THE CONTENT OF POLYPHENOLS AND ANTIRADICAL ACTIVITY OF PROPOLIS, POLLEN AND HONEY

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Abstract. The correlation between total polyphenol content and antiradical activity in propolis, pollen and honey in aspect of their therapeutic uses and the elaboration of new healthy products was investigated. It has been established that the antioxidant potential of the studied bee products largely depends on the content of the polyphenols that determine their antiradical activity. Thus, a high correlation between the polyphenols content and the antiradical activity was established for the ethanol and aqueous extract of propolis. In terms of both total polyphenol content and antiradical capacity, the greatest values are found in acacia pollen, followed by poly flower, then sunflower pollen. The investigation of the polyphenol content and the antiradical activity of honey samples revealed the greater antioxidant potential of the acacia honey, in terms of antiradical activity and of sunflower honey, in terms of polyphenol content. The data obtained allow us to state the possibility of targeted use of the varieties of beekeeping products in the elaboration of new healthy remedies with predetermined content and properties by modelling the content of their active components.

Keywords: propolis, pollen, honey, polyphenols, antiradical activity.

Rezumat. Conținutul polifenolilor și activitatea antiradicalică a propolisului, polenului și mierii. A fost investigată corelația dintre conținutul total al polifenolilor și activitatea antiradicalică în propolis, polen și miere în aspectul utilizării acestora în scopuri curative și în elaborarea de noi produse sanogene. S-a stabilit că potențialul antioxidant al produselor apicole studiate în mare parte depinde de conținutul polifenolilor ce determină activitatea antiradicalică a acestora. Astfel, o corelație înaltă între conținutul polifenolilor și activitatea antiradicalică a fost stabiltă pentru extractul etanolic și apos de propolis. Atât după conținutul total al polifenolilor, cât și după capacitatea antiradicalică, cea mai mare activitate manifestă polenul de salcâm, urmat de cel poliflor, apoi de polenului de floarea soarelui. Investigarea conținutului polifenolilor și a activității antiradicalice în mostrele de miere studiate a relevat un potențial antioxidant mai mare al mierii de salcâm, în ceea ce privește activitatea antiradicalică și al mierii de floarea soarelui, după conținutul de polifenoli. Datele obținute ne permit să afirmăm despre posibilitatea utilizării direcționate a varietăților de produse apicole în elaborarea de noi remedii sanogene cu conținut predeterminat și acțiune direcționată prin modelarea conținutului componentelor lor active.

Cuvinte cheie: propolis, polen, miere, polifenoli, activitate antiradicalică.

INTRODUCTION

The study of substances with antioxidant activity from various natural products and the detection of their mechanisms of action are of interest in the elaboration of new products beneficial to health, with predetermined composition and directed action, as an alternative to pharmacological therapy. Their elaboration must first of all be based on the prospecting of natural products, which are already being used for therapeutic purposes, by modelling the content of their active components.

Beekeeping products such as honey, propolis and pollen are natural therapeutic remedies used by humans for hundreds and thousands of years. Research in recent decades has revealed that their healthy (sanogenic) properties are largely determined by the content of antioxidant substances – enzymes, amino acids, organic acids, polyphenols, flavonoids, vitamins etc. (BUIA & BARAC, 1989; KOMOSINSKA-VASSEV et al., 2015). However, the most important antioxidants in bee products are phenols and polyphenols. It is known that polyphenols in plants represent an important class of substances both for the normal functioning of the plant organism and in the aspect of their use for nutritional and curative purposes (OZCAN et al., 2014). The antioxidant capacity of the polyphenols is determined: by the redox properties that enable them to act as reducing agents, hydrogen donors and singlet oxygen saturation (LEJA et al., 2007; MĂRGHITAŞ et al., 2009a, 2009b); the possibility to form metal-chelated compounds (RICE-EVANS et al., 1996) and react with free radicals and genotoxic or carcinogenic substances (BLUESTONE & TANG, 2005; CARPES et al., 2007), and it is considered to be much higher than the one of essential vitamins, contributing significantly to the healthy effects of natural products, in which they are contained (HENDRICH, 2006). Polyphenols also have the property of binding and precipitating protein macromolecules, carbohydrates and digestive enzymes (OZCAN et al., 2014).

The total concentration of phenols in bee products is highly dependent on the floral source, the place where nectar and pollen are collected. Pollen itself is a primary source of polyphenols, especially flavonoids. However, the antioxidant activity of pollen collected by bees can be provided not only by phenolic compounds, but also by other substances in its composition such as amino acids, vitamins etc. (DE ARRUDA et al., 2013). About 250 substances have been identified in the pollen composition collected by bees (NOGUEIRA et al., 2012).

It has been established that the chemical composition (polyphenols, terpenoids, steroids, and amino acids) of propolis – a bee product, which comes from the plant resins collected by honey bees from different plants – varies depending on the region and the plant-dominated species (FABRIS et al., 2013; SILVA-CARVALHO et al., 2015). In Europe, the main plant source of propolis is black poplar *(Populus nigra* L.), in Russia – silver birch (*Betula pendula*

Roth. (*Betula verrucosa* Ehrh)). Flavonoids and phenolic acids are among the phenolic compounds in propolis in European countries, representing about 10-15% by weight (BANKOVA et al., 2002; BANKOVA, 2005).

Also, phenols and polyphenols are some of the most important substances in natural honey which, in addition to enzymes, organic acids, amino acids, Maillard reaction products, ascorbic acid, carotenoids, attribute its antioxidant activity (GHELDOF et al., 2002).

SCHRAMM D. et al. (2003) established the increase of the total plasma phenol content, as well as the antioxidant and reducing capacity of the plasma as a result of honey consumption according to the therapeutic recommendations. These data confirm the concept that phenolic antioxidants from honey are bioavailable and contribute to increased plasma antioxidant activity. It has also been established that the consumption of natural products with a high content of antioxidants of phenolic origin is associated with a reduced risk of cardiovascular diseases and chronic diseases (GRIBEL & PASHINSKI, 1990; OZCAN et al., 2014).

Based on the correlation between the content of polyphenols in various natural products and their antioxidant activity with curative effects, the purpose of this paper is to reveal the correlation of polyphenol content and antiradical activity in bee products – propolis, pollen and honey.

MATERIAL AND METHODS

Three types of pollen – acacia, poly flower and sunflower and three types of honey – acacia, lime and sunflower, most commonly found on the territory of the Republic of Moldova were investigated. Also, aqueous and ethanolic solutions of propolis samples were analysed.

Samples of pollen, honey and propolis were collected from the central area of the Republic of Moldova.

In order to determine the antioxidant properties and the content of the polyphenols in the studied samples, ethanol-based propolis extracts (70%), water-based propolis extract and ethanol-based pollen extract (60%) were prepared. The samples were stored in special glass vessels at 4°C in the dark until analyses were performed. Honey solutions (10%) were prepared immediately prior to the analysis.

The total phenol content was determined with the Folin-Ciocalteu reagent, and the results were expressed as mg $_{\text{gallic acid}}$/g of sample, using the gallic acid calibration curve, within the limits of 0.2 to 1.2 mg /ml (Fig. 1).

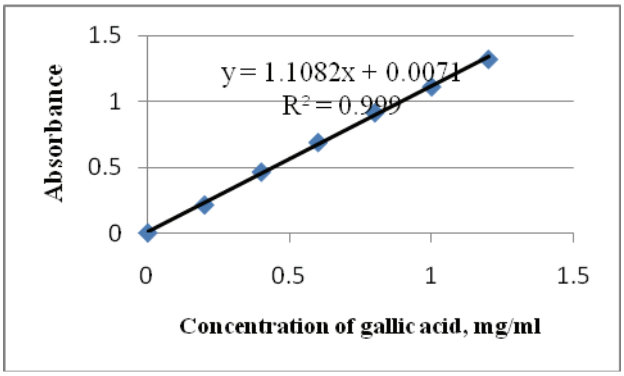

Figure 1. Calibration curve of gallic acid (SINGLETON et al., 1999).

Various methods, both enzymatic and non-enzymatic, are used to determine antioxidant activity. Of the non-enzymatic methods, the most commonly applied are indirect ones such as DPPH$^{\bullet}$, ABTS +, FRAP, and direct – the ORAC method. The DPPH method (BRAND-WILLIAMS et al., 1995) is widely used for the preliminary selection of substances capable of capturing active oxygen species and reflects the content of antioxidants in the analysed samples. The free radical spectrophotometric method DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) is based on the lower absorbance of the radical in the presence of antioxidants.

The antiradical activity was expressed as a percentage of reduction of DPPH$^{\bullet}$ (*AA* %), where:

A_0 – absorbance of DPPH$^{\bullet}$ solutions at time t = 0 s;

A_{30} – absorbance of DPPH$^{\bullet}$ solutions after 30 minutes.

The low A_{30} value in the studied extract indicates an increased antioxidant activity.

RESULTS AND DISCUSSIONS

Polyphenol content in bee products.

The data obtained revealed that the content of polyphenols significantly differs in the investigated bee products – propolis, pollen and honey, (Table 1).

The analysis of the content of polyphenols in the ethanolic and aqueous extract of propolis revealed an extremely large amount in the alcoholic extract, which constitutes 375.35 mg $_{\text{gallic acid}}$/g, as opposed to the water-based one – 64.14 mg gallic acid/g (Table 1).

Table 1. The content of polyphenols in alcoholic and aqueous solution of propolis, pollen and honey.

Samples	The total content of polyphenols, $mg_{gallic\ acid}/g$
Alcoholic solution of propolis	373.35±0.09
Aqueous solution of propolis	67.14±0.18
Acacia pollen	25.39±0.01
Poly flower pollen	23.61±0.03
Sunflower pollen	17.21±0.04
Acacia honey	0.16±0.01
Lime honey	0.23±0.01
Sunflower honey	0.26±0.01

The obtained results show that propolis contains a significant amount of polyphenols, which gives them remarkable biological properties, used for therapeutic purposes (SELAMOGLU et al., 2015; SILVA-CARVALHO et al., 2015; BERETTA et al., 2017). Although, the literature contains convincing evidence about the high physiological potential of aqueous propolis solutions (VOLPERT & ELSTNER, 1993; BERETTA et al., 2017), as the obtained data eloquently demonstrate the superiority of alcoholic propolis extract compared to aqueous in terms of polyphenol content. Because a greater quantity of polyphenols was detected in the alcoholic extract, we can assume that ethanol is a better solvent for the extraction of the active components of propolis. It is more difficult to obtain aqueous propolis extract, and special conditions are required in order to isolate compounds with antioxidant activity as fully as possible.

The content of polyphenols is pollen is lower compared to propolis. The bioactive properties of pollen extract may vary depending on the solvent used to isolate the active components. Ethanol is most commonly used to extract polyphenols from pollen (CARPES et al., 2007). We used 60% ethanol solution in the study. Data on the content of polyphenols in acacia, poly flower and sunflower pollen are presented in Table 1.

Thus, a higher polyphenol content was established in acacia pollen (25.39 $mg_{gallic\ acid}/g$), compared to poly flower pollen (23.61 $mg_{gallic\ acid}/g$) and sunflower pollen (17.21 $mg_{gallic\ acid}/g$), although, according to the data from literature, poly flower pollen is considered to be richer in phenolic substances. Since the study of polyphenols in pollen is most commonly used to establish the botanical source of its origin, we can assume that the acacia pollen, besides the basic source, may contain pollen from other botanical species, which gives an increased amount of polyphenols. Thus, the composition and quantity of the polyphenols in pollen may vary depending on the botanical and geographical origin and the plant species, environmental conditions and age of the plant (MĂRGHITAŞ et al., 2009a; MORAIS et al., 2011; DE ARRUDA et al., 2013).

Polyphenols were detected in all the analysed honey samples: acacia honey, lime honey and sunflower honey (Table 1), but honey has the lowest polyphenol content from all studied bee products.

A comparative analysis of polyphenols in the investigated honey samples revealed a higher amount of polyphenols in sunflower honey (0,26 mg gallic acid / g) and lime honey (0,23 mg gallic acid / g) compared to acacia honey (0,16 mg gallic acid / g). Thus, we can assume that sunflower and lime honey are more beneficial in terms of higher polyphenol content.

It is known that the quality of propolis, its high and varied biological activity (antifungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anticancer, immunostimulating, and local analgesic) is determined by its polyphenolic profile, especially the content of phenolic acids and flavonoids (MARCUCCI, 1995). Although propolis from different regions of the world has a different composition of polyphenols, it has been established that its pharmacological properties are identical (MOREIRA et al., 2008).

Pollen collected by bees contains significant quantities of polyphenolic substances, in particular flavonoids, which are considered the main pollen ingredients that can be used in establishing the quality standard in relation to their nutritional and physiological properties and in controlling the quality of commercial pollen preparations (KROYER & HEGEDUS, 2001; BARTH et al., 2010; DA SILVA et al., 2014).

Although the lowest quantitative level of polyphenols – which are mostly of vegetal nature and derived from pollen, nectar and sometimes from propolis (JASICKA-MISIAK et al., 2012) – has been detected in honey, there are data that indicate that phenolic compounds in honey act as natural antioxidants, and honey is commonly used due to this property of polyphenols. Phenolic compounds such as quercetin, caffeic acids, caffeic acid phenethyl ester (CAPE), acacetin, kaempferol, galangin, chrysin, pinocembrin, apigenin, which have a promising effect in the treatment of chronic diseases, have been detected in honey (ISTASSE et al., 2016). It has been demonstrated that phenolics from honey manifest protective action of erythrocyte membranes against oxidative damage (ALVAREZ-SUAREZ et al., 2012) and honey consumption contributes to an increased level of phenols in human blood plasma (SCHRAMM et al., 2003). Generally, most phenolic compounds in honey are flavonoids, phenolic acids and their derivatives (GHELDOF et al., 2002; EREJUWA & SULAIMAN, 2012; PÉREZ-PÉREZ et al., 2013).

Therefore, the importance of studying the quantity and classes of polyphenols in bee products is due to the fact that they are functional products and a natural source of biologically active molecules beneficial for health.

Antiradical activity of bee products.

Various studies have shown the positive correlation between the composition of the polyphenols and the high antioxidant potential of the bee products (MIHAI et al., 2011; FABRIS et al., 2013). The antioxidant activity of phenolic compounds is due to their chemical structure and is manifested by different mechanisms. Several methods, such as FRAP, DPPH•, ABTS analysis, are used to determine the antioxidant activity of a substance. The DPPH• method was applied to determine the antiradical activity using free radicals, to detect the ability of water-soluble compounds that act as free radical scavengers. The DPPH• method is also commonly used because the antioxidant potential of bee products is directly associated with the content of polyphenols and flavonoids (BERETTA et al., 2005). The antiradical activity of propolis, determined with the DPPH• method, revealed the high antioxidant capacity of this product in both ethanol and water extract (Fig. 2).

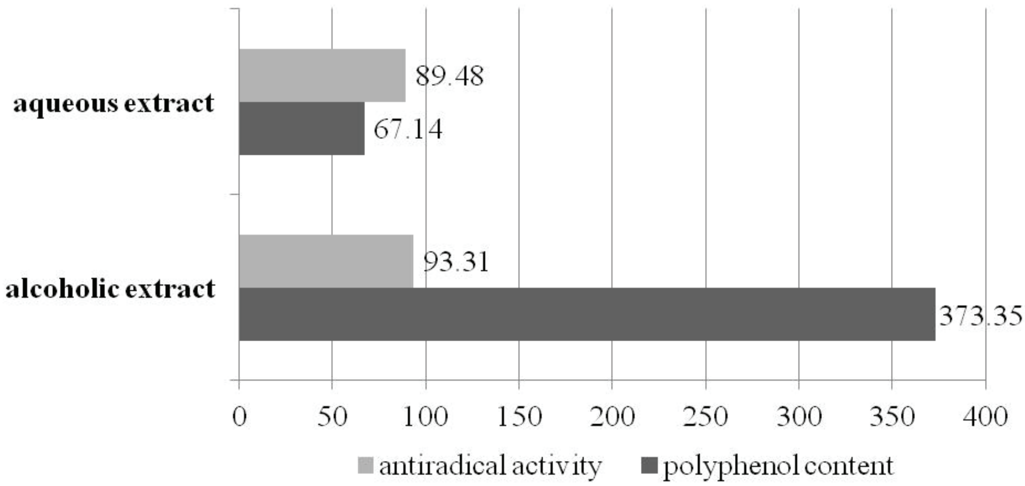

Figure 2. Correlation of antiradical activity and polyphenol content of propolis alcoholic and aqueous extracts.

The higher activity of the alcoholic extract is due, as mentioned above, to the ability of the alcohol to dissolve lipid substances, which means the extraction of many antioxidant molecules. Although in the aqueous extract of propolis it was not possible to fully extract the polyphenolic compounds, it exhibits a lower difference (3.8%) compared to antiradical activity of ethanolic extract (Fig. 2).

Ethanol propolis extract is most commonly used in research regarding biological (biochemical) properties of propolis, as opposed to the less studied aqueous propolis extract, although its constituents have a quite high antioxidant activity, which is also evident in our research (VOLPERT & ELSTNER, 1993; BERETTA et al., 2017).

Also, it has been established that the aqueous propolis solution also has relevant antimicrobial and antioxidant, activity, immunomodulatory action and as an apoptosis inducer, acting against tumours and is more harmless. Recently, the immunomodulatory potential of the aqueous extract of propolis and its protection against gram-negative bacteria has been established (SILVA-CARVALHO et al., 2015; BERETTA et al., 2017). Such properties also are found in the ethanolic extract of propolis, which makes it possible to apply both solutions in the prophylaxis and treatment of various diseases, especially of the skin, oral cavity, gastrointestinal tract and of some tumours (SELAMOGLU et al., 2015; SILVA-CARVALHO et al., 2015). It is considered that the mechanism of propolis action is achieved by the activation of macrophages (VOLPERT & ELSTNER, 1993; BERETTA et al., 2017; SILVA et al., 2017).

The antioxidant capacity of propolis has been shown to inhibit proinflammatory prostaglandins, aggregation and thromboxane formation induced by arachidonic acid (VOLPERT & ELSTNER, 1993; SELAMOGLU et al., 2015; SILVA-CARVALHO et al., 2015; BERETTA et al., 2017; SILVA et al., 2017), proving the immunomodulatory properties of ethanolic and aqueous propolis extract.

The determination of the antiradical activity of the pollen samples was carried out based on the same principles as in the propolis samples. The analysis of the obtained results revealed a higher antiradical activity of acacia and poly flower pollen, respectively 91.44 and 91.04% compared to that of sunflower – 89.64% (Fig. 3).

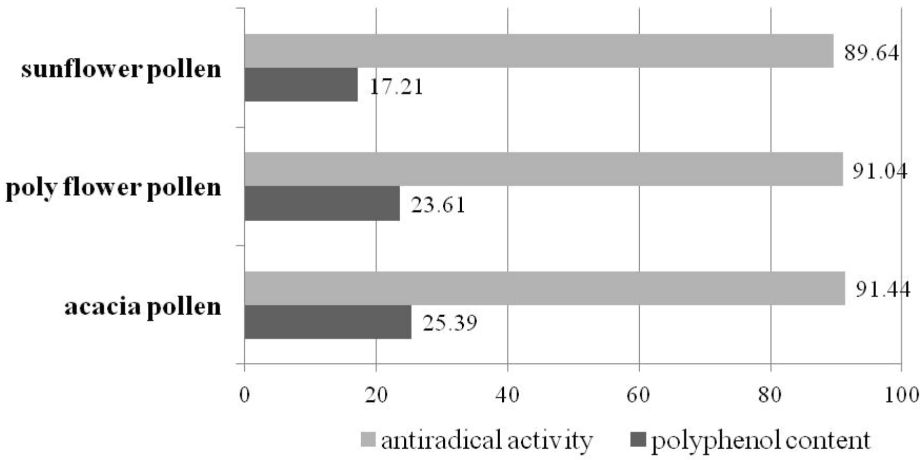

Figure 3. The correlation of the antiradical activity (%)
with the total polyphenol content (mg $_{\text{gallic acid}}$/g) in alcoholic extract (60%) of pollen.

Pollen, as well as propolis, shows quite high antioxidant activity. This capacity is largely due to the quantitative and qualitative pattern of polyphenols (MĂRGHITAŞ et al., 2009a). The data obtained in our investigations clearly demonstrated the correlation of the antiradical activity with the total polyphenol content in the analysed pollen samples (Fig. 3).

The antioxidant capacity of pollen collected by bees, as well as other physicochemical properties, depends, as mentioned above, on its botanical (floral source) and geographical origin and correlates with the total phenolic content (PASCOAL et al., 2013). This correlation could substantiate the recommendations for the use of pollen for pharmaceutical, nutritional and functional purposes.

Thus, based on the analysed pollen samples, the highest antioxidant activity is seen in acacia pollen, followed by polyflower, then by sunflower pollen (Fig. 3).

Due to its significant antioxidant capacity (polyphenol content) and significant antiradical activity, pollen is now considered a natural product with a high therapeutic potential, possessing antioxidant, bioactive, antimicrobial activity, and can be used to prevent diseases involving free radicals (CAMPOS et al., 2003; LEJA et al., 2007; PASCOAL et al., 2013).

Based on the analysis of total polyphenol content and antiradical activity determined with DPPH$^{\bullet}$ method, we can affirm that all investigated types of pollen (especially acacia pollen) can be used for nutritional, therapeutic and pharmaceutical purposes, due to the high polyphenol content and high antioxidant capacity.

The study of the antiradical activity of honey samples showed a lower value compared to propolis and pollen (Fig. 4).

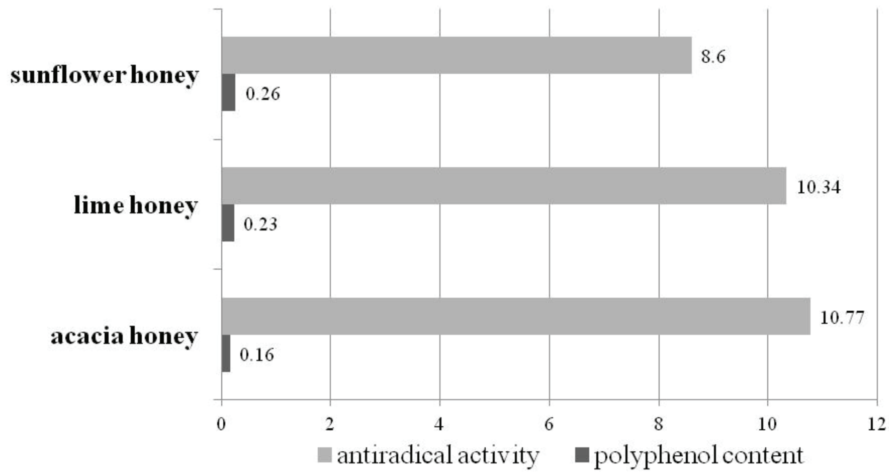

Figure 4. The correlation of the antiradical activity (%) with the total polyphenol content (mg $_{\text{gallic acid}}$/g) in honey samples.

Although the antioxidant activity of acacia honey, lime honey and sunflower honey harvested in the central area of the Republic of Moldova is lower according to the data obtained by other authors (ALZAHRANI et al., 2012; PERNA et al., 2013), there are, however, differences between the samples. Thus, the greatest antiradical activity was seen in acacia honey and lime honey, 10.77% and 10.34% respectively, compared to sunflower honey – 8.6%. The obtained results regarding the antiradical activity partly coincide with the data regarding the polyphenols content, except for acacia honey which has a lower polyphenol content, but a higher antiradical activity, compared to the other types of honey (Fig. 4).

Considering that the differences between the samples in terms of antiradical activity and polyphenol content are not significant, we can suppose that other antioxidants, such as enzymes, amino acids, some vitamins, are involved in the antioxidant activity of acacia honey. The data of our research correlates with the results obtained by other authors. The higher antiradical activity of acacia honey may be associated with the high content of ascorbic acid and proline content in this type of honey (CHUA et al., 2013). According to the same authors, acacia honey has a higher antioxidant activity using the FRAP (Ferric Reducing / Antioxidant Power Assay) method. The analysis of different types of honey in the eastern territory of Romania in terms of antiradical activity and polyphenol content revealed higher values of these indices in lime honey (DOBRE et al., 2010; MĂRGHITAȘ et al., 2009b). Thus, the results obtained in similar investigations in other countries and regions largely coincide with the data obtained in this study regarding antiradical activity, determined with the DPPH$^{\bullet}$ method.

Thus, based on the obtained data regarding the antiradical activity, determined with the DPPH$^{\bullet}$ method, we can assume that acacia honey is more beneficial. The differences between the analysed samples considering the antioxidant activity and the content of the polyphenols can be attributed to the natural variation of the composition, the floral source and different harvesting periods.

CONCLUSIONS

The high antiradical capacity of the ethanolic and aqueous extract of propolis, as well as the significant content of polyphenolic substances, suppose the use of both extracts in the targeted elaboration of the substances with a curative effect.

Both according to the total polyphenol content and the antiradical capacity, the greatest activity is seen in acacia pollen, followed by polyflower, then sunflower pollen, which allows their use as natural products with a high therapeutic potential, with antioxidant, bioactive, antimicrobial activity, as well as to prevent diseases in which free radicals are involved.

The investigation of the polyphenol content and the antiradical activity of honey samples revealed the greater antioxidant potential of the acacia honey, in terms of antiradical activity and of sunflower honey, in terms of polyphenol content. This allows modelling both characteristics in order to obtain products with a high therapeutic potential.

The data obtained regarding the total polyphenol content and the antiradical activity in beekeeping products – propolis, pollen and honey – allow us to affirm about the possibility of the targeted use of the varieties of bee products in the elaboration of the healthy products with predetermined content and directed action.

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