Borozan AB *et al.* (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 2, Article number 12826
DOI:10.15835/nbha51212826
Research Article

Comparative study on the antimicrobial activity of propolis, catechin, quercetin and gallic acid

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Abstract

Propolis, considered one of the most effective natural broad-spectrum antibiotics, which do not induce resistance or destroy the organism's commensal flora, together with catechin, quercetin and gallic acid (at different concentrations), have been tested against Gram positive bacteria *Streptococcus pyogenes* ATTC 19615, *Stapylococcus aureus* ATCC 25923 and Gram negative *Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 14028, *Shigella flexneri* ATTC 12022, as well as the yeast *Candida albicans* ATTC 10231, using the agar diffusion method. Propolis and the three compounds showed antimicrobial potential at most concentrations. The highest sensitivity to propolis (conc. 0.001-1%) was shown by the Gram-positive bacterial strain *S. pyogenes*, followed by the Gram-negative bacterium Escherichia coli, while the other species had an equal and reduced reaction. Gallic acid showed high antibacterial activity on *S. aureus, P. aeruginosa* and *S. enterica*, and a reduced effect on other bacterial strains. The antibacterial efficiency of 3.0224% quercetin was high against the bacterium *S. pyogenes* and catechin (2.9028%) proved to be the best antifungal, followed by propolis 1%, and quercetin 3.0224%. At certain concentrations, propolis and the three compounds could supplement gentamicin and ampicillin, as they have shown similar or even higher antibacterial efficacy than conventional drugs.

Keywords: antibacterial activity; antifungal activity; catechin; gallic acid; propolis; quercetin

Keywords: DMSO – dimethyl sulfoxide; cfu – colony-forming units; CLSI – Clinical and Laboratory Standards Institute; MIC – Minimum Inhibition Concentration; MBC – Minimum Bactericide Concentration

Received: 01 Aug 2022. Received in revised form: 20 Mar 2023. Accepted: 03 May 2023. Published online: 06 Jun 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

In recent years, researchers have tried to find alternative solutions due to the alarming increasing of the microorganism's resistance to antibiotics (Sevindik *et al*., 2017). Nowadays, in the EU, one in five bacterial infections occurs due to antibiotic resistance. The social impact is high considering that more than 670,000 antibiotic-resistant bacterial infections are reported, causing the death of 33,000 people. The economic impact is also high, as it has been estimated that 1.5 billion euro / year are spent to solve these problems (EC 1). In Romania approximately 40% of bacterial infections are resistant to antibiotics, and the anticipation for 2030 are not encouraging (OECD, 2019).

One of the alternatives to antibiotics is propolis, a bee product that contains compounds derived from spontaneous flora or supplied directly by bees. Propolis, the subject of several studies, is considered a health promoter, due to its promising therapeutic properties based on the compound's bioactivity (Touzani *et al*., 2019). These natural sources exploiting would reduce the costs involved in the healthcare system. So far, more than 300 compounds have been identified in propolis (Anjum *et al*., 2019), with pharmacological properties: medical (Touzani *et al*., 2019, (Pasupuleti *et al*., 2017), antiviral (Münstedt, 2019), antimicrobial (Lavinas *et al*., 2017; Béji-Srairi *et al*., 2020), protective, healing, antiseptic (Sun *et al*., 2015), cosmetics (Barros *et al*., 2019), conservative and promising functional potential (Mohdaly *et al*., 2015; Bankova *et al*., 2016). However, the benefits of applied research in this area are blocked, as there is no consensus on the international recognition for the application of propolis in the medical field. The EU Commission rejected the requests for the use of propolis as an antibacterial agent considering that the chemical composition depends on the geographical area, botanical origin, and working methodologies (EC 2). The disagreement is based on studies which prove that the chemical composition and biological characteristics of propolis are influenced by geographical area, plant source, season (Anjum *et al*., 2019) and climatic factors (Bankova *et al*., 2016; EC 2).

As a confirmation for the influence of the geographical area on the biological activity of propolis, it was highlighted, after the evaluation of more than 600 bacterial strains that propolis originated from the Middle East has a higher antimicrobial effect on Gram positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), compared to that from some European countries (Germany, Ireland) and Korea (Przybyłek and Karpiński, 2019).

The use of compounds common to propolis worldwide could be a solution that would eliminate these differences. A similar suggestion was also taken into account by Sforcin in 2016 (EC1; Pasupuleti *et al*., 2017). Considering our certainty regarding the benefits offered by propolis, this study focused on three compounds, in their pure state and in different concentrations, catechin, quercetin and gallic acid, from the classes of flavonoids and polyphenolic acids. According to studies developed over time, the two classes are among the dominant com-pounds in propolis regardless of its area and botanical origin. Bogdanov and the collaborators (2019) (EC1; Münstedt, 2019) have recently demonstrated that in general, propolis contains 40-70% flavonoids and phenolic acids, wax (20–35%), essential oils (1-3%), minerals, proteins and polysaccharides (5%) (EC1; Münstedt, 2019). In the case of Romanian propolis botanical sources are generally represented by deciduous forests and the biochemical content is similar to that of other geographical areas, namely flavonoids, polyphenolic acids and their esters in large quantities (Mărghitaş *et al*., 2013; Graikou *et al*., 2016). Also, in most cases, the therapeutic and antimicrobial properties are determined by representatives of these classes of compounds (Cauich-Kumul and Campos, 2019). Besides, based on additional sources of Oses *et al*. (2020), it appears that the three studied compounds have a high antioxidant capacity and they could be involved in the increase of the antioxidant status of the various systems of human body defence.

Catechin is a promising flavonoid, considered by Reygaert (2018) to have antimicrobial properties and may play a key role in both preventing and treating diseases caused by pathogens. Depending on the concentration, it also has immune effects, being observed the stimulation of autoantibodies that can produce

undesirable effects (NCBI3). Catechin in propolis has also been associated with the inhibitory effect of hyaluronidase, blocking its conversion process (Osés *et al*., 2020).

Quercetin is another flavonoid, ubiquitous in propolis, known for its antioxidant capacity (Gopu *et al*., 2015) and protein inhibition (OECD, 2019; Pasupuleti *et al*., 2017). It has been studied and proposed as an antimicrobial agent for the food industry, due to its disruptive potential in microbial signalling mechanisms, an important step in regulating gene expression, but also in microbial multiplication. Its antimicrobial role cannot be disputed, nor can its importance as an anticancer agent be limited (Gopu *et al*., 2015). Also, the same authors mention that quercetin enhances the antimicrobial activity of antibiotics, with a key role in their synergism (Siriwong *et al*., 2016).

Gallic acid is a polyphenolic acid, part of hydrolysable tannins, with mutagenic and teratogenic potential (NCBI1). It cannot be excluded from the list of antimicrobial agents, but due to its mutagenic effects, caution is required when the concentration is established. As always, for these compounds, the beneficial effects are associated with the adverse ones. For this reason, we considered, even if this was not our objective that the establishment of toxicities has a key role in these compounds using.

Although there are many researches on propolis worldwide, the studies in Romania are sporadic and limited only to certain areas (e.g., northwest, center). To compare the antimicrobial activity of the three compounds, we selected propolis from an unexplored area, southwest of the country. Antimicrobial activity is a simple but important test, because the reaction of microorganisms to propolis can be similar and can be taken into account by international regulations, requiring only an adjustment of the working methodology and the establishment of mutagenic or antimutagenic activity. A similar conclusion was drawn by Bridi *et al*. (2015). They considered that in addition to antimicrobial testing, radical scavenging capacity must also be evaluated. Although the range of propolis tests is broad, it has not yet been completed. Sarıçoban and Sabire (2016) considered that all compounds and application modalities of propolis should be identified.

Some research related to the antimicrobial activity of propolis has been performed in Romania, but as we specified earlier, they were limited to certain areas. These studies have shown that propolis has a strong biological activity on Gram-positive bacteria, compared to Gram-negative ones (Mărghitaș *et al*., 2013). Most of the global results showed a higher sensitivity of Gram-positive bacteria to propolis (Przybyłek *et al*., 2019). In contrast to these results, Mohdaly *et al*. (2015), for the first time, demonstrate that propolis also has a pronounced biological activity in relation to some Gram-negative bacteria. Studies limited to certain areas do not provide a broad perspective on the quality of propolis. Our study has been extended to the south-western part of Romania, where no data were recorded on the antimicrobial potential of propolis.

Why this extension is considered necessary? According to reports elaborated by the EU and EEA countries, the contribution of resistant bacteria to health damage is different from country to country. Therefore, these international forums emphasize the necessity to find and apply prevention and control strategies adapted to the needs of each country. Propolis, through its complex polyphenolic compounds, can be considered an "effective weapon" against pathogens in each country, also through the activity of "radical scavenger", can disrupt the activity of free radicals, and eliminate the effects of oxidative stress, the basis of the pathogenesis for many diseases (Arct and Pytkowska, 2008; Kocot *et al*., 2018).

The effectiveness of natural products against oxidative stress and the correlation between antimicrobial activity and Free-Radical Scavenging is a topic of interest to the scientific world (Mohammed *et al*., 2018; Mohammed *et al*., 2019). In this context, Rayan *et al*. (2020) emphasized that plants from spontaneous flora have antimicrobial potential and satisfactory free radical scavenging which may be a solution for managing oxidative stress. In this study, microorganisms relevant to medical practice were chosen (Ayatollahi *et al*., 2018; Sharma *et al*., 2018; Türk *et al*., 2018; del Mar Cendra *et al*., 2019; Rayan *et al*., 2020). For the selection of antibiotics used in experiments, the statistics made by the European Center for Disease Prevention and Control, Stockholm (ECDC, 2018) and Tosisa *et al*. (2020) were taken into account, which showed that large-spectrum antibiotics are the most used. Thus, antibiotics from penicillin and cephalosporin groups were chosen

as controls. The two groups of antibiotics also are on the first positions in Romania (ECDC, 2018). For example, cefotoxime is part of the third generation of antibiotics, the group of cephalosporins and is still included in the WHO list, because it is considered effective and necessary for the health system (Popescu *et al*., 2016), and nystatin is a fungicidal polyene antibiotic targeted by many antifungal studies (WHO, 2020). Ampicillin, from the penicillin group, is considered stable against the hydrolysis of beta-lactamases (Zhang *et al*., 2018) and is recommended for both Gram-positive and Gram-negative bacteria (NCBI 4), and gentamicin is still included in some studies on *Pseudomonas aeruginosa* or other bacteria (Shama *et al*., 2018; DHHS, 2019). All these antibiotics are therefore still of interest to the antimicrobial tests today (NCBI 4; Shama *et al*., 2018; Mohanty *et al*., 2020). The aim of this study was to compare the antimicrobial properties of catechin (conc. 0.002903 - 2.902800%), quercetin (conc. 0.003022 - 3.022400%), and gallic acid (conc. 0.001881 - 1.8813%), with the extract in DMSO (dimethyl sulfoxide) of some samples of propolis (0.00001 - 1%), originated from the southwestern part of Romania (Caras-Severin and Timis county).

Materials and Methods

Preparation of propolis extract and dilutions

Propolis samples obtained from persons who practice migratory beekeeping in the area of Banat's county, South-West part of Romania were used. Propolis extract was obtained by applying classical maceration. This has been found to be the most efficient method of extracting bioactive substances from propolis (Mărghitaș *et al*., 2007). Active principles of propolis were extracted with 70% (w/v) ethanol, 1:30 ratio for 24 h at room temperature. For easier handling of propolis, it was first frozen by freezing at -20 °C and then ground into a fine powder. The extract was filtered directly into a volumetric flask of 100 ml capacity and the residue was submitted to the second extraction in the same conditions as the first one. Then the filtered extracts were united and the volume was filled up with 70% ethanol. The third extraction was no longer needed since it gave negative reaction with $FeCl_3$ (10%). Then, the extract was evaporated to dryness. About 1g from this residue rich in bioactive compounds (flavonoids and polyphenolic acids) were mixed with 10 ml of DMSO, mixed and sonicated until completely dissolved (Ultrasonic Bath, Aqua Wave-Barnstead Labline, Germany). From this stock solution (10% w/v concentrations) were prepared by successive dilutions 1:10, the following (w/v) concentrations in DMSO: 1%, 0.1%, 0.01%, 0.001%, 0.0001% and 0.00001%.

In our experiments, serial dilutions (0.00001 - 1%) from a propolis sample originated from the western part of Romania were used. Its antimicrobial activity against six bacterial and a yeast strain was analyzed in comparison with quercetin, catechin and gallic acid (with the structures fungus shown in the Figures 2 and 3).

Their range of concentrations was equivalent to 10-2-10-6 M but expressed as percentage: gallic acid 0.001881-1.8813%, quercetin 0.003022-3.022400% and catechin 0.002903-2.902800% respectively. For propolis it was not possible to determine the molecular weight, therefore we considered some values of the percentage concentration close to the used substances.

Preparation of flavonoids (quercetin, catechin) and gallic acid solutions and dilutions

Chemically pure substances obtained from manufacturing companies.

1.Quercetin hydrate purum ($C_{15}H_{10}O_7 \cdot H_2O$, 95%, 33,795-1 code Aldrich, Germany), molecular weight = 302.24.

2.(+/-) Catechin hydrate purum (> 96% HPLC, 22130 code Fluka, USA), molecular weight 290.28.

3.Gallic acid, $(HO)_3C_6H_2CO_2H \cdot H_2O$ (> 99% HPLC, 27645 code Sigma Aldrich, Germany), molecular weight: 188.13.

4.DMSO (dimethyl sulfoxide): ACS Reagent Grade, 317275 code Sigma Aldrich, Germany.

From the mentioned pure substances (quercetin, catechin, gallic acid) stock solutions of w/v concentration 0.1 M (10-1) were prepared. They were successively diluted in DMSO (Dimethyl sulfoxides) 1:10 and solutions of w/v concentration 0.01 (10^{-2}), 0.001 (10^{-3}), 0.0001 (10^{-4}) and 0.00001 (10^{-5}) M were prepared. They were considered in the statistical analysis as percentages as follows:

- quercetin: 3.022400% (0.01 M), 0.302240% (0.001 M), 0.030224% (0.0001 M) and 0.003022% (0.00001 M);
- catechin: 2.902800% (0.01 M), 0.290280% (0.001 M), 0.029028% (0.0001 M) and 0.002903% (0.00001 M);
- gallic acid: 1.8813% (0.01 M), 0.18813% (0.001 M), 0.018813% (0.0001 M) and 0.001881% (0.00001 M).

The substances have been weighed with analytical balance (0.1 mg precision) (Kern, Germany).

Antibacterial and antifungal activities

Microbial strains

The propolis extract and the dilutions associated with each compound have been tested against reference bacterial strains, Gram positive: *Streptococcus pyogenes* ATTC 19615, *Stapylococcus aureus* ATCC 25923 and *Gram negative: Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 14028, *Shigella flexneri* ATTC 12022 as well as the yeast *Candida albicans* ATTC 10231.

For testing, the bacterial strains were sub-cultured on Nutrient Broth (HiMedia Laboratories Pvt. Limited, Mumbai, India) at 37 °C, and the yeast was grown on liquid media Sabouraud-Dextrose Broth (Oxoid Limited, UK) at the same temperature, in aerobic conditions, for 24 hours, The indicated growth conditions were applied, with small modifications.

Agar disk diffusion method

The antibacterial and antifungal activity was determined by Agar Disk Diffusion method according to Kirby-Bauer procedure recommended by the Institute of Clinical and Laboratory Standards (CLSI, 2011; CLSI, 2004, with some modifications (incubation time was similar for all microorganisms and the Mueller Hinton agar has been substituted by Nutrient agar).

The nutrient media were distributed in Petri dishes with a diameter of 100 mm and incubated for 30 minutes at a temperature of 37 °C, to drain the surface for a better adhesion of microbial cells (Raba *et al*., 2015).

The microbial suspensions were adjusted according to the McFarland standard 0.5, to 10^6 colony-forming units, i.e., cfu/mL for bacteria (Akinpelu *et al*., 2015) and 10^4 cfu/mL for yeast (Pessini *et al*., 2005). The bacteria were inoculated on Nutrient Agar (HiMedia Laboratories Pvt. Limited, Mumbai, India) and the fungal strain on Sabouraud Dextrose Agar (Oxoid Limited, UK).

Sterile absorbent paper discs (D 6 mm, Whatman No.1) (Merck, Damstadt, Germany) were placed on the surface of the inoculated media at equal distances from the edges of the Petri dish. Each disc was impregnated with 10 µL of extract / dilution. Antimicrobial tests were performed in three repetitions.

The incubation temperature was 37 °C (Incubator, Biobase, China) for both bacteria and *Candida albicans*. As controls DMS was used for all microorganisms because this was the substance used for extraction and dilutions. Besides, ampicillin (10 mcg/disc, HiMedia Laboratories Pvt. Limited, Mumbai, India), cefotaxime (30 µg / disc, Oxoid Laboratories, UK) and gentamicin (10 µg/disc, Uniparth Limited Basingstoke, Hampshire, England) were used as controls for bacteria. Nystatin (100 units/disc, HiMedia Laboratories Pvt. Limited, Mumbai, India), propionic acid (2%) and formic acid (0.5%) were used as controls for *Candida albicans* ATTC 10231.

Sensitivity of microbial strains to propolis and the 3 compounds, respectively controls was determined by measuring the diameter of the inhibition zone (D), including the diameter of the disc. The diameter of the inhibition zones was measured to an accuracy of 1 mm, immediately after the incubation period.

Statistical analysis

The data were statistically processed using ANOVA, and the means were compared using Multiple Range Test (Ciulca, 2006). The significance was expressed based on letters, being considered as significant the differences between treatments marked with different letters.

To display the response of each bacterial species to different treatments in a single graph, the basic principle of biplot technique was used (Yan and Kang, 2002).

Results

Following the application of the agar disk diffusion method, a variation of the antimicrobial activity of propolis, gallic acid, catechin and quercetin was observed, depending on their concentration but also on the nature of the studied microorganisms.

The results of the Kirby Bauer test were statistically analysed and included in Tables 1-5 and Figure 1-2.

Table 1. Antibacterial activity of propolis, catechin, quercetin and gallic acid on *E. coli, S. pyogenes* and *P. aeruginosa*

No	Treatments	Inhibition area (mm) of *E. coli*	Inhibition area (mm) of *S. pyogenes*	Inhibition area (mm) of *P. aeruginosa*
1.	Ampicillin	17.00+1.15 b	18.67+1.33 ab	7.33+0.33 f
2.	Cefotaxim	24.00+1.15 a	21.00+1.15 a	19.33+1.20 a
3.	Gentamicin	10.33+0.88 de	13.33+2.40 c	15.67+0.33 b
4.	Propolis 1%	13.67+1.20 c	17.33+1.45 b	8.67+0.88 def
5.	Propolis 0.1%	7.67+0.67 gh	13.67+3.28 c	8.33+0.67 def
6.	Propolis 0.01%	7.00+0.00 h	10.00+1.53 de	7.67+0.33 ef
7.	Propolis 0.001%	7.67+0.67 gh	7.00+0.00 f	7.33+0.33 f
8.	Propolis 0.0001%	7.33+0.33 gh	7.33+0.33 ef	7.67+0.67 ef
9.	Propolis 0.00001%	0.00+0.00 i	0.00+0.00 g	0.00+0.00 g
10.	Gallic acid 1.8813%	9.00+0.58 defg	0.00+0.00 g	10.67+1.33 c
11.	Gallic acid 0.1881%	8.33+0.33 efgh	0.00+0.00 g	10.00+0.58 cd
12.	Gallic acid 0.0188%	8.00+0.00 fgh	0.00+0.00 g	9.67+0.88 cd
13.	Gallic acid 0.0018%	7.67+0.33 gh	0.00+0.00 g	9.33+0.67 cde
14.	Quercetin 3.0224%	9.67+0.88 def	11.67+0.88 cd	10.67+0.67 c
15.	Quercetin 0.3022%	8.00+0.00 fgh	0.00+0.00 g	8.67+0.67 def
16.	Quercetin 0.0302%	7.33+0.33 gh	0.00+0.00 g	7.33+0.33 f
17.	Quercetin 0.0030%	7.33+0.33 gh	0.00+0.00 g	0.00+0.00 g
18.	Catechin 2.9028%	10.67+0.33 d	9.67+0.33 def	9.67+0.67 cd
19.	Catechin 0.2902%	10.00+0.58 de	8.33+0.88 ef	9.33+1.20 cde
20.	Catechin 0.0290%	9.00+1.00 defg	9.00+1.00 def	8.67+0.33 def
21.	Catechin 0.0029%	8.67+0.33 efgh	0.00+0.00 g	8.67+0.88 def
22.	DMSO	7.33+0.33 gh	0.00+0.00 g	7.00+0.00 f
	LSD 5%	1.80	2.96	1.97

Data are mean ±SE, n=3; Different letters indicates significance at $p < 0.05$

For antibacterial activity on *E. coli* (Table 1), amplitude of the inhibited area of 24 mm between the treatments was observed. Therefore, treatments with ampicillin (10 mcg) and cefotaxime (30 µg) showed antibacterial activity against *E. coli*, significantly superior to other treatments. A very important effect was also found for the treatment with 1% propolis, compared to DMSO. Catechin treatments in different concentrations, as well as gallic acid (1.8813%, 0.1881%), and quercetin 3.0224%, respectively, had an antibacterial effect on *E. coli* significantly equal to gentamicin (10 µg). In general, the antimicrobial activity of the studied compound decreased with reduced concentration. *E. coli* was sensitive to propolis 1% > catechin 0.2902 - 2.9028% > quercetin 3.0224% > gallic acid 1.8813% = catechin 0.2902%.

Considering the antibacterial effect of different treatments on *S. pyogenes*, it was noted that cefotaxime (30 µg) was most effective with a significantly higher inhibitory area compared with the other treatments. 1% propolis also had a high activity, significantly equal to ampicillin (10 mcg), while 0.1% propolis treatments and 3.0224% quercetin showed a gentamicin-like effect (10 µg). The antibacterial effect of propolis was maintained even at the lowest concentration compared to DMSO. *S. pyogenes* has shown resistance to gallic acid treatments, regardless of the used concentration. Resistance of these bacteria to 0.3022-0.003% quercetin and 0.0029% catechin treatments was also observed. Catechin treatments 2.9028-0.029% showed an antibacterial effect on this specie significantly inferior to the three antibiotics, but higher compared to DMSO. Thus, propolis 1% showed the strongest antimicrobial activity against *S. pyogenes* (D 17.33 mm), followed by 0.1% propolis > quercetin 3.0224% > propolis 0.01% > catechin (0.0290-2.9028%).

Treatments with cefotaxime (30 µg) and gentamicin (10 µg) had the highest efficacy against *P. aeruginosa*, considering the area of inhibition significantly higher compared with the rest of the treatments. Experiments with propolis in dilutions between 0.0001 and 1% showed an antibacterial effect equal to each other and to ampicillin (10 mcg), and compared to DMSO. The antibacterial effect of gallic acid (all concentrations) was significantly higher compared to DMSO. The reduction in quercetin concentration was associated with a progressive decrease in the antibacterial effect which was counteracts at 0.003% dilution. Treatment with quercetin 3.0224% was significantly superior to most other treatments excepting cefotaxime (30 µg) and gentamicin (10 µg). *P. aeruginosa* showed significantly higher sensitivity to catechin treatments at concentrations of 2.9028 and 0.029%, compared to DMSO and ampicillin (10 mcg) treatments. Regarding *P. aeruginosa*, an inhibitory effect was observed for gallic acid 1.8813% = quercetin 3.0224% > gallic acid 0.1881% > gallic acid 0.0188% = catechin 2.9028% > propolis 1%.

Considering the antibacterial activity against *S. enterica* (Table 2), amplitude of the inhibited surface of 20.67 mm was observed between the treatments, with values in the range of 0 mm for quercetin and 20.67 mm for cefotaxim (30 µg). Therefore, treatment with cefotaxime (30 µg) showed significantly superior antibacterial activity to other treatments. Also, a very important effect was found for the treatments with the other antibiotics, respectively gentamicin (10 µg) and ampicillin (10 mcg). Treatments with catechin 0.0290-2.9028%, as well as those with gallic acid (0.1881-1.8813%), had an antibacterial effect against *S. enterica* significantly equal to ampicillin (10 mcg) and superior to DMSO. The effect of 0.01-1% propolis had a higher effect compared to DMSO. *S. enterica* showed total resistance to quercetin-based treatments, regardless of the used concentration. *S. enterica* developed inhibition zones for first three concentrations of propolis (0.01-1%) and for all gallic acid and catechin concentrations.

Compared to DMSO, propolis had an inhibitory effect on microbial strains, which was also observed for catechin, quercetin and gallic acid (Tables 1, 2 and Figure 2).

Table 2. Antibacterial activity of the propolis, catechin, quercetin and gallic acid on *S. enterica, S. flexneri* and *S. aureus*

No	Treatments	Inhibition area (mm) of *S. enterica*	Inhibition area (mm) of *S. flexneri*	Inhibition area (mm) of *S. aureus*
1.	Ampicillin	11.33+1.45 bc	16.67+0.33 b	14.67+0.88 b
2.	Cefotaxim	20.67+2.19 a	21.00+1.15 a	27.67+1.20 a
3.	Gentamicin	13.33+0.88 b	12.67+1.45 c	14.33+1.67 b
4.	Propolis 1%	9.00+0.58 def	9.00+1.15 ef	9.67+0.88 cd
5.	Propolis 0.1%	8.67+0.88 def	8.67+0.67 ef	8.67+0.88 cde
6.	Propolis 0.01%	8.33+0.33 def	8.67+0.88 ef	7.67+0.67 de
7.	Propolis 0.001%	7.67+0.33 ef	8.33+0.67 ef	7.67+0.33 de
8.	Propolis 0.0001%	7.33+0.33 ef	8.00+0.58 f	0.00+0.00 f
9.	Propolis 0.00001%	7.00+0.00 f	7.67+0.33 f	0.00+0.00 f
10.	Gallic acid 1.8813%	10.00+0.00 cd	9.33+0.33 ef	11.00+2.08 c
11.	Gallic acid 0.1881%	10.00+0.58 cd	8.00+0.00 f	8.00+0.00 de
12.	Gallic acid 0.0188%	8.67+0.33 def	8.00+0.00 f	8.00+1.00 de
13.	Gallic acid 0.0018%	8.00+0.58 def	0.00+0.00 g	7.67+0.33 de
14.	Quercetin 3.0224%	0.00+0.00 g	9.33+0.33 ef	10.67+1.20 c
15.	Quercetin 0.3022%	0.00+0.00 g	9.00+0.58 ef	9.67+0.33 cd
16.	Quercetin 0.0302%	0.00+0.00 g	8.67+0.88 ef	9.00+0.58 cde
17.	Quercetin 0.0030%	0.00+0.00 g	8.67+0.88 ef	7.00+0.00 e
18.	Catechin 2.9028%	10.00+0.58 cd	11.33+0.67 cd	9.00+1.15 cde
19.	Catechin 0.2902%	10.00+0.00 cd	10.00+0.00 de	8.67+0.33 cde
20.	Catechin 0.0290%	9.33+0.33 cde	10.00+1.00 de	7.67+0.33 de
21.	Catechin 0.0029%	9.00+0.00 def	9.33+0.67 ef	0.00+0.00 f
22.	DMSO	7.67+0.33 ef	7.67+0.33 f	7.33+0.33 cde
	LSD 5%	*2.05*	*1.91*	*2.45*

Data are mean ±SE, n=3; Different letters indicates significance at $p < 0.05$

Following the antibacterial effect of different treatments against *S. flexneri*, it was found that cefotaxime (30 μg) was the most effective, with a significantly higher inhibition than the other products. This bacterium showed a higher sensitivity to ampicillin (10 mcg) compared to gentamicin (10 μg), but both antibiotics had a significantly higher antibacterial effect than treatments with propolis, quercetin and gallic acid. Propolis showed an antimicrobial effect against *S. flexneri* higher than DMSO but without statistically significance. The rest of the treatments had a significantly equal efficiency in terms of inhibiting this bacterium except catechin which showed a significantly higher antibacterial effect on this species than DMSO. The catechin highest concentration 2.9028% had an effect significantly higher compared to gentamicin. Biological activity against *S. flexneri* was observed for all concentrations of quercetin and catechin, 0.0188 - 1.8813% gallic acid and propolis at most concentrations.

Treatments with cefotaxime (30 μg), ampicillin (10 mcg) and gentamicin (10 μg) had the highest efficacy on *S. aureus*, considering the significantly higher inhibitory area. Treatments with 1% and 0.1% propolis had an antibacterial higher compared to DMSO. 1.8813% gallic acid and the high concentrations of quercetin and catechin had a significant antibacterial effect compared to DMSO. *S. aureus* had the highest inhibition area (D 11 mm) for 1.8813% gallic acid, followed by 1% propolis > quercetin (conc. 0.0302 - 3.0224%) > catechin (two concentrations) > gallic acid (0.0188 - 0.0018%).

Considering the results of the variance analysis (Table 3) it was pointed out that there are significant and statistically significant differences ($p < 0.01$) between the different treatments in terms of antibacterial activity on the six bacterial strains. The variation between repetitions showed a small and insignificant influence on the antibacterial activity of the 22 treatments.

Table 3. Analysis of variance (ANOVA) for antibacterial activity of the propolis, catechin, quercetin and gallic acid

Variation sources	DF	MS	F value	Variation sources	DF	MS	F value
E. coli				*S. enterica*			
Replication	2	2.29	1.92 ns	Replication	2	0.95	0.61 ns
Treatment	21	59.73	50.08**	Treatment	21	68.35	44.10**
Error	42	1.19		Error	42	1.55	
S. pyogenes				*S. flexneri*			
Replication	2	9.33	2.88 ns	Replication	2	3.45	2.52 ns
Treatment	21	150.71	46.49**	Treatment	21	43.70	32.37**
Error	42	3.24		Error	42	1.35	
P. aeruginosa				*S. aureus*			
Replication	2	1.88	1.31 ns	Replication	2	1.77	0.88 ns
Treatment	21	47.98	33.45**	Treatment	21	96.44	43.49**
Error	42	1.43		Error	42	2.22	

** - Significant at $p < 0.01$; ns - non significant at $p < 0.05$; DF - degree of freedom; MS - mean square

The two-dimensional diagram (biplot) based on the first two main components expresses 81.91% of the sensitivity variability for the six bacterial strains to the 22 treatments (Figure 1).

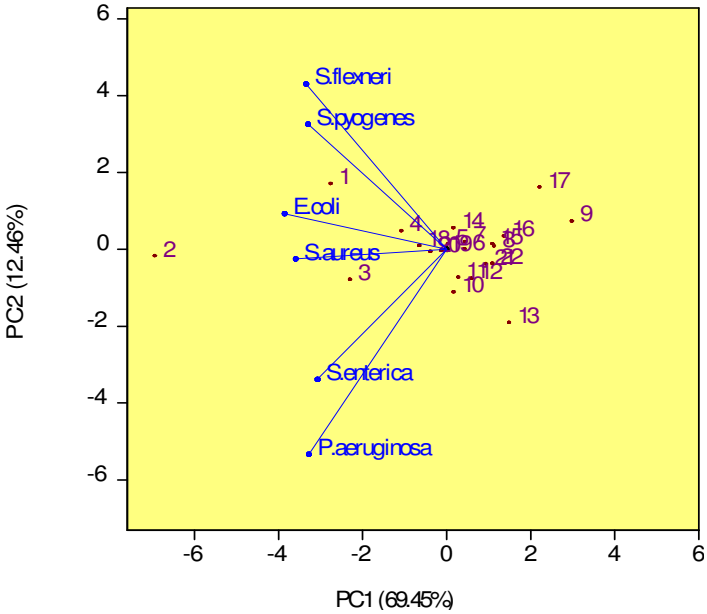

Figure 1. Biplot for antibacterial activity of the propolis, catechin, quercetin and gallic acid on different bacterial species

Considering the angles between the vectors of different species, it was found that in general the species *P. aeruginosa* and *S. enterica, E. coli* and *S. aureus*, respectively *S. flexneri* and *S. pyogenes*, showed a similar sensitivity to the applied treatments. It was also observed that ampicillin treatment (10 mcg) had the strongest antibacterial activity on *S. flexneri, S. pyogenes, E. coli* and *S. aureus* species, and an average efficiency on *P. aeruginosa* and *S. enterica* species. Treatment with gentamicin (10 μg) had the highest antibacterial effect on *P. aeruginosa* and *S. enterica* species, in association with superior antibacterial activity for the other species.

The variant 1% propolis had a high inhibition of *S. pyogenes*, *E. coli*, *S. aureus* and an average efficiency on *S. flexneri*, *P. aeruginosa* and *S. enterica*. The four gallic acid treatments showed a high antibacterial activity on *P. aeruginosa* and *S. enterica* and a reduced effect on the other species.

Quercetin had a high effect on *S. pyogenes*, *P. aeruginosa* and *S. aureus*. The influence of catechin was higher on *S. flexneri*, *E. coli* and *S. enterica*.

For a better evaluation of different compounds, the CLSI recommendations were taken into account.

Although in our experiments bacterial species showed high susceptibility to antibiotics, in relation to the CLSI it was observed that only four were in the sensitive category, namely: susceptibility to ampicillin (D ≥ 17 mm) - *S. pyogenes* (D 18.67 mm) and *E. coli* (D 18.67 mm); susceptibility to cefotaxime (D ≥ 23 mm) - *E. coli* (D 24.00) and *S. aureus* (D 27.67 mm) and susceptibility to gentamicin (D ≥ 15 mm) - *S. aureus* (D 15.67 mm) (Table 4).

Table 4. The effect of antibiotics on bacteria and yeast stains, compared to CLSI

Antibiotics	Bacterial and yeast strains / Inhibition zone (mm)					
	A	B	C	*D*	E	F
1. Diam. (mm) [Ampicillin]	17 (S)	18.67 (S)	7.33 (R)	11.33 (R)	16.67 (I)	14.67 (I)
2. Diam. (mm) [Cefotaxime]	24 (S)	21 (I)	19.33 (I)	20.67 (I)	21 (I)	27.67 (S)
3. Diam. (mm) [Gentamicin]	10.33 (R)	13.33 (I)	15.67 (S)	13.33 (I)	12.67 (R)	14.33 (I)
4. Diam. (mm) [Nystatin]	-	-	-	-	-	-

Legend: S - susceptible; R - resistant; I - intermediate.
Break point values (bacteria): ampicillin ≥ 17 mm S, 14-16 mm I, ≤ 13 mm R; cefotaxime ≥ 23 mm S, 15-22 mm I, ≤ 14 mm R; gentamicin ≥ 15 mm S, 13-14 mm I, ≤ 12 mm R (CLSI, 2011);
Bacterial strains: A - *E. coli*; B - *S. pyogenes*; C - *P. aeruginosa*; D - *S. enterica*; E - *S. flexneri*; F - *S. aureus*; G - *C. albicans*.

Biological activity against bacteria was also observed for propolis at the highest concentrations, 1% and 0.1% (Table 1, 2 and 3). According to these standards the bacterium *S. pyogenes* was considered sensitive for 1% propolis because its inhibition zone (17.33 mm) ranged into ampicillin sensitivity class (D ≥ 17 mm). The 0.1% propolis concentration also negatively influenced this bacterial strain (D = 13.67 cm) within the range of 13-14 mm, corresponding to the intermediate class of gentamicin. In the same class was included *E. coli*, with D = 13.37 mm.

Further on, *C. albicans* species was evaluated. It was shown that the inhibited area had amplitude of 14.33 mm, between 14.33 mm for nystatin treatment (100 units) and 0 mm for 10 treatments. Therefore, it was observed that nystatin (100 units) showed a significantly higher efficacy compared to the other treatments. A high inhibitory activity was also observed for the treatments with: catechin 2.9028%, propolis 1%, formic acid (0.5%) and quercetin 3.0224%. *C. albicans* species has been shown to be resistant to 10 of the treatments (Table 5).

Considering the propolis treatment, it was observed that the progressive decrease of the concentration determines a significant reduction of the inhibitory activity, the effect being cancelled at 0.001-0.00001% concentration. Also, in the case of gallic acid treatments the reduction of the concentration was associated with a significant decrease of the inhibition, which is completely cancelled at 0.0018%. For quercetin treatments, the same trend was observed, the antifungal effect being zero at 0.0302-0.003%. Catechin treatment showed an inhibitory effect only at concentrations of 2.9028 and 0.2902%.

Table 5. The effect of flavonoids, gallic acid and propolis on *C. albicans*

No	Treatments	Inhibition area (mm)	No	Treatments	Inhibition area (mm)
1.	Nystatin	14.33+0.88 a	12.	Gallic acid 0.0188%	7.67+0.33 f
2.	Formic acid	11.00+0.58 bc	13.	Gallic acid 0.0018%	0.00+0.00 g
3.	Propionic acid	0.00+0.00 g	14.	Quercetin 3.0224%	11.00+0.58 bc
4.	Propolis 1%	11.67+0.88 bc	15.	Quercetin 0.3022%	7.33+0.33 f
5.	Propolis 0.1%	9.33+0.33 de	16.	Quercetin 0.0302%	0.00+0.00 g
6.	Propolis 0.01%	8.33+0.88 ef	17.	Quercetin 0.0030%	0.00+0.00 g
7.	Propolis 0.001%	0.00+0.00 g	18.	Catechin 2.9028%	12.00+1.00 b
8.	Propolis 0.0001%	0.00+0.00 g	19.	Catechin 0.2902%	10.33+0.88 cd
9.	Propolis 0.00001%	0.00+0.00 g	20.	Catechin 0.0290%	0.00+0.00 g
10.	Gallic acid 1.8813%	10.33+0.67 cd	21.	Catechin 0.0029%	0.00+0.00 g
11.	Gallic acid 1.8813%	8.67+0.67 ef	22.	DMSO	0.00+0.00 g
	LSD 5%	*1.43*		*LSD 5%*	*1.43*

Data are mean ±SE, n=3; Different letters indicates significance at $p < 0.05$

Regarding yeast, the report to CLSI showed that the values of the inhibition zones determined by nystatin (D 14.33 mm), propolis (D 8.33-11.67 mm) and the three compounds were not within the standard limits (D 15-23 mm). But it should be noted that both propolis and compounds have antifungal activity against *C. albicans*, compared to DMSO (Table 4, Figure 2).

Thus, propolis and gallic acid showed biological activity for the first three concentrations, and quercetin and catechin for the first two concentrations. Catechin (conc. 2.9028%) had the highest antifungal effect (D 12 mm) followed by propolis 1% (D 11.67 mm) ≥ quercetin 3.0224% (D 11 mm) ≥ gallic acid 1.8813% and catechin 0.2902% (D 10.33 mm) ≥ propolis 0.1% (D 9.33 mm) ≥ propolis 0.01% (D 8.33 mm). The highest areas of inhibition were observed at the first concentrations of catechin, propolis and quercetin, and the lowest at gallic acid 0.0188% and quercetin 0.3022%. Besides nystatin, a good antifungal was 0.5% formic acid (D 11 mm) (Figure 2).

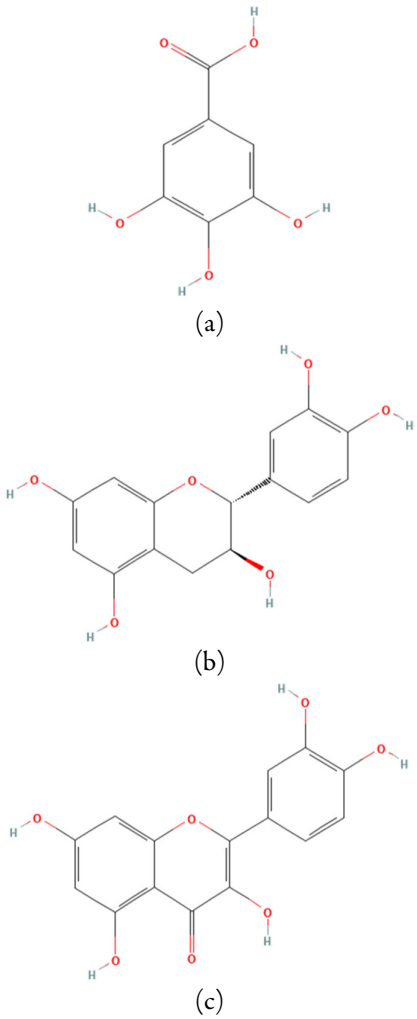

Figure 2 Chemical structure of gallic acid (a) (NCBI2) catechin (b) (NCBI3) and quercetin (c) (NCBI1)

Discussion

Propolis, catechin, gallic acid and quercetin are natural antimicrobial products that carry out the same influence on microorganisms as synthetic antibiotics (e.g., they interfere with the cell structures and metabolic processes of microorganisms, affecting their viability and growth). Abachi *et al.* (2016) mentioned that natural antimicrobial products do not necessarily have a bactericidal effect but their actions determine the suppression of microorganisms.

In line with the literature data, our study shown that propolis have an inhibitory effect on both groups of studied microorganisms (Ramanauskiene *et al.*, 2009; Huang *et al.*, 2014). The efficacy of the oily propolis extract (where MIC was 0.8%) in DMSO against Gram-positive and Gram-negative bacteria (e.g., S. aureus ATCC 25923, *P. aeruginosa* (PAO1), *E. faecalis* ATCC 29212, *S. aureus* S3) was relieved by Ghasemi *et al.* (2017). Contrary to previous statements, other authors have observed that propolis is more active against Gram-positive bacteria (Mohdaly *et al.*, 2015) and it has less influence on Gram negative ones, which can even be resistant (Ghasemi *et al.*, 2017). The same effect was observed by Al-Ani *et al.* (2018) on propolis samples originated from different parts of Europe. They showed that propolis has an antibacterial effect against Gram-

positive bacteria (MIC between 0.08 mg / ml to 5 mg / ml). The decreasing scheme of the bactericidal action of propolis is as follows: Irish propolis > Czech propolis > German propolis.

The antimicrobial effect, including the low concentrations, was also supported by Mohdaly *et al*. (2015). In their studies, they referred to a MIC between 0.20 - 0.63 mg/mL for Gram negative and 0.20 - 0.78 mg/ml for Gram-positive bacteria. Although in this study the significance of the propolis inhibitory activity was maintained even at lower concentrations, it can be considered that the best biological activity was observed at the first three concentrations (0.01-1%).

In line with our observations, Bayram *et al*. (2017), have highlighted the sensitivity of Gram positive and Gram-negative microorganisms (exception *K. pneumoniae*) to propolis (the MIC varied between 25 to 200 mg/mL).

Therefore, the application of 1% propolis determined a high inhibition of Gram-positive and Gram-negative microbial species *S. flexneri*, *S. pyogenes*, *E. coli*, *C. albicans* and *S. aureus*, and had an average efficiency on Gram-negative species *P. aeruginosa* and *S. enterica*. This lower efficiency on Gram-negative bacteria has also been attributed to the more complex composition of the cell wall and the higher lipid content (Stepanović *et al*., 2003; Veiga *et al*., 2017).

The sensitivity of *S. pyogenes* and *E. coli* was maintained at concentrations of 0.001-0.1% propolis, but there was an insignificant regression in the case of other microbial species, the effect being cancelled for *E. coli* at 0.00001% concentration. A possible explanation for the different levels of bacterial and fungal sensitivity to propolis would be the propolis phytochemical profile, variation and synergistic action of bioactive components (phenolic compounds) (Onlen *et al*., 2007; Jug *et al*., 2014; Przybyłek and Karpiński, 2019; Kharsany *et al*., 2019), but also the nature of the microorganism. Studies on European propolis have concluded that flavonoids and phenolic acids quantitatively dominate, explaining the antimicrobial activity observed in our experiments (De Groot *et al*., 2013; Berwal *et al*., 2019). This section does not look like Results.

S. pyogenes and *B. subtilis* were Gram-positive microorganisms extremely sensitive to propolis extract. The explanation was based on the structural differences of the cell wall. Some authors focus on the specific structure of the outer membrane of Gram-negative bacteria and the large amount of hydrolytic enzymes that break down the active ingredients of propolis (Sforcin *et al*., 2016; Al-Ani *et al*., 2018).

The increased biological activity of propolis against *S. pyogenes* and *E. coli* mentioned in the above scheme it is also supported by the researches carried out by Buriol *et al*. (2009) and Sawaya *et al*. (2004).

This knowledge is of particular importance, considering that *S. pyogenes* is one of the infectious microbial agents with antibiotic resistance (Berwal *et al*., 2019] that causes significant morbidity and records a high mortality of 10-30% worldwide, (Carapetis *et al*., 2005; Walker *et al*., 2014; Laabei and Ermert, 2019). Likewise, *E. coli* is considered by the WHO to be one of the bacteria that threatens human health (WHO, 2017).

There are some literature data referring to the reduced or non-existent activity of propolis against *E. coli* (Kosalec *et al*., 2005; Gonsales *et al*., 2006; Seidel *et al*., 2008). Regarding the antibacterial effect of propolis against *S. pyogenes*, our studies are in accordance with those presented by Fokt *et al.* (2010).

Like our studies, Özkalp and Özcan (2010) showed that propolis extract has a greater inhibitory effect on tested bacteria compared to pollen. The same authors point out that propolis (400 and 600 ppm) has an antibacterial effect comparable or even higher to some antibiotics (Özkalp and Özcan, 2010). The sensitivity of *S. pyogenes* and *E. coli* was maintained at concentrations of 0.001-0.1% propolis, but there was an insignificant regression in the case of other microbial species, the effect being cancelled for *E. coli* at 0.00001% concentration. A possible explanation for the different levels of bacterial and fungal sensitivity to propolis would be the propolis phytochemical profile, variation and synergistic action of bioactive components (phenolic compounds) (Onlen *et al*., 2007; Jug *et al*., 2014; Przybyłek and Karpiński, 2019; Kharsany *et al*., 2019), but also the nature of the microorganism.

Also, the antifungal effect of propolis is confirmed by Saeed *et al*. (2016). In addition, the results obtained by AL-Ani *et al*. (2018) on a wide range of clinically isolated or reference strains of the Candida genus attest an antifungal MIC in the range of 0.6–2.5 mg/mL or fungicide effect of propolis (MFC in the range of 0.1 - 2.5 mg/mL), which supports the idea of propolis interference with yeast virulence factors (Bezerra *et al*., 2020). In terms of antimicrobial efficacy, 1% propolis was especially noticeable in our studies. For a clearer picture of the microbial strains position on the sensitivity scale to propolis 1% a descending order was made: *S. pyogenes* > *E. coli* > *C. albicans* > *S. aureus* > *S. flexneri* > *P. aeruginosa* and *S. enterica*.

Although the areas of inhibition produced by the three compounds (gallic acid, quercetin, catechin) were smaller compared to propolis, their antimicrobial potential is not negligible, but the concentration should be taken into account, because the biological action is reducing proportionally to the concentration.

Due to their complex structure, all three studied polyphenolic compounds and propolis produce irreversible changes in membrane properties (charge, intra and extracellular permeability, and physicochemical properties) through hydrophobicity changes, decrease of negative surface charge, and occurrence of local rupture or pore formation in the cell membranes with consequent leakage of essential intracellular constituents (Borges *et al*., 2013; Araya-Cloutier *et al*., 2018).

From the structural formulas of the three studied compounds, it is observed that gallic acid and catechin (Figure 2) are distinguished by the multitude of phenolic hydroxyl groups that are recognized to have antiproliferative and proapoptopic effect in different cell types. The phenolic hydroxyl groups of catechins and gallic acid are primarily responsible for scavenging free radicals, whereas the galloyl moiety is involved in chelating metal ions. The galloyl moiety appears to be required both for the antiproliferative, apoptotic and antioxidant effects, but there is no clear structure-activity relationship (Braicu *et al*., 2011).

Gallic acid is the subject of biological and pharmacological reports due to its antimicrobial and antiviral action determined by some structural parameters (chemical structure, position and number of hydroxyl groups) (Borges *et al*., 2013; Choubey *et al*., 2015).

The inhibitory effect of gallic acid on Gram-negative bacteria is attributed to the capacity of the outer cell membrane disintegration (Nohynek *et al*., 2006). *P. aeruginosa* and *S. enterica* showed high sensitivity to all concentrations of gallic acid. The antibacterial activity of gallic acid against *P. aeruginosa* was also reported by Borges *et al*. (2013), at MIC of 500 μg/mL. The high sensitivity of bacterial species can be determined by irreversible changes in membrane properties, loss of mobility, blockage of surface fixation and biofilm formation (Kang *et al*., 2008; Borges *et al*., 2012; Shao *et al*., 2015).

Although gallic acid can destroy the integrity of Gram-positive and Gram-negative bacterial membranes (Shao *et al*., 201), *S. pyogenes* (Gram-positive bacteria) is resistant to gallic acid. Contrary to these observations, Tafesh *et al*., (2011) noted that gallic acid was effective against Gram-positive bacteria *S. pyogenes* and *S. aureus* at concentrations of 200-400 μg mL^{-1}, but had no inhibitory effect on Gram negative bacteria.

Quercetin (Figure 2), present mainly in propolis and onions has chemoprotective properties. According to Gopu *et al*. (2015) it may be a promising antibacterial agent against some Gram-negative species, due to interrupting of the quorum sensing signalling and biofilm formation.

Our studies emphasize that *S. enterica* (Gram negative bacterium) was resistant to all concentrations of quercetin, and *S. pyogenes* only to the last three concentrations (0.3022% - 0.003%). Instead, this last bacterial strain was sensitive to the highest concentration of quercetin (3.0224%), as evidenced by the following scheme: *S. pyogenes* > *C. albicans* > *P. aeruginosa* > *E. coli* > *S. flexneri* and *S. aureus*.

Actually, Siriwong *et al*. (2015) state that quercetin at MIC 128 g/mL interferes with the cytoplasmic membrane of the bacterium *S. pyogenes* and modifies its selective permeability, a hypothesis proven by a slight inhibition of growth.

Research done by Wang *et al*. (2018) showed that quercetin had a stronger inhibitory effect on Gram-positive bacteria compared to Gram-negative bacteria (*S. enterica*, *P. aeruginosa*, *E. coli*). On the contrary, Božič

et al. (2012) consider that quercetin has a high bacteriostatic effect against Gram-negative bacteria *S. enterica* and *E. coli*, arguing the ability of quercetin to inhibit D-alanine ligase, preventing bacterial growth (Wu *et al*., 2008).

The antimicrobial image given by 2.9028% catechin highlights the following descending order: *C. albicans* > *S. flexneri* > *E. coli* and *S. enterica* > *S. pyogenes* and *P. aeruginosa* > *S. aureus*, considering that the antibacterial and antifungal properties of this compound are also demonstrated by other authors (Hirasawa and Takada, 2004; Sitheeque *et al*., 2009; Reygaert, 2018).

Catechin is the best antifungal at 2.9028% concentration and a high bacteriostatic action against *S. flexneri* and *S. enterica* was observed at 0.2902%. In accordance to our research, the strong antibacterial activity of catechin (MIC between 0.025 and 0.1, respectively MBC 0.05–0.2 mg/mL) against enteric bacteria (*S. enterica*, *S. flexneri*, *E. coli*) was also demonstrated by the results obtained by Bhattacharya *et al*. (2016).

Catechin maintains its inhibitory effect on yeast even at 0.2902% concentration. With the reduction of the propolis concentration, gallic acid and quercetin, the antifungal effect is significantly reduced, until the complete cancellation at the last 2-3 concentrations (the case of propolis), a fact also proved by Awale *et al*. (2005).

Conclusions

The antibacterial and antifungal activity of propolis, catechin, gallic acid and quercetin was relevant (at some concentrations) even compared to conventional antibiotics.

Propolis and the three compounds can be considered natural antibiotics, due to their antimicrobial effect at the concentrations mentioned in the results chapter and they can supplement the antibiotics ampicillin and gentamicin.

The report of the results to the CLSI data confirms that the first two concentrations of propolis (0.1 - 1%) showed antimicrobial activity against *S. pyogenes* and *E. coli*, comparable to ampicillin (D ≥ 17 mm.) and gentamicin (D 13-14 mm) respectively. This result is of particular importance, since the two species represent a real problem for human health. Therefore, propolis has a higher antimicrobial activity compared to catechin, quercetin and gallic acid.

Authors' Contributions

Conceptualization, A.B.B. and I.G.; methodology, A.B.B. and I.G.; Analyzed the data: A.C.; investigation, A.B.B. and I.G.; resources, A.B.B. and I.G.; writing - original draft preparation, A.B.B., I.G. and C.M.; writing - review and editing and translation, A.B.B., I.G. C.M. and S.P.; supervision, A.B.B., S.P. and E.M.; funding acquisition, E.M.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research paper is supported by the project "Increasing the impact of excellence research on the capacity for innovation and technology transfer within USAMVB Timisoara", project code 6PFE, submitted in the competition Program 1—Development of the national system of research-development, Subprogram 1.2-Institutional performance, Institutional development projects-Development projects of excellence in R.D.I.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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