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CHEMICAL DIVERSITY OF POLYPHENOLS FROM BEE POLLEN AND PROPOLIS

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Abstract

Bee pollen and propolis represent the bee products with the highest concentration of phenolic substances. These products contains a variety of chemical compounds but phenolic compounds are considered the main bioactive constituents. The chemical composition in both cases varies with botanical sources available to bees and extraction conditions. Despite chemical diversity, most of the samples share a similar phenolic profile. Only a part of phenolic constituents like flavonoid glycosides and pigments have been demonstrated the use as markers to discriminate bee pollen or propolis from different botanical origins.This paper highlights the main factors that contributes to chemical diversity of polyphenols from bee pollen and propolis.

Key words: *bee pollen, propolis, phenolic compounds.*

INTRODUCTION

Bee pollen and propolis are popular natural products which have gained the reputation as important functional foods. Like other bee products, bee pollen and propolis are considered as pleiomorphic substances with multiple biochemical effects (Munstedt and Bogdanov, 2009) due to more than 300 compounds (Martos et al., 2008) and 250 compounds in propolis, respectively bee pollen composition. The main chemical components of bee pollen are proteins, amino acids, carbohydrates, lipids and fatty acids, phenolic compounds, enzymes, coenzymes, vitamins and bio-elements (Vassev et al., 2015). Propolis contains of variety of chemical compounds, generally being represented by 50% resin and vegetable balsam, 30% wax, 10% essential oil and aromatics, 5% pollen and 5% other substances (Sforcin, 2007; Coneac et al., 2008). Among these chemical compounds, phenolic substances are common chemical components found in high concentration. In general, the average content of bee pollen phenolic compounds is 1.6% (Vassev et al., 2015) and minimum of total phenolic content is 21% for European propolis (Popova et al., 2007; Bankova, 2008). Based on chemical structure, phenolic compounds can be group into: phenols, phenolic acids, coumarins and isocoumarins, xanthones, naphtoquinones, stilbens, anthraquinones, flavonoids and lignins (Wollgast et al., 2000). Phenolic compounds are considered as secondary metabolites of plants with an extensive number of flavonoids and phenolic acids (Ulusoy, 2014), the bioactive constituents of bee pollen and propolis (Stojko et al., 2015; Bankova et al., 2000). The most important polyphenolic class is represented by flavonoids, with more than 5000 compounds. From chemical point of view, flavonoids are a group of benzo-γ-pyrone derivatives classified as chalcones, flavan-3-ols, flavanones, flavones, flavonols, isoflavones and bioflavonoids (Kim et al., 2004).

The botanical sources and geographic origin of bee pollen and propolis are the major determinants of the diversity of phenolic substances (Ulusoy, 2014; Bankova et al.,

2000). Beside those, there are several other important factors influences phenolic content in propolis, such as, age of bees, conditions of the beehives, strength of the colony and method used to collect the sample (Can et al., 2015). Previous studies found that bee pollen and propolis present a heterogenous phenolic content (Carpes et al., 2009; Miguel and Antunes, 2011). Nowadays, various techniques such as maceration with different solvents, ultrasound-assisted and supercritical fluid extraction are the most used methods for extraction of bioactive constituents from bee pollen and propolis (Miha et al., 2013). Significant amounts of bee pollen polyphenols were obtained by enzymatic extraction with pepsin (Stojko et al., 2010). In case of propolis, have been developed other methods with good yield extraction like: microwave and Soxhlet extraction (Margeretha et al., 2012; Trusheva et al., 2007). Also, the commonly used solvents for phenolic compounds extraction from bee pollen and propolis are methanol, ethanol, water (Damir et al., 2014).

Since ancient times, both bee products demonstrated to possess many health benefits: anti-inflammatory, antimicrobial, antioxidant, antitumor, immunemodulatory, hepato-protective and wound healing. All these biological activities of bee pollen and propolis are related with their high polyphenol contents (Gabriele et al., 2015).

Chemical diversity of phenolic compounds

All hive products are rich sources of phytochemicals, but propolis and bee pollen are considered the main bee products with the highest amounts of phenolic compounds.

Various techniques for extraction, separation, identification and quantification of phenolic compounds have been developed over time to capitalize and characterized these biologically active constituents from bee pollen and propolis. Extraction procedures applied for phenolic compounds in both cases do not present significant differences. The only differences arise from bee pollen composition rich in polar compounds (sugars) which requires chemical or enzymatic hydrolysis to remove glucose molecules. (Ferreres et al., 1994; Barberán et al., 2013; Carpes et al., 2013). Enzymatic hydrolysis of flavonoid glycosides occurs in vivo by hydrolytic enzymes α- and β-glucosidases from bee saliva to free aglycone (Sameer et al., 2014; Yen et al., 2011) Most commonly flavonoids forms occur in bee pollen and propolis are flavonoid glycosides and free aglycones.

Bonvehi, 2001, confirmed that the major classes of phenols in honeybee collected pollen are flavonol glycosides.

The same class of constituents being determined in propolis (Marcucci, 1995). Some flavonoids from bee pollen, quercetin and kaemferol are derivatives of flavonol glycosides and can be considered as markers for pollen quality (Sameer et al., 2014).

Substantial developments in research focused on extraction of bioactive compounds from bee pollen and propolis have proved that polar solvents are more effective in the extraction of bioactive compounds.

The research findings of other authors reveal that flavonoid glycosides and free aglycones are richer in ethanolic extracts comparative with aqueous extracts (Yen Tin Kao et al., 2011) but a maximum concentration of bee pollen flavonoids and terpenoids was obtained when using water as solvent followed by ethanol and methanol (Kaur et al., 2013).

The results obtained by Chunli S. noted that the extraction yields of propolis are tended to elevate with increasing of the ethanol concentration (Chunli et al., 2015), specifically, phenolic compounds such as methyl gallate, rutin and myricetin were more abundant in the ethanol extract. Moreover, rutin amount was found to be direct proportionally with ethanol concentration (Yen Tin Kao et al., 2011). The using of various concentrations of ethanol as solvent allowed extraction of the highest amounts of different flavonoids: 80% ethanol extracted especiallly kaempferide, acacetin and isorhamnetin, while 60% ethanol and 70% ethanol extracted isosakuranetin, quercetin and kaempferol, respectively pinocembrin and sakuranetin (Gómez-Caravaca et al., 2006).

Less polar flavonoids (isoflavones, flavanones, methylated flavones, flavonols) are extracted with nonpolar solvents, instead flavonoid glycosides and polar aglycones are extracted using alcohols or alcohol-water mixtures, presented in Table 1. The water-solvent

mixtures such ethanol-water and methanol-water in various proportions are suitable solvents for the extraction of polar phenolic acids (cinnamic acids, benzoic etc.) (Mojzer et al., 2016).

Propolis extracts obtained by extraction with aqueous ethanol contain higher concentrations of phenolic substances than extracts obtained by extraction with absolute ethanol (R.G. Woisky et al., 1998).

Additional, water propolis extract has also demonstrated a substantially lower capacity to extract phenols, flavones, flavonols, flavanones and dihydroflavonols compared with methanol (Miguel et al., 2014). Despite the methanol ability to extract polar compounds, the use of methanol extracts in pharmaceutical industry can raise problems due to its toxicity and most frequently is replaced with water or ethanol extraction (Delfanian et al., 2015).

Tabel 1. The main phenolic compounds from various geographic origin identified by used of different extraction solvents

Bee product	Botanic origin	Geographic origin	Identified analytes	Extraction solvent	References
Bee pollen	-	Egypt	Hydroxycinnamic Acid (3,4-Dimethoxycinnamic)	Methanol	Mohdaly A.A.A. and colab., 2015
	Polyfloral	Algeria	Hydroxybenzoic acid (Gallic), Hydroxycinnamic acid (Caffeic)	Methanol	Rebiai A. and colab., 2014
	Castanea sp.	Italia	Hydroxybenzoic acid (Gallic, 4-hydroxybenzoic, Hydroxycinnamic acids (caffeic, p–coumaric)	Ethanol 95%	Gabriele M. and colab., 2015
	Almond (*Prunus amygdalus*)	Spain	Flavonols (8-Methoxykaempferol 3-glycoside, kaempferol 3-diglucosides. quercetin)	Methanol	Barberán T.F. and colab., 1988
	Cistus sp.	Spain	Flavonols (Quercetin 3-glycoside, quercetin 3-glucoside, kaempferol, myricetin 3-glycosides, isorhamnetin 3-glycoside, isorhamnetin 3-glucoside).	Methanol	Barberán T.F. and colab., 1988
	Echium sp.	Spain	Flavonol (Kaempferol 3-glycoside).	Methanol	Barberán T.F. and colab., 1988
	Chrysanthemum sp.	Spain	Flavonols (Kaempferol 3-glycoside, quercetin 3-glycoside, myricetin 3-glycoside, isorhamnetin 3-glycoside, isorhamnetin 3-glucoside) Flavone (apigenin 7-glycoside).	Methanol	Barberán T.F. and colab., 1988
	Brassica napus/Trifolium repens/ Carum carvi L.	Lithuania	Flavonols (quercetin 3-*O*-sophoroside, 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4-oxo-4H-chromen-3-yl 6-*O*-β-d-glucopyranosyl-β-d-glucopyranoside, quercetin dihexoside, isorhamnetin 3-glucoside), dicarboxylic acid (azelaic acid)	Methanol	Čeksterytė V. and colab., 2016
	Brassica juncea	India	Flavone (chrysin), Flavonols (kaempferol, rutin, quercetin).	Mixture: ethyl acetate, diammonium sulfate, meta-phosphoric acid, methanol	Sameer S.K. and colab., 2014
	Polyfloral	Turkey (Anzer)	Hydroxybenzoic acids (p-OH benzoic, benzoic acid, gallic, protocatechuic acid, syringic, vanillic acids), Hydroxycinnamic acids (trans-cinnamic acid, p-coumaric acid, chlorogenic, ferulic acid, caffeic acid, o-coumaric acid), Flavonols (rutin, quercetin) Flavan-3-ols (epicatechin, catechin)	Methanol	Ulusoy E., Kolayli S., 2014
	Cecropia	Bahia, northeastern Brazil	Flavonols (Isoquercetin, isorhamnetin, quercetin, kaempferol, myricetin) Flavone (Tricetin)	Ethanol	Freire K.R.L. and colab., 2012
	Eucalyptus	Bahia, northeastern Brazil	Flavonols (Isoquercetin, myricetin, kaempferol, isorhamnetin, quercetin)	Ethanol	Freire K.R.L. and colab., 2012
	Elaeis	Bahia, northeastern Brazil	Flavonols (Isoquercetin, quercetin, isorhamnetin)	Ethanol	Freire K.R.L. and colab., 2012
	Mimosa pudica	Bahia, northeastern Brazil	Flavonols (Isoquercetin, isorhamnetin, quercetin, kaempferol, myricetin) Flavone (Tricetin)	Ethanol	Freire K.R.L. and colab., 2012

Bee product	Botanic origin	Geographic origin	Identified analytes	Extraction solvent	References
	Scoparia	Bahia, northeastern Brazil	Flavonol (myricetin)	Ethanol	Freire K.R.L. and colab., 2012
	Camelia sinensis	Taiwan	Hydroxycinnamic acids (Caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid).	Cold water	Yen T. K. and colab., 2011
	Camelia sinensis	Taiwan	Flavan-3-ol (Catechin), Hydroxybenzoic acid (Gallic acid), Hydroxycinnamic acids (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid).	Hot water	Yen T. K. and colab., 2011
	Camelia sinensis	Taiwan	Flavan-3-ol (Catechin), Hydroxybenzoic acid (Gallic acid), Methyl ester of gallic acid (methyl gallate), Hydroxycinnamic acids (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid), Flavonol (myricetin, rutin).	Ethanol 50%	Yen T. K. and colab., 2011
	Camelia sinensis	Taiwan	Flavan-3-ol (Catechin), Hydroxybenzoic acid (Gallic acid), Methyl ester of gallic acid (methyl gallate) Hydroxycinnamic acids (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid Flavonols (myricetin, rutin).	Ethanol 95%	Yen T. K. and colab., 2011
	Phoenix dactylifera L.	Tunisia (Tozeur)	Hydroxycinnamic acids (caffeic, coumaric) Hydroxybenzoic acids (gallic, vanilic acid), Flavonols (quercetin, rutin), Flavan-3-ol (catechin, epicatechin)	Ethyl acetate	Daoud, A. and colab., 2015
	Phoenix dactylifera L.	Tunisia (Tozeur)	Hydroxycinnamic acids (caffeic, coumaric) Hydroxybenzoic acids (gallic, vanilic acid), Flavan-3-ol (epicatechin)	Acetone	Daoud, A. and colab., 2015
	Phoenix dactylifera L.	Tunisia (Tozeur)	Hydroxybenzoic acids (gallic, vanilic acid) Hydroxycinnamic acids (caffeic acid coumaric), Flavonols (quercetin, rutin, Flavan-3-ols (catechin, epicatechin)	Ethanol	Daoud, A. and colab., 2015
	Phoenix dactylifera L.	Tunisia (Tozeur)	Hydroxybenzoic acids (gallic, vanilic acid) Hydroxycinnamic acids (caffeic acid coumaric), Flavonols (quercetin, rutin), Flavan-3-ols (catechin, epicatechin)	Water	Daoud, A. and colab., 2015
	Phoenix dactylifera L.	Tunisia (Kerkennah)	Hydroxybenzoic acids (gallic, vanilic acid) Hydroxycinnamic acids (caffeic acid coumaric), Flavonols (quercetin, rutin, Flavan-3-ols (catechin, epicatechin)	Ethyl acetate	Daoud, A. and colab., 2015
	Phoenix dactylifera L.	Tunisia (Kerkennah)	Hydroxybenzoic acid (gallic), Hydroxycinnamic acids (caffeic acid, coumaric), Flavonols (quercetin, rutin), Flavan-3-ols (epicatechin)	Acetone	Daoud, A. and colab., 2015
	Phoenix dactylifera L.	Tunisia (Kerkennah)	Hydroxybenzoic acid (gallic), Hydroxycinnamic acid (caffeic acid), Flavonols (rutin), Flavan-3-ols (epicatechin, catechin)	Ethanol	Daoud, A. and colab., 2015
	Phoenix dactylifera L.	Tunisia (Kerkennah)	Hydroxybenzoic acids (gallic, vanilic acid) Hydroxycinnamic acids (caffeic acid coumaric), Flavonols (quercetin, rutin), Flavan-3-ols (epicatechin)	Water	Daoud, A. and colab., 2015
	Heterofloral (dominant *Brassicaceae*)	southern Brazil	Hydroxybenzoic acids (Benzoic acid, methyl ester), Flavonols (myricetin, rutin).	Hot ethanol 70%	Carpes S.T. and colab., 2013
	Heterofloral (dominant *Myrtaceae eucalyptus*)	southern Brazil	Hydroxybenzoic acids (Benzoic acid, methyl ester), Flavonol (rutin).	Hot ethanol 70%	Carpes S.T. and colab., 2013
	Crataegus pinnatifida	China (Jilin)	Hydroxybenzoic acids (Gallic acid, protocatechuic acid, vanillic acid), Hydroxycinnamic acid (p-Coumaric acid), flavonols (Quercetin, kaempferol, galangin) flavanones (Hesperetin)	Hot water	Cheng N. and colab., 2013

Bee product	Botanic origin	Geographic origin	Identified analytes	Extraction solvent	References
	Zea mays L.	Various regions	Flavonols (quercetin-3,7-O-glucoside, quercetin-3,7,3'-O-diglucoside, quercetin-3,3'-O-diglycoside), quercetin-3-O-glucoside-3'-O-diglucoside, isorhamnetin-3-O-glycosides, quercetin-3-O-dyglycoside, quercetin-3-O-glucoside)	Ethanol-water 50%	Campos M.G. and colab., 2015
Poplar propolis	*Populus* spp. (especcilaly *P. nigra*)	continental Europe, North America, West Asia, New Zealand	Flavanons (pinocembrin, pinostrobin), flavonols (galangin, pinobanksin, rutin), flavons (chrysin), aromatic acids and their esters (ferulic acid, p-coumaric acid, isoferulic acid, cinnamic acid, caffeic acid)	Ethanol	Bankova and colab., 2000, Bankova and colab., 2008, Fokt and colab., 2010; Mohdaly A.A.A. and colab., 2015
Medite-rranean propolis	*Ferula* spp. *(*most often *F. communis) Cupressaseae family (ex. Cupressus sempervirens)*	Croatia, Sicilia, Greece, Malta, Algeria and Cyprus	Diterpenes, sesquiterpene esters of benzoic acids, aliphatic hydroxyacids, aromatic and fatty acids, triterpenes, anthraquinones	Ethanol	Popova and colab., 2011; Miguel and Antunes, 2011 Righi and colab., 2013
Birch propolis	*Betula verrucosa*	Northern Russia, Azerbaijan	Flavones (acacetin, apigenin), flavonols (ermanin, rhamnocitrin, fisetin, quercetin, kaempferol, isorhamnetin, rutin), hydroxybenzoic acids (*p*-OH Benzoic acid, Protocatechuic, Vanillic acid, Gallic acid), hydroxycinnamic acids (Caffeic acid, Ferulic acid, *p*-Coumaric acid, chlorogenic acid), Flavan-3-ol (catechin) phenolic glycerides (dicoumaroyl acetyl glycerol, diferuloyl acetyl glycerol, feruloyl coumaroyl acetyl glycerol, caffeoyl coumaroyl acetyl glycerol)	Ethanol	Popravko, 1978, Bankova V and colab., 2002, Can Z. and colab., 2015
Green propolis	*Baccharis* spp., (predominantly *B. dracunculifolia*)	Brasil	Phenylpropanoids, prenylated phenylpropanoids (e.g., artepillin C), and sesqui- and diterpenoids, lignans, benzofurans, benzopirans	Chloroform, methanol	Bankova and colab., 2000, Rusak, 2008 Miguel and Antunes, 2011 Righi and colab., 2013
Red propolis	*Dalbergia ecastaphyllum*	Brasil	Chalcones, pterocarpans and other isoflavonoids	Ethanol	Fokt and colab., 2010 Miguel and Antunes, 2011 Righi and colab., 2013, Nunes and colab., 2013
Red propolis	*Clusia* spp	Brasil	polyprenylated benzophenones		Miguel and Antunes, 2011 Righi and colab., 2013
Red propolis	*Clusia* spp	Venezuela, Cuba	Isoflavons, isoflavans, isoprenylated flavonoids and benzophenones		Bankova and colab., 2000, Bankova, 2005 Rusak, 2008, Fokt and colab., 2010, Miguel and Antunes,2011, Cardinault and colab., 2012
"Pacific" propolis	*Macaranga tanarius*	Okinawa, Taiwan, Indonesia	Prenylflavanones (propolins)	Ethanol, chloroform	Bankova and colab. 2000, Bankova and colab., 2008, Trusheva, 2011, Miguel and Antunes,2011
"Canarian" propolis	unknown	Canary Islands	Furofuran lignans, carbohydrates	Ethanol	Bankova and colab., 2000, Bankova and colab., 2008, Miguel and Antunes, 2011, Pujirahaiu and colab., 2014
Stilbene propolis	*Ambrosia deltoidea Encelia farinose*	Australia and equatorial regions in South America	Prenylated stilbenes	Ethyl acetate	Bankova and colab., 2000 Fokt and colab., 2010, Miguel and Antunes,2011 Abu-Mellal and colab.,2012 Righi and colab., 2013

The phenolic compounds present in propolis and bee pollen were extracted and analyzed from complex matrices by various analytical methods.

Several analytical methods have been developed for separation and identification of phenolic compounds from bee pollen and propolis.

Liquid chromatography tandem mass spectroscopy (LC-MS), sometimes coupled with electrospray ionization detection (ESI), or light-scattering detection (LSD) and gas chromatography (GC) coupled with mass spectroscopy (GC-MS) are usually applied for identification of flavonoids like specific possible markers from a plant source (Oman et al., 2013; Ciulu et al., 2016). The most used HPLC detection system for measuring the phenolic profile of bee pollen or propolis are: UV-VIS detector (Can et al., 2015), PDA (photodiode array) detector (Sameer et al., 2014) and MS (mass spectrometry) detector.

The analytical conditions employed should be suitable for the diverse group of phenolics due to different chemical structures and the variate sensitivity of the compounds to pre-treatment (Häkkinen, 1998) describe in Table 2.

Table 2. Different conditions for identification of the common phenolic compounds from bee pollen and propolis

Bee product /type	Geographic origin/type	Phenolic compound	Min.	Max.	Procedure/conditions	References
Pollen	North and south Algeria	Gallic acid (mg/g)	0.321	0.406	RP-HPLC with linear gradient elution mode, mobile phase: mixture CH_3- COOH 0,1% - ACN, volume of injection: 20 µL, effluent detected at 300 nm.	Rebiai A. and colab., 2014
	Crataegus pinnatifida		0.0112		HPLC-DAD with linear gradient elution, C-18 column, mobile phase: methanol- 2% aqueous acetic acid, volume of injection: 10 µL, effluent detected at 360 nm.	Cheng N. and colab., 2013
	Camellia sinensis (Taiwan)		0.69	2.55	HPLC-DAD with linear gradient elution, RP column, mobile phase: methanol- trifluoroacetic acid, volume of injection: 10 µL, effluent detected at 280 nm.	Yen T.K. and colab., 2011
	Polyfloral		5,9 mg/mL		HPLC using Lichrosorb RP18 Lichrocart and Lichrocart RP 18 pre-column, gradient elution, water-methanol, flow rate 50 µL/min., effluent detected at 280 nm	Mohdaly A.A.A. and colab., 2015
Propolis	Polish	Caffeic acid (mg/g)	3.90		HPLC-DAD, reverse phase Cadenza 5CD-C18 column, mobile phase: formic acid in water-acetonitrile, gradient elution, the injection volume was 20 µL, detection at 290 nm, 325 nm, and 370 nm.	Szliszka E. and colab., 2013
	Chilean		12.3		HPLC equipped with a UV-visible detector, RP-18 column, mobile phase: formic acid 5% in water-methanol, isocratic-0 to 10 min-run, followed by a gradient up to 100% B at 70 min, detection at 290 nm, the injection volume was 10µL	Barrientos L. and colab., 2013
	Egyptian		0.13	10.16	HPLC on a C-18 reverse phase column, using a UV detector, Elution with water/formic acid and acetonitrile, Gradient elution at 35 min, and then the system became isocratic, detection at 340 and 290 nm	Abd El-Hady F.K. and colab., 2007
	Korean		1.0	8.7	HPLC with PDA and MS detection, C18 column, mobile phase: 0.1% formic acid in water-0.1% formic acid in acetonitrile, the injection volume was 10µL	Choi S.J. and colab., 2013
	Azerbaijan		0.03	5.32	HPLC-DAD, reverse phase C18 column, mobile phase: 2% acetic acid in water-70:30 acetonitrile/water mixtures, gradient elution, injection volume was 25 mL, detection at 280 and 315 nm.	Can Z. and colab., 2015
	Polish	Ferulic acid (mg/g)	22.01		HPLC-DAD, reverse phase Cadenza 5CD-C18 column, mobile phase: formic acid in water-acetonitrile, gradient elution, the injection volume was 20 µL, detection at 290 nm, 325 nm, and 370 nm.	Szliszka E. and colab., 2013
	Egyptian		0.30	0.56	HPLC on a C-18 reverse phase column, using a UV detector, Elution with water/formic acid and acetonitrile, Gradient elution at 35 min, and then the system became isocratic, detection at 340 and 290 nm	Abd El-Hady F.K. and colab., 2007
	Korean		0.50	1.9	HPLC-PDA and MS detection, C18 column, mobile phase: 0.1% formic acid in water-0.1% formic acid in acetonitrile, the injection volume was 10µL	Choi S.J. and colab., 2013
	Azerbaijan		0.005	3.675	HPLC-DAD, reverse phase C18 column, mobile phase: 2% acetic acid in water-70:30 acetonitrile/water mixtures, gradient elution, injection volume was 25 mL, detection at 280 and 315 nm.	Can Z. and colab., 2015

Bee product /type	Geographic origin/type	Phenolic compound	Min.	Max.	Procedure/conditions	References
	Polish	Galangin (mg/g)	0.47		HPLC-DAD, reverse phase Cadenza 5CD-C18 column, mobile phase: formic acid in water-acetonitrile, gradient elution, the injection volume was 20 µL, detection at 290 nm, 325 nm, and 370 nm.	Szliszka E. and colab., 2013
	Egyptian		0.63	5.0	HPLC on a C-18 reverse phase column, using a UV detector, Elution with water/formic acid and acetonitrile, Gradient elution at 35 min, and then the system became isocratic, detection at 340 and 290 nm	Abd El-Hady F.K. and colab., 2007
	Korean		4.9	26.3	HPLC-PDA and MS detection, C18 column, mobile phase: 0.1% formic acid in water-0.1% formic acid in acetonitrile, the injection volume was 10µL	Choi S.J. and colab., 2013
	Chinese		2.785		LC-DAD system, EclipseXDB-C18 column, mobile phase: mixture of phosphate buffered saline (pH=4.5) in water and methanol, isocratic mode, detection at 260 nm.	Yang l. and colab., 2013
	Egyptian	Kaempferol (mg/g)	0.47	2.0	HPLC on a C-18 reverse phase column, using a UV detector, Elution with water/formic acid and acetonitrile, Gradient elution at 35 min, and then the system became isocratic, detection at 340 and 290 nm	Abd El-Hady F.K. and colab., 2007
	Korean		0.7	2.5	HPLC-PDA and MS detection, C18 column, mobile phase: 0.1% formic acid in water-0.1% formic acid in acetonitrile, the injection volume was 10µL	Choi S.J. and colab., 2013
	Azerbaijan		0.124	2.159	HPLC-DAD, reverse phase C18 column, mobile phase: 2% acetic acid in water-70:30 acetonitrile/water mixtures, gradient elution, injection volume was 25 mL, detection at 280 and 315 nm.	Can Z. and colab., 2015

For example, the use of hydrophobic resin, Amberlite XAD2 for bee pollen sample purification determined a decrease in the content of phenolic compounds (Carpes et al., 2013). The presence of glycosidic flavonoids in bee pollen was reported in many studies (Ferreres et al., 1994; Ulusoy, 2014; Sameer et al., 2014) and the most frequent compound found is identified as quercetin-3-sophoroside by various methods regardless of the pollen type. Capillary electrophoresis coupled with electrospray ionization time-of-flight-mass spectrometry (CE–ESI–TOF–MS) showed the presence of quercetin-3-sophoroside in *Ranunculus petiolaris* bee pollen extract including acetin-glucoside, 7-O-methylherbacetin-3-sophoroside, galloyl-glucose, apigenin-6,8-di-C-glycoside, quercetin-3-rutinoside, genistein-7-O-β-D-glucoside, luteolin-7-O-glucoside, apigenin-7-O-glucoside and 2′,4′,6′-trihydroxy-3′-formyldihydrochalcone (Arráez-Román et al., 2007). HPLC-PDA combined with UHR-QTOF mass spectrometer used to analyze methanolic extracts of Lithuanian pollen also confirms that quercetin 3-o-sophoroside is a glycosidic flavonoid as well as other compounds like quercetin dihexoside and isorhamnetin 3-glucoside (Čeksterytė et al., 2016). Study carried out by Freire (2012), showed that bee pollen from Brazil contained mainly isoquercetin, quercetin and isorhamnetin. These flavonoids appeared in more than 90% of the sample and may be not considered a marker to plant species (Freire et al., 2012).

The chemical diversity of propolis depends mainly by geographic and plant origin (Bankova et al., 2000). The differences between propolis of temperate and tropical regions are determined by vegetal source: in temperate zones the main visited plant species are poplar trees, while in tropical zones the dominant propolis source is *Baccharis dracunculifolia*. In other geographical regions, such as in Australia and equatorial regions in South America, propolis have botanical origin in *Ambrosia deltoidea* and *Encelia farinose*, whereas the major sources for propolis from Venezuela are represented by *Clusia minor* and *Clusia major*. Based on plant origin it were estabilished the main chemical types of propolis: Poplar propolis from Europe, North America, and the non-tropical regions of Asia, Birch propolis from Russia, Mediterranean propolis from Croatia, Sicilia, Greece, Malta, Algeria and Cyprus (Popova et al., 2011), Green Propolis from Brazilia, Red propolis from Cuba and Venezuela, “Pacific” propolis from Okinawa and Taiwan and “Canarian” propolis from Canarian Islands (Bankova, 2005).

In the case of propolis, also the chemical pattern is directly related to the species of bee. Over 30 compounds were identified by ESI-MS/MS in green and brown Brazilian propolis and the results showed that ESI (-) -MS fingerprints of the extracts vary in relation with bee species (Marcucci et al., 2008).
More recent, six samples of propolis (green, red, brown Brazilian and Cuban yellow propolis) were analysed by UPLC-ESI (-) MS/MS to identify some ions observed by ESI (-)-MS fingerprints.
The author mentioned that yellow propolis from Cuba was abundant in triterpenoids and had a small proportion of phenolics and flavonoids comparing with green and red propolis (Machado et al., 2016).
Novel, advanced and traditional methods used for phenolic profile characterization are presented in (Figure 1).

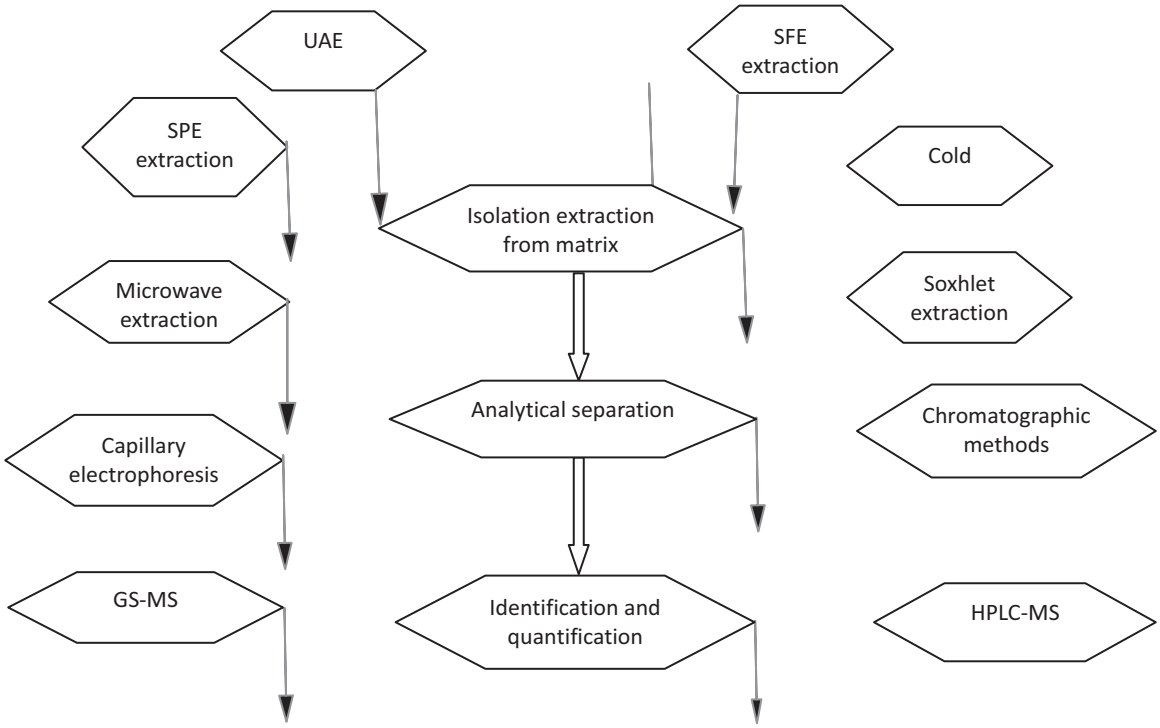

Figure 1. General extraction procedures and analytical methods for separation, identification and quantification of phenolic compounds from bee pollen and propolis

The flavonoids present in pollen are sometimes characteristic of botanical and geographical origin (Soler et al., 1994) but only in few cases were identified a species-specific compound, such as 8-Methoxykaempferol 3-Sophoroside in almond pollen (*Prunus amygdalus, Rosaceae).* (Ferreres F. and colab., 1989) Although some flavonoid glycosides quercetin-3-O-β-D-glucosyl-(2→l)-b-glucoside, kaempferol-3,4'-di-O-β-D-glucoside and kaempferol-3-O-β-D-glucosyl-(2→l)-β-D-glucoside have been demonstrated the use as markers to discriminate pollen from different floral origins (Zhou et al., 2015).
On the other hand, some flavones like chrysin and tectochrysin are considered characteristic compounds of propolis and beeswax (Soler C. et al., 1994).
Due to chemical diversity of phenolic compounds, flavonoid profile could be used as chemical marker to evaluate botanical origin, quality of product and nutritional and biological properties (Sameer et al., 2014; Ulusoy, 2014).
Phenolic compounds are responsible also for colour variations in bee pollen and propolis due to presents of two classes of pigments such as flavonoids and/or carotenoids (Owayss et al., 2004). In agreement with the distribution and abundance, the most important pigments in both bee products were anthocyanins, carotenoids and xantophylls (Webby and Bloor, 2000; Montenegro et al., 1997; Naranjo et al., 2004; Owayss et al., 2004).

CONCLUSIONS

Based on papers presented in current work, it can be concluded that propolis as well as bee pollen contain a high amount of phenolic constituents. Chemical diversity of these bee products is influenced by botanical origin and extraction methods.

These factors can also induced variations in phenolic compounds concentrations. High levels of some polyphenols can explain the biological activities of bee pollen and propolis.

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