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GC-MS analysis of honeybee products derived from medicinal plants

Maha Montaser[1], Ahmed M. Sayed[1], Mokhtar M. Bishr[2], Ehab W. Zidan[3], Mohamed A. Zaki[4], Hossam M. Hassan[1,4*], Rabab Mohammed[4] and Mohamed S. Hifnawy[5]

Abstract

Background Honeybees provide a wealth of valuable natural products containing health-promoting bioactive compounds, including honey, bee bread, bee venom, bee pollen, propolis, and royal jelly. In the present study, we investigated the chemical composition of four honeybee products (bees, honey, royal jelly, and bee bread) derived from three medicinal plants (marjoram, trifolium, and citrus) using headspace GC-MS.

Results GC-MS analysis coupled with the headspace method resulted in identification of 24 volatile compounds in marjoram honey, 14 volatile compounds in trifolium honey, and 25 volatile compounds in citrus honey, e.g., some of these compounds appeared in all three types of honey aroma, which are 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 2-furancarboxaldehyde, 5-(hydroxymethyl) and other unique compounds specific for each type where 23 compounds were from marjoram bees, 38 compounds from trifolium bees, and about 37 compounds were identified in citrus bees where 2,4-decadienal, (E, E) and methyl N-methyl anthranilate were common in all. Furthermore, the volatile compounds of all three types of royal jelly aroma were acetic acid, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 8-nonen-2-one and furfural where one compound appeared in both marjoram and trifolium royal jelly that is 2-furancarboxaldehyde,5-(Hydroxymethyl) and the volatile compounds in marjoram and citrus are 2,3-butanediol and 5-methylfurfural also only one volatile compound appear in both trifolium and citrus royal jelly that is furfur alcohol, Finally, 3 compounds from marjoram bee bread, 30 volatile compounds from trifolium bee bread, and 3 volatile compounds in citrus bee bread were identified.

Conclusions A detailed metabolomic analysis of the four honey product groups revealed an intriguing chemical diversity, with each sample exhibiting its own chemical fingerprint.

Keywords Honeybees, Multivariate analysis, GC-MS, Headspace

*Correspondence:
Hossam M. Hassan
hossam.mokhtar@nub.edu.eg
[1] Department of Pharmacognosy, Faculty of Pharmacy, Nahda University, Beni-Suef 62513, Egypt
[2] Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco-Medifood), Cairo 11361, Egypt
[3] Department of Bee Research, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt
[4] Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt
[5] Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11787, Egypt

1 Background

The honey bee *Apis mellifera* L. is an important pollinator species ecologically and economically worldwide, especially in Egypt [28]; it is essential because of its complex anatomy to collect plant nectar and chemically transform it into honey [6, 14].

Honey, bee bread, bee venom, bee pollen, propolis, and royal jelly are just a few of the valuable natural products produced by honeybees colonies and are attractive ingredients for healthy foods that include bioactive and beneficial chemicals for health [4, 5]

From ancient times, honey has been used as part of traditional medicine [9, 23]; it is a sweet natural substance produced by honey bees from nectar [3, 8].

Honey is a typical food used as an energy source; it contains simple sugars taken without being digested directly into the bloodstream. Nearly all foods pair nicely with honey since it works so well as a sweetener in hot and cold drinks and honey's ability to absorb moisture prolongs the freshness of cakes, cookies, bread, and candies [1]. Numerous elements of this usage indicate that it also serves additional purposes, such as being an antioxidant, an antibacterial, an anti-inflammatory, an anticancer, and an antiviral. The biological activity of honey is influenced mainly by its floral or geographic origin. Additionally, honey has been revealed to have some promising antibacterial and antiviral activities. As a result, honey is essentially a nutraceutical food [20].

Sugars are the primary constituent of honey [22], containing more than 200 additional components. Glucose and fructose are the most prevalent sugars, and water is the most abundant ingredient [29]. Minerals, vitamins, proteins, lipids, amino acids, phenolic compounds, enzymes, pigments, waxes, and pollen grains are examples of minor components [14, 33]. The bee species, raw material, edaphoclimatic conditions, floral source, processing, packaging, and storage conditions all affect the honey's composition, aroma, color, and flavor [13, 33].

Due to the existence of specific volatile organic components derived from the original nectar sources, the aroma of honey is the main feature [2, 7, 21, 30, 34]. It is possible to determine the honey's botanical and geographic origins by analyzing the volatile fraction of the honey. The flavor and aroma of honey vary significantly from variety to variety. Depending on the floral origin, the volatile chemicals considerably contribute to the distinctive flavor of honey. Honey aroma is quite complex, containing many tens of volatile components, and studies of the aroma composition of honey, as in all foods, traditionally utilize aromatic extracts. Different methods are employed to separate the volatile portion of honey, including hydro distillation, the method most commonly used to separate volatile components from a matrix [37].

Using GC-MS analysis, the following major chemical constituents were identified as 2,4-dimethyl-1-pentanol, 3- dihydro-4H-pyran-4-one, 3, 5-dihydroxy-6-methyl-2, 2-furancarboxaldehyde 5-hydroxy methyl, 2-butoxyethyl acetate. These substances are recognized to have some biological activities, including anti-inflammatory, antifungal, antioxidant properties, and makeup wound healing power activity [5].

Most European and Australian honey from various floral sources have also been documented to include certain benzene derivatives, such as benzaldehyde, benzyl alcohol, and 2-phenylethanol [8, 12, 35]. In addition, one of the substances that are thought to add to the aroma of honey is phenylacetaldehyde [7, 11].

Higher concentrations of linalool derivatives, linalool oxide, limonyl alcohol, 4-dimethyl-3-cyclohexene-1-acetaldehyde, a-terpineol, terpineal, and isomers of lilac aldehyde and lilac alcohol were found in the volatile compounds extracted from Spanish citrus honey using GC-MS and sensory analysis. Since sinensal isomers are found uniquely in this floral source, they are also suggested as potential chemical markers for citrus honey. It has been hypothesized that these chemicals significantly contributed to the characteristic aroma of citrus honeys [10, 11].

One of the most abundant volatile compounds in citrus honey is 4-dimethyl-3-cyclohexene-1-acetaldehyde; it has recently been reported in Greek citrus honeys [3].

Egyptian citrus honey was characterized by a higher concentration of lilac aldehyde C (isomer III), dill ether, methyl anthranilate [32], and herb oxide (isomer II). In earlier studies, citrus honey, among other varieties of honey, has been shown to have a volatile profile dominated by these substances. Also, the presence of suitable concentrations of 2-methylbutanal, 3-methylbutanal, 3-methyl-1-butanol, and 2-methyl-1-butanol was found in Egyptian clover honey. These volatile substances give food a fruity, malty flavor [17].

The sugars, acids, and other volatile honey ingredients are also thought to be responsible for honey's aroma and flavor. Various C1-C5 aldehydes and alcohols are among these volatile substances. In honey, methyl and ethyl formats have also been found when many phenylacetic esters have been shown to taste and smell similar to honey [31].

Young worker honeybees of the species A. mellifera L. generate royal jelly, which is a milky-white, extremely acidic (pH 3.1–3.9) fluid in their hypopharyngeal and mandibular glands (called nurses) [4].

Water (50–70%), proteins (9–18%), carbohydrates (7–18%), lipids (3–8%), mineral salts (1.5%), vitamins, polyphenols, enzymes, and hormones were determined to comprise the majority of royal jelly [19]. The lepidic fraction of R.J. is a distinctive and chemically exciting property. About 80–85% of this fraction are unusual short-chain hydroxy and dicarboxylic free fatty acids with 8–12 carbon atoms [4]. More than 50% of the free fatty acid content is made up of the primary compound, (E)-10-hydroxy-2-decanoic acid (10-HDA), which has not been found in any other naturally occurring products or even in products that are not directly associated with bees. In contrast to R.J.'s characterization as a mixture of proteins, lipids, carbohydrates, and phenols, which has received much study, its volatile portion has received less attention [4]. In addition, R.J. has been shown to possess some pharmacological effects, including antioxidant [19], anti-inflammatories [25, 36], antiaging, neuroprotective [25], antimicrobial [25, 26], anti-allergic, and antitumoral properties [25]. These qualities have led to the usage of R.J. in the food, cosmetics, and pharmaceutical industries [27].

Bee bread is a beehive product made from pollen that bees have collected. Proper hive management encourages pollen collecting to market for human consumption because it contains various nutrients and can be considered a valuable diet supplement. Bees treat pollen by adding honey and digestive enzymes, which are then stored in the honeycomb cells, where an anaerobic fermentation process changes its biochemistry. The amount of protein, amino acids, fatty acids, carbohydrates, mineral salts, polyphenols, and flavonoids in bee bread varies on the pollen's botanical source. Bee bread is currently being collected and consumed as a food supplement [18, 24]. Bee bread extract has antioxidant, antibacterial, anti-inflammatory, and neuroprotective properties [15].

Herein, we investigated the chemical composition of four honeybee products (bees, honey, royal jelly, and bee bread) raised upon three medicinal plants (marjoram, trifolium, and citrus) using GC-MS with headspace analysis followed by multivariate analysis.

2 Methods

2.1 Samples collection

Three samples each of honey, worker honeybees, bee bread, and royal jelly under investigation were obtained from apiaries located in different monofloral honey production regions in Egypt in each season of 2019 seasons during the flowering period of the following plants: Citrus sp (*Murcott tangerine* L. and *Jaffa orange* L.) at Wadi Al-Mollak region in Ismailia governorate in April, marjoram (*Origanum majorana* L.) from Sawiris Al-Gali manor, Tamiya district, Fayoum governorate, and Egyptian clover (*Trifolium alexandrinum* L.) in Mansoura region, Dakahlia Governorate, end of June 2019. The monospecificity of the samples was confirmed by examining the color uniformity and observing the shape and size of pollen grains under the microscope in all types of samples.

2.2 Specimen preparation

The different samples (honey, worker honeybees, bee bread, and royal jelly) from marjoram, trifolium, and citrus were used separately and directly for GC-MS analysis using the headspace technique.

2.3 Equipment

Gas chromatography–mass spectrometry was used to analyze the volatile compounds in the honey, worker honeybees, propolis, and bee bread for marjoram, trifolium, and citrus.

2.4 Experimental

Sample preparation: A small amount from each sample was used directly for the GC/MS analysis.

GC/MS analysis: The GC/MS analysis was carried out using Shimadzu GC-MS, Model QP-2010 Ultra, equipped with headspace AOC-5000 auto-injector, under the following condition: Column: Rtx-5 MS 30 m length, 0.25 mm ID, 0.25 mm film thickness (Cross bond 5% diphenyl/ 95% dimethyl polysiloxane), detector: mass spectroscopy, carrier gas: helium, oven temperature program: start temperature = 40 °C with a hold time = 2 min, rate of 3 °C/minute, while the final temperature = 220 °C with a hold time = 5 min at GC-MS program as follows: ion source temperature = 200 °C, interface temperature = 250°C solvent cut time = 2 min, run time = 60 min, ACQ mode is Scan, event time = 0.30 s, scan detector gain mode is relative, detector gain = 1.08 kv + 0.00 kv, speed = 1.666, start m/z = 35.00 and end m/z is 500.0.

2.5 Multivariate analysis

Metabo Analyst software [38] was used to perform multivariate analysis (MVA) on GC-MS-derived data. First, principal component analysis (PCA) was performed to determine the samples' metabolite composition variations and identify the characteristic metabolites in each product. Then, $\log_{10}$ transformation was applied to the signal intensity of all variables.

3 Results

3.1 GC-MS analysis

GC-MS analysis coupled with the headspace method for the three types of honey (marjoram honey, trifolium honey, and citrus honey) was applied and resulted in the quantification of 45 volatile compounds. Twenty-four volatile compounds were identified in marjoram honey, 14 volatile compounds in trifolium honey, and 25 volatile compounds in citrus honey; furthermore, it can be seen that some compounds appeared in all three types of honey aroma, which are 2,3-dihydro-3,5-dihydroxy-6-methyl -4H-pyran-4-one, 2-furancarboxaldehyde,5-(hydroxymethyl) [5], furfural and 2-acetylfuran. Also, we can see that hydroxy acetone and methyl furoate appear in marjoram and trifolium honey only. Moreover, 1-p-menthen-9-al and fenchyl acetate appear in marjoram and citrus honey without trifolium honey, trans-beta-ionone-5,6-epoxide, and ethanol in trifolium and citrus honey.

In addition, some unique volatiles such as methane tetranitro, formic acid, 2-furanmethanol, hyacinthin, linalool oxide, and dodecanol were presented in marjoram honey. Moreover, dimethyl ether, 1,2-benzenedicarboxylic acid diethyl ester and hexacosane were found in trifolium honey. While, methane amine N-methyl (silane methyl) [10], 2-furacarboxylic acid methyl ester, n-pentadecanoic acid, tetradecano, palmitic, stearic acid, methyl stearate, methyl anthranilate [17] and nonyl methoxy acetate were presented in citrus honey, as shown in Additional file 1: Table S1.

Consequently, about 85 volatile compounds were identified by the headspace technique when applied on Egypt's marjoram, trifolium, and citrus worker honeybees. Twenty-three compounds were identified from marjoram bees, 38 compounds from trifolium bees, and about 37 compounds identified by headspace in citrus bees.

2,4-Decadienal, (E,E) and methyl N-methyl anthranilate were present in the three types of bees marjoram, citrus and trifolium bees, where butanal, 2-methyl and camphene or (Limonene) were present in marjoram and trifolium bees only and acetic acid, propanoic acid, 2-methyl, 2,3-butanediol, oxime, methoxy-phenyl, 2-pyrrolidinone and E,E-2,4-dodecadienal were present in marjoram and citrus bees. H.D. analysis also demonstrated that methane, tetranitro, pyrazine, methyl, 2-heptanon, pyrazine, 2,5-dimethyl, gamma-valero lactone and trisulfide, dimethyl were present in trifolium and citrus bees, while every bee has its unique volatile compounds as in marjoram bee. It has l-alanine ethyl amide, ammonium acetate, hexanoic acid, cyclobutene-3,4-dione, 1-dimethylamino -2-hydroxy, butanal 3-methyl, formamide, 2-hydroxymethyl-3-methyl-oxirane, butanoic acid, 3-methyl, 1,2,3-propanetriol, pentanamide, iso amyl phenyl acetate, 9-hydroxy linalool, 2-undecenal, 1-hydroxylinalool, carbamic acid, (1-phenylethyl)-, 2-methyl-5-(1-methylethyl) cyclohexyl ester, tricosane and octadecamethylcyclonon, and the volatile compounds unique for trifolium are piperazine, amylene dichloride, disulfide, dimethyl, 2-pentanol, 3-methyl, 2-heptanol, propanal, 3-(methylthio), 9,12-octadecadien-1-ol, octanal, 1-pentanol, 2-ethyl-4-methyl, 1-butanamine, 2-methyl-n-(2-methy butylidene), hyacinthin, isoartemisia ketone, carveol 1, pyrazine, 3-ethyl-2,5-dimethyl, 2-nonanone, isoborneol, decanal, dimethyl anthranilate, isobornyl acrylate, 1-nonen-3-ol and 6-methyl-bicyclo[4.2.0]octan-7-one, and citrus bee has 11 volatile compounds as shown in Additional file 1: Table S2.

Furthermore, 53 volatile compounds were presented in the royal jelly of marjoram, trifolium, and citrus. 23 volatile compounds were identified through GC-MS with Head space in marjoram royal jelly, 22 volatile compounds were identified in trifolium royal jelly, and 20 volatile compounds were presented in citrus royal jelly.

The volatile compounds of all three types of royal jelly aroma were acetic acid, 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one, 8-nonen-2-one and furfural where one compound appeared in both marjoram and trifolium royal jelly that is 2-furancarboxaldehyde, 5-(hydroxymethyl) and the volatile compounds in marjoram and citrus are 2,3-butanediol and 5-methylfurfural, also only one volatile compound appeared in both trifolium and citrus royal jelly that is furfur alcohol, and the rest of the volatile compounds appearing in the headspace analysis are specialized for every type of royal jelly analyzed as trans-beta-ionon-5,6-epoxid, methane tetranitro, oxirane, isobutane, formamidine acetate, d-alanine, 2,3-dihydro-3,5-dihydroxy-6-methyl-1-piperazinecarboxylic acid, 2,3-butylene glycol and propiolic acid are specialized for marjoram propolis, octanoic acid, 8-hydroxy, 1-dodecanol, lauric acid, myristic acid, myristic alcohol and pentadecanoic acid for trifolium propolis and tetranitromethane, 2-pentanone, 4,4-dimethyl, hydroxy acetone, acetoin, 2,3-butanediol, 3,4,5,6,7,8-hexahydro-2h-chromene, octanoic acid, 2,4-decadienal, methyl n-methyl anthranilate and methyl linolenate for citrus propolis as shown in Additional file 1: Table S3.

Finally, the appearance of 34 volatile compounds in headspace analysis of marjoram, trifolium, and citrus bee bread resulted in 3 compounds for marjoram bee bread, 30 volatile compounds for trifolium bee bread, and 3 volatile compounds in citrus bee bread. Dimethylamine has appeared in marjoram and citrus bee bread, and carbamic acid and monoammonium salt are present in trifolium and citrus bee bread. In contrast, l-alanine ethyl amide and ethylene oxide are specialized for marjoram bee bread, pyridine-3-carboxamide, 1,2-dihydro-4,6-dimethyl-2-thioxo is specialized for citrus bee bread, while the rest or volatile compound in a table for representing the volatile compounds for trifolium or clover bee bread (Additional file 1: Table S4).

3.2 Multivariate analysis

The composition of volatile constituents in the studied honeybees and their products were investigated, and the GC-MS derived data were used to compare between them using multivariate analysis. The principal component analysis (PCA) scores plot of the GC-MS. Data (Fig. 1) showed a variation in the chemical composition of the three investigated bees and their related products (i.e., honey, royal jelly, and bread), indicating a different chemical makeup for each bee group according to the plant they raised beside.

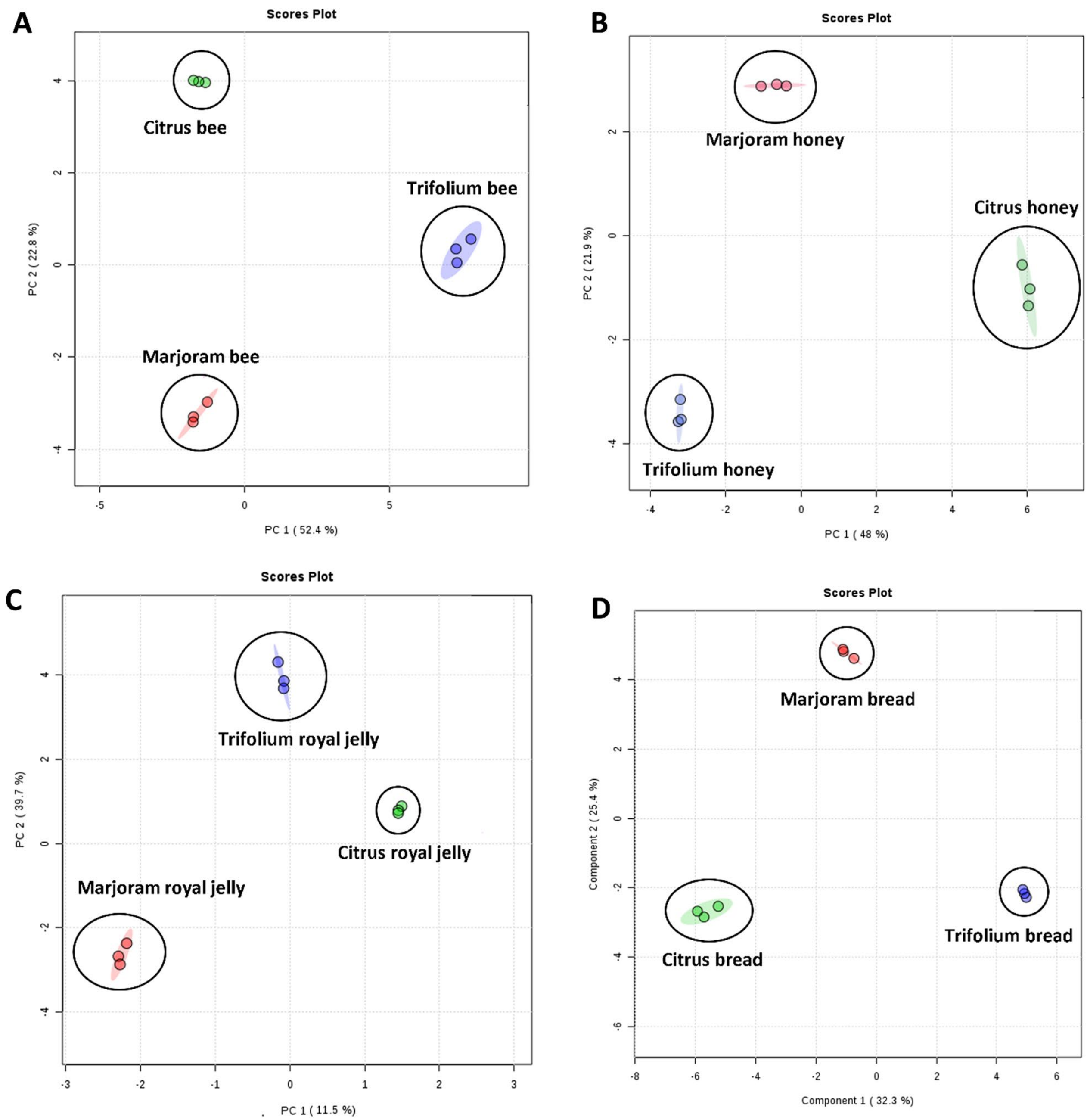

Fig. 1 Score plots representing PCA results based on the GC-MS data obtained for the studied honeybee groups (from marjoram, trifolium, and citrus) (**A**) and their related products: honey, royal jelly, and bread (**B**–**D**, respectively). Red circles are for marjoram products, blue circles are for trifolium products, and green circles are for citrus products

PCA-derived variable importance in projection (VIP) scores (Table 1) was used to investigate the characteristic volatile components in each variety within each group of honey-related products (i.e., honeybees, honey, royal jelly, and bread) (variables with VIP values > 1.2).

It is worth noting that there were a few by-product compounds that were detected and are highlighted in Table 1 [11], and these compounds indicate a possible exposure to high temperatures.

4 Discussion

By GC-MS analysis coupled with the headspace method for the analyzed honeybee products collected from the nectar of honeybees, especially fed on three

Table 1 Dereplication table of the characteristic volatile constituents in the studied honeybee groups (from marjoram, trifolium, and citrus) and their related products

No	Mass	Rt	Molecular formula	Compound name	VIP score*
Marjoram honey					
1	196	4.221	CN_4O_8	Methane, tetranitro	2.31
2	46	6.391	CH2O2	Formic acid	2.28
3	98	13.335	C5H6O2	Furfuryl alcohol*	2.26
4	98	13.749	C5H6O2	2-Furanmethanol*	2.25
5	98	13.954	C5H6O2	2(3H)-furanone, 5-methyl*	2.22
6	110	18.644	C6H6O2	5-Methyl furfural*	2.21
7	120	22.936	C8H8O	Hyacinthin	1.93
8	170	24.42	C10H18O2	Linalool oxide	1.91
9	186	43.33	C12H26O	Dodecanol	1.85
10	188	45.468	C13H16O	3-Buten-2-one,1-(2,3,6 trimethylphenyl	1.82
11	168	55.967	C12H24	1-Dodecene	1.77
12	254	59.22	C16H30O2	E-11-Tetradecen-1-ol acetate	1.72
Trifolium honey					
1	46	4.619	C2H6O	Dimethyl ether	1.95
2	194	42.833	C12H14O4	1,2-Benzenedicarboxylic acid	1.93
3	222	48.762	C12H14O4	1,2-Benzenedicarboxylic acid, diethyl ester	1.84
4	366	50.687	C26H54	Hexacosane	1.82
Citrus honey					
1	194	42.878		Methyl anthranilate	2.16
2	372	4.214	C22H28O5	7-Hydroxy-7-phenyl-3,9-diisopropyl-2,10-dioxadispiro[3.3.3.1]dodecan-1,11-dione	1.94
3	45	5.344	C2H7N	Methanamine, N-methyl-	1.92
4	126	25.024	C6H6O3	2-Furancarboxylic acid, methyl ester*	1.91
5	242	42.405	C15H30O2	*n*-Pentadecanoic acid	1.87
6	214	43.286	C14H30O	Tetradecano	1.85
7	256	43.555	C16H32O2	Palmitic acid	1.82
8	284	43.802	C18H36O2	Stearic acid	1.79
9	214	43.998	C13H26O2	Tridecanoic acid	1.74
10	216	50.100	C12H24O3	Nonyl methoxyacetate	1.73
11	242	53.267	C15H30O2	Methyl myristate	1.63
12	242	56.061	C16H34O	1-Hexadecanol	1.62
13	296	57.076	C21H44	Heneicosane	1.61
14	296	57.303	C19H36O2	Methyl oleate	1.57
15	282	57.715	C18H34O2	γ-Stearolactone	1.53
16	298	59.036	C19H38O2	Methyl stearate	1.52
Marjoram bee					
1	116	4.649	C5H12N2O	l-Alanine ethyl amide	1.99
2	77	5.580	C2H7NO2	Ammonium acetate	1.94
3	116	5.795	C6H12O2	Hexanoic acid	1.92
4	141	6.270	C6H7NO3	Cyclobutene-3,4-dione, 1-dimethylamino -2-hydroxy	1.83
5	86	6.999	C5H10O	Butanal, 3-methyl	1.81
6	74	9.300	C3H6O2	2-Propanone, 1-hydroxy	1.79
7	45	10.440	CH3NO	Formamide	1.75
8	76	10.860	C3H8O2	1,2-Propanediol	1.74
9	88	12.208	C4H8O2	2-hydroxymethyl-3-methyl-oxirane	1.72
10	102	14.090	C5H10O2	Butanoic acid, 3-methyl	1.68
11	92	20.495	C3H8O3	1,2,3-Propanetriol	1.65
12	101	21.383	C5H11NO	Pentanamide	1.63

Table 1 (continued)

No	Mass	Rt	Molecular formula	Compound name	VIP score*
13	206	22.696	C13H18O2	Iso amyl phenyl acetate	1.62
14	186	32.561	C12H26O	1-Decanol-2-ethyl	1.59
15	170	37.473	C10H18O2	9-hydroxy linalool	1.55
16	168	38.114	C11H20O	2-Undecenal	1.52
17	170	38.349	C10H18O2	1-hydroxy linalool	1.51
18	303	40.110	C19H29NO2	Carbamic acid, (1-phenylethyl)-, 2-methyl-5-(1-methylethyl) cyclohexyl ester	1.46
19	324	55.691	C23H48	Tricosane	1.44
20	914	56.010		Octadecamethylcyclonon	1.41
Trifolium bee					
1	86	4.154	C4H10N2	Piperazine	2.36
2	140	8.040	C5H10Cl2	Amylene dichloride	2.22
3	94	9.039	C2H6S2	Disulfide, dimethyl	2.17
4	102	11.064	C6H14O	2-Pentanol, 3-methyl	2.12
5	116	15.225	C7H16O	2-Heptanol	2.05
6	104	15.630	C4H8OS	Propanal, 3-(methylthio)	1.94
7	106	18.407	C7H6O	Benzaldehyde	1.92
8	130	19.561	C8H18O	1-Pentanol, 2-ethyl-4-methyl	1.85
9	128	20.308	C8H16O	Octanal	1.84
10	155	22.045	C10H21N	1-Butanamine, 2-methyl-N-(2-methy butylidene)	1.76
11	120	22.685	C8H8O	Hyacinthin	1.73
12	152	23.354	C10H16O	Isoartemisia ketone	1.71
13	152	23.898	C10H16O	Carveol 1	1.68
14	136	24.570	C8H12N2	Pyrazine,3-ethyl-2,5-dimethyl	1.67
15	142	24.930	C9H18O	2-Nonanone	1.63
16	142	25.543	C9H18O	Nonanal	1.61
17	154	28.685	C10H18O	Isoborneol	1.55
18	156	30.654	C10H20O	Decanal	1.52
19	142	34.301	C9H18O	1-Nonen-3-ol	1.49
20	138	35.880	C9H14O	6-Methyl-bicyclo[4.2.0]octan-7-one	1.47
21	208	39.087	C13H20O2	Isobornyl acrylate	1.43
22	165	40.325	C9H11NO2	Dimethyl anthranilate	1.41
23	260	42.782	C16H33Cl	Hexadecane, 1-chloro	1.37
24	268	43.800	C15H32	Pentadecane	1.35
25	266	48.539	C19H40	Nonadecane	1.33
26	886	50.698	C18H34O	9,12-Octadecadien-1-ol	1.32
Citrus bee					
1	208	4.171	C13H20O2	Trans-beta-ionon5,6-epoxid	2.32
2	102	4.784	C5H10O2	Ethene,(2-methoxyethoxy)	2.26
3	59	11.442	C2H5NO	Acetamide	2.21
4	76	13.200	C3H8O2	1,3-Propanediol	2.14
5	102	14.421	C5H10O2	Butanoic acid, 2-methyl	2.11
6	116	15.240	C7H16O	2-Hexanol, 5-methyl	2.05
7	116	20.043	C6H12O2	Hexanoic acid	1.93
8	122	20.392	C7H10N2	Pyrazine, 2-ethyl-6-methyl	1.91
9	122	20.730	C7H10N2	Pyrazine, 2-ethyl-3-methyl	1.86
10	153	21.346	C9H15NO	Benzenemethanamine, N-(4-methylphenyl)	1.84
11	144	26.480	C13H13NO2	Acrylic acid, 3- [1-hydroxy-N-(3-phenylpropyl) formimidoyl]-, cyclic anhydride	1.82
12	156	29.480	C8H16O2	Octanoic acid	1.78
12	99	30.049	C10H20O	2-Decanone	1.74

Table 1 (continued)

No	Mass	Rt	Molecular formula	Compound name	VIP score*
13	150	30.919	C5H9NO	Piperidinone	1.73
14	130	31.414	C10H14O	Berbenon	1.71
15	180	31.711	C6H10O3	4-(1-hydroxy-ethyl) gamma-butanolactone	1.64
16	166	34.446	C12H20O	TRANS,TRANS-2,4-DODECADIENAL	1.62
17	170	36.139	C11H18O	2,4-Undecadienal	1.58
18	158	38.373	C10H18O2	9-Hydroxy linalool	1.53
19	113	38.554	C9H18O2	2-Nonanone, 9-hydroxy	1.52
20	144	42.736	C7H15N	Piperidine, 2,3-dimethyl	1.47
Marjoram Royal jelly					
1	208	4.204	C13H20O2	Trans-beta-ionon-5,6-epoxid	2.18
2	196	4.356	CN4O8	Methane, tetranitro	2.13
3	44	4.515	C2H4O	Oxirane	2.07
4	70	4.555	C3H2O2	Propiolic acid	2.02
5	208	4.580	C13H20O2	Trans-beta-ionon-5,6-epoxi	2.01
6	58	4.625	C4H9D	Isobutane	1.95
7	104	4.697	C3H8N2O2	Formamidine acetate	1.93
8	45	4.794	C2H6DN	Dimethylamine-D1	1.92
9	89	5.070	C3H7NO2	d-Alanine	1.88
10	144	5.271	C6H8O4	2,3-Dihydro-3,5-dihydroxy-6-methyl-1-Piperazinecarboxylic acid	1.83
11	46	5.510	C2H6O	Ethanol	1.81
12	74	8.438	C3H6O2	2-Propanone, 1-hydroxy	1.78
13	90	11.318	C4H10O2	1,3-Butylene glycol	1.74
14	90	11.651	C4H10O2	2,3-Butylene glycol	1.72
15	96	12.610	C5H4O2	2-Furancarboxaldehyde*	1.67
16	142	25.224	C9H18O	2-Nonanone	1.62
Trifolium Royal jelly					
1	78	4.141	CH6N2O2	Ammonium carbamate	2.11
2	250	4.932	C3H2Br2ClF	Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro	2.02
3	46	5.740	C2H3F	Vinyl fluoride	1.94
4	160	42.391	C8H16O3	Octanoic acid, 8-hydroxy	1.93
5	194	42.963	C10H10O4	Methyl phthalate	1.89
6	186	43.333	C12H26O	1-Dodecanol	1.85
7	200	47.244	C12H24O2	Lauric acid	1.83
8	222	48.276	C12H14O4	Ethyl phthalate	1.76
9	222	49.028	C12H14O4	Diethyl phthalate	1.74
10	214	51.525	C14H30O	1-Tetradecanol	1.73
11	214	53.599	C14H30O	Myristic alcohol	1.71
12	228	54.686	C14H28O2	Myristic acid	1.68
13	242	56.519	C16H34O	2-Hexadecanol	1.65
14	242	58.181	C15H30O2	Pentadecanoic acid	1.63
15	242	58.841	C16H34O	1-Hexadecanol	1.62
Citrus Royal jelly					
1	196	4.213	CN4O8	Tetranitromethane	1.98
2	124	5.816	C2H8N2O4	Ammonium oxalate	1.91
3	114	6.850	C7H14O	2-Pentanone, 4,4-dimethyl	1.88
4	74	7.500	C3H6O2	Hydroxyacetone	1.85
5	88	8.488	C4H8O2	Acetoin	1.83
6	90	11.041	C4H10O2	2,3-Butanediol	1.82
7	138	26.754	C9H14O	3,4,5,6,7,8-Hexahydro-2H-chromene	1.81

Table 1 (continued)

No	Mass	Rt	Molecular formula	Compound name	VIP score*
8	144	29.490	C8H16O2	Octanoic acid	1.77
9	152	35.593	C10H16O	2,4-Decadienal, (E, E)	1.74
10	152	36.000	C10H16O	trans, trans-2,4-Decadienal	1.73
11	165	40.355	C9H11NO2	methyl N-methyl anthranilate	1.71
12	214	42.968	C14H30O	2-Hexyl-1-octanol	1.69
13	252	54.872	C17H32O	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	1.63
14	292	56.289	C19H32O2	Methyl linolenate	1.62
Marjoram bee bread					
1	116	4.283	C5H12N2O	l-Alanine ethyl amide, (S)	1.96
2	44	4.475	C2H4O	Ethylene oxide	1.92
Trifolium bee bread					
1	72	5.781	C4H8O	Oxirane, 2,3-dimethyl-, cis	2.23
2	60	7.071	C2H4O3	Acetic acid	2.17
3	74	7.800	C3H6O2	2-Propanone, 1-hydroxy	2.13
4	100	10.895	C6H12O	Hexanal	2.08
5	100	11.298	C5H8O2	3(2H)-Furanone, dihydro-2-methyl*	2.03
6	96	12.370	C5H4O2	Furfural*	1.97
7	98	13.714	C5H6O2	Furfuryl alcohol*	1.94
8	98	3.995	C5H6O2	2(3H)-Furanone, 5-methyl*	1.91
9	96	14.771	C5H4O2	2-cyclopentene-1,4-dione	1.88
10	110	16.029	C6H6O2	2-Furyl methyl ketone*	1.85
11	110	18.612	C6H6O2	5-Methyl-2-furfural*	1.81
12	158	19.667	C9H18O2	Nonanoic acid	1.79
13	109	23.951	C6H7NO	Methyl pyrrol-2-yl ketone	1.74
14	128	25.068	C6H8O3	2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	1.72
15	142	25.592	C9H18O	Nonanal	1.69
16	144	28.105	C6H8O3	2,3-Dihydro-3,5-dihydroxy-6-methyl–4H-pyran-4-one	1.64
17	156	30.682	C10H20O	Decanal	1.62
18	194	35.544	C14H26	1-Tetradecyne	1.58
19	152	36.120	C10H16O	2,4-Decadienal, (E, E)	1.58
20	196	39.101	C12H20O2	Acetic acid, 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl ester	1.54
21	184	40.139	C12H24O	Dodecanal	1.52
22	194	42.151	C13H22O	(Z)-Geranylacetone	1.47
23	222	46.800	C15H26O	Nerolidol 1	1.46
24	260	50.310	C50H102O	1-Pentacontanol	1.42
25	260	50.310	C16H33Cl	Cetyl chloride	1.41
26	386	50.575	C27H46O	Cholest-14-en-3-ol,	1.36
27	280	50.948	C20H40	9-EICOSENE, (E)	1.33
28	268	51.690	C19H40	NONADECANE	1.32
29	286	58.028	C20H30O	4-EPIDEHYDROABIETOL	1.28
Citrus bee bread					
1	182	4.501	C8H10N2OS	Pyridine-3-carboxamide, 1,2-dihydro-4,6-dimethyl-2-thioxo	

*sight for by-product compounds

plants with special medicinal values in Egypt namely Marjoram, Trifolium, and Citrus, the analyzed species gave different number of components present in the bees and its product as well as different from those present in the corresponding products of the other species. The analyzed Marjoram species (*Origanum majorana* L.) gave total number of 73 components in the bee, and it is products (honey, royal jelly and bee breed), of which 23 components were found in the bee, 24 components were found in the honey, 23 components in the royal jelly and 3 components in the marjoram bee breed, while the analyzed (*Trifolium alexandrium* L) gave total number of 104 components in the bee and its three products, of which 38 components were present in the bee, 14 components present in its honey, 22 components in its royal jelly and 30 components in its bee bread. The third species (*Murcott tangerine* L. and *Jaffa orange* L.) gave a total number of 85 components in the citrus bee and its three products, of which 37 components were present in the bee itself, 25 components present in citrus honey, 20 components in the royal jelly and 3 components in citrus bee bread.

Obviously, there are similar components between the bee products from the same species. As in marjoram, some volatile compounds appear in the bee and its product as royal jelly such as Acetic acid, 2-Propanone, 1-hydroxy and 2,3-Butanediol. While some compounds are similar in bee and bee bread as l-Alanine ethyl amide.

Also, some compounds appear in honey and royal jelly of marjoram: methane tetranitro, furfural, and 2-furancarboxaldehyde, 5-(hydroxymethyl).

We can see compounds in all bee products of Trifolium species as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and furfural, 2-furancarboxaldehyde in honey and royal jelly, acetic acid, furfural alcohol and ammonium carbamate in royal jelly and bee bread of Trifolium, also we can see decanal, 2,4-decadienal and nonadecane in bee bread and Trifolium bee.

For citrus bee products, some volatile compounds were present in the bee and its products as a citrus royal jelly that are acetic acid, octanoic acid, 2,3-butanediol, methane tetranitro, methyl N-methyl anthranilate, and 2,4-decadienal, trans-beta-ionon-5,6-epoxide present in citrus bee and its honey.

At the end of our study, we recognize that: the chemical composition and floral source of the bees and its bee product such as honey, royal jelly and bee bread all effect by their floral, geographic, and climatic characteristics of the place and all have a significant impact on their polyphenolic content.

It is quite interesting to identify the polyphenols in bee products. Additionally, polyphenols like phenolic acids supply them with an important antioxidant potential. These antioxidants have been found to improve human health. Consuming these bee products has been noted to aid in the treatment of stomach ulcers, sore throats, wounds, and burns. Their diverse pharmacological actions, including antibacterial, antifungal, antiviral, anti-inflammatory, hepatoprotective, antioxidant, and anticancer effects, have been demonstrated in several investigations [37]; as a result, a variety of analytical techniques have been used to determine the full phenolic profile of honeybee products such as GC-MS [16].

5 Conclusion

Honeybees are a source of numerous valuable natural products with health-promoting bioactive compounds, such as honey, bee bread, bee venom, bee pollen, propolis, and royal jelly. Using the headspace GC-MS analysis, we analyzed the chemical composition of four honeybee products (bees, honey, royal jelly, and bee bread) produced from three medicinal plants (marjoram, trifolium, and citrus).

Detailed metabolomic analysis of the 4 groups of honey products revealed an intriguing chemical diversity, with each strain exhibiting a distinct chemical fingerprint.

Abbreviations

GC-MS	Gas chromatography coupled with mass spectrometry
MVA	Multivariate analysis
PCA	Principal component analysis
Supp.	Supplementary data
Fig.	Figure
Rt.	Retention time
No.	Number

Supplementary Information

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Additional file 1. Supplementary file showing GC-MS tables and figures of the different honeys' products.

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Author contributions

MM was involved in data curation, formal analysis, investigation, funding acquisition, resources, and writing—original draft. AMS was responsible for metabolomic analysis. MMB contributed to GC-MS analysis. EWZ was responsible for samples collection. MAZ was a supervision. HMH was involved in data curation, resources, writing—review & editing and in supervision. RM was a supervision. MSH was a conceptualization and a supervision. All authors have read and approved the manuscript.

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Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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