

Preliminary Studies on the Antibacterial Effect of the Assortment of Polyfloral Honey from the Western Part of Romania

Alexandru Nan¹, Roxana Vasluianu¹, Ion Valeriu Carabă¹,
Nicoleta Marioara Filimon², Gabi Dumitrescu^{1*}

¹Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Calea Aradului, 119, 300645-Timisoara, Romania

²West University of Timisoara, Faculty Chemistry-Biology-Geography, Pestalozzi, 16, RO 300315-Timisoara, Romania

Abstract

Honey is a bee product that has antimicrobial activity, between different types of honey there being differences in terms of effectiveness and mechanisms of action against the same type of microorganism. Given the constant increase in the number of antibiotic-resistant bacteria and the need to find natural alternatives to them, the purpose of this study was to evaluate the antibacterial activity of polyfloral honey in western Romania. Honey was tested in the following concentrations: 80%; 60%; 40%; 20%; 10%; 5%. Two Gram+ bacterial strains (*Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619) and three Gram- strains (*Enterococcus faecalis* ATCC 29213, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028) were used in the study. The results of this study show that polyfloral honey from the western part of Romania has an antiproliferative effect on the bacterial strains tested, depending on the applied concentration. The highest inhibition rates were recorded on *Salmonella typhimurium*, *Streptococcus pneumoniae* and *Staphylococcus aureus*, and the lowest values were recorded in the bacterial strains *Enterococcus faecalis* and *Escherichia coli*.

Keywords: antimicrobial activity, Gram+ bacteria, Gram- bacteria, honey, inhibition rate

1. Introduction

Bee honey is a bee product obtained after harvesting nectar by bees, processed and stored by them in honeycomb cells, representing food for the hive population. This bee product has a complex chemical composition, containing about 200 substances represented mainly by water (about 20%), sugars (80-85%) and other substances such as: proteins (enzymes), organic acids, vitamins (especially vitamin B6, thiamine,

niacin, riboflavin and panthenic acid), minerals (calcium, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), pigments, phenolic compounds and a variety of volatile compounds [1, 2].

Honey is well known as a natural antioxidant, the components responsible for the redox properties of honey being fulfilled by phenolic acids, flavonoids, vitamins and enzymes, as well as a small amount of minerals, especially copper and iron. In addition, in the last two decades, research in the field of apitherapy has intensified, so that new valences of these products with various pharmacological properties, with antioxidant potential have been discovered [3], antitumor, antimicrobial, antifungal, antiallergic, anti-inflammatory, hepatoprotective, in the treatment

* Corresponding author: Gabi Dumitrescu
Tel. 0256 277115
Email: gdumitrescu@animalsci-tm.ro

of cardiovascular diseases, digestive diseases, diabetes, etc. [4-8].

At present, worldwide, antibiotic resistance is a serious problem, as a result of which new compounds with natural antimicrobial properties are constantly being sought [9, 10]. Numerous in vitro studies have shown the antimicrobial activity of various types of honey against a wide range of skin colonizing bacteria and food bacterial species, including antibiotic-resistant bacteria [11-15]. It has also been shown in vivo that honey has beneficial actions against wound infections [16]. The antimicrobial activity of honey is an extremely complex process, determined by the physico-chemical properties of honey [17, 18], such as low pH, high osmolarity, hydrogen peroxide [19, 20], defensin - 1 and polyphenols [14]. Most often honey is acidic (pH below 7), the pH of flower honey ranging between 3.03 and 5.5-6.0 [21-23], depending on the botanical source, the pH of the nectar, the combination of soil or plants and the concentration of various acids and minerals such as calcium, sodium, potassium and other constituents of ash. In general, the lower the pH value of honey, the higher the activity of inhibiting the activity of microorganisms [17]. The high concentration of sugars (about 80% by weight of this product) is an important factor in the elimination of microorganisms, mainly bacteria sensitive to high osmotic pressure. Defensin-1, a peptide secreted by bee's hypopharyngeal glands, has activity against Gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Paenibacillus*. This protein could be used to treat infections and in the development of new drugs that could fight antibiotic-resistant bacteria [24].

Another constituent of honey, with a strong antibacterial effect, is the enzyme glucose oxidase, an oxidoreductase that catalyzes the oxidation of glucose into gluconic acid. The by-product of this reaction, hydrogen peroxide (H_2O_2), is a powerful antimicrobial agent. However, the concentration of hydrogen peroxide varies depending on the floral source and the geographical area [25], respectively on the environmental conditions, and these differences are reflected in the value of the minimum inhibitory concentration (MIC) required for inhibit different bacteria (<3% - \geq 50%) [26]. Recent studies [27, 28] showed that H_2O_2 is the main compound involved in conferring the antibacterial effect of European honey. However,

some studies have shown that H_2O_2 levels in different types of honey have not been directly correlated with antibacterial activity [24]. More importantly, by degrading H_2O_2 from various honeys, hydroxyl radicals are generated, involved in the generation of oxidative stress in bacterial cells [25].

In addition, phenolic compounds or polyphenols in the structure of honey may contribute to its antimicrobial activity [5]. They represent a heterogeneous chemical group, containing about 10,000 compounds, which include phenolic acids and flavonoids, secondary metabolites, derived from plants [29]. Phenolic compounds such as quercetin, caffeic acids, caffeic acid phenethyl ester (CAPE), acacetin, kaempferol, galangin, chrysin, pinocembrine, pinobanksin apigenin have been found in honey and have a promising effect in the treatment of chronic diseases [30]. Phenolic compounds have an antioxidant effect, acting by eliminating free radicals and inhibiting lipid oxidation [31], but much research has shown that they are also responsible for the antimicrobial properties of honey.

Hydrolysis of H_2O_2 generates oxygen, which can accelerate the self-oxidation process of honey polyphenols. These, in turn, becoming pro-oxidizing agents, can lead to the generation of other H_2O_2 molecules, which, in the presence of transition metals, can generate hydroxyl radicals [32]. In this way, polyphenolic compounds can significantly contribute to the modulation of the antibacterial effects of honey.

Given the context presented and that the antibacterial potential of honey becomes increasingly important as the number of antibiotic-resistant bacteria steadily increases, which poses a health threat, the purpose of this study was to evaluate the antibacterial activity of polyfloral honey from the western part of Romania.

2. Materials and methods

Biological material tested

The biological material tested was an assortment of polyfloral honey certified as organic, from a beehive located in the mountainous area of western Romania, in the area of Făget. The honey was collected in sterile culture tubes and was stored at 2-8°C until use.

Experimental variants

Honey was diluted with sterile distilled water and the following 5 concentrations were achieved: c1 - 80%; c2 - 60%; c3 - 40%; c4 - 20%; c5 - 10%; c6 - 5%.

Microorganisms used in the study

The study of the effect of polyfloral honey of different concentrations was performed by using 6 standardized bacterial strains: two bacterial strains Gram+ (*Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619) and three strains Gram- (*Enterococcus faecalis* ATCC 29213i, *Escherichia coli* and *Salmonella typhimurium* ATCC 14028).

Testing the viability of microorganisms

100 µl of microbial culture and 50 µl of polyfloral honey solution from each tested concentration were introduced into the wells of the microplates. The microplates were incubated for 6 hours at a temperature of 37°C. After this time interval 10 µl 0.5% TTC solution was introduced into each well and incubation was continued for another 2 hours at the same temperature of 37°C. During this period of time the TTC solution interacts with the living, viable bacterial cells, not killed by the

biologically active compounds from the tested polyfloral honey. The results were read on the TECAM SUNRISE micro spectrophotometer at a wavelength of 460 nm. Based on the values of the inhibition rates recorded for each bacterial strain and concentration of polyfloral honey tested, the values of the final inhibition rates were calculated [33], as all tests were performed in triplicate.

3. Results and discussion

Following the antiproliferative effect of polyfloral honey on the *Staphylococcus aureus* bacterial strain, it is found that once with the decrease of the tested honey concentration, also decreases the effect of inhibiting the development of the bacterial strain. Inhibition rates ranged from 67.20% to 47.45%, therefore the results of the antiproliferative test suggest that the inhibitory effects of honey on *S. aureus* are manifested in a concentration-dependent manner. In general, values of inhibition rates of over 50% show an antibacterial effect for the biological product tested. The antiproliferative effect of honey on this bacterial strain is present at all concentrations tested [Figure 1].

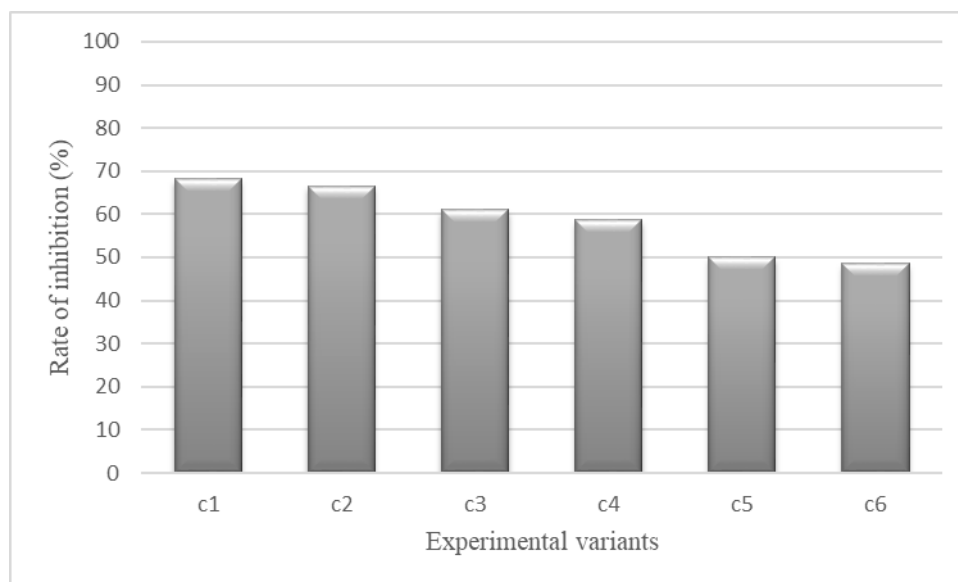


Figure 1. Rate of inhibition of the action of polyfloral honey on the bacterial strain *S. aureus*

In the case of the bacterial strain *Streptococcus pneumoniae*, the added polyfloral honey led to the recording of values of inhibition rates between 70.20% and 50.60%. Because the values of inhibition rates are over 50%, regardless of the

concentration of honey tested, we can say that the antibacterial effect is manifested with a moderate sensitivity, which increases with increasing concentration of polyfloral honey tested [Figure 2].

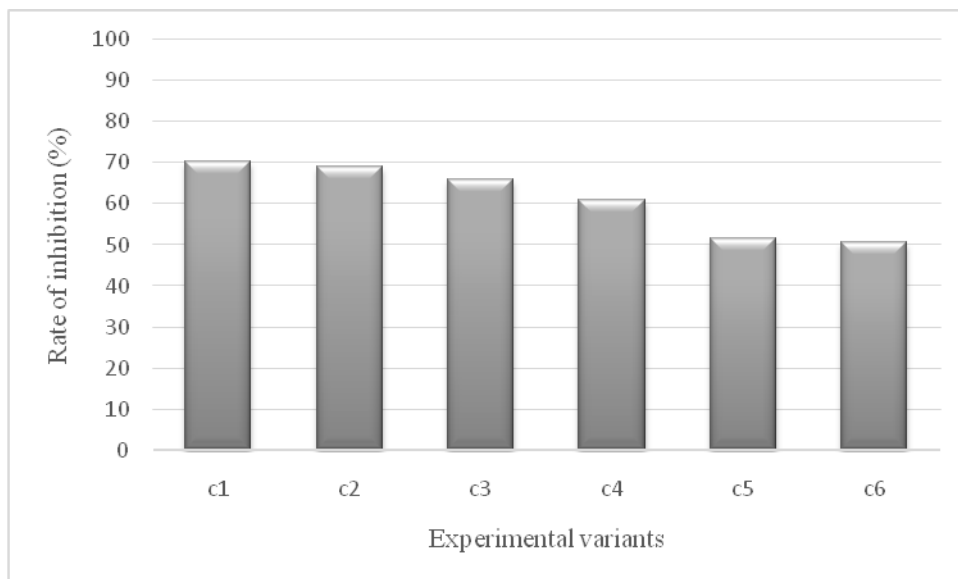


Figure 2. Rate of inhibition of the action of polyfloral honey on the bacterial strain *S. pneumoniae*

In the case of the bacterial strain *Enterococcus faecalis*, the values of the inhibition rates were between 51.50 and 16.30%. The decrease of the tested polyfloral honey concentration determined a decrease of the bacterial antiproliferative effect. The values recorded for the inhibition rates showed an antibacterial effect with moderate

intensity only when applying the concentrations c1, c2, c3 [Figure 3]. Analyzing comparatively the antiproliferative effect determined by polyfloral honey on the bacterial strains of interest, the lowest values for the inhibition rate were registered in the case of the bacterial strain *E. faecalis*.

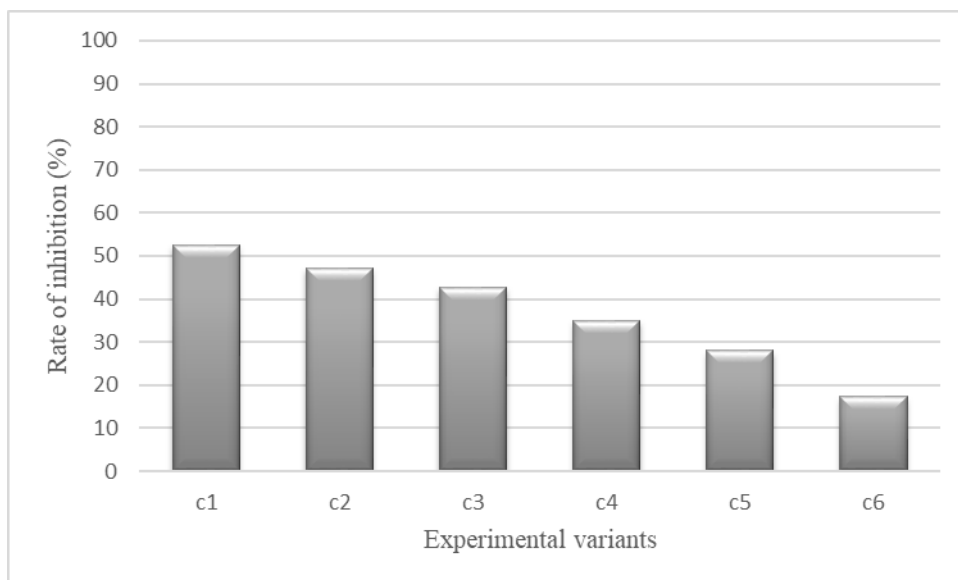


Figure 3. Rate of inhibition of the action of polyfloral honey on the bacterial strain *E. Faecalis*

In the case of the bacterial strain *Escherichia coli*, the values of the inhibition rates were between 55.45-25.21%. The antibacterial effect was manifested only when applying polyfloral honey in concentrations of 80%, 60%, 40% (c1-c3), and

starting with the concentration of 20% (c4), the decrease of the antibacterial effect of honey on the strain of interest was registered with decrease in the concentration of polyfloral honey tested [Figure 4].

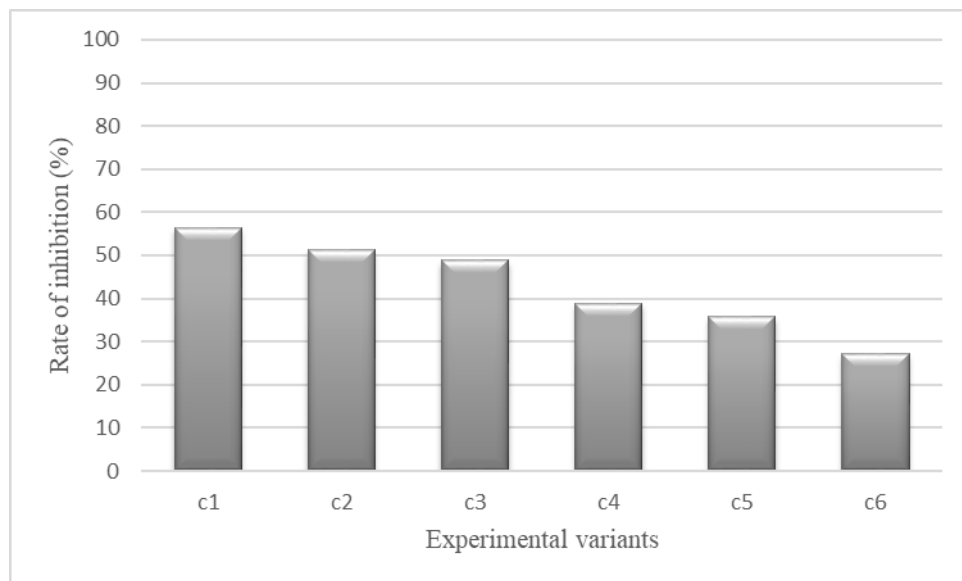


Figure 4. Rate of inhibition of the action of polyfloral honey on the bacterial strain *E. Coli*

Following the antiproliferative effect of polyfloral honey on the bacterial strain *Salmonella typhimurium*, it is found that the antiproliferative effect is manifested at all tested concentrations, but with the decrease of the tested honey concentration decreases the inhibitory effect of

bacterial strain development. Inhibition rate values ranged from 74.90-49.75% [Figure 5]. In general, values of inhibition rates of over 50% show a bacterial antiproliferative effect for the biological product tested.

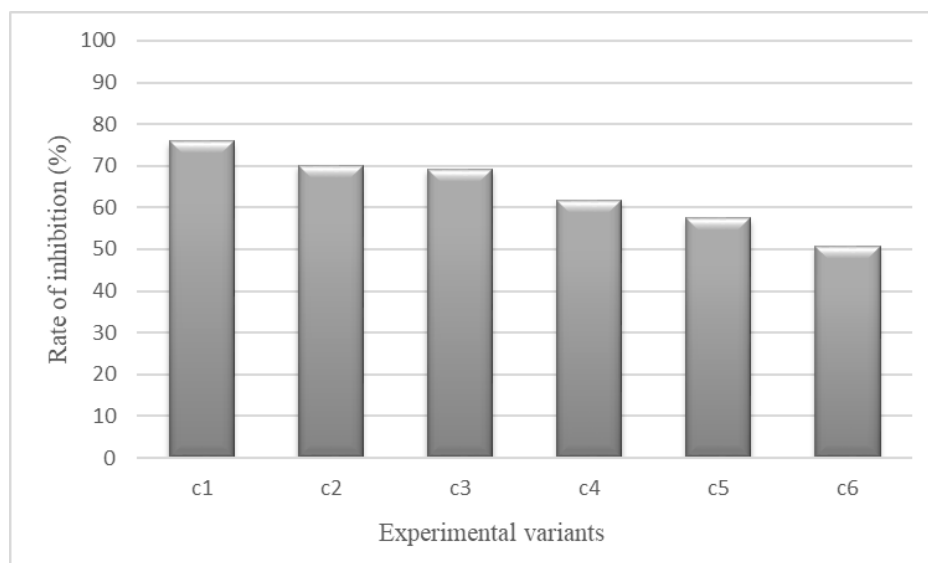


Figure 5. Rate of inhibition of the action of polyfloral honey on the bacterial strain *S. typhimurium*

Analyzing the results of our study, it is found that polyfloral honey, from the western part of Romania, has an antiproliferative effect on the tested bacterial strains, in a dose-dependent manner. The highest inhibition rates recorded, as a result of the application of polyfloral honey, were obtained at concentrations of 80%, 60% and 40% (c1, c2, c3) on the strains of *S. typhimurium*, *S.*

pneumoniae and *S. aureus*. In the case of the bacterial strain *E. faecalis* and *E. coli*, the values of inhibition rates were between 51.50-16.30% and 55.45-25.21%, respectively, an antibacterial effect with moderate intensity of polyfloral honey, being registered only when applying the concentrations c1, c2 and c3, respectively 80%, 60% and 40%. Analyzing, by comparison, the

lowest antiproliferative effect was recorded in the case of the bacterial strain *E. faecalis*. Studies by Lusby et al., [34], Wilkinson and Cavanagh [35], have shown that the antimicrobial activity of honey can vary from concentrations lower than 3% to 50% and higher. Adeleke et al., [2006] report that the bactericidal effect of honey depends on both the concentration of honey used and the bacterial strain tested. The concentration of honey administered has an impact on antibacterial activity, so that, at high concentrations, the chances of honey being a very good antibacterial agent increase [36]. Taormina et al. [37], reported that the concentration of honey required to completely inhibit the growth of the bacterial strain *S. typhimurium* is <25%. Studies on bacterial species that cause gastric infections: *S. typhi*, *S. flexneri* and *E. coli*, have shown that Tualang honey is highly effective against these bacteria and can help accelerate recovery in infections caused by them [38], when administered orally in its pure undiluted form. Another study showed that honey has a fairly pronounced inhibitory effect (85.7%) on Gram-bacterial strains such as *Pseudomonas aeruginosa*, *Enterobacter spp.*, *Klebsiella spp.* compared to a number of commonly used antimicrobial agents. A 100% inhibition was also observed in the methicillin-resistant bacterial strain Gram+ *S. aureus* compared to commonly used antibiotics. A synergistic effect was obtained when honey was applied together with antimicrobial agents in both Gram- and Gram+ bacteria [39]. There are differences between the different types of honey in terms of effectiveness and mechanisms of action against the same type of microorganism. In this sense, Manuka honey has the best antimicrobial performance. This is in line with what has been reported by other authors who have used honey as an agent to inhibit the form of *S. aureus* biofilm [40]. It has also been reported that the use of honey has a beneficial effect against both Gram + bacterial strains: *S. aureus*, *Bacillus cereus*, *Bacillus subtilis*, *E. faecalis*, *Micrococcus luteus* and Gram- bacteria: *E. coli*, *P. aeruginosa*, *Salmonella typhi* [41]. Mohammedaman Mama et al., [42] report values of the 70% inhibition rate of a higher number of microorganisms tested, at a honey concentration of 18.75% v/v.

This aspect is similar to the results obtained in our study, where the sensitivity of bacteria to

polyfloral honey depends very much on the concentration at which it was applied.

In general, the unpredictable antibacterial activity of non-standard honey can prevent its introduction as an antimicrobial agent due to the variation of the in vitro antibacterial activity of different varieties of honey. Carrying out in vitro studies, well-founded and scientifically documented, may lead to the proposal of microbiological certification of honey and other bee products.

4. Conclusions

The tested polyfloral honey showed antibacterial effect on all bacterial strains of interest, the values of inhibition rates being correlated with the applied dose. The antibacterial effect manifested at all 6 concentrations of polyfloral honey tested was registered in the case of the bacterial strains Gram + *S. aureus* and *S. pneumoniae*, respectively on the bacterial strain Gram- while in the case of the bacterial strains Gram- *E. coli* and *E. faecalis* had the lowest values of the inhibition rate.

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