SUPPLEMENTARY MATERIAL

Analysis of Volatile Components from *Melipona beecheii* Geopropolis from Southeast Mexico by Headspace Solid-Phase Microextraction

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Abstract

A head space solid-phase microextraction (HS_SPME) method combined with gas chromatography-mass spectrometry (GC/MS) was developed and optimised to extract and analyze 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 β -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.

Experimental

Raw geopropolis (131.6819 g) from a hive was manually collected in Coatepec, Veracruz state, Mexico, in July 2011, and was stored at -40 °C. The sample was stored in the Propolis Collection of College of Chemistry at National Autonomous University of Mexico under the accession number GM-2011.

500 mg of geopropolis, 100 mg of sodium chloride and 10 mL of deionized water were placed in a 25 mL vial with PTFE/Silicone septum. The Stableflex® 50/30 µm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS; 1 cm), 100 µm polydimethylsiloxane (PDMS; 1 cm) and 85 µm carboxen-polydimethylsiloxane (CAR/PDMS; 1 cm) fibers supplied by Supelco (Bellefonte, PA, USA) were employed and before the GC-MS analysis. The sample was analyzed in triplicate and the relative proportions of individual components adsorbed to the DVB/CAR/PDMS and PDMS fibers were calculated based on the total ion chromatogram (TIC) peak areas as a percentage of the sum of all peak areas.

GC-MS analyses were performed on an Agilent 6890 N (Agilent Technology, Palo Alto, CA, USA) series gas chromatograph coupled to a LECO time of flight mass spectrometer (LECO Corporation, St. Joseph, MI, USA) and fitted with a 5 % diphenyl-95 % dimethyl polysiloxane (20 m × 0.18 mm i.d.; 0.18 µm film thickness) capillary column (Bellefonte, PA, USA). Helium was used as the carrier gas at a constant flow of 1 mL/min. The column initial temperature was 40 °C. It was then raised to 300 °C with a rate of 20 °C/min and was held for 5 min. The ionization electron energy was 70 eV and the mass range scanned was

40-400 m/z . Injector and MS transfer were set at 300 and 250 °C, respectively. The volatile constituents of propolis were identified based on their Kovats Index, calculated in relation to the retention times of a series of alkanes (C₈ – C₂₀), in comparison with those of the chemical compounds gathered by Adams (2007) and by comparing their MS fragmentation patterns with those of pure compounds, the spectral database of the National Institute of Standards and Technology MS (Linstrom & Mallard, 2014) or from literature data (Adams, 2007).

Figure S1. Analytical ion current chromatogram of the volatile components from geopropolis by HS-SPME-GC using 50/30 μ m divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS).

Table S1. Volatile components identified by HS-SPME and GC-MS technique in geopropolis from *Melipona beecheii*.

Compound ^a	Area $\pm$ SD (%)	R. T. (s)	RI _{exp} ^b	RI _{lit} ^c	Method of identification ^d
Hexanal	0.64 $\pm$ 0.12	104	803	805	a,b,c,d
(2 <i>E</i>)-hexenal	0.07 $\pm$ 0.03	158	855	855	a,b,c
Styrene	9.35 $\pm$ 0.63	204	898	895	a,b,c
Heptanal	2.45 $\pm$ 0.27	218	900	902	a,b,c
α -pinene	4.32 $\pm$ 0.34	240	939	939	a,b,c,d
β -fenchene	14.99 $\pm$ 0.46	244	944	948	a,b,c
Camphene	1.35 $\pm$ 0.17	254	955	954	a,b,c
Artemisol	0.62 $\pm$ 0.06	259	960	960	a,b,c
Benzaldehyde	7.63 $\pm$ 0.19	274	977	978	a,b,c,d
β -pinene	2.36 $\pm$ 0.21	278	981	979	a,b,c,d
<i>tert</i> -butylbenzene	0.24 $\pm$ 0.07	288	992	992	a,b,c
(1-methylpropyl)-benzene	0.12 $\pm$ 0.05	299	1009	1009	a,b,c
Octanal	0.98 $\pm$ 0.12	301	1012	1012	a,b,c
<i>o</i> -Cymenene ^e	0.19 $\pm$ 0.03	302	1014	-	c
α -terpinene	0.05 $\pm$ 0.00	307	1023	1019	a,b,c
<i>p</i> -cymene	0.28 $\pm$ 0.07	308	1026	1026	a,b,c,d
<i>m</i> -cymene	4.24 $\pm$ 0.34	313	1035	1037	a,b,c,d
Limonene	2.78 $\pm$ 0.12	315	1039	1039	a,b,c,d
1-(2-furanyl)-3-butanone ^e	0.46 $\pm$ 0.09	316	1040	-	c
santoline alcohol	0.26 $\pm$ 0.05	317	1041	1040	a,b,c
3-methyl-phenol	0.02 $\pm$ 0.00	331	1067	1070	a,b,c
4-methyl-1-(1-methylethenyl)-cyclohexene	0.20 $\pm$ 0.07	332	1070	1072	a,b,c
2-octenal	0.19 $\pm$ 0.03	335	1075	1075	a,b,c
Unknown	1.48 $\pm$ 0.35	341	1085	-	-
<i>cis</i> -thujone	0.08 $\pm$ 0.00	350	1102	1102	a,b,c
<i>m</i> -cymenene ^e	2.19 $\pm$ 0.14	352	1106	-	c
5-methyl-2-hexanone ^e	0.44 $\pm$ 0.03	353	1108	-	c
1,2,4,5-tetramethyl-benzene	0.26 $\pm$ 0.04	355	1112	1114	a,b,c
4-(1-methylethyl)-cyclohexanol	0.44 $\pm$ 0.02	360	1122	1126	a,b,c
<i>allo</i> -ocimene	0.97 $\pm$ 0.01	372	1143	1144	a,b,c
α -campholenal ^e	2.22 $\pm$ 0.13	372	1143	-	c
<i>trans</i> -pinocamphone	2.12 $\pm$ 0.07	381	1159	1162	a,b,c
<i>p</i> -mentha-1,5-dien-8-ol	1.09 $\pm$ 0.09	382	1162	1165	a,b,c
<i>cis</i> -pinocamphone	1.15 $\pm$ 0.03	390	1176	1175	a,b,c
<i>m</i> -cymenol	0.97 $\pm$ 0.07	391	1178	1179	a,b,c
<i>trans</i> -isocarveol	2.80 $\pm$ 0.09	397	1189	1189	a,b,c
santalone ^e	1.98 $\pm$ 0.04	398	1191	-	c
Dihydrocarveol	1.98 $\pm$ 0.06	398	1192	1193	a,b,c
(4 <i>E</i>)-decenal	3.28 $\pm$ 0.12	401	1197	1196	a,b,c
3,4-dimethyl-phenol ^e	0.08 $\pm$ 0.00	403	1201	-	c

Table S1. Volatile components identified by HS-SPME and GC-MS technique in geopropolis from *Melipona beecheii* (continuation).

Compound ^a	Area $\pm$ SD (%)	R. T. (s)	RI _{exp} ^b	RI _{lit} ^c	Method of identification ^d
bicyclo[2.2.1]hept-2-en-7-ol ^e	0.27 $\pm$ 0.01	404	1202	-	c
(6Z)-nonen-1-ol ^e	0.27 $\pm$ 0.02	404	1203	-	c
Verbenone	2.59 $\pm$ 0.13	407	1210	1205	a,b,c
<i>trans</i> -pulegol	2.06 $\pm$ 0.17	410	1216	1214	a,b,c
2-(3-methyl-2-butenyl)cyclopentanone	0.79 $\pm$ 0.06	413	1224	1227	a,b,c
(Z)-ocimenone	5.50 $\pm$ 0.17	416	1230	1229	a,b,c
neral	0.41 $\pm$ 0.03	420	1239	1238	a,b,c
<i>cis</i> -anethole	0.07 $\pm$ 0.00	428	1259	1258	a,b,c
D-carvone ^e	0.46 $\pm$ 0.01	429	1260	-	c
α -citral	0.13 $\pm$ 0.00	433	1270	1267	a,b,c
thymol	0.05 $\pm$ 0.00	441	1288	1290	a,b,c
<i>trans</i> -verbenyl acetate	1.36 $\pm$ 0.10	445	1296	1292	a,b,c
2,3,5,6-tetramethyl-phenol	0.05 $\pm$ 0.00	456	1323	1319	a,b,c
α -longipinene	0.16 $\pm$ 0.01	470	1355	1352	a,b,c
modhephene	0.44 $\pm$ 0.01	482	1383	1383	a,b,c
β -bourbonene	0.69 $\pm$ 0.01	486	1391	1390	a,b,c
1,4-dimethyl-naphthalene	0.12 $\pm$ 0.04	503	1437	1436	a,b,c
aromadendrene	0.12 $\pm$ 0.00	503	1438	1440	a,b,c
<i>allo</i> -aromadendrene	0.41 $\pm$ 0.04	514	1465	1462	a,b,c
γ -gurjunene	0.39 $\pm$ 0.08	515	1470	1473	a,b,c
1,5- <i>cis</i> -aromadendr-9-ene	0.57 $\pm$ 0.01	521	1484	1484	a,b,c
α -muurolene	0.60 $\pm$ 0.02	527	1500	1499	a,b,c
β -bisabolene	0.67 $\pm$ 0.12	529	1507	1509	a,b,c
δ -cadinene	0.34 $\pm$ 0.07	535	1524	1524	a,b,c
<i>trans</i> -dauca-4(11),8-diene	0.31 $\pm$ 0.01	537	1528	1529	a,b,c
α -ionene ^e	0.98 $\pm$ 0.09	539	1533	-	c
(3S- <i>cis</i>)-3,3 α ,4,5-tetrahydro-3 α -methyl-2h-benz[e]inden-3 α -ol ^e	0.11 $\pm$ 0.08	543	1544	-	c
γ -calacorene	0.02 $\pm$ 0.00	546	1551	1554	a,b,c
guaia-6,9-dien-4 β -ol	0.13 $\pm$ 0.03	549	1561	1565	a,b,c
1a,10a-epoxiamorph-4-ene	0.03 $\pm$ 0.00	556	1577	1569	a,b,c
β -funebrene epoxide	0.39 $\pm$ 0.01	560	1590	1591	a,b,c
bisabola-2,10-diene [1,9] oxide	0.62 $\pm$ 0.09	561	1592	1592	a,b,c
β -bisabolol	0.74 $\pm$ 0.02	588	1675	1672	a,b,c
Kusinol	0.67 $\pm$ 0.10	588	1675	1674	a,b,c
4-isopropyl-1,6-dimethyl-naphthalene	0.16 $\pm$ 0.01	593	1690	1690	a,b,c

Table S1. Volatile components identified by HS-SPME and GC-MS technique in geopropolis from *Melipona beecheii* (continuation).

Compound ^a	Area $\pm$ SD (%)	R. T. (s)	RI _{exp} ^b	RI _{lit} ^c	Method of identification ^d
Alcohols	1.24				
Aromatic compounds	19.07				
Terpenoids	58.17				
Ketones	1.69				
Aldehydes	17.87				
Hydrocarbons	0.48				
Total identified	98.91				

^aCompounds are listed in order of elution on the DB-5 column

^bRetention indices experimentally determined on the DB-5 column

^cRetention indices of literature on non polar columns reported from NIST (Linstrom & Mallard, 2014) and Adams (2007)

^da: retention time; b: retention index; c: mass spectrum; d: peak enrichment

^eTentatively identified by mass spectral library search only

- not identified