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ASSESMENT OF THE ANTIOXIDANT POTENTIAL OF PROPOLIS FROM DIFFERENT GEOGRAPHICAL ORIGINS AND POSSIBLE MEDICAL APPLICATION

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ABSTRACT

Propolis is a natural resinous substance produced by bees by collecting substances from plant parts. Propolis has antibacterial, antifungal, anti-inflammatory, antitumor effects on the human organisms. Flavonoids are the one of basic component of propolis, but propolis also contains a number of organic acids, mineral substances, vitamins, amino acids, terpenes and hydrocarbons. Antioxidants are substances with function of protection cells from the oxidizing action of free radicals. The most important antioxidants are: vitamins A, E, C, zinc, selenium, polyphenols. Polyphenols have large antioxidant activity. They include about 8000 compounds with different chemical structures. Flavonoids are polyphenolic compounds and act as the main biologically active compounds of propolis. In this research werw used spectrophotometric methods, FRAP and FC for determination of antioxidant capacity of propolis and the concentration of total polyphenols in propolis. The FRAP method is based on the ability of antioxidants to donate electrons in an acidic medium (pH = 3.6) to reduce the yellow complex of ferric iron (Fe3+) with TPTZ into a blue-colored Fe2+-TPTZ complex. The intensity of the blue color at 593nm is measured. The intensity of the color is proportional to the concentration of antioxidants. The results are expressed in μMFe(II)/L. The FC method is based on oxidation and reduction reactions, i.e. on the colorimetric reaction of phenol with the Folin-Ciocalteu reagent, during which the color changes from yellow to dark blue. Total phenols are determined as a good indicator of antioxidant activity. The values

are expressed in mg/L and were measured at 750nm wavelength. It was established that propolis has a significant antioxidant capacity and that it is largely correlated with the concentration of polyphenols in them.

Keywords: propolis, antioxidant capacity, polyphenols

1. INTRODUCTION

Contemporary trends and advances in society bring with them great benefits for the human population, but they are certainly accompanied by the accelerated and unhealthy way of life and nutrition of people. Man's awareness of the importance of a healthy diet for overall health is increasing every day. There is an increasing interest in scientific and research related to the causal connection of the quality of certain food substances as well as the method of food preparation with the possible risk of the occurrence and development of some chronic diseases. Degenerative processes that lead to aging and the appearance of various diseases such as cancer, atherosclerosis and diabetes include oxidation and other reactions in which the formation of free radicals occurs. Reactive oxygen species (ROS) are constantly formed in living organisms during the process of cellular respiration or induced by exogenous sources such as pollution, ionizing radiation and drugs. Living organisms protect themselves from oxidative damage with an endogenous antioxidant defense system or with antioxidants that are taken in through food. Antioxidants are chemical compounds that, in the simplest terms, can prevent or slow cell damage. They act to protect cells from the oxidative action of free radicals. The most important antioxidants that can be ingested with food and nutritional supplements are: vitamin A, vitamin E, vitamin C, zinc, selenium, carotenoids, polyphenols - flavonoids, and endogenous antioxidants that are produced in the body, but can also be ingested with food such as: glutathione, coenzyme Q10, alpha lipoic acid. As time goes on, the human population is increasingly based on a healthy diet, and antioxidants in the diet are being mentioned more and more. Recently, natural food products, such as propolis, have been particularly important. Following the trend of using what nature directly offers, bee products (honey, propolis, royal jelly, etc.) are increasingly important as essential natural products in a healthy diet. Propolis is an aromatic resin of plant origin, which bees collect from the buds, leaves and bark of plants, enriched with enzymes from the bee's digestive tract. Propolis has attracted attention in recent years due to its beneficial effects, which make it a potential preventive and therapeutic agent, as well as a useful additive in food and cosmetics. Flavonoids are also present in large quantities in bee propolis. Propolis is known as a medicine in "tradicional medicine", and due to its biological and pharmacological properties, such as, for example, antimicrobial, antiviral properties and cytotoxic activity, it is considered a medicine of the 21st century.

2. THEORETICAL FRAMEWORK

Propolis is a natural resinous substance produced by bees by collecting substances from plant parts, buds and tissue fluids. Propolis protects bee colonies from diseases with antiseptic and antibacterial properties. (Ghisalberti, 1979) The characteristic property of propolis is that it is a lipophilic material, rigid and brittle in the cold (temperature below 15°C), while at temperatures between 25°C and 45°C it becomes soft,

suitable for molding and sticky. (Krell, 1996) Propolis cannot be used as a raw material. It must be purified by extraction with suitable solvents, in order to remove the unwanted material, preserving the active components, for example, the polyphenolic fractions. Several solvents are used for these purposes, such as water, ethanol, methanol, hexane, acetone and chloroform, with ethanol being the most common, especially at a concentration of 70%. (Gómez-Caravaca et al., 2006)

In order to determine the composition of raw propolis, after extracting it, it will be the most often use thin-layer chromatography with spectrophotometric procedures or high-efficiency liquid chromatography. (Bonamigo at al., 2017) The chemical and physical properties of propolis depend on various factors: the geographical areas of collection, the time when it was collected, the type of plants from which it was collected, (Ghisalberti, 1979) as well as the species of bees that have a great influence on the chemical composition and propolis quality. (https://www.jpbsonline.org 2021) The main components of propolis are: resin (50%-70%), oil and wax (30%-50%), pollen (5%-10%) and other chemical compounds including: amino acids, minerals, sugars, vitamins B, C and E, flavonoids, phenol, as well as aromatic compounds. (Bankova, de Castro, Marcucci, 2000); (Russo, Longo, Vanella, 2002)

In the human body, a series of intermediate compounds are formed in the tissue between the cells, the intercellular space and within the cells themselves, which are unnecessary or even harmful to the body. Among the most important harmful intermediates are the free radicals. A free radical is any molecule that contains one or more unpaired electrons in its outer shell. That unpaired electron has a tendency to create an electron pair, therefore free radicals are very reactive, but short-lived. They are formed in the body under normal conditions during metabolic reactions. The action of free radicals is necessary and useful in some cases in the human body. Antioxidants ensure that they are not in excess and that they are short-lived. http://www.zzjzpgz.hr/nzl/12/radikali.htm (October 2021)

Antioxidants work to protect cells from the oxidizing action of free radicals. Antioxidants are microelements that bind to themselves particles of free radicals, thus preventing their oxidation and the creation of a destructive chain reaction. We often hear that these are miracle-working and healing substances that cure any disease, but the word itself denotes a whole group of different compounds. It is mainly about vitamins and some minerals. (https://www.krenizdravo.hr/ 2021) The most important antioxidants that can be ingested with food and food supplements are: vitamin A, vitamin E, vitamin C, zinc, selenium, carotenoids, polyphenols - flavonoids, and endogenous antioxidants that are produced in the body, but can also be ingested with food, such as: glutathione, coenzyme Q10, alpha lipoic acid. (Jašić, 2010)

Antioxidants are widely used in the dietary supplement industry, which aims to prevent various diseases, from heart disease to the treatment of various types of cancer. Although initial research into the impact of antioxidants in nutritional supplements on the prevention and treatment of various types of diseases has yielded positive results, excessive use can have a detrimental effect on health. In addition to the pharmaceutical industry, antioxidants are also used as preservatives in the food and cosmetic industries

for the purpose of extending the product's durability. (Kolankaya et al., 2002) The most abundant active components of propolis are polyphenols. (Jašić, 2010) Polyphenols are a large family of natural plant products that are very widely distributed in plant foods, including fruits, vegetables, nuts, seeds, flowers and barks. The content of polyphenols is the highest in the bark. (Bušić, 2015)

Many polyphenols have strong antioxidant, anticancer, antiatherosclerotic, antibacterial, antiviral and anti-inflammatory activity. Polyphenols include more than 8,000 compounds with different chemical structures, from simple hydroxymethyl acids and anthocyanins (plant pigments) to more complex flavonoids and tannins, whose basic feature is the presence of one or more hydroxylated benzene rings. (Jašić, 2017)

Polyphenols are one of the most numerous and widespread groups of substances in the plant world. Their most characteristic feature is their aromatic ring and its associated alcohol group (−OH). Polyphenols are further divided into at least 18 classes: simple phenols, benzoquinones, phenolic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, phenylpropenes, coumarins and isocoumarins, chromones, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoligns, lignans and condensed tannins. (Jašić, 2010)

3. METHOD AND MATERIALS

In this research, thirteen propolis samples were analyzed, of which 8 samples were obtained from individual beekeepers. Out of those 8 samples, 5 samples come from beekeepers from the "Association of beekeepers from Zavidovići" who keep their bees in the geographical location of Zavidovići, and 3 samples were collected in cooperation with individual beekeepers from the area of Bosnia and Herzegovina. Table 1 shows data on samples obtained from individual beekeepers from a survey filled out by beekeepers. The table 1 contains data of the year of propolis collection, the method of collection, bee nutrition, the geographical area where the bee grazed, the used solvent, and the name and surname of the beekeeper. The other 5 samples were collected from commercial use that we can find every day on the market in Bosnia and Herzegovina. Data on samples from commercial use can be found in Table 2. There are manufacturers and the solvents used to prepare the propolis tincture are listed. All collected propolis samples were stored in a cool and dark place in closed vials. Beekeepers filled out a questionnaire for each of the samples and the data from the survey can be found in table 1. The research was carried out using the FRAP and FC method in the scientific research laboratory of the Faculty of Science and Mathematics in Tuzla. Thirteen samples of propolis from the territory of Bosnia and Herzegovina were analyzed and their antioxidant potential and the total proportion of polyphenols in propolis were determined.

Table 1. The table shows the data from the surveys filled out by the beekeepers for each analyzed sample

No sample	Name and surname of	Year of collection	The method of collection	Bee nutrition	Solvent	Geografical area
1.	Fikret Kadrić	2020	By means of a mesh which is placed over the frames	yes	ethanol	Kamenica - near mountine Tajan
2.	Adnan Alagić	2020	Struganjem sa ramova, nastavaka i hranilica	yes	ethanol	Near mountine Grmeč
3.	Asim Bašić	2020	Struganjem sa okvira	yes	ethanol	Stavci - Zavidovići
4.	Zejnil Karajbić	2000	Struganjem sa okvira	yes	ethanol	Stošnica – near Zavidovići
5.	Enver Gagulić	2021	struganjem	yes	ethanol	Pepelari –near Zenice
6.	Edin Kovačević	2020	Sa najlona	yes	ehtanol	Vikovići and Sinanovići – near Zavidovići
7.	Sead Arnautović	2020	struganjem	yes	ethanol	Badre - Tuzla
8.	Mirsad Bošnjaković	2021	Struganjem sa ramova	yes	96% ethanol	Kovači – near Zavidovići

Table 2. The table contains data of analyzed commercial propolis samples

No sample	Manufacturer	Solvent
9.	Esena d.o.o. Beograd	ethanol
10.	Krnjevac	ethanol
11.	Diapharm	ethanol
12.	Medex	propylene glycol
13.	Api Med – Sanski most	ethanol

Absorption spectrophotometry and spectrophotometric methods

Spectrophotometry is a part of electromagnetic spectroscopy. It is an instrumental method, which deals with quantitative measurements of reflective or transmissive properties of materials as a function of wavelength. Absorption spectroscopy is based on ultraviolet (UV) and visible radiation (VIS) and represents the most popular technique in quantitative chemical analysis due to its accuracy, selectivity, simplicity, wide applicability, high sensitivity and speed of execution. UV-VIS spectrophotometry is a technique used to determine the concentration of a sample by measuring the intensity of radiation absorbed by the examined sample. Basically, these are the energies that cause a certain electronic transition with respect to the types of bonds in the molecules of the sample. The listed energies actually represent ultraviolet or visible radiation that causes the transition of an electron from a filled orbital of lower energy to an unfilled orbital of higher energy.

FRAP method

Ferric Reducing Antioxidant Power (FRAP) is one of the methods of absorption spectrophotometry. The FRAP method is based on the ability of antioxidants to donate electrons in an acidic medium (pH 3.6) to reduce the yellow complex of ferric iron (Fe^{3+}) with TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) to a blue-colored Fe^{2+}-TPTZ complex. The intensity of the resulting blue color is measured spectrophotometrically at 593 nm. The intensity of the color is proportional to the reducing ability (concentration) of the antioxidant. The reaction is not specific. The results are expressed as μmol of Fe^{2+} equivalent (Fe) per L of sample.

Methods based on the transfer of electrons are somewhat slower than methods based on the transfer of hydrogen atoms and are very sensitive to changes in the solvent and pH of the solution. In most methods that measure antioxidant activity based on electron transfer, the antioxidant reacts with a colored blank instead of a free radical that causes a color change that can be detected by a spectrophotometer. Immediately before the measurement, it was necessary to prepare the FRAP reagent. The FRAP reagent consisted of 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) and 0.3M acetate buffer ($CH_3COONa \times 3H_2O$) pH=3.6 in a ratio of 1:1:10 (5ml TPTZ:5ml $FeCl_3 \cdot 6H_2O$: 50ml acetate buffer).

The next thing that is necessary is to prepare solutions for the standard curve, with which the FRAP value was calculated, and the results were expressed in μM Fe (II) of a 10% propolis solution. To construct the calibration curve, it is necessary to prepare ten solutions of 20 mM $FeSO_4 \times 7H_2O$ (iron (II) sulfate hexahydrate) of different concentrations and measure the absorption at a wavelength of 593 nm.
$FeSO_4 \times 7H_2O$ solutions ranging from 50 to 1600 μM were used to construct the calibration curve.

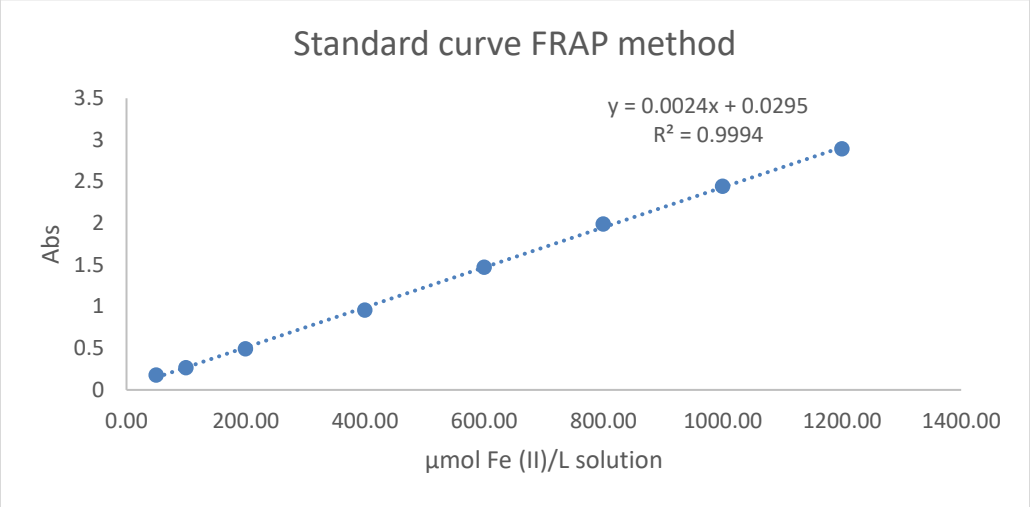

Figure 1. Standard curve for measuring the concentration of antioxidants in propolis

The concentration of antioxidants in the measured samples based on the obtained values is calculated according to the expression:

$$X = y - 0{,}0295 / 0{,}0024\ \mu MFe\ (II)/L$$

Where is:

X – antioxidant concentration

Y– average sample absorption

For measuring the concentration of antioxidants in propolis, we diluted the samples to 10%. We pipetted 1 ml of propolis sample into previously numbered glass beakers and diluted to the mark (10 mL) with 96% ethanol. In this way, we obtained 10% of propolis samples that are ready for analysis. To analyze the samples, that is to measure the absorbance of the propolis samples, it is necessary to transfer 200 µL of the sample from the cups to the cuvettes with an automatic pipette and 1.8 ml of the freshly prepared FRAP reagent with another automatic pipette. Leave it for 10 minutes to react and a blue coloration of the solution is obtained as proof of the presence of antioxidants in the sample. After 10 minutes, the absorbance of the sample is measured in a spectrophotometer at 593 nm compared to a blank sample. For the blank test, 200µL of distilled water was pipetted into two cuvettes to which 1.8ml of FRAP reagent was added. The blank should show zero (0) absorbance at 593nm and each time before measuring the absorbance of the sample, measure the absorbance of the blank

Folin – Ciocalteu (FC) method

The Folin-Ciocalteu (FC) method is the best known and most widely used spectrophotometric method. It was developed by Folin and Ciocalteu (1927), and is based on oxidation and reduction reactions, i.e. on the colorimetric reaction of phenol with the Folin - Ciocalteu reagent, during which the color changes

from yellow to dark blue. It is widely used in determining the total phenol content in natural products. Total phenols are determined as a good indicator of antioxidant activity. Research has shown a connection between the proportion of phenolic components (primarily flavonoids and phenolic acids) and antioxidant capacity. Folin-Ciocalteu reagent consists of phosphotungstic and phosphomolybdic acids. The reagent is prepared by boiling for 10 h a mixture of sodium tungstate ($Na_2WO_4 \times 2H_2O$, 100 g), sodium molybdate ($Na_2MoO_4 \times 2H_2O$, 25 g), concentrated hydrochloric acid (100 mL), 85% phosphoric acid (50 mL) and water (700 mL). After boiling, lithium sulfate ($Li_2SO_4 \times 4H_2O$, 150 g) is added to the mixture to obtain an intensely yellow colored FC reagent solution. Contamination of the reductant results in a green color, and the addition of an oxidant such as bromine can restore the desired yellow color. The FC reagent oxidizes phenolic compounds in an alkaline medium, after that they are reduced to oxides. The reaction takes place through the mechanism of electron transfer, and the blue-colored compounds formed by the reaction of phenolate and FC reagent are independent of the structure of phenolic compounds, thus excluding the possibility of coordination of complexes formed between the metal center and phenolic compounds. The intensity of the resulting coloring is measured spectrophotometrically by determining the absorbance at a wavelength of 745 to 765 nm, and is proportional to the proportion of phenolic compounds in the tested sample. Gallic acid is most commonly used as a standard and results are expressed in mg of gallic acid equivalents (GAE) per gram or liter of sample. The greatest advantage of the FC method is its simplicity, precision, reproducibility and economy. (Zanko, 2020); (Buljeta, 2015)

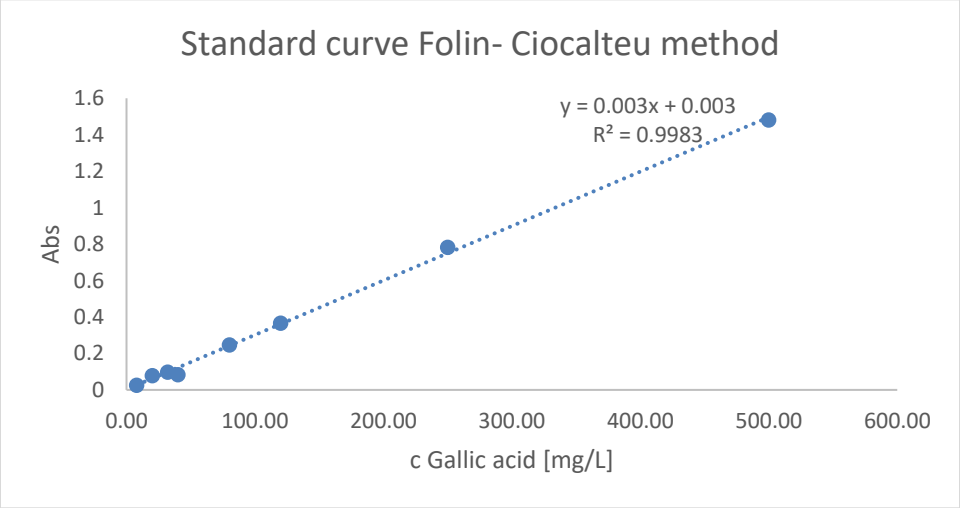

Figure 2. Standard curve for measuring the concentration of total polyphenols in propolis

The concentration of polyphenols in the measured sample based on the obtained values is calculated according to the expression:

$$X = y - 0{,}003 / 0{,}003 \text{ mg GA/100g propolisa}$$

Where:

X – polyphenol concentration

Y – average sample absorption

4. RESULTS AND DISCUSSION

The results obtained from the research are presented in a table in the order of analysis. Table 3 shows the results of antioxidant concentration and polyphenol content in propolis samples collected from individual beekeepers (the first 8 samples) and in samples from commercial use (samples 9 to 13).

Table 3. Shows comprehensive results of antioxidant capacity and total phenol concentrations of all propolis samples that were analyzed

Number sample	Antioxidant capacity µM Fe(II) / L	Concentration of total polyphenols in propolis mg GA/100g propolis
1.	618,805	344,11
2.	821,041	230,966
3.	746,208	548,382
4.	737,083	586,816
5.	465,083	299,34
6.	581,375	442,266
7.	480,402	383,33
8.	481,472	367,888
9.	496,541	415,613
10.	1447,916	1136,06
11.	192,645	112,4
12.	1455,54	1003,2
13.	720,562	578,233

It is known that the products of bee work have provided man with many health benefits. There are different types of propolis and therefore different medicinal properties, such as antibacterial, antifungal, anti-inflammatory and antitumor. Since ancient times, people have started using propolis for various health purposes, which is still present. Now, special work is being done on researching propolis as well as other bee products and obtaining results on their effectiveness on our health. The antioxidant capacity of propolis was analyzed using the FRAP method and depends on a number of factors, both from the

botanical and geographical origin, as well as from the chemical composition, the method of propolis collection, the bee's diet, the method of extraction, the means used for dissolution, etc. collected samples, all propolis samples dissolved in ethanol. All bee colonies are fed with bee cakes and/or bee syrup. The results of the antioxidant capacity of propolis collected from individual beekeepers, which were analyzed by the FRAP method, are shown in Figure 3.

Antioxidant capacity

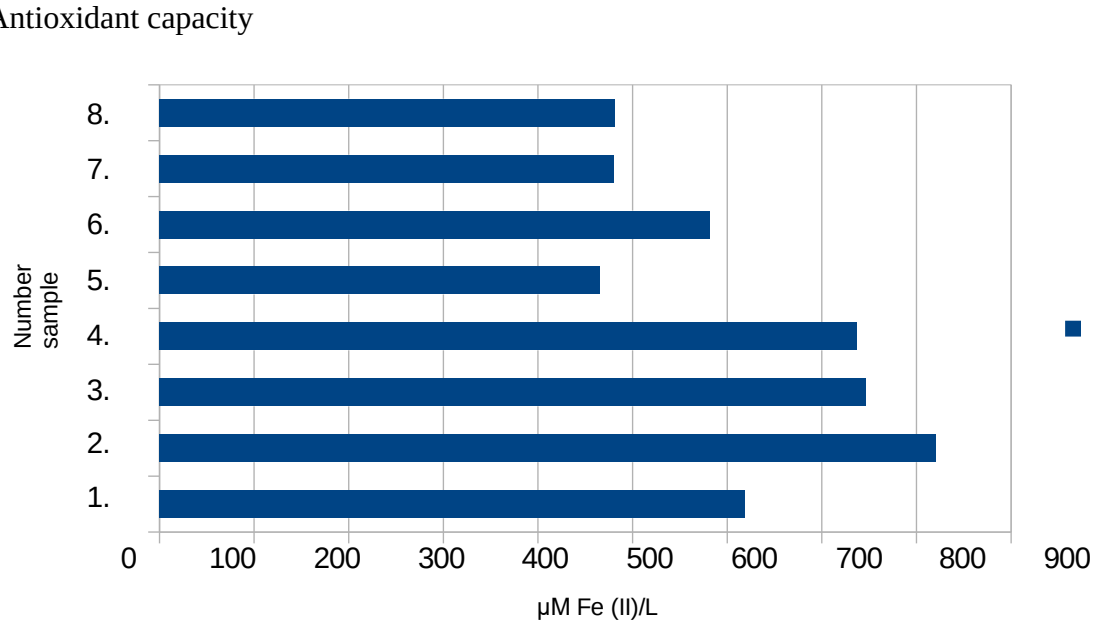

Figure 3. The antioxidant capacity of propolis samples that were collected from individual beekeepers and analyzed by the FRAP method. The samples come from different geographical areas of Bosnia and Herzegovina.

The antioxidant capacity of propolis collected from individual beekeepers varies from 465 µMFe (II)/L to 821 µMFe (II)/L. The mean value of the antioxidant capacity of these eight samples is 616.433 µMFe (II)/L. Sample number 5, which has the lowest antioxidant activity, which is 465.083 µMFe (II)/L, was taken from a colony of bees that graze at Pepelari in Zenica, which is located near the Tvrtkovica mountain, whose altitude is 770 m. Pepelari are surrounded by a dense pine forest, then a mixed forest of deciduous trees and conifers. There are many mushrooms and medicinal herbs. It is a known fact that Zenica is a place of great pollution. Sample number two, which has the highest antioxidant capacity of 821,041 µMFe (II)/L, (almost twice the value of sample number 5) comes from bees that grazed near Mount Grmeč, which is at 1605m above sea level. Grmeč is located in the north-western part of Bosnia and Herzegovina. The Grmeč forest complex consists of a variety of coniferous and coniferous trees. Nature in that area is still well preserved and clean. Because of the excellent conditions and the preserved nature, the population is mostly engaged in beekeeping, animal husbandry and agriculture. Sample number

two in terms of antioxidant capacity is followed by samples from Zavidovići, which were taken from different locations. Locations that are geographically located in places rich in honey plants and unpolluted air. In Zavidovići, a large number of the population is engaged in beekeeping and they have an established association of beekeepers. Zavidovići is an area rich in coniferous and coniferous forests. They are surrounded by nature, mountains, rivers and rich flora. The antioxidant capacity of propolis samples from commercial use, which was analyzed by the FRAP method, is shown in Figure 4.

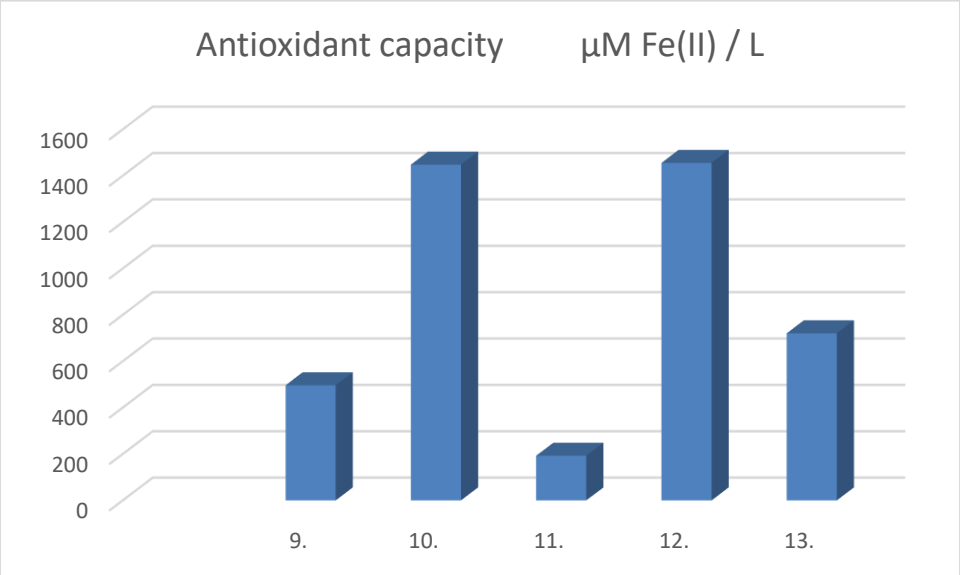

Figure 4. The results of measuring the antioxidant capacity of propolis samples from commercial use and analyzed by the FRAP method

The antioxidant capacity of five commercial samples measured by the FRAP method is at a higher level than expected. The measured antioxidant potential of a commercial propolis sample dissolved in propylene glycol showed the highest antioxidant capacity. The value of antioxidant capacity of sample number 12 is 1455.54 µM Fe (II)/L. It is a sample produced by Medex d.o.o. Of the samples dissolved in ethanol, sample number 10 showed the highest antioxidant capacity. The value of its antioxidant capacity is 1447.916 µM Fe (II)/L. The manufacturer of that sample is Krnjevac d.o.o. Serbia. Sample number 11 showed the lowest antioxidant activity, with a value of 192,645 µM Fe (II)/L. The producer of sample number 11 is DIAPHARM Serbia. In the commercial offer, sample number 11 was the cheapest, and samples number 10 and 12 were among the more expensive propolis products offered to customers. The mean value of the antioxidant capacity of commercial samples dissolved in to ethanol is 714.416 µM Fe(II)/L. If we calculate the mean value of commercial samples with with the non-ethanol sample, it amounts to 862,641 µM Fe (II)/L. Figure 5 shows the results of the antioxidant capacity of all propolis samples that were analyzed in the research. In Figure 6.

there is a graphic representation of the mean values of antioxidant capacity sorted by propolis samples

taken from individual beekeepers, propolis samples collected from commercial use and a sample of propolis dissolved in propylene glycol is shown.

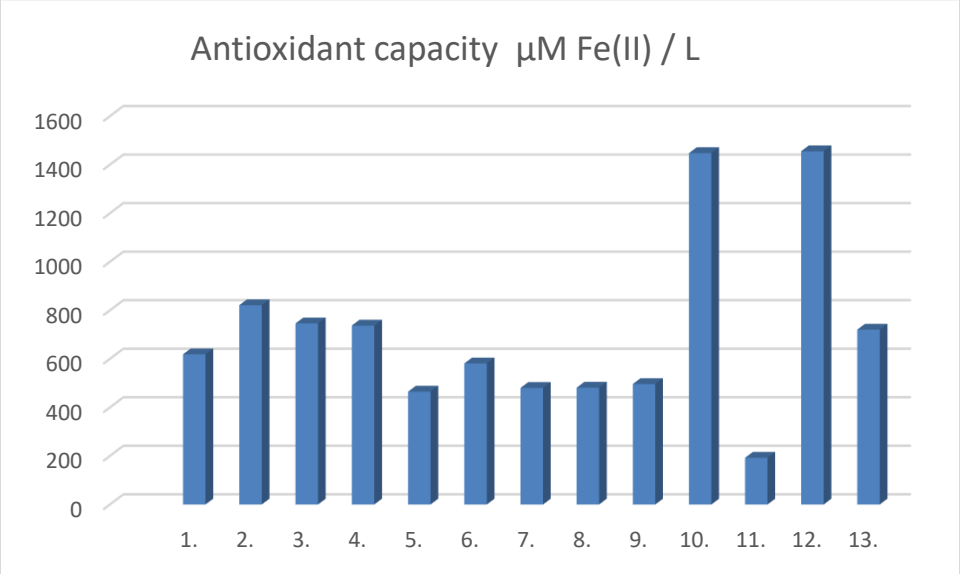

Figure 5. The antioxidant capacity of all propolis samples used in this research analyzed by the FRAP method. Propolis samples number 10 and 12 show the highest values.

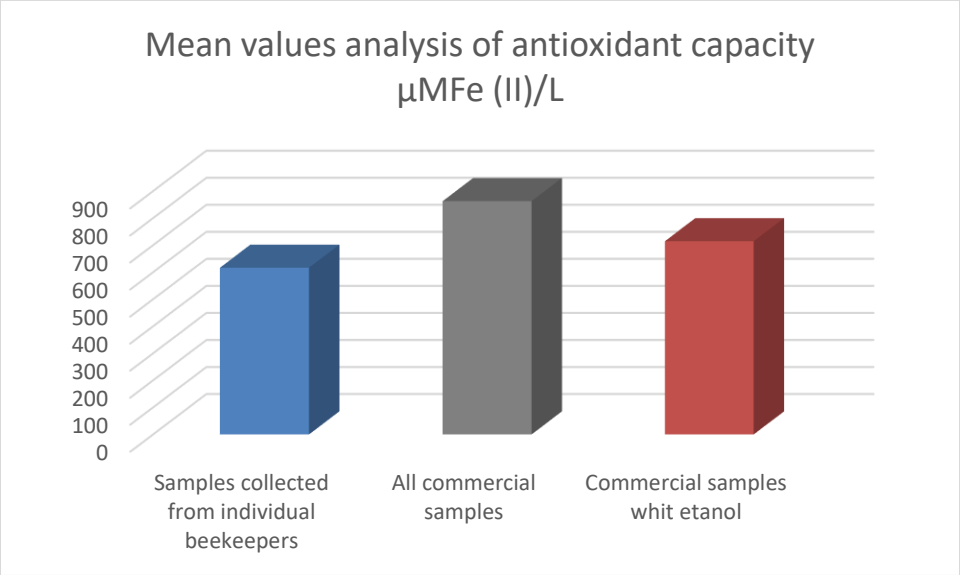

Figure 6. The mean values of antioxidant capacity

The blue color indicates the mean value of the antioxidant capacity of propolis samples collected from individual beekeepers and it is 616,433 µMFe (II)/L. The red column represents the mean value of

commercial propolis samples dissolved in ethanol and is 714.416 μM Fe(II)/L. This value does not include sample number 12, which was not dissolved in ethanol. The commercial sample that was dissolved in propylene glycol was included in the mean value of the antioxidant capacity of propolis, which is marked in gray. It represents the highest value of the antioxidant potential of commercial propolis samples and is 862,641 μM Fe(II)/L. According to the obtained results, we can see that the propolis that was procured from individual beekeepers does not represent the result of a higher mean concentration of antioxidants than commercial samples. Which means that when buying propolis samples in order to preserve our health, we can also rely on commercial samples that undergo food safety analysis, with the fact that, observing these results, we should not take propolis whose monetary value is lower than the others. It is better to spend more money for a better quality product, because research has shown that the more expensive sample of propolis has a higher antioxidant capacity. should buy propolis that is dissolved in propylene glycol because it has shown the highest antioxidant capacity.

Propolis samples collected from individual beekeepers showed slightly lower antioxidant capacity, which does not mean that we should reject their propolis products. All samples showed a certain antioxidant capacity, with the fact that when buying propolis from individual beekeepers, the geographical area should be considered. The closer the bee pasture is to the mountain, the further away from the inhabited place and without pollution, the higher the antioxidant capacity. None of the propolis samples that were collected from individual beekeepers showed such a low antioxidant capacity as the commercial sample number 11, which is 192,645 μM Fe(II)/L. The antioxidant capacity of propolis samples collected from beekeepers did not show a value lower than 465,083 μM Fe(II)/L. All bee societies had a diet, which means that the diet of the bees has a positive effect on the antioxidant capacity of propolis.

In this work, the concentration of polyphenols in propolis was also investigated; in order to find out how much influence polyphenols have on the antioxidant capacity of propolis. In the following displays, the results obtained by the FC method follow.

Figure 7. shows a graphic representation of the results of the research on the concentration of total polyphenols in propolis samples collected from individual beekeepers and analyzed by the FC method.

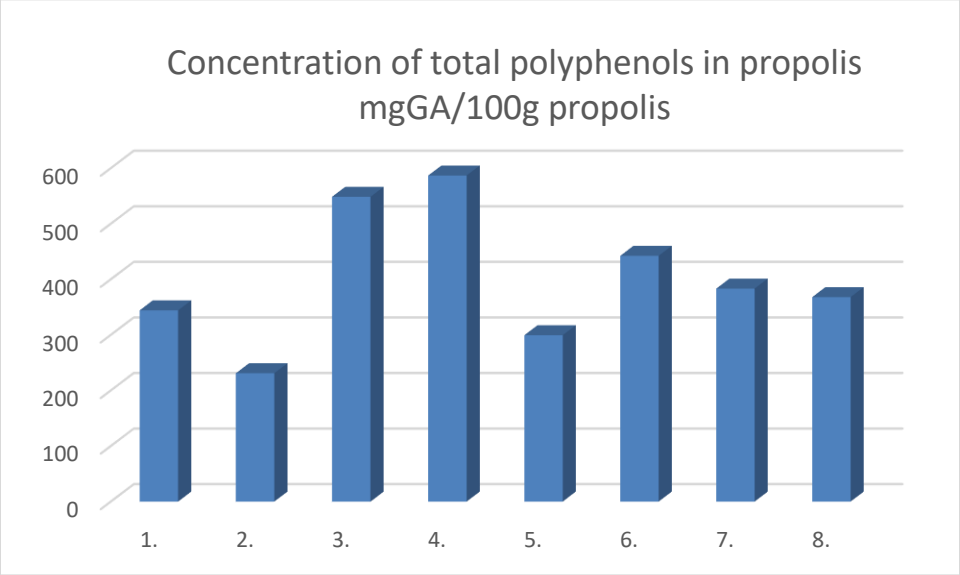

Figure 7. Shows the results of the total concentration of polyphenols in propolis samples collected from individual beekeepers

The concentration of total polyphenols ranges from 230,966 mgGA/100g propolis to 586,816 mg GA/100g propolis. The value of 230,966 mg GA/100g obtained from the analysis of sample number 2, which was obtained from the Una-Sana Canton, is calculated as the lowest value of total polyphenols in the samples collected from individual beekeepers. Sample number 4, which has the highest concentration of total polyphenols in the propolis sample, was taken from a beekeeper whose bees graze in the town of Stošnica in the vicinity of Zavidovići. The mean value of the total concentration of polyphenols is 400,386 mgGA/100g.

Figure 8. shows a graphic representation of the obtained results of the research on the concentration of total polyphenols of propolis samples that were collected from commercial use and analyzed by the FC method.

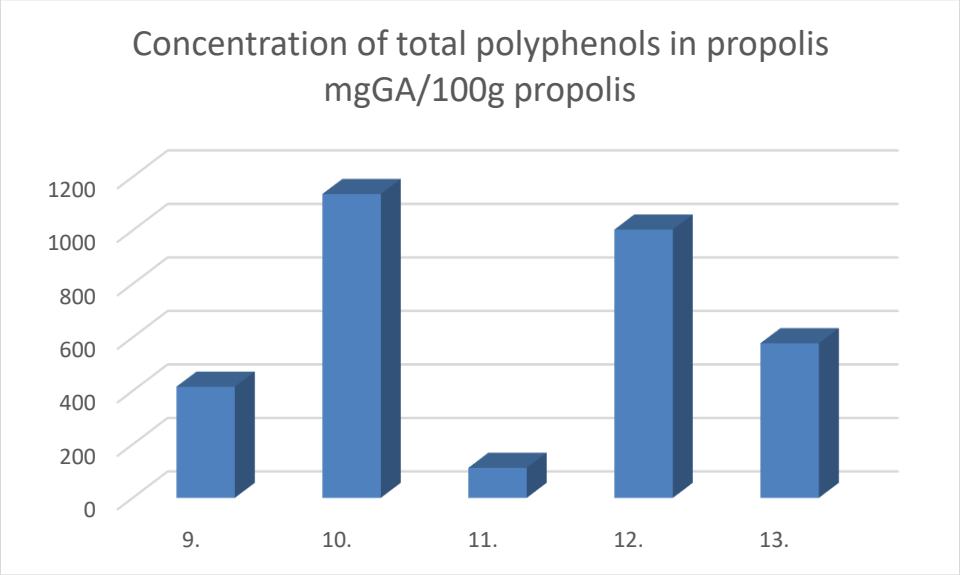

Figure 8. shows the results of the concentration of total polyphenols in propolis from commercial use

Grafički su prikazani rezultati koncentracije ukupnih polifenola uzoraka propolisa iz komercijalne upotrebe. Najmanja vrijednost dobijena je analizirajući uzorak broj 11. koji je otopljen u etanolu i iznosi 112,4 mg GA/100g. Najveću vrijednost je pokazao uzorak broj 10. koji je otopljen u etanolu i iznosi 1136,06 mg GA/100g. Vrijednost koncentracije ukupnih polifenola kod neetanolnog uzorka je 1003,2 mg GA/100g. Srednja vrijednost koncentracije ukupnih polifenola kod komercijalnih uzoraka je 649,102 mg GA/100g. Izračunata srednja vrijednost koncentracije ukupnih polifenola u propolisu iz komercijalnih uzoraka ne uzimajući u proračun komercijalni uzorak sa propilenglikolom je nešto manja i iznosi 560,576 mg GA/100g. Grafički prikaz koncentracije ukupnih polifenola svih analiziranih uzoraka FC metodom nalazi se na slici 9., a srednja vrijednost koncentracije ukupnih polifenola svih uzoraka je prikazana na slici 10.

The results of the concentration of total polyphenols of propolis samples from commercial use are shown graphically. The lowest value was obtained by analyzing sample number 11, which was dissolved in ethanol and is 112.4 mg GA/100g. The highest value was shown by sample number 10, which was dissolved in ethanol and amounted to 1136.06 mg GA/100g. The concentration value of total polyphenols in the non-ethanol sample is 1003.2 mg GA/100g. The mean concentration of total polyphenols in commercial samples is 649.102 mg GA/100g. The calculated mean value of the concentration of total polyphenols in propolis from commercial samples without taking into account the commercial sample with propylene glycol is slightly lower and amounts to 560,576 mg GA/100g. The graphic representation of the concentration of total polyphenols of all analyzed samples by the FC method can be found in Figure 9, and the mean value of the concentration of total polyphenols of all samples is shown in Figure 10.

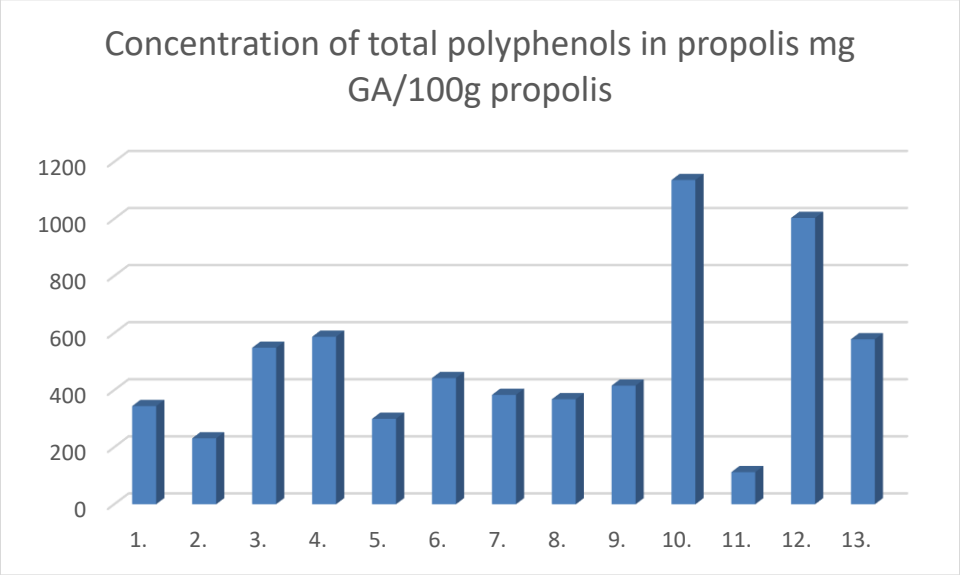

Figure 9. Shows the concentrations of total polyphenols expressed in mg GA/100g of propolis in all analytical samples used in this research

Looking at the graphically displayed values of all the analyzed samples, it is noticeable that sample number 10 and sample number 12 show far higher values of the concentration of total polyphenols than the other samples. Sample number 10 and 12 are samples from commercial use and their purchase price was higher than sample number 11 which was much cheaper. Sample number 11 had a light brown color, while other commercial propolis samples were darker brown in color, especially dark samples number 10 and 12.

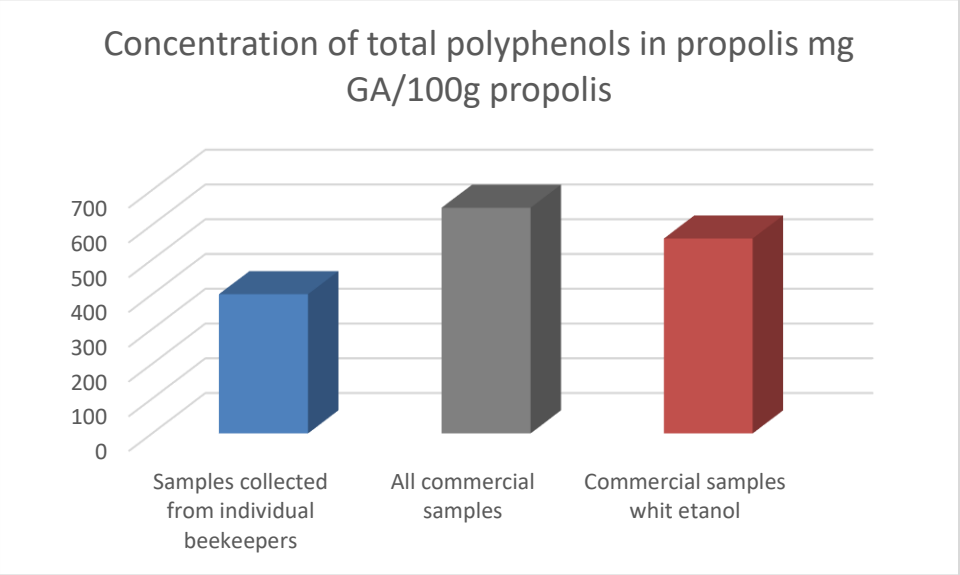

Figure 10. Shows the mean concentration values of total polyphenols in the analyzed propolis

samples

The graph shows three mean values. The mean value shown in blue represents the mean value of the concentration of total polyphenols of the samples collected from individual beekeepers and represents the smallest amount of the mean value of 400.386 mg GA/100g. The mean value expressed in red represents the mean values of commercial propolis samples without non-ethanol sample and is 560,576 mg GA/100g. The gray column of the graph which has the highest value and which is 649.102 mg GA/100g represents the mean value of the concentration of total polyphenols of commercial samples. The mean value of commercial samples is higher, but no sample from individual beekeepers showed such a low value of polyphenol concentration as commercial sample number 11.

Figure 11. shows a graphic representation of the results of all propolis samples analyzed using the FRAP and FC methods, where we can see the dependence of the antioxidant capacity on the concentration of total polyphenols.

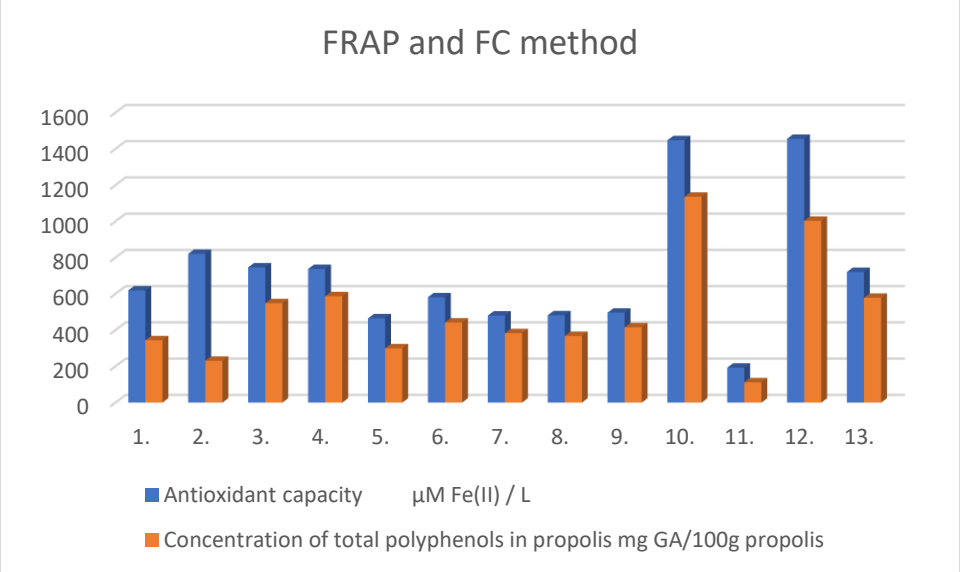

Figure 11. Shows the results of samples analyzed by FRAP and FC method and graphically shows the dependence of antioxidant capacity on the concentration of total polyphenols

The values of antioxidant capacity of propolis samples are indicated in blue, and the concentration of total polyphenols in propolis samples is indicated in orange. The antioxidant capacity of propolis depends on the concentration of polyphenols. The antioxidant capacity of propolis is higher than the concentration of total polyphenols. Display of the deviation of the value of antioxidative capacity from the concentration of total polyphenols:

2 > 12 > 10 > 1 > 3 > 5 > 4 > 13 > 6 > 8 > 7 > 9 > 11

The samples are ordered from the largest to the smallest deviation value. Sample number 2 showed the greatest deviation in antioxidant activity. We can say that its antioxidant activity does not depend to a large extent on the concentration of polyphenols, but on other antioxidant parameters such as vitamins A, E, C and minerals Zn, Mn, Fe, Se. The smallest difference in the ratio of antioxidant capacity to the capacity of total polyphenols was shown by samples number 9 and 11, where we believe that their antioxidant activity depends on polyphenols.

Figure 12. shows a graphic representation of the mean value of total polyphenols in propolis that was collected and analyzed in Greece.

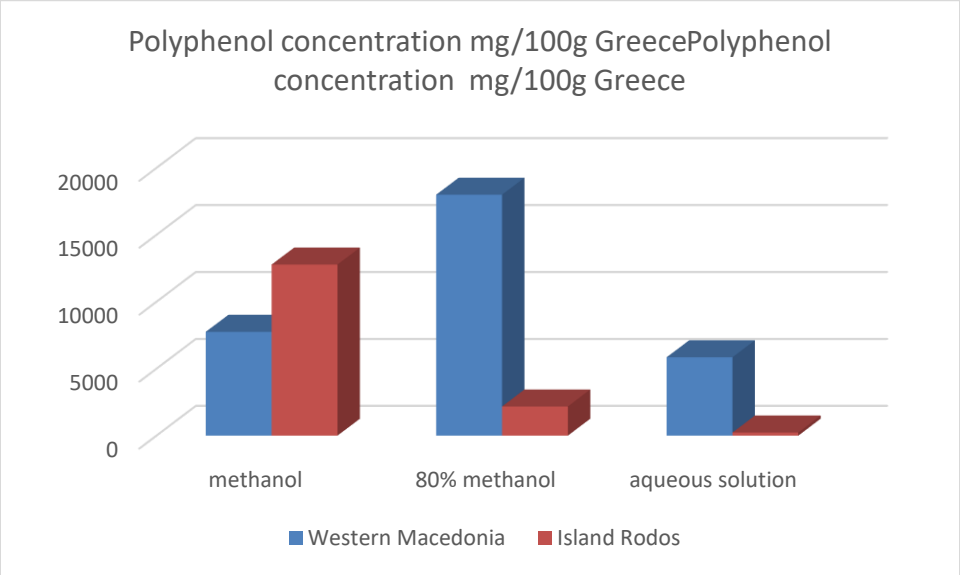

Figure 12. Shows the content of total polyphenols (mg GA/100g propolis) in propolis samples collected and analyzed in Greece. Data shown are mean values of triplicate determinations with different solvents.

The scientists who conducted the research used three types of solvents: methanol, 80% methanol and an aqueous solution. The research was conducted in two regions: Western Macedonia and the island of Rhodes. Propolis from Western Macedonia had the highest concentration of total phenols (mean value 105.36 mg/g). Specifically, the 80% methanol extract from Western Macedonia showed the highest amount (179.99 mg/g), while the water extract from Rhodes showed the lowest (2.33 mg/g). The obtained results showed that methanolic and 80% methanolic extracts of propolis from both geographical regions (Western Macedonia, Rhodes) show higher radical removal and reduction activities than aqueous extracts. Geographical areas affected the antioxidant activity as well as the number of phenolic compounds more than the extraction solvent. (Lagouri, Prasianaki and Krysta, 2014)

In Figure 13, we have a graphic representation of the comparison of total polyphenols in propolis obtained by analysis in different areas: Greece, Azerbaijan, Croatia and Bosnia and Herzegovina.

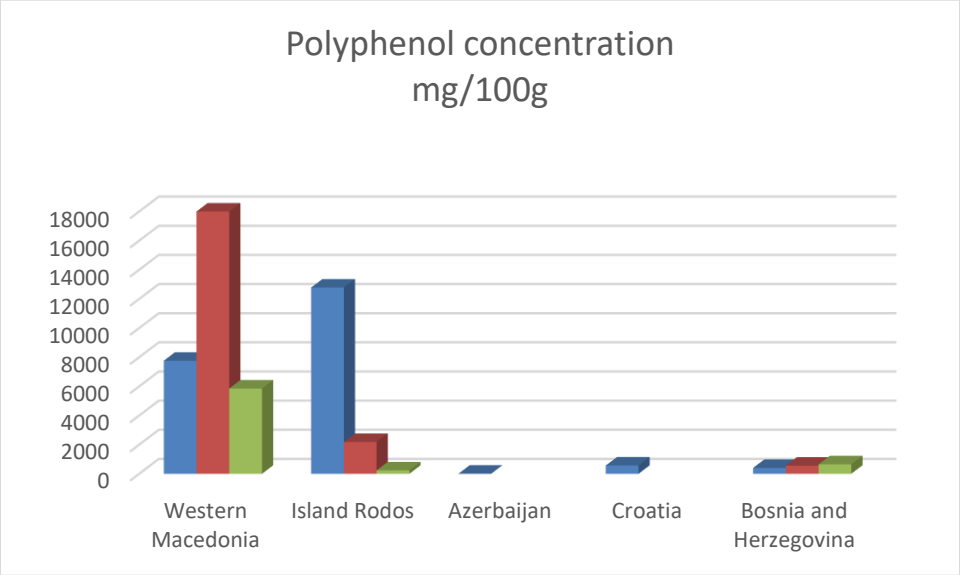

Figure 13. Shows the values of total polyphenols in propolis that was analyzed in different areas: Greece (island of Rhodes and Western Macedonia), Azerbaijan, Croatia (Nova bukovica, Slatina, Mošćenica and the area of Hungary) and Bosnia and Herzegovina. (Lagouri, Prasianaki and Krysta, 2014); (Can, Yildiz and Şahin, 2015); (Ernjes, 2017)

The presented results show that the total polyphenols are the highest in propolis samples from Greece (Western Macedonia), especially in propolis dissolved in 80% methanol, and the lowest concentration of polyphenols is found in propolis originating from Azerbaijan. Propolis from Croatia has the smallest deviations in the values of total phenols compared to propolis from Bosnia and Herzegovina. Research by scientists from these reactions has shown that the total share of polyphenols is most influenced by the geographical area where the societies grazed. The results of our research showed that the proportion of polyphenols as well as the antioxidant potential is most influenced by the geographical area where the bee colonies grazed.

In Figure 14, the mean values of the antioxidant capacity of propolis from Azerbaijan and Bosnia and Herzegovina are shown, so that the difference in the quality of propolis from those two areas can be seen on the graphic display.

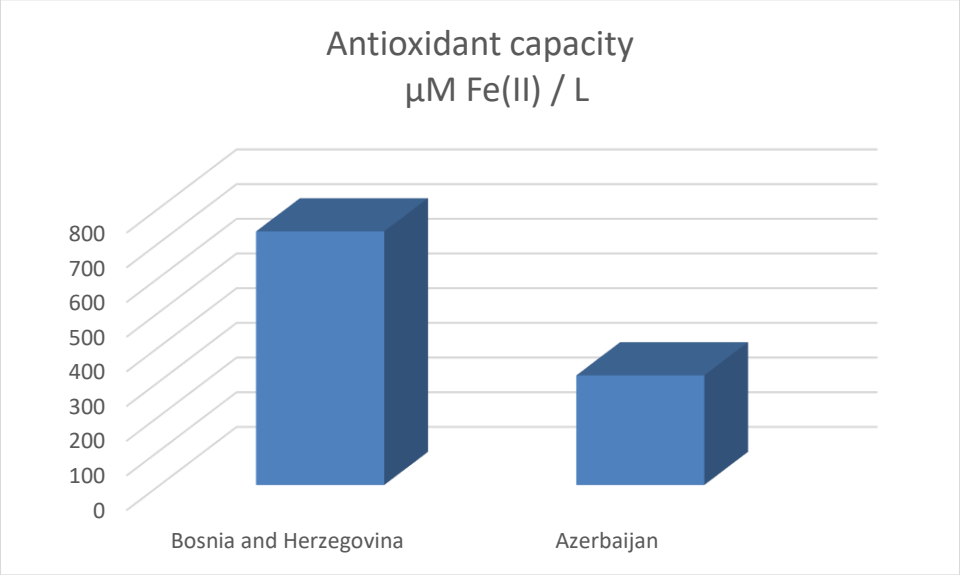

Figure 14. Shows the mean values of the antioxidant capacity of propolis from Bosnia and Herzegovina and Azerbaijan.

Fifteen samples from different cities of Azerbaijan were analyzed. The values of antioxidant capacity of propolis from Azerbaijan ranged from 170 to 438 µM/g. The scientists in this paper concluded that the antioxidant capacity of propolis samples depends on the phenol content, and the phenol content on the flora of the region, age and conditions in the hive, as well as the strength of the society and the method used for collecting samples. (Can, Yildiz, and Şahin, 2015)

Propolis from Azerbaijan has far lower values of antioxidant activity than propolis from Bosnia and Herzegovina. Scientists who analyzed Azerbaijani propolis found that the bioactive potential of propolis depends on the geographical area, the flora present, which is one of the conclusions from our work. Therefore, it is necessary to collect samples from several regions.

The research conducted in Bosnia and Herzegovina entitled "The influence of the chemical composition of honey on its antioxidant activity" gave results on the antioxidant activity of honey, the mean value of which is 423.196 mg/L and the mean value of the content of total polyphenols in honey, which is 16.108 mgGA/100g of propolis. The obtained research results are shown in Figure 15, where we also have a graphic comparison with the antioxidant capacity and content of total polyphenols of propolis. (Kesić, 2010)

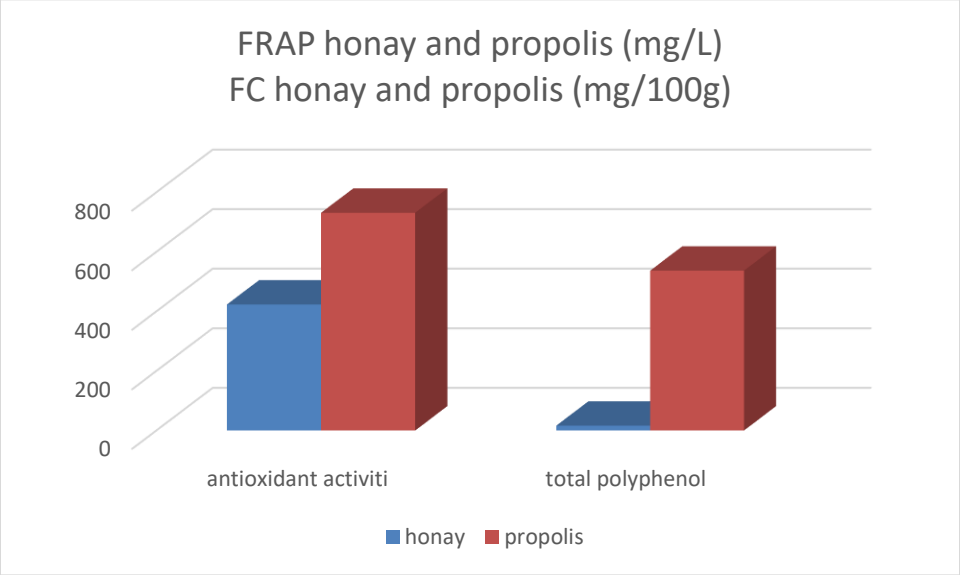

Figure 15. Graph showing the mean value of antioxidant capacity of honey and propolis, mean value of total polyphenols of honey and propolis

Based on the graphic representation, we can conclude that propolis from BiH, which was analyzed using the same methods as honey from BiH, has a much higher proportion of polyphenols as well as antioxidant activity.

5. CONCLUSIONS

The antioxidant capacity of samples collected from individual beekeepers showed a lower mean value than samples collected from commercial use. The samples that were collected from the geographical area where there is more pollution, lower altitude and worse distribution of honey flora showed a lower value of antioxidant activity than samples that were collected from areas with less pollution, less population, which are rich in honey flora and which are at a higher altitude near the Bjelogorica forest.

More expensive commercial propolis products also showed stronger antioxidant activity, which also indicates their higher quality during preparation.

The propolis sample that was dissolved in propylene glycol showed higher antioxidant activity than the propolis samples that were dissolved in ethanol.

The antioxidant capacity of propolis depends to the greatest extent on the total concentration of polyphenols.

The mean value of polyphenol concentration measured by the FC method in samples collected from individual beekeepers is lower than the value of samples from commercial use.

No sample collected from individual beekeepers showed such a low value as commercial sample number 11. The values obtained on samples collected from individual beekeepers did not show large deviations in measurement, as happened with commercial samples. Samples whose propolis had a darker color showed higher antioxidant activity and polyphenol content.

Sample number 2, which showed the highest antioxidant potential of the samples collected from individual beekeepers, showed a lower proportion of polyphenols, so we can conclude that its antioxidant activity does not depend only on the proportion of polyphenols, but also on other antioxidant parameters such as vitamins A, E, C and minerals Zn, Mn, Se, Fe.

For the samples that showed a smaller deviation of the concentration of total polyphenols from the antioxidant activity, we can say that to a large extent their antioxidant capacity depends on the concentration of polyphenols.

The results of research from Greece showed that propolis from the area of Western Macedonia and the island of Rhodes has a higher proportion of polyphenols and antioxidant activity than propolis from Bosnia and Herzegovina.

Propolis from Azerbaijan shows lower values of total polyphenols and antioxidant activity compared to propolis from BiH. Propolis from Croatia shows minimal deviation from propolis from Bosnia and Herzegovina in terms of polyphenol content. Propolis showed a much higher proportion of polyphenols and antioxidant activity compared to honey from Bosnia and Herzegovina. The antioxidant potential and content of total phenols of propolis depends on several factors, most of all on the geographical area and the presence of honey-bearing flora on which the societies grazed. Further research is needed to identify other phenolic components present in propolis and to investigate the contribution of minor phenolic compounds to the overall antioxidant activity of propolis extracts.

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