ECO-CONSCIOUS EXTRACTION METHODS IN UNLOCKING THE CHEMICAL PROFILES AND BIOACTIVE POTENTIAL OF MONOFLORAL BEE POLLEN

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Introduction

Bee pollen is a natural nutritional product reported for its bioactive properties, including antioxidant and anticancer activities.[1] Green sample preparation methods such as ultrasonic extraction (USE) have been successfully applied to the extraction of bee products, including bee pollen.[2] The aim of this study was to demonstrate the application of some eco- conscious extraction methods in the characterization of monofloral bee pollen (BP). *Castanea sativa* bee pollen was selected for comprehensive characterization.

Experimental

The monofloral bee pollen of *Castanea sativa* obtained from the Croatian region (Glina) was selected on the basis of its visual appearance and further ascertained by palynological analysis.[3]

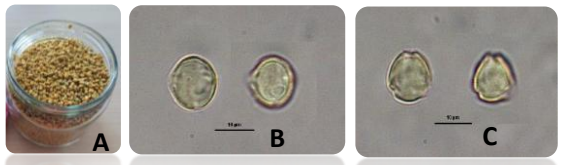


Figure 1. Pollen grains of *Castanea sativa* (**A**) and pollen grains under a light microscope (**B** and **C**).

Headspace solid-phase microextraction (HS-SPME) and GC/MS analysis

Headspace (HS) volatiles from 3 g of crude (native) bee pollen grains were extracted by solvent-free headspace solid-phase microextraction (HS-SPME) using an AOC-6000 autosampler system followed by gas chromatography-mass spectrometry (GC-MS). Extraction was performed at 50 °C for 40 minutes using a divinylbenzene/polydimethylsiloxane (DVB/PDMS)-coated fiber.

Ultrasonic extraction (USE) with pretreatment step

- ***GC/FID analysis of fatty acids***
- ***Determination of cytotoxic activity***

USE was applied as green method for the extraction of bioactive compounds to evaluate the cytotoxic potential of the obtained bee pollen extract. Prior to ultrasonic extraction with methanol, the 10 grams of crude pollen was defatted using hexane (100 mL) in a Soxhlet apparatus[4], by 5 cycles of solvent regeneration. The oil extracted during this pretreatment was analyzed for its fatty acid composition.

Results

- ***The Headspace Composition of Castanea sativa BP***

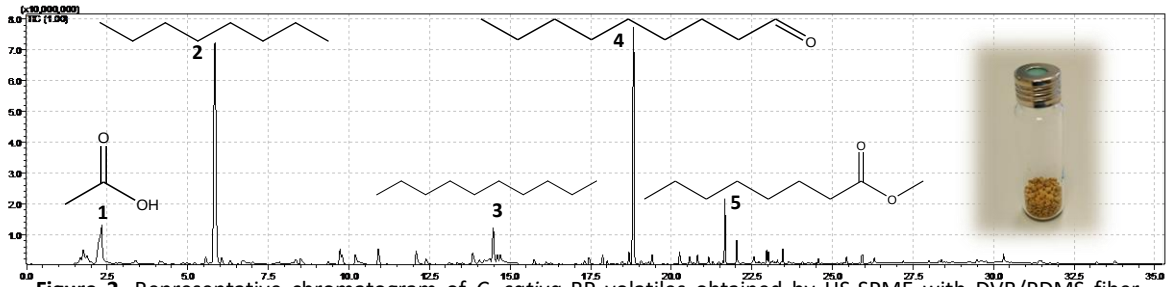


Figure 2. Representative chromatogram of *C. sativa* BP volatiles obtained by HS-SPME with DVB/PDMS fiber. The major HS compunds identified in headspace of *C. sativa* BP were **(1) *acetic acid**, **(2) *octane**, **(3) decane**, (**4) nonanal**, **(5) methyl- nonanoate;** **Tentatively identified*

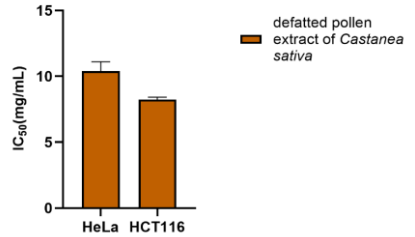
- ***Fatty acid composition of Castanea sativa BP***

Table 1. Major fatty acids identified in BP extract as methyl esters after the derivatization step, using gas chromatography with flame ionization detection (GC-FID)

Fatty acid	Average content ± SD (%)
Palmitic acid, C16:0	13.77±0.58
Oleic acid, C18:1n9c	41.44±2.51
Linoleic acid, C18:2n6c	12.14±0.70
Linolenic acid, C18:3n3	7.95±0.18
Tricosanoic acid, C23:0	7.05±0.44

- ***Cytotoxic activity of Castanea sativa BP:***

Figure 3. Cytotoxic activity of defatted pollen extract of C. sativa on cervical cancer cell line (HeLa), and colon cancer cell line (HCT116) determined by MTS-based cell proliferation test. Analysis of the cytotoxic activity of the pollen extract showed that the extract exhibited weak cytotoxic activity on both Hela (IC_{50} =10.391 ± 0.72mg/ml) and HCT116 cell line (IC_{50} =8.232 ± 0.18 mg/ml).



Conclusion

In this study, HS-SPME and USE were used as suitable eco-conscious extraction methods for the characterization of monofloral bee pollen from Croatia. Furthermore, this study underlines the need for further development of sustainable green practices for comprehensive characterization of regional natural products.

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3. O. M. Barth et al. Annals of Brazilian Academy of Sciences 82 (2010), 893–902.
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