Screening bioactivity and bioactive constituents of Nordic unifloral honeys

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Abstract: The objective of this study was to screen the antibacterial and antioxidant activity of thirty nine honey samples from Finland, Sweden, Norway and Denmark. Their physicochemical properties were analysed, antioxidant activity was evaluated by DPPH assay and antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was assessed by microdilution assay. The honey samples obtained: buckwheat, caraway, clover, dandelion, fireweed, heather, lime tree, lingonberry, rape, raspberry, sweet clover, willow, mire, honeydew and polyfloral. Eleven honey samples showed high antioxidant activity. With 15% honey dilution, three unifloral honeys had over 85% inhibition against the growth of *P. aeruginosa* and ten honey samples against *S. aureus*. The buckwheat, raspberry and honeydew honeys showed the highest antibacterial and antioxidant activity. Unexpected high amount of methylglyoxal was

found in mire and forest honeys. Some phenolic compounds can be shown to be plant species-specific floral markers due to their appearance in specific unifloral honey samples.

Keywords: honey, antioxidant activity, antibacterial activity, hydrogen peroxide, methylglyoxal, *Pseudomonas aeruginosa*, *Staphylococcus aureus.*

1. Introduction

The antibacterial and antioxidant activity of honey is a result of multiple synergistically functioning compounds or factors, which originate from the nectar of plants, honeybees, and the chemical and physical properties of honey (Bogdanov, 2016). The nectar has been shown to contain e.g. phenolic and volatile compounds, organic acids, pyruvaldehyde called methylglyoxal (MGO) and an enzyme called catalase (Bogdanov, 2016). While evaporating water from the nectar, honeybees add the enzymes glucose oxidase, invertase and diastase, as well as organic acids, antibiotic-like antifungal peptide compounds and defensin 1-protein to the raw honey. In addition, compounds originating from lactic acid bacteria growing in the honeybees' stomach and the antibacterial 10-Hydroxy-2-Decenoic acid originating from royal jelly can contribute to the bioactivity of the honey (Bogdanov, 2016; Fujiwara, Imai, Fujiwara, Yaeshima, Kawashima, & Kobayashi, 1990; Kwakman & Zaat, 2012; Olofsson, Butler, Markowicz, Lindholm, Larsson & Vásquez, 2014). The high carbohydrate content of honey brings out certain chemical factors, such as high osmotic pressure (Osato, Reddy & Graham, 1999) and Maillard reaction products (Brudzynski & Miotto, 2011), which make the honey environment unviable for microorganisms. Bees use propolis, an antibiotic and phenolic rich bee glue, to create a hygienic environment inside the hive. Propolis is spread on every surface of the hive, and its components are mixed with honey (Salonen, 2011)

The Nordic countries, Finland, Sweden, Norway and Denmark, are situated in four different ecoregions, namely the Scandinavian taiga, Sarmatic mixed forests, Baltic mixed forests, and Scandinavian montane birch forests and grasslands (Hogan, 2011). The production of unifloral honeys in these areas is challenging due to the short summers, plant species blooming at the same time and changing weather conditions. The unifloral honey varieties collected are partially similar to those of Central Europe. However, some local

specialities, such as honeys collected from wild berries, fireweed and mire biotopes can also be found (Salonen, 2011). Only a few studies on the antibacterial activity of Nordic unifloral honeys have been carried out; e.g. Huttunen, Riihinen, Kauhanen and Tikkanen-Kaukanen (2012) found that some Finnish unifloral honeys had high antimicrobial activity against human pathogens *Streptococcus pneumoniae*, *S. pyogenes*, *Staphylococcus aureus* and methicillin-resistant *S. aureus*.

The objectives of this study were to screen the antibacterial and antioxidant activity of unifloral honeys from Nordic countries and to measure the amounts of carbohydrates, MGO, hydrogen peroxide (H_2O_2) and phenolic compounds in these honeys. To our knowledge, this is the first time that the antioxidant activity, H_2O_2 and MGO content of Nordic unifloral honeys, as well as their antibacterial activity against *Pseudomonas aeruginosa* have been analysed.

2. Materials and methods

2.1. Honey samples

Thirty nine honey samples were received directly from beekeepers, either with the help of the national beekeepers' organisations or from shops. Five samples came from Denmark, six from Sweden, twelve from Norway and twenty-one from Finland. The honey samples were from the year 2014, with the exception of the samples from Denmark, which were from the year 2015. Antibacterial analyses were not conducted on the Danish samples due to the unavailability of the samples. The honey samples came from the following plant species and origins: buckwheat (n=2), caraway (1), clover (2), dandelion (5), fireweed (5), heather (7), lime tree (2), lingonberry (1), rape (2), raspberry (3), sweet clover (1) willow (1) honeydew (1) and mire (2), (Table 1). Mire honey is collected from plants growing in a mire biotope. The group of polyfloral honeys contained three samples from Sweden and four typical Finnish polyfloral honey samples from honey packers. In addition, the botanical origin of two samples had not been defined correctly by the beekeeper, and they were transferred to the polyfloral group. The samples were stored at + 5°C in the dark until analysis. Artificial honey was prepared by copying the sugar content of Finnish honey using 40.5% fructose, 33.5% glucose, 7.5% maltose 1.5% sucrose and 17% of MilliQ water. The pH was set to 3.5 with 1 M HCl.

2.2. Moisture, electrical conductivity, pH and pollen samples

Moisture, electrical conductivity, pH and pollen content were analysed as an average of three individual subsamples. Before the moisture % was measured using a Pocket Refractometer (Atago), the honey samples were heated at 40° C for 50 minutes in order to remove all crystals and then allowed to cool to room temperature. Electrical conductivity and pH were measured and pollen samples were prepared for each honey by the three-step method, slightly modified, of the International Honey Commission's harmonised methods (International Honey Commission, 2016). Step one: Based on the results of moisture content, 5 g of dry matter of honey was measured and dissolved in 20 ml of MilliQ water. The volume of the honey solution was then set to give 20% honey solution. Electrical conductivity was measured using ECTest 11+ Multi Range equipment. Step two: The honey-water solution used in step one was diluted to 10% honey solution with MilliQ water. pH was measured with a pH meter pHep by Hanna. Step three: The honey-water solution from step two was centrifuged (3000 rpm for 10 minutes, Eppendorf centrifuge 5810 R), the pellet was re-suspended in water and centrifuged again and pollen samples were prepared from the pellets according to Sawyer (1981).

2.3. Colour coordinates

As advised by González-Miret, Terrab, Hernanz, Fernández-Recamales, Francisco & Heredia (2005), the honey samples were kept in an oven at 50°C before measurement in order to dissolve the crystals. The samples were then centrifuged to remove the air bubbles, as bubbles scatter light and reduce the transmittance level. Colour coordinate measurements of the honey samples were conducted according to the CIE L*a*b* method. The transmittance of the honey samples was measured using the spectrophotometer PerkinElmer lambda 1050 for the wavelength range from 360 nm to 830 nm, using 1 nm steps. The colour coordinates, L*, a* and b* were calculated according to CIE L*a*b* 1931. The L* value represents the brightness of the honey (0 indicates dark, 100 indicates bright), a* represents the amount of red (+a) or the amount of green (-a), and b* represents the amount of blue (-b) or orange (+b).

2.4. Analyses of carbohydrate content

The sugar analyses were based on the high-performance liquid chromatographic (HPLC) method (Salonen, Hiltunen & Julkunen-Tiitto, 2011). The 3% honey-water-acetonitrile (VWR) solution (1:1) was eluted using isocratic aq. 75% acetonitrile elution solvent with HPLC (Agilent, Series 1100, Germany, containing binary pump (G1316A), thermostated autosampler (G1329A), thermostated column oven (G1316A) and refractive index detector (RID) (G1362A), HP Chem Station Software and Zorbax column carbohydrate, 4.6 x 1500 mm with 5 µm particle size). Commercially available standards (Sigma-Aldrich: D-(-)-fructose, D-(+)-glucose, D-(+)-sucrose, D-(+)-maltose, palatinose hydrate, D-(+)-cellobiose, D-turanose, isomaltose, erlose, D-(+)-melezitose hydrate, β-gentiobiose, D-(+)-raffinose, D-panose; ICN Biomedicalsinc: D-(+)-trehalose) were used for qualification and quantification of the carbohydrates. Each sample was analysed in duplicate. A standard sample with all carbohydrate standards was run after every sixth sample, and the chromatograms of the honey samples were compared with that of the nearest standard sample.

2.5. Extraction and analyses of phenolic compounds

Phenolic compounds were extracted by a slightly modified method of Sergiel, Pohl and Biesaga (2014). Briefly, Strata X SPE cartridges (500 mg/6 ml, surface area 800 $m2g^{-1}$, particle size 33 µm, average pore size 85 A, Phenomenex) were used for purification and concentration of the phenolic compounds. A 10% honey solution was prepared with acidified water (pH set to 2 using 1.0 mol l^{-1} HCl solution) and filtered through a piece of cotton to remove the solid particles. Each honey sample was analysed in duplicate. The SPE Cartridges were conditioned with 1 ml of methanol and 1 ml of acidified water, as advised by the manufacturer. The honey solution was passed through the cartridge at 2 ml min^{-1} in order to separate the phenolic compounds from sugars and peptides. After this, the cartridge was rinsed with 10 ml of acidified water. The phenolic compounds were eluted from the cartridge matrix with 10 ml of methanol (flow 2 ml min^{-1}). The sample was concentrated at 45°C using an Eppendorf 270 concentrator (Hamburg, Germany). The final volume of the sample was adjusted to 3 ml with methanol. Samples were analysed using an HPLC instrument with a Diode Array Detector (DAD) (G1315B) and the column Zorbax, SB-C18, 4.6 x 75 mm with 3.5 µm particle size were used for the analyses. The eluent solvents were 1.5% tetrahydrofuran + 0.25%

123 *ortho*-phosphoric acid in water (=A) and 100% methanol (=B, VWR), and the flow rate was 2.0 ml min $^{-1}$. The
124 gradient and the identification of phenolic compounds were carried out according to Salonen et al. (2011).

125 2.6. H_2O_2 analyses

126 The Amplex® Red Hydrogen Peroxide Assay Kit (Life Technologies Europe BV) was used in H_2O_2 analysis. The
127 assay was conducted according to the manufacturer's instructions. Amplex red reagent reacts with H_2O_2 in
128 a 30% honey solution diluted with a phosphate buffer, producing a red fluorescent oxidation product,
129 resorufin. The fluorescence was measured with a multidetection microplate reader (FLUOstar Omega,
130 Ordior BMG LabTech) at an excitation wavelength of 530 and an emission wavelength of 590 nm. The
131 standard curve was set using dilutions of 20 µmol H_2O_2 standard solution and was used for calculating the
132 results with Omega Software (V3.00 R3). Each sample was analysed in triplicate.

133 2.7. MGO measurements

134 The amount of MGO in the honey samples was analysed according to Mavric, Wittmann, Barth & Henle
135 (2008). MGO was converted to quinoxaline with orthophenylendiamine (OPD, Sigma-Aldrich) as follows: 1
136 ml of 30% honey solution (in phosphate buffer) was mixed with 0.6% OPD and incubated in the dark at
137 room temperature for 16 hours. The samples were then centrifuged for three minute at 4°C (13 000 rpm,
138 Eppendorf 5415R centrifuge, Hamburg, Germany) in order to remove cloudiness. After this the samples
139 were run at 220 nm using HPLC as described above for phenolic compound analysis (chapter 2.5). The
140 results were calculated on the basis of the external standard (MGO standard solution, Sigma-Aldrich). Each
141 sample was analysed in triplicate.

142 2.8. DPPH radical scavenging assay

143 The 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) assay was carried out by the slightly modified
144 method of Ferreira, Aires, Barreira & Estevinho (2009). 1 g of honey was dissolved with methanol in a 2 ml
145 volumetric flask. This stock solution was further diluted to three concentrations with methanol. 500 µl of
146 each concentration was mixed with 1000 µl of DPPH (75 mg l^{-1}) and incubated at room temperature for 30

147 minutes. The samples were then centrifuged for three minutes at +4°C (13 000 rpm, Eppendorf 5415R
148 centrifuge, Hamburg, Germany) in order to remove cloudiness. The absorbance was measured at 517 nm
149 using a spectrophotometer (Spectronic 20 Genesys, Thermo Electron Inc., Madison, USA). To eliminate the
150 impact of the native honey colour, honey samples without DPPH were used as a blank for each
151 concentration. The percentage of DPPH discolouring was used to calculate the radical-scavenging assay
152 (RSA) percentage and to draw a graph of these percentages against various concentrations. The results are
153 expressed as IC_{50} mg ml^{-1} (required concentration of honey sample for 50% inhibition of free radicals). Each
154 sample was analysed in triplicate.

155 2.9. Antibacterial analysis

156 2.9.1. Microbial strains and culture conditions

157 For antimicrobial screening, gram-negative strain *P. aeruginosa* (ATCC 27853) and gram-positive strain *S.*
158 *aureus* (ATCC 25923) were obtained from Microbiologics Inc. The bacterial strains were grown on Mueller
159 Hinton II Agar (MHA) plates and incubated at 37°C for 16–18 h. For antimicrobial screening, a bacterial
160 suspension was prepared by culturing the bacteria in Mueller Hinton II broth (MHB) and incubating it at
161 37°C for 16–20 h, with 100 rpm continuous agitation prior to the assay.

162 2.9.2. Microdilution assay to assess antimicrobial activity

163 The antimicrobial screening assay was performed by the broth microdilution method after the standards of
164 the Clinical and Laboratory Standards Institute (2013). Briefly, bacterial suspensions were diluted with MHB
165 in order to obtain an inoculum with 5x105 colony-forming units (cfu) ml^{-1} for all the bacteria in the assay.
166 Analyses were conducted in clear 96-well microtiter plates using 10 µl of microbial suspension and 190 µl of
167 sample solution diluted in the assay medium. Ciprofloxacin was used as a positive control. The plates were
168 incubated, with agitation, for 24 h at 37°C (Biosan Thermo-Shaker PST-60HL-4). Absorbance was measured
169 at 620 nm using a plate reader (Thermo Fisher Scientific Multiskan GO using program SkanIt RE, version 3.2)
170 at the beginning of the assay and after 4, 8 and 24 hours' incubation. The antimicrobial activity of the
171 samples was calculated from the absorbance values by comparing them to the controls, and the activity

was expressed as inhibition percentage of growth. All the honey samples were first tested at concentrations of 30% and 15% dilution (w/v, with three replicates). Honey samples that showed inhibitory activity higher than 85% in the preliminary screening were selected for dose-response experiments in order to determine the minimum inhibitory concentration (MIC_{90}) (with two-fold dilution, using eight concentrations from 30 to 0.23%). The MIC_{90} value was expressed as the lowest concentration (%) of honey that inhibited microbial growth by ≥ 90%. The MIC_{90} value was measured in two independent experiments. Ciprofloxacin was used as a positive control.

2.9.3. Determination of the minimum bactericidal concentration (MBC)

The MBC assay was carried out for samples with no visible bacterial growth after the MIC_{90} assay. From 96-well microtiter plates, a 50 µl sample of each concentration with no growth was taken and plated on MHA plates. The plates were incubated for 24 h at 37°C, and the number of the colonies was counted. The MBC assay was carried out with two replicates.

2.10. Statistical analysis

Correlation analysis was carried out to determine the relationships between the main components. As the data were not normal, nonparametric Spearman's rank correlation analysis was used (IBM® SPSS® Statistics 22.)

3. Results and discussion

3.1. Honey samples

Defining unifloral honeys is not easy, and it is difficult to obtain references for pure unifloral honeys as bees collect nectar non-selectively from all plant species growing around their hive (Oddo & Bogdanov, 2004). Unifloral honey samples for this study were collected directly from beekeepers (Table 1). The unifloral properties of the samples were identified by beekeepers based on the location of the hives and their personal experiences. In Nordic countries the growing season is very short, and the blooming periods of many plant species overlap. This combined with inadequate knowhow in identifying the unifloral honeys,

means that the definition of unifloral honeys may be unreliable. Thus the botanical origin of two samples was not defined correctly by the beekeeper, and they were transferred to the polyfloral group.

3.2. Moisture, electrical conductivity, pH and pollen analysis

The physico-chemical properties of the Nordic unifloral honey samples found in this study corresponded to those of our previous research about Finnish unifloral honeys (Salonen, 2011). The moisture percentages of honey should be under 20%, as it was with all the samples in this study. Variation was between 14.0 and 19.9% (Table 1). Electrical conductivity is a very useful tool in the classification of unifloral honeys, and its values correlate with the mineral and ash content of the honey (White, 1975). Electrical conductivities measured from honey samples in this study varied between 136 and 1663 µS cm^{-1}. Light fireweed honeys (#2, 7, 16, 19 and 21) had the lowest values, while mire (#3 and 4), honeydew (#27), heather (#28, 29, 31, 32, 34, 36 and 52) and one polyfloral sample (#5) had the highest values (Table 1). The pH of the honey samples exhibited values between 3.5 and 5.2 (Table 1). Mire honey (#3 and 4) and one polyfloral honey (#5) with high electrical conductivity also had high pH. In all honey samples, the electrical conductivity values and pH values correlated positively (0.72) but pH showed a negative correlation (-0.48) with total sugar amounts (Table 2). In European honey samples, the amount of pollen from the dominating plant species in unifloral honeys should be over 45% of all the pollen counted (Von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004). In Nordic honey samples, this is not the case. For example, in many Nordic unifloral honeys, e.g. fireweed, pollen of botanical origin is highly under-presented (Salonen, 2011). In this study, a 45% share is met only in the clover #50 and 51, rape #48 and 49, heather #29, and raspberry #33 honey samples (Table 1). Nevertheless, all unifloral honey samples had the organoleptical properties as proposed for the honey type and accordingly they were accepted for this study as unifloral honeys.

3.3. Colour

For the colour of the honey samples, the CIELAB L* a* b* colour coordinates were calculated (Table 1). The botanical origin and storage conditions affect the colour coordinates L* a* b* of the honey (Brudzynski, & Kim, 2011), and the dark colour of honeydew and heather honeys has been shown to correlate with the

mineral content of the honey (Gonzales-Miret et al, 2005). In this study, low a* values of Nordic honey samples indicate that there is little red colour in these honey varieties. The negative a* value indicates that the honey presents some green components, as was the case in the rape (#48 and 49) and the artificial (#45) honey samples. Most of honey samples had a high level of yellow colour as the high parameter b* shows. The L* value ranged between 50.2 and 96.6 meaning that all Scandinavian unifloral honey samples were rather bright or pale, while dark honeys are more rare. In our previous studies, fireweed honey has been reported to be very light coloured (Salonen et al., 2011).

3.4. Carbohydrate content

The HPLC-method used was capable of separating fructose, glucose, sucrose, trehalose, isomaltose, raffinose, panose and gentiobiose, while turanose/palatinose, maltose/cellobiose and erlose/melezitose were eluted as overlapping peaks and could not be identified or quantified separately. Fructose is the main sugar in unifloral honeys (Table 3), with the exception of the samples dandelion # 26 and rape #48 and 49, where the amount of glucose was higher. Sucrose was absent from dandelion, heather, honeydew, lime tree, lingonberry, mire and rape honeys, while the highest sucrose content was found in buckwheat honey (2.12 g 100 g^{-1}, Table 3). The amount of disaccharide turanose/palatinose varied between 0.22 and 1.51 g 100 g^{-1}, maltose/cellobiose between 0.35 and 1.97 g 100 g^{-1}, trehalose between 0.18 and 1.30 g 100 g^{-1}, and isomaltose between 0.11 and 1.35 g 100 g^{-1}. Oligosaccharide raffinose was found only in mire honey (#3 and 4) and panose in only one polyfloral honey (#5). The amount of erlose/melezitose varied between 0.11 and 2.30 g 100 g^{-1}. Gentiobiose, which has been found in lime tree honey (Cotte, Casabianca, Chardon, Lheritier & Grenier-Loustalot, 2004), was not found in lime tree honey samples (#30 and 37) in this study. This may be due to the different varieties and/or different growing area of the lime trees. The amount of oligosaccharides was the lowest in rape honeys (#48 and 49) and the highest in caraway honey (#6) (Table 3).

3.5. MGO

MGO is a component of honey which originates from nectar, but is not found in fresh honey. It is formed during honey storage from dihydroxyacetone, which is converted non-enzymatically into MGO (Mavric et al, 2008). In this study, the amount of MGO was measured for the first time from Nordic unifloral honeys. It was one of the most important finding of this study, that the amount of MGO was as high as 166 mg kg^{-1} in some honey samples (Figure 1A). The highest producers of MGO were honeys collected from mire #3 and 4 or polyfloral honey #5. MGO was not found in caraway #6, sweet clover #8 or dandelion honeys #25, 26 and 35 from Sweden and Norway, nor in any heather honey samples.

Arena, Ballistreri, Tomaselli & Fallico (2011) have measured the amounts of MGO in Italian uni- and polyfloral honeys. They found MGO in all honey samples, but the amounts were very low, ranging from 0.2 to 2.9 mg kg^{-1}. In Finnish polyfloral honey samples, MGO amounts of 22–27 mg kg^{-1} have been found (Oinaala, Lehesvaara, Lyhs & Tikkanen-Kaukanen, 2015). In manuka honey, which is known for its exceptionally high MGO levels, the amounts of MGO have been shown to vary between 38.4 and 761 mg kg^{-1} (Mavric et al., 2008).

3.6 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) has been considered to have an important role in the antibacterial activity of honey. In this study, the amounts of hydrogen peroxide were measured from 30% honey dilutions. The results covered a wide range, from 0.1 - 619.6 µMol g honey $^{-1}$ (Figure 1B). The level of H_2O_2 in honey should be measured from fresh honey samples, as its amount decreases in the course of time. In this study, the H_2O_2 levels were measured about 15 months after the honey samples had been harvested, which may partly explain the wide range of H_2O_2 amounts in our results.

3.7. Phenolic compounds

In the phenolic compound assay, the Phenomenex Strata X SPE cartridges were easy to use and gave comparable results to those of our earlier studies (Salonen et al. 2011). Identification of the phenolic compounds was based on commercial standards, retention times and HPLC-DAD/MS-identification of the UV-spectrum and MS-ions (Table supplementary). The phenolic profiles of the honey samples varied

considerably according to botanical origin. We identified 33 phenolic compounds from our Nordic honey samples, namely, 14 cinnamic acid derivatives, 6 phenolic acids and 13 flavonoids (Table 4). The total amount of phenolics in the samples varied from 9.4 mg kg^{-1} (#24 willow) to 55.2 mg kg^{-1} (mean heather honeys). The number of individual phenolic compounds detected in the honey samples ranged between 7 and 18. Some phenolic compounds can be shown to be plant species specific floral markers. In Nordic honey samples, high benzoic acid content seems to be typical for heather honeys and the high content of tetragalloylglucose is typical for caraway honey (Table 4). In fact, it has been shown in the previous study (Salonen, 2011), that tetragalloylglucose, high number of phenolic and cinnamic acid derivatives and the marked amounts of benzoic acid are typical for Nordic honeys. Some of these compounds may originate from the Nordic phenolic-rich propolis, in which these are the main components (Salonen, Saarnio & Julkunen-Tiitto, 2012). Fireweed honey had the largest quantities of a flavonoid called kaempferol 3-O-rhamnoside, which has also been found in the nectar of fireweed (Salonen, 2011) but not in American fireweed honey (Gheldof, Wang, & Engeseth, 2002). Rape honey was the only honey variety containing unknown myricetin derivative. In our study, a high amount of coumaric acid was found in buckwheat honey, which agrees with the content of phenolic acids analysed from buckwheat honey originating from Lithuania (Ramanauskiene, Stelmakiene, Briedis, Ivanauskas, & Jakštas, 2012) and America (Gheldof, et al., 2002). However, we did not find any chlorogenic acid in our raspberry honeys which was the main phenolic acid in Lithuanian raspberry honeys (Ramanauskien et al., 2012). Ellagic acid is another typical phenolic compound in raspberry honey (Escuredo, Silva, Valentão, Seijo, & Andrade, 2012; Salonen et al., 2011), and in this study, it was also found in the lingonberry honey (Table 5).

3.8. Antioxidant activity

Antioxidant activity was measured by DPPH assay, which is an easy spectrophotometric method for screening and measuring the antioxidant activity of honey samples. The results are presented as IC_{50} values (Figure 1C). Buckwheat #14 and clover #50, two heather, #28 and 52, honeydew #27, two mire honeys #3 and 4, raspberry #23 and surprisingly, polyfloral honeys #5, 39 and 40 had high antioxidant activity with IC_{50} values lower than 5 mg ml^{-1}. Beretta, Granata, Ferrero, Orioli, & Facino (2005) measured the antioxidant

activity of Italian unifloral honeys, finding high antioxidant activity in buckwheat honey. They found moderate antioxidant activity (IC_{50} value from 5 to 50 mg ml^{-1}) in dandelion and clover honeys, which agrees with our results (Figure 1C). These results suggest that honeys of the same botanical origin from different countries have similar antioxidant activity, and that the antioxidant capacity of the honey may be defined by the floral origin.

Light coloured honeys such as fireweed and rape possessed very low antioxidant activity (IC_{50} higher than 100 mg ml^{-1}). In many studies it has been observed that dark coloured honeys have stronger antioxidant powers than lighter honeys (e.g. Blasa, Candiracci, Accorsi, Piacentini, Albertini, & Piatti, 2006). In our study we had no dark honeys (see chapter 3.3). Artificial honey had no antioxidant activity which indicates that the carbohydrates in honey have no effect on antioxidant activity.

Pichichero, Canuti and Canini (2009) studied the total phenolics and antioxidant activity of Italian honeys. They found a strong correlation between phenolic content and DPPH value, concluding that the phenolic content of the honey samples is involved in the antioxidant activity of the honey. A similar correlation was also found by Gheldof et al. (2002). They suggested that phenolic compounds are important antioxidant factors, but without any doubt, many other compounds are also involved in the oxidation processes. In our study, no correlation (-0.26) was found between total phenolic content and antioxidant activity (Table 2).

3.9. Antibacterial activity

The antibacterial activity of the honey samples was tested against gram- bacterium *P. aeruginosa* and gram+ bacterium *S. aureus.* As 30% dilutions, all the honeys and the artificial honey showed 65–100% inhibition against *P. aeruginosa*. Only five honeys (#6, 7, 15, 35 and artificial honey) had an inhibition lower than 80% (Figure 1D). When 15% honey dilution was used, the inhibition of most of the honey samples was reduced, and only buckwheat #14, raspberry #23 and honeydew #27 had over 85% inhibition against the growth of *P. aeruginosa* (Figure 1E). The results for gram-positive *S. aureus* were different. All the 30% honey dilutions exhibited inhibition from 13 to 96%, while ten unifloral honeys also displayed high inhibition against *S. aureus* as 15% dilutions (Figure 1F and G).

321 MIC_{90} values were determined for the honey samples showing inhibitory activity higher than 85% in the
322 primary screening. A 15% dilution level was the MIC_{90} value for buckwheat #14, raspberry #23 and
323 honeydew #27 honeys (Table 5) against *P. aeruginosa*. Buckwheat #14, heather #31 and polyfloral #40
324 honeys had the lowest MIC_{90} values (7.5%) against *S. aureus*. For other honeys screened, the MIC_{90} value
325 against *S. aureus* was 15% (Table 5). Interestingly, the MIC_{90} value of Manuka honey against *S. aureus* was
326 15–30% (Mavric et al., 2008). Thus, the antibacterial activity of some Nordic honeys against *S. aureus*
327 corresponds to that of Manuka honey.

328 One of the most important findings in this study was that all the honeys with the highest antibacterial
329 activity proved to be bactericidal, as well. The MBC results (Table 5) indicate that seven unifloral honey
330 samples were able to kill *S. aureus* as 15% dilutions (sweet clover #8, polyfloral #17, buckwheat #22,
331 raspberry #23, honeydew #27, and heather #29 and #36). Buckwheat #14, heather #31 and polyfloral #40
332 honeys had the lowest MBC value at 7.5% dilution. Buckwheat #14 and honeydew #27 were able to kill *P.*
333 *aeruginosa* at 15% dilution (Table 5). As shown by Henriques, Jenkins, Burton, & Cooper (2010 a and b),
334 Manuka honey treatment affected the cell division and structure of gram-negative *P. aeruginosa* and gram-
335 positive *S. aureus*, but with different mechanisms. *P. aeruginosa* cells exhibit irregular cell structure and
336 lysis while in *S. aureus* the cell division fails.

337 It has been shown that the less acidic honeys inhibit the growth of bacteria more than do the acidic
338 varieties (Gallardo-Chacón, Caselles, Izquierdo, & Rius, 2008). In contrast to these findings, our study
339 showed that, at 15% honey dilution, the least acidic mire #3 and 4 and polyfloral #5 samples had very low
340 inhibition against both bacteria in this study.

341 The honey samples buckwheat #22, heather #29, 31 and 36, sweet clover #8 and polyfloral #17 and 40 had
342 high antibacterial activity against *S. aureus* as 15% dilutions, and they also showed high or moderate
343 antioxidant activity. The phenolic content of these samples was high and there is also a high positive
344 correlation (0.57) between total phenolic content and inhibition against *S. aureus* with 15% honey dilution
345 (Table 2). Estevinho, Pereira, Moreira, Dias, & Pereira, (2008) have reported that *S. aureus* is the most

346 sensitive bacterium to the phenolic compounds of honey. In our study, five individual phenolic compounds,
347 namely benzoic acid, tetragalloylglucose derivative, rhamnetin derivative 1, galangin derivative 2 and
348 apigenin correlated significantly with inhibition results of 15% honey dilutions on *S. aureus*, while
349 corresponding results of *P. aeruginosa* correlated significantly only with kaempferol derivative and
350 quercetin 3-O-rhamnoside (Table 4).

351 Brudzynski (2006) tested the antibacterial properties of buckwheat, clover, sweet clover, dandelion and
352 fireweed honeys from Canada using *Escherichia coli* and *Bacillus subtilis.* Their dandelion and fireweed
353 honeys gave similar inhibition results as those found in our study (Figure 1D). However, the inhibition
354 properties of their sweet clover samples were different. In our study, sweet clover honey had a stronger
355 inhibiting effect against the growth of gram-positive bacteria, but in the Canadian study, its inhibition effect
356 was stronger against gram-negative bacteria. Escuredo et al. (2012) studied the properties of *Rubus* honeys
357 from Portugal and they found antibacterial inhibition that was higher against *P. aeruginosa* than against *S.*
358 *aureus*. Our findings were similar.

359 Buckwheat honey was one of the honeys which had the high antibacterial and antioxidant activity honey
360 varieties in our study. Same results were found by Huttunen et al. (2012) who tested the antibacterial
361 activity of buckwheat honey, and found out that the 20 and 40% dilutions displayed high inhibition against
362 *S. aureus*. Brudzynski, Abubaker & Wang (2012) have also proved that buckwheat honey has powerful
363 bactericidal properties. They stated that H_2O_2 was one active component in the bacteria-killing mechanism
364 of buckwheat honey, causing oxidative damage and bacterial DNA degradation. However, they concluded
365 that H_2O_2 does not accomplish this alone but the bactericidal properties of buckwheat honey result from
366 several honey components.

367 Unifloral honey specialities from the Nordic ecoregions are mire, fireweed and lingonberry honeys. As
368 shown in our earlier study (Salonen, & Julkunen-Tiitto, 2012), mire honeys had high pH and electrical
369 conductivity and low content of phenolic compounds. In this study, we found that the MGO content in mire
370 honey samples was the highest, they were able to inhibit the growth of *S. aureus* as 15% dilutions, and they

371 also exhibited high antioxidant activity. Fireweed honey samples had high inhibition against both bacteria
372 as a 30% dilution. Huttunen at al. (2012) found high inhibition against the growth of *Streptococcus*
373 *pneumoniae* and *S. pyogenes* for 20% fireweed honey dilutions. Our lingonberry honey had moderate
374 antioxidant activity and high inhibition against both bacteria as a 30% dilution.

375 4. Conclusions

376 Seventeen out of thirty nine Nordic honey samples had high antibacterial and/or antioxidant activity.
377 Nordic unifloral honeys derived from fourteen floral origins exhibited antibacterial activity against *P.*
378 *aeruginosa* and/ or *S. aureus.* In addition, the polyfloral honeys in this study were also antibacterial.
379 Moreover, all the honeys tested in the MBC assay were bactericidal. Although surprisingly high levels of
380 MGO were found in Nordic forest honeys, the mechanisms of antibacterial and antioxidant activities are as
381 yet unknown and hard to explain by one or few factors. The multifactorial origin of the mechanisms is a
382 benefit, as it lowers the risk of bacterial resistance.

383 Our data indicate that it is possible to find Nordic unifloral honeys which have high antibacterial and
384 antioxidant activity. They could be utilised for several purposes. When honey is used in special contexts,
385 such as in the chemical industry or in clinical practice, the selection of the honey variety is important. As
386 presented here, there is great variation in the bioactivity of individual unifloral honeys, and with reliable
387 research data, it is possible to choose a right honey variety for different uses.

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395 References

396 Arena, E., Ballistreri, G., Tomaselli, F., & Fallico, B. (2011). Survey of 1,2-dicarbonyl compounds in
397 commercial honey of different floral origin. *Journal of Food Science*, *76*, C1203-10. doi: 10.1111/j.1750-
398 3841.2011.02352.x.
399 Beretta, A., Granata, P., Ferrero, M., Orioli, M., & Facino, R.M. (2005). Standardization of antioxidant
400 properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics.
401 *Analytica Chimica Acta, 533*, 185–191.
402 Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M.P., Albertini, M.C., & Piatti, E. (2006). Raw Millefiori
403 honey is packed full of antioxidants. *Food Chemistry, 97*, 217–222.
404 Bogdanov , S. (2016) *The Honey Book*. www.bee-hexagon.net/honey accessed 20.04.16
405 Brudzynski, K. (2006). Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. *Canadian*
406 *Journal of Microbiology, 52*, 1228–1237.
407 Brudzynski, K., & Kim, L. (2011). Storage-induced chemical changes in active components of honey de-
408 regulate its antibacterial activity. *Food Chemistry, 126,* 1155–1163.
409 Brudzynski, K., & Miotto, D. (2011). The relationship between the content of Maillard reaction-like products
410 and bioactivity of Canadian honeys. *Food Chemistry, 24*, 869-874.
411 Brudzynski, K., Abubaker, K., & Wang, T. (2012). Powerful bacterial killing by buckwheat honeys is
412 concentration-dependent, involves complete DNA degradation and requires hydrogen peroxide. *Frontiers*
413 *in Microbiology, 3,* Article242, 1-9.
414 Clinical and Laboratory Standards Institute (2013). Performance Standards for Antimicrobial Susceptibility
415 Testing; Twenty-Third Informational Supplement. CLSI document M100-S23
416 Cotte, J. F., Casabianca, Æ H., Chardon, Æ S., Lheritier, Æ J., & Grenier-Loustalot, M. F. (2004).
417 Chromatographic analysis of sugars applied to the characterisation of monofloral honey. Anal Bioanal Chem
418 (2004) 380: 698–705
419 Escuredo, O., Silva, L.R., Valentão, P., Seijo, M.C., & Andrade, P.B. (2012). Assessing *Rubus* honey value:
420 Pollen and phenolic compounds content and antibacterial capacity. *Food Chemistry, 130,* 671–678.

421 Estevinho, L., Pereira, A.P., Moreira, l., Dias, L.G., & Pereira, E. (2008). Antioxidant and antimicrobial effects
422 of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology, 46,* 3774-
423 3779.
424 European Commission. (2002). Council Directive 2001/110/EC concerning honey*. Official Journal of*
425 *European Communities,* Jan 12th 2002. L10/47-52.
426 Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese
427 honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, *114,*
428 1438-1443.
429 Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T., & Kobayashi, K. (1990). A potent
430 antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *The*
431 *Journal of Biological Chemistry, 265,* 11333-11337*.*
432 Gallardo-Chacón, J. J., Caselles, M., Izquierdo, M., & Rius, N. (2008). Inhibitory activity of monofloral and
433 multifloral honeys against bacterial pathogens. *Journal of Apicultural Research and Bee World, 47*, 132–137.
434 Gheldof, N., Wang, X-H., & Engeseth, N.J. (2002). Identification and Quantification of Antioxidant
435 Components of Honeys from Various Floral Sources. *Journal of Agricultural and Food Chemistry***,** *50,* 5870-
436 5877.
437 González-Miret, M. L., Terrab, A., Hernanz, D., Fernández-Recamales, M. A., Francisco J., & Heredia, F. H.
438 (2005). Multivariate Correlation between Color and Mineral Composition of Honeys and by Their Botanical
439 Origin. *Journal of Agricultural and Food Chemistry, 53,* 2574-2580.
440 Henriques, A. F., Jenkins, R. E., Burton, N. F., & Cooper, R. A. (2010 a). The intracellular effects of manuka
441 honey on *Staphylococcus aureus*. *European Journal of Clinical Microbiology and Infectious Diseases, 29,* 45–
442 50.
443 Henriques, A. F., Jenkins, R. E., Burton, N. F., & Cooper, R. A. (2010 b). The effect of manuka honey on the
444 structure of *Pseudomonas aeruginosa*. *European Journal of Clinical Microbiology and Infectious Diseases*,
445 *30,* 167-171.
446 Hogan, C. (2011). Ecoregions of Sweden.
447 http://www.eoearth.org/view/article/51cbed887896bb431f692b3d/ Accessed 13.04.16

448 Huttunen, S., Riihinen, K., Kauhanen, J., & Tikkanen-Kaukanen, C. (2012). Antimicrobial activity of different
449 Finnish monofloral honeys against human pathogenic bacteria. *Acta Pathologica, Microbiologica et*
450 *Immunologica Scandinavica, 12,* 827-834.
451 International honey commission (2016). Harmonised methods of the international honey commission.
452 http://www.ihc-platform.net/ Accessed 18.06.16
453 Kwakman, P. H. S., & Zaat, S. A. J. (2012). Antibacterial Components of Honey. *IUBMB Life, 64,* 48–55.
454 Mavric, E., Wittmann, S., Barth, G., & Henle, T. (2008). Identification and quantification of methylglyoxal as
455 the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand.
456 *Molecular Nutrition & Food Research, 52,* 483 – 489.
457 Oddo, L. P., & Bogdanov, S. (2004). Determination of honey botanical origin: problems and issues.
458 *Apidologie 35*, S2-S3.
459 Oinaala, D., Lehesvaara, M., Lyhs, U., & Tikkanen-Kaukanen, C. (2015). Antimicrobial activity of organic
460 honeys against food pathogenic bacterium *Clostridium perfringens*. *Organic Agriculture, 5,* 153-159.
461 Olofsson, T. O., Butler, E., Markowicz, P., Lindholm, C., Larsson, L., & Vásquez, A. (2014). Lactic acid bacterial
462 symbionts in honeybees – an unknown key to honey's antimicrobial and therapeutic activities. *International*
463 *wound journal*, DOI: 10.1111/iwj.12345.
464 Osato, M. S., Reddy, S. G., & Graham, D. Y. (1999). Osmotic Effect of Honey on Growth and Viability of
465 *Helicobacter pylori. Digestive Diseases and Sciences, 44,* 462-464.
466 Pichichero, E., Canuti, L., & Canini, A. (2009). Characterisation of the phenolic and flavonoid fractions and
467 antioxidant power of Italian honeys of different botanical origin. *Journal of the Science of Food and*
468 *Agriculture, 89,* 609–616.
469 Ramanauskiene, K., Stelmakiene, A., Briedis, V., Ivanauskas, L., & Jakštas, V. (2012). The quantitative
470 analysis of biologically active compounds in Lithuanian honey. *Food Chemistry, 132,* 1544–1548.
471 Salonen, A. (2011). *Boreal unifloral honeys: screening properties and composition*. Publications of the
472 University of Eastern Finland. Dissertations in Forestry and Natural Science: 51.
473 Salonen, A., & Julkunen-Tiitto, R. (2012). Characterisation of two unique unifloral honeys from the boreal
474 coniferous zone: lingonberry and mire honeys. *Agricultural and Food Science, 21*,159-170.

475 Salonen, A., Hiltunen, J., & Julkunen-Tiitto, R. (2011). Composition of Unique Unifloral Honeys from the
476 Boreal Coniferous Forest Zone: Fireweed and Raspberry Honey. *Journal of ApiProduct and ApiMedical*
477 *Science, 3,* 128-136.
478 Salonen, A., Saarnio, S., & Julkunen-Tiitto, R. (2012). Phenolic compounds of propolis from the boreal
479 coniferous zone. *Journal of Apicultural Science*, *56*, 5-11.
480 Sergiel, I., Pohl, P., & Biesaga, M. (2014). Characterisation of honeys according to their content of phenolic
481 compounds using high performance liquid chromatography/tandem mass spectrometry. *Food Chemistry,*
482 *145,* 404–408.
483 Sawyer, R. (1981). *Pollen Identification for Beekeepers.* Cardiff Academy press.
484 White, J. W. jr. (1975). Physical characteristics of honey. In Crane, E. *Honey, A comprehensive survey.*
485 Heinemann: London.
486 Von der Ohe, W., Persano Oddo, L., Piana, M. L., Morlot, M., & Martin, M. (2004) Harmonized methods of
487 melissopalynology. *Apidologie, 35*, S18-S25.
488
489 Figure captions.
490 Figure 1.
491 Quantity of MGO and H_2O_2, antioxidant activity and inhibition activity of honey dilutions against *P.*
492 *aeruginosa* and *S. aureus* in individual honey samples.
493 A. Amount of methylglyoxal mg kg^{-1} of honey
494 B. Amount of H_2O_2 µMol g honey $^{-1}$
495 C. IC_{50} values in DPPH assay mg ml^{-1}. (Arrow; high antioxidant activity with IC_{50} values < 5 mg ml^{-1}.)
496 D. Inhibition of 30% honey dilutions against *P. aeruginosa*
497 E. Inhibition of 15% honey dilutions against *P. aeruginosa (black bars; inhibition > 85%)*
498 F. Inhibition of 30% honey dilutions against *S. aureus*
499 G. Inhibition of 15% honey dilutions against *S. aureus (black bars; inhibition > 85%)*

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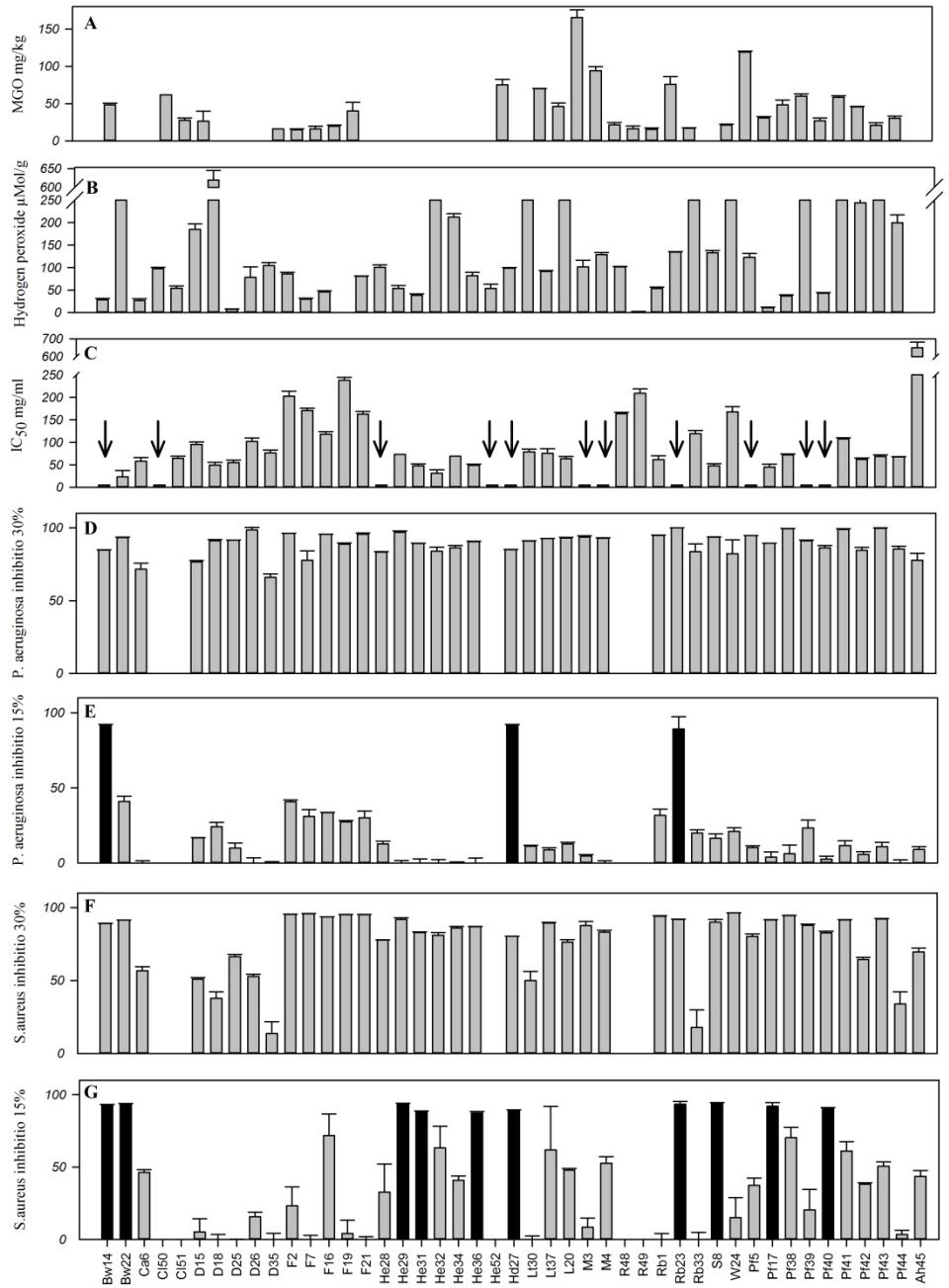

503

504 Table 1. Honey samples: botanical and geographical origin, year of production, moisture, electrical conductivity, pH, pollen content and CIELAB colour
505 coordinates. (s.e.= standard error)

Botanical origin	#	Country	Year of production	Water-% Average ± s.e.	Electrical conductivity µS cm^{-1} Average ± s.e.	pH Average[a]	Dominant pollen	Pollen of botanical origin	L*	a*	b*
Unifloral											
Buckwheat	14	F	2014	17.3 ± 0	497 ± 1.2	3.8	Rosaceae 57.5%	*Fagopyrum* 8.8%	81.94	2.24	37.94
	22	F	2014	16.9 ± 0.1	278 ± 4.2	3.9	Rosaceae 28.1%	*Fagopyrum* 7.2%	91.64	0.05	17.52
Caraway	6	F	2014	19.9 ± 0.2	378 ± 2.0	3.9	Rosaceae 69.2%	Apiaceae 7.2%	79.37	5.53	55.64
Clover	50	D	2015	18.5 ± 0.2	438 ± 3.8	3.5		*Trifolium* 76.2%	69.74	16.56	73.10
	51	D	2015	16.0 ± 0.1	713 ± 0.6	4.1		*Trifolium* 83.6%	87.26	-0.21	41.21
Dandelion	15	F	2014	17.8 ± 0.1	379 ± 0.6	4.0	Rosaceae 36.9%	*Taraxacum* 4.1%	50.19	34.68	81.85
	18	F	2014	16.1 ± 0.1	362 ± 2.2	4.4	*Salix* 56.6%	*Taraxacum* 0.9%	76.89	3.36	50.83
	25	N	2014	16.5 ± 0	708 ± 0.3	4.3	Mixed 61.7%	*Taraxacum* 1.1%	87.59	0.40	25.21
	26	N	2014	18.2 ± 0	599 ± 0.9	4.7	Mixed 46.9%	*Taraxacum* 6.4%	75.68	3.07	55.69
	35	S	2014	17.6 ± 0	599 ± 1.5	4.2	Mixed 47.8%	*Taraxacum* 10.6%	81.58	2.10	84.58
Fireweed[b]	2	F	2014	16.4 ± 0.3	161 ± 0.2	3.9	*Filipendula* 32.5%	*Epilobium* 2.0%	81.64	-0.22	14.80
	7	F	2014	16.9 ± 0.1	137 ± 0.3	3.8	Mixed 78.4%	*Epilobium* 1.7%	96.65	-0.22	11.29
	16	F	2014	17.0 ± 0.2	161 ± 1.1	3.8	Rosaceae 44.5%	*Epilobium* 0%	81.77	3.16	61.61
	19	F	2014	16.8 ± 0	147 ± 0.2	3.8	Mixed 67.4%	*Epilobium* 1.3%	70.07	3.78	58.29
	21	F	2014	16.0 ± 0.1	136 ± 0.5	3.9	Rosaceae 47.1%	*Epilobium* 0.2%	77.85	2.32	45.88
Heather	28	N	2014	17.0 ± 0.3	1087 ± 2.1	4.3	Mixed 80.8%	*Calluna* 5.3%	60.16	19.95	76.86
	29	N	2014	18.5 ± 0	880 ± 3.2	4.7		*Calluna* 76.5%	54.37	20.96	75.62
	31	N	2014	18.9 ± 0.1	1071 ± 3.1	4.2	Mixed 73.9%	*Calluna* 26.1%	81.73	3.52	54.41
	32	N	2014	19.0 ± 0.1	734 ± 5.0	4.3	Mixed 75.6%	*Calluna* 6.4%	66.84	12.63	70.48
	34	N	2014	14.0 ± 0	1214 ± 1.8	4.7	Mixed 78.8%	*Calluna* 21.2%	90.43	-0.50	35.01
	36	S	2014	18.9 ± 0.1	733 ± 1.0	4.3	*Trifolium* 47.8%	*Calluna* 4.5%	67.42	12.20	68.34
	52	D	2015	18.3 ± 0.1	967 ± 2.1	4.4	*Trifolium* 65.2%	*Calluna* 3.1%	62.45	22.04	82.93
Honeydew	27	N	2014	16.1 ± 0.1	1155 ± 0.9	4.2	Mixed 74.2%	honeydew elements	84.29	1.29	84.06
Lime tree	30	N	2014	17.3 ± 0.3	585 ± 1.3	4.1	Mixed 62.0%	*Tilia* 0.8%	64.37	16.36	73.95
	37	S	2014	17.9 ± 0.2	914 ± 2.6	4.5	Mixed 65.1%	*Tilia* 10%	84.80	0.54	43.62
Lingonberry	20	F	2014	16.4 ± 0.1	367 ± 0.3	4.3	Rosaceae 48.0%	*Vaccinium* 37.2%	96.63	-0.15	11.86

Mire	3	F	2014	19.8 ± 0	1663 ± 1.5	4.9	*Vaccinium* 49.3 %		79.30	10.66	78.26
	4	F	2014	16.3 ± 0.3	1180 ± 4.9	5.2	*Vaccinium* 79.6%		78.86	10.76	78.03
Rape	48	D	2015	16.2 ± 0.1	256 ± 0.6	4.0		*Brassica* 86.8%	89.13	-1.04	23.15
	49	D	2015	16.2 ± 0	193 ± 0.3	3.9		*Brassica* 95.6%	94.04	-1.03	16.04
Raspberry	1	F	2014	16.9 ± 0.2	187 ± 0.5	3.9		Rosaceae 87.9%	88.74	0.002	33.75
	23	N	2014	16.4 ± 0.1	515 ± 1.0	3.8		Rosaceae 36.1%	74.34	14.25	72.42
	33	N	2014	18.4 ± 0	174 ± 0.4	4.0		Rosaceae 74.7%	62.44	14.27	65.35
Sweet clover	8	F	2014	16.7 ± 0.2	374 ± 0.3	4.0	Rosaceae 28.4%	*Melilotus* 25.1%	95.84	-0.15	11.69
Willow	24	N	2014	17.7 ± 0.1	191 ± 0.7	4.1		*Salix* 81.1%	78.84	7.85	63.15
Polyfloral											
Polyfloral	5	F	2014	17.7 ± 0.2	1408 ± 0.6	5.0	Rosaceae 37.0%		88.49	-0.01	25.80
	17	F	2014	16.8 ± 0	319 ± 0.6	3.9	*Brassica* 65.0%		79.07	7.06	68.08
	38	S	2014	17.7 ± 0	593 ± 0.7	4.8	Mixed 59.1%		84.43	1.16	52.61
	39	S	2014	16.8 ± 0.1	262 ± 0.6	4.0	Rosaceae 73.3%		80.86	3.11	48.60
	40	S	2014	18.4 ± 0.4	570 ± 1.5	4.1	*Brassica* 64.0%		70.26	10.56	64.80
	41	F	2014	17.6 ± 0.1	292 ± 4.5	3.9	Mixed 31.3%		72.68	5.47	51.17
	42	F	2014	17.9 ± 0.3	307 ± 2.3	3.9	Rosaceae 43.6%		74.72	3.84	48.21
	43	F	2014	18.5 ± 0	296 ± 0.3	4.0	Rosaceae 32.6%		79.32	2.59	44.93
	44	F	2014	18.1 ± 0	250 ± 0.6	3.8	*Brassica* 64.1%		73.01	9.76	59.71
Artificial honey	45			17.6 ± 0.1	422 ± 8.4	3.2			91.11	-1.48	3.26

[a] s.e. for pH was < 0.05 in all samples

[b] *Epilobium* pollen is highly under-represented in Fireweed honey (Salonen et al., 2011)

Table 2. Correlation coefficient results with Spearman's rank correlation analysis. (IC_{50}= antioxidant activity, *P.a* 15= inhibition% of 15% honey dilution against *P. aeruginosa*. *S.a* 15= inhibition% of 15% honey dilutions against *S. aureus*. Elec.cond..= electrical conductivity).

	total sugars	H_2O_2	MGO	IC_{50}	*P.a* 15	*S.a* 15	elec.cond.	pH	total phenols
total sugars	1								
H_2O_2	0.18	1							
MGO	0.04	0.32*	1						
IC_{50}	0.29	0.14	-0.17	1					
P.a 15	0.23	0.07	0.25	0.09	1				
S.a 15	-0.35 *	-0.11	0.08	-0.44	-0.08	1			
elec.cond.	-0.54**	-0.02	0.04	-0.58**	-0.55**	0.28	1		
pH	-0.48**	0.24	0.11	-0.30*	-0.51"	0.02	0.72**	1	
total phenols	-0.21	-0.19	-0.17	-0.26	-0.37*	0.57**	0.17	-0.03	1

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 5. MIC_{90} and MBC values of the most antibacterial honey samples (>85% inhibition). MIC_{90} and MBC value was expressed as the lowest concentration (%, w/v) of honey that inhibited or prevented microbial growth.

Botanical origin	#	*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
		MIC_{90} %	MBC %	MIC_{90} %	MBC %
Sweet clover	8			15	15
Buckwheat	14	15	15	7.5	7.5
Polyfloral	17			15	15
Buckwheat	22			15	15
Raspberry	23	15	30	15	15
Honeydew	27	15	15	15	15
Heather	29			15	15
Heather	31			7.5	7.5
Heather	36			15	15
Polyfloral	40			7.5	7.5

Table 3. Amounts of mono- and polysaccharides in honey samples (g 100 g $^{-1}$).

Honey variety	#	fructose	glucose	sucrose	turanose[a]	maltose[b]	trehalose	isomaltose	erlose[c]	raffinose	panose	total
Buckwheat	14	39.9	31.3	0.0	1.2	1.5	1.2	0.8	0.0	0.3	0.0	76.3
	22	42.2	30.0	0.0	1.6	2.0	1.3	1.4	0.0	0.0	0.0	78.5
Caraway	6	41.3	34.5	0.0	1.1	1.4	1.2	0.8	0.1	0.0	0.0	80.3
Clover	50	42.8	34.0	0.5	0.6	1.4	0.6	0.8	0.5	0.0	0.0	81.1
	51	40.3	31.2	0.0	1.4	2.5	1.2	2.1	0.3	0.0	0.0	79.0
Dandelion	15	40.8	34.7	0.0	1.2	1.2	0.8	0.7	0.4	0.0	0.0	79.7
	18	40.5	35.6	0.0	1.4	1.4	0.7	0.9	0.3	0.0	0.0	80.7
	25	40.2	33.2	0.0	1.3	1.1	1.3	1.4	0.0	0.0	0.0	78.5
	26	38.3	39.2	0.0	1.0	1.0	0.5	1.8	0.3	0.0	0.0	82.1
	35	39.6	38.3	0.0	1.1	0.9	0.6	0.6	0.2	0.0	0.0	81.2
Fireweed	2	42.8	30.0	2.3	1.4	2.2	0.8	0.9	0.7	0.0	0.0	81.0
	7	42.3	30.5	0.7	0.9	2.0	0.8	0.8	0.6	0.0	0.0	78.5
	16	42.8	32.7	0.9	1.2	0.2	0.7	2.4	0.4	0.0	0.0	81.2
	19	45.0	32.5	1.5	1.7	2.4	0.9	1.1	0.7	0.0	0.0	86.0
	21	42.8	31.0	0.5	1.9	2.3	1.0	1.0	0.6	0.0	0.0	81.0
Heather	28	40.7	31.8	0.0	1.2	1.4	1.2	0.9	0.8	0.0	0.0	78.1
	29	40.8	31.3	0.0	1.2	1.2	0.7	0.7	0.5	0.0	0.0	76.3
	31	40.8	31.9	0.0	1.1	1.1	0.8	0.9	0.4	0.0	0.0	76.9
	32	43.0	31.7	0.0	1.1	1.1	0.7	0.7	0.0	0.0	0.0	78.2
	34	46.7	31.1	0.0	1.0	1.7	0.9	0.7	0.0	0.0	0.0	82.1
	36	41.3	32.6	0.0	1.2	1.2	0.8	0.8	0.0	0.0	0.0	77.8
	52	44.1	33.4	0.0	0.8	1.0	0.6	0.8	0.0	0.0	0.0	80.6
Honeydew	27	39.3	32.9	0.0	1.3	1.4	1.2	1.1	0.6	0.0	0.0	77.9
Lime tree	30	41.3	34.4	0.0	1.0	1.3	0.8	0.9	0.0	0.0	0.0	79.7
	37	38.6	32.3	0.0	1.7	1.6	1.0	1.8	0.4	0.0	0.0	77.4
Lingonberry	20	42.5	33.4	0.0	1.4	1.9	1.3	1.1	0.2	0.0	0.0	81.8
Mire	3	43.2	29.4	0.0	1.0	1.5	1.0	0.9	0.0	0.0	0.0	77.0
	4	41.8	30.4	0.0	1.1	2.2	1.2	1.2	0.2	0.8	0.0	79.0
Rape	48	38.5	40.5	1.0	0.3	0.4	0.0	0.4	0.1	0.0	0.0	81.1
	49	37.8	39.8	0.9	0.2	0.3	0.4	0.0	0.0	0.0	0.0	79.4

Raspberry	1	42.5	35.6	0.8	0.9	1.2	0.7	0.7	0.5	0.0	0.0	82.7
	23	42.1	34.6	0.0	1.2	1.7	1.0	1.0	0.2	0.0	0.0	81.8
	33	45.0	38.6	0.0	0.6	0.5	0.4	1.1	0.0	0.0	0.0	86.3
Sweet clover	8	41.0	38.0	0.0	1.1	1.2	0.8	1.7	0.4	0.0	0.0	84.1
Willow	24	39.6	31.3	0.8	1.5	1.6	0.7	0.7	2.3	0.0	0.0	78.5
Polyfloral	5	39.2	29.2	0.6	1.9	2.5	1.4	2.3	0.3	0.3	0.7	78.3
	17	41.0	32.1	0.0	1.6	2.2	1.5	1.5	0.0	0.0	0.0	79.9
	38	38.7	33.8	0.0	1.4	1.9	0.6	1.4	0.2	0.0	0.0	78.0
	39	43.7	37.1	0.0	1.0	1.5	0.8	2.0	0.0	0.0	0.0	86.2
	40	39.9	36.8	0.0	1.0	1.1	1.0	1.0	1.2	0.0	0.0	82.0
	41	43.6	38.2	0.0	1.0	1.7	1.1	1.8	0.5	0.0	0.0	87.9
	42	42.4	36.5	0.0	1.1	1.8	1.0	1.7	0.3	0.0	0.0	84.8
	43	40.7	34.0	0.0	1.0	1.8	1.3	1.6	0.7	0.0	0.0	81.0
	44	40.1	38.1	0.0	1.2	1.2	1.0	0.8	0.0	0.0	0.0	82.5
Artificial honey	45	41.4	38.3	0.4	0.0	5.5	0.0	0.0	0.0	0.0	0.0	85.6

[a] turanose and/or palatinose, [b] maltose and/or cellobiose, [c]erlose and/or melezitose

Table 4. Phenolic compounds in unifloral honey samples mg kg^{-1} (mean ± s.e. Standard error was not counted if there was only one sample presenting the honey variety. Der = derivative)

	Buck-wheat	Cara-way	Clo-ver	Dandelion	Fireweed	Heather	Honey-dew	Lime-tree	Lingon-berry	Mire	Rape	Rasp-berry	Sweet clover	Willow	Polyfloral
Phenolic acids															
Protocatechuic acid der	0	0	0.82	0.14±0.07	0.05±0.03	0.85±0.29	1.83	1.86	0	0.15	0	0.04±0.04	0.29	0	0.21±0.13
Vanillic acid	0.25	2.53	0.77	0.95±0.45	0.34±0.10	0.16±0.16	0	0	0.77	0	0.3	0.67±0.11	1.41	0	0.98±0.14
Benzoic acid[c]	6.15	4.1	6.33	3.55±0.7	2.06±0.24	41.26±10.21	2.7	3.9	4.95	10.9	4.29	2.81±1.33	4.07	3.18	4.96±0.36
Tetragalloylglucose[d]	1.73	25.43	0.8	0.28±0.19	0.20±0.05	1.37±0.15	1.18	0.57	0	0.28	7.69	0.84±0.38	4.36	0.3	4.31±1.22
Benzoic acid der	0	0	0	0.04±0.04	0	0.21±0.21	0.2	0	0	0.3	0	0	0	0	0
Ellagic acid	0	0.21	0	0.07±0.07	0	0	0	0	0.48	0	0	0.39±0.24	0	0	0.10±0.10

Tot. phenolic acids	8.13	32.27	8.72	5.03±1.22	2.64±0.36	43.85±10.19	5.91	6.33	6.2	11.63	12.28	4.75±1.65	10.13	3.48	10.56±0.39
Cinnamic acids															
Cinnamic acid der 1	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cinnamic acid der 2	0.63	0	0	0	0	0.32±0.12	0	1.23	0.79	0	0	0	0	0	0.09±0.09
Cinnamic acid der 3	0	0	0.84	0	0	0	0	0	0	0	0	0	0	0	0
Chlorogenic acid der 1	0.97	1.33	0	0.51±0.32	0.26±0.19	0.14±0.14	0.2	0	0.56	0	0	0	1.1	0	0.86±0.22
ρ-coumaric acid	5.69	2.16	1.36	1.73±0.17	1.11±0.24	0.69±0.44	1.28	0.61	1.57	6.46	2.41	1.05±0.32	1.71	1.69	2.70±0.39
ferulic acid	1.38	2.01	0	1.03±0.36	1.09±0.24	0	0.53	0.3	1.59	0	0.39	0.45±0.24	1.27	0.79	2.00±0.21
Methyl-cinnamic acid der	0	0	0	0	0.24±0.05	0	0	0	0	0	0	0.21±0.21	0.1	0	0.31±0.09
Cinnamic acid der 4	0.64	0.42	0.62	0.53±0.12	0.24±0.02	2.93±0.44	0.56	0.85	0.36	0.82	0.12	0.34±0.20	0.22	0	0.26±0.05
ρ-OH-cinnamic acid der 2	0	0	0	0	0	0.14±0.14	0	0	0.56	0.21	0	0	0	0	0
Cinnamic acid der 5	0	0.39	2.76	3.72±1.62	0	0.12±0.12	0	0.66	0	0	0	0.16±0.16	0.19	0	0.05±0.04
ρ-OH-cinnamic acid der 3	0.25	0	0	0.06±0.06	0	0	0	0	0	0	0	0	0	0	0
ρ-OH-cinnamic acid der 4	0	0	0	0	0	0.07±0.05	0	0	0	0	0	0	0	0	0
Chlorogenic acid der 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.48	0
Caffeic acid der	0.39	0	0.35	0.18±0.14	0.42±0.04	0.71±0.21	0.15	0.09	0	0	0	0.11±0.11	0	0	0.40±0.10
Tot. cinnamic acids	10.18	6.31	5.93	7.75±1.16	3.36±0.70	5.11±0.23	2.72	3.74	5.43	7.49	2.92	2.31±0.40	4.59	2.96	6.69±0.22
Flavonoids															
Myricetin der	0	0	0	0	0	0	0	0	0	0	1.26	0	0	0	0
Kaempferol glycoside	0	0	0	0	0.24±0.07	0	0	0	0	0	0	0.18±0.18	0.46	0	0.14±0.09
Kaempferol der[a]	0	0	0	0.96±0.53	0	0	0	0.1	0	0	1.39	0	0.81	1.63	0.04±0.04
Quercetin 3-O-rhamnoside[b]	0.91	0	0	0	1.79±0.13	0	0	0	0	0	0	0.44±0.44	0	0	0.14±0.14
Kaempferol 3-O-rhamnoside	1.73	0	0	0.08±0.08	5.20±0.44	0.25±0.16	0.32	0	0.43	0.24	0	1.12±1.12	0	0	0.76±0.41
Flavonoid der 2	0	0	0	0	0	0	0	0	0	0	2.43	0	0	0.89	0
Rhamnetin der 1[e]	7.53	0	3.46	0	1.88±1.15	3.15±1.14	6.34	0	0	0	7.81	4.49±3.10	7.33	0	6.53±1.47
Galangin der 1	0.26	0.52	0.56	0.87±0.25	0.30±0.03	0.47±0.10	0.82	0.59	0.33	0.08	0.64	0.48±0.15	1.08	0	0.52±0.05
Galangin der 2[f]	0.23	0.34	0.33	0.25±0.05	0.18±0.0	0.24±0.06	0.63	0.24	0.24	0	1.63	0.27±0.12	0.79	0.18	0.75±0.18
Apigenin[g]	0.15	0.08	0.19	0	0.04±0.02	0.40±0.07	0	0.08	0.09	0	0	0.16±0.11	0.12	0.18	0.09±0.02
Rhamnetin der 2	0	0	0	0.27±0.12	0.04±0.02	0	0	0	0	0	0	0	0.46	0	0.34±0.09

Methyl-naringenin	0.77	0	0.75	1.13±0.16	0.55±0.06	1.62±0.60	0.92	0.99	0.49	0.81	1.89	0.48±0.48	0.85	0	0.80±0.15
Acacetin	0.04	0	0	0.06±0.02	0	0.12±0.04	0.1	0.06	0	0	0	0	0	0	0
Tot. flavonoids	11.62	0.94	5.29	3.62±0.59	10.22±1.36	6.26±1.17	9.13	2.06	1.58	1.13	17.05	7.62±3.80	11.9	2.88	10.09±0.61
Total phenolics	29.9	39.5	19.9	16.4±1.40	16.2±1.21	55.2±9.45	17.8	12.3	13.2	20.2	32.2	14.7±5.65	26.6	9.4	27.3±2.89

[a and b] positive significant (**) correlation with *P.a* 15= inhibition% against *P. aeruginosa* with 15% honey dilution. Correlations: a= 0.42, b= 0.48

[c, d, e, f and g] positive significant (**) correlation with *S.a* 15= inhibition% against *S. aureus* with 15% honey dilution. Correlations: c= 0.49, d= 0.52, e= 0.44, f= 0.42, g= 0.62

Table Supplementary. Identification of phenolic compounds: retention times and HPLC/MS-identification of the MS-ions (M=mass, Na= natrium, H=hydrogen).

Phenolic compound	tR	Identification of MS-ions
Cinnamic acid der 1	3.1	*
Protocatechuic acid der	4	155 (M+H)
Cinnamic acid der 2	7.5	*
Vanillic acid	8.5	169 (M+H), 191 (M+Na)
Cinnamic acid der 3	9.2	*
Chlorogenic acid der 1	10.4	455
ρ-OH-cinnamic acid der 1 (ρ-coumaric acid)	13.3	165 (M+H)
Benzoic acid	13.5	123 (M+H), 145(M+Na)
4-hydroxy-3-methoxy cinnamic acid (ferulic acid)	14.4	195(M+H), 217(M+Na)
Methyl-cinnamic acid der	14.6	179(M+H), 201(M+Na)
Tetragalloylglucose	16.2	*
Benzoic acid der	17.1	*
Myricetin der	17.1	*
Kaempferol glycoside	17.5	*
Kaempferol der	20.4	*
Ellagic acid	21.7	303 (M+1)
Cinnamic acid der 4	22.4	*
ρ-OH-cinnamic acid der 2	23.9	*
Quercetin 3-O-rhamnoside	23.9	471 (M+H)
Cinnamic acid der 5	25.4	*
Kaempferol 3-O-rhamnoside	27.4	455 (M+H)
Flavonoid der 2	28.1	*
Rhamnetin der 1	29.1	*
ρ-OH-cinnamic acid der 3	31.3	*
Galangin der 1	33.2	271 (M+H)
Galangin der 2	33.8	271 (M+H)
Apigenin	34.4	271 (M+H)
Rhamnetin der 2	34.7	*
ρ-OH-cinnamic acid der 4	35.8	355, 179
Methyl-naringenin	38.5	287 (M+H)
Chlorogenic acid der 2	40.1	*
Acacetin	42.9	*
Caffeic acid der	46.1	307 (M+Na)

*Due to lack of ions detected in mass analyses, identification is based on retention time and LC-DAD spectrum