African Journal of Agricultural Research Vol. 6(18), pp. 4327-4334, 12 September, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.754
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Identification of volatile compounds in solvent extracts of honeys produced in South Africa

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Accepted 5 July, 2011

Volatile organic compounds in honey are derived from numerous biosynthetic pathways and contribute in the organoleptic and aromatic properties of honey as well as aid in its floral and geographical origin determination. They are usually extracted from the sugar matrix using various methods associated with varying degree of selectivity and effectiveness. In this study, the volatile composition of three local South African honeys was explored by solvent extraction and identified by a gas chromatograph equipped with a mass spectrometry detector. Thirty-two volatile compounds were identified and classified as hydrocarbons (3), acids (3), aldehydes (3), ketones (3), benzene derivatives (4), terpenes and its derivatives (3), alcohols (6), furans (2) and pyran (1) derivatives and others (4). The compounds found in the relatively highest percentage of area were hexane, methanamine hydrochloride, butanal and acetic acid. Astoundingly, thiophene and N-methyl-D3-Aziridine, essential precursors used for the synthesis of natural products and pharmaceuticals with vital biomedical properties, plus methanamine hydrochloride were the additional compounds identified in these honeys.–However, the botanical identification of a honey is based on plant-derived metabolites such as norisoprenoids, terpenes, benzene compounds and their derivatives. Further studies are needed to characterize the aroma constituents as well as to determine the botanical and geographical origins of these honeys in a bid to standardize their quality, to avoid fraud and to authenticate them.

Key words: Solvent extraction, volatile compounds, honey, South Africa.

INTRODUCTION

Honey is the natural sweet substance produced by honey bees of the genera *Apis* and *Meliponini*, from nectar or blossoms or from the secretion of living parts of plants or excretions of plants, which they collect, transform, and combine with specific substances of their own to ripen and mature. It can also be defined as the nectar and saccharine exudation of plants, gathered, modified and stored as honey in the honeycomb by honeybees (Chauhan et al., 2010).

This product of economical importance has a long history of traditional use as an active medicinal compound in a large number of cultures, where they are used either alone or in combination with other substances (El-Gendy, 2010).In South Africa, the medicinal use of honey is in vogue, and people are still using honey as a source of folkloric treatment for certain ailments, with the belief that it boost the immune system and is good for wound healing. Besides, its medicinal properties honey is being used for cosmetic production.

Wholly, honey is made up of about 181 compounds classified as carbohydrates, enzymes, amino acids, minerals, vitamins, trace elements, volatiles, and polyphenols; all of these dissolved in water (Alvarez-Saurez et al., 2009). Yao et al. (2003) reported that the chemical composition of honey depends on the floral source used to collect nectar, seasonal and environmental factors, as well as processing and storage conditions. Notwithstanding, the organoleptic and aromatic properties of honey is being influenced by its volatile constituents given that they represent specific "fingerprint" of a particular honey, depending on its

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floral source (Barra et al., 2010).

Volatile compounds in honey have been reported to be derived from diverse sources such as plant constituents, transformation of plant constituents by honeybee, direct generation of constituents by the honeybee, thermal processing of honey, action of micro-organisms or environmental contamination (Jerković et al., 2006; Jerković and Marijanović, 2009). They are grouped in different chemical families such as alcohols, ketones, aldehydes, hydrocarbons, acids, esters, terpenes and its derivatives, norisoprenoids, benzene derivatives and sulphur compounds (Iglesias et al., 2004; Cuevas-Glory et al., 2007; Jerković et al., 2009).

However, the concentration of volatile compounds in honey is very low, therefore before their isolation it is highly recommended to remove sugars which are the major honey components (Kaškonienė et al., 2008). Accordingly, these compounds are extracted with low polarity solvents including hexane, diethyl ether, chloroform and dichloromethane. Nevertheless, their composition depends on the extraction methods (Alissandrakis et al., 2005).

Various methods have been employed in the extraction of volatile compounds in honey which included solvent extraction, simultaneous distillation-extraction, head-space, ultrasound-assisted extraction, hydro distillation and solid-phase micro extraction techniques. Cuevas-Glory et al. (2007) review these methods, and indicated their advantages and drawbacks as well as pointed out recent advances in the extraction methods. After fractionation of the volatiles from the sugar matrix, gas chromatography-mass spectrometry (GC-MS) technique is employed to identify the components in the fraction.

Several studies have been published on volatile compounds in honey elsewhere (Castro-Várquez et al., 2007; Cuevas-Glory et al., 2007). On the other hand, several authors have carried out antimicrobial studies with honeys produced in South Africa (Basson and Grobler, 2008; Manyi-Loh et al., 2010) but there is no available information about their volatile constituents. It is worth mentioning that the country has a large floral biodiversity with many unique plants indigenous to the region. The majority of these plants are used by the bees to collect honey nectar; consequently plant originated bioactive components can be transferred to honey (Baltrusaitytė et al., 2007). Therefore, it can be expected that the properties of these honeys including their volatile composition from different locations should be different due to different vegetative flowers and plants flourishing in the different locations as a result of variation in the climatic conditions.

Characterizations of these honey varieties based on volatile composition would present data relating to honey botanical source since the volatile compounds are part of the chemical constituents of the nectar from which the honeys were derived and would also provide information about the compounds responsible for its aroma, an important factor that influences consumers' choice. Thus, it is important for consumers to know what their foods are composed of, as well as for producers to know the quality that their product possesses. It is on these bases that we decided to conduct this study to determine the volatile constituents of three locally produced South African honeys by using solvent extraction method and the resulting volatiles were identified by Gas Chromatography-Mass spectrometry.

MATERIALS AND METHODS

Honey sources and solvents

The floral sources of Pure honey (PH) and Goldcrest (GC) were mainly *Citrus limon* and *Citrus sinesis*, while the floral source of Champagne royal train (CRT) was vineyards. Our previous study on antimicrobial activity of selected South African honeys which included PH from Eastern Cape Province, CRT from Western Cape Province and GC from Gauteng Province, revealed that the chloroform extracts of PH and CRT as well as the n-hexane extract of GC honey demonstrated antibacterial activity (Manyi-Loh et al., 2010).

It is noteworthy that the volatile compounds (essential oils, waxes, fatty acids and hydrocarbons) could be extracted with non-polar solvents such as n-hexane and chloroform (Stalikas, 2007), thus they may be part of these extracts. In addition, it has been reported that essential oils are primarily composed of terpenes which are a class of volatiles occurring in honey and they do demonstrate significant biomedical activities including antimicrobial activity (Trombetta et al., 2005).

Solvents used in this study: water, n-hexane, chloroform, methanol, acetic acid and ethyl acetate were obtained from Merck, South Africa.

Solvent extraction of honey

Soria et al. (2003) stated that volatiles in honey are extracted with low polarity solvents such as n-hexane and chloroform, since they extract neither sugars nor water from honey. Therefore, based on our previous antimicrobial study, PH and CRT honeys were extracted with chloroform while GC honey was extracted with n-hexane. This was carried out according to the method of Manyi-Loh et al. (2010). Briefly, 100 g of crude honey was placed in a 500 ml separating funnel, diluted with 150 ml of sterile distilled water and extracted with 150 ml of the stated solvent for each honey type. This was performed as three successive extractions using 50 ml of solvent each time. The shaking time for each extraction process was 15 min, after which the mixture was allowed to stand to permit the solvent layer to separate. The three layers were collected; mixed and water contaminating extracts was removed by filtration over anhydrous sodium sulphate. The organic solvent extract was concentrated by evaporating the extract under reduced pressure using a rotary evaporator (Steroglass, Strike 202, Padua, Italy) at 40°C for n-hexane and 50°C for chloroform, respectively.

Purification

The separation and purification of the compounds in the extracts was conducted over Silica gel by column chromatographic technique with the solvent systems; S1, n-hexane: ethyl acetate: acetic acid (7.8:3.5:1.25) for PH and CRT honeys and S2, methanol: acetic acid: water (10:7:3) for PH and GC honeys, as per

the method of Hassan et al. (2007) and Shrivastav et al. (2009). Depending, on the solvent system used for elution, slurry of silica gel (MN Kieselgel 60, 0.063 to 0.2 mm, Darmstadt, Germany) was prepared with either n-hexane (when elution was done with S1) or chloroform (in the situation where elution was done with S2) and was used to pack separate columns of dimension 40 × 2.5cm (125 ml), then labeled PH, CRT and GC extracts. The columns were gently tapped to ensure uniform packing of the particles of the stationary phase as well as to eliminate air bubbles. They were equilibrated with the respective solvents that were used in the gel preparation (that is, n-hexane or chloroform) and a small quantity of the solvent was allowed to remain at the top of each column (about 4 cm). Each extract (6 g) was mixed separately with silica gel (12 g) in n-hexane or chloroform; they were gently mashed until the extract was adsorbed on the silica gel and allowed to dry. Each mixture in a powder form was loaded to the top of its corresponding column and eluted at a flow rate of 3 ml/min with the appropriate solvent system. Fractions were collected separately from the columns based on the polarity of the solvent system (S1) as well as color separation (S2).

Chromatographic conditions

The GC-MS analyses of the volatiles in the column fractions were carried out using Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP-6890 gas chromatograph with an HP5 column (Wiley, New York, USA). The following conditions were used: initial temperature 70°C, maximum temperature 325°C, equilibration time 3 min, ramp 4°C/min, final temperature 240°C; inlet: split less, initial temperature 220°C, pressure 8.27 psi, purge flow 30 ml/min, purge time 0.20 min, gas type helium; column: capillary, 30 m × 0.25 mm i.d., film thickness 0.25 µm, initial flow 0.7 ml/min, average velocity 32 cm/s; MS: EI method at 70 eV. The volatile compounds were identified by matching their mass spectra and retention indices with those of the Wiley 275 library (Wiley, New York) in the computer library and literature (Joulain et al., 2001; Abd El-Moaty, 2010). The yield of each component was calculated per kg of the honey material, while the percentage composition was calculated from the summation of the peak areas of the total oil composition.

RESULTS

GC-MS analysis of the solvent extracts of three local honey varieties from different areas in South Africa enabled the identification of thirty-two volatile compounds (Table 1). The compounds identified included hydrocarbons (3), acids (3), aldehydes (3), ketones (3), benzene derivatives (4), terpenes and its derivatives (3), alcohols (6), furans (2) and pyran (1) derivatives and others (4).

Common among the three extracts (PH, CRT and GC) were the volatile compounds hexane, butanal and methanamine hydrochloride with different area percentages that depended on the extract. The extracts of PH and GC had the following in common: 1, 3-benzenediamine, pyran, furfuryl alcohol, acetic acid and acetone (Tables 2 and 3). Taking into consideration the chloroform extracts, PH presented the highest number of volatile compounds while only four compounds were identified in CRT.

DISCUSSION

It is well established that the types of organic compounds present in a honey sample is dependent on its floral origin, foraging habits and physiology of the bees as well as on the isolation and detection techniques. More than 400 compounds have been identified and described as volatiles in honeys of different floral types (Bentivenga et al., 2004). To the best of our knowledge this is the first study to determine and identify the volatile compounds in solvent extracts of South African honeys. Most of these compounds identified as volatiles in these extracts have been previously reported by several other studies, although their proportions can be different as a result of the different methods involved (Castro-Várquez et al., 2006; Jerković et al., 2009).

Compounds integrated in all the extracts were: hexane with an area percentage between 4.56 to 96.80%, butanal with an area percentage between 5.55 to 71.22% and methanamine hydrochloride with an area percentage between 31.75 to 89.89%. In addition to the three common compounds, methanol was another compound present in CRT; GC-MS identified just four volatile compounds (i.e. hexane, butanal, methanamine hydrochloride and methanol) for this extract (Table 4).

Aldehydes identified in the analyzed extracts included: butanal, succinaldehyde and 3-cyclohexene-1-acetaldehyde. Butanal was common in all the extracts with maximum abundance of 71.22% in GC honey but hexanal and heptanal with a higher number of carbon atoms have been reported as the major flavor compounds in lavender honey (Bouseta et al., 1992). Succinaldehyde and 3-cyclohexene-1-acetaldehyde were present only in PH with area percentages of 0.62% and 0.13%, respectively. α-4-dimethyl-3-cyclohexene-1-acetaldehyde has been proposed for differentiating citrus honeys (Alissandrakis et al., 2005).

Alcohols identified in this study included; 1-butanol, methanol, 1-propanol, 1, 3 butanediol, oxiranemethanol, and 1-(1-cyclopentenyl)-propanol. They represent an important class of compounds in honey (Barra et al., 2010). Methanol had the highest area percentage of 45.17% and was present only in CRT. More than half of these identified alcohols were present in GC. Aldehydes and alcohols however, reflect product quality and are a consequence of microbiological activity, heat exposure, and honey aging (Cuevas-Glory et al., 2007).

Hexane, octane and cyclohexane were the hydrocarbons identified in the studied extracts. Hexane was the most predominant hydrocarbon in all the samples especially in PH. Kaskonienė et al. (2008) also determined linear as well as branched chain hydrocarbons in Lithuanian honey samples. Diketones, alkanes and sulphur compounds have been reported as characteristic compounds of eucalyptus honeys (Castro-Várquez et al., 2007).

Ketones such as 2-cyclopentanone, acetone and 2, 5-furandione were identified. Acetone was present in both

Table 1. Volatile compounds identified in three local honey varieties in South Africa.

Organic compounds	Availability of organic compounds in different solvent extracts		
	Eastern PH(chloroform)	Gauteng GC(n-hexane)	Western CRT(chloroform)
Hydrocarbons			
Hexane	+	+	+
Octane	+	-	-
Cyclohexane	+	-	-
Acids			
Acetic acid	+	+	-
Propanoic acid	-	+	-
Hexadecanoic acid	+	-	-
Aldehydes			
Butanal	+	+	+
Succinaldehyde	+	-	-
3-cyclohexene-1-acetaldehyde	+	-	-
Ketones			
2-cyclopentanone	+	-	-
Acetone	+	+	-
2,5-Furandione	+	-	-
Benzene derivatives			
Toluene	+	-	-
1,2-dimethyl benzene	+	-	-
1,3-dimethyl benzene	+	-	-
1,3-benzenediamine	+	-	-
Terpenes and its derivatives			
Epoxylinalool	+	-	-
Linalool	+	-	-
1-Octen-7-methylocta-1,3(Z),5(E) triene	+	-	-
Furan derivatives			
Furfuryl alcohol	+	+	-
5-Hydroxymethylfurfural	-	+	-
Alcohols			
1-Butanol	+	-	-
Methanol	-	-	+
1-Propanol	-	+	-
1,3-Butanediol	-	+	-
Oxiranemethanol	-	+	-
1-(1-cyclopentenyl)-propanol	-	+	-
Pyran derivative			
2,3-dihydro-3,5-dihydroxyl-6-methyl-4H-pyran-4-one	+	+	-
Others			
Thiophene	+	-	-
Methanamine hydrochloride	+	+	+
N-methyl-D3-Arizidine	+	-	-
Propanenitrile	-	+	-

+, Present; -, absent; PH, pure honey; GC, Goldcrest; CRT, champagne royal train.

Table 2. Volatile compounds present in chloroform extract of PH obtained from the Eastern Cape province of South Africa.

Organic compounds	Area percentage of each compound identified in each fraction						
	Chloroform extract of PH						
	S1				S2		
	F1	F2	F3	F4	F1	F2	F3
Hexane	89.40	7.75	-	96.80	-	19.98	-
Octane	0.37	-	-	-	-	-	-
Cyclohexane	5.97	-	-	-	-	-	-
2-Propanone	-	-	-	-	10.06	1.63	6.47
2,5-Furandione	-	-	-	-	2.21	-	1.72
Butanal	-	-	-	-	-	23.66	-
Succinaldehyde	-	-	-	-	0.62	-	-
3-cyclohexene-1-acetaldehyde	0.13	-	-	-	-	-	-
Acetic acid	-	11.12	29.26	-	70.57	54.73	80.63
Hexadecanoic acid	0.45	-	-	-	-	-	-
Methyl benzene	1.52	-	-	1.69	-	-	-
1,2-dimethyl benzene	0.27	-	-	0.23	-	-	-
1,3-dimethyl benzene	1.36	-	-	1.29	-	-	-
1,3-benzenediamine	-	-	-	-	0.95	-	-
1-Butanol	-	-	-	-	-	-	1.42
Furfuryl alcohol	-	-	-	-	4.22	-	3.02
Epoxy linalool	0.20	-	-	-	-	-	-
Linalool	0.17	-	-	-	-	-	-
1-Octen-7-methylocta-1,3(Z),5(E)-triene	0.15	-	-	-	-	-	-
2,3-dihydro-3,5-dihydroxy-6-methylpyran-4-one	-	-	-	-	9.2	-	6.75
N-methyl-D3-Arizidine	-	-	-	-	0.43	-	-
Thiophene	-	-	-	-	1.74	-	-
Methanamine HCl	-	81.13	70.74	-	-	-	-

S1, n-hexane: ethyl acetate: acetic acid (7.8:3.5:1.25); S2, methanol: acetic acid: water (10:7:3); F, fractions; HCl, hydrochloride.

n-hexane extract of GC and chloroform extract of PH (Tables 2 and 3). Its area percentage was between 0.44 to 10.06%. Cuevas-Glory et al. (2007) identified acetone as a relevant compound of acacia and rosemary unifloral honey. Bastos and Alves (2003) mentioned that acetone compound is typical of fir honey (*Abies* spp), produced by one of the most common introduced tree species in Chile. Though not found in our samples, 3-hydroxy-2 butanone is a ketone that has been identified as a distinctive feature of eucalyptus honey and is considered to be a floral marker for this type of honey (Pérez et al., 2002).

Organic acids are also common in honeys of different sources. They are chemical compounds that have different aromas, ranging from spicy to rancid depending on the length of the carbon chain. Short chain carboxylic acids such as acetic acid was identified in PH and GC honeys and had an area percentage between 8.09 to 89.13%. It is however, among those acids that naturally occur in honey, synthesized during bee metabolism (Bastos and Alves, 2003). Other acids detected in these honey extracts were propanoic acid and hexadecanoic (palmitic) acid. These acids have been identified as volatile compounds from two unifloral honeys of *Robinia pseudoacacia* L. and *Castanea sativa* L. in a study conducted by Jerković et al. (2007), although different methods were used. Consequently, there could be differences in the proportion of these acids occurring in the honeys employed in the different studies.

Furan derivatives identified in the honey samples were: furfuryl alcohol (2-furanmethanol) and 5-hydroxymethylfurfural. They were both present in GC extract whereas furfuryl alcohol occurred as the only furan compound in PH with an area percentage that ranged between 3.02 to 4.22%. Furfuryl alcohol detected in the n-hexane extract of GC had an area percentage that ranged between 1.56 to 3.56% while the area percentage of 5-hydroxymethylfurfural was 0.17%.

The presence of furan derived compounds in the extracts has purportedly been ascribed to sugar degradation during long storage, heating process and/or distillation process. They are however, considered to be indicators of thermic processes and storage (Barra et al., 2010). These compounds indicate a possible loss of freshness due to prolonged storage or exposure to high temperatures; their concentrations increase over time with increase in temperature (Castro-Várquez et al., 2007).

Table 3. Volatile compounds identified in n-hexane extract of GC honey obtained from Gauteng.

Organic compounds	Area percentage of compound in each fraction		
	n-hexane extract of GC honey		
	F1	F2	F3
Hexane	-	21.26	-
2-propanone	8.05	0.44	5.54
Cyclopentanone	-	-	0.64
Butanal	71.22	-	-
Acetic acid	11.58	8.07	89.13
Propanoic acid	0.52	-	-
1-propanol	-	37.85	-
1-(1-cyclopentenyl)-propanol	0.40	-	-
1,3 Butanediol	0.27	-	-
Oxiranemethanol	-	0.18	-
Furfuryl alcohol	3.56	-	1.56
5-Hydroxymethylfurfural	0.17	-	-
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	0.80	0.48	3.13
1,3-benzenediamine	1.49	-	-
Methanamine hydrochloride	-	31.75	-
Propanenitrile	1.94	-	-

GC, Goldcrest; F, fractions.

Table 4. Volatile compounds identified in chloroform extract of CRT honey obtained from Western Cape Province.

Organic compounds	Area percentage of compound in each fraction		
	Chloroform extract of CRT honey		
	F1	F2	F3
Butanal	-	-	5.55
Hexane	54.83	17.86	4.56
Methanol	45.17	-	-
Methanamine hydrochloride	-	82.14	89.89

CRT, Champagne royal train; F, fractions.

Despite the negative implications due to the presence of these compounds, furfuryl alcohol was identified as a relevant compound in lavender unifloral honey (Cuevas-Glory et al., 2007). Moreover, Barra et al. (2010) mentioned the presence of these furan derivatives in fresh citric honey.

Pyran derivative, example, 2, 3-dihydro-3, 5-dihydroxyl-6-methyl-4H-pyran-4-one was identified in both extracts of PH and GC and recorded an area percentage between 0.48 to 9.2%. Pyran derived compounds are produced by Maillard reaction and are indicative of loss of freshness, which could negatively influence the sensory properties of honey (Castro-Várquez et al., 2006).

Terpenic compounds such as epoxy linalool, linalool and 1-octen-7-methylocta-1, 3(Z), 5(E)-triene were detected only in one extract (PH) with area percentages of 0.20, 0.17 and 0.15 respectively. Castro-Várquez et al. (2007) reported that Spanish citric honey extracts were rich in terpene and its derivatives therefore they are indicated to be the most significant floral markers for citrus honey.

Benzene derivatives such as toluene, 1, 2-dimethyl and 1, 3 dimethyl benzene and 1, 3-benzenediamine were identified in PH with an area percentage that ranged from 0.23 to 1.69%. It was only 1, 3-benzenediamine that was detected in GC extract and it recorded an area percentage of 0.95%.Though not identified in this study, some benzene derived compounds such as benzene acetaldehyde and benzaldehyde have been reported as relevant compounds of lavender, acacia and rosemary honey and are said to be present in most European and Australian honey from a wide variety of floral origin (Castro-Várquez et al., 2007).

Honey is greatly appreciated by consumers, not only for its nutritive properties but also for its sweet taste and characteristic aroma (Soria et al., 2003). Aroma is one of the most sought-after properties of honey that is produced by the complex mixtures of volatile compounds of different functionality and relatively low molecular weight (Soria et al., 2003). Not all volatile compounds

have a significant impact on honey's aroma; however, some compounds even though present in low concentrations can equally contribute significantly to honey's aroma (Castro-Várquez et al., 2007). Clearly, the contribution of a given compound depends on the extent to which the concentration exceeds its odor threshold and it is determined by calculating its odor activity value (OAV).This is done by dividing the concentration of the compound by its perception threshold (Castro-Várquez et al., 2007).

For example Wardencki et al. (2009) described the sweet, citrus, forest geranium flavor of honey to be due to the presence of linalool compound. Seemingly, the ripe fruit and spicy aroma of heather honey has been attributed to the occurrence of benzene and phenolic compounds (Castro-Várquez et al., 2009).Castro-Várquez et al. (2007) equally demonstrated that the floral, fresh and orange-like aroma of Spanish citrus honeys was due to available sinensal isomers and linalool derivatives.

Furthermore, it is most probable that the volatile fraction could be useful in identifying the botanical and geographical origin of honey samples. This is because typical volatile components can be identified for honeys from some definite floral sources; such compounds are specified as floral markers of the corresponding honey (Kaskonienė et al., 2008). Also, the floral origin could be determined by a greater concentration of certain compounds in some types of honey than in others or by the absence of determined compounds. However, there is discrepancy over which volatile compounds should serve as markers for a given honey because of differences between plant varieties, geographical origins, or beekeeping practices. Notwithstanding, the floral origin of honey should be determined based on plants derived compounds and their metabolites including norisoprenoids, terpenes and its derivatives and benzene and its derivatives (Castro-Várquez et al., 2006).

However, it is clear that the methods used in extracting the volatile fraction may show varying degree of selectivity and effectiveness depending upon the compounds involved (Alissandrakis et al., 2005). In our study, solvents were used to extract and purify the volatile compounds. These methods were simple to operate but hazardous since large quantities of toxic and expensive solvents were used as well as being labor intensive and time-consuming (Pontes et al., 2007).

It is believed that the solvents have a negative implication on the volatile composition; they could have covered some of the compounds causing them to elude the GC column without being detected as well as solubilized non-volatile compounds that could have contaminated the GC port. Moreover, some volatile compounds could have been lost during solvent removal (Cuevas-Glory et al., 2007). In addition, heat was employed in an attempt to remove the solvents from the extracts and fractions collected and as such it could generate artifact (Alissandrakis et al., 2005).

Albeit, volatile compounds identified in honeys obtained from South Africa have been reported in other honey studies but with the exception of these three compounds; thiophene, N-methyl-D3-Arizidine and methanamine hydrochloride. Thiophenes are important heterocyclic compounds endowed with numerous biomedical activities and as such are used as synthetic intermediates in the pharmaceutical industry for the production of drugs with an array of biological activities including antibacterial, antifungal, anti-viral and anti-inflammatory effects (Lednicer, 1999; Bondock et al., 2010). They equally serve as building blocks of products for agrochemical, dyestuffs and electronic applications.

Similarly, aziridines, the nitrogenous analogs of epoxides, are a versatile class of natural/synthetic organic compounds sharing the aziridine functional group which is a three-membered heterocycle (Padwa and Murphree, 2006). They are highly reactive due in part to ring strain and serve as useful intermediates in the synthesis of organic compounds, natural products and pharmaceuticals such as kainoids, actinomycin, feldamycin, azinomycin, mitomycin and a host of others. The biological properties including antibiotic and antitumour properties of several of these compounds are well known (Ismail et al., 2009).

These newly identified compounds could be part of the antibacterial components responsible for the potent antibacterial activity revealed by the chloroform extract of PH (Manyi-Loh et al., 2010), even though the extracts of PH and GC presented compounds of same chemical groups. Further studies are needed to characterize the aroma and floral origins of these honeys in South Africa in order to standardize their quality, to avoid fraud and to authenticate them.

ACKNOWLEDGEMENTS

We are grateful to the Govan Mbeki Research and Development Centre, University of Fort Hare, South Africa for funding. We also thank Mrs Okoh O and Mr. Okoh S for technical assistance.

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