

Correlation between Polyphenolic Profile and Antioxidant Activity of Propolis from Transylvania

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Abstract

Propolis samples used for the present study were collected in Transylvania. Our study had the purpose to investigate the amount of total polyphenols and the correlation that it may exist between the mentioned class of compounds and antioxidant properties. Polyphenolic profile was determined using Folin-Ciocalteu colorimetric method, while antioxidant activity was investigated by means of free radical scavenging activity (DPPH assay) and ferric reducing antioxidant power (FRAP assay). The content in polyphenols for analyzed propolis samples ranged between 24.46 and 62.39 g standard mixture/ 100 g propolis. All propolis samples showed antioxidant activity. The correlation between polyphenolic profile of propolis and its antioxidant properties was found to be positive.

Keywords: DPPH assay, FRAP assay, polyphenols, propolis.

1. Introduction

Propolis is a natural honeybee product known to have beneficial effects on human health. It has a complex composition, which highly depends upon the flora available at the site of collection [1, 2]. Among the years, more than 300 compounds have been reported as constituents of propolis: flavonoids, phenolic acids and their esters, terpenoids, steroids. These compounds are considered responsible for the pharmacological activities of propolis such as: antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and immunomodulatory [3, 4].

At present a wide variety of synthetic antioxidants are being used, even though their use has been restricted by legislation due to doubts over their toxic and carcinogenic effects. The interest in natural antioxidant has been increased mostly for those containing flavonoids and phenolic acids which prevent free radical damage [5]. The antioxidant activity of phenolics is mainly due to

their redox properties, which allows them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [6].

Phenolic compounds play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals [7].

Propolis, due to its pharmacological activities already proven has been used since ancient times. Nowadays it is also incorporated in toothpaste, mouthwash solutions, ointments, creams and lotions. It can also be found as tablets in pharmacies.

In the present work we have investigated the polyphenolic profile and antioxidant activity of propolis harvested in Transylvania in order to identify possible correlations between the mentioned characteristics. The amount of total phenolics was determined using Folin-Ciocalteu colorimetric method, DPPH and FRAP assays were performed also by spectrophotometric measurements.

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2. Materials and methods

All propolis samples (20) used for the present study were obtained directly from beekeepers in Transylvania and were kept in the freezer (-20°C) until analysis. Origin of the propolis samples subjected to the present study is shown in table 1.

Table 1. Origin of the studied propolis samples

Sample code	Location	Sample code	Location
PS 1	Tarnaveni, Mures county	PS 11	Bulz, Bihor county
PS 2	Iernut, Mures county	PS 12	Valea lui Mihai, Bihor county
PS 3	Targu Mures, Mures county	PS 13	Ariniş, Maramureş county
PS 4	Ludus, Mures county	PS 14	Dej, Cluj county
PS 5	Fersig, Maramures county	PS 15	Corund, Harghita county
PS 6	Targu Lapus, Maramures county	PS 16	Mărtiniş, Harghita county
PS 7	Blaj, Alba county	PS 17	Teaca, Bistriţa-Năsăud county
PS 8	Blajel, Sibiu county	PS 18	Josenii Bârgăului, Bistriţa-Năsăud county
PS 9	Izvoarele, Alba county	PS 19	Bistriţa, Bistriţa-Năsăud county
PS 10	Livada, Satu-Mare county	PS 20	Agriş, Bistriţa-Năsăud county

Propolis samples were grounded into fine powders. The active principles were then extracted with ethanol 70%. 1 g of each propolis sample was mixed with 30 ml ethanol and left over night with continuous stirring at 400 rpm. The suspension was then filtered using qualitative filter paper (\varnothing 90 mm). The extraction was repeated two times, the extracts were combined and the volume was adjusted to 100 ml into a volumetric flask (initial extract, 1% concentration). All extracts were kept in the dark until further analysis. Initial propolis extract was diluted to the concentrations needed for the determination of proposed characteristics.

Total polyphenolic content was determined by the Folin-Ciocalteu colorimetric method with minor

modifications [2]. A reference mixture of pinocembrin and galangin (2:1, w/w) of appropriate concentrations range was used for the calibration curve. 5 μ l of propolis extract 0.06% was added over 195 μ l of distilled water. The Folin-Ciocalteu reagent was added over the solution and was mixed for 5 minutes. 30 μ l of sodium carbonate were added and the resulted mixture was incubated for 2 hours in the dark. The absorbance was measured at 760 nm using a multi-channel detection UV-Vis spectrophotometer Biotek Synergy 2 (BioTek Instruments, USA). Results are expressed as g standards mixture/ 100 g of propolis.

For the antioxidant activity two methods were employed: DPPH and FRAP assays.

For the DPPH assay, the Brandt-Williams method [8] was employed with some modifications. Radical scavenging activity of a 1000 μ l/ml propolis solution was measured against 2,2-diphenyl-picrylhydrazyl radical (DPPH) by spectrophotometric means. Briefly, 5 μ l of propolis solution was added over 295 μ l 0.01 M DPPH solution. Samples were kept in the dark for 20 minutes and the absorbance was measured at 517 nm against a blank. The inhibition percentage was calculated using the following equation:

$$\text{Inhibition (\%)} = (A_{\text{DPPH}} - A_{\text{sample}}) / A_{\text{DPPH}} * 100,$$

where A_{DPPH} is the absorbance of DPPH solution and A_{sample} is the absorbance of the sample. The extent of the inhibition of propolis samples was compared with a standard curve obtained from the corresponding readings of Trolox solutions (0.2 – 1.5 mM). Radical scavenging activity of propolis is expressed as mmol Trolox/ g propolis.

“Antioxidant power” of studied samples was evaluated using Ferric Reducing Antioxidant Power (FRAP) as described by Benzie & Strains [9]. FRAP reagent was prepared by mixing 25 ml of 300 mM acetate buffer at low pH=3.6 (3.1 g sodium acetate and 16 ml glacial acetic acid per liter of buffer) with 2.5 ml of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution (0.031 g TPTZ in 10 ml of 40 mM HCl) and 2.5 ml of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (3.24 g ferric chloride per liter of distilled water). FRAP reagent was freshly prepared each time before use and was incubated at 37°C for 15 minutes [10]. Initial propolis extracts were diluted up to the concentration of 1000 μ l/ml. The method employed [10] was modified so it can be applied using a multi-channel detection UV-Vis

spectrophotometer Biotek Synergy 2. Aliquots (10 μ l) of each diluted ethanolic extracts of propolis were mixed with 300 μ l freshly prepared FRAP reagent. FRAP value are obtained by measuring the absorbance at 593 nm. Aqueous solutions of known ferrous sulphate concentrations (0.1-1 mmol/L) were used to construct the calibration curve. The results are expressed as mmol $\text{Fe}^{\text{II}}\text{SO}_4/\text{g}$ propolis.

3. Results and discussion

Most propolis samples were dark brown, with amber – reddish pieces and intense aromatic resin flavor. Table 2 shows the content in total polyphenols and antioxidant activity (DPPH and FRAP values) for the studied propolis samples.

Table 2. Total polyphenols content and antioxidant activity of studied propolis samples

Sample code	Total polyphenols [g/100 g propolis]	DPPH [mmol Trolox/ g propolis]	FRAP [mmol $\text{Fe}^{\text{II}}\text{SO}_4/\text{g}$ propolis]
PS 1	55.92 \pm 1.91	0.98 \pm 0.05	1.34 \pm 0.02
PS 2	41.98 \pm 2.30	0.86 \pm 0.02	1.20 \pm 0.03
PS 3	31.22 \pm 1.11	0.57 \pm 0.02	1.16 \pm 0.04
PS 4	45.68 \pm 1.83	0.92 \pm 0.01	1.34 \pm 0.04
PS 5	62.39 \pm 2.02	1.10 \pm 0.02	1.91 \pm 0.06
PS 6	55.01 \pm 1.54	1.09 \pm 0.03	2.15 \pm 0.01
PS 7	58.16 \pm 0.93	1.23 \pm 0.06	2.36 \pm 0.04
PS 8	37.53 \pm 1.13	0.68 \pm 0.03	1.60 \pm 0.05
PS 9	61.34 \pm 3.08	0.97 \pm 0.12	2.54 \pm 0.10
PS 10	24.46 \pm 0.91	0.29 \pm 0.10	0.74 \pm 0.05
PS 11	54.67 \pm 1.87	0.93 \pm 0.01	1.33 \pm 0.04
PS 12	40.20 \pm 1.11	0.73 \pm 0.03	1.90 \pm 0.14
PS 13	59.79 \pm 2.54	1.24 \pm 0.01	2.37 \pm 0.04
PS 14	46.46 \pm 1.58	0.88 \pm 0.05	1.86 \pm 0.07
PS 15	36.04 \pm 5.87	0.51 \pm 0.03	1.21 \pm 0.13
PS 16	53.68 \pm 4.75	0.85 \pm 0.02	1.35 \pm 0.06
PS 17	40.70 \pm 0.67	0.65 \pm 0.02	1.68 \pm 0.04
PS 18	49.31 \pm 1.25	0.94 \pm 0.02	1.85 \pm 0.08
PS 19	34.57 \pm 0.60	0.70 \pm 0.02	1.46 \pm 0.04
PS 20	51.41 \pm 1.13	0.91 \pm 0.01	1.85 \pm 0.08

* Results are expressed as mean of three determinations \pm standard deviation

The content in polyphenols for analyzed propolis samples ranged between 24.46 and 62.39 g standard mixture/ 100 g propolis. Differences in the polyphenols content may be due to the different locations where propolis has been harvested. It is reported in the literature that differences in chemical composition of propolis

are due to the local flora available to bees at the site of collection [2, 11].

The ferric reducing antioxidant power (FRAP) assay was used for the determination of “antioxidant power” of propolis samples gathered in the region of Transylvania. In this method the ferric reducing ability of antioxidants is measured. At low pH, ferric-tripyridyltriazine (Fe^{3+} -TPTZ) complex is reduced to the ferrous (Fe^{2+}) blue color complex with an absorption maximum at 593 nm [10]. FRAP values have been calculated by comparing the absorbance at 593 nm of the investigated propolis samples with the one of ferrous solutions of known concentrations in the range 0.1-1 mmol/L. The comparison between the two antioxidant methods used for the present study is shown in figure 1.

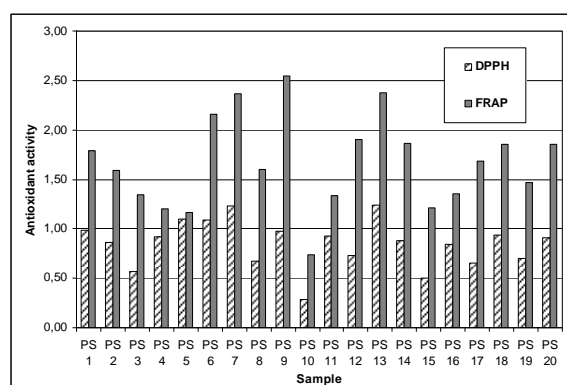


Figure 1. Comparison between DPPH and FRAP values for the investigated propolis samples

All propolis samples showed antioxidant activity. By comparing the two methods used for the determination of antioxidant activity, they seem to be well correlated. Propolis with high radical scavenging activity seems to have also high “antioxidant power”. The relationship between DPPH radical scavenging activity of propolis samples and the amount of total polyphenols, and FRAP values of propolis samples and the amount of total polyphenols was investigated, and a positive correlation was observed for both of them (Figure 2 and 3).

Due to differences that we have found with regards to antioxidant activity of propolis, it could be concluded that not only geographical region where propolis is harvested influences the chemical composition, but also the antioxidant properties.

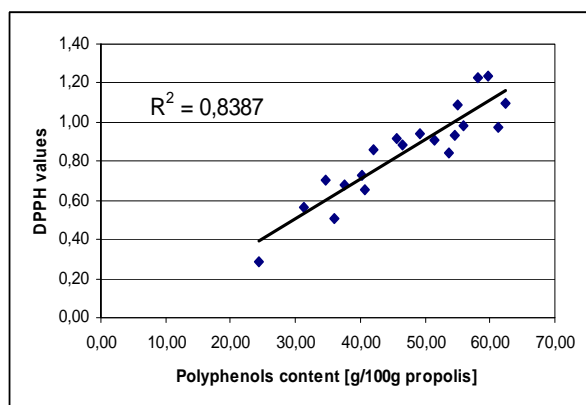


Figure 2. Correlation between DPPH values and total polyphenols

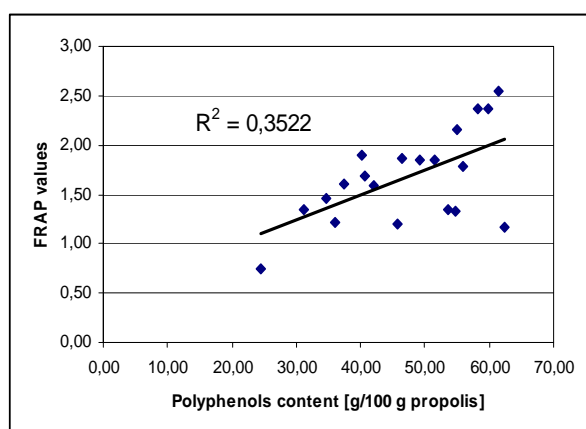


Figure 3. Correlation between FRAP values and total polyphenols

In conclusion, samples with the highest amount of polyphenols showed high antioxidant activity. Further investigations regarding the identification of the main compounds responsible for the antioxidant properties of propolis are necessary.

4. Conclusions

Propolis is a natural product which has been used since ancient times and continues to attract more interest. However the difference in its general composition creates a problem with regards to its use as a pharmaceutical compound.

In conclusion, antioxidant activity of the investigated propolis has been demonstrated for all samples, by means of both methods used. Due to its important therapeutically properties, propolis could be the answer to our need for natural products, but this matter requires more investigations.

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