Research Article

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Presence of short and cyclic peptides in Acacia and Ziziphus honeys may potentiate their medicinal values

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Abstract: Acacia honey is characterized by high nutritional, antioxidant, antibacterial and immuno-modulatory values. This work investigated the presence of short and cyclic peptides in Acacia and Ziziphus honey samples. Acacia honey samples (*Acacia tortilis* and *Acacia hamulosa*) and three Ziziphus honeys (*Ziziphus spina-christi*) were screened for their short and cyclic peptide contents using the LC-MS and the chemical structure databases. Moreover, the total protein content was determined using the Bradford method. The *A. tortilis* honey contained three short peptides; HWCC, DSST, and ECH, and the *A. hamulosa* honey sample contained five short peptides and one cyclic peptide. The short peptides of the *A. hamulosa* honey were Ac-GMGHG-OH (Ac-MGGHG-OH), Boc-R(Aloc)2-C(Pal)-OH, H-C (1)-NET2-H-C (1)-NET2, APAP (AAPP), and GAFQ (deamino-2-pyrid-4-yl-glycyl-

DL-alanyl-DL-norvalyl-DL-asparagine). The cyclic peptide of the *A. hamulosa* honey was *cyclo*[Aad-RGD-D-F] (*cyclo* [Aad-Arg-Gly-Asp-D-Phe]). The Ziziphus honey was characterized by the presence of either Almiramide B or Auristatin-6-AQ. *A. tortilis*, *A. hamulosa*, and Ziziphus honeys are characterized by the presence of short and cyclic peptides which may contribute to their medicinal values.

Keywords: bioactive peptides, LC-MS, PubChem, ChemSpider, Molbase

1 Introduction

Nutritionally, honey is considered as energy food since it is majorly composed of carbohydrates and it is used in infants and children feeding to boost their growth. Moreover, honey contains some amounts of vitamins, minerals, amino acids, enzymes, and phenolic compounds which qualify it to act as antioxidant and to boost the athletic performance, immune system, and digestion and absorption. Medicinally, honey is used for wound healing due to its high sugar content, low moisture percentage, hydrogen peroxide, gluconic acid, and dicarbonyl molecules including the methylglyoxal. Furthermore, honey is used to treat disorders of hematology and immunity, metabolism and cardiovascular system, oral health, ophthalmology, and gastrointestinal tract beside its usage as anticancer (chemotherapy) and antimicrobial [1,2].

Honey is characterized by proteins such as the enzymes which originate from the honeybees or the plants' nectars and secretions. The protein content of honey is associated with its medical and pharmaceutical value [3,4].

Short or bioactive peptides are composed of small number of amino acids in foods. The bioactive peptides are mostly produced by enzymatic hydrolysis of large proteins from animal and plant origins. Milk and its

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products, eggs, meat, marine organisms, spinach, soybeans, and cereal grains are the best examples of bioactive peptides containing foods. Some bioactive peptides are chemically synthesized and added to foods for the purpose of increasing their medicinal value. Short peptides affect different body systems including the cardiovascular, endocrine, immune, nervous, and digestive systems. The presence of short peptides in foods prevents their oxidation and degradation by microbes [5–8].

Cyclic peptides are short peptides with ring structure due to the binding of its amino terminal to its carboxyl terminal by amide bond or other chemical bonds such as the ether, disulfide, and lactone bonds. Cyclic peptides are reported to be found in roasted coffee, cocoa, and malt beside their presence in milk, beverages, chicken, and fermented foods. Biological activities of cyclic peptides include antibacterial, antitumor, immunosuppressive and antioxidant activities. Known functions of cyclic peptides are the *cyclo* (–Phe–Phe) of chicken essence which inhibits the serotonin transporter and the acetylcholinesterase and the *cyclo* (–His–Pro) which inhibits rat's food intake and reduces their body weight [5,9].

This article measured the concentration of total proteins and investigated the presence of short and cyclic peptides in Acacia and Ziziphus honey samples after they were authenticated with regard to their floral origin and some quality parameters.

2 Materials and methods

2.1 Study design

This study is an observational descriptive study. The disadvantages of descriptive studies include the small number of samples and the difficulty in deriving a general conclusion. However, descriptive studies are useful since they highlight research areas for survey studies [10].

2.2 Honey samples and their authentication

Two Acacia and three Ziziphus honey samples were collected directly from the bee farms and their hives. The samples were involved in this study after confirming their floral origin and their conformance to some of the international standards for honey. The two Acacia honeys were collected during the flowering seasons of *A. tortilis* and *A. hamulosa*, while the dominant Ziziphus tree in the

study area was *Z. spina-christi*. All the honey samples were collected from Asir region at the southwestern part of Saudi Arabia.

The Ziziphus 3 honey samples were collected from bee farms at sea level altitude, while the *A. hamulosa*, Ziziphus 1, and Ziziphus 2 samples were harvested from bee farms at 900 m above sea level. *A. tortilis* was harvested at 2,000 m above sea level. The flowering season of the *Z. spina-christi* and *A. hamulosa* ranges from September to November, while the flowering season of *A. tortilis* is from March to July [11,12].

The floral origin of the honey samples was determined following the method published by Louveaux et al. [13]. The moisture, pH and acidity, conductivity, and diastase activity were determined according to the methods of International honey commission, (2009) [14], while the glucose, fructose, sucrose, and HMF were measured following the methods of Agilent company.

2.3 Determination of total protein concentration

The total protein concentration was determined in the studied samples using the spectrophotometric method of Bradford (1976) [3]. The protein in the samples (50% W/V; 100 µL) was reacted with coomassie brilliant blue (5 mL) and the absorbance was measured at a wavelength of 595 nm. Albumin was used in preparation of standard curve (0–500 µg/mL).

2.4 Liquid chromatography-mass spectrometry (LC-MS)

The chemical constituents and the presence of short peptides in honey samples were investigated using the LC-MS. Reverse phase elution was used (Waters Symmetry LC18 column 250 × 4.6 mm, 5 µm) on Agilent 6500 Series Accurate-Mass Quadrupole Time-of Flight (Q-TOF; Agilent CA, USA); Chemical structure databases search was carried out to identify the molecular formula and structure of the spectra obtained. LC-MS system with Agilent 1200 Series Diode Array Detector (module G1315B; detection type: 1,024-element photodiode array; light source: deuterium and tungsten lamps; wavelength range 190–950 nm). The mobile phase was composed of (A) formic acid (0.1%, v/v); (B) acetonitrile + 0.1% formic acid; gradient (in solvent B): (i) 20%, from 0 to 20 min, (ii) 95%, from 20 to 27 min, and (iii) 35%, at 27–30 min of total run time; flow rate was 0.2 mL/min; and injection

volume was 3 L. The ESI parameters were both negative and positive ion modes, mass range 100–1,200 m/z, spray voltage 4 kV, gas temperature 325°C, gas flow 10 L/min, and Nebulizer was 40 psi. The Agilent technologies Mass Hunter software was used to analyze the mass. Tuning and optimization are carried out before any run and on each single day as recommended by the manufacturer.

2.5 Chemical structure databases search

The molecular formulas obtained from the LC-MS were searched in the PubChem, ChemSpider, and Molbase databases to investigate the possible isomers. However, one short peptide is published by the University of Dortmund-Germany.

2.6 Statistical analysis

The agglomerative hierarchical Cluster analysis of the Statistical Package for Social Sciences (SPSS) was used to group the honey samples according to the values of the physicochemical parameters and the total proteins.

3 Results

The honey microscopic pollen analysis showed that all the honey samples were mono-floral with dominance of one pollen type by more than 60% (Figure 1).

The results of the measured quality parameters were within their ranges in the Codex Alimentarius standards of honey [15] (Table 1). However, some honeys were with marginal diastase activity compared to the standards which may be due to the geographical and climatic conditions.

3.1 Total protein concentration

The R^2 was 0.976, while the equation of the standard curve line was $Y = 0.00142X + 0.042$. The *A. hamulosa* and the *Ziziphus* 2 honey samples had the highest concentration of proteins (Table 1).

3.2 The agglomerative hierarchical clustering

The honey samples were divided into three groups (levels). Level one was composed of *A. hamulosa*, *Ziziphus* 2, and *Ziziphus* 3 honey samples and level 2 contained the honey samples of *A. tortilis* and *Ziziphus* 1. Level 3 involved all the honey samples except *Ziziphus* 1 honey (Figure 2). The clustering analysis grouped the honey samples according to their flowering time in the case of group one (*A. hamulosa*, *Ziziphus* 2, and *Ziziphus* 3). Moreover, the clustering analysis grouped the honey samples according to their altitude (level 3); all the honey samples were from farm at 900–2,000 m above sea level except the *Ziziphus* 3 sample which was from farms at the sea level.

Figure 1: Representative pollens of the studied honey samples.

Table 1: The results of the quality parameters of the honey samples

SN	Quality parameter	<i>A. tortilis</i>	<i>A. hamulosa</i>	<i>Ziziphus 1</i>	<i>Ziziphus 2</i>	<i>Ziziphus 3</i>	Range in CODEX standards
1	Moisture %	14.7 ± 0.51	16.2 ± 0.2	14.8 ± 0.35	16.9 ± 0.49	17 ± 0.5	Less than 20%
2	pH	4.5 ± 0.2	4.2 ± 0.31	5.7 ± 0.21	4.7 ± 0.07	5 ± 0.2	3.4–6.1
3	Acidity (meq acid/100 g)	39 ± 1.7	40 ± 2.1	25 ± 7.1	27.5 ± 3.5	30 ± 0.25	Not more than 50 meq acid/100 g
4	Fructose %	51.4 ± 0.32	43 ± 0.5	54 ± 0.7	41.2 ± 2.1	37.1 ± 0.16	NA
	Glucose %	33.5 ± 0.47	30 ± 0.1	35.6 ± 0.1	35.7 ± 3.4	29 ± 0.5	
	Fructose + glucose %	84.9	73	89.6	76.9	66.1	Not less than 60 g/100 g
5	Sucrose %	0.08 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	1.01 ± 0.21	0.83 ± 0.8	Not more than 5 g/100 g
6	HMF (mg/kg)	0.0 ± 0.0	0.0 ± 0.0	10 ± 1.5	1.3 ± 0.4	15 ± 0.5	Not more than 40 mg/kg
7	Diastase (diastase number – DN)	12 ± 2.6	15 ± 1.8	20 ± 2.5	7.5 ± 0.7	7.9 ± 0.8	Not less than 8 DN (Schade)
8	Total protein (µg/g)	222.54 ± 23.0	560.56 ± 28.1	307.04 ± 21.5	560.56 ± 32.7	518.30 ± 49.8	NA

All the quality parameters were within the CODEX ranges except the diastase which was with marginal activities in the *Ziziphus 2* and 3 samples.

Figure 2: The agglomerative hierarchical clustering of the studied honey samples. The honey samples were clustered according to the values of their quality parameters and protein concentration.

3.3 LC-MS and the search in chemical structure databases

The LC-MS showed that the *Ziziphus* honey samples had one short peptide. Three short peptides were found in the *A. tortilis* honey, while five short peptides and one cyclic peptide were reported for the *A. hamulosa* honey.

3.4 The short peptides of the *A. tortilis* honey

- The three short peptides of the *A. tortilis* honey were:
- (1) HWCC (His-Trp-Cys-Cys) with the molecular formula of $C_{23}H_{29}N_7O_5S_2$ which is retrieved from the ChemSpider database (Table 2 and Figure 3) [16].
 - (2) DSST (Asp-Ser-Ser-Thr) which was obtained from the Molbase database and it had the molecular formula of $C_{14}H_{24}N_4O_{10}$ (Table 2 and Figure 3) [17].
 - (3) The search in the PubChem for the molecular formula of $C_{14}H_{22}N_6O_5S$ showed two short peptide isomers containing three amino acids with different sequences; Gln-Cys-His (ECH) and Cys-His-Gln (CHE) (Table 2 and Figure 3) [18,19].

3.5 The short and cyclic peptides of the *A. hamulosa* honey

The *A. hamulosa* honey was characterized by five short peptides and one cyclic peptide.

Table 2: The short and cyclic peptides of the *Acacia* and *Ziziphus* honey samples

Peak	Analyte peak name	RT min	Precursor m/z	Formula	MS/MS fragment	Proposed peptide fragment	Score	Honey sample	Database	
1	47	548.1764/4.31	4.33	548.1764	C ₂₃ H ₂₉ N ₇ O ₅ S ₂	548.1745 549.1732	His-Trp-Cys-Cys HWCC	98.557	<i>A. tortilis</i>	ChemSpider
2	375	409.1556/9.98	10.01	409.1556	C ₁₄ H ₂₄ N ₄ O ₁₀	408.1879 409.1110 409.1565 410.0937 410.1186 410.1349 410.1595 411.1980 411.1254 412.1389	L-Aspartic acid, L-seryl-L-seryl-L-threonyl-DSST	99.686	<i>A. tortilis</i>	Molbase
3	393	387.1454/10.33	10.32	387.1453	C ₁₄ H ₂₂ N ₆ O ₅ S	386.2884 387.1087 387.1445 387.1984 388.1460 388.1819 388.3217 389.1444 390.1211	Gln-Cys-His (ECH) Cys-His-Gln (CHE)	98.413	<i>A. tortilis</i>	PubChem
4	135	500.1933/5.16	5.15	500.1933	C ₁₉ H ₂₉ N ₇ O ₇ S	499.1383 499.1976 500.1411 500.1340 500.1922 501.1951 502.2071 502.3851 503.0817 503.3925	Ac-Gly-Met-Gly-His-Gly-OH Ac-Met-Gly-Gly-His-Gly-OH Ac-GMGHG-OH Ac-MGGHG-OH	99.811	<i>A. hamulosa</i>	PubChem
5	274	784.4526/9.58	9.58	784.4526	C ₃₈ H ₆₅ N ₅ O ₁₀ S	784.3932 784.4528 784.7388 785.4589 785.5045 786.4598 787.4499	Boc-Arg(Aloc)2-Cys (Pal)-OH Boc-R(Aloc)2-C(Pal)-OH <i>N</i> α- <i>tert</i> -Butyloxycarbonyl-(<i>N</i> δ,ω-diallyloxycarbonyl)-L-arginyl-(<i>S</i> -palmitoyl)-L-cysteine [21].	97.161	<i>A. hamulosa</i>	University of Dortmund
6	709	351.2083/14.54	14.42	351.2083	C ₁₄ H ₃₀ N ₄ O ₂ S ₂	349.8780 350.1882 350.9728 351.1882 351.2357 352.1916 352.2651 353.1786 353.1982 353.2395	L-Cysteine diethylamide (1 → 1')-disulfide compound with L-cysteine diethylamide H-C (1)-NEt2.H-C (1)-NEt2	98.603	<i>A. hamulosa</i>	PubChem

(Continued)

Table 2: Continued

Peak	Analyte peak name	RT min	Precursor m/z	Formula	MS/MS fragment	Proposed peptide fragment	Score	Honey sample	Database
7	939	372.2242/ 15.32M +NH4+	372.2242	C ₁₆ H ₂₆ N ₄ O ₅	353.3566		96.085	<i>A. hamulosa</i>	ChemSpider PubChem
					354.2020				
					371.2996	Ala-Pro-Ala-Pro			
					372.0578	Ala-Ala-Pro-Pro			
					372.2239	APAP			
					372.2960	AAPP			
					373.2275				
8	979	444.1856/ 15.37M +Na+	444.1856	C ₁₉ H ₂₇ N ₅ O ₆	373.3502		98.318	<i>A. hamulosa</i>	PubChem
					374.2260				
					375.2158				
					443.2626	Alfuzosin hydroxy acid			
					443.2857				
					444.1584	Deamino-2-pyrid-4-yl-			PubChem
					444.2676	glycyl-DL-alanyl-DL-			
9	1057	619.2838/ 15.62	619.2838	C ₂₇ H ₃₈ N ₈ O ₉	445.1865	norvalyl-DL-asparagine	98.108	<i>A. hamulosa</i>	PubChem
					445.2777	(Deamino-Gly(4-			
					446.2936	pyridyl)-DL-Ala-DL-Nva-			
						DL-Asn-OH)			
					446.9739	Deamino-G(4-pyridyl)			
					447.3131	DL-A-DL-NV-DL-N			ChemSpider
						Gly-Ala-Phe-Gln			
10	1345	725.4955/ 18.51	725.496	C ₄₀ H ₆₄ N ₆ O ₆	GAFAQ		99.94	<i>Ziziphus-2</i>	PubChem
					619.2836	Cyclo[Aad-Arg-Gly-Asp-			
						D-Phe]			
					620.2860	Cyclo[Aad-RGD-D-F]			
					621.2891	Cyclo[L-alpha-			
					622.5138	homoglutamyl-L-			
						arginyl-glycyl-L-alpha-			
						aspartyl-D-			
						phenylalanyl]			
					725.4441	Auristatin-6-AQ or			ChemSpider
					725.4960				
					725.6715	Almiramide B			
					726.5013				
					726.5954				
					727.5192				
					727.4981				
					727.5192				
					728.5191				

- (I) The five short peptides and their databases were:
1. The PubChem search showed that the molecular formula C₁₉H₂₉N₇O₇S is similar to the molecular formula of two short peptides Ac-GMGHG-OH (Ac-Gly-Met-Gly-His-Gly-OH) and Ac-MGGHG-OH (Ac-Met-Gly-Gly-His-Gly-OH) (Table 2 and Figure 4) [20].
 2. Eisele (2000) [21] did his diploma degree at the university of Dortmund and synthesized a short peptide with the molecular formula of C₃₈H₆₅N₅O₁₀S similar to the molecular formula reported in the *A. hamulosa*

- honey. The short peptide was Boc-R(Aloc)2-C (Pal)-OH (Boc-Arg(Aloc)2-Cys(Pal)-OH) or N α -tert-Butyloxycarbonyl-(N δ , ω -diallyloxycarbonyl)-L-arginyl-(S-palmitoyl)-L-cysteine (Table 2 and Figure 4) [21].
3. The third molecular formula was C₁₄H₃₀N₄O₂S₂ which is corresponding to H-C (1)-NEt2-H-C (1)-NEt2 (L-cysteine diethylamide (1 $\rightarrow$ 1')-disulfide compound with L-cysteine diethylamide) in the PubChem database (Table 2 and Figure 4) [22].

Figure 3: The LC-MS spectra of the short peptides in the *Acacia tortilis* honey sample. The three short peptides were HWCC, DSST, and ECH (CHE). The molecular structure of the HWCC were retrieved from the ChemSpider, Molbase, and PubChem databases [16–19].

4. APAP (Ala-Pro-Ala-Pro) in the ChemSpider database and AAPP (Ala-Ala-Pro-Pro) in the PubChem. The molecular formula was $C_{16}H_{26}N_4O_5$ (Table 2 and Figure 4) [23,24].
5. The fifth short peptide of the *A. hamulosa* honey was with the molecular formula of $C_{19}H_{27}N_5O_6$ which corresponds the sequence of deamino-G(4-pyridyl)-DL-A-DL-NV-DL-N (deamino-Gly(4-pyridyl)-DL-Ala-DL-Nva-DL-Asn-OH) in the PubChem and the sequence of GAFQ (Gly-Ala-Phe-Gln) in the ChemSpider database (Table 2 and Figure 4) [25,26]. Moreover, the $C_{19}H_{27}N_5O_6$ is the molecular formula of Alfuzosin hydroxy acid. However, the Alfuzosin and its metabolites are routine drugs for Benign Prostatic Hyperplasia (BPH) [27].

- (II) The cyclic peptide found in the *A. hamulosa* honey is the *cyclo*[L-alpha-homoglutamyl-L-arginyl-glycyl-L-alpha-aspartyl-D-phenylalanyl] (*cyclo*[Aad-RGD-D-F]) with the molecular weight of $C_{27}H_{38}N_8O_9$. The LC-MS molecular formula was searched in the PubChem database (Table 2 and Figure 4) [28].

3.6 The short peptide of the *Ziziphus* honey samples

The *Ziziphus* 2 honey sample contained a short peptide with the molecular formula of $C_{40}H_{64}N_6O_6$. In the PubChem, the short peptide is registered as Auristatin-6-AQ, a

Figure 4: The LC-MS spectra of the short and cyclic peptides in the *Acacia hamulosa* honey sample. Five short peptides were found; Ac-GMGHG-OH or Ac-MGGHG-OH, Boc-R(Aloc)₂-C(Pal)-OH, H-C (1)-NEt₂-H-C (1)-NEt₂, APAP or AAPP, and deamino-G(4-pyridyl)(DL-A-DL-NV-DL-N or GAFQ). The cyclic peptide: *cyclo*[L- α -homoglutamyl-L-arginyl-glycyl-L- α -aspartyl-D-phenylalanyl] (*cyclo*[Aad-Arg-Gly-Asp-D-Phe]). The molecular structures of the studied compounds were cited from [20–28].

dipeptide containing two methylated valine residues [29]. The ChemSpider and PubChem search showed that the molecular formula is for Almiramide B, a short peptide with phenylalaninamide, three methylated valine residues and one methyl alanine (Table 2 and Figure 5) [30,31].

4 Discussion

All the studied quality parameters of the honey samples were within the ranges of the Codex Alimentarius standards except the diastase activity of two *Ziziphus* honey samples which was in the lower margin. The marginal activity of the diastase enzyme may be due to the climatic conditions of Tehama (Geographical region). The *Ziziphus* honey had no short peptides, while the two *Acacia* honeys contained short and cyclic peptides.

The first short peptide of the *A. tortilis* honey is the (His-Trp-Cys-Cys) which is available in the ChemSpider database [16]. It proposed that presence of cross links between His-Cys and Trp-Cys play a regulatory role of host enzymes [32]. Dipeptides that possess tryptophan, tyrosine, cysteine, or methionine have antioxidant activities [33].

The L-aspartic acid-L-seryl-L-seryl-L-threonyl (DSST) (CAS No.: 748808-93-7) of the *A. tortilis* honey sample has the molecular formula of C₁₄H₂₄N and is registered in the Molbase database [17]. Proteins and short peptides containing Asp, Ser, and threonine are reported to act as binders and transporters of cations and their related substrates [34,35]. Thus, the presence of the DSST short peptide in the *A. tortilis* honey may be an indication for its mineral content.

The *A. tortilis* honey contained a third short peptide identified as Gln-Cys-His (ECH) [18] or Cys-His-Gln (CHE) [19]. No published article containing the three amino acids was retrieved. However, Cys-His are known to be responsible for acetyl transfer reactions in enzyme mimics [36].

Concerning the short peptides of the *A. hamulosa*, the *N*-acetyl-L-methionyl-glycyl-glycyl-L-histidyl-glycine is the first identified short peptide in the PubChem of the national center for biotechnology information [20]. Peptides that contain the sequence of Gly-Gly-His are known to act as copper binding proteins [37–39]. Moreover, it is reported that presence of tyrosine, tryptophan, methionine, lysine, cysteine, and histidine in a short peptide increases its antioxidant activity [40].

Figure 5: The short peptide of the *Z. spina-christi* honey. The short peptide was defined as Auristatin-6-AQ or Almiramide B [29–31].

N α -*tert*-Butyloxycarbonyl-(*N* δ , ω -diallyloxycarbonyl)-L-arginyl-(*S*-palmitoyl)-L-cysteine (Boc-Arg(Aloc)2-Cys(Pal)-OH) was the second short peptide of the *A. hamulosa* honey. A search of the literature showed that this short peptide is synthesized for the purpose of identifying the function of viral proteins and their modified forms [21]. Cysteine residues of short peptides and their position are well-known to possess an antioxidant activity because of the direct interaction between the SH and the radicals [41]. Some Arginine containing short peptides in royal jelly samples have been proved to possess antioxidant activities [42]. Since this short peptide contains both arginine and cysteine, it may qualify the *A. hamulosa* honey to act as antioxidant diet.

The third short peptide in the *A. hamulosa* was the L-cysteine diethylamide (1 $\rightarrow$ 1')-disulfide compound with L-cysteine diethylamide (H-C (1)-NEt2-H-C (1)-NEt2) [22], with score percentage of 98.6%. This dipeptide was produced and tested as inhibitor of L-cystine crystallization and a possible treatment for cystinuria and prevention of cystine renal stones [22]. The *A. hamulosa* honey may be a possible inhibitor of the formation of cystine renal stones.

The fourth supposed short peptide in the *A. hamulosa* honey have sequence of Ala-Ala-Pro-Pro in the PubChem [23] and the sequence of Ala-Pro-Ala-Pro in the ChemSpider database [24]. Short peptides rich in proline are known to possess an antioxidant activity [43,44]. Strong

capacity of hydroxyl radical scavenging was reported for the Alanine containing dipeptides [45].

The *A. hamulosa* fifth short peptide was reported as deamino-2-pyrid-4-yl-glycyl-DL-alanyl-DL-norvalyl-DL-asparagine (deamino-G(4-pyridyl)DL-A-DL-NV-DL-N) in the PubChem [25] and gly-ala-phe-gln in the ChemSpider [26]. Presence of hydrophobic amino acids such as alanine, glycine, and valine in the sequence of short peptides facilitates the binding of these short peptides to fatty acids leading to the inhibition of lipid oxidation [39]. Presence of phenylalanine in a short peptide indicates its radical scavenging capability [40], while short peptides containing Asparagine or glutamine in the mid exhibited antihypertensive activity [46,47].

The cyclic peptide of the *A. hamulosa* honey was the cyclo[L-alpha-homoglutamyl-L-arginyl-glycyl-L-alpha-aspartyl-D-phenylalanyl] (cyclo[Aad-RGD-D-F]) which is found in the PubChem database [28]. The cyclic peptide is used in bioassay for integrin receptor [28]. According to its cyclic nature and amino acid content, this peptide could be used as antioxidant, antimicrobial, antitumor and can also be used as weight loss inducing peptide [5,9].

The *Z. spina-christi* honey contained Almiramide B short peptide which acts as anti-*Leishmania donovani* and anti-*Trypanosoma brucei brucei* [48,49]. Auristatin-6-AQ is potently active against human cancer cell lines [50].

This study is limited due to the small number of honey samples. Future studies should be designed to involve more Acacia honey samples and the short peptides should be extracted or synthesized so as to investigate their biological and medicinal activities.

5 Conclusion

The *A. tortilis* honey contained three short peptides, while five short peptides and one cyclic peptide were found in the *A. hamulosa* honey. The *Ziziphus spina-christi* honey can be used as anticancer, anti-*Leishmania donovani*, and anti-*Trypanosoma brucei* brucei. The presence of the short and cyclic peptides in the Acacia honey qualifies it to act as a natural medicine such as the Manuka honey.

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Conflict of interest: The authors declare no conflict of interest.

Informed consent (if applicable): The bee keepers did not agree to publish the latitude and longitude of their bee farms, because they believe that if their location is published, many beekeepers will bring their bee farms to the location. The geographical location of the samples is mentioned as wide areas such as Bisha and Tehama.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The research data are available upon request.

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