

NATURAL DRUGS

ANTIRADICAL AND ANTIMICROBIAL ACTIVITY OF PHENOLIC FRACTIONS OBTAINED FROM HONEYS

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Abstract: Honey is a natural product consisting of multiple components which determine its dietary and medicinal properties. In this work there were studied methanol fractions obtained from seven honeys from Lower Silesia (Poland) collected in different seasons of three successive years. Melissopalynologic studies revealed that two of them were polyfloral, and five were classified as monofloral (two buckwheat and three rapes). The amount of phenolic compounds in honeys varied from 0.09 to 0.38 mg per g of honey. Honeys harvested in 2010 were the richest in phenolic compounds and especially rich was buckwheat honey, comparing to 2011–2012. Determination of antioxidant potential with the DPPH radical revealed that the strongest antiradical activity was exhibited by extracts obtained from polyfloral (1.22 TAU_{515mg}) and buckwheat (1.06 TAU_{515mg}) honeys, while the highest number of antiradical units was observed for rape honey (3.64 TAU_{515g}). Polyphenolic fractions exhibited various bactericidal activities against *Klebsiella pneumoniae* and *Staphylococcus aureus* and weak or no activity was observed against *Pseudomonas aeruginosa*.

Keywords: antimicrobial agents, antioxidants, honey, DPPH, phenols, polyphenols

Honey is a natural product with dietetic and medicinal activity, produced by honey bees (*Apis mellifera*) from plant excretion and nectar. It is an aqueous solution with high concentrations primarily of carbohydrates such as glucose, fructose, maltose, sucrose and other oligo- and polysaccharides. They represent 95% of the dry mass of honey. After honey consumption mainly carbohydrates are transported to the blood and they can be used by the human organism to meet the demand for energy.

Besides carbohydrates, honey contains compounds such as proteins, enzymes (α - and β -glycosidase, catalase, phosphatase), organic acids, lipids, vitamins, flavonoids and other polyphenols as well as minerals (1).

Studies show that honey has a beneficial effect in many diseases (2, 3). It is known for its antibacterial, antiinflammatory and antioxidant activity (4). Honey is one of the oldest natural foods with anti-

bacterial features and can be used to treat many diseases of the gastrointestinal tract and infected wounds (5). The antioxidant activity of honey is affected by the physical features of honey and biologically active compounds. Many researchers consider that the main substance with antibacterial activity in honey is hydrogen peroxide, formed with the participation of glucose oxidase in the presence of water. It was noted that antibacterial activity is a result of the action of many components including phenolic compounds (6).

Phenolic compounds such as flavonoids, benzoic acid, and cinnamic acid found in honeys exhibit antibacterial activity but much lower than hydrogen peroxide (7, 8). Antibacterial activity of honeys depends on botanical and geographical origin and the amount of bee metabolic products (9).

Today's consumers willingly use natural products which have specific health benefits. It has been

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suggested that oxidative stress could be a cause of many diseases associated with the progress of civilization (10, 11).

Honey is a natural source of antioxidants preventing inflammatory processes, heart diseases, cancers, chronic diseases of the respiratory tract and diabetes. The natural antioxidants in honey are glucose oxidase, catalase and non-enzymatic compounds such as ascorbic acid, organic acids, products of Maillard reactions, proteins, and amino acids. Compounds which are responsible for this antioxidant activity also include phenolic acids, which come from plants. Chemical composition and antioxidant features depend on the botanical origin of nectar and environmental agents such as climate, type of soil, seasonal factors and methods of storage of honey (12-15).

Research has shown that by the consumption of food ingredients one can prevent the typical diseases for industrialized countries such as diseases of the circulatory system and cancers. Research showed that a diet rich in antioxidant compounds is a basic agent preventing oxidative damage in the human organism. Antioxidants in food are a heterogeneous group of compounds including polyphenolic compounds which are strong antioxidants (16).

Honey as a natural product is also known for its nutritionally valuable features, prophylactic and therapeutic factors. There are many scientific reports on honey as effective in therapy of wounds, burns, bacterial and fungal infections, and inflammatory acute or chronic states of the stomach.

The aim of this work was to define the type of honey by determination grains of pollen and measurement of the amount of phenolic compounds, antioxidant and antibacterial activity of honeys and in their methanol extracts.

MATERIALS AND METHODS

Materials

Honeys

The materials used for the research comprised seven honeys obtained from an apiary situated in a village situated in the central-western part of Poland in different seasons described below:

H1 - honey harvested in July 2010;

H2 - honey harvested in August 2010;

H3 - honey harvested at the beginning of June 2011;

H4 - honey harvested at the end of June 2011;

H5 - honey harvested in May 2012;

H6 - honey harvested in June 2012;

H7 - honey harvested in July 2012.

Apparatus

Microscope Axio Lab. A1 Zeiss, Germany; Baker system spe-12G (J.T. Baker USA), columns Bakerbond spe C18, USA; ultrasonic bath Bandelin, Germany; spectrophotometer UV/VIS Hitachi model U-5100; fluorescence microplate reader Victor², Wallac, Perkin Elmer, USA.

Methods

Pollen analysis

Pollen analysis was performed with the method approved by the International Commission for Plant-Pollinator Relationships (I.C.P.P.R.) and described by Louveaux et al. (17). Honeys were classified by frequency of appearance of pollen grains of nectaring plants. The appearance of pollen of a specific taxon with a frequency above 45% was called very frequent and honey was classified as monofloral. When the pollen grain appeared with a frequency from 16 to 45% it was frequent, pollen grain which appeared with a frequency of 3 to 15% was called unique, and pollen grain which appeared with a frequency below 3% was called rare.

Preparation of phenolic fraction with the method of SPE (solid phase extraction)

One gram of honey was dissolved in 10 mL of 0.01 mol/dm³ HCl and extraction was carried out in an ultrasonic bath (Badelin, Germany) for 15 min at 20°C. The Bakerbond spe column C18 (Mallinckrodt Baker, Inc., USA) was equilibrated with 20 mL of 0.01 mol/dm³ HCl. Obtained extract was filtered with a filter (Mallinckrodt Baker, Inc., USA) and applied onto the column. The column was washed with 0.01 mol/dm³ HCl until there was a complete lack of carbohydrates in the effluent. Then fractions rich in phenolic compounds were eluted with methanol (18). The phenolic fraction was condensed to dryness under reduced pressure.

Measurement of amount of phenolic compounds

Total amount of phenolic compounds in methanolic extracts was measured with colorimetric methods using Folin-Ciocalteu's phenol reagent (19).

Seven milliliters of water was added to the test tubes, then 0.5 mL of Folin-Ciocalteu's phenol reagent, and 0.5 mL of methanol extract of the phenolic fraction at 2.9 mg/mL. After 3 min, 2.0 mL of 20% aqueous extract of sodium carbonate was added. The reaction mixture was heated with a boiling water bath for 1 min. After cooling, absorbance was measured at 685 nm, in a glass cuvette with the optical path length of 1 cm.

The amount of phenolic compounds was given in mg/g as gallic acid equivalents.

Measurement of antiradical activity of methanol extract

The measurement of antioxidant activity of extracts was performed using DPPH radical with the method described by Brand-Williams et al. (20).

Fifty microliters of extract solution in methanol at the concentration of 2.9 mg/mL was added to 2 mL of DPPH solution in methanol at 0.037 mg/mL and the reaction was carried out at 20°C. The absorbance of the reaction mixture was measured in a glass cuvette with 1 cm optical path. A control sample was also made for 50 µL of methanol added instead of methanol extract. Absorbency was measured at 515 nm in glass cuvette with 1 cm optical path.

Antiradical activity was presented as the number of antiradical units per mg of extract ($TAU_{515/mg}$) and per g of honey ($TAU_{515/g}$), and the values were calculated according to equations (I) and (II), respectively. One unit of antiradical activity is the

amount of substance in 1 mL of reaction mixture that causes the change of absorbency of 1 at 515 nm, in 1 min, at 20°C.

$$TAU_{515/mg} = \frac{A_0 - A_1}{c} \quad (I)$$

where: $TAU_{515/mg}$ is the number of antiradical units calculated per mg of extract; A_0 is absorbency of DPPH solution at the beginning of the reaction; A_1 is absorbency of the DPPH solution 1 min after extract addition; c is the concentration of extract in the cuvette [mg/mL].

$$TAU_{515/g} = m_e \cdot TAU_{515/mg} \quad (II)$$

where: $TAU_{515/g}$ is the number of antiradical units calculated per g of honey; m_e is the amount of extract [mg] in 1 g of honey.

Antimicrobial activity of extracts

The antimicrobial activity was determined with the microplate Alamar Blue assay according to (21). The experiments were performed using strains obtained from the Polish Collection of Microorganisms (PCM) of the Institute of Immunology and Experimental Therapy, Polish Academy of

Table 1. The amount of pollen of nectaring plants in honeys.

Honey	Type of honey	Pollen grains occurred very often (>45%)	Pollen grain occurred often (16-45%)	Pollen grain occurred between (3-15%)	Pollen grain occurred rarely
H1	Polyfloral honey		<i>Fagopyrum</i> (32.4)	<i>Brassica napus</i> , <i>Salix</i> , <i>Centaurea cyanus</i> , <i>Echium</i> , <i>Malus</i> type, <i>Trifolium repens</i>	<i>Aesculus</i> , <i>Melilotus</i> type, <i>Achillea</i> type, <i>Robinia</i> , <i>Rubus</i> type, <i>Centaurea jacea</i> , <i>Solidago</i> type
H2	Buckwheat honey	<i>Fagopyrum</i> (64.5)		<i>Robinia</i> , <i>Trifolium repens</i> , <i>Centaurea cyanus</i> , Brassicaceae	<i>Achillea</i> type, <i>Solidago</i> type, <i>Echium</i> , <i>Rubus</i> type, <i>Cirsium</i> type, <i>Lotus</i> , <i>Helianthus</i> type, <i>Cichorium</i> type, <i>Galium</i>
H3	Rape honey	<i>Brassica napus</i> (73.3)		<i>Fagopyrum</i> , <i>Tilia</i>	<i>Trifolium repens</i> , <i>Centaurea cyanus</i> , <i>Malus</i> type, <i>Rubus</i> type, <i>Frangula alnus</i> , <i>Centaurea jacea</i>
H4	Rape honey	<i>Brassica napus</i> (55.4)		<i>Fagopyrum</i> , <i>Frangula alnus</i> , <i>Galium</i> , <i>Malus</i> type, <i>Tilia</i> , <i>Cirsium</i> type	
H5	Polyfloral honey		<i>Brassica napus</i> (31.3)	<i>Malus</i> type, <i>Salix</i> , <i>Prunus</i> type, Rosaceae, <i>Centaurea cyanus</i> , <i>Trifolium repens</i> , <i>Rubus</i> type	<i>Viola tricolor</i> type, <i>Echium</i> , <i>Medicago sativa</i> , <i>Frangula alnus</i> , <i>Acer</i> , <i>Taraxacum officinale</i>
H6	Rape honey	<i>Brassica napus</i> (62.1)		<i>Fagopyrum</i> , <i>Malus</i> type	<i>Taraxacum</i> type, <i>Centaurea cyanus</i> , <i>Galium</i> , <i>Viola tricolor</i> type, <i>Anthriscus</i> type, <i>Robinia</i> , <i>Frangula alnus</i>
H7	Buckwheat honey	<i>Fagopyrum</i> (51.7)	Brassicaceae	<i>Tilia</i>	<i>Anthriscus</i> type, <i>Achillea</i> type, <i>Cirsium</i> type, <i>Taraxacum</i> type, <i>Helianthus</i> , <i>Centaurea cyanus</i>

Sciences: *Escherichia coli* (PCM 1144), *Klebsiella pneumoniae* (PCM 57), *Pseudomonas aeruginosa* (PCM 2563), *Staphylococcus aureus* (PCM 2602), *Candida albicans* (PCM 2566). Microorganisms were cultivated on liquid broth medium with sugar at 37°C for 24 h under aerobic conditions. Then, cells were diluted with sugar broth to obtain a suspension of about 2×10^5 cfu/mL of each strain. Methanol fractions were dissolved in DMSO solution (1 : 100) in phosphate buffered saline, pH 7.4 (PBS) in proportions 5 mg of extract in 5 mL of DMSO/PBS solution. The solubility of extracts was

determined on a three-stage scale (insoluble, moderately soluble, soluble). Fractions obtained from **H3**, **H4**, **H6** and **H7** were dissolved in DMSO/PBS. Then, dilutions were prepared exponentially on a plate for cell growth (Packard Instrument Company, USA). Samples of stock solutions were diluted eight times with sterile PBS to obtain the concentrations of investigated samples from 1 mg/mL to 0.0078 mg/mL. For fractions and their dilutions three repetitions were made. The antibiotic streptomycin was used as a positive control. To the wells containing 100 μ L of extracts, aliquots of 100 μ L of the diluted

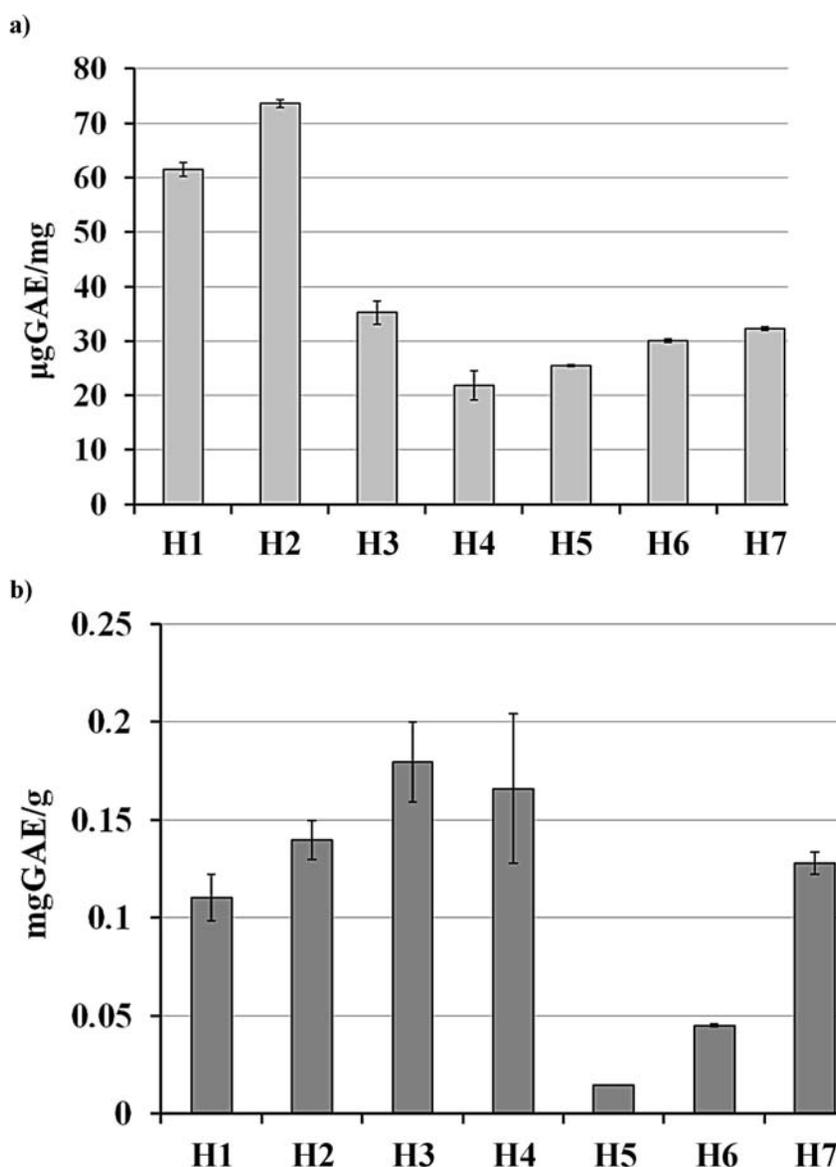


Figure 1. Amount of phenolic compounds expressed as gallic acid equivalents (GAE): (a) per mg of extracts (μ gGAE/mg) and (b) per g of honey (mgGAE/g)

Table 2. Weight of honey, weight of extract, number of antiradical units per mg of extract ($TAU_{515/mg}$), amount of phenolic compounds per mg of extracts [$\mu\text{gGAE/mg}$], number of antiradical units per g of honey ($TAU_{515/g}$) and amount of phenolic compounds per g of honey [mgGAE/g].

Honey	Honey weight [g] (± 0.01)	Extract weight [mg] (± 0.1)	$TAU_{515/mg}$	Amount of phenolic compounds [$\mu\text{gGAE/mg}$]	$TAU_{515/g}$	Amount of phenolic compounds [mgGAE/g]
H1	7.13	12.8	1.22 ± 0.11	61.5 ± 1.3	2.20 ± 0.27	0.110 ± 0.0119
H2	7.10	13.5	1.06 ± 0.08	73.6 ± 0.7	2.02 ± 0.20	0.140 ± 0.0099
H3	7.13	36.4	0.56 ± 0.08	35.2 ± 2.2	2.91 ± 0.63	0.180 ± 0.0204
H4	7.12	54.2	0.48 ± 0.05	21.8 ± 2.7	3.64 ± 0.53	0.166 ± 0.0381
H5	10.06	06.0	0.76 ± 0.14	25.5 ± 0.2	0.45 ± 0.06	0.014 ± 0.0001
H6	10.08	15.0	0.70 ± 0.12	30.1 ± 0.3	1.05 ± 0.15	0.045 ± 0.0007
H7	10.05	40.0	0.52 ± 0.06	32.3 ± 0.3	2.07 ± 0.40	0.128 ± 0.0055

Table 3. Minimal Inhibitory Concentration (MIC mg/mL). Value MIC for streptomycin (positive control) was: *Escherichia coli* (0.5 ± 0.073), *Klebsiella pneumoniae* (0.25 ± 0.002), *Pseudomonas aeruginosa* (0.25 ± 0.029), *Staphylococcus aureus* (0.5 ± 0.065), *Candida albicans* – no inhibition was detected.

Bacteria strain	H1	H2	H3	H4	H5	H6	H7
<i>Escherichia coli</i>	0.25 ± 0.004	0.5 ± 0.026	1 ± 0.032	1 ± 0.035	-	-	0.0625 ± 0.009
<i>Klebsiella pneumoniae</i>	0.5 ± 0.04	0.125 ± 0.018	0.125 ± 0.053	0.125 ± 0.006	0.25 ± 0.009	0.25 ± 0.004	0.25 ± 0.002
<i>Pseudomonas aeruginosa</i>	1 ± 0.027	1 ± 0.026	1 ± 0.008	1 ± 0.261	-	-	-
<i>Staphylococcus aureus</i>	0.25 ± 0.001	1 ± 0.001	1 ± 0.021	0.5 ± 0.022	1 ± 0.088	1 ± 0.098	1 ± 0.01
<i>Candida albicans</i>	0.5 ± 0.099	1 ± 0.142	0.5 ± 0.027	1 ± 0.028	-	-	0.0625 ± 0.004

– no antibacterial activity was observed

suspension of the strain were added. The control wells consisting of either bacteria only or medium only and those containing different fraction concentrations (100 μL) were inoculated with 100 μL of the diluted bacterial cells. Plates were incubated at 37°C for 48 h and after that 20 μL of Alamar Blue (10 \times diluted) and 12.5 μL of 20% Tween 80 solutions were added to the wells and incubation was continued at 37°C for 2 h. Fluorescence was measured with the reader Victor² (Wallac, Perkin Elmer) at wavelength 570 nm. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of investigated extract which prevented a color change from blue to pink, inhibiting the growth of = 90% of bacteria.

Statistical analysis

The statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA). Samples were analyzed using multivariate procedures based on the

relationship between the variables antiradical activity, total amount of phenolic compounds and antimicrobial activity.

Principal component analysis was applied to determine similarities and differences among honeys.

RESULTS

Pollen analysis

In **H1** honey samples there were found the types of pollen belonging to 34 taxa including 25 taxa of nectaring plants and 9 non-nectaring. Among nectaring plants there was often observed pollen of *Fagopyrum* (32.4%, Table 1). The honey was classified as polyfloral because it did not contain dominating pollen which could qualify the honey as monofloral. In **H2** honey samples there were noted types of pollen belonging to 32 taxa including 23 taxa of nectaring plants and 9 non-nectaring. Very

often pollen belonging to the genus *Fagopyrum* (64.5%) was observed. This allowed the honey to be classified as buckwheat honey. In **H3** honey samples there was noted the presence of pollen of 21 taxa of plants, namely 16 taxa belonging to nectaring plants and 5 to non-nectaring. Among nectaring plants very often pollen belonging to the species *Brassica napus* (73.3%) was observed. Honey **H3** was classified as rape honey. In samples of honey **H4** there were observed 18 taxa of plants including 12 taxa of nectaring and 6 taxa of non-nectaring

plants. There was very often observed pollen of *Brassica napus* (55.4%) that qualified this honey as rape. In honey **H5** samples there were identified 21 taxa of nectaring plants and 7 of non-nectaring and wind-pollinated plants. The most represented pollen was *Brassica napus* (31.3%). In the absence of dominating pollen, honey **H5** was qualified as polyfloral. Samples of honey **H6** contained 20 taxa of nectaring plants and 6 non-nectaring. Among nectaring very often there were grains of pollen of *Brassica napus* (62.1%). Pollen of *Fagopyrum* and *Malus* appeared

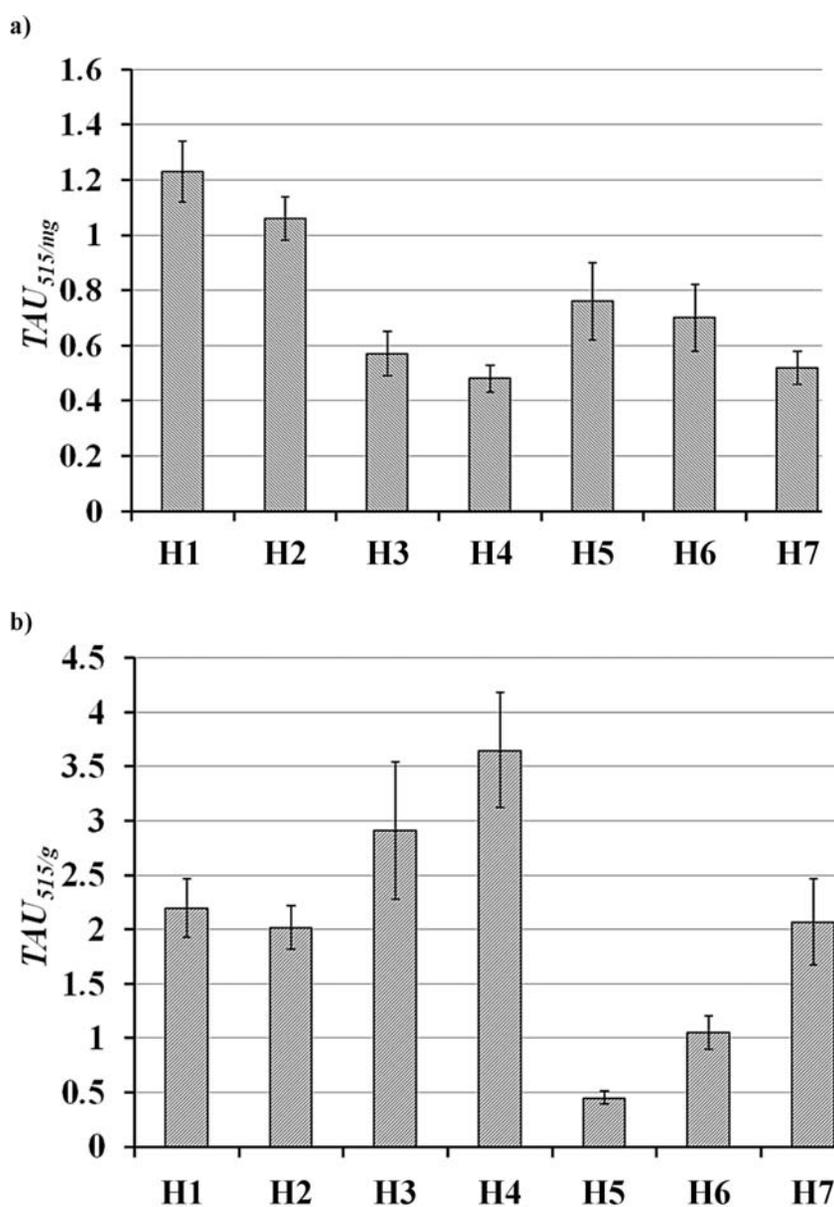


Figure 2. Antiradical activity expressed as number of antiradical units (a) per mg of extracts ($TAU_{515/mg}$) and (b) per g of honeys ($TAU_{515/g}$)

Table 4. Measured parameters for honey samples taken for PCA analysis: number of antiradical units per g of honeys ($TAU_{515/g}$), amount of phenolic compounds per g of honeys (mgGAE/g), and the reverse of antibacterial activity for 5 strains.

Honey	$TAU_{515/g}$	Amount of phenolic compounds [mg/g]	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
H1	2.20	0.110	4	2	1	4	2
H2	2.02	0.140	2	8	1	1	1
H3	2.91	0.180	1	8	1	1	2
H4	3.65	0.166	1	8	1	2	1
H5	0.45	0.014	0	4	0	1	0
H6	1.05	0.045	0	4	0	1	0
H7	2.07	0.130	16	4	0	1	16

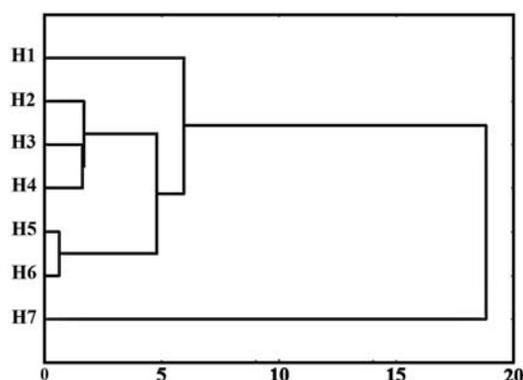


Figure 3. Dendrogram of the honey samples, based on number of antiradical units per g of honeys ($TAU_{515/g}$), amount of phenolic compounds per g of honeys (mgGAE/g) and antimicrobial activity for 5 strains

as a single grain. This honey was qualified as monofloral, rape honey.

In samples of honey **H7** there were specified 24 taxa of nectaring plants and 6 non-nectaring. Very often there was noted the presence of pollen belonging to *Fagopyrum* (51.7%). Honey **H7** was categorized as monofloral buckwheat honey.

The amount of phenolic compounds and antioxidant activity of honeys

The amount of phenolic compounds measured in extracts and honeys is demonstrated in Table 2 and Figure 1. The highest content of phenols was identified in extracts obtained from buckwheat honey **H2** ($74 \mu\text{gGAE}/\text{mg} \pm 0.07$). Among the tested honeys, the buckwheat one in different years was characterized by the highest amount of phenolic compounds. For honey **H2** the amount of phenols was $0.14 \text{ mgGAE}/\text{g} \pm 0.0099$. Our results are con-

sistent with the results of other researchers who have found that dark honeys, such as buckwheat honey, contain more phenols than light ones such as rape (22).

Phenolic fractions were tested for antioxidant activity with a DPPH radical assay according to the method of Brand-Williams et al. (20). Antiradical activity was expressed as the number of activity units per mg of extract $TAU_{515/mg}$ and g of honey $TAU_{515/g}$ (Table 2, Fig. 2). The strongest antioxidant activity was observed for extracts from polyfloral honeys **H1** ($TAU_{515/mg} = 1.22 \pm 0.11$) and buckwheat **H2** ($TAU_{515/mg} = 1.06 \pm 0.08$). The highest number of antiradical activity units per g of honey ($TAU_{515/g}$) was observed for rape honey **H4** (3.64 ± 0.53) and rape honey **H3** (2.91 ± 0.63). Socha et al. (23) investigating the Polish honey found that buckwheat honeys exhibited stronger antioxidant features than rape honey.

On the basis of obtained results it was confirmed that there is a positive correlation between the amount of phenolic compounds and antioxidant activity of extracts ($r = 0.85$) and honeys ($r = 0.93$). Bertoncelj et al. and Escuredo et al. (24, 25) also observed the dependence of antioxidant features of honeys on the amount of phenolic compounds.

Antimicrobial activity of honeys

Polyphenol extracts were tested for antimicrobial activity using microplate Alamar Blue assay with three strains of Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), a strain of Gram-positive bacteria (*Staphylococcus aureus*) and a fungus (*Candida albicans*) (Table 3). Streptomycin was used as a positive control sample. The minimal concentration of extract which inhibits bacterial growth was defined

as the minimal inhibitory concentration (MIC). Antimicrobial activity was measured for eight different concentrations in the range from 0.0078 to 1 mg/mL.

Extracts from polyfloral honey **H1**, buckwheat honey **H2**, and from rape honeys **H3** and **H4** exhib-

ited antibacterial activity against all bacterial strains at the concentration range 0.125 mg/mL to 1 mg/mL (Table 3). Phenolic fractions from polyfloral honey **H5** and rape honey **H6** were the less active against microorganisms. Two strains of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aerugi-*

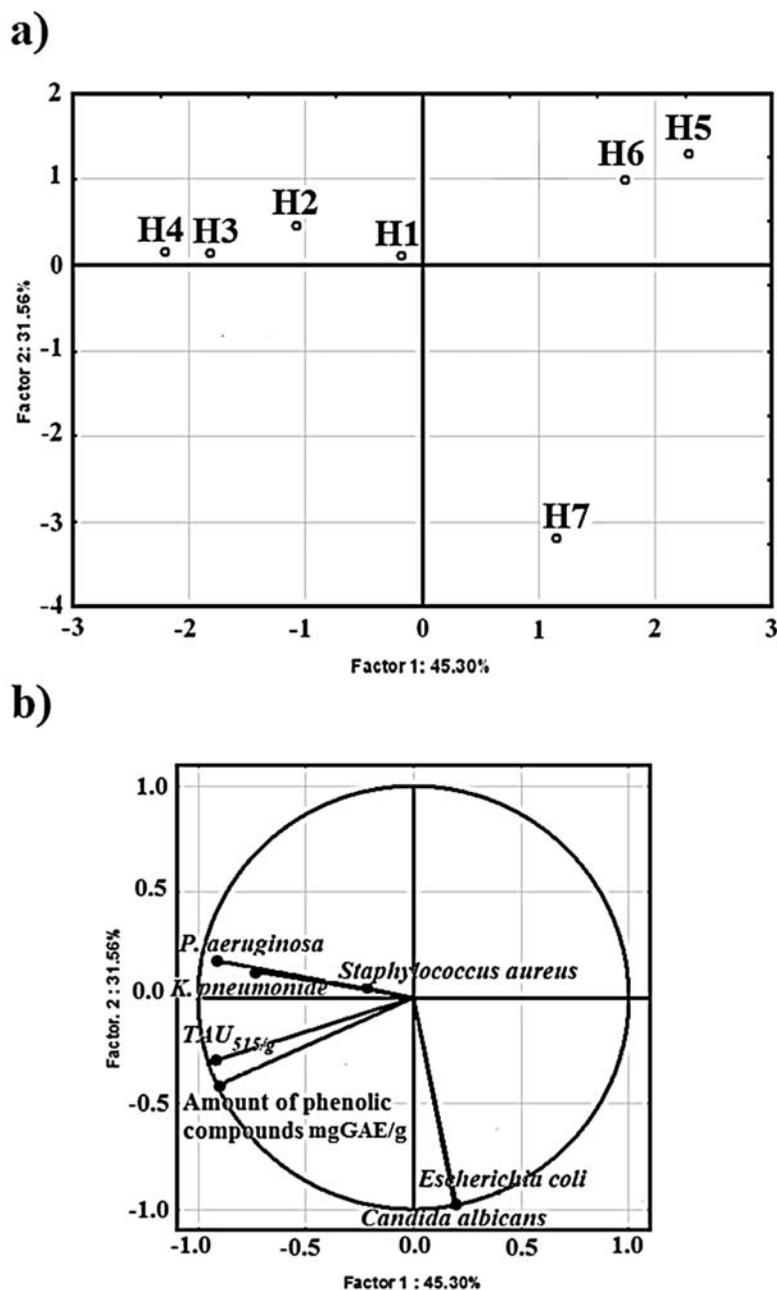


Figure 4. (a) Principal component analysis (PCA) with distribution of honeys; (b) PCA analysis with distribution of analyzed parameters: amount of phenolic compounds, $TAU_{515/g}$ and antimicrobial activity of honeys against strains: *Klebsiella pneumoniae* (PCM 57) (*K. pneumoniae*), *Pseudomonas aeruginosa* (PCM 2563) (*P. aeruginosa*), *Staphylococcus aureus* (PCM 2602), *Escherichia coli* (PCM 1144), *Candida albicans* (PCM 2566)

nosa) and the fungus *Candida albicans* were resistant to the action of honey extracts **H5** and **H6**. The action of extracts on *Klebsiella pneumonia* was similar to streptomycin (MIC 0.25 mg/mL). All extracts from the investigated honeys exhibited bactericidal activity against *Klebsiella pneumoniae* but the action against *Pseudomonas aeruginosa* was very weak. Estevinho et al. (26), examining methanol extracts from light and dark honey from Portugal, observed a lack of inhibition of the growth of *Pseudomonas aeruginosa* in the presence of extracts. The buckwheat honey **H2** and rape honeys **H3** and **H4** acted on *Klebsiella pneumoniae* more strongly than other honeys. These honeys also exhibited stronger action on bacterial strains than streptomycin. They completely inhibited the growth of bacteria at the concentration 0.125 mg/mL, but streptomycin and other honeys at 0.25 and 0.5 mg/mL. An extract obtained from the buckwheat honey **H7** exhibited the strongest action on *Escherichia coli* and on *Candida albicans*. The inhibition of growth took place at 0.062 mg/mL and was stronger than the action of streptomycin. However, this extract does not inhibit growth of *Pseudomonas aeruginosa*. The investigated honeys inhibited growth of *Staphylococcus aureus* from 0.25 mg/mL to 1 mg/mL. The polyfloral honey **H1** caused complete inhibition of growth of bacteria at 0.25 mg/mL, rape honey **H4** at 0.5 mg/mL, while other honeys did so at the concentration of 1 mg/mL. The experiments showed that phenolic fractions of polyfloral, rape and buckwheat honeys have a great influence on antimicrobial activity of honeys, especially against *Klebsiella pneumoniae*.

Hierarchical cluster analysis and principal component analysis

Figure 3 shows the grouping of the honeys according to the hierarchical cluster analysis based on $TAU_{515/g}$, amount of phenolic compounds and antibacterial activity against 5 strains. The first group delimited by Euclidian distance lower than 1.0 including honeys **H5**, **H6** with the lowest $TAU_{515/g}$ unit, the lowest amount of phenolic compounds (< 0.05 mg/g) and identical low antibacterial activity. They next group is formed (Euclidian distance < 3) with honeys **H2**, **H3** and **H4**, which has similar value of the $TAU_{515/g}$ unit ($TAU_{515/g} > 2.0$), the highest amount of phenolic compounds (≥ 0.14 mgGAE/g) and high antibacterial activity.

The results of Principal Component Analysis (PCA) are shown in Figure 4 (a, b) and parameters of honey samples taken for PCA are demonstrated in Table 4. The main dimensions explained 76.8% of

the total variation. The first factor is dependent on $TAU_{515/g}$ unit, amount of phenolic compounds and antibacterial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The second factor is strictly dependent on antibacterial activity against *Escherichia coli* and *Candida albicans*.

Projection honey samples on the surface of two main factors is shown on Figure 4 (b). Two groups of similar honeys could be observed. First one contains **H5** and **H6** honeys and the second **H3**, **H4** and **H2**. **H7** sample is strongly different from all other honeys.

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