Antioxidant activity and constituents of ethanolic propolis extracts from Romanian market

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Introduction

In Romania, propolis ethanolic extracts are widely used accordingly or in combination with other herbal extracts. The main objective of the present study was to analyse and to compare the bioactive compounds and free radical scavenging activity of several samples of ethanolic propolis extracts from Romanian market.

Materials and Methods

▪ *The samples was ethanolic propolis extracts* : Comercial soft extract of propolis –Bioremed (C1), Tincture of propolis –Favisan (C2), Tincture of propolis- Flavasol (C3), Hydroalcoholic extract of propolis-Dacia Plant (C4), Tincture of propolis – Plant extract (C5), Tincture of propolis –ICDPA (C6), Tincture of propolis-Fabiol (C7), Tincture of propolis –Santo Rafael (C8), Tincture of propolis- Larix (C9).

▪ *Total phenolic substances.* The phenolic components were determined by using the Folin-Ciocalteau reagent solution [1]. A reference mixture of pinocembrin and galangin (2:1, w/w) was employed as the standard for calibration [2].

▪ *Flavone and flavonol content.* The flavone and flavonol content content was determined by the method described by Dowd [3], employing AlCl3 to form a complex, which was measured spectrophotometrically at 425 nm. Galangin was employed as the standard.

▪*Flavanone and dihydroflavonol content.* The flavanone and dihydroflavonol content was determined by the procedure described by Nagy and Grancai [4], employing 2,4-dinitrophenylhydrazine wich reacts with ketones and aldehydes to form dinitriphenylhydrazones. Absorbance was measured at 495nm. Pinocembrin was employed as the standard.

▪ *Free radical scavenging activity on DPPH.* The free radical-scavenging capacity of samples was tested by its ability to bleach the stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) [5]. The reaction of DPPH radical with antioxidants was determined spectrphotometrically at 517 nm. Trolox water-soluble derivative of vitamin E, was used as a standard.

▪ *Scavenging activity of ABTS radical cation.* The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) scavenging activity was measured according to the method described by Erel with some modifications [6]. ABTS was dissolved in water to a 7 mM concentration. The ABTS radical cation was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate.The reaction of ABTS radical with antioxidants from samples was determined spectrphotometrically at 734nm. The equivalent of antioxidant capacity was expressed as equivalent Trolox(6-hydroxy-2,5,7,8-

tetramethylchroman-2-carboxylic acid).

▪*FRAP assay.* The FRAP values are obtained by comparing the absorbance change of blue coloured ferrous-tripyridyltriazine complex at 593 nm in diluted ethanolic

extracts of propolis samples with those containing ferrous ions in known concentrations [7]. Aqueous solutions of known ferrous sulphate concentrations in the were used for calibration. In order to make comparison,Trolox was also tested under the same conditions as a standard antioxidant compound.

▪*HPLC separations* for method development were carried with a LC-10AD system from Shimadzu (Japan), consisting of LC-10Dvp pumps, a diode array detector (SPD-M20A) and a MS detector Shimadzu 2010 equipped with ESI interface. Solvents were degassed with a degasser (DGU-20A5) and the temperature was kept constant at 25 degree by column oven (CTO-10Svp). Gradient control , data acquisition and analysis were provided running LC-Solution software. The column was Kromasil 150nm x 4.6 mm with 3µm packing.

▪ *A HPLC method* was deveolped with a linear gradient elution, solvent A was formic acid in water (pH 2.65) and solvent B was acetonitrile. Gradient elution was from 2% B in a linear gradient over 5 min followed by 50% B during 35 min and 1% B after 10 min. Stock standard solutions were prepared in methanol (1x10-3 g/mL). Injection volume was 20 µL and flow rate was 0.2mL/min. Polyphenolic individual compounds (caffeic acid, caffeic acid phenethyl ester-CAPE, p-coumaric acid, ferulic acid, chrysin, luteolin, naringenin, isorhamnetin, acacetin, kaempferol, galangin, pinocembrin, pinostrobin) were quantified in our samples using the RP HPLC method. The SIM (selected ion monitoring) mode was used when a search for some particular ions shoul be done.

RESULTS

Table 1. Results of quantification of main biologically active compounds in propolis samples.

Sample	Phenolics[a,b] mg/mL	Flavones and flavonols[a,c] mg/mL	Flavones and dihydroflavonols[a,d] mg/mL
C1	519.64±0.03	30.78±0.02	11.66±0.01
C2	59.31±0.01	1.98±0.04	1.16±0.01
C3	45.57±0.04	1.53±0.01	0.78±0.01
C4	63.61±0.04	3.80±0.02	1.20±0.01
C5	14.89±0.01	0.52±0.01	0.30±0.04
C6	92.30±0.02	7.69±0.01	1.83±0.02
C7	58.81±0.02	4.80±0.02	1.89±0.02
C8	94.43±0.08	5.69±0.01	1.97±0.01
C9	83.67±0.03	7.18±0.01	2.42±0.04

[a] Values are means ±SD(n=3).
[b] As pinocembrin:galangin (2:1,w/w).
[c] As galangin.
d As pinocembrin.

Figure 1. Trolox Equivalent Antioxidant Capacity of samples using DPPH and ABTS methods

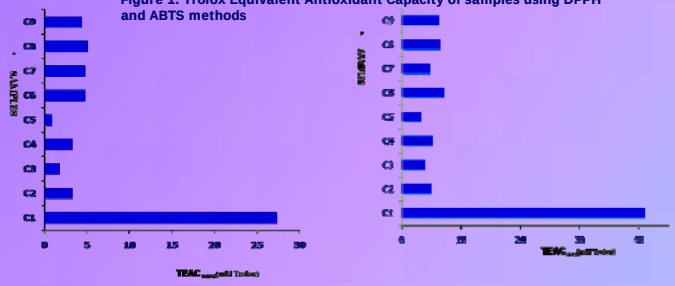

Figure 2. FRAP value (µM) for propolis ethanolic extracts and Trolox at concentration of 1000 µg/ml.

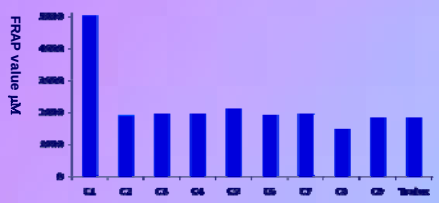

Table 2. Individual phenolic content of samples determined by RP HPLC

Samples	Caffeic acid mg/L	Coumaric acid mg/L	Ferulic acid mg/L	Naringenin mg/L	Kaempherol mg/L	Isorhamnetin mg/L	Pinocembrin mg/L	CAPE mg/L	Chrysin mg/L	Acacetin mg/L	Galangin mg/L	Pinostrobin mg/L
C1	7.14	9.97	11.11	17.10	-	0.43	9.77	8.16	1.50	2.65	0.35	13.68
C2	0.06	1.19	1.26	1.76	-	0.32	5.48	3.45	3.62	1.57	1.20	1.05
C3	0.05	1.84	1.62	1.98	-	0.19	1.91	4.77	2.50	1.49	0.91	1.26
C4	0.88	2.50	2.23	1.10	-	0.01	1.07	1.33	0.38	0.41	0.14	0.18
C5	0.19	0.74	0.62	0.43	-	0.01	1.34	1.80	0.93	0.42	0.30	0.34
C6	0.13	2.21	2.20	4.45	0.076	0.64	10.68	6.79	0.68	3.82	2.68	2.73
C7	1.12	1.59	1.63	3.35	-	0.27	6.41	3.66	1.88	1.24	0.61	0.37
C8	1.24	5.78	5.39	2.31	-	0.01	0.89	1.95	0.83	0.57	0.23	0.04
C9	1.53	1.58	1.21	4.13	0.033	0.29	3.46	4.64	0.97	1.08	0.43	-

Figure 3. Comparative cromatogram s of sample C3 and C5

Conclusions

▪ These results suggest that propolis ethanolic extracts might be used as active pharmaceuticals for patients with various diseases such as cancer, cardiovascular diseases, and diabetes.

▪ The HPLC method allow to obtain a very good peak-to-peak separation and a good linearity for all the analytes (R^2>0.9978).

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