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Chemical Composition of Propolis from the Baha Region in Saudi Arabia

Ohood Hasan ALMOHAMMADI[1], Abdulsallam BAKDASH[1,2], Nael Abu TAHA[3], Ali ABU-RUMMAN[1] and Sachil KUMAR[2]*

[1]Department of Forensic Chemistry, College of Forensic Sciences and [2]Arab Society for Forensic Sciences and Forensic Medicine, Naif Arab University for Security Sciences, Riyadh, Saudi Arabia; [3]Department of Zoology, Bioproducts Research Chair (BRC), College of Science, King Saud University, Riyadh, Saude Arabia

**Corresponding author: a.bakdash@nauss.edu.sa*

Abstract

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The chemical composition of organic compounds in the extractable organic matter of propolis collected from the Baha region of Saudi Arabia was investigated. The propolis samples were extracted with methanol followed by column chromatography and collected in fractions (15 ml), then analysed by gas chromatography-mass spectrometry (GC-MS). Samples were injected with and without derivatization using BSTFA simultaneously. The results showed that a total of 61 chemical compounds were characterized and identified by analysing each of the fractions. Out of these, 33 compounds have not been reported previously. The Baha sample contains a high amount of aromatic acids, alcohol and phenol aldehydes. In addition to these, some other compounds (aliphatic acids, sugar derivatives, steroid derivatives and flavone derivatives) were also present. Some of the identified compounds have shown diverse biological activities: antimicrobial, anti-inflammatory, and analgesic activities. Methanolic extraction of propolis followed by column chromatography and GC-MS resulted in the separation of a high number of compounds compared with propolis samples from different geographical regions. The new identified compounds were found related to the flora of the Baha region. Therefore, the composition of propolis differs according to the plants found in the area in which it is produced. This may be used in the determination of a geographical area as the source of a propolis sample.

Keywords: chemical analysis; chromatography; methanol extraction; Baha propolis; bees

Propolis (derived from the Greek pro-, for or in defence, and polis-, the city, that is, defence of the city, or the hive) (Ramos 2007) is a natural resinous mixture produced by honeybees from various plant sources (Elnakady *et al.* 2014). Due to its adhesive nature and mechanical properties, bees use propolis to seal holes in their honeycombs, smoothing out the internal walls and as a protective barrier against external invaders. They also use it to embalm dead invaders and prevent the occurrence and spread of microbial diseases.

Propolis has a long history of medicinal properties, dating back to 350 BCE. The ancient Greeks, Romans, and Egyptians were familiar with the healing properties of propolis and made immense use of it as a medicine. Propolis is said to have antibacterial, antiseptic, astringent, antimycotic, spasmolytic, anaesthetic, anti-inflammatory, antioxidant, antitumoural, antifungal (Quiroja 2006), anticancer, antiulcer, and immunomodulatory effects. It has been used in a variety of applications, which include oint-

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ments and creams used in wound healing, treatment of burns, ulcers and skin problems. Various propolis preparations have been applied in the treatment of laryngological problems, gynaecological diseases, asthma, and diabetes (Salomao 2008). Due to its medicinal uses, it has recently gained popularity as a food supplement in numerous countries to improve health and prevent diseases (Bankova *et al*. 2000; Banskota *et al*. 2001).

The chemical composition of propolis is not fixed and differs according to hive, region, and season (Toreti *et al*. 2013). As a result, more than 300 different compositions have been recognized so far (Park *et al*. 2002), among which phenolic compounds, including flavonoids, are major components. The main constituents of propolis are vegetable balsams and resins, cinnamic acid and its derivatives, prenylated compounds, coumaric acid, artepillin C (50%), beeswax (30%), essential oils (10%), bee pollen (5%), minerals, polysaccharides, amino acids, proteins, amides, amines, and organic debris (5%). It has been suggested that the analysis of these propolis varieties may be used in the determination of the geographical origin of honey (Toreti *et al*. 2013).

Most of the studies on propolis composition and pharmacological effects have been carried out on samples from Europe and Latin America (de Barros *et al*. 2007; Daugsch *et al*. 2008). There is a small number of studies about propolis samples from Saudi Arabia (Elnakady *et al*. 2014); however, neither the chemical composition nor the properties of Saudi propolis have been reported so far. In this study, we investigated the chemical composition of organic compounds in the extractable organic matter of propolis collected from the Baha region of Saudi Arabia, using GC-MS.

MATERIAL AND METHODS

Samples. Propolis samples were collected from the Baha region of Saudi Arabia during the spring of 2014. The samples were purified and 100 g propolis was stored at –20°C in a plastic container until their processing.

Chemicals and reagents. Methanol and ethanol (99.9%) of HPLC grade were purchased from Thermo Fisher Scientific (USA). Bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS), and pyridine was purchased from the Sigma-Aldrich chemical company (USA).

Methanolic extraction. Powdered propolis (100 g) was extracted with 300 ml of methanol (three times, in total 900 ml methanol) and kept on a shaking incubator (30°C) at 200 *g* for 24 hours. The three extracts were combined and filtered using cotton, then centrifuged at 2000 *g* for five minutes. After centrifugation, the filtrate was evaporated under reduced pressure using a rotary evaporator (Heidolph, Germany) at 45°C. The crude extract was stored at 4°C until use.

Column chromatography. The crude extract was first fractionated by vacuum quick silica gel 60 (Merck, Germany) and column chromatography (Vacuum Quick Column, 500 ml in size). Four grams of the crude extract were dissolved in methanol (3 ml) and combined with 10 g of rough silica gel 60, left to dry and then sprinkled evenly over the surface of the packed column. This was then covered with silica gel and a piece of filter paper and cotton in order to protect the crude layer. The packed chromatography column was then eluted with methanol, collecting 15 ml fractions, a total of 25 fractions. Each 15 ml fraction (in some cases more than 2 fractions were gathered together) was collected in a 250 ml flask and the solvent was evaporated under reduced pressure using a rotary evaporator at 45°C.

Instrumental conditions. The analyses were performed on a 450 gas chromatograph equipped with an IT mass 240 spectrometer (Varian, USA). Separation was carried out on a VF-5ms capillary column 5% phenyl, 95% dimethylpolysiloxane (30 m × 0.25 mm i.d. and 0.25 µm film thickness). Operating GC conditions within a run time of 30 min were as follows: temperature of injection port 250°C, temperature programming: initial temperature at 80°C, rising to 280°C at 10°C/min, then maintained for 9 min, sample amount 1 µl; carrier gas helium (purity > 99.99%), flow velocity 1.0 ml/min, splitless.

The MS operating parameters were as follows: data acquisition was performed using full scan mode within mass range from 40 to 50 *m/z*; transfer line temperature 270°C; ion trap temperature 200°C; electron impact ionization used with filament delay 4.5 min; ionization potential 70 eV.

Mass spectra and relative retention time (RRT) of compounds detected by GC-MS analysis were based on peaks marked by signal to noise S/N greater than 10, and matching with reference NIST/EPA/NIH Mass Spectral Library (NIST 08) and Wiley 8th Ed., by matching factors higher than 80%. RRT was calculated using a mixture of internal standards in accordance with a modified method.

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Analysing method. One mg/ml in methanol from each fraction (in some cases more than 2 fractions were gathered together) were prepared as stock solution. 100 µl stock solution was evaporated in a GC vial and then derived using 100 µl BSTFA + 1% TMCS and 50 µl of pyridine at 70°C for 30 min, the derivatization agent was evaporated. 100 µl stock solution was added to make sure that the analysis of derivatized and underivatized components had taken place in one injection.

RESULTS AND DISCUSSION

Numerous scientific studies on propolis have been conducted all over the world so far; the composition of propolis is complex and varies considerably from region to region depending on the local flora and phenology of the source plants (Silici & Kutluca 2005). Due to this, further research is required to determine the composition of propolis samples from the Baha region of Saudi Arabia.

Fractionation of Baha propolis was carried out using a two-step sequential extraction with methanol followed by column chromatography (Table 1) then analysed by gas chromatography-mass spectrometry (GC-MS).

The chemical compounds identified and characterized are shown in Table 2 according to their elution order. A total of 61 chemical compounds were identified by analysing each of the fractions with distinct chemical structures from the following classes: aliphatic acids, aromatic acids, phenol-alcohols/aldehydes, flavonoids, sugars, and others (Table 3).

The major compounds identified included 19 phenol/alcohol/aldehydes, 10 aromatic acids, 8 esters, 6 aliphatic acids, 4 flavonoids, 4 ketones, 4 fatty acid esters, 3 terpenes 2 sugars, and 1 steroid. Recent studies have shown that diterpenoids were the major compounds in propolis samples from Greece and Sicily (Trusheva *et al*. 2003; Popova *et al*. 2009, 2010). Propolis samples from Egypt contained caffeate esters, triterpenoids with major diterpenoids, but no aromatic acids and flavonoids (Hegazi & El-Hady 2002). Some compounds are frequent in all propolis samples and determine its characteristic properties. We found 28 out of 60 compounds that were detected and identified by other researchers. The first studies to identify the active elements of propolis (vanillin, cinnamic acid, and alcohol) were performed in 1911 by researchers in Germany (Veronese *et al*. 2012).

Five aliphatic acids, decanoic acid, oleic acid, dodecanoic acid, docosanoic acid and nonanoic acid, identified in this study are common with previous research results. Dodecanoic acid was identified as a component of propolis in studies undertaken by Kartal *et al*. (2002), Said *et al.* (2006) and Wiryowidagdo *et al*. (2009). Oleic acid was identified as a component of propolis like in studies undertaken in the U.A.E. (Dubai) and Egypt (Wiryowidagdo

Table 1. Characteristics of fractions collected from propolis samples after methanolic extraction followed by column chromatography

Fraction	Volume (ml)	Dried quantity (mg)	Colour	Fraction	Volume (ml)	Dried quantity (mg)	Colour
F1	30	5	yellow	F13	15	112	transparent
F2	15	13	yellow	F15	15	350	transparent
F3	15	2	yellow	F16	15	242	transparent
F4	15	70	yellow	F17	15	236	transparent
F5	15	62	yellow	F18	15	86	transparent
F6	15	35	yellow	F19	15	18	transparent
F7	15	54	yellow	F20	15	15	transparent
F8	15	63	light green	F21	15	15	transparent
F9	15	33	transparent	F22	15	2	transparent
F10	15	41	transparent	F23	15	5	brown
F11	15	50	transparent	F24	15	2	brown
F12	15	75	transparent	F25	15	5	brown

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Table 2. Chemical compounds identified and characterized from Baha propolis

Name of compounds	Retention time (min)	Library formula	CAS No.	MW (*m/z*)	Mass spectrum principle ions (*m/z*)
Fractions 1–5					
1,2-Benzenedicarboxylic acid, butyl octyl ester	15.606	$C_{20}H_{30}O_4$	84-78-6	334	149, 150, 41
12-Hydroxy methyl ester dodecanoic acid	14.017	$C_{13}H_{26}O_3$	71655-36-2	230	74, 55, 87
Oleic acid amide	19.277	$C_{18}H_{35}NO$	301-02-0	281	59, 72, 55
Cis 13-octadecenoic acid, methyl ester	16.921	$C_{19}H_{36}O_2$	56554-47-3	296	55, 41, 69
9-Oxomethyl ester nonanoic acid	9.732	$C_{10}H_{18}O_3$	1931-63-1	186	74, 87, 55
β-Lactose	12.435	$C_{12}H_{22}O_{11}$	5965-66-2	342	73, 43, 60
14-Methylpentadecanoic acid methyl ester	15.252	$C_{17}H_{34}O_2$	5129-60-2	270	74, 87, 270
Oleic acid*	16.779	$C_{18}H_{34}O_2$	112-80-1	282	41, 55, 43
Dodecanoic acid*	11.315	$C_{12}H_{24}O_2$	143-07-7	200	73, 60, 43
4-Hydroxybenzoic acid*	10.987	$C_7H_6O_3$	99-96-7	138	121, 138, 93
Fractions 6–7					
Benzoic acid*	6.296	$C_7H_6O_2$	65-85-0	122	105, 122, 77
Fractions 8–11					
4-Hydroxybenzoic acid*	10.987	$C_7H_6O_3$	99-96-7	138	121, 138, 93
Dodecanoic acid*	11.315	$C_{12}H_{24}O_2$	143-07-7	200	73, 60, 43
Fractions 12–13					
Hydroquinone*	7.645	$C_6H_6O_2$	123-31-9	110	110, 81, 55
2-Methoxy-4-vinylphenol*	8.208	$C_9H_{10}O_2$	7786-61-0	150	135, 150, 107
Fraction 14					
2-Methoxy-4-vinylphenol*	8.218	$C_9H_{10}O_2$	7786-61-0	150	135, 150, 107
4-Hydroxy-2-methylacetophenone	8.24	$C_9H_{10}O_2$	875-59-2	150	135, 150, 107
2-Propyl phenol	8.97	$C_9H_{12}O$	644-35-9	136	107, 136, 77
4-Hydroxy benzene ethanol	9.84	$C_8H_{10}O_2$	501-94-0	138	107, 138, 77
2-Hydroxy-5-methyl benzaldehyde	9.805	$C_8H_8O_2$	613-84-3	136	136, 107, 77
2-Napthalene methanol, decahydro, α,α,4α-trimethyl-8 (β-Selinenol)	12.633	$C_{15}H_{26}O$	473-15-4	222	59, 149, 108
Ferulic acid methyl ester	14.485	$C_{11}H_{12}O_4$	2309-07-1	208	208, 177, 145
3-α,5-pregnan-20-one*	18.56	$C_{21}H_{34}O_2$	516-54-1	318	43, 55, 84
Fraction 15					
Vanillin*	9.030	$C_8H_8O_3$	121-33-5	152	151, 81, 109
2-Hydroxy-6-methyl benzaldehyde	9.81	$C_8H_8O_2$	18362-36-2	136	135, 90, 77
2,3,5-Trimethylphenol	9.852	$C_9H_{12}O$	697-82-5	136	121, 136, 91
D-Mannose*	10.03	$C_6H_{12}O_6$	3458-28-4	180	73, 60, 43
Dodecanoic acid*	11.26	$C_{12}H_{24}O_2$	143-07-7	200	73, 60, 43
3-Hydroxy-dodecanoic acid	12.073	$C_{12}H_{24}O_3$	1883-13-2	216	43, 55, 69
γ-Selinene*	12.307	$C_{15}H_{24}$	515-17-3	204	189, 133, 204
α-Eudesmol*	12.310	$C_{15}H_{26}O$	473-16-5	222	59, 149, 189
β-Selinenol*	12.609	$C_{15}H_{26}O$	473-15-4	222	59, 149, 108
Caryophyllene*	12.76	$C_{15}H_{24}$	87-44-5	204	93, 133, 41
Ferulic acid*	18.522	$C_{10}H_{10}O_4$	1135-24-6	194	194, 179, 133
Docosanoic acid*	20.07	$C_{22}H_{44}O_2$	112-85-6	340	340, 57, 129
Pinocembrin*	20.52	$C_{15}H_{12}O_4$	480-39-7	256	256, 179, 152

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Table 2 to be continued

Name of compounds	Retention time (min)	Library formula	CAS No.	MW (*m/z*)	Mass spectrum principle ions (*m/z*)
Pinostrobin chalcone*	19.808	$C_{16}H_{14}O_4$	18956-15-5	270	193, 270, 166
Coniferyl alcohol*	13.430	$C_{10}H_{12}O_3$	32811-40-8	180	137, 180, 124
4-Hydroxy-2-methoxycinnamaldehyde*	13.390	$C_{10}H_{10}O_3$	127321-19-1	178	178, 135, 77
Glycine methyl ester	5.275	$C_3H_7NO_2$	616-34-2	89	30, 89, 33
Nonanoic acid*	7.464	$C_9H_{18}O_2$	112-05-0	158	60, 73, 57
Simiarenol*	24.181	$C_{30}H_{50}O$	1615-94-7	426	274, 55, 95
Decanoic acid*	7.433	$C_{10}H_{20}O_2$	334-48-5	172	60, 73, 41
Chrysine*	22.245	$C_{15}H_{10}O_4$	480-40-0	245	245, 152, 226
Fraction 16					
Gentisic acid	8.608	$C_7H_6O_4$	490-79-9	154	136, 154, 108
Cembrene	10.256	$C_{20}H_{32}$	1898-13-1	272	272, 93, 81
Ficusin	14.567	$C_{11}H_6O_3$	66-97-7	186	186, 158, 1012
Angecin	14.547	$C_{11}H_6O_3$	523-50-2	186	186, 158, 102
Fractions 17–18					
4-Hydroxy benzoic acid*	10. 671	$C_7H_6O_3$	99-96-7	138	121, 138, 93
2,4-Dihydroxy benzaldehyde	9.919	$C_7H_6O_3$	95-01-2	138	137, 138, 81
Phloretin*	13.674	$C_{15}H_{14}O_5$	60-82-2	274	153, 120, 274
Methyl 13-methylpentadecanoate	15.250	$C_{17}H_{34}O_2$	5487-50-3	270	74, 87, 270
2-Ethylidene-6-methyl-3,5-heptadienal	8.238	$C_{10}H_{14}O$	99172-18-6	150	107, 91, 79
Sugiol*	21.594	$C_{20}H_{28}O_2$	511-05-7	300	285, 300, 217
Geranyl isovalerate	22.1	$C_{15}H_{26}O_2$	109-20-6	238	85, 43, 69
Methyl 11-octadecenoate	16.937	$C_{19}H_{36}O_2$	1937-63-9	296	55, 41, 69
Psoralen	16.785	$C_{11}H_6O_3$	66-97-7	186	186, 158, 102
Bergaptan	16.9	$C_{12}H_8O_4$	484-20-8	216	216, 173, 145
Fraction 19					
Totarol*	19.134	$C_{20}H_{30}O$	511-15-9	286	271, 175, 286
2,5-Dihydroxy benzene acetic acid	11.623	$C_8H_8O_4$	451-13-8	168	122, 94, 150
4-Propyl-1,3-benzenediol	11.816	$C_9H_{12}O_2$	18979-60-7	152	123, 152, 67
2,3,5-Trimethylphenol	10.021	$C_9H_{12}O$	697-82-5	136	121, 136, 91
1-(2-Hydroxy-4,6-dimethoxyphenyl)-ethanone	13.907	$C_{10}H_{12}O_4$	90-24-4	196	181, 196, 43
3,4-Dihydroxy-methyl ester benzenepropoanoic acid	13.482	$C_8H_8O_4$	2150-43-8	168	137, 168, 109
3,5,7-Trimethoxyflavone	21.318	$C_{18}H_{16}O_5$	26964-29-4	312	312, 77, 105
2-Methylaminomethyl-5-nitrobenzophenone	22.639	$C_{14}H_{12}N_2O_3$	4958-56-9	256	256, 255, 77
1,2-Benzenediol	6.605	$C_6H_6O_2$	120-80-9	110	110, 64, 92
Resorcinol*	7.752	$C_6H_6O_2$	108-46-3	110	110, 82, 55
Fractions 20–25					
D-Mannose*	10.494	$C_6H_{12}O_6$	3458-28-4	180	73, 60, 43
Sclerone	10.536	$C_{10}H_{10}O_3$	22332-51-0	178	160, 131, 121

CAS No. – chemical abstracts service registry number; *compounds which match with other studies

et al. 2009), Greece (Kalogeropoulos *et al*. 2009), Turkey (Kartal *et al*. 2002) and Canada (Christov *et al*. 2006). Docosanoic acid, nonanoic acid and decanoic acid compounds were identified, this result was similar to the studies conducted in Canada (Garcia-Viguera *et al*. 1993), Portugal (Miguel

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Table 3. Compounds identified in propolis similar to previous studies

Chemical name	Geographical location	Reference
Aliphatic acids		
Decanoic acid	Turkey, Jawa	Kartal (2002), Wiryowidagdo (2009)
Oleic acid	Egypt, Greece, Turkey, Canada	Kartal (2002), Wiryowidagdo (2009), Kalogeropoulos (2009) & Christov (2006)
Dodecanoic acid	Canada	Garcia-Viguera (1993)
Docosanoic acid	Portugal	Migueal (2014)
Nonanoic acid	India	Thirugnanasampandan (2012)
Aromatic acids		
4-Hydroxybenzoic acid	Canada	Christov (2006) & Huang (2014)
Benzoic acid	Brazil, Canada, Indonesia, Turkey	Wiryowidagdo (2009), Kalogeropoulos (2009) & Garcia-Viguera (1993)
Hydroquinone	Canada	Christov (2006)
Vainllin	Turkey	Kartal (2002)
Ferullic acid	Turkey, Brazil, Canada, Turkey	Kartal (2002), Wiryowidagdo (2009), Kalogeropoulos (2009) & Christov (2006)
Phenols, alcohols and aldehydes		
2-Methoxy-4-vinylphenol	Egypt	Wiryowidagdo (2009)
β-Selinenol	Poland	Isidorov (2013)
α-Eudesmol	Poland	Isidorov (2013)
Simiarenol	Saudi Arabia, Brazil	Sawaya (2011)
Totarol	Malta	Zammit (2013)
Coniferyl alcohol	Poland	Kurek-Górecka (2013)
Sugiol	Brazil	Patricio (2002)
Resorcinol	Poland	Kurek-Górecka (2013)
Phloretin*		
4-Hydroxy-2-methoxy cinnamaldehyde	Turkey	Kartal (2002)
Flavonoids		
Pinocembrin (5,7-dihydroxy flavanone)	Greece, Cyprus, Canada	Wiryowidagdo (2009), Kalogeropoulos (2009) & Huang (2014)
Chrysine (5,7-dihydroxy flavone)	Greece, Cyprus, Canada	Wiryowidagdo (2009) & Kalogeropoulos (2009)
Pinostrobin chalcone	Canada, Brazil	Garcia-Viguera (1993) & Park (2002)
Steroids		
3-α,5-Pregnan-20-one	Turkey	Kartal (2002)
Sugars		
D-Mannose	Greece	Kalogeropoulos (2009)
Others		
Cembrene	East Mediterranean	Wiryowidagdo (2009)
γ-Selinene	Poland	Isidorov (2013)
Caryophyllene	Poland	Isidorov (2013)

et al. 2014), and India (Thirugnanasampandan *et al*. 2012), respectively.

Chemical analysis of propolis extracts indicated that it had a high concentration of aromatic acids. Aromatic acids detected in this study are consistent with other researches. 4-Hydroxybenzoic acid was reported in propolis samples from Canada (Christov *et al*. 2006; Huang & Zhang 2014). Benzoic

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acid was reported in propolis samples from Brazil, Canada, Indonesia and Turkey (Garcia-Viguera *et al*. 1993; Kalogeropoulos *et al*. 2009; Wiryowidagdo *et al*. 2009). The presence of hydroquinone (Chrisitov *et al*. 2006), vanillin (Kartal *et al*. 2002) and ferulic acid (Garcia-Viguera *et al*. 1993; Kartal *et al*. 2002; Kalogeropoulos *et al*. 2009; Wiryowidagdo *et al*. 2009) has been identified by previous studies.

Chemical analysis of propolis collected from the Baha region has highlighted 10 other terphenyl and alcohol aldehyde compounds. The compound 2-methoxy-4-vinylphenol is compatible with the study carried out in Egypt (Wiryowidagdo 2009). Isidorov *et al*. (2003) confirmed the presence of β-selinenol, α-eudesmol, caro phenylene, γ-selinene in propolis (Isidorov & Vinogorova 2003). On the other hand, in a study conducted in Turkey (Garcia-Viguera *et al*. 1993), a composite 4-hydroxy-2-methoxy cinnamaldehyde was detected. Totarol was reported in propolis samples from the Baha region, which is consistent with another study carried out on Maltese propolis where totarol was one of the most abundant compounds; it was found in all the Maltese propolis samples (Zammit *et al*. 2013). Simiarenol was detected only in Baha and Brazil (Sawaya *et al*. 2011). Other compounds like resorcinol, coniferyl alcohol (Kurek-Górecka *et al*. 2013) and sugiol (Patricio *et al*. 2002) have been reported in propolis samples from other regions of the world.

Flavonoids are among the major groups of phenol components in propolis and are a key component to assess the quality of propolis. Flavonoids in propolis include aglycones (without a sugar component). The concentration of flavonoids in propolis depends on the region of origin and ecosystems (plant source of propolis) (Chang *et al*. 2002) as well as the extraction method used. Some flavonoids like pinocembrin (5,7-dihydroxy flavanone) (Park *et al*. 2002; Thirugnanasampandan *et al*. 2012; Huang *et al*. 2014), chrysin (5,7-dihydroxy flavone) (Christov *et al*. 2006; Kalogeropoulos 2009) and pinostrobin chalcone (Garcia-Viguera *et al*. 1993; Park *et al*. 2002) have been reported in propolis samples from different countries.

Steroids are a group of cyclic organic compounds representing highly concentrated energy stores. Steroids like 3-α,5-pregnan-20-one and androstan-1,17-dimethyl-17-hydroxy-3-one have been reported (Kartal 2002) in a propolis sample from Turkey like in this study.

The sugar content of propolis may be derived from hydrolysed flavonoid glycosides in propolis, and plant mucilage may be the source of sugars (Hegazi *et al*. 2002). D-Mannose was reported in the Baha propolis and in propolis samples from Greece (Kalogeropoulos *et al*. 2009).

Cembrene, γ-selinene, and caryophyllene are also some other propolis compounds identified in the Baha samples. This finding is identical to other studies (Park 2002; Sahinler 2005).

Propolis is the explanation of the manifold chemical profiles. These chemical differences might lead to different biological activities; however, the studies dedicated to the bioactivity of propolis are relatively scarce. Available research has shown that certain volatile compounds found in propolis, such as pinocembrin, were active against non-pathogenic fungi and fungal human pathogens (Kuropatnicki *et al*. 2013; Toreti *et al*. 2013). In addition, other available research has suggested that chrysin and totarol may be considered a potential compound for both cancer prevention and treatment (Samarghandian *et al*. 2011; Kuropatnicki *et al*. 2013). Furthermore, chyrisin is a flavonoid with inflammatory and pain relieving properties.

Studies regarding the plant sources of Baha propolis are very scarce. The chemical composition of Baha propolis samples was compared with samples of plant exudates. This approach demands detailed bee observations to be able to know which plants are visited by bees for resin collection. This observation is very important in order to determine and collect samples from possible plant sources and compare them with samples of propolis from a beehive located in the same area.

Baha propolis contains some compounds such as sclerone, 3,5,7-trimethoxyflavone, brevifolin, bergaptan, psoralen, ethyl-11-octadecenoate, 2-ethylidene-6-methyl-3,5-heptadienal, phloretin, and these compounds are present in only a few species in the vegetal kingdom. This confirmed that these plant species are a source of resin for the production of Baha propolis.

However, there are some compounds present in Baha propolis samples that are absent in the other samples, suggesting that there are other botanical sources.

The same study also analysed results of other studies, whose samples showed compositions similar to the Baha propolis; therefore, the botanical origin might be the same. This theory was confirmed by

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comparing the composition of the Baha propolis sample with a sample of chamomile (*Chamomilla recutita* L. Rauschert) root (Szoke *et al*. 2004) or roots of *Ficus hirta* (Yi *et al*. 2013).

The composition of propolis obviously varies between different samples. These results are in agreement with those found by other authors in different countries**.** A total of 20 major compounds were noted in the fractions from the U.A.E., 22 major compounds from Behera (Huang & Zhang 2014), 65 major compounds from Dakahlia, 46 major compounds from Sharkia and 27 compounds from Ismailia (Hegazi 2002), 24 compounds from Kazan (Turkey) and 18 compounds from Marmaris (Turkey) (Kartal *et al*. 2002), 19 compounds from Indonesia (Wiryowidagdo *et al*. 2009), 35 compounds from Poland (Socha *et al*. 2015), and 75 compounds from Portugal (Miguel *et al*. 2014). Similarly, propolis from several regions of Brazil shows different chemical compositions depending on the local flora at the site of collection. Figure 1 shows 36 compounds from Sydenham, 10 from southern, 9 from northeastern and 10 from southeastern Brazil (Park *et al*. 2002).

It is important to note that propolis from each region contains a different composition of chemical compounds. These studies have shown that aromatic acids, alcohol and phenol aldehydes were the major compounds in propolis samples collected from the Baha area. The Egyptian samples contain a high amount of aliphatic and aromatic acids. The UAE sample is characterized by the presence of a high content of aliphatic acids and a low content of aromatic acids. In addition to these, some other compounds (high molecular weight alkanes, sugar derivatives, anthraquinone derivatives and flavone derivatives) were also present (Figure 1).

The constituents of propolis vary widely due to climate, season, location and year, and its chemical formula is not stable. In a study carried out in Pernambuco, Brazil, the author noted a small variation

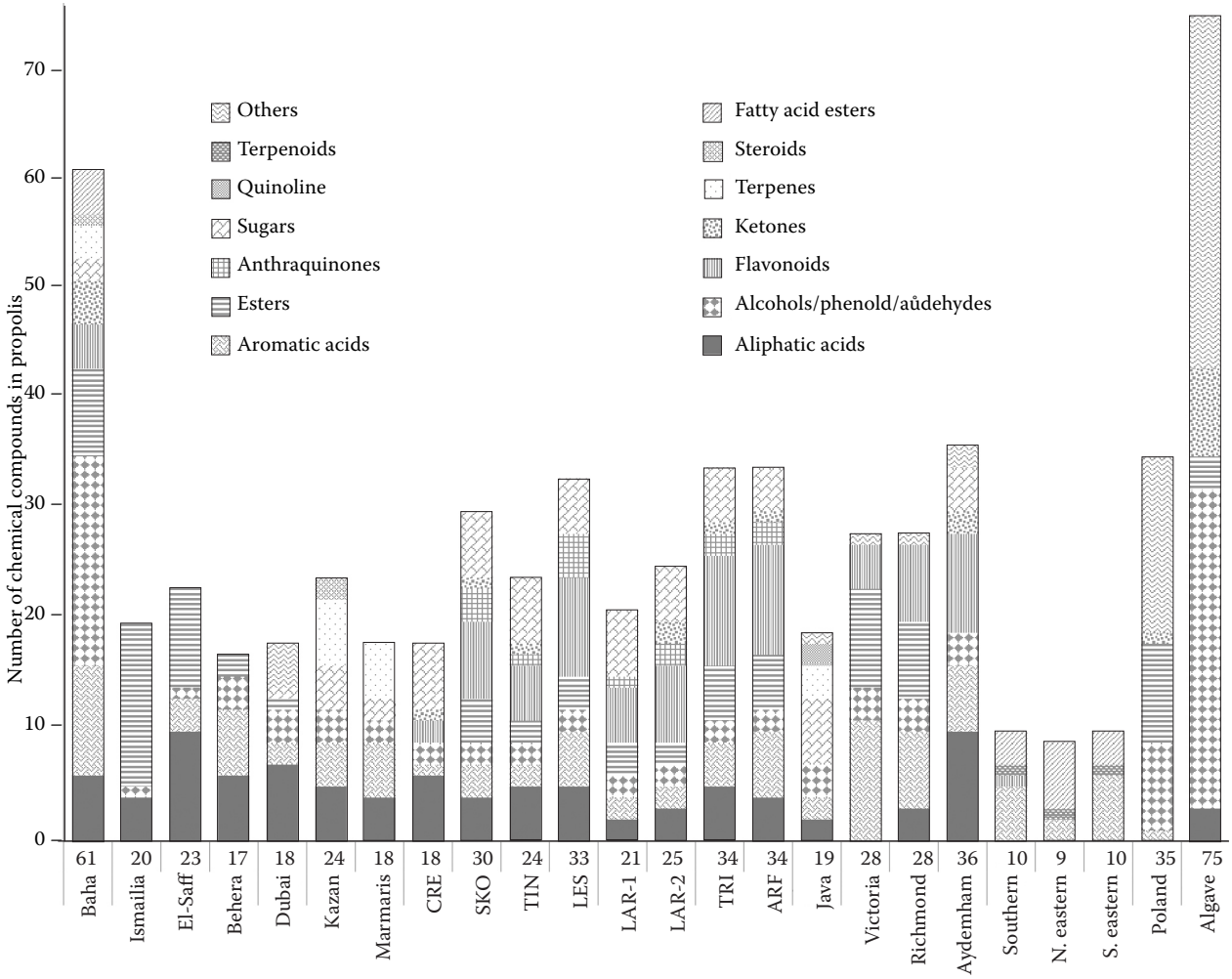

Figure 1. Propolis compound distribution according to geographical regions and chemical structures

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in the propolis volatile compound profile (Salatino *et al*. 2005). These chemical changes in propolis due to environmental factors may alter the biological activity of some propolis compounds which may make it less useful or more toxic.

CONCLUSIONS

The chemical composition of propolis samples collected from the Baha area of Saudi Arabia was investigated by GC-MS after methanolic extraction. A total of 61 chemical compounds were identified and characterized by analysing each fraction with distinct chemical structures. Some compounds are frequent in all propolis samples and determine its characteristic properties. Certain compounds in propolis reflect the plant exudates found in the area where it is produced; therefore, as a forensic application of this article, there is a possibility to identify a geographical area based on the composition of propolis found in a sample.

In addition to the above forensic application, Baha propolis shows diverse biological properties: antimicrobial, antiparasitic, anti-inflammatory and analgesic properties were all detected. These results indicate that this natural product deserves to be better studied for its promising therapeutic effects.

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