ORIGINAL ARTICLE

Comparative evaluation of the polyphenol composition and antioxidant capacity of propolis and *Echinacea purpurea*

Silviya S. Georgieva[1], Valentina L. Christova-Bagdassarian[2], Maria S. Atanassova[3]

[1]Institute of Chemical Engineering, Bulgarian Academy of Sciences;
[2]National Center of Public Health and Analysis, Department "Food Contact Materials";
[3]Metallotechnica Ltd.; ***Sofia, Bulgaria***

Received May 28, 2013
Accepted September 5, 2013

Published Online November 1, 2013

DOI 10.5455/jeim.050913.or.089

Corresponding Author
Valentina L. Christova-Bagdassarian
National Center of Public Health and Analyses,
15, Akad. Iv. Ev. Geshov Blvd,
1431 Sofia, Bulgaria.
v.hristova@ncpha.government.bg

Key Words
DPPH; *Echinacea purpurea*; Propolis; Total flavonoids; Total phenolics

Abstract
Objective: This study was undertaken to evaluate and compare total phenolics and total flavonoides, and antioxidant capacity of propolis and *Echinacea purpurea* ethanol extracts.
Methods: Propolis and dried *Echinacea purpurea* extracts were obtained by extraction methods. The extracts were tested for total phenol and total flavonoid contents and antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.
Results: The content of total phenolics and total flavonoids of propolis were found to be higher than *Echinacea*. The *E.purpurea* extract exhibited nearly 10 times lower antioxidant content in comparison with the propolis extracts. The 70% ethanol extract of propolis presented maximum antioxidant content, and the IC_{50} concentration was found to be higher in the 96% ethanol extract. However, both of the propolis extracts exhibited similar antioxidant activities.
Conclusions: In the present study, the extracts of *Echinacea purpurea* and propolis were studied as sources of natural antioxidants. The results from the antioxidant assays show that all extracts can act as radical scavengers to a certain extent. They exhibit potent antioxidant activity in different solvent concentrations. Further studies in this area are in progress.

INTRODUCTION

Recent years have seen growing interest on the part of consumers, the food industry, and researchers into food and the ways in which it may help maintain human health. Crude extracts of herbs rich in phenolic compounds are of increasing interest in the food industry, because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. *Echinacea*-based phytomedicines are among the most popular herbal products sold in North America and Europe [1]. Diversity of products, species, and phytochemicals is a key feature of this herbal medicine. There are over 800 *Echinacea purpurea* products in Europe alone which are based on different plant parts and widely different extraction or formulation techniques. There is a large number of other species and varieties in the genus. The phytochemistry of the genus is diverse (several classes of phytochemicals) and redundant (many phytochemical analogues per class) and these phytochemicals provide several types of medicinally important biological activities. All of the above make the understanding and quality control and improvement of *Echinacea* products challenging [2].

Species of the genus *Echinacea* show a high level of phytochemical redundancy with at least six different classes of secondary metabolites and multiple derivatives within any class [2]. Most biological activities have been associated with three classes of *Echinacea* phytochemicals: the caffeic acid derivatives (phenolics), the alkamides, and cell-wall derived polysaccharides. The main active compounds of the aerial parts of *Echinacea purpurea* are alkamides and polyacetylenes, caffeic and ferulic acid derivatives, polysaccharides (such as 4-O-methylglu-curonylarabinoxylans, rhamnoarabinogalactans and acidic arabinogalactan) and glycoproteins. Among the caffeic acid derivatives, only the cichoric acid, which is found to be the main phenolic compound in *Echinacea purpurea*, shows immunostimulatory properties, promoting phagocyte activity *in vitro* and *in vivo*, antihyaluronidase activity and has a protective effect on the free-radical-induced degradation of collagen. Beside cichoric acid, typical constituents of *Echinacea purpurea* extracts are echinacoside, chlorogenic acid, cynarine and caftaric acid. All of them are able to inhibit free radical production and lipid peroxidation, involved in the development of inflammation [3].

The antioxidant activity of *Echinacea purpurea* extracts has been already shown. Generally, the tested *Echinacea purpurea* extracts showed medium to low activity compared to the other investigated medicinal and aromatic plants. The antioxidant activity could be ascribed to the polyphenolic components, such as flavonoids, phenolic acids or phenolic diterpenes [4].

The benefits of effects of propolis on human health were recognized thousands of years ago. Its chemical composition is very complex and varies with geographic origin, depending on the local flora and phenology of the source plants. Propolis is well known for its antibacterial, antifungal, antitrypanosomal, antimicrobial, antioxidant, antiinflammatory, and antihepatotoxic activities. Propolis is used in a number of commercial dietary supplements, as a food preservative and as a germicide and insecticide. The chemical composition and biological activities of propolis depend mainly upon the local flora, the geographic region, and the climate. Thus, development of analytical methods to evaluate the antioxidant capacity and to discriminate the floral origin of propolis is necessary [5]. The major flavonoids, comprising about 25-30% of the ethanol extract of propolis on a dry weight basis, are galangin, isalpinin, kaempferol, kaemferid, rhamnocitrin, rhamnetin, quercitin, pinocembrin, pinostrobin and pinobanksin [6].

The antioxidant activity of the propolis and *Echinacea purpurea* extracts is mainly attributed to their phenolic compounds such as flavonoids, phenolic acids and polyphenolic compounds which neutralize free radicals including hydrogen peroxide (H_2O_2), superoxide ($O_2•^-$), hydroxyl ($•OH^-$) and peroxyl (ROO•) radicals by different mechanism including metal chelation and electron donation as reducing agent [6, 7]. The objectives of this study were to evaluate and compare total phenolics and total flavonoides, and antioxidant capacity of propolis and *Echinacea purpurea* ethanol extracts.

MATERIALS AND METHODS

Chemical reagents

HPLC methanol, 70% and 96% ethanol, gallic acid, (+)-catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, Na_2CO_3, $NaNO_2$, $AlCl_3$, NaOH, ascorbic acid were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Propolis and *Echinacea purpurea* were kept in dry and cold conditions.

Sample preparation

The study covered propolis and *Echinacea purpurea* varieties of species from different regions of Bulgaria. The samples were mixed and the mean sample was used for analysis. The sampling was carried out during one year according to the seasonality of harvesting for individual species. All samples data are stated in the sampling protocol and were kept in a dry place until further use.

Propolis extract was obtained by applying two different concetration of ethanol as the solvent. Two ethanolic propolis extracts, *i.e.* 1:100 w/v in 96% ethanol and 1:100 w/v in 70% ethanol, were worked up by maceration for 24 h at room temperature with continuous agitation.

Dried *Echinacea purpurea* was ground to a powder. *Echinacea purpurea* was extracted (1:100 w/v in 96% ethanol) at room temperature for 24 h.

Total phenolic assay

The total phenolic content of propolis and *E.purpurea* ethanol extracts were determined by using the modified Folin-Ciocalteu assay [8]. An aliquot (1 ml) of extracts or standard solution of gallic acid (10, 20, 40, 60, 80, 100 and 120 mg/l) was added to 25 ml volumetric flask, containing 9 ml of distilled deionised water (dd H_2O). A reagent blank using dd H_2O was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. To the solution the dd H_2O was added up to volume of 25 ml and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-VIS spectrophotometer (Cary; Varian Australian Pry. Ltd., Mulgrave, VIC, Australia). Total phenolic content of extracts were expressed as mg gallic acid equivalents per 100 ml (GAE/100 ml). All samples were analyzed in duplicates.

Total flavonoid assay

The total flavonoid contents were measured by a modified aluminum chloride colorimetric assay [8]. An aliquot (1 ml) of extracts or standard solution of catechin (10, 20, 40, 60, 80, 100 and 120 mg/l) was added to 10 ml volumetric flask, containing 4 ml of distilled dd H_2O. 0.3 ml 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. At 6^{th} min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with dd H_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid contents of extracts were expressed as mg catechin equivalents per 100 ml (CE/100 ml). All samples were analyzed in duplicates.

Determination of free radical scavenging activity

The $DPPH•^+$ assay provides information on the reactivity of test compounds with a stable free radical. Because of its odd electron, $DPPH•^+$ gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is steichiometric with respect to the number of electrons taken up.

 DOI 10.5455/jeim.050913.or.089

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•$^+$) has been widely used to evaluate the antioxidant capacity (AOC) of natural antioxidants. DPPH•$^+$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

The AOC of tested samples was determined photometrically using the DPPH•$^+$ method [9]. Briefly, 1 ml diluted extract sample was added to 4 ml 0.004% solution of DPPH•$^+$ in methanol (0.004 mg/ 100 ml). After 60 min solution retention in the dark, the light absorption was measured in 1 cm standard cuvette at 517 nm with a UV-VIS-spectrophotometer (Unicam®-Helios β). The reference liquid was methanol. The free radical inhibition (scavenging) was calculated by following formula:

$$IC(\%) = \frac{(A_0 - A_s)}{A_0} \times 100$$

-$\mathbf{A_0}$; absorption of the reference liquid not containing extracts (it's 0.004 % solution of DPPH•$^+$ in methanol)

-$\mathbf{A_s}$; absorption of the extract containing sample.

The DPPH• solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4ºC before measurements. The IC_{50} is a parameter representing the extract concentration, able to inhibit 50% of the used DPPH•$^+$ amount. It was determined by drawing a graph with sample concentration C_w (ml exract/l solvent) on the abscissa and free radical inhibition capacity I [%] as ordinate. A series of samples was prepared as already described. The initial extract sample was diluted in a manner to obtain a linear graph which lies in the zone of 0 to over 50% radical scavenging capacity. Dilutions were as follows:

-*Exinacea purpurea*; 1, 2, 3, 4 and 5 ml/l

-Propolis (1 and 2); 0.1, 0.2, 0.3, 0.4 and 0.5 ml/l

This means 1-5 ml of *Exinacea purpurea* or 0.1-0.5 ml) of propolis extracts were dissolved in 1 liter extractant (ethanol).

The extract concentration which reduces 50% of free radicals (IC_{50}) can be calculated by using graph equation or can be simply determined from the graph. Each sample was analyzed at least three times, and a mean value was calculated [10]. The correlation coefficient for *Echinacea purpurea* graph is $R^2 = 0.9848$; $R^2 = 0.9699$ for propolis 1 and R^2=0.9541 for propolis 2.

RESULTS

The contents for total phenolics and flavonoids of propolis were found to be much higher than and *E.purpurea* extract as shown in Table 1. These results indicate that a higher antioxidant activity of the propolis extracts compared to the *E.purpurea* extract may correlate to the phenolic and flavonoid content of the respective extracts.

To evaluate the scavenging effect of DPPH•$^+$ in ethanol extracts of *E.purpurea* and propolis, DPPH•$^+$ inhibition was investigated and these results are shown as relative activities against control. The extracts manufactured by *E.purpurea* and propolis showed different antioxidant activities. The antioxidant activity of the 70% ethanol extract of propolis was higher than all the others. The graph in Fig.1 (inhibition/concentration) present the results obtained to the method described above. The exact IC50% values of analyzed extracts are shown in Table 2.

The 70% ethanol extract of propolis were found the richest source of antioxidants among the samples investigated. The IC_{50} value of the 70% extract was also found to be lower than that of the 96% extract (Table 2).

Table 1. Total phenolics and total flavonoids in propolis and *Echinacea purpurea* extracts

Sample	Total phenolics	Total flavonoids
Echinacea purpurea	353.56 (RSD 4.7%)	10.85 (RSD 5.6%)
Propolis (96%)	2180.45 (RSD 3.9%)	415.03 (RSD 4.3%)
Propolis (70%)	2312.44 (RSD 3.8%)	494.23 (RSD 4.2%)

Total phenolic content is expressed as mg GAE/100 ml and total flavonoid content as mg CE/100 ml. RSD, relative standard deviation; n = 3 for all experiments.

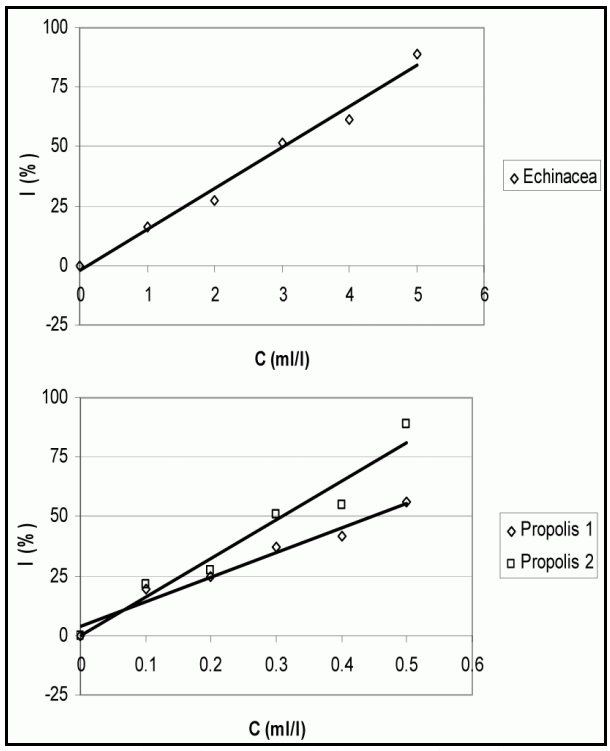

Figure 1. Graph equations used to find the IC_{50} value of the extracts

Table 2. The values of IC_{50} of the studied samples

Sample	IC_{50} (ml/l)
Ascorbic acid	15.36 (RSD 7.2%)
Echinacea purpurea	3.02 (RSD 7.7%)
Propolis (96%)	0.44 (RSD 8.7%)
Propolis (70%)	0.32 (RSD 7.9%)

IC_{50}, the extract concentration which reduces 50% of DPPH•$^{+}$; RSD, relative standard deviation; n = 3 for all experiments.

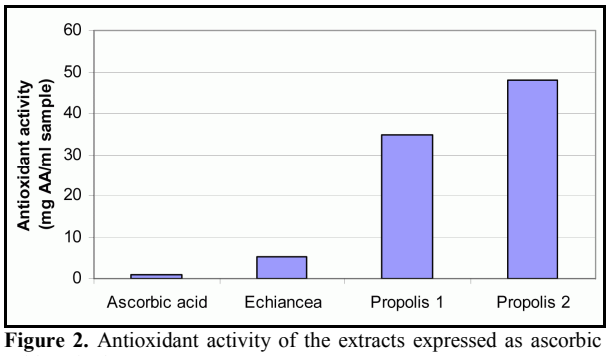

Figure 2. Antioxidant activity of the extracts expressed as ascorbic acid equivalent

In addition, we compared the antioxidant capacity of our samples with that of vitamin C (ascorbic acid). The same procedure was applied to vitamin C, and its IC_{50} value was determined. As result, the sample from *Echinacea* exhibited nearly 10 times lower antioxidant content in comparison with propolis extracts (Fig.2).

DISCUSION

Flavonoids in propolis have been studied frequently in the last years due to their biological effect on health. Not all propolis samples are the same, although they present similar biological activity. The vegetation available to bees in order to create propolis is very important and determinant for propolis composition. Flavonoids and phenolics are the major complementary compounds of propolis that have beneficial effects as natural antioxidants and prevent oxidative damage of DNA caused by reactive oxygen species [11]. The antioxidant effects may be a result of a combination of radical scavenging and an interaction with enzyme functions. Some components of propolis are absorbed and circulate in the blood and behave as hydrophilic antioxidant and save vitamin C [12]. Large amounts of cichoric and caftaric acids, which are largely recognized in the inhibition of hyaluronidase secreted by *Streptococci* and other bacteria to enable penetration into tissue, have been demonstrated with *Echinacea* plant juice [13]. *E.purpurea* was used to treat dizziness, snake bites and as an anti-infective agent until the advent of modern antibiotics [3]. Additionally, root extracts of *E.purpurea* were found to contain antioxidant compounds [14], to be capable of scavenging hydroxyl radicals and to suppress the oxidation of human low-density lipoprotein [15].

Polyphenolic compounds have been found to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants [16-19]. They are able to act as antioxidants in a number of ways, mainly as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelating agents [19-21].

The antioxidant properties of propolis extracts are certainly related to its chemical composition. Many studies have evidenced the presence of flavonoids (including flavones, flavonols, flavanones and dihydroflavonols) and other phenolics (mainly substituted cinnamic acids and their esters) as main active constituents of propolis possessing potent antioxidant activities [19, 22-24]. Flavonoids represent the main group of chemical compounds present in the European propolis samples and are well-known to possess antioxidant activity, mainly via their free radical scavenging activity and metal chelating properties [19, 20, 25, 26]. Many studies have already shown strong antioxidant properties for different propolis samples using different chemical assays, such as scavenging of DPPH•$^{+}$ [12, 19, 27, 28], scavenging of superoxide anion [19, 29], β-carotene bleaching assay [19, 23], and inhibition of DNA cleavage induced by hydrogen peroxide UV-photolysis [19, 29]. The antioxidant potential of propolis samples from northeast and center regions of Portugal was evaluated by their ability to inhibit the 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH)-induced oxidative hemolysis and lipid peroxidation in human erythrocytes [19].

Under the experimental conditions described, the three selected samples exhibited good antioxidant activity. It was interesting to note that the antioxidant activity of the propolis extract with 70% ethanol was higher compared to that with 96% ethanol. This decrease in antioxidant activity might be due to the solubility of some antioxidants compounds with a potential of better solubility in water.

In the existent studies on the chemical composition of European propolis it have only been determined and identified the phenolic compounds and flavonoids [12, 30-34]. The total quantity of polyphenols and flavonoids in propolis are important parameters to evaluate the quality and biological potentialities of the product [12, 34]. The specific phenolic composition of propolis is extremely dependent on the plants found around the hive, as well on the geographic and climatic characteristics of the place [34, 35].

According to the literature [33, 35, 36], the majority of compounds already identified in propolis are polyphenols. Globally, the present results are in

 DOI 10.5455/jeim.050913.or.089

agreement with the data obtained by Moreira *et al* [12] and Silva *et al* [33], who studied propolis from the northeast of Portugal. However, Miguel *et al* [37] obtained inferior values when analyzing propolis from the south of the same country. This discrepancy may be due to the great distances between the origin and the different apicultural practices. In fact, our data suggest that propolis from different places have different concentrations of polyphenols. The values obtained for catechin and gallic acid, which were used as standards, were bellow the concentration obtained in this study for flavonoids. This is in agreement with other reports [30, 31, 33] that refer the minor importance of gallic acid in propolis from temperate zones. This phenolic acid is mostly found in tropical samples. In propolis from the Mediterranean region flavonoids and esters of caffeic and ferulic acids prevailed. Considering that the hydro-alcoholic extract was the most effective, it was used in all the assays performed after [33].

In conclusion, the present study suggests that ethanolic extracts of *E.purpurea* and propolis are potential sources of active natural and non-toxic substances, which have functions as antioxidants, antimicrobials and antibiotics. The experiment shows that propolis extracts have 35 to 50-fold higher activity than ascorbic acid, examined by the same mean. These activity results were in accordance with the results of total phenols found in the two different propolis origins. The radical scavenging ability of *E.purpurea* flower extract could be attributable to the caffeic acid derivatives. The order of potency against DPPH•$^{+}$ radicals was the following: propolis (70% ethanol) > propolis (96% ethanol) > *Echinacea purpurea* > Ascorbic acid. So, the total phenols content was considered the main responsible for the antioxidant activity of different extracts.

REFERENCES

1. Brevoort P. The booming U.S. botanical market: A new overview. HerbalGram 1998; 44:33-47.
2. Bauer R. *Echinacea*: biological effects and active principles. ACS Symposium Series 1998; 12:140-57.
3. Abouelella AMK, Shahein YE, Tawfik SS, Zahran AM. Phytotherapeutic effects of *Echinacea purpurea* in gamma-irradiated mice. J Vet Sci 2007; 8:341-51.
4. Stojicevic S, Stanisavljevic A, Velickovic D, Veljkovic V, Lazic M. Antioxidant and antimicrobial activities of *Echinacea* (*Echinacea purpurea* L.) extracts obtained by classical and ultrasound extraction. Chin J Chem Eng 2009; 17:478-83.
5. Mot AC, Silaghi-Dumitrescu R, Sarbu C. Rapid and effective evaluation of the antioxidant capacity of propolis extracts using DPPH bleaching kinetic profiles, FT-IR and UV-vis spectroscopicdata. J Food Comp Anal 2011; 24:516-22.
6. Nagai T, Inoue R, Inoue H, Suzuki N. Preparation and antioxidant properties of water extract of propolis. Food Chem 2003; 80:29-33.
7. Hu C, Kitts DD. Studies on the antioxidant activity of Echinacea root extract, J Agric Food Chem 2000; 48:1466-72.
8. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. JUCTM 2005; 40:3:255-60.
9. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method. LWT Food Sci Technol 1997; 30:609-15.
10. Brand-Williams W, Cuvelier ME, Berset C. Use of a Free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995; 28:25-30.
11. Marghitas LA, Laslo L, Dezmirean D, Moise A, Maghear O. Total phenolics and antioxidant activity of Romanian propolis. International Conference Agricultural and Food Sciences, Progresses and Technologies, Sibiu, Romania, pp 209-212, 2007.
12. Moreira L, Dias LG, Pereira JA, Estevinho L. (2008). Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal, Food Chem Toxicol 2008; 46:3482-85.
13. Melchart D, Clemm C, Weber B, Draczynski T, Worku F, Linde K, Weidenhammer W, Wagner H, Saller R. Polysaccharides isolated from Echinacea purpurea herba cell cultures to counteract undesired effects of chemotherapy--apilot study. Phytother Res 2002; 16:138-42.
14. Kligler B. *Echinacea*. Am Fam Physician 2003; 67:77-80.
15. Pellati F., Benvenuti S., Melegari M., Lasseigne T. Variability in the composition of anti-oxidant compounds in Echinacea species by HPLC. Phytochem Anal 2005; 16:77-85.
16. Kaviarasan S, Vijayalakshmi K, Anuradha C. Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. Plant Foods Hum Nutr 2004; 59:143-7.
17. Asgary S, Naderi G, Askari N. Protective effect of flavonoids against red blood cell hemolysis by free radicals. Exp Clin Cardiol 2005; 10:88-90.
18. Biswas S, Bhattacharyya J, Dutta A G. Oxidant induced injury of erythrocyte-role of green tea leaf and ascorbic acid. Mol Cell Biochem 2005; 276:205-10.
19. Valente M J, Baltazar AF, Henrique R, Estevinho L, Carvalho M. Biological activities of Portuguese propolis: protection against free radical-induced erythrocyte damage and inhibition of human renal cancer cell growth *in vitro*. Food Chem Toxicol 2011; 49:86-92.

20. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radic Biol Med 1997; 22:749-60.

21. Rice-Evans C, Miller N, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996; 20:933-56.

22. Moreno M, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. J Ethnopharmacol 2000; 71:109-14.

23. Kumazawa S, Hamasaka T, Nakayama T. Antioxidant activity of propolis of various geographic origins. Food Chem 2004; 84:329-39.

24. Banskota AH, Tezuka Y, Kadota S. Recent progress in pharmacological research of propolis. Phytother Res 2001; 15:561-71.

25. Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 2009; 53:75-100.

26. Rice-Evans C. Flavonoid antioxidants. Curr Med Chem 2001; 8:797-807.

27. Izuta H, Narahara Y, Shimazawa M, Mishima S, Kondo S, Hara H. 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity of bee products and their constituents determined by ESR. Biol Pharm Bull 2009; 32:1947-51.

28. Banskota AH, Tezuka Y, Adnyana IK, Midorikawa K, Matsushige K, Message D, Huertas AA, Kadota S. Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. J Ethnopharmacol 2000; 72:239-46.

29. Russo A, Cardile V, Sanchez F, Troncoso N, Vanella A, Garbarino JA. Chilean propolis: antioxidant activity and antiproliferative action in human tumor cell lines. Life Sci 2004; 76:545-58.

30. Popova M, Bankova V, Butovska D, Petkov V, Nikolova-Damyanova B, Sabatini A, Marcazzan G, Bogdanov S. Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phytochem Anal 2004; 15:235-40.

31. Falcao SI, Vilas-Boas M, Estevinho LM, Barros C, Domingues MR, Cardoso S. Phenolic characterization of Northeast Portuguese propolis: usual and unusual compounds. Anal Bioanal Chem 2010; 396:887-97.

32. Salonem A, Saarnio S, Julkunen-Tiitto R. Phenolic compounds of propolis from Boreal Coniferous zone. J Apicult Sci 2012; 56:13-22.

33. Silva JC, Rodrigues S, Feas X, Estevinho LM. Antimicrobial activity, phenolic profile and role in the inflammation of propolis. Food Chem Toxicol 2012; 50:1790-5.

34. Dias LG, Pereira AP, Estevinho LM. Comparative study of different Portuguese samples of propolis: Pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. Food Chem Toxicol 2012; 50:4246-53.

35. Bankova VS, De Castro SL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. Apidologie 2000; 31:3-15.

36. Gomez-Caravaca AM, Gomez-Romero M, Arraez-Roman D, Segura-Carretero A, Fernández-Gutiarrez A. Advances in the analysis of phenolic compounds in products derived from bees. J Pharm Biomed Anal 2006; 41:1220-34.

37. Miguel MG, Nunes S, Dandhlen SA, Cavaco AM, Antunes MD. Phenols and antioxidant activity of hydro-alcoholic extracts of propolis from Algarve, South of Portugal. Food Chem Toxicol 2010; 48:3418-23.

 DOI 10.5455/jeim.050913.or.089