

Verification of Chemical Composition of Commercially Available Propolis Extracts by Gas Chromatography–Mass Spectrometry Analysis

Urszula Czyżewska,¹ Joanna Konończuk,¹ Joanna Teul,¹ Paweł Drągowski,¹ Renata Pawlak-Morka,² Arkadiusz Surazyński,³ and Wojciech Milyk¹

¹Department of Pharmaceutical Analysis, Medical University of Białystok, Kilinskiego, Białystok, Poland.

²Pharmaceutical Company BIOTON S.A, Staroscinska, Warsaw, Poland.

³Department of Medicinal Chemistry, Medical University of Białystok, Kilinskiego, Białystok, Poland.

ABSTRACT Propolis is a resin that is collected by honeybees from various plant sources. Due to its pharmacological properties, it is used in commercial production of nutritional supplements in pharmaceutical industry. In this study, gas chromatography–mass spectrometry was applied for quality control analysis of the three commercial specimens containing aqueous-alcoholic extracts of bee propolis. More than 230 constituents were detected in analyzed products, including flavonoids, chalcones, cinnamic acids and their esters, phenylpropenoid glycerides, and phenylpropenoid sesquiterpenoids. An allergenic benzyl cinnamate ester was also identified in all tested samples. This analytical method allows to evaluate biological activity and potential allergenic components of bee glue simultaneously. Studies on chemical composition of propolis samples may provide new approach to quality and safety control analysis in production of propolis supplementary specimens.

KEY WORDS: • gas chromatography • propolis • retention indices • trimethylsilyl derivatives

INTRODUCTION

PROPOLIS, KNOWN AS BEE GLUE, is a natural resinous mixture produced by honeybees (*Apis mellifera*) from substances collected from various species of plants, buds, and exudates. Bees use propolis to seal and to protect the hive against external intruders. A wide range of illnesses was treated with propolis in ancient times especially in folk medicine. Nowadays, a return to natural medicine is observed. Bee glue is used for self-treatment of many diseases.¹

Propolis became the focus of great scientific interest during the last 30 years due to its biological activity. Pharmacological properties of bee glue, including antimicrobial, antifungal, anti-inflammatory, anticancer, and antioxidative activity, have been described.² Dermatological preparations of propolis are currently used in wound healing, treatment of burns, and acne. It is used in natural medicine and in cosmetology. Effectiveness of biological activity of propolis is usually established as a sum of all constituents. It has been proved that individual compounds of bee glue reveal lower pharmacological activity comparing to propolis as a whole. Propolis is available in pharmacies and healthcare stores in

various formulations: capsules, mouthwash solutions, creams, powders, and aqueous-alcoholic extracts.

Biological activity of propolis is related to its phytochemical origin. The main sources of bee glue are the bud exudates of various plants. In Europe, North America, and nontropic regions of Asia, honeybees collect propolis mainly from poplar trees, (*Populus* spp.).³ In Russia a typical bee glue originates from birch (*Betula verrucosa* Ehrh.).³ The most popular Brazilian propolis, called “green,” is collected from leaf resin of *Baccharis* (spp.).^{4,5} Red propolis is typical for North Brazil, Cuba, and Venezuela.^{6,7} It was identified that *Clusia nemorosa* and *Clusia scrobiculata* belong to main propolis sources in these regions. Nowadays, the most important biologically active compounds of propolis and their source species from various geographic locations have been determined.

The use of propolis as a raw material for production of drugs requires procedure of standardization.⁸ Manufacturers producing propolis specimens should characterize concentrations of its constituents that could provide a reasonable degree of bioactivity. There are several methods of analysis of total biologically active compounds: phenolics, flavones/flavonols, and flavanones/dihydroflavonols contents, based on nonseparation techniques.⁹ These methods are useful in routine control of propolis samples, but cannot guarantee effective and safe pharmacotherapy. In this context, development of analytical methods for evaluation of chemical profile of propolis samples is of the great interest.

Manuscript received 24 April 2014. Revision accepted 28 July 2014

Address correspondence to: Urszula Czyżewska, MS, Department of Pharmaceutical Analysis, Medical University of Białystok, Kilinskiego 1, 15-089 Białystok, Poland.
E-mail: urszula.czyzewska@umb.edu.pl

Many analytical separation methods, including gas chromatography (GC), high performance liquid chromatography, high performance capillary electrophoresis, and high performance thin layer chromatography, have been used for identification and quantitative determination of phenolic propolis constituents.⁹ The majority of these investigations have been focused on characterization of group of compounds or individual components.^{10,11}

The aim of this study was to investigate the chemical profile of propolis extracts obtained from commercially available propolis specimens with their gas chromatographic and mass spectrometric characterization. A gas chromatography–mass spectrometry (GC-MS) technique was applied for screening the complex mixtures. This article presents reliable methods for identification of propolis compounds by using mass spectra combined with calculated retention indices. This approach allows to evaluate biologically active and allergenic components of bee glue simultaneously. Studies on chemical composition of propolis samples may provide new quality and safety in production of propolis supplementary specimens.

MATERIALS AND METHODS

Materials and chemicals

Three commercial preparations containing the aqueous-alcoholic extracts of propolis were purchased from local pharmacies in Poland in 2012. As indicated by the manufacturers in the label, the extraction solvent used for these preparations was aqueous EtOH at concentration ranging 60–93% (Table 1). These samples were stored at 4°C temperature, protected from light. Pyridine and the silylation reagent N,O-Bis(trimethylsilyl)trifluoroacetamid (BSTFA) with 1% trimethylchlorosilane (TMSC) were obtained from Sigma-Aldrich (Steinheim, Germany). Caffeine (purity 99%) applied as internal standard (IS) was obtained from Pharma Cosmetic (Kraków, Poland). Methanol (GC grade) used for the preparation of working solute of IS was procured from Honeywell Burdick & Jackson (Morrison, NJ, USA). Mixture of C₇–C₄₀ *n*-alkanes in hexane was purchased from Supelco (Steinheim, Germany). All chemicals and reagents used in this study were of analytical grade.

TABLE 1. CHARACTERISTICS OF COMMERCIAL PREPARATIONS CONTAINING THE HYDROALCOHOLIC EXTRACTS OF PROPOLIS ACCORDING TO INFORMATION CLAIMED ON THE LABEL

Manufacturer	Form of preparation	Lot no.	Other information	
			Concentration (mg/mL)	EtOH content (%)
Farmapia	Drops	11041	58	60
Apipol Farma	Spray	70611	25	93
Farmapia	Spray	12068	58	60

Sample preparation and GC-MS analysis

An aliquot of 250 or 500 μ L of each commercially available propolis aqueous-alcoholic extract was filtered through a 0.45- μ L PTFE filter into a vial of 2 mL in volume. The extract was mixed with 100 μ L of IS (caffeine = 0.24 mg/mL) and solvents were evaporated to dryness under nitrogen. The dry residue was dissolved in 220 μ L of pyridine, and 80 μ L of BSTFA was added into the vial. The reaction mixture was sealed and heated during 45 min at 80°C to obtain trimethylsilyl (TMS) derivatives. All sample procedures were carried out in triplicate.

GC-MS analyses were performed on an Agilent Technologies 5970C VL quadrupole mass spectrometer connected directly to an Agilent Technologies 7890A gas chromatograph and to an autosampler 7693 (Agilent Technologies, Wilmington, DE, USA). Samples were separated on a 30 m $\times$ 0.25 mm i.d., 0.25- μ m film thickness, HP-5MS capillary column J&W (Agilent Technologies). The column temperature was initially held at 50°C for 10 min, and then the temperature was raised to 310°C at rate of 2°C/min, followed by an isothermal period of 10 min. Ultrapure helium with an inline oxygen and moisture trap was used as carrier gas at a flow rate of 1.2 mL/min. Aliquots of 1 μ L were injected in the split (50:1) mode. The injector was kept at 280°C; mass spectrometry (MS) source and MS quad temperatures were 230°C and 150°C, respectively. The mass selective detector was acquiring data in the full-scan mode (mass range = 40–750 amu) at 2.9 scan/sec, with multiplier voltage of 1300 V and ionization energy of 70 eV. GC-MS analysis was conducted according to the modified method described by Isidorov *et al.*¹²

Qualitative and semiquantitative analyses

Mass spectral data and calculated retention indices (RI) values were used to identify the components. Therefore, hexane solution of C₇–C₄₀ *n*-alkanes was previously separated under the aforementioned conditions. Linear-temperature-programmed retention indices were calculated from Equation (1) from the results of the separation of silylated propolis extracts.

$$RI_x = 100 \left(\frac{t_{R(x)} - t_{R(C_z)}}{t_{R(C_{z+1})} - t_{R(C_z)}} n + z \right) \quad (1)$$

Where $n \geq 1$ $t_{R(x)}$ is the retention time of compound x , $t_{R(C_z)}$ is the retention time of the n -alkane eluting directly before the compound x , $t_{R(C_{z+1})}$ is the retention time of the n -alkane eluting directly after the compound x , Z is the number of carbon atoms for the n -alkane eluting directly before the compound x . The chromatographic data were recognized with the aid of an automatic system of processing data of GC-MS supplied by NIST 08 mass spectra and retention index library. The MS library search was performed by using probability-based matching algorithm. Other literatures were used to identify individuals.^{13–15}

The percentage relative amount of individual components was expressed as percent peak area relative to total peak

area (%). Semiquantitative data were mean of three analyses ($n=3$) with standard deviations.

RESULTS AND DISCUSSION

Fast-screening GC-MS method was applied to analyze the commercial preparation of aqueous-alcoholic extracts of propolis. The analysis of samples indicated extremely complex composition, as shown in Figure 1. About 230 individual components were detected and results with chromatographic data were presented in Table 2. GC profiles of tested extracts exhibited a very similar chromatogram, but the values of percentage relative peak area of examined ingredients were significantly different. The main identified components include flavonoids and chalcones (28.66–17.42%), cinnamic acids (21.77–14.97%) and their esters (14.13–10.68%), phenylpropenoid glycerides (2.00–3.05%), and phenylpropenoid sesquiterpenoids (0.77–0.22%).

Results of this study, supplied by GC-MS data, may provide information about vegetal origins of propolis samples. High content of flavonoids, cinnamic acids, and their esters found in all analyzed samples (Table 2) is characteristic for propolis obtained from black poplar trees. Minor presence of glycerides indicates that bees collected propolis from aspen trees. Identification of traces of sesquiterpenoids suggests that the source of propolis is also birch exudates. In

this study, we identified triterpenoids in all of the samples. Their specific mass spectra and fragments at m/z 189 are typical for birch.^{13,14} All analyzed samples presented a common phytochemical profile. The results of this study indicate that three samples of propolis are poplar type with a minor participation of birch exudates. High content of components from *Populus* spp. proves great medicinal properties of propolis. It is well known that flavonoids and cinnamic acids are antioxidative factors, which define pharmacological activity of poplar propolis.

In this experiment we detected compounds responsible for antitumor and cytotoxic activity, including phenylethyl caffeate caffeic acid phenethyl ester (CAPE) (1.38–1.01%) and flavonoid galangin (5.46–2.87%). CAPE has been investigated by many researchers as one of the major active compounds in propolis with chemopreventive and antitumor properties that are not associated with cytotoxic activity affecting normal cells.¹⁶

Results of this study showed presence of phenylpropenoid glycerides in all tested samples. This group of chemical compounds consists of fragments derived from cinnamic acids, acetyl groups, and glycerol.¹³ It has been shown that some of these compounds exhibit anti-inflammatory¹⁷ and antioxidant¹⁸ activity. The percentages of relative abundance of total glycerides in samples were identified on level from 2.00% to 3.05%.

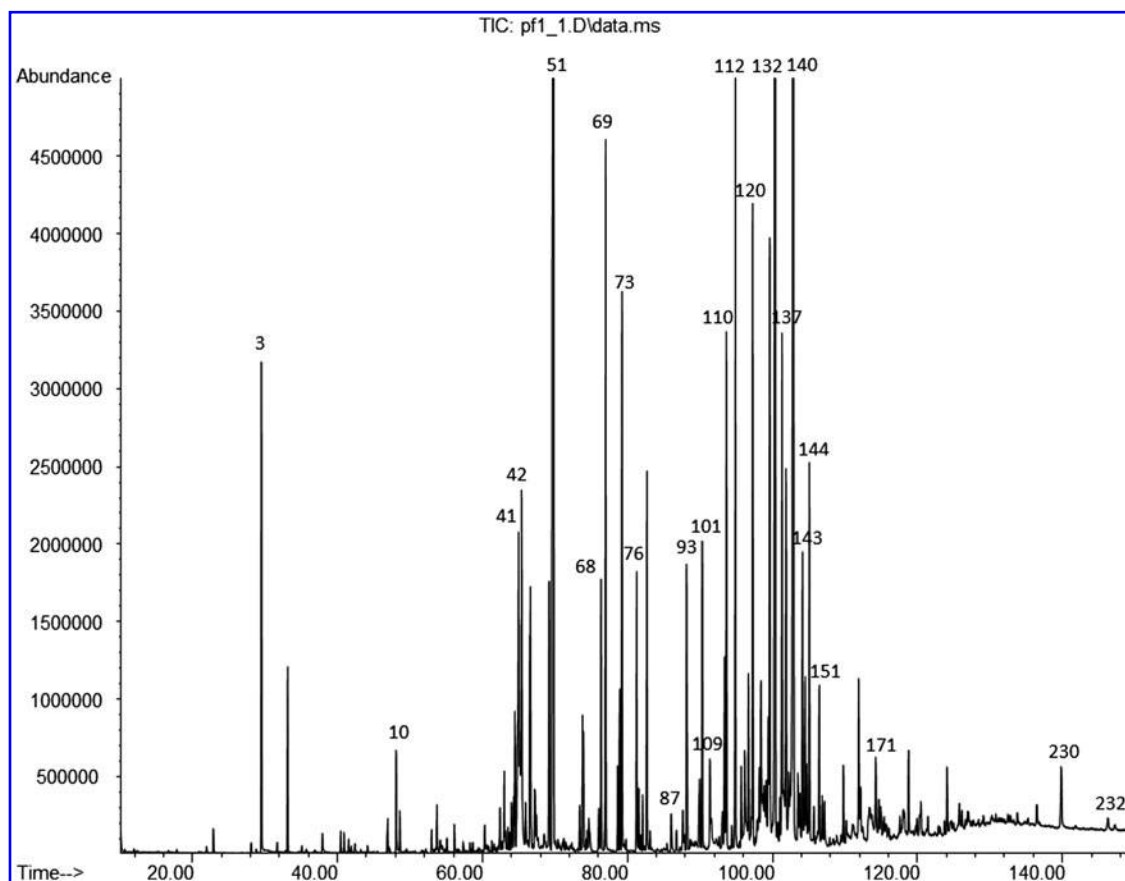


FIG. 1. Total ion current (TIC) chromatogram obtained by gas chromatography–mass spectrometry analysis of the trimethylsilyl (TMS) derivatives from propolis (sample pf1_1). Numbers correspond with compounds presented in Table 2.

TABLE 2. CHEMICAL COMPOSITION OF COMMERCIAL PREPARATIONS OF PROPOLIS (DATA ARE EXPRESSED AS AVERAGE OF RELATIVE COMPOSITION $\% \pm$ STANDARD DEVIATIONS)

No.	Compound, TMS	RI_{exp}	RI_{lit}	Target ions, m/z	% %		
					pf_1	pf_2	pf_3
1	Benzyl alcohol	1156	1153	165.91.135	0.090 $\pm$ 0.003	0.101 $\pm$ 0.002	0.168 $\pm$ 0.019
2	2-Phenylethanol	1227	1223	73.179.103	0.040 $\pm$ 0.003	0.076 $\pm$ 0.004	0.035 $\pm$ 0.002
3	Benzoic acid	1247	1248	179.105.135	1.847 $\pm$ 0.050	2.294 $\pm$ 0.024	5.356 $\pm$ 0.298
4	Glycerol	1298	1295	73.147.205	0.667 $\pm$ 0.015	0.577 $\pm$ 0.007	0.604 $\pm$ 0.025
5	1,2-Benzenediol	1322	1321	73.254.74	n.d.	0.019 $\pm$ 0.004	n.d.
6	Succinic acid	1328	1322	147.73.148	0.025 $\pm$ 0.002	0.028 $\pm$ 0.008	0.040 $\pm$ 0.002
7	2-Methylsuccinic acid	1337	1333	175.147.73	n.d.	0.042 $\pm$ 0.001	0.031 $\pm$ 0.001
8	4-Hydroxybenzaldehyde	1371	1373	179.194.151	0.088 $\pm$ 0.007	0.094 $\pm$ 0.008	0.156 $\pm$ 0.007
9	Hydroquinone	1410	1410	239.254.73	0.084 $\pm$ 0.007	0.110 $\pm$ 0.002	0.128 $\pm$ 0.009
10	Hydrocinnamic acid	1417	1418	104.75.207	0.080 $\pm$ 0.003	0.076 $\pm$ 0.003	0.048 $\pm$ 0.003
11	Cinnamyl alcohol	1427	1428	117.206.73	0.058 $\pm$ 0.003	0.047 $\pm$ 0.001	0.024 $\pm$ 0.002
12	<i>p</i> -Hydroxyacetophenone	1469	1471	193.73.77	0.034 $\pm$ 0.003	0.042 $\pm$ 0.002	n.d.
13	Malic acid	1516	1506	73.147.233	0.195 $\pm$ 0.019	0.051 $\pm$ 0.007	0.198 $\pm$ 0.005
14	Adipic acid	1519	1522	73.147.103	0.022 $\pm$ 0.017	0.024 $\pm$ 0.019	n.d.
15	Vanilline	1536	1530	194.193.209	0.436 $\pm$ 0.003	0.537 $\pm$ 0.011	1.001 $\pm$ 0.051
16	Cinnamic acid	1544	1540	205.131.103	0.197 $\pm$ 0.003	0.241 $\pm$ 0.014	0.225 $\pm$ 0.003
17	Dodecanoic acid, ethyl ester	1596	1597	88.101.55	n.d.	n.d.	0.024 $\pm$ 0.001
18	<i>m</i> -Hydroxybenzoic acid	1621	1623	73.267.282	0.107 $\pm$ 0.003	0.077 $\pm$ 0.002	0.133 $\pm$ 0.022
19	<i>p</i> -Hydroxybenzoic acid	1631	1635	267.223.193	0.217 $\pm$ 0.009	0.258 $\pm$ 0.005	0.422 $\pm$ 0.018
20	D-Ribofuranose	1640	—	73.217.215	0.077 $\pm$ 0.009	n.d.	n.d.
21	α -Eudesmol	1642	1643	161.204.189	0.024 $\pm$ 0.003	n.d.	n.d.
22	<i>p</i> -Hydroxyphenylacetic acid	1646	1648	73.95.121	0.038 $\pm$ 0.014	n.d.	0.064 $\pm$ 0.005
23	Dodecanoic acid	1658	1657	121.134.73	0.073 $\pm$ 0.001	0.081 $\pm$ 0.004	0.092 $\pm$ 0.002
24	Sesquiterpene alcohol	1677	—	73.75.156	0.101 $\pm$ 0.001	0.165 $\pm$ 0.003	0.250 $\pm$ 0.013
25	3-(4-Hydroxyphenyl)-1-propanol	1700	—	73.280.281	0.067 $\pm$ 0.004	0.096 $\pm$ 0.003	0.098 $\pm$ 0.007
26	Suberic acid	1718	1710	73.91.155	0.041 $\pm$ 0.000	0.074 $\pm$ 0.003	0.089 $\pm$ 0.008
27	NN	1726	—	73.250.205	0.042 $\pm$ 0.002	0.054 $\pm$ 0.001	0.140 $\pm$ 0.007
28	Benzoic acid, benzyl ester	1758	1760	105.91.77	0.142 $\pm$ 0.004	0.211 $\pm$ 0.002	0.197 $\pm$ 0.012
29	Ribitol	1764	1766	217.73.147	0.050 $\pm$ 0.010	n.d.	n.d.
30	4-Hydroxyhydrocinnamic acid	1769	1762	179.192.310	0.029 $\pm$ 0.002	n.d.	0.032 $\pm$ 0.005
31	Vanillic acid	1776	1776	297.267.312	0.049 $\pm$ 0.002	0.083 $\pm$ 0.008	0.099 $\pm$ 0.003
32	Edesmol acetate	1783	1778	91.131.43	0.043 $\pm$ 0.004	0.065 $\pm$ 0.007	0.066 $\pm$ 0.001
33	(<i>Z</i>)- <i>p</i> -Coumaric acid	1798	1794	219.293.73	0.216 $\pm$ 0.025	0.201 $\pm$ 0.005	0.273 $\pm$ 0.012
34	(<i>E</i>)-9-Tetradecenyl acetate	1803	1801	149.59.109	0.059 $\pm$ 0.003	n.d.	0.083 $\pm$ 0.005
35	Monosaccharide	1817	—	73.217.75	0.025 $\pm$ 0.004	n.d.	n.d.
36	Monosaccharide	1820	—	217.73.147	0.104 $\pm$ 0.009	0.150 $\pm$ 0.001	0.117 $\pm$ 0.006
37	NN	1825	—	73.210.143	0.081 $\pm$ 0.004	0.110 $\pm$ 0.002	0.143 $\pm$ 0.007
38	4-Methoxycinnamic acid	1830	1832	161.235.191	0.244 $\pm$ 0.027	0.111 $\pm$ 0.003	0.183 $\pm$ 0.017
39	Occidol	1836	1832	128.157.231	0.238 $\pm$ 0.003	0.296 $\pm$ 0.011	0.176 $\pm$ 0.011
40	Galactofuranoside, methyl	1839	1830	217.73.218	0.561 $\pm$ 0.017	n.d.	0.453 $\pm$ 0.016
41	α -D-Fructofuranose	1850	1846	217.73.147	2.527 $\pm$ 0.004	2.333 $\pm$ 0.048	2.602 $\pm$ 0.045
42	β -D-Fructofuranose	1858	1854	217.73.147	2.363 $\pm$ 0.101	1.175 $\pm$ 0.050	2.389 $\pm$ 0.056
43	Ethyl (<i>m</i>)-coumarate?	1869	—	264.219.249	0.338 $\pm$ 0.018	n.d.	n.d.
44	α -D-Galactofuranose	1871	1867	217.73.147	n.d.	0.184 $\pm$ 0.023	n.d.
45	β -D-Fructopyranose	1876	—	204.218.246	0.061 $\pm$ —	n.d.	0.063 $\pm$ 0.014
46	α -D-Mannofuranose	1882	1874	217.73.218	1.766 $\pm$ 0.015	n.d.	1.593 $\pm$ 0.052
47	α -D-Galactopyranose	1894	1895	217.73.218	0.409 $\pm$ 0.136	0.318 $\pm$ 0.024	0.435 $\pm$ 0.018
48	β -D-Sorbopyranose	1899	1900	73.204.147	0.174 $\pm$ 0.010	n.d.	0.357 $\pm$ 0.020
49	NN	1922	—	73.133.103	0.071 $\pm$ 0.051	0.103 $\pm$ 0.035	0.135 $\pm$ 0.024
50	α -D-Glucopyranose	1936	1932	73.204.147	1.437 $\pm$ 0.048	1.983 $\pm$ 0.073	1.228 $\pm$ 0.020
51	(<i>E</i>)- <i>p</i> -Coumaric acid	1948	1947	293.219.73	10.433 $\pm$ 0.090	8.495 $\pm$ 0.181	12.189 $\pm$ 0.411
52	Coniferyl alcohol	1953	1943	324.250.280	0.049 $\pm$ 0.009	0.060 $\pm$ 0.008	0.183 $\pm$ 0.014
53	Gulonic acid, γ -lactone	1961	1958	217.73.305	0.052 $\pm$ 0.010	n.d.	0.095 $\pm$ 0.009
54	NN	1964	—	399.355.281	0.029 $\pm$ 0.002	n.d.	0.066 $\pm$ 0.004
55	<i>n</i> -Propyl-(<i>E</i>)- <i>p</i> -coumarate	1974	1976	73.278.219	0.040 $\pm$ 0.002	n.d.	0.053 $\pm$ 0.002
56	NN	1977	—	73.75.81	0.063 $\pm$ 0.004	n.d.	0.114 $\pm$ 0.008
57	Sedoheptulose	1983	1972	204.147.73	0.045 $\pm$ 0.002	0.070 $\pm$ 0.001	0.122 $\pm$ 0.003
58	NN	1985	—	251.217.91	0.018 $\pm$ 0.005	0.309 $\pm$ 0.039	0.041 $\pm$ 0.002

(continued)

TABLE 2. (CONTINUED)

No.	Compound, TMS	RI_{exp}	RI_{lit}	Target ions, m/z	%		
					pf_1	pf_2	pf_3
59	Ethyl palmitate	1996	1993	88.101.43	0.028 ± 0.005	0.065 ± 0.010	n.d.
60	(Z)-Caffeic acid	2001	2000	73.219.396	0.041 ± 0.011	n.d.	0.029 ± 0.002
61	NN	2024	—	91.92.285	0.201 ± 0.002	0.234 ± 0.001	0.140 ± 0.003
62	3,4-Dimethoxycinnamic acid	2033	2032	191.280.265	0.626 ± 0.000	0.462 ± 0.003	0.439 ± 0.008
63	β -D-Glucopyranose	2036	2031	204.73.217	0.479 ± 0.020	2.081 ± 0.040	0.330 ± 0.016
64	Gluconic acid	2048	2037	73.147.219	0.089 ± 0.008	0.110 ± 0.002	0.072 ± 0.012
			2043				
65	Palmitic acid	2052	2052	73.117.313	0.176 ± 0.009	0.497 ± 0.003	0.193 ± 0.122
66	NN	2054	—	333.143.156	0.112 ± 0.014	n.d.	0.133 ± 0.111
67	Benzyl cinnamate	2082	2092	91.131.192	0.216 ± 0.006	0.284 ± 0.004	0.539 ± 0.025
68	Isoferulic acid	2089	2088	338.73.308	1.120 ± 0.015	0.669 ± 0.000	0.907 ± 0.020
69	(E)-Ferulic acid	2103	2104	338.323.73	2.879 ± 0.038	2.914 ± 0.041	5.638 ± 0.178
70	Myo-inositol	2129	2129	73.305.217	n.d.	0.057 ± 0.002	n.d.
71	NN	2141	—	73.75.157	0.306 ± 0.008	0.204 ± 0.001	0.083 ± 0.002
72	Isoamyl-(E)-p-coumarate	2148	2153	236.219.73	0.668 ± 0.013	0.593 ± 0.009	0.103 ± 0.001
73	(E)-Caffeic acid	2155	2156	219.396.73	2.277 ± 0.030	1.805 ± 0.031	1.775 ± 0.039
74	NN	2160	—	344.131.73	n.d.	0.029 ± 0.009	n.d.
75	Ethyl oleate	2168	2168	55.88.84	n.d.	0.030 ± 0.002	n.d.
76	2-Methyl-2-butenyl-(E)-p-coumarate	2201	2209	219.73.192	1.095 ± 0.013	0.922 ± 0.014	0.162 ± 0.006
77	3-Methyl-2-butenyl-(E)-p-coumarate	2208	2214	221.73.236	0.244 ± 0.003	0.195 ± 0.003	0.105 ± 0.000
78	Linoleic acid	2215	2215	73.75.67	0.076 ± 0.002	0.094 ± 0.003	0.094 ± 0.002
79	Oleic acid	2221	2222	73.75.117	0.215 ± 0.005	0.391 ± 0.003	0.268 ± 0.003
80	NN	2228	—	91.373.329	0.021 ± 0.001	0.029 ± 0.002	n.d.
81	3-Hydroxyhexadecanoic acid	2235	2237	73.156.75	1.456 ± 0.031	1.149 ± 0.010	0.258 ± 0.005
82	3-Trimethylsiloxy palmitic acid	2239	2240	233.147.285	n.d.	0.073 ± 0.003	0.032 ± 0.002
83	Isobutyl-(E)-caffeate	2246	2245	219.380.73	0.078 ± 0.004	0.060 ± 0.002	0.107 ± 0.003
84	Octadecanoic acid	2250	2250	341.117.73	n.d.	0.058 ± 0.003	0.026 ± 0.003
85	NN	2291	—	374.344.73	n.d.	0.021 ± 0.011	0.039 ± 0.013
86	2-Methyl-2-butenyl-(E)-isoferulate	2302	2304	236.249.334	0.039 ± 0.001	0.063 ± 0.006	0.026 ± 0.004
87	3-Methyl-3-butenyl-(E)-ferulate	2316	2319	236.334.249	0.223 ± 0.008	0.093 ± 0.005	0.106 ± 0.001
88	Benzyl ester	2318	—	91.326.192	n.d.	0.096 ± 0.004	0.139 ± 0.011
89	15-Hydroxyhexadecanoic acid	2334	2333	73.117.75	0.113 ± 0.003	0.102 ± 0.003	0.090 ± 0.006
90	3-Methyl-2-butenyl-(Z)-isoferulate	2346	2240	236.249.334	0.018 ± 0.003	0.020 ± 0.002	n.d.
91	1-o-Coumaroyl glycerol	2356	2355	219.351.73	0.205 ± 0.002	0.140 ± 0.002	0.182 ± 0.000
92	3-Methyl-2-butenyl-(E)-isoferulate	2360	2365	236.334.249	0.096 ± 0.002	0.126 ± 0.001	0.028 ± 0.001
93	3-Methyl-3-butenyl-(E)-caffeate	2369	2370	219.392.73	1.180 ± 0.018	0.827 ± 0.835	0.703 ± 0.017
94	3-Methyl-2-butenyl-(E)-ferulate	2371	2374	236.334.266	0.382 ± 0.005	0.245 ± 0.254	0.317 ± 0.011
95	4-Hydroxyanthraquinone-2-carboxylic acid?	2382	—	397.239.73	0.080 ± 0.014	0.085 ± 0.029	0.032 ± 0.013
96	NN	2391	—	73.147.233	0.050 ± 0.023	0.033 ± 0.017	0.046 ± 0.005
97	NN	2399	—	129.131.156	0.045 ± 0.016	0.050 ± 0.019	0.018 ± 0.008
98	NN	2402	—	339.311.340	0.041 ± 0.001	0.058 ± 0.039	n.d.
99	2-Methyl-2-butenyl-(Z)-caffeate	2412	2412	73.219.392	0.198 ± 0.010	0.333 ± 0.005	0.130 ± 0.011
100	2',6'-Dihydroxy-4'-methoxydihydrochalcone	2414	2418	311.401.312	0.289 ± 0.018	0.259 ± 0.011	0.020 ± 0.004
101	3-Methyl-2-butenyl-(E)-caffeate	2423	2425	219.73.392	1.253 ± 0.004	0.999 ± 0.013	0.778 ± 0.021
102	3-Trimethylsilyloxystearic acid	2431	2406	73.233.147	n.d.	0.025 ± 0.010	0.041 ± 0.001
103	2',4',6'-Trihydroxydihydrochalcone	2451	2457	251.328.327	0.472 ± 0.018	0.465 ± 0.031	0.326 ± 0.031
104	3-Methyl-3-butenyl-(E)-3-acetyloxycaffeate	2456	2455	369.459.370	0.262 ± 0.004	0.163 ± 0.050	0.150 ± 0.061
105	NN	2473	—	239.73.212	0.133 ± 0.004	0.130 ± 0.049	n.d.
106	NN	2484	—	73.75.117	0.090 ± 0.008	0.068 ± 0.022	0.046 ± 0.011
107	NN	2495	—	73.75.81	0.129 ± 0.005	n.d.	n.d.
108	NN	2496	—	73.117.75	0.158 ± 0.006	0.274 ± 0.007	0.240 ± 0.004
109	Pinostrobin chalcone	2503	2506	399.400.73	0.862 ± 0.013	1.127 ± 0.027	0.279 ± 0.010
110	Benzyl (E)-p-coumarate	2510	2515	91.192.326	2.258 ± 0.029	2.472 ± 0.059	3.761 ± 0.170
111	1-p-Coumaroyl glycerol	2530	2528	219.73.117	0.115 ± 0.002	0.108 ± 0.012	0.125 ± 0.001
112	Pinocembrin	2545	2544	385.386.73	5.296 ± 0.059	4.677 ± 0.123	3.155 ± 0.108
113	1-Acetyl-2-p-coumaroyl glycerol	2560	2557	219.236.297	0.039 ± 0.005	0.086 ± 0.002	0.038 ± 0.001
114	NN	2567	—	303.73.304	0.353 ± 0.005	0.255 ± 0.003	0.361 ± 0.001
115	NN	2573	—	268.73.239	0.106 ± 0.013	0.088 ± 0.004	0.048 ± 0.021
116	Chalcone?	2579	—	192.73.219	0.800 ± 0.095	0.611 ± 0.023	0.790 ± 0.122

(continued)

TABLE 2. (CONTINUED)

No.	Compound, TMS	RI_{exp}	RI_{lit}	Target ions, m/z	%		
					pf_1	pf_2	pf_3
117	2-Acetyl-1- <i>p</i> -coumaroyl glycerol	2593	—	236.219.221	0.803 ± 0.015	0.748 ± 0.026	0.250 ± 0.008
118	2-Phenylethy- <i>p</i> -coumarate	2600	2601	238.342.239	0.065 ± 0.002	0.091 ± 0.003	0.075 ± 0.007
119	<i>p</i> -Coumaric acid derivative	2604	—	219.117.115	0.151 ± 0.007	0.099 ± 0.009	n.d.
120	Pinobanksin	2609	2610	296.369.73	2.770 ± 0.040	2.208 ± 0.054	1.536 ± 0.039
121	NN	2620	—	342.343.369	0.053 ± 0.007	0.027 ± 0.007	n.d.
122	NN	2626	—	225.224.326	0.177 ± 0.007	0.282 ± 0.029	0.238 ± 0.003
123	NN	2631	—	217.361.103	0.127 ± 0.020	n.d.	0.085 ± 0.015
124	Coniferyl benzoate	2635	2639	297.253.73	0.444 ± 0.004	0.592 ± 0.018	1.332 ± 0.027
125	Chrysin, mono-TMS	2641	2645	326.311.327	1.118 ± 0.006	1.073 ± 0.033	1.282 ± 0.042
126	NN	2649	—	253.326.217	0.350 ± 0.024	0.668 ± 0.031	0.506 ± 0.019
127	2-Phenylethyl x-acetyloxycaffeate	2654	2659	356.341.284	0.508 ± 0.001	0.469 ± 0.022	0.305 ± 0.010
128	NN	2661	—	238.385.325	0.424 ± 0.012	0.452 ± 0.018	0.352 ± 0.036
129	NN	2669	—	296.443.73	0.688 ± 0.021	0.320 ± 0.019	0.280 ± 0.018
130	Chalcone?	2675	—	192.311.356	2.890 ± 0.041	2.272 ± 0.062	2.754 ± 0.117
131	NN	2685	—	355.296.443	0.156 ± 0.003	0.109 ± 0.010	0.078 ± 0.072
132	Pinobanksin-3-acetate	2694	2695	296.443.73	5.088 ± 0.047	4.516 ± 0.100	3.032 ± 0.083
133	NN	2702	—	205.73.217	0.203 ± 0.031	0.211 ± 0.026	0.184 ± 0.034
134	Tectochrysin	2712	2700	399.400.401	0.278 ± 0.036	0.180 ± 0.026	0.202 ± 0.033
135	NN	2721	2718	73.414.91	2.408 ± 0.032	2.501 ± 0.057	1.781 ± 0.054
136	Benzyl (E)-caffeate	2727	2724	91.192.73	0.378 ± 0.175	0.544 ± 0.024	0.544 ± 0.049
137	Chrysin, di-TMS	2738	2745	383.413.384	2.050 ± 0.081	1.964 ± 0.050	0.952 ± 0.052
138	5,7-Dihydroxy-3-methoxyflavone	2750	2754	413.414.383	0.641 ± 0.025	0.611 ± 0.036	0.359 ± 0.061
139	2-Phenyl-(E)-isoferulate	2756	—	236.413.121	0.289 ± 0.014	0.356 ± 0.033	n.d.
140	Galangin	2766	2773	471.472.473	5.459 ± 0.143	4.490 ± 0.129	2.868 ± 0.156
141	NN	2784	—	357.238.358	0.424 ± 0.043	0.602 ± 0.045	0.406 ± 0.078
142	Pinobanksin 3-isobutanoate	2791	2795	296.471.73	0.349 ± 0.044	0.602 ± 0.045	0.281 ± 0.054
143	2-Phenylethyl-(E)-caffeate (CAPE)	2802	2805	73.428.105	1.324 ± 0.036	1.378 ± 0.069	1.008 ± 0.041
144	Isosakuranetin	2813	2815	415.296.73	0.879 ± 0.048	0.811 ± 0.041	0.242 ± 0.040
145	NN	2818	—	413.414.73	0.383 ± 0.026	0.431 ± 0.033	0.242 ± 0.028
146	2',4',6'-Trihydroxy-4-methoxychalcone	2824	2824	487.488.73	0.091 ± 0.019	0.078 ± 0.016	n.d.
147	(E)-Cinnamyl (E)- <i>p</i> -coumarate	2830	—	219.117.73	1.689 ± 0.036	1.627 ± 0.073	0.586 ± 0.044
148	NN	2848	—	222.73.296	0.243 ± 0.067	0.726 ± 0.091	0.192 ± 0.085
149	NN	2859	—	192.204.177	0.061 ± 0.011	0.110 ± 0.035	n.d.
150	NN	2864	—	179.73.205	0.082 ± 0.011	0.116 ± 0.015	0.145 ± 0.026
151	Sakuranetin	2870	2880	415.238.73	0.777 ± 0.011	1.067 ± 0.047	0.826 ± 0.013
152	Pinobanksin 3-isopentanoate	2877	2885	73.285.147	0.041 ± 0.006	n.d.	n.d.
153	Pinobanksin 3-pentanoate	2882	2891	296.485.73	0.259 ± 0.025	0.388 ± 0.055	0.306 ± 0.006
154	Naringenin	2892	2894	73.473.474	0.278 ± 0.003	0.236 ± 0.027	0.229 ± 0.003
155	NN	2914	—	280.73.281	0.047 ± 0.002	0.087 ± 0.005	0.106 ± 0.002
156	NN	2929	—	280.73.281	0.051 ± 0.027	0.097 ± 0.019	0.066 ± 0.017
157	Triterpenoid	2937	—	189.73.75	0.018 ± 0.002	0.029 ± 0.009	0.058 ± 0.005
158	Triterpenoid	2944	—	73.189.75	0.092 ± 0.007	0.104 ± 0.027	0.249 ± 0.014
159	Flavonoid	2962	—	371.296.73	0.071 ± 0.005	0.118 ± 0.050	0.079 ± 0.002
160	(E)-Cinnamyl (E)-ferulate	2972	2973	117.249.73	0.392 ± 0.010	0.302 ± 0.057	0.345 ± 0.010
161	NN	2984	—	73.247.426	0.126 ± 0.004	0.202 ± 0.028	0.481 ± 0.067
162	Caffeic acid derivative	2989	—	356.308.310	0.086 ± 0.007	0.097 ± 0.031	n.d.
163	(Z)-Cinnamyl (Z)-ferulate?	2995	—	117.249.115	0.042 ± 0.005	0.147 ± 0.030	n.d.
164	NN	3011	—	73.429.305	0.165 ± 0.023	0.483 ± 0.040	0.264 ± 0.010
165	NN	3015	—	443.308.207	0.212 ± 0.074	0.125 ± 0.043	0.151 ± 0.017
166	(E)-Cinnamyl (E)-caffeate	3038	3048	73.117.356	0.858 ± 0.030	0.592 ± 0.030	0.614 ± 0.017
167	1,2,8-Trihydroxy-6-methoxy-3-methylanthraquinone	3046	—	501.502.73	0.398 ± 0.015	0.670 ± 0.032	0.330 ± 0.014
168	NN	3055	—	297.413.298	0.154 ± 0.005	0.250 ± 0.036	0.139 ± 0.010
169	NN	3085	—	487.488.489	0.562 ± 0.020	0.541 ± 0.033	0.190 ± 0.010
170	NN	3095	—	338.443.444	0.315 ± 0.027	0.423 ± 0.036	0.238 ± 0.109
171	Kaempferol	3112	3114	559.560.73	0.643 ± 0.031	0.658 ± 0.059	0.402 ± 0.063
172	<i>p</i> -Coumaric acid derivative	3127	—	219.73.103	0.259 ± 0.022	0.349 ± 0.035	0.417 ± 0.031
173	1,6-Dihydroxy-3-(hydroxymethyl)-8-methoxyanthraquinone	3135	—	501.73.502	0.315 ± 0.010	0.346 ± 0.035	0.561 ± 0.048

(continued)

TABLE 2. (CONTINUED)

No.	Compound, TMS	RI_{exp}	RI_{lit}	Target ions, m/z	%		
					pf_1	pf_2	pf_3
174	Apigenin	3150	3154	471.472.473	0.235 ± 0.002	0.262 ± 0.033	0.157 ± 0.042
175	NN	3157	—	73.514.471	0.155 ± 0.048	0.132 ± 0.020	0.292 ± 0.089
176	14-Hydroxy- β -caryophyllene <i>p</i> -coumarate	3160	3165	219.236.220	0.062 ± 0.001	n.d.	n.d.
177	<i>p</i> -Coumaric acid derivative	3161	—	219.338.471	n.d.	0.164 ± 0.014	n.d.
178	<i>p</i> -Coumaric acid derivative	3172	—	73.219.338	0.118 ± 0.006	n.d.	0.227 ± 0.041
179	<i>p</i> -Coumaric acid derivative	3172	—	219.73.267	n.d.	0.176 ± 0.041	n.d.
180	Triterpenoid	3183	—	189.73.143	0.022 ± 0.003	0.093 ± 0.025	0.086 ± 0.024
181	NN	3195	—	517.518.519	0.036 ± 0.023	n.d.	0.056 ± 0.025
182	NN	3195	—	455.73.456	n.d.	0.120 ± 0.074	n.d.
183	NN	3216	—	517.518.593	0.022 ± 0.009	n.d.	0.053 ± 0.023
184	NN	3222	—	103.73.575	0.125 ± 0.009	0.061 ± 0.024	0.112 ± 0.031
185	NN	3239	—	647.589.648	0.267 ± 0.050	0.384 ± 0.054	n.d.
186	NN	3244	—	605.297.606	0.143 ± 0.013	0.260 ± 0.045	0.172 ± 0.022
187	NN	3263	—	73.103.301	0.418 ± 0.047	0.396 ± 0.042	0.288 ± 0.031
188	NN	3295	—	589.189.129	0.056 ± 0.009	0.120 ± 0.030	0.126 ± 0.091
189	NN	3301	—	297.298.299	0.092 ± 0.002	0.179 ± 0.023	0.312 ± 0.032
190	6-Hydroxy- β -caryophyllene <i>p</i> -coumarate	3313	—	253.254.219	0.095 ± 0.002	0.264 ± 0.029	0.353 ± 0.037
191	NN	3320	—	103.355.271	0.197 ± 0.004	n.d.	0.290 ± 0.059
192	6-Hydroxycaryophyllene ferulate	3321	3327	253.73.197	n.d.	0.354 ± 0.058	n.d.
193	14-Hydroxy- β -caryophyllene ferulate	3346	3342	297.73.223	0.063 ± 0.013	0.155 ± 0.018	0.055 ± 0.029
194	NN	3354	—	73.301.103	0.105 ± 0.007	0.107 ± 0.023	0.084 ± 0.024
195	Triterpenoid	3367	—	189.73.267	n.d.	0.052 ± 0.017	0.055 ± 0.018
196	Triterpenoid	3376	—	189.73.143	n.d.	0.086 ± 0.027	0.082 ± 0.013
197	NN	3390	—	341.73.342	n.d.	0.087 ± 0.032	0.053 ± 0.001
198	NN	3405	—	73.103.129	0.055 ± 0.009	0.076 ± 0.025	0.094 ± 0.005
199	NN	3414	—	253.103.189	0.060 ± 0.020	0.102 ± 0.015	0.133 ± 0.018
200	NN	3434	—	297.73.298	0.083 ± 0.004	0.191 ± 0.056	0.357 ± 0.045
201	NN	3447	—	73.253.301	0.373 ± 0.011	0.383 ± 0.042	0.581 ± 0.022
202	NN	3462	—	297.296.533	0.075 ± 0.011	n.d.	n.d.
203	NN	3468	—	121.311.81	0.091 ± 0.008	n.d.	0.059 ± 0.016
204	NN	3479	—	253.73.355	0.075 ± 0.006	0.185 ± 0.026	0.129 ± 0.035
205	Triterpenoid	3508	—	189.73.133	0.219 ± 0.012	0.306 ± 0.037	0.069 ± 0.025
206	NN	3515	—	663.664.665	n.d.	0.086 ± 0.026	0.058 ± 0.027
207	NN	3521	—	203.73.408	0.133 ± 0.007	n.d.	n.d.
208	Triterpenoid	3541	—	95.189.444	n.d.	0.161 ± 0.029	n.d.
209	Triterpenoid	3539	—	73.189.301	n.d.	n.d.	n.d.
210	NN	3549	—	393.129.134	0.105 ± 0.007	0.245 ± 0.042	0.084 ± 0.024
211	NN	3555	—	605.73.606	0.083 ± 0.004	0.153 ± 0.065	n.d.
212	Oleanolic acid	3561	3570	203.202.156	0.037 ± 0.006	n.d.	0.059 ± 0.016
213	NN	3570	—	267.73.91	0.035 ± 0.006	0.142 ± 0.025	n.d.
214	NN	3579	—	73.193.189	0.059 ± 0.004	n.d.	0.129 ± 0.035
215	NN	3583	—	131.215.132	n.d.	0.204 ± 0.069	n.d.
216	Ursolic acid	3612	3620	320.203.202	0.023 ± 0.007	0.153 ± 0.052	0.069 ± 0.025
217	NN	3623	—	603.73.604	0.034 ± 0.001	0.090 ± 0.043	n.d.
218	NN	3630	—	73.301.103	0.059 ± 0.006	0.122 ± 0.073	0.058 ± 0.027
219	NN	3669	—	267.203.635	0.037 ± 0.010	0.142 ± 0.025	n.d.
220	NN	3674	—	253.311.156	0.071 ± 0.018	n.d.	0.123 ± 0.037
221	NN	3694	—	267.375.235	0.088 ± 0.010	0.196 ± 0.069	0.191 ± 0.081
222	NN	3709	—	147.197.192	0.066 ± 0.006	0.192 ± 0.064	0.187 ± 0.098
223	NN	3749	—	341.92.132	n.d.	0.150 ± 0.083	n.d.
224	NN	3750	—	73.253.311	0.093 ± 0.028	n.d.	n.d.
225	NN	3761	—	73.355.91	0.074 ± 0.020	n.d.	n.d.
226	NN	3762	—	297.320.203	n.d.	0.167 ± 0.078	n.d.
227	NN	3793	—	311.73.91	0.073 ± 0.005	0.096 ± 0.032	0.042 ± 0.008
228	NN	3836	—	297.73.253	0.058 ± 0.005	0.089 ± 0.026	0.167 ± 0.003
229	1,3-Di- <i>p</i> -coumaroyl glycerol	3870	3867	219.73.351	0.162 ± 0.006	0.211 ± 0.011	0.459 ± 0.014
230	2-Acetyl-1,3-di- <i>p</i> -coumaroyl glycerol	3955	3960	219.73.220	0.487 ± 0.022	0.796 ± 0.006	1.543 ± 0.063
231	1- <i>p</i> -Coumaroyl-3-feruloyl glycerol	>4000	—	219.249.73	n.d.	n.d.	0.036 ± 0.024
232	2-Acetyl-1- <i>p</i> -coumaroyl-3-feruloyl glycerol	>4000	—	219.73.249	0.138 ± 0.007	0.190 ± 0.012	0.317 ± 0.002
233	1-Acetyl-2-caffeoyl-3- <i>p</i> -coumaroyl glycerol	>4000	—	219.73.335	0.048 ± 0.013	0.060 ± 0.004	0.102 ± 0.015

n.d., not detected; NN, unidentified compound (no name); pf, sample name; RI_{exp} , experimental retention indices; RI_{lit} , literature retention indices; TMS, trimethylsilyl.

Despite of many reports in literature regarding biological activity of “poplar” propolis, it is not entirely safe to use this type of propolis due to its sensitizing contents. Results of this study indicate that all three samples contain benzyl cinnamate (0.54–0.22%), which is a well-known allergen. Other studies have suggested that benzyl salicylate and 1,1-dimethylallyl caffeic ester are a group of propolis compounds that cause allergies.¹⁹ They were not detected in tested samples.

In some cases, identification of compounds in this study was defined on fragmentation patterns only. Then, the identified constituents were classified to a group of compounds. TMS of cinnamic acid derivatives presents characteristic fragmentation pathways. The most useful information obtained from electron impact mass spectrometry (EI-MS) analysis occurred in mass spectrum following ions 219 *m/z* for *p*-coumaric, 249 *m/z* for ferulic, and 308 *m/z* for caffeic acid derivatives. Other class of compounds, triterpenoids, showed in mass spectra an intense peak at 189 *m/z*. EI of compounds leads to characteristic fragmentation, which allows us to qualify a molecule to a certain class of compounds.

Literature presents qualitative composition of propolis from different regions of the world. It has been demonstrated that the chemical composition of propolis and its biological activity depend on the plant source. Propolis is used as a raw material in the pharmaceutical industry, which produces many forms of nutritional supplements. There should be introduced quality tests of the propolis specimens due to its sensitizing contents and potential contamination with fabricated products, such as asphalt and mineral oils. The use of simple separation technique may allow analyzing not only the sum of the propolis compounds but also each component individually. In this study, gas chromatography coupled with mass spectrometry enabled us to analyze the quality of available pharmacy specimens containing hydroalcoholic extracts of propolis. More than 230 compounds were detected in tested samples, including flavonoids, phenylpropenoid, cinnamic acids, their esters, and glycerides. The used GC-MS method provided information about plant sources, botanical precursors, and active principles in propolis extracts. We identified allergenic ester in all tested samples of propolis. Fast sample-preparation procedure and GC-MS methods introduced by us may be used as a simple quality control test of propolis pharmaceutical supplements.

ACKNOWLEDGMENTS

This study was conducted with the use of equipment purchased by Medical University of Białystok as part of the OP DEP 2007–2013, Priority Axis I.3, contract No. POPW.01.03.00-20-008/09.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Krol W, Bankova V, Sforzin JM, Szliszka E, Czuba Z, Kuropatnicki AK: Propolis: properties, application, and its potential. *Evid Based Complement Alternat Med* 2013;2013:807578.
2. Gazzani G, Daglia M, Papetti A: Food components with anti-caries activity. *Curr Opin Biotech* 2012;23:153–159.
3. Bankova VS, de Castro SL, Marcucci MC: Propolis: recent advances in chemistry and plant origin. *Apidologie* 2000;31:3–15.
4. Nunes CA, Guerreiro MC: Characterization of Brazilian green propolis throughout the seasons by headspace GC/MS and ESI-MS. *J Sci Food Agr* 2012;92:433–438.
5. Guimaraes NSS, Mello JC, Paiva JS, *et al.*: *Baccharis dracunculifolia*, the main source of green propolis, exhibits potent antioxidant activity and prevents oxidative mitochondrial damage. *Food Chem Toxicol* 2012;50:1091–1097.
6. Ribeiro Cabral IS, Cadorin Oldoni TL, Prado A, *et al.*: Phenolic composition, antibacterial and antioxidant activities of Brazilian red propolis. *Quim Nova* 2009;32:1523–1527.
7. Trusheva B, Popova M, Bankova V, *et al.*: Bioactive constituents of Brazilian red propolis. *Evid Based Complement Alternat Med* 2006;3:249–254.
8. Bankova V, Marcucci MC: Standardization of propolis: present status and perspectives. *Bee World* 2000;81:182–188.
9. Gomez-Caravaca AM, Gomez-Romero M, Arraez-Roman D, Segura-Carretero A, Fernandez-Gutierrez A: Advances in the analysis of phenolic compounds in products derived from bees. *J Pharm Biomed* 2006;41:1220–1234.
10. Valencia D, Alday E, Robles-Zepeda R, *et al.*: Seasonal effect on chemical composition and biological activities of Sonoran propolis. *Food Chem* 2012;131:645–651.
11. Pellati F, Orlandini G, Pinetti D, Benvenuti S: HPLC-DAD and HPLC-ESI-MS/MS methods for metabolite profiling of propolis extracts. *J Pharm Biomed* 2011;55:934–948.
12. Isidorov VA, Czyzewska U, Isidorova AG, Bakier S: Gas chromatographic and mass spectrometric characterization of the organic acids extracted from some preparations containing lyophilized royal jelly. *J Chromatogr B* 2009;877:3776–3780.
13. Isidorov VA, Brzozowska M, Czyzewska U, Glinka L: Gas chromatographic investigation of phenylpropenoid glycerides from aspen (*Populus tremula* L.) buds. *J Chromatogr A* 2008;1198:196–201.
14. Isidorov VA, Szczepaniak L, Bakier S: Rapid GC/MS determination of botanical precursors of Eurasian propolis. *Food Chem* 2014;142:101–106.
15. Szczepaniak L, Walejko P, Isidorov VA: Gas chromatographic and mass spectrometric characterization of trimethylsilyl derivatives of some terpene alcohol phenylpropenoids. *Anal Sci* 2013;29:643–647.
16. Wu J, Omene C, Karkoszka J, *et al.*: Caffeic acid phenethyl ester (CAPE), derived from a honeybee product propolis, exhibits a diversity of anti-tumor effects in pre-clinical models of human breast cancer. *Cancer Lett* 2011;308:43–53.
17. Shi H, Yang H, Zhang X, Sheng Y, Huang H, Yu L: Isolation and characterization of five glycerol esters from wuhan propolis and their potential anti-inflammatory properties. *J Agr Food Chem* 2012;60:10041–10047.
18. Luo J, Li L, Kong L: Preparative separation of phenylpropenoid glycerides from the bulbs of *Lilium lancifolium* by high-speed counter-current chromatography and evaluation of their antioxidant activities. *Food Chem* 2012;131:1056–1062.
19. Aliboni A, D’Andrea A, Massanisso P: Propolis specimens from different locations of central Italy: chemical profiling and gas chromatography-mass spectrometry (GC-MS) quantitative analysis of the allergenic esters benzyl cinnamate and benzyl salicylate. *J Agr Food Chem* 2011;59:282–288.