Journal of Agricultural Science and Technology B 10 (2020) 350-364
doi: 10.17265/2161-6264/2020.06.004

# The Polyphenol Content and Antimicrobial Activity of Selected Propolis' Extracts

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**Abstract:** Propolis is a resinous natural product, produced by bees (*Apis mellifera*), from vegetable parts and plant secretions. Propolis' samples A, B, C and D were extracted with phosphate buffer saline (PBS) or with 70% EtOH at pH values 8.0, 7.2 and 6.4 followed by: (1) reverse-phase high-performance liquid chromatography (RP-HPLC) on Purospher® Star RP-18 column, the quantity of caffeic acid, chrysin, pinocembrin and galangin was determined; (2) determination of total flavonoids in both extracts; (3) antimicrobial tests of both extracts against (a) Gram-positive bacteria: methicillin-resistant *Staphylococcus aureus* (MRSA), *St. aureus*, *Streptococcus pyogenes*, *Str. agalactiae*, (b) Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Acinetobacter baumanii* and (c) yeast: *Candida albicans*. The antimicrobial activity of propolis' PBS extracts against Gram-positive bacteria shows the lowest minimal inhibitory concentration (MIC, mg/mL) at pH 8.0 in sample C, followed by A, B and D. In sample C, MICs at pH 8.0 were 0.007 (*Str. agalactiae*), 0.015 (MRSA), 0.015 (*Str. pyogenes*) and 0.007 (*St. aureus*). The polyphenol content of sample C is: flavonoid content 5.47 ± 0.62 mg/mL, caffeic acid 1.33 ± 0.92 mg/mL, chrysin 41.02 ± 4.22 μg/mL, pinocembrin 2.93 ± 0.33 mg/mL and galangin 41.87 ± 4.23 mg/mL. PBS extracts against Gram-negative bacteria show the lowest MIC (mg/mL) at pH 8.0 in sample D, followed by B, C and A. In sample D, MICs at pH 8.0 were 0.003 (*Acin. baumanii*, *Pr. mirabilis*, *Ps. aeruginosa*) and 0.007 (*E. coli*). Polyphenol content of sample D is: flavonoids 8.28 ± 0.92 mg/mL, caffeic acid 3.56 ± 0.32 mg/mL, chrysin 677.42 ± 68.42 μg/mL, pinocembrin 146.49 ± 13.89 mg/mL and galangin 59.81 ± 5.86 mg/mL. The strongest anti *C. albicans* activity, with the lowest MIC (mg/mL), at pH 8.0 was in the sample C, followed by samples D, A and B. In sample C, the MIC at pH 8.0 is 0.001 (PBS extract). The antimicrobial activities of selected propolis samples correlate with their polyphenol content, more precisely, flavonoid, caffeic acid, chrysin, pinocembrin and galangin content.

**Key words:** Propolis, pH, phosphate buffer saline extract, 70% EtOH extract, antimicrobial activity.

## 1. Introduction

"Propolis", is the generic name for a natural complex mixture of resinous substances, sometimes referred as bee glue, collected from plants by bees, being used in the bee hive to coat the inner walls, to protect the entrance against intruders, and to inhibit the growth of fungi and bacteria [1]. For propolis production, bees add their salivary enzymes to the plant resin and this material is then partially digested, followed by the addition of waxes, found in most bee species. It is usually composed of 45% resins, 30% waxes and fatty acids, 10% essential oils, 5% pollen, and 10% organic compounds and minerals [2, 3]. There are more than 300 compounds, among them are flavonoids, glycans, phenolic acids and their esters, phenolic aldehydes, alcohols, ketones, terpenes, steroids, sugars and amino acids, all in the raw propolis. The proportion of each compound significantly differs because of botanical and geographical factors, and the collection season [4, 5].

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*Paper is devoted to the remembrance of Dr. Klemen Rihar who passed away in December 2018.

The compounds found in propolis are responsible for most of its biological activities, like antimicrobial and antioxidant one. Because of a variety of propolis activities, it became a big interest of pharmaceutical industry and health-food stores, where it is used in foods, beverages, cosmetics and medicine to make better a general health and prevent spread of different diseases [6, 7]. One of the most important components of raw propolis is flavonoid. It can represent around 50% of the propolis contents, depending on the region where it is collected. Its characteristics are influenced by botanical, geographical and climate factors. The propolis' properties were investigated regarding its antibacterial, antiviral and antifungal activities. Due to its antimicrobial activity, propolis ethanol extract is more active against Gram-positive bacteria than Gram-negative ones [8, 9]. The usual way of propolis preparation is powdering the resin, followed by extraction in an alcoholic or aqueous medium.

The pH variation could have a positive or a negative effect on extraction, depending on the interaction of the polyphenols with other constituents of each plant. The quantification of the extracted propolis components shows that only these with a more neutral pH (7.0 and 8.0) show an increase in the concentration of the main functional component. This behavior was the same for phenolic compounds. Samples with different pH values were tested to water as a solvent, and compared with samples without pH variation. Analyses of antimicrobial activities of propolis extract are the strongest at pH = 8.0 [10].

The experiments presented herein were aimed to make the propolis extracts with phosphate buffer saline (PBS) at pH values 8.0, 7.2 and 6.4 instead of water, and to test their antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and yeast *Candida albicans*. In comparison, the propolis extraction was made with 70% EtOH at pH values 8.0, 7.2, 6.4, and compared with the PBS extract. Additionally, the analyses of the polyphenol content of selected propolis samples were made.

## 2. Materials and Methods

### *2.1 Used Species of Microorganisms*

Different clinical isolates of Gram-positive bacteria: methicillin-resistant *Staphylococcus aureus* (MRSA), *St. aureus*, *Streptococcus pyogenes* and *Str. agalactiae*, were used in the experiments. The Gram-negative clinical isolates were: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Acinetobacter baumanii*. Clinical isolate of the yeast was *C. albicans*. All clinical isolates [11] used in the experiments were obtained from "Microbe collection" of the Institute for Microbiology and Immunology, Medical Faculty in Ljubljana, Slovenia. All microbes were cultivated at first on Mueller-Hinton agar at 37 °C for 48 h and afterward, transferred to Mueller-Hinton broth, until the concentration of 0.5 McFarland was obtained.

### *2.2 Propolis Samples*

The origin of propolis samples was from different Slovenian beekeepers and from Veterinary Faculty in Ljubljana, Slovenia. The sample A was from beekeeper Hunjadi, sample B was from beekeeper Pušnik (Pušnik's sample B), sample C was from beekeeper Pušnik (Pušnik's sample A) and sample D was from Veterinary Faculty, University of Ljubljana, Slovenia, a propolis sample "Ljubljana Center". All propolis samples were stored at -20 °C until use.

### *2.3 Propolis Extracts*

2.3.1 PBS Extracts of Propolis at pH 8.0

(1) Propolis sample of 10 g was frozen at -30 °C for 3 d; (2) Defrozen sample was grounded in a mortar and resuspended in 100 mL PBS with pH 8.0; (3) 10% suspension was shaken at 37 °C for 5 d; (4) 10% suspension was grounded in mortar and shaken at 37 °C for additional 3 d; (5) 10% suspension was put into 50 mL tubes and exposed in microwave oven at 300 MW for 2 min [12, 13]; (6) 10% suspension of propolis

was filtered through Whatman Filter Paper No. 1 and centrfuged at 2,000 rpm for 20 min at 4 °C; (7) The supernatant was sterilised by filtration through 0.2 μm syringe filters and stored at -20 °C until use.

2.3.2 PBS Extract of Propolis at pH 7.2

Sample was prepared by the same way as in section 2.3.1 with the exception that PBS with pH 7.2 was used.

2.3.3 PBS Extract of Propolis at pH 6.4

Sample was prepared by the same way as in section 2.3.1 with the exception that PBS with pH 6.4 was used.

2.3.4 Propolis Extracts by 70% EtOH at pH 8.0

(1) The raw propolis was cleaned of wax, paint, wood, crushed and cut into small pieces, and put in the freezer at -30 °C for 3 d; (2) Defrozen propolis samples were grounded in a mortar; (3) 28 g of grounded propolis sample was mixed with 100 mL mixture of 70% ethanol:PBS pH 8.0 (at ratio 75:25); (4) The propolis suspension was shaken at 37 °C for 5 d; (5) The exact at pH 8.0 was adjusted by adding 1 N NaOH and controlled with the pH meter; (6) The propolis suspension was put into 50 mL tubes and exposed in microwave oven at 300 MW/3 × 2 min; (7) The propolis suspension was filtered through Whatman Filter Paper No. 1 and centrfuged at 2,000 rpm for 20 min at 4 °C; (8) The supernatant, propolis extract of pH 8.0 was filtered through 0.2 μm syringe filters and stored at -20 °C until use.

2.3.5 Propolis Extracts by 70% EtOH at pH 7.2

The propolis sample was prepared by the same way as in section 2.3.4, with the exception that the PBS of pH 7.2 was used in the mixture 70% EtOH:PBS pH 7.2 (75:25). The exact pH 7.2 of the propolis suspension was adjusted by adding 1 N NaOH.

2.3.6 Propolis Extracts by 70% EtOH at pH 6.4

The propolis sample was prepared by the same way as in section 2.3.4, with the exception that the PBS of pH 6.4 was used in the mixture of 70% EtOH:PBS pH 6.4 (75:25). The exact pH 6.4 of the propolis suspension was prepared by adding 1 N HCl.

*2.4 Methods*

2.4.1 Amount of Caffeic Acid, Chrysin, Pinocembrin and Galangin in the Propolis Samples

The amounts of the caffeic acid, chrysin, pinocembrin and galangin in propolis samples were determined by the method of Urushisaki *et al.* [14] as follows: 1.0 mg of caffeic acid, chrysin, pinocembrin and galangin were put and diluted to 10.0 mL with methanol. From this solution, 150 μL of the sample was transferred into a vial and loaded with 1.350 μL of methanol. Samples were filtered through a 0.45 μm filter and 20 μL was injected into the reverse-phase high-performance liquid chromatography (RP-HPLC) column Purospher® Star RP-18 end-capped (5 μm). Their separation was achieved with Acetonitrile gradient in RP-HPLC column. The separation was measured at 290 nm, with Acetonitrile gradient. The quantity of total flavonoids, caffeic acid, chrysin, pinocembrin and galangin in the experimental samples of propolis were calculated in comparison to standards.

2.4.2 Colorimetric Estimation of Total Flavonoid Content by Folin-Ciocalteu (FC)

The FC was performed by the method of Blainski *et al.* [15] as follows: the phenol compounds react with an FC reagent in a basic medium and colored product is formed. The absorbance is measured at 750 nm. Results are expressed as 1 g of caffeic acid per 100 g of the sample (g/100 g). Procedure goes like: pipette 200 μL of sample or standard and add 1.0 mL of FC reagent and 1.0 mL of 10% $Na_2CO_3$. Mix well, and after 1 h, measure the absorbance at 750 nm against water. Each sample and standard should be analyzed in duplicate. The results are expressed as caffeic acid in mg/mL.

2.4.3 Determination of the Minimal Inhibitory Concentration (MIC) in mg/mL

The MIC value determinations were performed according to "Resazurin method" described by Filipič *et al.* [16] as follows. The 96-well "U" profile microtitre plates (8 × 12 wells) were used. Into the 2nd

column (eight wells) until the 12th column, the 50 µL of saline was put. Into the 1st column (eight wells), 100 µL of samples was put as follows: in the 1st plate samples, in wells 1-4 and into wells 5-8 in the 1st column; in the 2nd plate samples, in wells 9-11 + untreated control in the 1st column; in the 3rd plate, Penicillin, Streptomycin, Gentamycin and Nystatin were put into wells in duplicated in the 1st column. Then, 50 µL was transferred from the 1st column into the next and until 11th, where 50 µL was discharged. Into each plate on each well, 50 µL of test microorganisms in the concentration of 0.5 McFarland was added, and the plate was wrapped into aluminum foil and incubated for 48 h at 37 °C. After the incubation is finished, 20 µL of resazurin (Sigma-Aldrich; 0.0028 g of resazurin dissolved in 10 mL of distilled water, to which 90 mL of Eagle's minimal essential medium (EMEM) was added) was added into each well in the micro titer plate. The plates were then wrapped into aluminums foil and incubated at room temperature for additional 4 h. The absence of microbe's growth inhibition results in the change of the color of resazurin from blue to pink. MIC was determined as the highest dilution, resulting in no or the minimal change in color. For example, in the 7th well, there is a change from pink to blue. So, color changes in the 7th well with dilution 1:64, MIC = 1 × 10/64 = 0.156 mg/mL.

2.4.4 Statistical Analyses

The statistical significance was calculated by the two-tailed Student's *t*-test, in order to compare the difference in efficiency between PBS extracts and 70% EtOH extract. The MICs for different propolis samples were pooled.

2.4.5 Scheme of the Experiments

During the experiments, shown in Fig. 1, the propolis samples A, B, C and D were extracted with PBS or with 70% EtOH at pH values 8.0, 7.2 and 6.4, followed by (1) RP-HPLC on Purospher® Star RP-18 end-capped column, the quantity of caffeic acid, chrysin, pinocembrin and galangin was determined; (2) determination of total flavonoids in the both extracts: PBS or 70% EtOH; (3) antimicrobial activity of all extracts (PBS or 70% EtOH) against (a) Gram-positive bacteria: MRSA, *St. aureus*, *Str. pyogenes* and *Str. agalactiae*; (b) Gram-negative bacteria: *E. coli*, *Ps. aeruginosa*, *Pr. mirabilis* and *Acin. baumanii*; (c) yeast: *C. albicans*.

## 3. Results

### *3.1 The Polyphenol Content of Different Propolis' Samples at pH Values 8.0, 7.2 and 6.4*

During the experiments, the polyphenol contents of different propolis' samples were analysed. The results are summarized in Table 1.

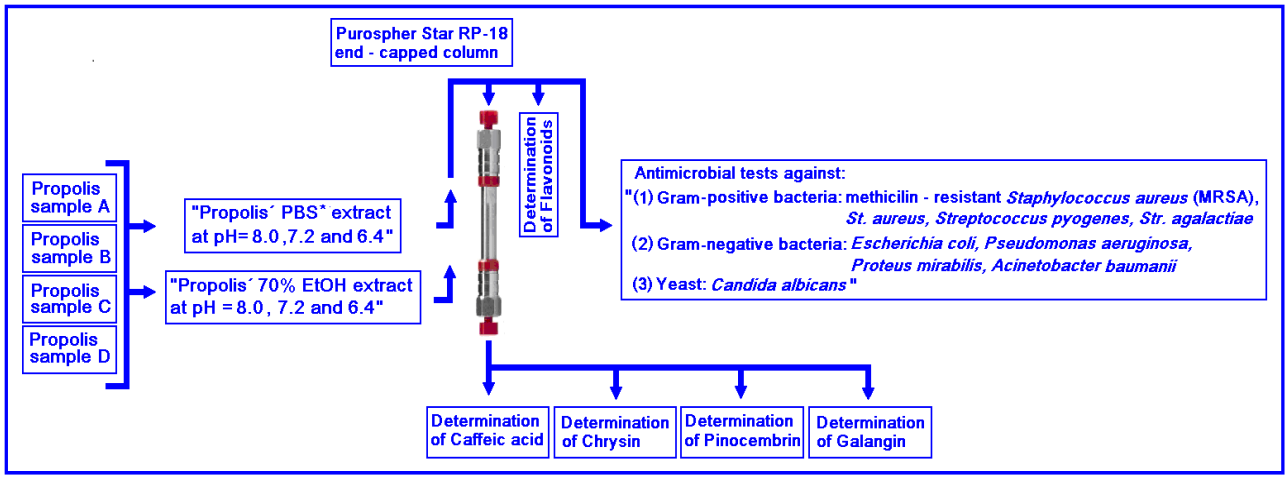


**Fig. 1 Scheme of the experiments.**
PBS* = phosphate buffer saline.

**Table 1 The polyphenol content of phosphate buffer saline (PBS) or 70% EtOH extracts of propolis' samples A to D at pH values 8.0, 7.2 and 6.4.**

| Propolis' sample | Flavonoid content (mg/mL) | | Caffeic acid (mg/mL) | | Chrysin (μg/mL) | | Pinocembrin (mg/mL) | | Galangin (mg/mL) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PBS-extract | 70% EtOH extract | PBS-extract | 70% EtOH extract | PBS-extract | 70% EtOH extract | PBS-extract | 70% EtOH extract | PBS-extract | 70% EtOH extract |
| Sample A pH = 8.0 | 1.11 ± 0.12 | 9.58 ± 0.89 | 0.36 ± 0.04 | 2.89 ± 0.29 | 71.55 ± 6.96 | 572.44 ± 51.44 | 15.95 ± 1.62 | 95.71 ± 8.98 | 17.91 ± 1.81 | 119.63 ± 10.98 |
| Sample A pH = 7.2 | 1.73 ± 0.15 | 13.09 ± 1.24 | 0.61 ± 0.05 | 4.89 ± 0.48 | 101.61 ± 10.11 | 812.91 ± 82.22 | 14.64 ± 1.51 | 87.89 ± 8.88 | 9.71 ± 0.98 | 648.02 ± 63.97 |
| Sample A pH = 6.4 | 1.40 ± 0.13 | 11.26 ± 1.10 | 0.50 ± 0.04 | 4.00 ± 0.45 | 65.92 ± 6.61 | 527.42 ± 50.72 | 17.90 ± 1.86 | 107.43 ± 11.44 | 12.81 ± 1.29 | 89.72 ± 8.86 |
| Sample B (1) pH = 8.0 | 1.57 ± 0.14 | 12.57 ± 1.34 | 0.44 ± 0.06 | 3.56 ± 0.42 | 236.34 ± 21.46 | 1,890.73 ± 180.2 | 68.48 ± 5.97 | 410.88 ± 40.88 | 11.39 ± 1.14 | 79.75 ± 7.86 |
| Sample B (1) pH = 7.2 | 1.41 ± 0.13 | 11.34 ± 1.14 | 0.61 ± 0.05 | 4.897 ± 0.52 | 204.86 ± 19.34 | 1,638.93 ± 160.8 | 58.59 ± 5.66 | 351.58 ± 34.21 | 12.81 ± 1.31 | 89.72 ± 8.82 |
| Sample B (1) pH = 6.4 | 1.61 ± 0.17 | 12.90 ± 1.35 | 1.39 ± 0.14 | 11.129 ± 1.22 | 329.30 ± 31.44 | 2,634.43 ± 258.33 | 61.91 ± 6.21 | 371.48 ± 36.02 | 11.39 ± 1.14 | 79.75 ± 7.99 |
| Sample C (2) pH = 8.0 | 0.68 ± 0.07 | 5.47 ± 0.62 | 0.16 ± 0.009 | 1.33 ± 0.92 | 5.12 ± 0.49 | 41.02 ± 4.22 | 5.48 ± 0.49 | 32.93 ± 3.21 | 5.98 ± 0.61 | 41.87 ± 4.23 |
| Sample C (2) pH = 7.2 | 1.34 ± 0.14 | 10.75 ± 1.26 | 0.13 ± 0.01 | 1.11 ± 0.95 | 36.62 ± 3.59 | 293.014 ± 29.13 | 12.37 ± 1.33 | 74.22 ± 7.51 | 9.96 ± 0.98 | 69.78 ± 6.67 |
| Sample C (2) pH = 6.4 | 1.43 ± 0.16 | 11.38 ± 1.28 | 0.70 ± 0.06 | 5.64 ± 0.52 | 52.22 ± 5.14 | 417.78 ± 42.22 | 16.92 ± 1.72 | 101.57 ± 11.05 | 12.53 ± 1.24 | 87.72 ± 8.92 |
| Sample D pH = 8.0 | 1.03 ± 0.11 | 8.28 ± 0.92 | 0.44 ± 0.03 | 3.56 ± 0.32 | 84.67 ± 8.39 | 677.42 ± 68.42 | 24.41 ± 2.39 | 146.49 ± 13.89 | 8.54 ± 0.86 | 59.81 ± 5.86 |
| Sample D pH = 7.2 | 1.36 ± 0.14 | 10.94 ± 1.11 | 0.26 ± 0.03 | 2.137 ± 0.26 | 178.76 ± 1.69 | 1,430.12 ± 143.1 | 48.18 ± 4.17 | 289.08 ± 28.92 | 4.27 ± 0.39 | 29.90 ± 2.89 |
| Sample D pH = 6.4 | 1.70 ± 0.15 | 13.61 ± 1.42 | 0.26 ± 0.03 | 2.137 ± 0.22 | 240.86 ± 23.88 | 1,926.89 ± 191.8 | 49.48 ± 4.88 | 296.89 ± 28.88 | 9.95 ± 0.95 | 69.78 ± 6.86 |

(1) = Beekeper Pušnik's sample B; (2) = Beekeper Pušnik's sample A.

*3.2 The Antimicrobial Activity of Propolis' PBS Extracts at pH Values 8.0, 7.2 and 6.4 against Gram-Positive Bacteria*

The strongest antimicrobial activity, the lowest MIC (mg/mL), at pH 8.0 was found in sample C, followed by samples A, B and D (Table 2, Fig. 2). In sample C, the MICs at pH 8.0 were: 0.007 (*Str. agalactiae*), 0.015 (MRSA), 0.015 (*Str. pyogenes*) and 0.007 (*St. aureus*). It is interesting that the "Ratio to Gentamycin" is 1.75 (*Str. agalactiae* and *St. aureus*), 3.75 (MRSA and *Str. pyogenes*), meaning that the MIC values are higher, with weaker values than for Gentamycin (0.004 mg/mL). The ratios of MIC at pH values 6.4 to 8.0 were 8.85 (*Str. agalactiae*), 4.13 (MRSA and *Str. pyogenes*) and 2.14 for *St. aureus*.

In sample A, the MICs at pH 8.0 were 0.001 (*Str. agalactiae*), 0.007 (MRSA), 0.031 (*Str. pyogenes*) and 0.015 (*St. aureus*). The "Ratio to Gentamycin" was 0.25 (*Str. agalactiae*), 1.75 (MRSA), 7.75 (*Str. pyogenes*) and 3.75 for *St. aureus*. The strongest was "Ratio to Gentamycin", 0.25 with the MIC 0.001. The ratios of MIC at pH values 6.4 to 8.0 were 15.00 (*Str. agalactiae*), 4.42 (MRSA), 4.03 (*Str. pyogenes*) and 16.61 for *St. aureus*.

In sample B, the MICs at pH 8.0 were 0.007 (*Str. agalactiae*), 0.007 (MRSA), 0.031 (*Str. pyogenes*) and 0.125 (*St. aureus*). The "Ratio to Gentamycin" was 1.75 (*Str. agalactiae* and MRSA), 7.75 (*Str. pyogenes*) and 31.25 for *St. aureus*. The strongest was 1.75 for *Str. agalactiae* and MRSA. The ratios of MIC at pH values 6.4 to 8.0 were 4.42 (*Str. agalactiae*), 8.85 (MRSA), 4.03 (*Str. pyogenes*) and 4.00 for *St. aureus*.

In sample D, the MICs at pH 8.0 were 0.001 (*Str. agalactiae*), 0.007 (MRSA), 0.031 (*Str. pyogenes*) and 0.125 for *St. aureus*. The "Ratio to Gentamycin" was 0.25 (*Str. agalactiae*), 1.75 (MRSA), 7.75 (*Str. pyogene*s)

**Table 2 The antimicrobial activity of propolis' PBS extracts against Gram-positive bacteria at pH 8.0.**

| PBS extract | Sample A | | | Sample B | | | Sample C | | | Sample D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram-positive bacteria | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin |
| *Staphylococcus aureus* | 0.015 ± 0.0013 | 16.61 | 3.75 | 0.125 ± 0.013 | 4.00 | 31.25 | 0.007 ± 0.00069 | 2.14 | 1.75 | 0.125 ± 0.013 | 4.16 | 30.00 |
| *Streptococcus pyogenes* | 0.031 ± 0.0029 | 4.03 | 7.75 | 0.031 ± 0.0029 | 4.03 | 7.75 | 0.015 ± 0.0013 | 4.13 | 3.75 | 0.031 ± 0.0029 | 4.03 | 7.75 |
| Methicillin-resistant *St. aureus* (MRSA) | 0.007 ± 0.00069 | 4.42 | 1.75 | 0.007 ± 0.00069 | 8.85 | 1.75 | 0.015 ± 0.0013 | 4.13 | 3.75 | 0.007 ± 0.00069 | 4.42 | 1.75 |
| *Str. agalactiae* | 0.001 ± 0.0009 pH 8.0 | 15.00 | 0.25 | 0.007 ± 0.00069 pH 8.0 | 4.42 | 1.75 | 0.007 ± 0.00069 pH 8.0 | 8.85 | 1.75 | 0.001 ± 0.0009 pH 8.0 | 15.00 | 0.25 |

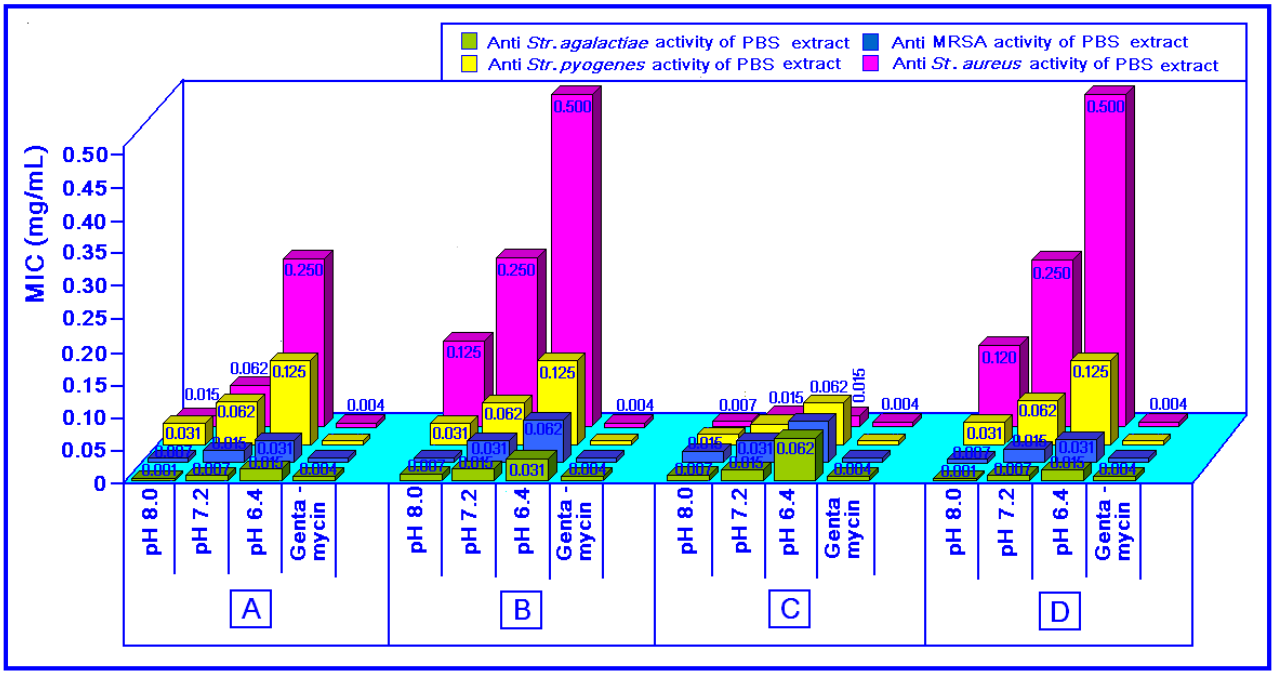


**Fig. 2 Anti Gram-positive bacteria activity of PBS extracts at pH values of 8.0, 7.2 and 6.4 with the Gentamycin as a control.**

and 30.00 for *St. aureus*. The strongest was "Ratio to Gentamycin", 0.25 with the MIC 0.001 for *Str. agalactiae*. The ratios of MIC at pH values 6.4 to 8.0 were 15.00 (*Str. agalactiae*), 4.42 (MRSA), 4.03 (*Str. pyogenes*) and 4.16 for *St. aureus*.

*3.3 The Antimicrobial Activity of Propolis' 70% EtOH Extracts at pH Values 8.0, 7.2 and 6.4 against Gram-Positive Bacteria*

The strongest antimicrobial activity, with the lowest MIC (mg/mL), at pH 8.0 was found in sample D, followed by C, A and B (Table 3, Fig. 3). In sample D, the MICs at pH 8.0 were 0.003 for *St. aureus*, MRSA, *Str. pyogenes* and *Str. agalactiae*. The "Ratio to Gentamycin" was 0.75 for *St. aureus*, MRSA, *Str. pyogenes* and *Str. agalactiae*. The ratios of MIC at pH values 6.4 to 8.0 were 5.00 (*St. aureus*, MRSA), 20.62 (*Str. pyogenes*) and 10.33 (*Str. agalactiae*).

In sample C, the MICs at pH 8.0 were 0.007 (*St. aureus*), 0.001 (MRSA), 0.015 (*Str. pyogenes*) and 0.007 (*Str. agalactiae*). The "Ratio to Gentamycin" was 1.75 (*St. aureus*), 0.25 (MRSA), 3.75 (*Str. pyogenes*) and 1.75 (*Str. agalactiae*). The ratios of MIC at pH values 6.4 to 8.0 were 4.42 (*St. aureus*), 15.00 (MRSA), 2.06 (*Str. pyogenes*) and 4.42 (*Str. pyogenes*).

**Table 3 The antimicrobial activity of Propolis' 70% EtOH extracts against Gram-positive bacteria at pH 8.0.**

| 70% EtOH extract | Sample A | | | Sample B | | | Sample C | | | Sample D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram-positive bacteria | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin |
| *Str. agalactiae* | 0.015 ± 0.0013 | 4.13 | 3.75 | 0.015 ± 0.0013 | 4.13 | 3.75 | 0.007 ± 0.00069 | 4.42 | 1.75 | 0.003 ± 0.00029 | 10.33 | 0.75 |
| *Str. pyogenes* | 0.015 ± 0.0013 | 4.13 | 3.75 | 0.015 ± 0.0013 | 4.13 | 3.75 | 0.015 ± 0.0013 | 2.06 | 3.75 | 0.003 ± 0.00029 | 20.62 | 0.75 |
| MRSA | 0.003 ± 0.00029 | 5.00 | 0.75 | 0.003 ± 0.00029 | 20.66 | 0.75 | 0.001 ± 0.0009 | 15.00 | 0.25 | 0.003 ± 0.00029 | 5.00 | 0.75 |
| *St. aureus* | 0.007 ± 0.00069 pH 8.0 | 2.14 | 1.75 | 0.007 ± 0.00069 pH 8.0 | 4.42 | 1.75 | 0.007 ± 0.00069 pH 8.0 | 4.42 | 1.75 | 0.003 ± 0.00029 pH 8.0 | 5.00 | 0.75 |

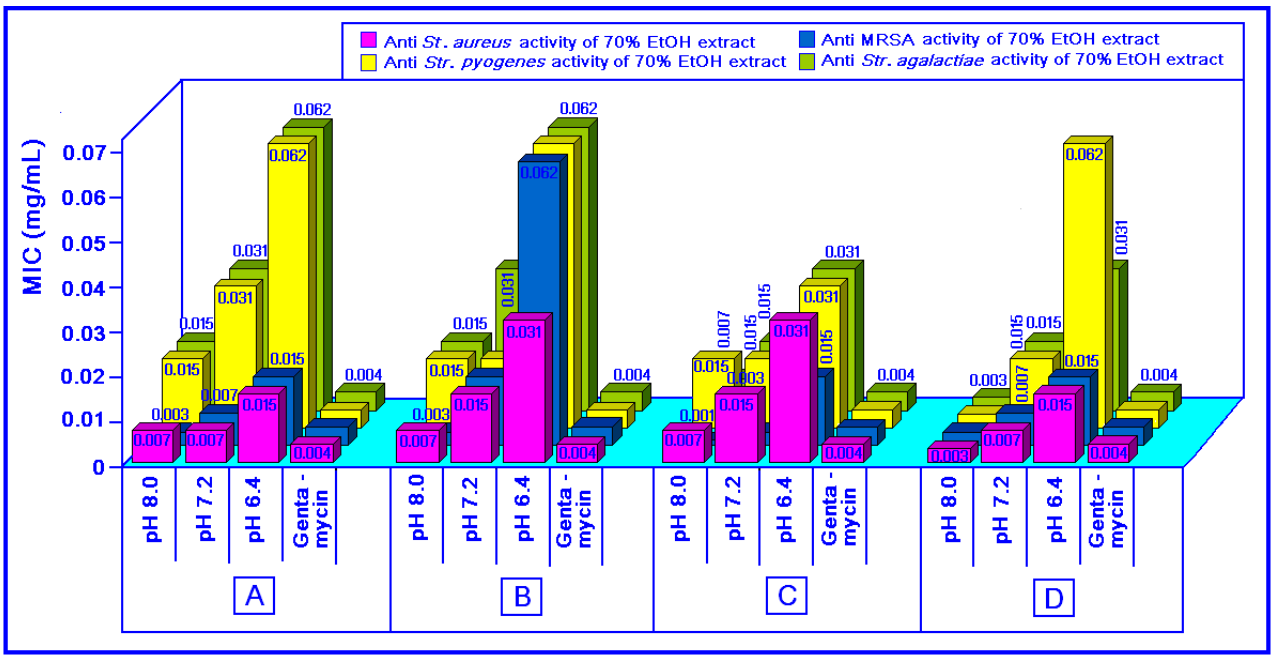


**Fig. 3 Anti Gram-positive bacteria activity of 70% EtOH extracts at pH values of 8.0, 7.2 and 6.4 with the Gentamycin as a control.**

In sample A, the MICs at pH 8.0 were 0.007 (*St. aureus*), 0.003 (MRSA) and 0.015 for *Str. pyogenes* and *Str. agalactiae*. The "Ratio to Gentamycin" was 1.75 for *St. aureus*, 0.75 for MRSA, 3.75 for *Str. pyogenes* and *Str. agalactiae*. The ratios of MIC at pH values 6.4 to 8.0 were 2.14 (*St. aureus*), 5.00 (MRSA), 4.13 for *Str. pyogenes* and *Str. agalactiae*.

In sample B, the MICs at pH 8.0 were 0.007 (*St. aureus*), 0.003 (MRSA), 0.015 (*Str. pyogenes*, *Str. agalactiae*). The "Ratio to Gentamycin" was 1.75 (*St. aureus*), 0.75 (MRSA) and 3.75 for *Str. pyogenes* and *Str. agalactiae*. The ratios of MIC at pH values 6.4 to 8.0 were 4.42 (*St. aureus*), 20.66 (MRSA) and 4.13 for *Str. pyogenes* and *Str. agalactiae*.

*3.4 Antimicrobial Activity of Propolis' PBS Extracts at pH Values 8.0, 7.2 and 6.4 against Gram-Negative Bacteria*

The strongest antimicrobial activity, the lowest MIC (mg/mL), at pH 8.0 was found in the sample D, followed by samples B, C and A (Table 4, Fig. 4). In sample D, the MICs at pH 8.0 were 0.003 (*Acin. baumanii*, *Pr. mirabilis*, *Ps. aeruginosa*) and 0.007 (*E. coli*). The "Ratio to Gentamycin" was 0.75 (*Acin. baumanii*, *Pr. mirabilis*,

**Table 4 The antimicrobial activity of propolis' PBS extracts against Gram-negative bacteria at pH 8.0.**

| PBS extract | Sample A | | | Sample B | | | Sample C | | | Sample D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram-negative bacteria | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin |
| *Escherichia coli* | 0.007 ± 0.00069 | 8.85 | 1.75 | 0.015 ± 0.0016 | 4.13 | 3.75 | 0.007 ± 0.00069 | 8.85 | 1.75 | 0.007 ± 0.00069 | 8.85 | 1.75 |
| *Pseudomonas aeruginosa* | 0.007 ± 0.00069 | 8.85 | 1.75 | 0.007 ± 0.00069 | 8.85 | 1.75 | 0.003 ± 0.00029 | 10.33 | 0.75 | 0.003 ± 0.00029 | 5.00 | 0.75 |
| *Proteus mirabilis* | 0.001 ± 0.0009 | 31.00 | 0.25 | 0.001 ± 0.0009 | 7.00 | 0.25 | 0.007 ± 0.00069 | 4.42 | 1.75 | 0.003 ± 0.00029 | 10.33 | 0.75 |
| *Acinetobacter baumanii* | 0.007 ± 0.00069 | 4.42 | 1.75 | 0.001 ± 0.0009 | 7.00 | 0.25 | 0.0004 ± 0.00003 | 2.51 | 0.11 | 0.003 ± 0.00029 | 5.00 | 0.75 |
| | pH 8.0 | | | pH 8.0 | | | pH 8.0 | | | pH 8.0 | | |

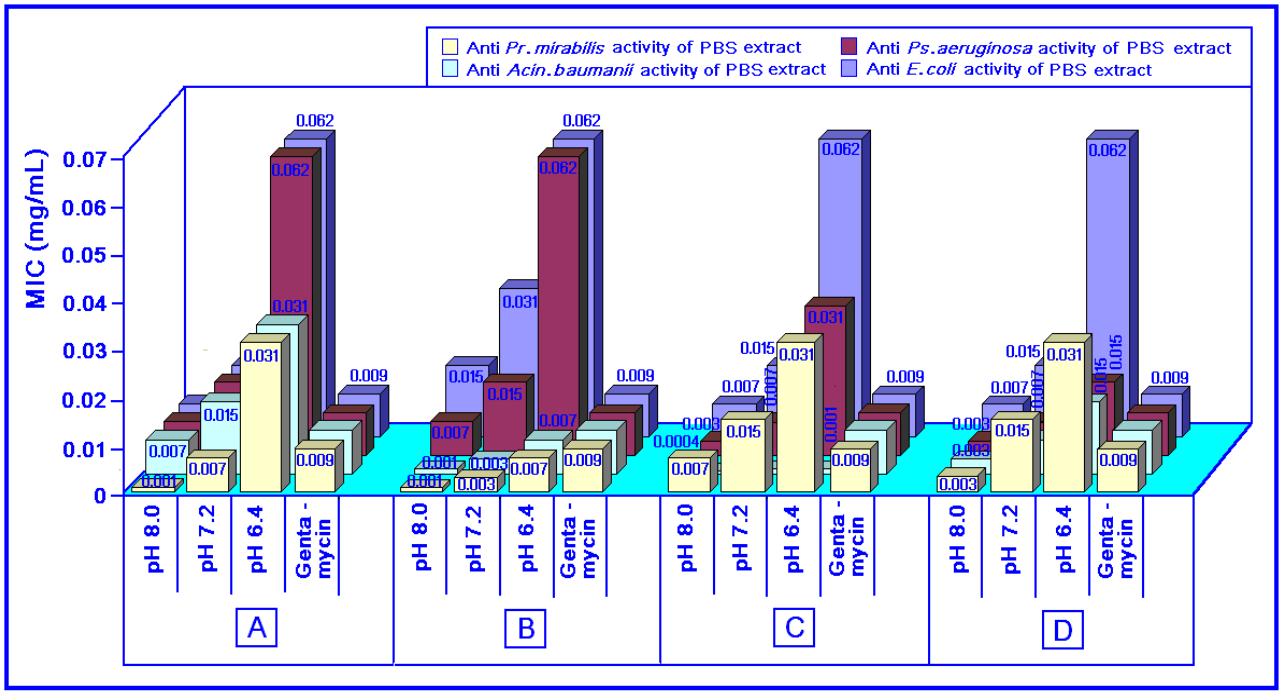


**Fig. 4 Anti Gram-negative bacteria activity of PBS extracts at pH values of 8.0, 7.2 and 6.4 with the Gentamycin as a control.**

*Ps. aeruginosa*) and 1.75 (*E. coli*). The ratios of MIC at pH values 6.4 to 8.0 were 5.00 (*Acin. baumanii*), 10.33 (*Pr. mirabilis*), 5.00 (*Ps. aeruginosa*) and 8.85 (*E. coli*).

In sample B, the MICs at pH 8.0 were 0.001 (*Acin. baumanii*, *Pr. mirabilis*), 0.007 (*Ps. aeruginosa*) and 0.015 for *E. coli*. The "Ratio to Gentamycin" was 0.25 (*Acin. baumanii*, *Pr. mirabilis*), 1.75 (*Ps. aeruginosa*) and 3.75 for *E. coli*. The ratios of MIC at pH values 6.4 to 8.0 were 7.00 (*Acin. baumanii*, *Pr. mirabilis*), 8.85 (*Ps. aeruginosa*) and 4.13 (*E. coli*).

In sample C, the MICs at pH 8.0 were 0.0004 (*Acin. baumanii*), 0.007 (*Pr. mirabilis*), 0.003 (*Ps. aeruginosa*) and 0.007 for *E. coli*. The "Ratio to Gentamycin" was 0.11 (*Acin. baumanii*), 1.75 (*Pr. mirabilis*), 0.75 (*Ps. aeruginosa*) and 1.75 for *E. coli*. The ratios of MIC at pH values 6.4 to 8.0 were 2.51 (*Acin. baumanii*), 4.42 (*Pr. mirabilis*), 10.33 (*Ps. aeruginosa*) and 8.85 (*E. coli*).

In sample A, the MICs at pH 8.0 were 0.007 (*Acin. baumanii*), 0.001 (*Pr. mirabilis*), 0.007 (*Ps. aeruginosa*, *E. coli*). The "Ratio to Gentamycin" was 1.75 (*Acin. baumanii*, *Ps. aeruginosa*, *E. coli*) and 0.25 (*Pr. mirabilis*).

The ratios of MIC at pH values 6.4 to 8.0 were 4.42 (*Acin. baumanii*), 31.00 (*Pr. mirabilis*) and 8.85 (*Ps. aeruginosa*, *E. coli*).

**Table 5 The antimicrobial activity of propolis' 70% EtOH extracts against Gram-negative bacteria at pH 8.0.**

| 70% EtOH extract | Sample A | | | Sample B | | | Sample C | | | Sample D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram-negative bacteria | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Genta-mycin |
| *Pr. mirabilis* | 0.007 ± 0.00069 | 8.85 | 1.75 | 0.015 ± 0.0016 | 4.13 | 3.75 | 0.001 ± 0.0009 | 15.00 | 0.25 | 0.0009 ± 0.00008 | 7.77 | 0.22 |
| *Acin. baumanii* | 0.003 ± 0.00029 | 5.00 | 0.75 | 0.007 ± 0.00069 | 8.85 | 1.75 | 0.001 ± 0.0009 | 15.00 | 0.25 | 0.003 ± 0.00029 | 5.00 | 0.75 |
| *Ps. aeruginosa* | 0.003 ± 0.00029 | 5.00 | 0.75 | 0.007 ± 0.00069 | 4.42 | 1.75 | 0.003 ± 0.00029 | 10.33 | 0.75 | 0.003 ± 0.00029 | 5.00 | 0.75 |
| *E. coli* | 0.003 ± 0.00029 | 10.33 | 0.75 | 0.031 ± 0.0029 | 5.00 | 0.75 | 0.003 ± 0.00029 | 5.00 | 0.75 | 0.003 ± 0.00029 | 5.00 | 0.75 |
| | pH 8.0 | | | pH 8.0 | | | pH 8.0 | | | pH 8.0 | | |

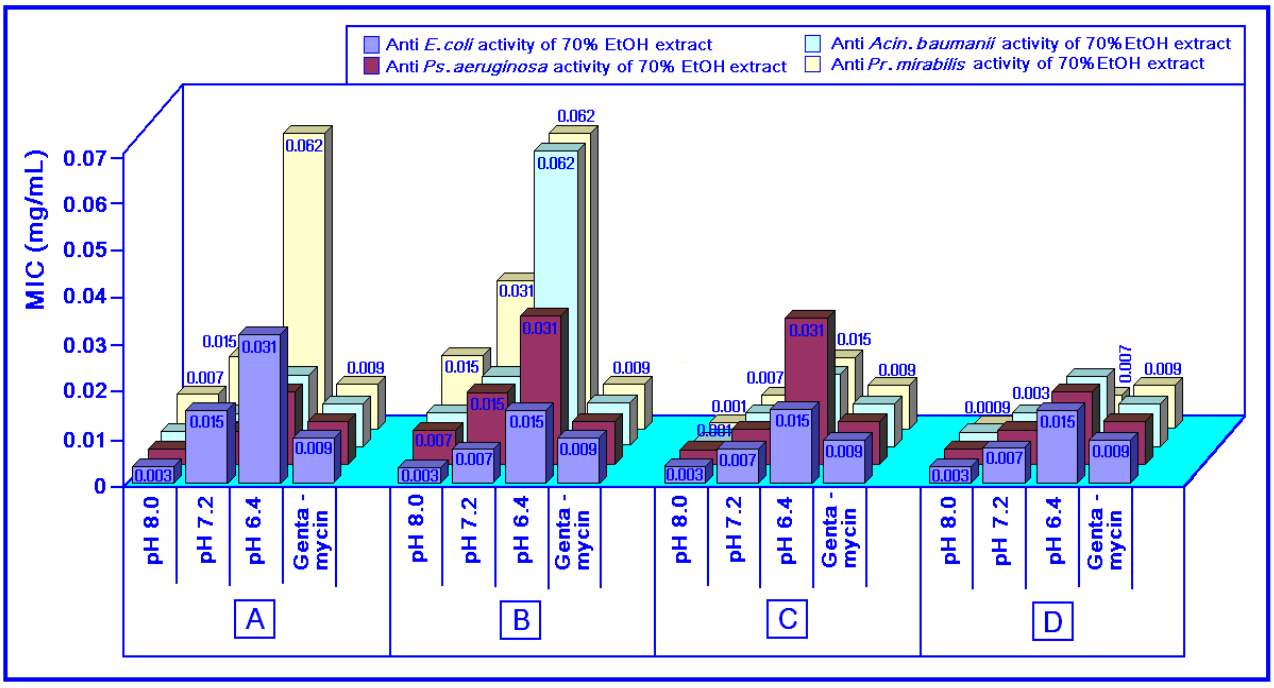


**Fig. 5 Anti Gram-negative bacteria activity of 70% EtOH extracts at pH values of 8.0, 7.2 and 6.4 with the Gentamycin as a control.**

*3.5 Antimicrobial Activity of Propolis' 70% EtOH Extracts at pH Values 8.0, 7.2 and 6.4 against Gram-Negative Bacteria*

The best antimicrobial activity, the lowest MIC (mg/mL), at pH 8.0 was found in sample C, followed by samples D, A and B (Table 5, Fig. 5). In sample C, the MICs at pH 8.0 were 0.001 (*Acin. baumanii*, *Pr. mirabilis*), 0.003 (*Ps. aeruginosa*, *E. coli*). The "Ratio to Gentamycin" was 0.25 (*Acin. baumanii* and *Pr. mirabilis)*, 0.75 (*Ps. aeruginosa* and *E. coli*). The ratios of MIC at pH values 6.4 to 8.0 were: 15.00 (*Acin. baumanii* and *Pr. mirabilis*), 10.33 (*Ps. aeruginosa*) and 5.00 (*E. coli*).

In sample D, the MICs at pH 8.0 were 0.003 (*Acin. baumanii*, *E. coli* and *Ps. aeruginosa*), 0.0009 (*Pr. mirabilis*). The "Ratio to Gentamycin" was 0.75 (*Acin. baumanii*, *E. coli* and *Ps. aeruginosa*) and 0.22 (*Pr. mirabilis*). The ratios of MIC at pH values 6.4 to 8.0 were 5.00 (*Acin. baumanii*, *E. coli* and *Ps. aeruginosa*) and 7.77 (*Pr. mirabilis*).

In sample A, the MICs at pH 8.0 were 0.003 (*Acin. baumanii*, *E. coli* and *Ps. aeruginosa*) and 0.007 (*Pr.*

*mirabilis*). The "Ratios to Gentamycin" were 0.75 (*Acin. baumanii*, *E. coli* and *Ps. aeruginosa*) and 1.75 (*Pr. mirabilis*). The ratios of MIC at pH values 6.4 to 8.0 were 5.00 (*Acin. baumanii*, *Ps. aeruginosa*), 8.85 (*Pr. mirabilis*) and 10.33 (*E. coli*).

In sample B, the MICs at pH 8.0 were 0.007 (*Acin. baumanii*, *Ps. aeruginosa*), 0.015 (*Pr. mirabilis*) and 0.031 (*E. coli*). The "Ratios to Gentamycin" were 1.75 (*Acin. baumanii*, *Ps. aeruginosa*), 3.75 (*Pr. mirabilis*) and 0.75 (*E. coli*). The ratios of MIC at pH values 6.4 to 8.0 were 8.85 (*Acin. baumanii*), 4.13 (*Pr. mirabilis*), 4.42 (*Ps. aeruginosa*) and 5.00 (*E. coli*).

### *3.6 Antimicrobial Activity of Propolis' PBS and 70% EtOH Extracts at pH Values 8.0, 7.2 and 6.4 against the Yeast C. albicans*

The strongest anti *C. albicans* activity, the lowest MIC (mg/mL), at pH 8.0 was found in sample C, followed by samples D, A and B (Table 6, Fig. 6). In sample C, the MICs at pH 8.0 were 0.001 (PBS extract), 0.001 (70% EtOH extract). The "Ratio to Nystatin" was 0.06 (PBS extract) and 0.06 (70% EtOH extract). The ratios of MIC at pH values 6.4 to 8.0 were 62.00 (PBS extract) and 31.00 (70% EtOH extract).

In sample D, the MICs at pH 8.0 were 0.001 (PBS extract) and 0.007 (70% EtOH extract). The "Ratio to Nystatin" was 0.06 (PBS extract) and 0.46 (70% EtOH extract). The ratios of MIC at pH values 6.4 to 8.0 were 15.00 (PBS extract) and 9.14 (70% EtOH extract).

In sample A, the MICs at pH 8.0 were 0.003 (PBS extract) and 0.007 (70% EtOH extract). The "Ratio to Nystatin" was 0.21 (PBS extract) and 0.46 (70% EtOH extract). The ratios of MIC at pH values 6.4 to 8.0 were 5.00 (PBS extract) and 8.85 (70% EtOH extract).

In sample B, the MICs at pH 8.0 were 0.031 (PBS extract) and 0.015 (70% EtOH extract). The "Ratio to Nystatin" was 2.06 (PBS extract) and 1.00 (70% EtOH extract). The ratios of MIC at pH values 6.4 to 8.0 were 4.03 (PBS extract) and 8.33 (70% EtOH extract).

### *3.7 The Effectiveness of 70% EtOH Extracts at pH 8.0 against Gram-Positive Bacteria, Gram-Negative Bacteria and the Yeast C. albicans versus PBS Extracts at pH 8.0*

The effectiveness of 70% EtOH extracts at pH 8.0 against Gram-positive bacteria, Gram-negative bacteria and yeast versus PBS extracts at pH 8.0 was calculated by the following equation:

$$\text{Effectiveness of 70\% EtOH extract} = \frac{\text{Value of PBS extract at pH = 8.00}}{\text{Value of 70\% EtOH extract at pH = 8.00}}$$

The results are very dispersed for each propolis sample (Fig. 7). For sample A, the effectiveness is between 0.06 and 2.33. The highest is 2.33 times better then PBS extract for MRSA. For sample B, this is between 17.85 and 0.06. The highest three are 17.85 times better then PBS extract for *St. aureu*s, 5.00 times better than PBS extract for *E. coli* and 2.33 times better than PBS extract for MRSA. For sample C, this is between 15.00 and 0.40. The highest three are: 15.00 times better than PBS extract for MRSA, 7.00 times better than PBS extract for *Pr. mirabilis* and 2.33 times better than PBS extract for *E. coli*. Finally, for sample D this is 10.33 times better, than PBS extract for *Str. pyogenes* and 3.33 times better than PBS extract. For

**Table 6 The antimicrobial activity of propolis' PBS and 70% EtOH extracts against yeast *Candida albicans* at pH 8.0.**

| | | Sample A | | | Sample B | | | Sample C | | | Sample D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Nystatin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Nystatin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Nystatin | MIC (mg/mL) | Ratios to pH 6.4/8.0 | Ratios to Nystatin |
| PBS extract | *C. albicans* | 0.003 ± 0.00029 | 5.00 | 0.21 | 0.031 ± 0.0029 | 4.03 | 2.06 | 0.001 ± 0.0009 | 62.00 | 0.06 | 0.001 ± 0.0009 | 15.00 | 0.06 |
| 70% EtOH extract | *C. albicans* | 0.007 ± 0.00068 | 8.85 | 0.46 | 0.015 ± 0.0021 | 8.33 | 1.00 | 0.001 ± 0.0009 | 31.00 | 0.06 | 0.007 ± 0.00068 | 9.14 | 0.46 |
| | | pH 8.0 | | | pH 8.0 | | | pH 8.0 | | | pH 8.0 | | |

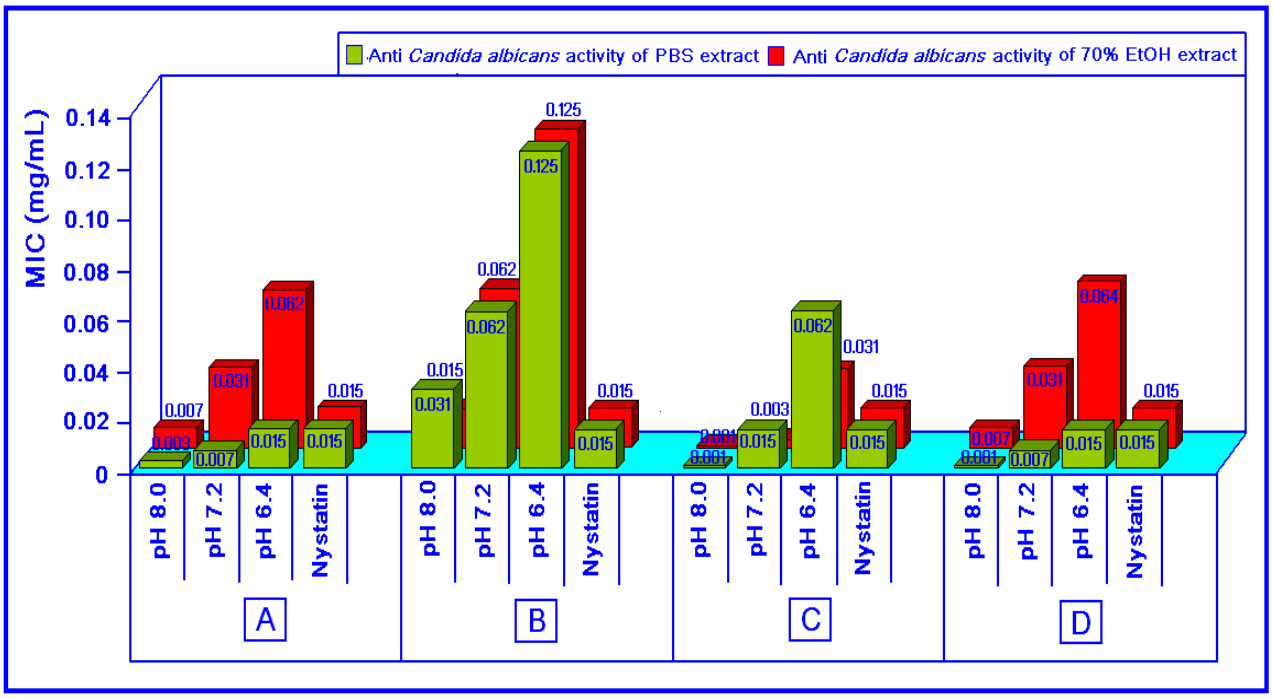


**Fig. 6 Antimicrobial activity of propolis' PBS and 70% EtOH extracts against the yeast *Candida albicans* at pH values of 8.0, 7.2 an d 6.4 with the Nystatin as control.**

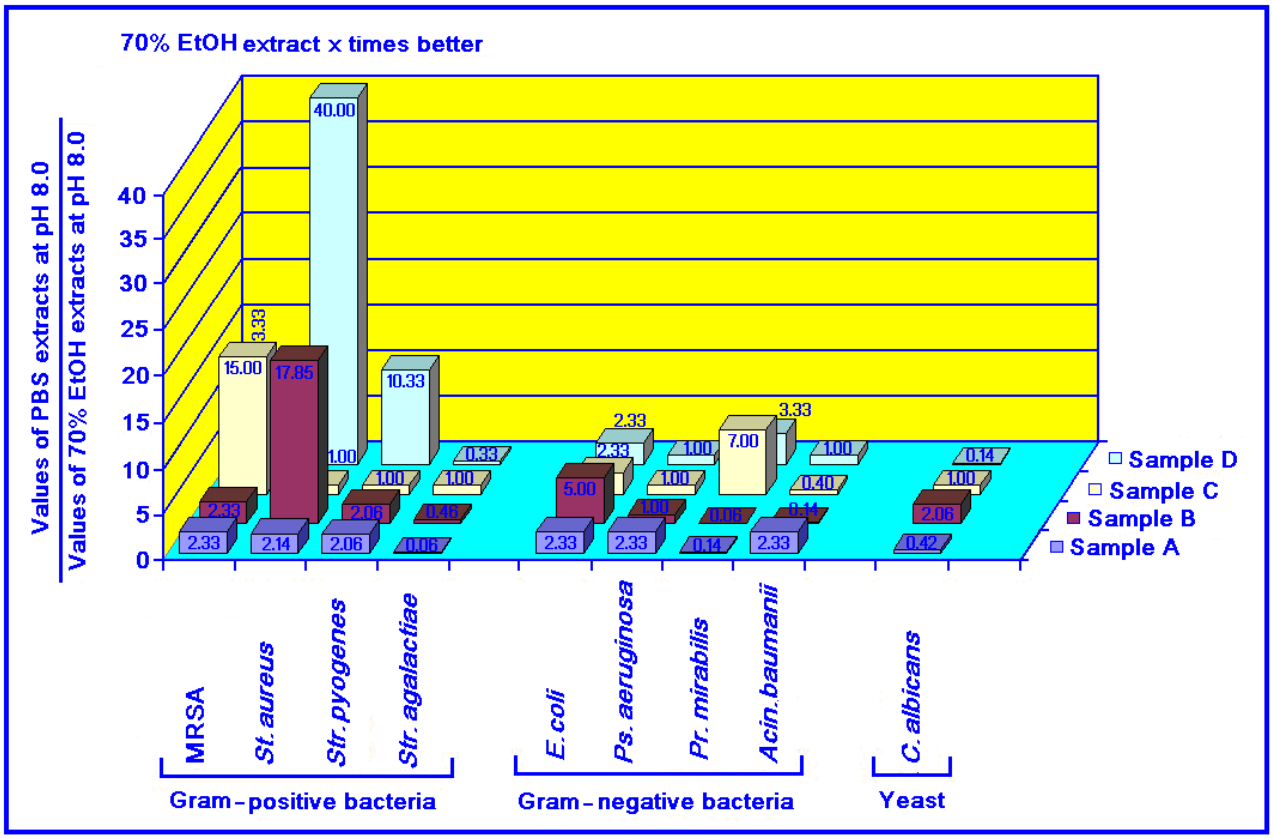


**Fig. 7 Effectiveness of 70% EtOH extracts at pH 8.0 against Gram-positive bacteria, Gram-negative bacteria and yeast versus PBS extracts at pH 8.0.**

sample D the effectiveness is between 40.00 and 0.14. The three best are: 40.00 times better, than PBS extract for *St. aureus*, 10.33 times better than PBS extract for *Str. pyogenes* and 3.33 times better than PBS extract for MRSA. Overall, the effectiveness of 70% EtOH extracts at pH 8.0 is bigger for Gram-positive bacteria than Gram-negative bacteria and yeast, where it is the lowest.

## 4. Discussion

A solvent and its ionic strength are very important for effective extraction of the bioactive compounds from propolis, and methanol was reported to be the effective one [17]. However, ethanol and water are still the solvents of choice, because they are considered as a green solvent [18]. The use of water as solvent is highly restricted because of the poor solubility of organic compounds during the extraction. So, Mello *et al.* [19] and De Moura *et al.* [20] revealed about ten-time lower total phenolics in a water extract compared to 70% EtOH extract, using raw propolis harvested from the beehives of *Apis mellifera*. Hence, ethanol is likely to be the most popular solvent for propolis' extraction according to Pietta *et al.* [21]. Nevertheless, the mode of extraction can be increased by changing the pH of the solvent. This is because the change of ionic strength affects the solubility of compounds. Yeo *et al.* [22] reported about the increased antioxidant activity, if extraction of propolis was conducted in an alkaline condition. However, Jug *et al*. [23] showed that acidic water and ethanol (at pH 3.00) produced a slightly higher total flavonoid amount in a propolis extract, in comparisons to their neutral solvent system (at pH 7.00). On the other hand, Kubiliene *et al.* [24] reported that the addition of polyethylene glycol in their extraction solvent enhanced the propolis' extraction.

In the performed experiments, water as a solvent was replaced with the PBS at pH values 8.0, 7.2 and 6.4, respectively. The PBS extraction is higher than it is in water. The four propolis samples extracted with PBS or 70% EtOH at pH values 8.0, 7.2 and 6.4, were tested against Gram-positive bacteria (MRSA, *St. aureus*, *Str. pyogenes* and *Str. agalactiae*), Gram-negative bacteria (*E. coli*, *Ps. aeruginosa*, *Pr. mirabilis* and *Acin. baumanii*) and the yeast (*C. albicans*). Moreover, literally in all tested microbes, the highest antibacterial activity, with the lowest value for MIC, was found at pH 8.0. Overall, the highest antibacterial activity with minimal values for MIC was obtained at pH 8.0, followed by 7.2 and 6.4, where the highest values for MICs, with the lowest antibacterial activity, were found.

When there are interests for the data, how much higher the MIC is at pH 6.4, regarding the pH 8.0, the ratio of MICs 6.4/8.0 is calculated. In the case of Gram-positive bacteria, effect of PBS extract on the MICs ratio 6.4/8.0 is 6.76. In the case of the effect of 70% EtOH extract on Gram-positive bacteria, the MICs ratio 6.4/8.0 is higher, and is 7.22. In comparison, the Gram-negative bacteria' effects of PBS extract on the MICs ratio 6.4/8.0 is 8.53. In the case of 70% EtOH extracts, the MICs ratio 6.4/8.0 is 7.74. When the yeast *C. albicans* is studied under the same conditions, the influence of PBS extracts on the MICs ratio 6.4/8.0 is 22.47. With the 70% EtOH extracts, this is about half-lower and is 14.33.

Important factor during the study of antimicrobial activity of PBS extracts or 70% EtOH extracts is the "Ratio to Gentamycin". It shows that some propolis' extracts are stronger than Gentamycin with the "Ratio to Gentamycin" being one and lower. This is calculated by dividing of MIC at pH 8.0 with MIC of Gentamycin.

Comparing the PBS extracts with 70% EtOH extracts' activity against Gram-positive bacteria, a big difference between samples and bacteria was found. In the case of PBS extracts against Gram-positive bacteria for *Str. agalactiae* "Ratio to Gentamycin", it is the same, 0.25 for both samples A and D. When the activity of 70% EtOH extracts against Gram-positive bacteria is studied, in samples A and B for MRSA, "Ratio to Gentamycin" is 0.75. In sample C for MRSA it is 0.25 and in sample D for MRSA is 0.75. In sample D for *Str. agalactiae* it is 0.75, for *Str. pyogenes* is 0.75, for MRSA is 0.75 and for *St. aureus* is 0.75.

The comparison of the activity of PBS extract to 70% EtOH extracts on the "Ratio to Gentamycin" in the Gram-negative bacteria, shows the activity of PBS extracts on *Pr. mirabilis* in the samples A and B is 0.25 and in sample D is 0.75. For *Ps. aeruginosa*, "Ratio to Gentamycin" is 0.75 in samples C and D. For *Acin. baumanii* the "Ratio to Gentamycin" is 0.10 for sample C and 0.75 for sample D.

The study of anti *C. albicans* effects of PBS extracts on the "Ratio to Nystatin" in sample A was 0.21, sample B 2.06, sample C 0.06 and for sample D it was 0.06. In average, the "Ratio to Nystatin" was 0.59. The 70% EtOH extracts show the next figure of the "Ratio to Nystatin": sample A 0.46, sample B 1.00, sample C 0.06 and sample D 0.46. The average is 0.49. Generally, the antimicotic influence of PBS or 70% EtOH extracts on the "Ratio to Nystatin" has the same trend as is valid for Gram-negative bacteria. Effectiveness of PBS extracts is even much better than Nystatin.

Finally, the results from the performed experiments are in good agreement with these of Tlak Gajger *et al.* [25] about components responsible for antimicrobial activity of propolis. It can be stated that the antimicrobial effects of different propolis' samples correlate with their polyphenol content, more precisely, with the content of flavonoids, caffeic acid, chrysin, pinocembrin and galangin at defined pH 8.0. Specifically, galangin should be emphasized as a substance with strong antimicrobial and anti-cancer activity for gastric cancer, hepatocellular carcinoma, promyelocytic leukemia [26, 27], and human glioblastoma cells where they suppress the cell growth [28]. Both the galangin and pinocembrin exert the additional significant antifungal activity [29], which puts them to the top priority in the propolis' further investigation.

## 5. Conclusions

From the performed experiments, the following conclusions can be drawn:

(1) The antimicrobial activity of propolis' PBS extracts against Gram-positive bacteria shows the lowest MIC (mg/mL), at pH 8.0 in sample C, followed by A, B and D. In sample C, MICs at pH 8.0 were 0.007 (*Str. agalactiae*), 0.015 (MRSA), 0.015 (*Str. pyogenes*) and 0.007 (*St. aureus*).

The "Ratio to Gentamycin" is 1.75 (*Str. agalactiae* and *St. aureus*) and 3.75 (*MRSA* and *Str. pyogenes*). The polyphenol content of sample C is flavonoid content 5.47 ± 0.62 mg/mL, caffeic acid 1.33 ± 0.92 mg/mL, chrysin 41.02 ± 4.22 μg/mL, pinocembrin 32.93 ± 3.21 mg/mL and galangin 41.87 ± 4.23 mg/mL.

(2) The propolis' PBS extracts against Gram-negative bacteria show the lowest MIC (mg/mL), at pH 8.0 in sample D, followed by B, C and A. In sample D, MICs at pH 8.0 were 0.003 (*Acin. baumanii*, *Pr. mirabilis*, *Ps. aeruginosa*) and 0.007 (*E. coli*). "Ratio to Gentamycin" was 0.75 (*Acin. baumanii*, *Pr. mirabilis*, *Ps. aeruginosa*) and 1.75 (*E. coli*). The polyphenol content of sample D is flavonoid content 8.28 ± 0.92 mg/mL, caffeic acid 3.56 ± 0.32 mg/mL, chrysin 677.42 ± 68.42 μg/mL, pinocembrin 146.49 ± 13.89 mg/mL and galangin 59.81 ± 5.86 mg/mL. This are the values for the 70% EtOH extract of propolis' sample D at pH = 8.0. The polyphenol's content of PBS extract at pH = 8.0 are flavonoids 1.03 ± 0.11 mg/mL, caffeic acid 0.44 ± 0.03 mg/mL, chrysin 84.67 ± 8.39 μg/mL, pinocembrin 24.41 ± 2.39 mg/mL and galangin 8.54 ± 0.86 mg/mL.

(3) The best anti *C. albicans* activity, the lowest MIC (mg/mL), at pH 8.0 was found in sample C, followed by samples D, A and B. In sample C, the MIC at pH 8.0 was 0.001 (PBS extract). Effects of PBS extracts on the "Ratio to Nystatin" are 0.06 in samples C and D.

(4) The antimicrobial effects of different' samples A to D, correlate with its polyphenol content, more precisely, with the flavonoid, caffeic acid, pinocembrin, chrysin and galangin content.

## Acknowledgments

The presented research was supported in the frame

of Croatian Institute for Experimental and Translational Oncology (CIETO), Koledinečka 03, 10040 Zagreb, Croatia. It was included in the Project "Oncolytic Newcastle Disease Virus in the Veterinary Medicine" and partially supported by Ivan Čermak from the Crodux-plin, Savska Opatovina, 10000 Zagreb, Croatia. Authors are indebted to Tomaž Velnar for English suggestions.

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