Food Chemistry 171 (2015) 84–88

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Short communication

Chemical composition and antimicrobial activity of Polish herbhoneys

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ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form 27 July 2014
Accepted 26 August 2014
Available online 6 September 2014

Keywords:
Herbhoney
Genuine honey
Chemical composition
Royal jelly acids
Antimicrobial activity

ABSTRACT

The present study focuses on samples of Polish herbhoneys (HHs), their chemical composition and antimicrobial activity. A gas chromatography–mass spectrometry (GC–MS) method was used to analyse eight samples of herbal honeys and three samples of nectar honeys. Their antimicrobial activities were tested on selected Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus schleiferi*) and Gram-negative (*Escherichia coli*) bacteria, as well as on pathogenic fungi *Candida albicans*. Ether extracts of HHs showed significant differences in composition but the principal groups found in the extracts were phenolics and aliphatic hydroxy acids typical of royal jelly and unsaturated dicarboxylic acids. In spite of the differences in chemical composition, antimicrobial activity of the extracts of HHs against all the tested microorganisms except *E. coli* was observed.

1. Introduction

Herbhoneys (HHs) are natural products of beekeeping obtained in the process of feeding honeybees with 50% sucrose syrup tinctured with medical and aromatic herbs or with sucrose syrup with additions of different fruit juices. Infrequently, the bees are also fed with water solutions of honey with additions of the above mentioned herbs and fruit juices. Initially, HHs were produced in 1939 by Jorish (Russia) who wanted to create a therapeutic and health-promoting food full of monosaccharides and enzymes, as found in honey, but also enriched with biologically active substances not commonly found in the floral nectar, such as vitamins, polyphenols and micro-elements (Jorish, 1976).

Production of HHs is widespread in some of the eastern European countries, predominantly Poland, Lithuania and Latvia (Baltrušaitytė, Venskutonis, & Čeksterytė, 2007; Juszczak, Socha, Rożnowski, Fortuna, & Nalepka, 2009). A large number of beekeepers in these countries produce HHs for their own use and also for retail. Large scale producers in Poland are the beekeeping enterprises such as Apipol-Kraków and Sadecki Bartnik®, which offer between six to ten different kinds of HHs depending on seasonal availability of plant raw material. On the Polish commercial market there are six kinds of herbhoneys available on a regular basis, namely pine, hawthorn, nettle, thyme, black chokeberry and aloe herbhoneys.

It has been shown (Juszczak et al., 2009) that Polish HHs satisfied most of the requirements concerning genuine honeys such as water, saccharose and hydroxymethylfurfural (HMF) content as well as free acidity and the diastase number. The analysed herbhoneys also exhibited a high antioxidant activity (Baltrušaityte et al., 2007; Socha, Juszczak, Pietrzyk, & Fortuna, 2009), which, according to the authors, was connected with a high concentration of phenolic compounds. It can also be presumed that due to their high phenolic compound content, HHs should possess a high antimicrobial potential. Up till now, however, this property has not been fully investigated. Moreover, there are no data available in the literature concerning the chemical composition of these bee products.

The aim of the present study is to investigate the chemical composition of the extracts of the most widespread types of herbhoneys and their antimicrobial activity and compare them with the activity of selected genuine nectar honeys.

2. Materials and methods

2.1. Materials

The study involved six herbhoneys and, for comparison, three genuine honeys (GHs). The herbhoneys included pine, hawthorn, nettle, thyme, black chokeberry and aloe varieties produced in 2012 and supplied by Apipol (Kraków, Poland) and Sadecki Bartnik® (Stroze, Poland). The genuine honeys included heather, buckwheat and multifloral honeys supplied by Sadecki Bartnik®. Prior to analysis, all samples were stored in a refrigerator at 4 °C.

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http://dx.doi.org/10.1016/j.foodchem.2014.08.112
0308-8146/© 2014 Published by Elsevier Ltd.

Pyridine, bis(trimethylsilyl)trifluoroacetamide (BSTFA) with an addition of 1% trimethylchlorosilane were purchased from Sigma–Aldrich (Poznań, Poland). Extraction Speedisks® C_{18}XF for solid-phase extraction were acquired from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). The microbiological media used in the study were supplied by Oxoid (Oxoid Ltd, Basingstoke, England).

2.2. Sample preparation and analysis

Extraction of acidic compounds from both herbhoneys and genuine honeys, as well as their GC–MS analysis and estimation of precision were carried out as described previously (Isidorov, Czyżewska, Jankowska, & Bakier, 2011). Briefly, herbhoneys or honey samples (10 g) were diluted in 50 ml of water and filtered through C_{18}XF extraction disks. The adsorbed compounds were eluted with 50 ml of diethyl ether. After removing the solvent, the dry residue (5–10 mg) was dissolved in 220 μl of dry pyridine and 80 μl of BSTFA was added to obtain trimethylsilyl (TMS) derivatives. All the experiments were performed in triplicate.

Separation of the TMS derivatives, as well as hexane solution of C_{10}–C_{40} *n*-alkanes was performed using a GC–MS apparatus (Agilent Technologies, USA). Injection of 1 μl of the sample was performed with using HP 7673 autosampler. Linear temperature programmed retention indices (I_T) were calculated from the results of the separation of *n*-alkanes solution and silanized honey extracts. To identify the components, both mass spectral data and the calculated retention indices were used. The identification was considered reliable if the results of computer search in the mass spectra library (NIST 98) were confirmed by the experimental I_T^{Exp} values, i.e., if their deviation from the literature values I_T^{Lit} (NIST Chemistry WebBook, 2013) did not exceed ±5 u.i.

2.3. Determination of antimicrobial activity

The extracts of both herbal and genuine honeys were tested against a set of microorganisms including bacteria from both worldwide and Polish collections, such as Gram-positive *Staphylococcus aureus* ATCC 29213 (American Type Culture Collection), *Bacillus cereus* ATCC 10987, *Staphylococcus schleiferi* CCM 4070 (Czech Collection of Microorganisms), Gram-negative *Escherichia coli* PCM 2268 (Polish Collection of Microorganisms), as well as pathogenic fungus *Candida albicans* PCM 2566. All the microorganisms were kept at −80 °C in the storage medium (LB broth and glycerol at a ratio of 1:1) and were inoculated onto nutrient agar (bacteria) or Sabouraud agar (fungi) and incubated overnight at 37 °C.

The antimicrobial activity of the extracts was assessed by determining the minimal inhibitory concentration (MIC) in accordance with the Clinical and Laboratory Standard Institute (2011) protocols. For this purpose, extracts were dissolved in DMSO at a concentration of 200 mg/ml, filtrated with 0.22 μm pore size Rotilabo-Spritzenfilter filter (Carl Roth GmbH and Co, Karlsruhe, Germany) and serially diluted twice in Mueller–Hinton broth, ranging from 0.01 to 5 mg/ml, in a U-shaped 96-well microtiter plate with final volumes of 100 μl. The cultures were suspended to a final optical density of 0.2–0.3 at 600 nm, measured with a V-670 spectrophotometer (Jasco, Japan). For the assay, 100 μl of the bacterial suspension was added to each well in the microtiter plate containing diluted honey extracts and incubated overnight at 37 °C. All the tests were repeated four times and the results were averaged. As a positive control, microorganisms cultured in Mueller–Hinton broth without the honey extracts, were applied. Mueller–Hinton broth supplemented with 10% DSMO was used as solvent control.

3. Results and discussion

3.1. Quality parameters of herbal honeys

The herbhoneys under investigation had a water content between 15.5% and 17.1%, i.e., lower than the limit allowed for genuine honeys (20%). Specific conductivity of the herbhoneys (0.43–0.83 mS/cm) only slightly exceeded the limit established for Polish nectar honeys (0.80 mS/cm). Hydroxymethylfurfural (HMF) content ranged from 6.7 to 28.5 mg/kg and the values were much lower than those defined by the Polish standards (40 mg/kg) for natural honeys. The diastase number varied from 10.9 to 13.9 J. u. and only the black chokeberry herbhoney had a value of 2.0 J. u., which is lower than the minimum (8 J. u.) allowed for genuine honeys. On the whole, all these figures remain in good agreement with recent findings (Juszczak et al., 2009).

3.2. Chemical composition of acid fractions of herbal and nectar honeys

In all eight samples of HHs, as many as 147 chemical compounds of different classes were detected. Due to the high number, their chemical composition is presented in this section (Table 1) by means of the main group of a compound and selected "specific" compounds identified. The complete list of compounds identified in the separate samples is presented in Table 1S in Supplementary information. In addition, the pine and hawthorn herbhoneys are represented by two separate samples from different producers. The purpose of creating the duplicates was to compare the same type of HHs produced, most likely, with different technologies. As can be seen, there are no substantial differences in the composition of aromatic and phenyl carboxylic acids. However, considerable variations can be observed in the composition of flavonoids and hydroxy acids, as well as some other individual compounds (for example, the relative concentration of hydroquinone in two pine honeys was equal to 0.3% and 2.2%). These variations may have resulted from different weather conditions prevailing in the area during the collection of plant materials (Čeksterytė, Kazlauskas, & Račys, 2006).

At present, the chemical composition of extractable components of the herbhoneys does not allow us to draw any conclusions concerning plant precursors of the products, with an exception of thyme HH containing typical compounds for *Thymus vulgaris* L. such as thymol and thymol β-D-glucopyranoside (Fig. 1). The chemical composition varies substantially between individual herbal honey samples; however it is possible to note some similarities in the group composition. The most numerous groups among the extracted compounds (46 substances in each) were phenolics and aliphatic acids. The former group can be divided into phenyl carboxylic acids (the dominant sub-group) and flavonoids.

In turn, the fraction of aliphatic acids can be divided into both fatty and hydroxy acids. Since even C_8–C_{12} hydroxy acids are characteristic of royal jelly (Isidorov, Bakier, & Grzech, 2012; Isidorov, Czyżewska, Isidorova, & Bakier, 2009; Melliou & Chinou, 2005; Wytrychowski et al., 2012), they are described in Table 1 as "royal jelly hydroxy acids". Overall, there are 14 acids characteristic of RJ in HHs. This group constitutes 30.6 ± 10.0% ($n = 8$) of all the extracted components from the herbhoneys in which the higher portion (19.6 ± 5.6%) belongs to 2-decene-1,10-dioic acid (2-DecDA).

The floral sources of the Polish nectar honeys analysed were confirmed by chemical analysis that showed the existence of characteristic compounds in each sample. For example, a combination of dehydrovomifoliol, β-phenyllactic and abscisic acids was found to be typical of heather honey (Guyot, Scheirman, & Collin,