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RESEARCH PAPER

Evaluation of The Phenolic Content of Multifloral Honey From Şanlıurfa, Türkiye

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Abstract

Honey is a sweet, natural product made by honeybees when they break flower nectar into smaller molecules using enzymes. It contains a variety of beneficial compounds, such as amino acids, saccharides, vitamins, minerals, and phenolic substances, which contribute to its health-promoting properties. The aim of this study was to determine the types and concentrations of phenolic compounds present in multifloral honey samples obtained from Şanlıurfa, Türkiye. A total of 25 phenolic compound contents of ten honey samples were analyzed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Of these, alizarin, luteolin, kaempferol, myricetin, 2-hydroxy-1.4-naphthoquinone, protocatechuic acid and thymoquinone were not detected in any of the samples. Hydroxycinnamic acid (1552.1 ± 1556.3 ng g^{-1}) and vanillic acid (522.1 ± 238.3 ng g^{-1}) were identified as the most abundant phenolic compounds. Conversely, acetohydroxamic acid (3.59 ± 0.101 ng g^{-1}) was present in the lowest quantity. Correlation analysis of phenolic compounds revealed a strong positive correlation (rho = 0.988) between curcumin and hydroxybenzoic acid. The results of this study provide valuable data on the phenolic composition of Şanlıurfa multifloral honey and contribute to our understanding of its potential nutritional and therapeutic significance.

Introduction

Honey is a natural sweetener produced by honeybees when they collect flower nectar and transform it using enzymes. As well as being sweet, honey is renowned for its numerous health benefits thanks to its abundance of bioactive compounds, including phenolic substances (Becerril-Sánchez et al., 2021). Phenolic compounds constitute the primary group of secondary plant metabolites. These aromatic (cyclic unsaturated) or aliphatic (branched) substances include phenol (hydroxybenzene), which is present in the human body as a result of digestion (Peñarrieta et al., 2014). The botanical source of the nectar greatly influences the type and concentration of phenolic compounds in honey (Becerril-Sánchez et al., 2021). The phenolic content is also influenced by geographic location, climatic conditions and seasonal changes. Other factors that can influence the phenolic profile of honey include soil composition, bee species and post-harvest processing techniques (Soares et al., 2017; Alshammari et al., 2022).

Studies have shown that phenolic compounds have a variety of biological properties, including anti-inflammatory, anti-cancer, antioxidant and antimicrobial effects. These properties mean honey can be classified as a functional food with healing potential (Zawawi et al., 2021; Ranneh et al., 2021). The bioavailability and biological activity of phenolic compounds depend on several factors, including their chemical structure, interaction with the food matrix and metabolism by gut microbiota (Cardona et al., 2013; Cianciosi et al., 2018).

Phenolic compounds found in honey play a key role in combating oxidative stress by neutralising free radicals produced during metabolism. Oxidative stress occurs when there is an imbalance between the body's antioxidant defences and reactive oxygen species (ROS),

which can lead to cell damage. This type of damage has been strongly linked to various chronic diseases, including different types of cancer, neurodegenerative diseases such as Alzheimer's and Parkinson's, and cardiovascular disorders (Pentoś et al., 2020). Other chemicals, such as caffeic acid, p-coumaric acid, quercetin and kaempferol, have been shown to inhibit ROS and metal ions involved in radical formation. They also increase the production of endogenous antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). These mechanisms reduce DNA damage, lipid peroxidation and protein oxidation, all of which play a central role in the development of chronic degenerative diseases (Kaurinovic & Vastag, 2019; Rao & Zheng, 2025).

Phenolic compounds found in honey are known to act through multiple biological pathways, in addition to exhibiting anti-inflammatory and antioxidant properties. For instance, they can inhibit the expression and activity of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β). They have also been observed to reduce key inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Zamani-Garmsiri et al., 2022). These effects alleviate tissue inflammation by suppressing the production of inflammatory mediators such as nitric oxide and prostaglandins. The potential of phenolic compounds such as quercetin, ellagic acid and apigenin in managing inflammatory diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis and asthma has been investigated and established in both *in vivo* and *in vitro* studies (Kassim et al., 2010; Khan et al., 2019; Ranneh et al., 2021).

A number of antimicrobial properties of honey have been linked to its phenolic content. The phenolic content can suppress the growth of a variety of microorganisms, such as fungi, Gram-negative bacteria, Gram-positive bacteria, and viruses. They can do this by disrupting the cell membranes and walls of microbes, which results in the loss of cell contents. They also inhibit nucleic acid synthesis and microbial signalling. Flavonoids such as galangin and pinocembrin inhibit pathogens, including *Candida albicans, Escherichia coli*, and *Staphylococcus aureus*. Inhibition has also been reported in antibiotic-resistant strains, one of which is MRSA (methicillin-resistant *Staphylococcus aureus*) (Kwakman et al., 2008; Mandal & Mandal, 2011; Bueno-Costa et al., 2016; Ecevit et al., 2022).

Additionally, various phenolic compounds found in honey have been shown to affect cellular metabolic processes, and these compounds have demonstrated anticancer properties. These include preventing the proliferation of cancer cells, promoting cell death (apoptosis), stopping the cell cycle in the G1 or G2/M phases, preventing the formation of new blood vessels and stopping the spread of cancer (Erejuwa et al., 2014). These compounds' anticancer effects are considered to result from their capacity to regulate signalling pathways such as PI3K/Akt, MAPK, and NF-κB, which are often altered in tumour cells (Oršolić & Jazvinšćak Jembrek, 2022). Research has shown that compounds such as quercetin, caffeic acid and resveratrol are highly effective in combating various types of cancer, including breast, colon and prostate cancer cell lines. They reduce oxidative DNA damage, affect mitochondrial membrane potential and enhance pro-apoptotic gene expression (Jaganathan & Mandal, 2009; Ahmed & Othman, 2013; Combarros-Fuertes et al., 2018; Afrin et al., 2020).

The biological activities of honey's phenolic compounds highlight their importance in terms of food quality and preservation, as well as their potential therapeutic role in preventing and managing various diseases. Therefore, the phenolic composition of honey is of vital importance. Beekeeping significantly impacts the economies of rural areas in developing countries. It provides income, food and employment opportunities. Thanks to its rich plant diversity and long flowering seasons, Türkiye is a major player in global beekeeping, ranking second only to China in terms of honey production (Burucu & Bal, 2017). Şanlıurfa Province, located in south-eastern Türkiye, is the third-largest province in terms of land area and ranks 12th in national honey production. It produces around 2,107 tonnes of honey each year from around 130,000 beehives (TEPGE, 2022). Given the importance of phenolic compounds in improving food quality and human health, this study aimed to identify and quantify the phenolic compounds present in multifloral honey samples collected in Şanlıurfa Province using LC-MS/MS techniques.

Material and Methods

Sample collection and storage

In this study, ten multifloral honey samples were purchased from the central and surrounding districts of Şanlıurfa Province of Türkiye. A total of 250 analyses were conducted on the honey samples to measure the content of 25 different phenolic compounds. The honey samples were stored in sterile Falcon tubes at room temperature in a dark place to prevent the degradation of phenolic compounds prior to analysis. Chemicals and reagents purchased from Merck (Darmstadt, Germany) were used in the study and were all of analytical grade.

Extraction of phenolic compounds

Phenolic compounds were extracted using a modified version of the method outlined in our previous study by Altun and Aydemir (2020). Briefly, 25 g of each honey sample was homogenized with 5 volumes of acidic water. The mixture was filtered through cotton to remove insoluble residues. The filtrate was subsequently passed through a glass chromatography column packed with Amberlite XAD-4 resin. Sugars and other polar compounds were washed with using acidic

water (100 mL) followed by purified water (300 mL), while phenolic compounds remained adsorbed on the resin.

Elution and concentration

Phenolic compounds were eluted from the column in the presence of methanol (400 mL) and then concentrated on a rotary evaporator under reduced pressure at 40 °C. The concentrated residue was dissolved in 5 mL of distilled water and extracted three times with 5 mL of diethyl ether. The pooled ether extracts were then evaporated at 30 °C and the resulting residue reconstituted in methanol (0.5 mL) for analysis. resin.

Sample preparation for LC-MS/MS analysis

Prior to LC-MS/MS analysis, the methanolic extract was filtered through a 0.45 µm PTFE membrane syringe filter to remove any remaining particulates.

Chromatographic and mass spectrometric analysis

A quantitative analysis of 25 phenolic compounds was conducted using a liquid chromatography system coupled with tandem mass spectrometry (LC-MS/MS). Specific multiple reaction monitoring (MRM) transitions were employed for each compound. Depending on the polarity of the compound, both negative and positive electrospray ionisation (ESI) modes were employed. Chromatographic separation was carried out using a C18 reversed-phase column. A mixture of acidified water (0.1% hydrochloric acid) and a methanol/acetonitrile mixture was used as the mobile phase, and a gradient elution programme ensured the optimal separation of the compounds. The column temperature, flow rate and injection volume were optimised based on preliminary studies.

Quantitative analysis was performed using specific Multiple Reaction Monitoring (MRM) transitions that had been identified for each phenolic compound. For instance, the m/z transition at 76.10 > 43.10 was analysed in positive ion mode for acetohydroxamic acid, and the m/z transition at 179.20 > 135.00 was analysed in negative ion mode for caffeic acid.

Method validation

We used a multifloral honey matrix to validate the analytical method. Calibration curves were generated using standard solutions of each compound prepared at various concentrations. The following parameters were evaluated:

Linearity: Linear regression analysis was performed on the calibration curves prepared using five to seven concentration levels. The obtained correlation coefficients (R^2) ranged from 0.9943 to 0.9995, demonstrating the method's high accuracy and precision.

Limit of detection (LoD) and limit of quantification (LoQ): We determined the LoD and LoQ values based on signal-to-noise ratios of 3:1 and 10:1, respectively. This demonstrates the method's high sensitivity. The LoD and LoQ levels were presented in Table 1.

Precision: Quality control samples were analysed in triplicate at low, medium and high concentrations over three continuous days to evaluate intra-day and inter-day precision. The reproducibility and repeatability of the method were confirmed by RSDs of below 15%.

Accuracy (recovery): Recovery studies were performed by adding various concentrations of known phenolic standards to honey matrices. The method was found to be highly effective in complex matrices, with recoveries ranging from 85% to 110%.

Stability: Various storage conditions were assessed to evaluate the stability of the phenolic compounds in the prepared extracts. These included short-term storage at room temperature, refrigeration, and freeze-thaw cycles. Analyte concentrations demonstrated remarkable stability, exhibiting an average variation of less than 10%.

Table 1 presents the calibration equations, MRM transitions, ion modes, R^2, LoD, and LoQ values obtained for each phenolic compound.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics software (version 16.0, SPSS Inc., Chicago, IL, USA). The mean and standard deviation levels of phenolic compounds were calculated using Microsoft Excel (Office 2016, Microsoft Corporation, USA). Spearman's rank correlation coefficient test was applied to investigate the relationships between phenolic compounds. The statistical significance of each correlation was assessed. P-values were calculated. These were based on the t-distribution with (n-2) degrees of freedom. For these analyses, non-detectable (ND) values were excluded, as were variable pairs for which data were unavailable from at least three samples. Additionally, scatter plot analyses were used to visualise the strongest correlations among phenolic contents.

Results

Phenolic compound concentrations in honey samples

The concentrations of phenolic compounds detected in the multifloral honey samples from Şanlıurfa are summarized in Table 2 and Table 3. Among the 25 target phenolic compounds analyzed, several were consistently detected across all samples, whereas others were either absent or found at trace levels. A total of 25 phenolic compounds were targeted in the study. Among them, 18 were detected in at least one of the honey samples, whereas 7 compounds (alizarin, luteolin, kaempferol, myricetin, 2-hydroxy-1.4-naphthoquinone,

Table 1. Analytical method validation parameters that belong to the LC-MS/MS method

Standards	Retention time	ESI Ion Mode	Equation	MRM	R^2
Acetohydroxamic acid	1.986	positive	Y = (150.982)X + (23.1833)	76.10>43.10	0.999363
Butein	6.084	negative	Y = (49.3543)X + (367.917)	271.10>135.00	0.9992028
Caffeic acid	5.283	negative	Y = (124.785)X + (-487.132)	179.20>135.00	0.9956162
Catechin hydrate	4.958	negative	Y = (79.2933)X + (-2406.22)	291.10>139.00	0.9991201
Curcumin	6.516	negative	Y = (227.706)X + (-10111.1)	367.00>149.00	0.9966966
Ellagic acid	5.895	negative	Y = (5.25903)X + (-1167.31)	300.90>145.10	0.9995757
Fumaric acid	3.674	negative	Y = (20.2986)X + (-762.592)	115.20>71.00	0.9989117
Gallic acid	4.134	negative	Y = (65.3835)X + (-2699.84)	169.20>125.00	0.998971
4-Hydroxycinnamic acid	5.738	negative	Y = (13.1516)X + (717.421)	163.20>119.00	0.9949564
Hydroxybenzoic acid	6.13	negative	Y = (735.804)X + (-498.102)	137.20>93.00	0.9987579
Naringenin	6.104	negative	Y = (317.241)X + (33733.3)	271.10>150.90	0.9955044
Oleuropein	5.643	negative	Y = (25.9240)X + (-558.916)	539.10>377.20	0.9989262
Phloridzin dihydrate	5.646	negative	Y = (33.4069)X + (-1396.90)	435.00>273.10	0.9986845
Quercetin	6.091	negative	Y=(13.7831)X+(-146.951)	301.1>151	0.9990363
Resveratrol	5.713	positive	Y = (46.4361)X + (-1314.61)	229.10>135.00	0.9978816
Salicylic acid	6.104	negative	Y = (746.369)X + (6072.41)	137.20>93.00	0.9989762
Silymarin	5.978	negative	Y = (31.9969)X + (-1823.79)	481.00>301.00	0.9947323
Vanillic acid	6.026	positive	Y = (48.0522)X + (-876.904)	168.80>93.00	0.9976425
Alizarin	6.8	negative	Y = (3.97487)X + (1614.23)	239.20>210.90	0.998357
Myricetin	5.858	negative	Y = (37.0934)X + (2684.23)	317.10>150.90	0.9992188
Protocatechuic acid	5.875	negative	Y = (526.954)X + (23026.1)	181.20>108.00	0.9943057
Thymoquinone	6.632	negative	Y = (60.4553)X + (2285.92)	164.20>149.00	0.9991933
Luteolin	6.19	negative	Y = (34.6668)X + (3721.79)	285.20>132.90	0.9976786
Kaempferol	6.288	negative	Y = (2.63905)X + (-206.494)	285.10>116.90	0.9993608
2-Hydroxy-1.4-naphthoquinone	6.058	negative	Y = (203.469)X + (29033.1)	173.20>144.90	0.9971801

protocatechuic acid, and thymoquinone) were not detected in any samples. Hydroxycinnamic acid was the most abundant phenolic compound (1552.1 ± 1556.3 ng g^{-1}), followed by vanillic acid (522.1 ± 238.3 ng g^{-1}), silymarin (119.36 ± 34.91 ng g^{-1}), and resveratrol (96.27 ± 57.3 ng g^{-1}). In contrast, acetohydroxamic acid exhibited the lowest concentrations (3.59 ± 0.10 ng g^{-1}), found in only three samples.

Phenolic compounds were detected in all samples

Vanillic acid, resveratrol, fumaric acid, caffeic acid, phloridzin dehydrate, hydroxycinnamic acid, silymarin, hydroxybenzoic acid, and salicylic acid were detected in all honey samples, indicating their prevalence in Şanlıurfa multifloral honey. The concentrations of these compounds varied significantly between samples, likely due to botanical and geographical influences. For example, the concentrations of caffeic acid ranged from

Table 2. Concentrations of phenolic compound of the Şanlıurfa honey samples (ng g^{-1})

Phenolic compounds	n	Minimum	Maximum	Mean±SD
Acetohydroxamic acid	3	3.79	3.68	3.59±0.101
Butein	6	0.23	37.59	16.42±16.50
Caffeic acid	10	56.98	371.82	174.17±102.01
Catechin hydrate	9	23.81	70.23	42.23±5.21
Curcumin	5	44.87	58.85	48.51±5.81
Ellagic acid	3	262.2	529.70	365.85±143.55
Fumaric acid	10	69.25	737.91	218.41±198.75
Gallic acid	6	44.39	95.38	61.88±25.8
Hydroxycinnamic acid	10	351.8	4817.52	1552.1±1556.3
Hydroxybenzoic acid	10	31.73	1069.32	208.12±308.26
Naringenin	2	30.81	119.62	75.22±62.80
Oleuropein	8	50.14	140.43	100.39±27.03
Phloridzin dihidrat	10	73.15	171.56	111.77±26.15
Quercetin	5	47.26	135.50	85.11±34.76
Resveratrol	10	35.70	219.15	96.27±57.3
Salicylic acid	10	23.17	1060.15	194.37±309.55
Silymarin	10	77.25	180.98	119.36±34.91
Vanillic acid	10	189.3	904.7	522.1±238.3

56.98 to 371.82 ng g^{-1}, with an average of 174.17 ± 102.01 ng g^{-1}. Resveratrol levels also showed considerable variability, ranging from 35.7 to 219.15 ng g^{-1} (mean: 96.27 ± 57.3 ng g^{-1}), reflecting the diverse floral sources.

Phenolic compounds not detected

None of the analyzed honey samples contained the following phenolic compounds: alizarin, luteolin, kaempferol, myricetin, 2-hydroxy-1.4-naphthoquinone, protocatechuic acid, and thymoquinone. The absence of these compounds in our samples may be explained by the floral composition and environmental conditions in the region where the samples were collected.

Flavonoid Content

Of the flavonoids identified in this study, quercetin was found in five samples with an average concentration of 85.11 ± 34.76 ng g^{-1} while naringenin was found in two samples with an average concentration of 75.22 ± 62.8 ng g^{-1}. Butein was present at lower concentrations (16.42 ± 16.50 ng g^{-1}) in 6 honey samples. The absence of other flavonoids, such as kaempferol and luteolin, suggests that the floral sources used in our study were not consistent.

Variability among samples

Significant standard deviations were observed in the concentrations of phenolic compounds due to the multifloral nature of the honey and the diverse geographical origins of the samples. There was a lot of variation in the levels of the compounds between the samples, especially for hydroxycinnamic acid (range: 351.8–4817.5 ng g^{-1}) and salicylic acid (range: 23.17–1060.15 ng g^{-1}). This is likely due to the multifloral origin of the samples, as different plant sources contribute distinct phenolic profiles. Flavonoid presence was inconsistent: quercetin was found in five samples (mean: 85.11 ± 34.76 ng g^{-1}), naringenin in two samples (75.22 ± 62.8 ng g^{-1}), and butein in six samples (16.42 ± 16.50 ng g^{-1}). The absence of other flavonoids may reflect either low natural abundance or concentrations below the detection limits.

Table 3. Phenolic composition of Şanlıurfa honeys samples (ng g^{-1})

Şanlıurfa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Center	N.D.	N.D.	240.34	47.7	N.D.	N.D.	130.59	N.D.	1206.91	44.52	N.D.	103.02	97.74	N.D.	90.43	23.18	180.98	794.31
Akçakale	N.D.	28.59	371.82	26.93	45.93	305.64	155.93	N.D.	885.38	124.98	119.63	98.06	115.83	N.D.	35.71	118.49	122.25	447.52
Siverek	N.D.	N.D.	118.52	26.24	N.D.	N.D.	125.21	N.D.	351.80	31.74	N.D.	50.15	111.97	N.D.	75.71	25.69	78.68	399.84
Siverek	N.D.	0.79	308.43	54.83	N.D.	N.D.	737.91	95.33	1316.24	1069.32	N.D.	N.D.	171.56	N.D.	94.63	1060.16	154.56	830.89
Siverek	N.D.	N.D.	84.07	48.21	N.D.	529.71	117.41	95.06	877.19	124.16	N.D.	N.D.	122.79	N.D.	105.47	113.32	135.41	904.74
Suruç	N.D.	4.31	161.53	27.02	44.87	N.D.	185.10	45.62	1260.83	167.84	0.81	129.06	73.15	47.26	61.43	144.52	86.36	524.06
Siverek	3.61	N.D.	56.92	N.D.	58.85	N.D.	76.07	44.72	404.48	68.05	N.D.	93.53	84.77	77.48	60.87	53.19	101.36	189.36
Harran	3.48	0.23	100.98	23.81	46.45	262.22	69.26	N.D.	414.58	73.43	N.D.	90.26	112.60	62.75	48.49	62.08	77.26	397.87
Bozova	3.68	27.01	133.65	70.23	N.D.	N.D.	281.10	44.39	3986.13	160.18	N.D.	98.68	110.84	102.57	219.15	141.62	146.66	352.19
Bozova	N.D.	37.59	165.51	55.11	46.45	N.D.	305.59	46.18	4817.53	216.98	N.D.	140.44	116.49	135.50	170.89	201.46	110.12	380.60

[1]Acetohydroxamicacid, [2]Butein, [3]Caffeic acid, [4]Catechinhydrate, [5]Curcumin, [6]Ellagic acid, [7]Fumaric acid, [8]Gallic acid, [9]Hydoxy cinnamic acid, [10]Hydroxybenzoic acid, [11]Naringenin, [12]Oleuropein, [13]Phloridzindihydrate, [14]Quercetin, [15]Resveratrol, [16]Salicylic acid, [17]Silymarin, [18]Vanillic acid, N.D.: Not Detected

Correlations between phenolic compounds

The strongest correlation is between curcumin and hydroxybenzoic acid (rho = 0.988). The scatter plot analysis for this pair is presented in Figure 1. The figure shows the levels of the two variables with dots and highlights the monotonic correlation.

The correlation between oleuropein and curcumin (rho = 0.905) indicates that these variables have an almost perfect positive monotonic correlation. Similarly, hydroxybenzoic acid and salicylic acid (rho = 0.821) exhibit a strong correlation. Pairs such as curcumin and hydroxybenzoic acid (rho = 0.988), oleuropein and resveratrol (rho = 0.867), and phloridzindihydrate and oleuropein (rho = 0.905) also exhibited a strong positive correlation. Spearman correlation heatmap is applied to show the relationship between phenolic compounds. Dark blue (rho = -1): Strong negative correlation. As one variable's ranks increase, the other's decrease (e.g., catechinhydrate and fumaric acid, rho = -1.0). Dark orange (rho = 1): Strong positive correlation. Both variables' ranks increase together (e.g., curcumin and hydroxybenzoic acid, *P* = 0.988). Cream (rho= 0): Weak or no correlation. No significant monotonic relationship between variables (e.g. Naringenin and Curcumin, *P* = 0.000).

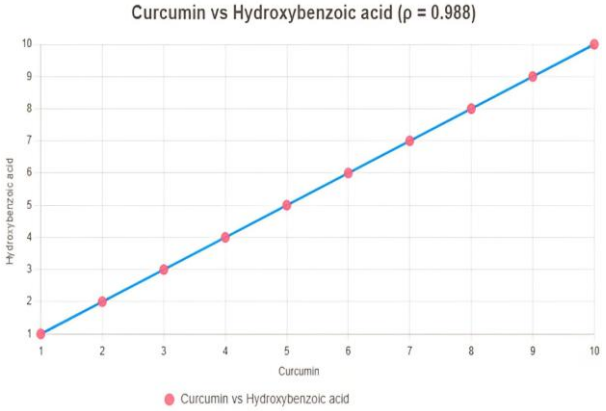

Figure 1. The scatter plot analysis between curcumin and hydroxybenzoic acid.

Discussion

This study presented a comprehensive analysis of phenolic compounds in multifloral honey samples from the Şanlıurfa region, particularly hydroxycinnamic acids and vanillic acid derivatives. These compounds are known to play an important role in the anticancer, anti-inflammatory, antioxidant, and antimicrobial properties of honey.

The high level of hydroxycinnamic acids in our samples (mean level: 1.552.1 ± 1.556.3 ng g^{-1}) is consistent with the findings of Alshammari et al. (2022), who discovered significant levels of phenolic acids and strong antioxidant activity in honey from the Hail Province of Saudi Arabia. This consistency highlights the importance of hydroxycinnamic acids, such as p-coumaric acid and caffeic acid, in honey's health benefits (Cianciosi et al., 2018). Similarly, Can et al. (2015) demonstrated that Turkish honeys exhibit significant antioxidant capacities, which are closely linked to their hydroxycinnamic acid content. Our findings highlight the fundamental function of phenolic acids found in honeys from this region in scavenging free radicals and reducing oxidative damage, thereby contributing to the alleviation of chronic diseases (Khalil & Sulaiman, 2010). Vanillic acid, detected at high levels in the samples (mean 522.1± 238.3 ng g^{-1}), has been the subject of extensive research in many studies for its antioxidant and anti-inflammatory properties (Kassim et al., 2010). The biological potential of Şanlıurfa honey is further supported by its presence. The anti-inflammatory properties of phenolic acids, including ellagic acid and flavonoids, were also highlighted in Malaysian honey by Kassim et al. (2010). These mechanisms are thought to be responsible for the therapeutic effects of honey in the treatment of inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis. The inhibitory effects of vanillic acid on proinflammatory cytokines have been reported in both in vivo and in vitro studies, and its amount in honeys from this region is well supported by our findings (Khalil &Sulaiman, 2010).

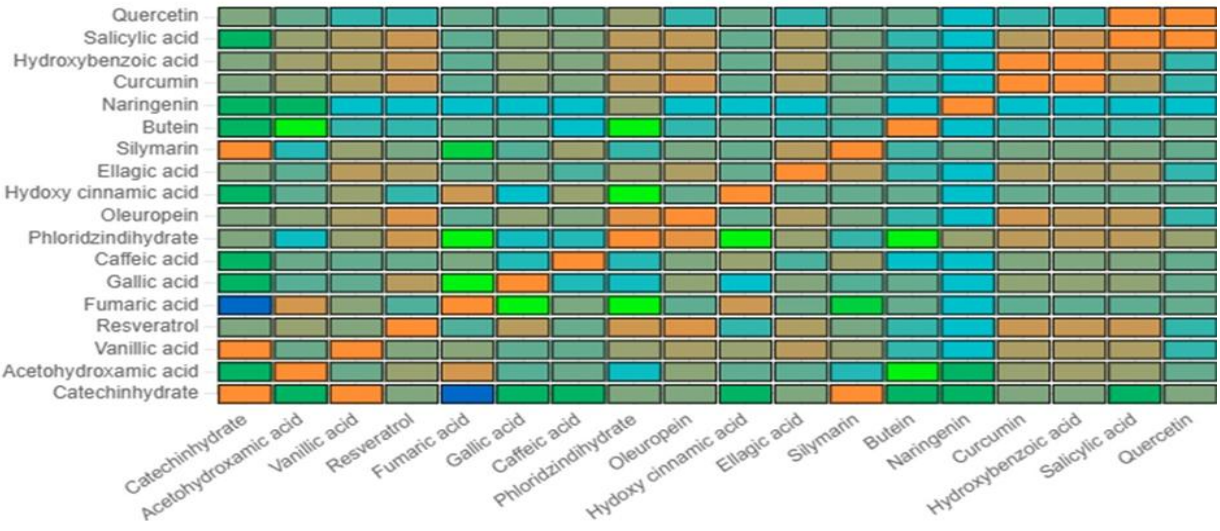

Figure 2. Heat map of Spearman Correlation

Notably, we did not detect the flavonoids myricetin, kaempferol, luteolin and thymoquinone in any of the honey samples we analysed. This agrees with the results of other regional studies. For instance, Altun and Aydemir (2020) documented significant geographical variation in the flavonoid composition of Anatolian propolis. Silici et al. (2014) reported the absence or very low presence of certain flavonoids in rhododendron honey collected from various regions of Türkiye, underlining the significant impact of botanical origin on phenolic profiles. The absence of these flavonoids in Şanlıurfa honey may be attributed to the local flora and environmental conditions, indicating that honey's therapeutic properties are closely related to its floral source.

Resveratrol, a polyphenol with powerful anti-cancer, anti-inflammatory and antioxidant properties, is a phenolic compound (Cianciosi et al., 2018). The antiproliferative effects of polyphenols in honey, including resveratrol, have been documented. Researchers have tested these effects on various cancer cell lines, including those from the colon, prostate and breast (Jaganathan & Mandal, 2009). This suggests that Şanlıurfa honey has the potential to be a functional food with chemopreventive properties. So, to really show this off, we need to do some more bioactivity analyses to check out these effects *in vitro* and *in vivo*.

The connection between phenolic content and antioxidant capacity is well-known and significant in a variety of research areas. Beretta et al. (2005) conducted a quantification of this relationship using standardised spectrophotometric and chemometric methods. Similarly, Zawawi et al. (2021) confirmed that the antioxidant activity of different honey types is strongly affected by flavonoids and phenolic compounds. Although this study primarily focused on phenolic profiling, Şanlıurfa honey's high levels of antioxidant phenolics, consistent with previous research, suggest that it is a significant source of antioxidants.

The phenolic composition of honey varies due to the influence of geographic and botanical factors. Karabagias et al. (2014) stated that monofloral Greek thyme honeys had considerably higher levels of flavonoids such as quercetin (up to 69.00 mg kg^{-1}), kaempferol, myricetin, and syringic acid compared to the Şanlıurfa multifloral honeys examined in this study. In our samples, quercetin was detected at much lower concentrations (mean: 85.11 ± 34.76 ng g^{-1}) and was present in only half of the samples. Furthermore, kaempferol, myricetin and syringic acid were not detected at all. These differences are probably due to the influence of floral diversity, environmental conditions and plant biosynthetic pathways. Şanlıurfa honeys are produced by bees using a mixture of floral sources grown in the region, which could potentially reduce the concentration of individual phenolic compounds. In contrast, thyme honeys are richer in phenolic compounds than flower honeys because they are derived from a single, phenolic-rich source of nectar. The use of phenolic profiles as indicators of botanical and geographical origin should be supported by comparative analyses. These profiles are also crucial for honey authentication, quality control, and regional product characterisation.

The phenolic profiles obtained in our study on multifloral honey samples collected from Şanlıurfa province showed both similarities and notable differences when compared with the findings reported by Kıvrak and Kıvrak (2016). In their study, the researchers analyzed 60 monofloral Turkish honey samples from nineteen different botanical origins. While Kıvrak and Kıvrak (2016) reported exceptionally high total phenolic contents in thyme honey (1051.58 mg kg^{-1}), pine honey (994.18 mg kg^{-1}), and carob honey (935.03 mg kg^{-1}), the total phenolic concentrations detected in this study were generally lower than theirs. This is probably due to the large number of different flower species in our samples and the unique vegetation of the Şanlıurfa region. Although there were differences in total phenolic content, several significant phenolic compounds were found in both studies. Both studies revealed that the following compounds were among the most prevalent: caffeic acid, vanillic acid, and hydroxycinnamic acid (trans-2-hydroxycinnamic acid). In this study, hydroxycinnamic acid was the dominant phenolic compound. Its concentrations ranged from 351.8 to 4817.5 ng g^{-1}. The mean value was 1552.1 ± 1556.3 ng g^{-1}. Similarly, vanillic acid was consistently detected in all Şanlıurfa honey samples, with a mean concentration of 522.1 ± 238.3 ng g^{-1}. These findings are consistent with the findings of another study that identified these compounds as the dominant phenolic compounds in various monofloral honeys. However, some phenolic acids, such as ferulic acid, syringic acid, and homogentisic acid, which were abundant in the data set of Kıvrak and Kıvrak (2016), were not detected in our study. The lack of these compounds could be explained by differences in the origin of the flowers specific to Şanlıurfa, climate, or soil characteristics. Furthermore, the multifloral structure of the analyzed samples may have led to a dilution effect that reduced the concentration of certain monofloral nutrients.

Phenolic profiles are not enough to show the full range of biological activity, so other methods should be used. Combarros-Fuertes et al. (2018) emphasise the importance of linking phenolic profiles to biological activity analyses in order to develop a comprehensive understanding of the functional potential of honey varieties. Therefore, future research is recommended to increase the size of the sample, analyse seasonal variation and include analyses of antioxidant, anti-inflammatory, antimicrobial and anticancer activities.

Limitations

This study provides useful insights into the phenolic composition of Şanlıurfa honey. However, the limited sample size may not cover all aspects of seasonal and geographical variation. A larger sample size, including monofloral honeys and samples taken in different seasons, would enable a more comprehensive study. Moreover, investigating the relationship between phenolic profiles and biological activities, such as anti-inflammatory or antimicrobial effects, would improve the practical value of these results.

Conclusion

This study investigated the phenolic profiles of multifloral honey samples from Şanlıurfa using LC-MS/MS. The results showed that the main phenolic compounds were hydroxycinnamic acid and vanillic acid, with significant variability in phenolic content observed among the samples. This reflects the influence of regional botanical resources and environmental factors on the region's biodiversity. The absence of certain flavonoids, such as myricetin and kaempferol, highlights the specific phytochemical profile of Şanlıurfa honey. The phenolic components of honey contribute to metabolic biological activities, including anti-inflammatory, antioxidant and antimicrobial effects. This is important for food quality control and has significant potential for therapeutic applications. Validation of our analytical approach confirmed its reliability and reproducibility, which is a crucial step in verifying the accuracy and consistency of the results. This comprehensive phenolic profiling provides baseline data for future research. These data can be used for honey identification and quality assessment. The assessment is based on geographic origin. Location is the factor that determines this. Honey produced in this region has functional properties and health benefits that remain largely unexplored. To enhance our data further, future research should focus on increasing sample sizes, considering seasonal variation and conducting bioactivity tests.

Ethical Statement

AI-assisted language tools were employed only for grammar improvement and text editing. The authors take full responsibility for the scientific content and affirm that all analyses, results, and conclusions are original.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Serap Kılıç Altun: Conceptualization (supporting); formal analysis (equal); data curation (equal); funding acquisition (supporting); methodology (equal); investigation (equal); project administration (supporting); resources (supporting); software (supporting); supervision (supporting); validation (supporting); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

Nilgün Paksoy: Conceptualization (supporting); formal analysis (equal); data curation (equal) funding acquisition (supporting); methodology (equal); investigation (equal); supervision (equal); resources (supporting equal); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal).

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