TOTAL POLYPHENOLS, FLAVONOIDS AND RADICAL SCAVENGING ACTIVITY OF BEEPOLLEN AND BEEBREAD COLLECTED FROM TRANSYLVANIA AREA

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Abstract: The content of total phenolic compounds and flavonoids was measured in methanol beepollens' and beebreads' extracts as well as their radical scavenging activity. Beepoleens' extracts were the most effective DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavengers, which contained the highest amount of polyphenols. The major contribution to the antiradical activity is due to the total polyphenolic compounds.

INTRODUCTION

The distructive action of free radicals is focused especially to the cells, particulary to polyunsaturated fatty acids, the major constituents of the cell membranes, but also to the proteins, especially to the DNA, to enzymes, to lipids. Therefore, the continuous action of the free radicals can lead to disturbances in membrane structure and function, and consequently to various degenerative deseases such as cardiovascular disease, various cancers and neurological diseases (Bagchi, 1998; Narayana, 2001).

The polyphenolic compounds are plant secondary metabolites widely spread throughout the plant kingdom (Nijveldt, R.J, 2001). Flavonoids belong to a group of polyphenolic compounds, which are classified as flavonols, flavonones, flavones, flavanols, flavan-3-ols and isoflavones according to the positions of the substitutes present on the parent molecule. Flavonoids of different classes have several pharmacological effects and therapeutic potential, such as antioxidant, anti-inflammatory, antineoplastic and antimicrobial activity (Narayana K R,, 2001).

Recent interest in developing analytical methodologies for their detection and measurement from plant sources is do to their potential as protective factors against cancer and heart diseases in part because of their potent antioxidative properties (Martin K, 2006).

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. One way is the direct scavenging of free radicals. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. (Nijveldt, 2001). Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities (Damintoti K., 1998).

There are many methods to determine the antioxidant capacity. These methods differ in terms of their assay principles and experimental conditions. Consequently, in different methods, particular antioxidants have varying contributions to total antioxidant

potential (Cao and Prior, 1998 cited by Katalinic V., 2006). The DPPH method (Brand-Williams, Cuvelier and Besnet, 1995) consist in the reaction of DPPH (2,2-diphenyl-1-picrylhydrazyl) a stable free radical, which accepts an electron or hydrogen radical to become a stable molecule, and, accordingly, is reduced in presence of an antioxidant (Kroyer G., 2001). DPPH radical are widely used for the preliminary screening of compounds capable to scavenging activated oxygen species since they are much more stable and easier to handle than oxygen free radical (Tominaga Y, 2005).

Previous study conducted on different plants revealed that antioxidants activities present in the extracts were essentially due to polyphenols, a good correlation ($r>0.9$) was found between polyphenol content and antioxidant activities (Damintoti K., 1998).

The total polyphenol content of bee-collected pollen and their extracts was previously determinated by Kroyer (2001). The average amount of polyphenols in beepollens was 8,2 mg/g, with some relative variation due to their botanical origin (7.4-9.4 mg/g). In the beepollens' extracts (ethanol, methanol-water 1:1 and water) the amount of total polyphenols was significantly increased (21.4-24.6 mg/g) with the highest content in the ethanolic extract (Kroyer, 2001). As a result, the best antiradical activity against the DPPH was pursued by the ethanolic extract (53 %).

The antioxidant activity of honeybee-collected pollen has been recognized as a free radical scavenger and as a lipid peroxidation inhibitor (Campos et al, 1997, cited by Almaraz-Abarca N., et al., 2004). The total flavonol content in a mixture of beepollen and constituent pollens, determined by Almaraz-Abarca (2004), ranged between 3.5-0.1 mg/g dry matter of pollen. In addition, using a modified Campos method (1997), the antiradical activity was expresed as the amount of antioxidant needed to decrease by 50% the initial DPPH concentration (EC_{50}). Comparing the antiradical activity and the flavonol content of the samples, no correlation seems to exist between the total extract of the mixture of beepollen and those of its costituent pollens, in terms of their flavonol content (Almaraz-Abarca, 2004).

High levels of antioxidant activity, as determined by ORAC (Oxygen Radical Absorbance Capacity), have been found to be present in blueberries 61 ORAC units (μmole TE/g) and black raspberries 164 ORAC units (Narayana, 2001). Bee pollen tested for ORAC antioxidant activity resulted in 247 ORAC units, the highest score ever recorded for any whole food. Thus, bee pollen has been shown to be at the top of the list of foods exhibiting antioxidant activity. The total polyphenol content of this product has the highest value (15.05 mg/g) of any food yet tested

MATERIAL AND METHOD

Chemicals:

Standards for gallic acid, quercetin were purchased from Sigma-Aldrich Chemie; methanol solvent; Folin-Ciocalteau reagent (2N) from Merk; anhydrous sodium carbonate, DPPH (2,2-diphenyl-picrylhydrazyl) from Sigma-Aldrich Chemie.

Pollen samples:

Polifloral mixture of honeybee collected pollen and beebread was purchased from local beekeepers.

Preparation of extracts:

One gram of the mixtures of beepollen and beebread were individually extracted using methanol solvent at the room temperature for 48 h. After maceration, the extracts

were separated by centrifugation for 15 min at 3000 rpm. The supernatant was evaporated to dryness under vaccum. The resulting dried extracts were dissolved in methanol and stored until analysis.

Determination of total polyphenols content

The content of total polyphenols was quantified according to the Folin-Ciocalteau spectrofotometric method (Singlenton, and col., 1999) using gallic acid as reference standard (cited by Meda, A, 2005). 2,5 ml of Folin-Ciocalteau reagent (0.2 N) was added to 0,5 ml of pollen extract and mixed for 5 min. After the addition of 2 ml sodium carbonate solution the extracts was incubated for 2 h. The absorbance at 760 nm was then measured against a methanol blanc.

A standard curve of gallic acid was created using an adequately range of gallic acid solutions from 1 to 25 µg/ml ($y = 21.96063x - 0.02337$, $r^2 = 0.99748$). The results were expresed as Gallic Acid Equivalent (mgGAE/g dry matter sample).

Determination of flavonoid content

The flavonols content was determined using a modified Dowd method (Meda, A., 2005) using quercetin as a reference compound. The beepollen methanol extract (5 ml) was mixed with 5 ml aluminum trichoride 2%. The absorbtion at 415 nm was readed after 10 min, using a methanol blanc solution.

The content of flavonols was calculated using a standard calibration curve prepared for quercetin (1 to 50 µg/ml).

Free radical scavenging activity

The scavenging activity of beepollen extracts for the DPPH was determined spectrophotometrically by a slightly modified method of Brand-Williams et al, 1995 (Meda, A., 2005). Extract solution of beepollen was mixed with 2.5 ml DPPH solution in methanol (0.02 mg/ml). Samples were kept for 15 min at room temperature and then the absorption was measured at 517 nm. Absorption of blanc sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The radical scavenging activity is determined in terms of PI-values, which was calculated by the ratio of the decrease of ansorbtion of the DPPH-pollen extract to the absorbtion value of the reference sample, according to the formula:

PI (% inhibition) = $[A_0 - A/A_0]x\ 100$, were A_0 is the absorbancy of the blanc solution and A_s is the absorbancy of the sample after 15 minutes.

All the experiments was carried out in triplicate.

Equipments

In order to perform the analysis the following equipments were used: centrifuge Sigma 2-5, rotary evaporator Heidolph, spectrofotometer UV-1700 Shimadzu.

RESULTS AND DISCUTIONS

The total polyphenolic contents in the two beehive products are shown in Table 1.

Table 1

Total phenolic content and free radical scavenging activity and of beepollen and beebread

Samples	Total phenolic content (mgGAE/g)	Flavonoids (mgQE/g)	PI-value (%)
Polifloral beepollen	25.66 ± 0.4	1.22 ± 0.2	87.95 ± 2
Beebread	15.33 ± 0.51	5.13 ± 0.3	53.84 ± 3

Data expressed as mean ± standard deviation of three samples analysed separately.

The content of polyphenols compounds (mg/g) in methanolic extracts, determined from regression equation of calibration curve (y =21.96063x -0.02337, r^2 = 0.99748) and expressed in gallic acid equivalents (GAE), varied between 25.66 in polifloral beepollen and 15.33 in beebread.

It can be observed that the content of phenolics in the extracts correlates with their antiradical activity, confirming that phenolic compounds are likely to contribute to the radical scavenging activity of these beehive products.

The highest amounts of flavonoids were found in extracts of beebread (5.13 mgQE/g), the beepollen contain a lower amounts of these compounds (1.22 mgQE/g), which contained the highest amount of phenolics.

CONCLUSIONS

It is known that only flavonoids of a certain structure and particularly hydroxyl position in the molecule, determine antioxidant properties; in general these properties depend on the ability to donate hydrogen or electron to a free radical. Detailed examination of phenolic composition in plant extracts is required for the comprehensive assessment of individual compounds exhibiting antioxidant activity. Howard et al. (2000) (citated by Karadenüz F. et al, 2005) reported that flavonoid concentrations were negatively correlated to the antioxidant activity of pepper cultivars.

In a previous estimation of total flavonoid content Chang C-C. (2002) of propolis, the convenient colorimetric method utilizing aluminium chloride reaction was proved to be specific only for flavones and flavonols, while another utilizing 2,4-dinitrophenylhydrazine reaction was specific for flavonones. Therefore the autors suggest both analysis as two complemetary colorimetric methods for establish the real content of total flavonoids.

As previous study indicate, the results of this preliminary study conducted on the beepollen and beebread from Transylvania area, demonstrated a good correlation between the total polyphenols content of their methanol extracts and the antiradical activity. In the case of the flavonoid content, no correlation seems to exist with their scavenging activity, the colorimetric method used has estimated partially the flavonoid real content of the samples.

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