, by inhibiting the analyzed microbial strains adherence capacity to eukaryotic cells and decreasing the production of soluble virulence factors, may be used in the development of effective strategies to prevent colonization and infectious process initiation and/or progress. The propolis extracts can be particularly used in the treatment of opportunistic infections caused by antibioresistant bacterial strains with the ability to develop microbial biofilms.

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