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Original article

Volatile aromatic composition and antimicrobial activity of different types of honey

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Summary. Total phenol contents and volatile aromatic compounds of five types of honey (pine, french lavender, chestnuts, thyme and milk vetch) were determined by Folin–Ciocalteau and gas chromatography-mass spectrometry (GC-MS) method, respectively. The honey samples were diluted in various concentrations (0%, 25%, 50%, 75% and 100%) and subjected to zone of inhibition test for their antimicrobial activity against eight common food-borne pathogens (*Enterococus faecalis* ATCC29212, *Staphylococcus aureus* ATCC29213, *Klebsiella pneumoniae* ATCC700603, *Camplylobacter jejuni* ATCC 33560, *Listeria monocytogenes* ATCC7677, *Aeromonas hydrophila* NCIMB 1135, Salmonella Parathypi A NCTC13 and *Yersinia enterocolitica* NCTC 11175). Total phenol contents of honeys were above 13.51 mg GA/g. A total of 64 compounds were identified, including 27 in French lavender honey, 19 in pine and thyme honey, 17 in chestnuts honey and 12 in milk vetch honey. From among the 64 identified constituents, only octane, trans-linalool oxide and cis-linalool oxide were found in all analysed samples. γ-decalactone were also present in all honey samples apart from French lavender and chestnuts honey. Chestnuts, pine and milk vetch honey showed a strong antimicrobial effect on food-borne pathogens. The highest inhibitory effects of honey were observed on *L. monocytogenes* (>12 mm) and *Staph. aureus* (>4 mm). *S*. Paratyphi A and *Y. enterocolitica* were inhibited only by milk vetch honey (8.33 mm) and thyme honey (2.83 mm) at doses of 75%, respectively. The inhibition of *C. jejuni* was solely observed by undiluted pine and chestnuts honey. The study results emphasized the potential importance and use of chestnuts, pine and milk vetch honey as food and ingredients in various food preparations due to their antimicrobial activity and chemical composition.

Key words: honey, chestnuts, pine, aromatic compounds, antimicrobials

Introduction

Honey is a sweet, sticky substance that is formed by bees following the collection of nectar and honeydew (1,2). The main chemical constituents of honey are sugars and water (>95%) (3). The major carbohydrates found in honey are also fructose (38.5%) and glucose (31%), with maltose, sucrose, and other sugars making up the remaining 12.9% (4). Proteins, flavors, pigments, vitamins, free amino acids, and numerous volatile compounds contribute minor components which is mainly responsible for honey's organoleptic and nutritional properties (3).

Volatile compounds in honey produced from plant components via the direct generation of aromatic compounds by bees, as well as thermo-generation of aromatic compounds and the action of microorganisms (3,5,6). As volatiles in honeys of different floral types, more than 400 compounds have been reported (7). The volatile compounds in honey includes hydrocarbon; aldehyde; alcohol; ketone; acid; ester; benzene and its derivatives, furan and pyran; norisoprenoids; terpenes and its derivatives and sulphur; and cyclic compounds (6,8). The composition of honey varies depending on mostly the floral region, as the nectar from different plants which contain different compositions of the

main sugars and trace elements (9). In addition, variations occur in the level of volatile components found in honey during storage as a result of the temperature at which it is exposed and also the period of exposure (8). Volatile compounds are useful in linking honeys to their floral source, as the volatile fraction of honey can originate from the plant from which it was produced (10). Some components are unique to particular types of honey only (11).

Honey is used as a food preservative (12) and a dressing for chronic wounds, burns, or skin ulcers because of its antibacterial activity (2). It has also some biological properties such as antioxidant, antiviral, anti-inflammatory, antiulcerous, immunomodulating, vasodilative, hypotensive, antihypercholesterolemic, antibrowning, disinfectant, and antitumour, and many of its applications, may be attributed to their minor components (13,14). Studies have shown the broad-spectrum antibacterial effect of honey for several bacteria including, aerobes and anaerobes and gram-positive and gram negative (15,16). The antimicrobial activity of honey is attributed largely to osmolarity, pH, hydrogen peroxide production and the presence of other phytochemical components such as flavonoids and phthalic acid (17). Many study results indicated 'non-peroxide' antibacterial activity of some honeys (1,18). The bactericidal effect of honey is reported to be dependent on concentration of honey used and the nature of the bacteria (9).

Campylobacter, *Salmonella*, *L. monocytogenes*, and *Escherichia coli* O157:H7 are known the most common bacteria to be responsible for majority of food-borne outbreaks, although there are various food borne pathogens that have been identified for food borne illness (19,20). *L. monocytogenes* is a gram-positive intracellular pathogen which has been implicated as the causative organism in several outbreaks of foodborne disease which is listeriosis, with a mortality rate of about 24%, found mainly among pregnant women, their fetuses, and immunocompromised persons (21). *Campylobacter* infections in humans orginate from poultry contamination (22). It cause bacterial food-borne diarrhoeal disease throughout the world (23). *K. pneumoniae* is also an important opportunistic pathogen that causes various types of extraintestinal infections in both the community and hospitals (24). The use of chemical preservatives to control of food borne pathogens in food has increased consumer concern, leading to a desire for more natural and minimally processed foods (25).

The aim of the current study was to investigate total phenol and volatile aromatic compounds present in five different types of honey (pine, french lavender chestnuts, thyme and milk vetch) as well as their antimicrobial activity against eight common food-borne pathogen (*Enterococus faecalis* ATCC29212, *Staphylococcus aureus* ATCC29213, *Klebsiella pneumoniae* ATCC700603, *Camplylobacter jejuni* ATCC 33560, *Listeria monocytogenes* ATCC7677, *Aeromonas hydrophila* NCIMB 1135, *Salmonella* Parathypi A NCTC13 and *Yersinia enterocolitica* NCTC 11175).

Material and Method

Honey samples

Five honey including pine, french lavender, chestnuts, thyme and milk vetch were provided by the beekeepers in July-Semptember 2018. Triplicate samples were taken to estimate chemical compositions and antimicrobial activity of honeys. The samples were stored at room temperature (25°C) and kept sealed until analysis.

Food-borne pathogens

Enterococus faecalis ATCC29212, *Staphylococcus aureus* ATCC29213, *Klebsiella pneumoniae* ATCC700603, *Camplylobacter jejun*i ATCC 33560 and *Listeria monocytogenes* ATCC19112 were puchased from the American Type CultureCollection (Rockville, MD, USA). *Aeromonas hydrophila* NCIMB 1135, *Salmonella* Parathyphi A NCTC13 and *Yersinia enterocolitica* NCTC 11175 were obtained from the National Collection of Type Cultures (London, UK) and the National Collections of Industrial Food and Marine Bacteria (Aberdeen, UK)

Total phenol content

Total phenol content of honeys was determined using a spectrophotometric Folin–Ciocalteau method (26) with minor modifications. The samples were prepared in triplicate for each analysis and the mean value of absorbance was measured. Results are reported at mg gallic acid equivalent (GAE)/g of honey samples.

Analysis of volatile aromatic compounds

Fifty grams of each honey samples were taken for solid phase microextraction (SPME). SPME fiber (Supelco) precoated with a 100 μm layer of polydimethylsiloxane (PDMS) was used. The SPME fiber was placed into a vial for 30 min at 30°C by stirring. The SPME syringe was then inserted into the injector port of the GC/MS instrument for analysis.

An a Shimadzu QP 5050A apparatus equipped with a CP Sil 5CB (25 mx0.25 mm *i.d.)* fused-silica capillary column were used to separate volatile compounds. Carrier gas was helium (1ml/min). The injector temperature was 250 °C, set for split less injection. The oven temperature were arranged to 50 °C for 1 min and then increased to 200 °C at a rate of 4 °C/min. Thermal desorption was set for 1.5 min. The detector temperature was 280 °C. The components were detected by comparison of mass spectra and retention time data with those of samples and complemented with a Wiley and GC-MS library.

Preparation of honey concentrations

For antimicrobial activity test, undiluted (100%) and diluted honey in different concentrations were used. Diluted honey samples were prepared at three concentrations: 25%, 50% and 75% (v/v). This was done by dissolving the respective volumes: 0.25mL, 0.5mL and 0.75mL of each honey into corresponding volumes of sterile distilled water to give a 1mL preparation.

Antibacterial activity

Antibacterial activities of honey were carried out using well diffusion method on Mueller-Hinton Agar (MHA, Merck 1.05437, Darmstadt, Germany). The test bacteria were incubated in Nutrient broth (Merck 1.05443, Darmstadt, Germany) at 37°C for 24 h. For diffusion method, petri plates were prepared by pouring 20 ml of MHA and inoculated with 24 h broth culture of pathogenic bacteria under aseptic conditions after matching the turbidity with 0.5 Mc Farland. After solidified, five wells were made. Fifty microliters of honey samples prepared at different concentrations (25%, 50%, 75% and 100%) were poured in each of the four wells of MHA plates. Fifth (control well) was filled with distilled water. The plates were kept for 1 h at room temperature to allow the diffusion into the medium and then incubated at 37°C for 24 h. After the incubation period, the inhibition zones formed around the wells were measured in milimeter.

Statistical Analysis

To find the average value and standard deviation, the data obtained from the three samples for each honey was used. The significance of differences ($P<0.05$) was determined using Duncan's multiple comparison test with SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL. USA).

Result and Discussions

Total phenol content

Total phenol contents of honeys were found as 14.47, 14.32, 13.75, 13.54 and 13.51 mg GA/g for pine, chestnuts, milk vetch, thyme and French lavender, respectively. Total phenolic content of honeys tested were considerable higher than that of values previously reported for honeys collected from different regions (27,28). Santana et al. (2014) found that total phenolic compounds in various honey samples varied from 5.82 to 15.25 mg GA/g of honey, with an average value of 10.28 mg GA/g. The lowest total phenolic content was reported for visually light-coloured Eucalyptus honey. The high total phenol content of honeys analysed may indicate their good antimicrobial and antioxidant properties. The contents of phenolic compounds were significantly affected by kind of honey, location, and date of the honey harvest (30). Honeys with dark colour was also shown to have a higher total phenolic content and thus, a higher antioxidant capacity (31).

Chemical composition

Identified volatile aromatic compounds in five different types of honey were given in Table 1. A total of 64 compounds were identified, including 27 in French lavender honey, 19 in pine and thyme honey, 17 in chestnuts honey and 12 in milk vetch honey. From among the 64 identified constituents, only octane, trans-linalool oxide and cis-linalool oxide- were found in all analysed samples. Moreover, γ-decalactone

Table 1. Volatile aromatic compounds in different types of honey

Compounds		Pine honey	French lavender honey	Chestnuts honey	Thyme honey	Milk vetch honey
	RT			Peak area (%)		
Methyl-butyrate	3.28	-	0.03	-	-	-
2-Butene, (Z)-	3.34	-	0.14	-	-	-
Ethanol	3.40	-	0.28	-	-	-
Formic acid	3.47	-	1.13	-	0.94	-
Isoamyl alcohol	3.54	-	-	-	-	-
Acetic acid	3.86	-	2.74	-	-	-
Iso amyl formate	4.54	-	0.12	-	-	-
Iso amyl alcohol	3.87	-	-	-	-	-
Acetic acid, methyl ester	4.03	-	-	0.4	-	-
Vinylbutanol	4.21	-	-	0.26	-	-
Octane	4.72	5.08	8.14	0.81	6.31	9.95
Butyl acetate	5.06	0.48	-	-	-	1.04
Trans-2-hexenol	6.48	-	4.05	-	-	-
Pimelic ketone	7.29	0.09	-	-	-	-
2-acetyl-furan	7.78	0.11	-	0.32	-	-
1-(2-furanyl)- Ethanone	7.80	-	-	-	0.15	-
2(5H)-furanone	7.83	-	-	0.89	-	-
4-Chloro-butanoic acid	8.06	-	-	-	0.17	-
Butyrolactone <gamma->	8.47	-	0.1	-	-	-
Furfural <5-methyl->	9.41	-	-	1.56	0.39	-
Benzaldehyde	9.43	-	0.48	-	-	-
Hexanoic acid (CAS)	10.12	-	-	1.1	-	-
Isocaproic acid	10.19	0.81	-	-	-	-
Hexanoic acid (CAS)	10.21	-	-	-	0.53	0.41
3-Methyl-valeric acid	10.35	-	1.62	-	-	-
Sulcatol	10.38	-	-	-	0.32	-
Pseudolimonene	10.87	-	-	0.4		-
Para-cymene	11.36	-	-	0.25		-
Benzeneacetaldehyde	11.91	-	-	-		0.08
Phenylacetaldehyde	11.92	-	0.36		0.72	-
γ-Hexalactone	12.50	-	0.1		-	-
α-Phenylethanol	12.53	-	-	0.16	-	-
trans-Linalool oxide	12.92	0.26	2.56	2.24	3.19	1.09
cis-Linalool oxide	13.43	0.2	1.1	1.27	1.18	0.33
Nonanal	13.86	0.41	-	-	0.41	0.4
Pelargonaldehyde	13.98	-	0.23	0.77	-	-
2-ethyl- Hexanoic acid,	14.42	-	-	-	0.16	0.07
2-Cyclohexen-1-one, 3,5,5-trimethyl	14.48	-	0.06	-	-	-
4-Hydroxy-5-Oxohexanoıc Acıd Lactone	14.59	-	-	0.38	-	-

Table 1. Volatile aromatic compounds in different types of honey

Compounds		Pine honey	French lavender honey	Chestnuts honey	Thyme honey	Milk vetch honey
	RT			Peak area (%)		
Oxophorone	15.09	-	-	-	-	0.11
4-Ketoisophorone	15.10	-	0.12		0.14	-
endo-Borneol	15.83	0.15		0.26	-	-
Isoborneol	15.85	-	-	-	0.12	-
Octanoic acid	16.02	-	0.1	-	0.16	-
5-methyl-2-(1-methylethyl)-Cyclohexanol	16,06	0.23	-	-	-	-
Pelargol	16.12	-	-	0.6	-	-
α-terpineol	16.60	0.34	-	-	-	-
Dodecane	16.79	0.31	-	-	-	-
Capraldehyde	16.98	-	-	-	0.14	0.09
Verbenone	17.24	0.05	-	-	-	-
Linalyl formate	17.39	-	0.25	-	-	-
Hydroxy methyl furfural	17.74	-	-	0.42	-	-
L-Citronellol	17.81	-	0.07	-	-	-
Heptylidene acetone	17.82	0.13	-	-	-	-
Verdox	19.63	0.15	-	-	-	-
Tridecane	19.73	-	0.02	-	-	-
3,4,5-trimethyl-Phenol	20.25	-	0.13	-	0.12	-
4-tert-Butylcyclohexanol	21.74	0.02	-	-	-	-
allyl-Pelargonate	22.18	-	0.02	-	-	-
γ-Decalactone	24.39	0.06	0.07	-	0.05	0.04
Viridiflorene	24.78	-	0.02	-	-	-
Butylated hydroxytoluene	25.55	0.1	-	-	-	-
Hexadecane	27.63	-	0.02	-	-	-
2-methyl-Propanoic acid	27.63	1.08	-	-	0.07	0.11

-, not identified; RT: retention time

were present in all honey samples apart from chestnuts honey. Interestingly, nonanal was also one of the flavor components found in most of honey samples except for French lavender and chestnuts honey at level of 0.4%. Pattamayutanon et al. (32) found that *cis*-linalool oxide, *trans*-linalool oxide, ho-trienol, and furan-2,5-dicarbaldehyde were in all the honeys studied, independent of their floral origin. Baroni et al. (3) identified six volatile organic compounds (octanal, benzeneacetaldehyde, 1-octanol, 2-methoxyphenol, nonanal, and 2-H-1-benzopyran-2-one (coumarin)) as the most representative to discriminate among different floral origin of honey samples. The compounds found in the highest percentage of area in ten samples of honey from different apiculturists were ethanol, acetic acid, 1-hydroxy-2-propane, 3-hydroxy-2-butane, and furfural (6).

The major components in pine honey were found as octane, 2-mthyl-propanoic acid and isocaproic acid with corresponding value of 5.08, 1.08 and 0.8%. Pine honey also consisted of butyl-acetate, γ-terpineol, dodecane and 5-methyl-2-(1-methylethyl)- cyclohexanol and minor amount of endo-borneol, verbenone, heptylidene acetone, verdox, 4-tert-butylcyclohexanol and butylated hydroxytoluene. Main component of pine honey was reported as nonanal, benzene, 4-hex-

en-3-ol, alpha-pinene, and 2-heptanone (33), which did not present in any samples analysed.

French lavender was characterized by its high contents of hexanal, heptanol, 2-phenylacetaldehyde and coumarin. In the current study, french lavender honey did not contain coumarin which is in good agreement with the results reported in previous studies (34). French lavender had more diversity of compound than that of other honey tested. The main compounds of the french lavender honey were octane (8.14%), trans-2-hexanol (4.05%), acetic acid (2.74%), trans-linalool oxide (2.56%), 3-methyl-valeric acid (1.62%), formic acid (1.13%) and *cis*-linalool oxide (1.1%). Benzaldehyde, phenylacetaldehyde, pelargonaldehyde, linalyl formate, 3,4,5-trimethyl phenol, 4-ketoisophorone and octanoic acid were other components found French lavender honey with a value ranging from 0.1% to 0.5%. Major flavor compounds in lavender honeys were linear aldehydes, nγhexanol, coumarin, and phenylacetaldehyde (35).

Chestnuts honey consisted of mainly *trans*-linalool oxide (2.24%), 5-methyl-furfural (1.56%), *cis*-Linalool oxide (1.27%), hexanoic acid (1.1%), 2 (5H) furanone (0.89%), octane (0.81%), pelargonaldehyde (0.77%) and pelargol (0.6%). Other compounds found in chestnuts honey at lower amounts were hydroxy methyl furfural, acetic acid, pseudolimonene, 2-acetylfuran, vinylbutanol, para-cymene and endo-borneol. Daher and Gulacar (2) found that cinnamic acid had the highest concentrations (148.8-260.1 μg/100 g of honey) in the two chestnut honey samples and 1-(2 or 3-aminophenyl)- 1-butanone was only present in this type of honey. In the current study, these two compounds was not identified in chestnuts honey.

Major components in thyme was octane, trans-linalool oxide (3.19%), cis-Linalool oxide (1.18%), formic acid (0.94%) and phenylacetaldehyde (0.72%). Sulcatol, 2-ethyl-hexanoic acid, 1-(2-furanyl) ethanone and isoborneol were specific compounds identified only thyme honey. Formic acid, phenylacetaldehyde, 4-ketoisophorone and 3,4,5-trimethyl phenol were also detected only in French lavender and thyme honey. Karabagias et al. (36) reported the existence of a different volatile fraction in commercial thyme honey produced in different Mediterranean countries. Composition of each honey is considerably dependent on the floral source, geographical region, and season, as well as the processing conducted after the harvest (14,37).

Milk vetch honey had the highest octane content (9.95%) that of other honeys. The main compounds of the milk vetch honey were also trans-linalool oxide, butyl acetate and hexanoic acid. Oxophorone and benzeneacetaldehyde were only identified milk vetch honey. This compound was not identified in milk vetch honey previously. Hexanoic acid and capraldehyde were found in two types of honey (thyme and milk vetch). Tian et al. (38) affirmed the presence of octanol, 2-ethylhexanol and isoprene as a marker in milk vetch honey.

Antimicrobial activity

Table 2 shows antibacterial activity of honey samples against eight food-borne pathogen. The antimicrobial efficacy of honey samples on food-borne pathogens changed depending on bacterial strains, type of honey and honey concentrations used, which is in agreement with other studies (9,16). Chestnuts, pine and milk vetch honey seemed to have a strong antimicrobial effect on growth of bacteria tested. Moreover, honey samples generally showed higher antimicrobial activity with higher honey concentrations (75 and 100%). The greatest inhibitory activity against the *Helicobacter pylori* was also reported by mountain honey at 75% concentration while the least was observed with Manuka and Eco- honeys at 20%v/v concentrations (39). Honey samples were generally ineffective against *S*. Paratyphi A, *Y. enterocolitica, E. faecalis* and *C. jejuni*. *S.* Paratyphi A and *Y. enterocolitica* were inhibited only by milk vetch hony (8.33 mm) and thyme honey (2.83 mm) at doses of 75%, respectively. In addition, the inhibition of *C. jejuni* was only observed by undiluted pine and chestnuts honey samples.

Pine, chestnuts and milk vetch were found to be the most active honeys against *K. pneumoniae*, although thyme honey did not have any effect on the growth of this bacteria. The highest inhibition zones were observed for pine honey in ranging value from 11.50 mm (25% honey) to 19.50 (100% honey). Chestnuts honey at concentration of 75% had the highest antimicrobial activity (14.33 mm) than that of other concentrations. de Queiroz Pimente et al. (40) found that the highest inhibition zones were when the samples were applied

Table 2. Diameter of inhibition zone (mm) of different types of honey against common food-borne pathogens

	Pine honey				Chestnuts honey				French lavender honey				Thyme honey				Milk vetch honey			
Concentrations (%)																				
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
K.pneumoniae	11.50^{*xd}	13.83^{c}	16.67^{b}	19.50^{a}	5.17^{d}	9.17^{c}	14.33^{a}	11.00^{b}	-	-	-	8.00	-	-	-	-	5.33^{d}	6.33^{c}	14.00^{b}	17.67^{a}
	0.50^{y}	1.04	0.58	0.50	0.29	0.76	0.29	1.00				0.50					0.29	0.58	0.50	0.58
Staph. aureus	5.83^{c}	14.50^{b}	18.50^{a}	13.50^{b}	-	4.33^{c}	13.67^{b}	15.83^{a}	-	10.67^{c}	13.33^{b}	19.50^{a}	-	-	8.33^{b}	15.33^{a}	5.33^{d}	7.67^{c}	15.67^{b}	22.67^{a}
	0.29	0.87	0.87	0.87	-	0.58	0.58	1.04	-	1.15	0.58	1.32	-	-	0.58	1.15	0.58	0.58	1.53	0.76
S. Paratyphi A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.33	-
																			0.58	
A. hydrophila	3.33^{d}	5.67^{c}	8.33^{b}	12.67^{a}	-	15.33^{b}	22.33^{a}	22.67^{a}	-	-	-	-	-	6.50^{b}	12.67^{a}	-	-	-	-	7.50
	0.58	1.15	0.58	0.58	-	0.58	0.58	0.58	-	-	-	-	-	0.87	0.58	-	-	-	-	0.87
E. faecalis	-	-	-	-	-	-	-	8.33	-	-	-	7.67	-	-	-	-	-	-	-	9.50
								0.58				0.58								0.87
L.monocytogenes	22.00^{d}	24.33^{c}	26.50^{b}	28.67^{a}	20.67^{d}	26.33^{c}	31.67^{b}	37.67^{a}	23.33^{c}	24.83^{c}	28.33^{b}	32.67^{a}	21.67^{c}	30.33^{b}	31.33^{b}	37.00^{a}	12.33^{d}	24.33^{c}	27.67^{b}	33.00^{a}
	1.00	0.58	0.87	1.53	1.15	0.58	0.76	0.58	1.15	0.76	2.31	1.15	1.15	1.15	1.15	1.73	0.58	1.15	1.15	1.00
Y.enterocolitica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.83	-	-	-	-	-
															0.29					
C. jejuni	-	-	-	8.33	-	-	-	4.50	-	-	-	-	-	-	-	-	-	-	-	-
				0.58				0.87												

*Data are expressed as mean value of three samples, [x]Mean value, [y]Standard deviation. [a–d] Indicate significant differences ($P < 0.05$) among groups in a column.

undiluted, except for honey from the stingless bees *M. compressipes manaosensis* against *Shigella sonnei*, which showed the greatest susceptibly when the honey was used at a dilution rate of 50%. In the present study, thyme honey did not affect *K. pneumoniae* growth, although antimicrobial effect of french lavender on this bacteria was found only at 100% concentration, with diameter zone of 8 mm.

Boateng and Diunase (41) found that manuka and cameroonian honeys exhibited 18.7 and 17.0 mm zones of inhibition against *Staph. aureus*. In the current study, *Staph. aureus* was one of the most susceptible bacteria against honey samples tested. The highest inhibitory effects were observed from milk vetch honey and french lavender at doses of 100% (22.67 vs. 19.50 mm), followed by pine honey at doses of 75% (18.50 mm). Apart from pine and milk vetch honey, 25% honey concentration did not inhibit bacterial growth, whilst other concentrations tested reflected good antimicrobial activity. Kgozeimeh et al. (42) indicated that milk vetch flower honey could be used as a natural antibiotic due to the reasonable antibacterial effect against *Staphylococcus mutans* and *Lactobacillus* spp. strains. In the current study, milk vetch honey showed best antimicrobial activity against *K. pneumoniae*, *Staph. aureus* and *L. monocytogenes*. Apart from *E. faecalis*, Gram positive *Staph. aureus* and *L. monocytogenes* were more sensitive to honey samples. Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan many times thicker than is found in the Gram-negatives (43). Thus, antimicrobials cannot pass through outer layer of Gram-negative bacteria easily (44).

Jayanthi and Asokan (9) reported that 100% dilution of honey sample obtained from local bee keepers in three different places showed maximum zone of inhibition (14 mm) against methicillin resistant *Staph. aureus*. Commercial honey was also effective to inhibit *Staph. aureus* at the concentration of 375 mg/ml (15). Chestnut honey showed high antimicrobial activity against *Staph. aureus* whilst thyme honey had no activity against *Staph. aureus* (45). However, in the current study, thyme honey exerted 8.33 and 15.33 mm inhibition zone

against *Staph. aureus* at doses of 75 and 100%, respectively. The average diameter of the inhibition zones of 100% and 75% acacia honey was 12.48 and 11.06 mm, while horse chestnut honey (100%) had 11.08 mm inhibition zone against *Streptococci* isolates (46).

L. monocytogenes was the one of the most sensitive microorganism against all honey samples used, while low inhibitory activities were evidenced against *E. faecalis* with respective diameter zone of 8.33, 7.67 and 9.50% for chestnuts, french lavender and milk vetch honey only at doses of 100%. Honey samples were effective in the inhibiton of *L. monocytogenes* growth at all doses used. Inhibition diameter zones of honey sampes were in range from 12.33 mm for milk vetch at doses of 25% to 37.67 mm for chestnuts honey at doses of 100%.

Chestnuts honey had stronger antimicrobial activity against *A. hydrophila* with ranging value from 15.33 mm for 50% honey to 22.67 mm for 100% than that of other honey. The inhibition zones of thyme and milk vetch against *A. hydrophila* were only observed when the samples were applied undiluted or at doses of 75%. French lavender honey did not possess any antimicrobial activity against *A. hydrophila*. The minimum concentration of a sugar solution for inhibition of the growth of most pathogenic bacteria required is reported as 29% (w/v) along with a water activity value (aw) of between 0.86 and 0.89, which are equivalent to a 22% honey concentration (40). Antibacterial activity of honey is also probably attributed to osmolarity, pH, hydrogen peroxide production and the ability of flavonoids to form complexes with soluble proteins and with the bacteria cell wall (17,40). Some studies reported that depending on the bacterial species, the honey's mechanism of action is linked with effects of the bacterial size (shorter or longer cells), morphology, cell division (incomplete division), surface (irregular), motility, injury to nucleic acids, and lysis (14).

Conclusion

The study results revealed that pine, chestnuts, french lavender, thyme and milk vetch honey were rich in volatile aromatic compounds. From among identified constituents, only octane, trans-linalool oxide and cis-linalool oxide- were found in all analysed samples. Chestnuts, pine and milk vetch honey seemed to have a strong antimicrobial activity against most of bacteria tested. *L. monocytogenes* and *Staph. aureus* were the most sensitive food-borne pathogens against honeys tested, whilst honey samples were generally ineffective against *S.* Paratyphi A, *Y. enterocolitica, E. faecalis* and *C. jejuni*.

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Conflict of interest statement

The author declare no conflicts of interest.

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