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HONEYDEW HONEY: CORRELATIONS BETWEEN CHEMICAL COMPOSITION, ANTIOXIDANT CAPACITY AND ANTIBACTERIAL EFFECT

MIEREA DE MANĂ: CORELATII ÎNTRE COMPOZIŢIA CHIMICĂ, CAPACITATEA ANTIOXIDANTĂ ŞI EFECTUL ANTIBACTERIAN

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Selected physico-chemical parameters, total polyphenols, flavonoids, antioxidant and antibacterial activity of honeydew honey samples from Romanian were determined. Regarding the chemical composition, analysed honey samples framed in this type of honey, phenolic content, determined as gallic acid equivalents, presented a mean value of 116.45mg GAE/100 g honey. Total flavonoid content expressed as quercetin equivalents, was 1.53 mg in honeydew honey. Antioxidant activity expressed as % inhibition of a solution of DPPH, ranged between 47.84 and 62.99%. The concentration of honey that inhibit with 50% the DPPH solution was established to be 16.16%. 10 strains of Staphylococcus aureus presented different inhibition percentages when were treatred with a solution of honey. In conclusion, Honeydew honey could be recommended to complement other polyphenol source in human diet and also used in medical treatment

Key words: honeydew honey, radical scavenging activity, DPPH, antioxidant power, FRAP, antibacterian effect, *Staphylococcus aureus*

Introduction

Even thow honey main constituents are sugars and water, there are some small quantity components in the composition of honey that are responsible for honey properties. The components of honey that have antioxidant properties are phenolic

acids and flavonoids (Ferreres et al., 1992; Andrade et al., 1997), enzymes like catalase and glucose-oxidase, ascorbic acid, organic acids, amino-acids and proteins .

Several studies made on honey show that European honeys have a rich phenolic profile, consisting of benzoic, cinnamic acids and flavonoid aglycones (Ferreres et al., 1992; Andrade et al., 1997; Ferreres et al., 1994; Martos et al., 2000, Tomas-Barberan et al., 2001).

Bees generally forage for plant nectar, but they also can take the sweet secretions of aphids, known as honeydew. The most healthful form of honey does not come from nectar, but rather from honeydew, a new Spanish study says (Sanz et al. 2005). Honeydew is created from the secretions of aphids and other bugs feeding on plant sap.

The main group of antioxidants in honey are the bioflavonoids pinocembrin, chrysin, pinobanksin, and galagin. Pinocembrin is found only in honey and bee propolis. Ascorbic acid (vitamin C), catalase, and selenium are also found in honey. According to the International Honey Commision and Food and Agriculture Organization of the United Nations honey contains many minerals in very small quantities, potassium being the most abundant. Dark honeys, particularly honeydew, are the richest in minerals. Other minerals found in honeydew include calcium, zinc, magnesium, copper, manganese, iron, phosphorus, selenium, chromium, and sodium. Honeydew honey contains larger amounts of oligosaccharides (about 5%) than nectar honey. The oligosaccharides present include erlose, theanderose, ketose, raffinose, melezitose, maltotriose, and panose. In general, the darker honeys, such as honeydew, have stronger antioxidant potential than other forms of honey. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals, which may damage cells. Antioxidants fight the damage caused by free radical molecules in the body. The increasing interest in the antibacterial properties of various types of honey is being supported by their well-known, remarkable therapeutic potential, mostly accepted in alternative medicine (Beretta et al., 2007). Honeydew honey has proven to be a powerful antibacterial, antiviral, and antifungal agent, killing bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Helicobacter pylori.*

Our research team has made studies on components that might have antioxidant power (total phenolisc and total flavonoids)(Bobiş et al., 2005, 2006, 2007), but it is at the beginning the studies regarding antibacterial activity of romanian honeys.

The main objective of this study was to evaluate, quantify and correlate the chemical composition, antioxidant capacity and antibacterial effect.

Materials and Methods

Materials. Honeydew honey samples of different locations from Transilvania were obtained from individual beekeepers, associations (raw honey) and commercial honey (processed).

Chemicals, reagents and standards used were analytical and chromatographic grade purity.

The botanical origin was confirmed by pollen analysis and classical quality determinations (water content, HMF, sugars).

Selective physicochemical parameters were determined according to Romanian standard (STAS 784/1...3-89) and Harmonized Methods of International Honey Commission. Water content was determined refractometrically (Abbe digital refractometer WYA-S Selecta Spain) and expressed as mg/100g; hydroxymethylfurfural was determined spectrofotometrically according to White method (expressed as mg/kg) on a Varian Cary UV50 Multicell apparatus. Sugar profile was determined by HPLC on a Shimadzu system equipped with a LC-10AD pump, DGU-14A degasser, SIL-10AV VP auto sampler, RID-10A refractive index detector, thermostatted at 30ºC with CTO-10AS VP temperature controller of separation column (Altima Amino 100 Å 5 µm, 250 mm x 4,6 mm) with a mixture of acetonitrile/water as mobile phase with 1.3 ml/min flow rate.

For the quantification of main sugars, a calibration curve in the range 40 – 0.5 g/100g, with regression coefficient of R^2=0.9982 for a mixture of standards (glucose, fructose and saccharose) was obtained. Results were expressed in g/100g honey.

Determination of total phenolics and total flavonoid content. The concentration of total phenolics and total flavonoids in diluted honey samples (10%, w/v in two different solvents) was determined with a modified method developed by Singleton et al. (1999)(Folin–Ciocalteu method) and Kim et al. (2003), respectively.

After studying the effect of five different solvents on the concentration of total phenolics and flavonoids from honey, the solvent used to dilute the honey samples was mixture methanol:water (pH=2) 1:1.

Each honey sample (2 g) was diluted to 20 ml with mixture 1:1 of methanol and deionised water (pH=2 with concentrated HCl (Fluka-Riedel de Haen Germany) and filtered through Whatman filter paper. An aliquot of the honey solution was mixed with 2,5 ml of 0,2 N Folin-Ciocalteu reagent (Sigma-Aldrich Chemie GmbH) for 5 minutes, and 2 ml of 75 g/l Na_2CO_3 (Acros Organics Geel Belgium) was added. After incubation at room temperature in the dark for 2 hours, the absorbance of the reaction mixture was measured at 760 nm against a blank (Cary UV-50 Spectrophotometer). Gallic acid (Sigma Aldrich Germany)(0,25-250µg/ml) was used as standard, to make the calibration curve (y=12,81x-0,0247, r^2=0,9968). The mean of three readings was

used and the total phenolic content was expressed as mg of Gallic acid equivalents (GAE)/100 g of honey.

Same honey solutions were subjected to total flavonoid content.

5 ml of honey sample was mixed with 5 ml of 2% $AlCl_3$ (Acros Organics Geel Belgium.) in methanol and the absorbance at 415 nm, was read after 10 min, against a blank, using quercetin (2,5 - 125 µg/ml)(Sigma Aldrich GmbH Germany) as standard for calibration curve (y=61,031x-0,0098, r^2=0,9944). The mean of three readings was used and expressed as mg quercetin equivalents (QE)/100 g of honey.

Determination of free radical –scavenging activity. The method used by Chen et al (2000) for the measurement of the antioxidant activity of honey was adapted and followed in this study. The purple colour of 2,2-diphenyl-1-picrylhydrazyl (DPPH) decays in the presence of an antioxidant, and the change in the absorbency can be monitored spectrophotometrically at 517 nm. Briefly, 0,75 ml of honey solution (0,1 mg/ml) in deionised water was mixed with 1,5 ml of a 0,03 mg/ml DPPH (Sigma Aldrich GmbH Germany) in methanol and shaken vigurously, incubated in the dark for 30 min, and the absorbance of the remaining DPPH was determined at 517 nm against a blank.

The radical scavenging activity was calculated as follows:

$$\% \text{ Inhibition} = [(Abs_{Blanc} - Abs_{Sample})/Abs_{Blanc}] \times 100$$

The IC_{50} value was also calculated for one honey sample to see the correlation between the two ways of expression for radical scavenging activity. For this was recorded the absorbance of several concentrations of honey against their own % of inhibition of the DPPH solution. The result show the concentration of honey that inhibit with 50% the concentration of the DPPH solution.

Determination of antibacterial activity. The present study aimed to assess the in vitro antibacterial activity of honeydew honey against antibiotic resistant animal pathogens (*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Pseudomonas aeruginosa*), considering the possible concordance with the chemical composition of the honeys.

The in vitro antibacterial potential of the honeys were studied against the animal pathogens by disk diffusion and well diffusion assays. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), were obtained .

Results and Discussions

The results of selected physico-chemical parameters, determined from honey samples is presented in table 1. All the investigated parameters framed in the standard limits for the honeydew honey.

Table 1. Water, HMF and main sugars from honeydew honey samples

Honey samples	Water content (%)	HMF content (mg/kg)	Fructose (%)	Glucose (%)	Sucrose (%)	Melezitoze (%)
Honeydew 1	18.2	4.59	41.19	34.43	0.35	1.08
Honeydew 2	17.9	3.14	39.64	37.65	0.15	2.67
Honeydew 3	19.8	15.2	38.68	34.30	0.09	0.51
Honeydew 4	16.9	6.23	34.92	32.05	0.09	0.75
Honeydew 5	18.5	5.45	40.65	35.30	0.08	2.78
Honeydew 6	17.3	3.41	40.68	33.52	1.02	2.05

Total phenolics and total flavonoid content. High content of total polyphenols were found in the analysed samples (Table 2)(93,5 – 144,94 mgGAE/100 g honey). The flavonoid content ranged between 1,26 and 2,05 mgQE/100 g honey.

Table 2. Total polyphenol and flavonoid content in honeydew honey samples

Honey samples	Total polyphenols (mgGAE/100g)	Total flavonoids (mgQE/100g)
Honeydew 1	93.5	1.26
Honeydew 2	95.8	1.31
Honeydew 3	120.06	1.82
Honeydew 4	114.08	2.05
Honeydew 5	130.32	1.26
Honeydew 6	144.94	1.49

Antioxidant activity. 6 honey samples of different locations were tested in order to asses their antioxidant properties. The results obtained shows that all tested samples were antioxidatively active, their RSA varying between 47,84 and 62,99 % Inhibition of the DPPH solution (Table 3).

Table 3. Radical scavenging activity for honeydew honey solution (10% v/v)

Sample	Abs_{DPPH}	$Abs_{honey+DPPH}$	Abs_{honey}	Difference in Abs	% Inhibition
Honeydew 1	0.5404	0.4589	0.2014	0.2575	52.35
Honeydew 2	0.5705	0.4308	0.2014	0.2294	59.79
Honeydew 3	0.6168	0.4297	0.2014	0.2283	62.99
Honeydew 4	0.5404	0.4968	0.2382	0.2586	52.15
Honeydew 5	0.5705	0.5358	0.2382	0.2976	47.84
Honeydew 6	0.6168	0.4844	0.2382	0.2462	60.08

An example of expressing radical scavenging activity as IC_{50} (the concentration of honey that reduce the DPPH concentration with 50% is presented in Table 4.

Table 4. IC_{50} value for one honeydew honey sample

Honey concentration	Abs_{DPPH}	$Abs_{honey+DPPH}$	Abs_{honey}	Difference in Abs	% Inhibition
17%	0.5647	0.3827	0.1018	0.2809	50.26
8,5%	0.5546	0.4544	0.0531	0.4013	27.64
4,25%	0.5535	0.4945	0.0264	0.4681	15.43
2,12%	0.5533	0.5266	0.0179	0.5087	8.06
1,6%	0.5560	0.5397	0.0116	0.5281	5.02
0,8%	0.5947	0.5858	0.0076	0.5782	2.77

Intercept: 1,478604 ⇒ **IC_{50} = 16,16**
Slope: 2,9234

Antibacterial activity. The MICs and MBCs values determined using a broth micro dilution method were presented as average values in table 5.

This study revealed some differences between the honey samples antibacterial activities. The level of induced bacterial growth inhibition, as established by the broth micro dilution assay, proved to be depending mostly of the product's type, and less of the bacterial strain.

Table 5. Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs) 10% v/v of honeydew honey solution

Strain	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
MIC	4%	2%	2%	4%	2%	4%	4%	2%	2%	2%
MBC	4%	2%	2%	4%	2%	4%	4%	2%	2%	2%

Conclusions

The study showed that the analyzed honeydew honey samples contain antioxidant substances like phenolics, flavonoids and other pigments.

The content of this bioactive compounds is very high, comparing to other types of honey.

Radical scavenging activity of honeydew honey is very high, the decrease in colour for the DPPH solution after 15 min. being in almost all the cases more than 50%, using a honey solution of 10%.

Antibacterial activity was showed by the bacterial growth inhibition for 10 strains of *Staphyloccocus aureus* .

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