SHORT COMMUNICATION

Analysis of volatile components from *Melipona beecheii* geopropolis from Southeast Mexico by headspace solid-phase microextraction

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A head space solid-phase microextraction method combined with gas chromatography–mass spectrometry was developed and optimised to extract and analyse volatile compounds of *Melipona beecheii* geopropolis. Seventy-three constituents were identified using this technique in the sample of geopropolis collected. The main compounds detected include \(\beta\)-fenchene (14.53–15.45%), styrene (8.72–9.98%), benzaldehyde (7.44–7.82%) and the most relevant volatile components presents at high level in the geopropolis were terpenoids (58.17%).

**Keywords:** *Melipona beecheii*; volatile compounds; headspace; solid-phase microextraction; mass spectrometry; geopropolis

1. Introduction

*Melipona beecheii*, a species distributed throughout much of South and Central America is of potential economic and ecological importance in Mexico (Ayala 1999; Moo-Valle et al. 2000), both for honey production and as a pollinator of crops. The stingless bee subfamily belongs to the Apidae family, of the Hymenoptera order of the insects. They are insects with four membranous wings, with buccal parts that form a structure in the form of tongue, through which liquid food is taken. These bees have small size, the majority between 15 mm long, relative absence of hair and abdomen not pointed (Pino et al. 2006). The growing interest in the honey and geopropolis produced by stingless bees proceeds from its composition, which has been

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associated with antiseptic, antimicrobial, anticancer, anti-inflammatory and wound healing properties and may provide defence for and promote cell function in erythrocytes (Silva et al. 2006; Liberio et al. 2011; Álvarez-Suárez et al. 2012). Geopropolis is a different kind of propolis because it presents wax and soil in its constitution, conferring unique characteristics to it (Dutra et al. 2008). In some countries, geopropolis has been used empirically by the population for wound healing, for the treatment of gastritis, and as an antibacterial agent (Liberio et al. 2011). The monoamino oxidase inhibition and antioxidant properties of propolis extracts were described by Yildiz et al. (2013). These results show that propolis possesses a sedative effect and may be effective in protecting humans against depression and similar diseases.

2. Results and discussion

In the present study, the powdered raw geopropolis collected in Coatepec, Veracruz State, Mexico was analysed by head space solid-phase microextraction (HS-SPME)-GC-TOF-MS. Pellati et al. (2013) have highlighted the importance of the effect of fibre coating on HS-SPME of volatile compounds and applied for the first time the HS-SPME-GC-TOF-MS to analyse Italian samples of raw propolis. Therefore, the first step was the selection of the best fibre coating for HS-SPME. In this study, three fibre types, including Divinylbenzene/Carbowax/Polydimethylsiloxane (DVB/CAR/PDMS), Polydimethylsiloxane (PDMS) and CAR/PDMS, were tested. These fibres have been frequently employed for the extraction of volatile components from natural products (Kataoka et al. 2000; Demir et al. 2014). The DVB-CAR-PDMS fibre allowed to obtain better profile for geopropolis volatile compounds, hence, it was selected for this study. The results showed that the optimal experimental conditions were 500 mg of geopropolis, 15 min of extraction, 45°C, 30 min of equilibrium and 30% sodium chloride under agitation. Seventy-three volatile compounds were identified as shown in Table S1 in order of their elution from the DB-5 column. At first, tentative identification on the selected compounds was performed by means of National Institute of Standards and Technology (Linstrom & Mallard 2011) and Adams (2007) libraries, and then confirmed by standards mass spectra acquisition. The main volatile compounds identified in the geopropolis from Veracruz, Mexico were \( \beta \)-fenchene (14.53–15.45%), styrene (8.72–9.98%) and benzaldehyde (7.44–7.82%). Another relevant class of volatile is represented by monoterpenes, such as (Z)-ocimenone (5.33–5.67%), \( \alpha \)-pinene (3.98–4.66%), \( m \)-cymene (3.90–4.58%), trans-isocarveol (2.71–2.89%), limonene (2.66–2.90%), verbenone (2.46–2.72%), \( \beta \)-pinene (2.15–2.57%), \( \alpha \)-campholenal (2.09–2.35%), \( m \)-cymenene (2.05–2.33%), trans-pinocamphone (2.05–2.19%) and trans-pulegol (1.89–2.23%) (Table S1). The total ion current is shown in Figure S1. The present study determined the chemical composition of geopropolis volatile fraction by means of HS-SPME-GC-TOF-MS. It is interesting to note that the content of the components differed from a prior study, in which one sample from Yucatan was extracted in a Likens–Nickerson microapparatus. In particular, the terpenes \( \beta \)-caryophyllene (11.8%), spathulenol (9.7%) and caryophyllene oxide (9.5%) were found in higher amount (Pino et al. 2006).

Geopropolis is a type of propolis collected by stingless bee species native to tropical countries, which, in addition to resin and wax, has soil in its composition, leading to low yield extracts. This type of propolis has been scarcely described in the literature, and little is known about its chemical composition and biological activity (da Cunha et al. 2013). The chemical composition of geopropolis depends on the local flora, the geographic and climatic characteristics of the area where the sample is collected (Pino et al. 2006). In Coatepec, Veracruz, the vegetation is composed primarily of Liquidambar styraciflua (ocozote), Populus tremula (alamillo), Ulmus mexicana (palo de baqueta), Populus mexicana (alamo) and Cedrela odorata (cedro). In previous studies, resins of Liquidambar (Hovaneissian et al. 2008) and
leaf-buds of *Populus nigra* (Jerković & Mastelić 2003) were found to be rich in styrene, β-fenchene, α-pinene, β-pinene and limonene.

3. Conclusions

In conclusion, HS-SPME-GC-TOF-MS using a DVB/CAR/PDMS fibre is a fast and useful method for the analysis of volatile compounds in raw geopropolis.

Supplementary material

Figure S1 relating to this paper is available online.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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