Caffeic Acid Phenethyl Ester (CAPE) in Romanian Propolis

Liviu Al. MĂRGHITAȘ, Daniel DEZMIREAN, Flavius DRĂGLĂ, Otilia BOBIȘ*

Department of Apiculture and Sericulture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăştur Street, 400372 Cluj-Napoca, Romania

*Corresponding author, email: *obobis@usamvcluj.ro*

Bulletin UASVM Animal Science and Biotechnologies 71(2) / 2014,
Print ISSN 1843-5262; Electronic ISSN 1843-536X
DOI: 10.15835/buasvmcn-asb:10557

Abstract

Propolis is an important bee product, rich in biologically active compounds. It is collected by the bees from buds and exudates of different trees and plants and it has been used since ancient times as a natural remedy or in embalming process. Caffeic acid phenethyl ester (CAPE) is one of the main medicinal components of propolis, possessing important biological activities, such as anti-bacterial, anti-oxidant anti-inflammatory and the most important anti-cancer properties. Taking into account that CAPE is an excellent compound in cancer, hepatitis and other oxidative stress diseases treatment, knowing the amount of this compound in Romanian propolis is a very challenging proposal. To identify and quantify the amount of CAPE in the samples, chromatographic determinations were made according to existing method developed in APHIS Laboratory. The analyzed propolis samples show that beside different phenolic acids and flavonoids, Romanian propolis is also rich in CAPE. Concentrations ranging from 0.86 to 3.87 mgCAPE/g was measured and correlated with high amounts of chrysin. High concentrations of caffeic acid (1.4 mg/ml) and pinocembrin (2.85 mg/g) were also determined in the analyzed samples. HPLC analysis of propolis samples from different locations in Romania, has highlighted the fact that they contain large amounts of CAPE. This propolis may be used successfully in the treatment of various diseases, including cancer.

Keywords: *CAPE, flavonoids, HPLC, propolis, polyphenols*

INTRODUCTION

Propolis is a resinous beehive product, collected by honeybees from different plant sources, including poplar buds, willow, plums, coniferous or alder (Mărghițaș, 2005). It has a pleasant aromatic flavor and different colors from yellow-green to dark brown, depending on its botanical source and geographical origin.

Propolis has a long history of being used in traditional medicine dating back at least to 300 BC and has been reported to have a broad spectrum of biological activities, including

antioxidant, antiinflammatory, antibiotic, antifungal and anticancer activities (Marcucci 1995; Kimoto *et al.*, 1998; Bankova *et al.*, 2000; Banskota *et al.*, 2001; Mărghițaș *et al.*, 2013).

Because of its large spectrum of biological activities and uses in different food supplements as well as in apitherapy, there is a renewed

interest in the composition of propolis and its biological activities. As a consequence, more than 300 constituents have been identified so far from propolis, which are well described by Marcucci (1995), Bankova *et al.* (2000), Mărghițaș *et al.* (2013) in their review articles.

As a natural compound bearing polyphenolic ring, caffeic acid phenethyl ester (CAPE) (*Fig. 1*), is originated from a this biologically active bee product, propolis.

Fig. 1. Chemical structure of caffeic acid phenethylester (CAPE)

It is known to have antitumoral, antiinflammatory, antineoplastic activity (Oršolic *et al.*, 2005; Lotfy 2006; Lin *et al.*, 2012; Markiewicz-Żukowska *et al.*, 2012; Omene *et al.*, 2012) and antioxidant properties to some extent.

The aim of our research was to identify and quantify the bioactive compounds from Romania propolis and to search for the specific compound (CAPE) with high antitumoral activity.

MATERIALS AND METHODS

For this experiment, thirteen samples of propolis were harvested from Transylvania Region (7 counties), from stationary apiaries. The samples were immediately transported to laboratory and stored at -20°C until analysis. Chemical analysis as well as bioactive constituents were measured from alcoholic extracts (1% w/v) of each sample.

Water content was measured from grounded samples dried in the oven until constant weight. Wax analysis was measured with Soxhlet equipment following a standard method, adapted for propolis (in terms of solvent used and extraction time).

Total phenolic content and total flavonoid content were assessed following literature methods (Folin Ciocâlțeu for total phenolics (Popova *et al.*, 2004) and total flavonoid content (Nagy and Grancai, 1996; Popova *et al.*, 2004).

Stock solutions of galangin for flavone/flavonols and pinocembrin for flavanone/dihydroflavonols quantification were prepared and diluted properly to construct calibration curves for total flavonoid determination. A mixture of pinocembrin and galangin (2:1 w/w) was prepared as stock solution for total polyphenols' determination. Propolis ethanolic extracts and serial dilutions of standards were prepared identically for absorbance reading and phenols quantification.

As propolis extracts are soluble in ethanolic and hydroalcoholic solutions, reverse phase High Performance Liquid Chromatography (HPLC), has become the most commonly used chromatographic method for propolis analysis. Phenolics and flavonoids absorb in the UV-Vis range and can easily be detected with a diode array detector (DAD) (Marcucci *et al.*, 2000; Marcucci *et al.*, 2008). HPLC-DAD has been applied to the characterization of Romanian propolis, following a method of Garcia-Viguera *et al.* (1993).

RESULTS AND DISCUSSION

Total polyphenols and total flavonoid content indicates the levels of bioactive compounds present in propolis samples. As a mean of the thirteen samples we quantified 73.66 mg/g phenols and 38.96 mg/g flavonoids (*Tab. 1*). These amounts are in accordance with literature data, obtained both from other Romanian propolis samples (Coneac *et al.*, 2008; Croci *et al.*, 2009) and from other European propolis (Bankova *et al.*, 1992; Bankova *et al.*, 2002). We made this specification, because it is generally known that Romanian propolis have the same pattern of bioactive compounds than most of all European propolis, all of them belonging to poplar type propolis. This type of propolis is characterized by high amounts of phenolics.

Populus species are considered to be the main source of propolis, the main phenolic compounds in propolis are flavonoids, such as pinocembrin, pinobanksin, chrysin and galangin (Bankova *et al.*, 1992; Bankova *et al.*, 2002). In our propolis samples pinocembrin, chrysin, galangin or quercetin were found, caffeic, p- and o-coumaric, ferulic acids were about 12.5% from total polyphenols (quantification based on HPLC/DAD-analyses) (*Fig. 2*).

Tab. 1. The content of total polyphenols and total flavonoids (mean $\pm$ S.E. in g) of Romanian propolis samples

Propolis samples (n = 13)	Total polyphenols (mg/g)	Total flavonoids (mg/g)
	73.66 $\pm$ 5.2	38.96 $\pm$ 2.42

Fig. 2. Chromatogram of one propolis sample with identified phenolic compounds including caffeic acid phenetylester (CAPE)

Tab. 2. Identified compounds using HPLC analysis of propolis samples (mg/g propolis)

Sample	Pinocembrin	Chrysin	Galangin	Caffeic acid	p-coumaric acid	Ferulic acid	CAPE
P1	2.08±0.11	3.91±0.85	2.66±0.15	0.78±0.01	0.51±0.02	0.57±0.02	0.87±0.02
P2	1.67±0.09	3.06±0.45	1.97±0.15	0.89±0.02	0.63±0.01	0.19±0.01	0.98±0.04
P3	1.00±0.08	0.55±0.04	0.97±0.05	0.74±0.04	0.87±0.00	0.64±0.01	0.95±0.02
P4	0.36±0.00	1.14±0.25	0.56±0.01	0.58±0.03	1.12±0.21	0.69±0.02	0.86±0.06
P5	0.63±0.01	2.51±0.25	0.60±0.02	0.97±0.02	1.20±0.30	0.15±0.00	2.26±0.25
P6	1.65±0.08	3.44±0.42	1.89±0.21	1.26±0.21	0.81±0.04	0.34±0.01	2.20±0.26
P7	0.49±0.01	2.47±0.31	0.81±0.04	1.07±0.31	0.84±0.06	0.29±0.04	2.05±0.42
P8	1.06±0.08	2.66±0.45	1.67±0.03	1.49±0.20	1.14±0.21	0.15±0.02	1.19±0.25
P9	0.13±0.00	1.14±0.21	-	0.48±0.02	1.33±0.04	1.98±0.04	1.66±0.21
P10	0.15±0.00	1.51±0.16	-	0.84±0.01	1.89±0.08	1.65±0.08	1.16±0.04
P11	0.69±0.02	2.70±0.12	-	0.73±0.04	0.79±0.02	1.06±0.04	3.87±0.21
P12	-	0.66±0.02	-	0.46±0.02	1.19±0.31	1.59±0.08	1.29±0.08
P13	1.72±0.06	2.04±0.42	1.90±0.06	0.51±0.01	0.50±0.04	0.15±0.01	2.37±0.14

Note: Results represent the mean ± standard deviation of three replicates separately analyzed.

Tab. 3. Location and amounts of bioactive compounds identified and quantified in propolis samples, analyzed by HPLC (mg/g propolis)

Propolis sample	Location	CAPE	Chrysin	Caffeic acid
P5	Alba	2.26 e	2.51 c	0.97 h,i
P6	Alba	2.20 e,f	3.44 c,d	1.26 g
P7	Arad	2.05 f	2.47 c	1.07 h
P11	Hunedoara	3.87 a	2.70 b	0.73 i
P13	Braşov	2.37 d,e	2.04 f	0.51 j

Note: Results represent mean of three replicates, individually analyzed. Different letters within the same column denote significant differences (Duncan test, $p < 0.05$).

Data presented in *Table 2*, show the amount of flavonoids, phenolic acids and caffeic acid phenethyl ester in analyzed propolis sample. Excepting P12, in all other samples pinocembrin was found and quantified in different amounts (0.15–2.08 mg/g propolis). Chrysin and caffeic acid phenethyl ester were found in all 13 samples, while galangin was quantified in nine from the thirteen samples. In all samples caffeic, p-coumaric and ferulic acids were identified and quantified (*Tab. 2*).

High amounts of CAPE were quantified in the sample with high amount of chrysin and caffeic acid (*Tab. 3*). No correlation between CAPE content and galangin was found, high amounts of flavonoid (galangin), does not show also high amounts of ester.

CONCLUSION

Romanian propolis posses different amounts of biologically active compounds, depending on the botanical source of the sample.

Cafeic acid phenethyl ester (CAPE) is found in high amounts in all tested propolis samples

High amounts of CAPE are correlated with high amounts of cafeic acid and chrysin

No correlation of CAPE content and geographical origin was observed, the conclusion being that CAPE as well as the other bioactive compounds, are correlated with the botanical origin of the resins collected by bees for producing propolis.

Acknowledgments. This research was made through the Partnerships in Priority Areas Programme - PN II, developed with the support of MEN - UEFISCDI, project no. 148/2014

REFERENCES

- Bankova C, Dyulgerov A, Popov S, Evstatieva L, Kuleva L, Pureb O, Zamjansan Z (1992). Propolis produced in Bulgaria and Mongolia: phenolic compounds and plant origin. *Apidologie* 23:79-85.
- Bankova V, deCastro SL, Marcucci MC (2000). Propolis: recent advances in chemistry and plant origin. *Apidologie* 31:3-15.
- Bankova V, Popova M, Bogdanov S, Sabatini AG (2002). Chemical composition of European propolis: expected and unexpected results. *Z Naturforsch* 57c:530-533.
- Banskota AH, Tezuka Y, Kadota S (2001). Recent progress in pharmacological research of propolis. *Phytother Res* 15:561-571.
- Coneac G, Gafițeanu E, Hădărugă DI, Hădărugă NG, Pînzaru IA, Bandur G, Urșica L, Păunescu V, Gruia A (2008). Flavonoid contents of propolis from the west side of Romania and correlation with the antioxidant activity. *Chem Bull "POLITEHNICA" Univ (Timișoara)* 53: 56-60.
- Croci AN, Cioroiu B, Lazăr D, Corciova A, Ivănescu B, Lazăr MI (2009). HPLC evaluation of phenolic and polyphenolic acids from propolis. *Farnacia* 57:1-6.
- Garcia-Viguera C, Ferreres F, Tomas-Barberan FA (1993). Study of Canadian propolis by GC-MS and HPLC. *Z Naturforsch* 48c:731-735.
- Kimoto T, Arai S, Kohguchi M et al. (1998). Apoptosis and suppression of tumor growth by Artepillin C extracted from Brazilian propolis. *Cancer Detect Prev* 22:506-515.
- Lin HP, Lin CY, Huo C, Su LC, Chuu CP (2012). Anticancer effect of caffeic acid phenethyl ester. *Pharmacologia* 3:26-30.
- Lotfy M (2006). Biological activity of Bee propolis in health and disease. *Asian Pac J Cancer Prev* 7:22-31.
- Marcucci MC (1995). Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie* 26:83-99.
- Marcucci MC, Ferrerez F, Custodio AR, Ferreira MMC, Bankova VS, Garcia-Viguera C, Bretz WA (2000). Evaluation of phenolic compounds in Brazilian propolis from different geographic regions. *Z Naturforsch* 55c:76-81.
- Marcucci MC, Sawaya ACHE, Custodio AR, Paulino N, Eberlin MN (2008). HPLC and ESI-MS typification: New approaches for natural therapy with Brazilian propolis. Scientific evidence of the Use of Propolis in Ethnomedicine Transworld Research Network, Kerala, India; 33-54.
- Mărghițaș L (2005). *Albinele și produsele lor*, Editura Ceres, București.
- Mărghițaș LA, Dezmirean DS, Bobiș O (2013). Important Developments in Romanian Propolis Research. *Evid Based Complem Alt Med*, 1:2013 DOI:10.1155/2013/159392
- Markiewicz-Żukowska R, Car H, Naliwajko SK, Sawicka D, Szynaka B, Chyżewski L, Isidorov V, Borawska MH (2012). Ethanol extract of propolis, chrysin, CAPE inhibit human astroglia cells. *Adv Med Sci* 57, doi 10.2478/v10039-012-0042-6
- Nagy M, Grancai D (1996). Colorimetric determination of flavanones in propolis. *Pharmazie* 51:100-101.
- Omene CO, Wu J, Frenkel K (2012). Caffeic Acid Phenethyl Ester (CAPE) derived from propolis, a honeybee product, inhibits growth of breast cancer stem cells. *Invest New Drugs* 30:1279-1288.
- Oršolic N, Terzić S, Mihaljević Ž, Šver L, Bašić I (2005). Effects of local administration of propolis and its polyphenolic compounds on tumor formation and growth. *Biol Pharm Bull* 28:1928-1933.
- Popova M, Bankova V, Butovska D, Petkov V, Nikolova-Damyanova, Sabatini AG, Marcuzzan GL, Bogdanov S (2004). Validated methods for the quantification of biologically active constituents of poplar-type propolis. *Phytochem Anal* 15:235-240.