



Optimization of Phenolic Compound Extraction from Bee Products via Solvent Systems and PCA Integration

Ceren Birinci¹ · Yakup Kara¹ · Betül Gıdık² · Sevgi Kolaylı¹ · Zehra Can³

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Abstract

To achieve a highly selective and sensitive analysis of phenolic compounds in bee products (honey, bee bread, and propolis), a simultaneous quantification of 25 phenolic compounds was performed using high-performance liquid chromatography coupled with a diode array detector. In the study, various solvents were utilized to enhance the phenolic profile of bee products, including pH 2 and 7 ultra-pure water, diethyl ether, and ethyl acetate. These included non-liquid–liquid extraction, pH 2 with 10 mL of pure water and 20 mL of diethyl ether; pH 2 with 10 mL of pure water and 20 mL of ethyl acetate; pH 2 with 10 mL of pure water, 10 mL of diethyl ether, and 10 mL of ethyl acetate; and pH 7 with 10 mL of pure water, 10 mL of diethyl ether, and 10 mL of ethyl acetate. Additionally, the integration of phenolic compounds identified through the enrichment method was pursued using principal component analysis (PCA). The extraction yields and recovery rates of phenolic compounds can be significantly improved by combining and integrating different extraction techniques. Our results demonstrated that using different solvent combinations significantly improved both the yield and diversity of phenolic compounds extracted from the complex matrices of bee products.

Keywords Bee products · Phenolic compounds · HPLC · Principal component analysis (PCA)

Introduction

Honeybee-derived products, such as honey, bee bread, and propolis, are increasingly recognized for their rich phenolic content, which plays a vital role in their biological

and therapeutic properties. Phenolic compounds, including flavonoids and phenolic acids, are natural antioxidants that are crucial in combating oxidative stress, reducing inflammation, and providing protection against microbial infections (Martinello and Mutinelli 2021). Numerous studies have demonstrated that these bioactive compounds exhibit various health benefits, including anti-inflammatory, antimicrobial, and anticancer activities, making them significant in both nutritional and medicinal contexts (Pasupuleti et al. 2017).

The composition and concentration of phenolic compounds in bee products can vary widely based on factors such as floral origin, geographic location, and processing methods (Puscas et al. 2013). For instance, the phenolic profile of honey can differ significantly depending on the type of flowers visited by bees, which influences the antioxidant capacity and overall health benefits of the honey produced. Similarly, variations in the phenolic content of bee bread and propolis can be attributed to the specific plant sources and environmental conditions affecting the bees.

To analyze these phenolic compounds effectively, high-performance liquid chromatography (HPLC) is commonly employed, often coupled with diode array detectors (DAD)

✉ Zehra Can
zehracan61@gmail.com

Ceren Birinci
cerendidar.birinci@gmail.com

Yakup Kara
yaakupkara@gmail.com

Betül Gıdık
betulgidik@gmail.com

Sevgi Kolaylı
skolayli61@yahoo.com

¹ Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080 Trabzon, Türkiye

² Department of Organic Farming Management, Bayburt University, Bayburt 69000, Turkey

³ Department of Emergency Aid and Disaster Management, Faculty of Applied Sciences, Bayburt University, 69000 Bayburt, Türkiye

(Tanleque-Alberto and Escriche 2020; Park et al. 2024) or electrochemical detectors (ECD) (Zhu et al. 2019; Wang et al. 2020; Abad-Gil et al. 2022). These methods allow for the precise quantification and identification of phenolic compounds. However, the complex matrices of bee products present challenges in achieving high sensitivity and accuracy in analytical results. During the process of saccharification, for example, efforts to enhance analytical sensitivity may inadvertently lead to longer sample preparation times and potential losses of analyte content, complicating the analysis (Gómez-Caravaca et al. 2006; Lawag et al. 2023).

This study primarily aims to combine advanced enrichment methods with principal component analysis (PCA) to improve both the quantitative and qualitative profiling of phenolic compounds in bee products like honey, bee bread, and propolis. By employing various solvent systems, the research seeks to optimize extraction efficiency and recovery of these bioactive substances. Additionally, the study intends to clarify the chemical variability in bee products and deepen understanding of their phenolic content.

The findings are expected to enhance scientific knowledge about the health benefits of phenolic compounds, supporting their use in nutritional and pharmaceutical applications. Ultimately, the results highlight the importance of tailored extraction techniques to maximize the bioactive potential of bee products.

Material and Methods

Collection of honey, Bee Bread, and Propolis Samples

The bee products (honey, bee bread, and propolis) used in the study were sourced from local beekeepers in the Eastern Black Sea region in 2024.

Sample Preparation of Honey, Bee Bread, and Propolis Samples

The extraction of phenolic compounds from the samples was conducted using a shaker (Heidolph Promax 2020, 200 rpm). Initially, 3 g of each sample was weighed, and 30 mL of 70% ethanol was added to 45 mL centrifuge tubes. The mixture was then agitated in a shaker for 24 h. Upon completion of the extraction period, the extracts were filtered using Whatman No.1 filter paper, followed by a 0.45- μ m filter. Following the determination of the final concentrations of the filtered samples, a division into five methods of analysis was made as follows:

1. NLLE: non-liquid–liquid extraction
2. DEE: pH:2 10 mL pure water, 20 mL diethyl ether

3. EA: pH:2 10 mL pure water, 20 mL ethyl acetate
4. DEE + EA: pH:2 10 mL pure water, 10 mL diethyl ether, 10 mL ethyl acetate
5. DEE + EA: pH:7 10 mL pure water, 10 mL diethyl ether, 10 mL ethyl acetate

The first method was directly analyzed at the HPLC. As for the other four, the final residue obtained through a rotary vacuum evaporator (IKA®-WerkeRV 05 Basic, Staufen, KG, Germany) at 40 °C was dissolved in 2 mL of methanol, filtered, and subsequently injected into the RP-HPLC (Shimadzu Corporation LC 20AT, Japan) coupled with a photodiode-array (PDA) device for analysis (Fig. 1).

Chromatographic Procedure and System Settings

The analysis of phenolic content was performed using a high-performance liquid chromatography (HPLC) system comprising an LC-20AT liquid chromatograph (Shimadzu), an SIL-20AC HT autosampler (Shimadzu), an SPD-M20A diode array detector (Shimadzu), and an InertSustain C18 analytical column (5 μ m particle size, 4.6 mm internal diameter, 250 mm length; GL Sciences). The optimized method consisted of 70% acetonitrile in ultrapure water (mobile phase A) and 2% acetic acid in ultrapure water (mobile phase B).

The gradient elution program 0.0 min, 82% (B); 5.0 min, 81% (B); 10.0 min, 73% (B); 14.0 min, 62% (B); 25.0 min, 35% (B); 40.0 min, 10% (B); 40.01 min, 82% (B); and 50.0 min, 82% (B) was conducted over a total duration of 50 min. The flow rate was maintained at 1.0 mL/min, the column temperature was set to 30 °C, and the injection volume was 20 μ L. The autosampler cell temperature was set at 20 °C. Detection of phenolic compounds was performed using four distinct wavelengths (250 nm, 280 nm, 320 nm, and 360 nm), selected based on the specific absorbance maxima of the standards (Kara et al. 2022).

Determination of Total Phenolic Content (TPC) and Ferric Reducing Antioxidant Power (FRAP Assay)

The total phenolic content (TPC) was determined using the method outlined by Slinkard and Singleton (1977) with minor modifications as follows. Briefly, 20 μ L of each sample was combined with 400 μ L of Folin–Ciocalteu reagent (0.25 N), 400 μ L of 7.5% sodium carbonate solution, and 750 μ L of distilled water. The reaction mixtures were incubated, and the absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Thermo Scientific Evolution TM 201, USA). The TPC was expressed of gallic acid equivalents mg GAE/g.

The ferric reducing antioxidant power (FRAP) of propolis samples was evaluated using the FRAP assay, as described

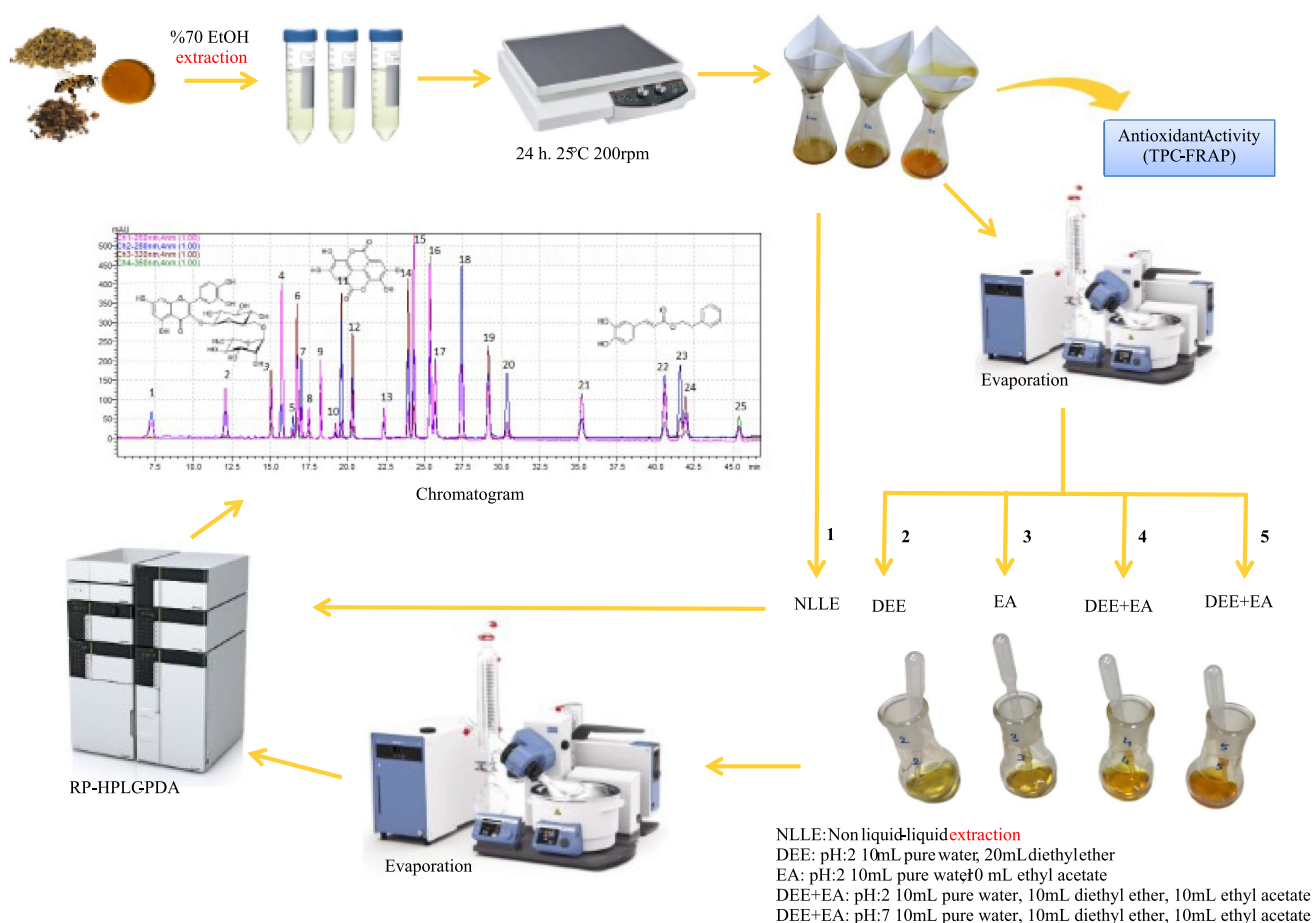


Fig. 1 Extraction conditions for the analysis of phenolic compounds in bee products

by Benzie and Strain (1996). The reaction mixture consisted of 10 mM TPTZ (prepared in 40 mM HCl), 20 mM ferric chloride hexahydrate, and 300 mM acetate buffer. 100 μ L from each sample was mixed with 3000 μ L of FRAP reagent, followed by incubation at room temperature and dark conditions for 4 min. Absorbance was measured at 593 nm using a spectrophotometer. A calibration curve was used with $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (31.25 to 1000 μ M), and the results were expressed as $\mu\text{mol Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O/g}$.

Chemicals

High-purity ($\geq 98.00\%$) standards of gallic acid, protocatechuic acid, chlorogenic acid, *p*-OH benzoic acid, epicatechin, caffeic acid, syringic acid, *m*-OH benzoic acid, rutin, ellagic acid, *p*-coumaric acid, ferulic acid, myricetin, resveratrol, daidzein, luteolin, quercetin, *t*-cinnamic acid, apigenin, hesperidin, rhamnetin, chrysin, pinocembrin, caffeic acid phenethyl ester (CAPE), curcumin. HPLC grade methanol and acetonitril, diethyl ether, ethyl acetate, Folin-Ciocalteu,

TPTZ (2,3,5-triphenyltetrazolium chloride), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) ferric chloride hexahydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Statistical Analysis

The statistical evaluation of the data obtained in this study was performed using the SPSS statistical analysis program, version IBM SPSS Statistics 25. Descriptive statistics were determined, and standard deviation errors of the data were calculated to determine the phenolic content of bee bread, honey, and propolis samples using five different solvents ((1) directly used, (70% ethanol), (2) (pH:2) 20 mL diethyl ether, (3) (pH:2) 20 mL ethyl acetate, (4) (pH:2) 10 mL diethyl ether, 10 mL ethyl acetate, (5) (pH:7) 10 mL diethyl ether, 10 mL ethyl acetate). In this study, principal component analysis (PCA) was applied to determine the principal components of phenolic content of the samples using five different solvents.

Table 1 Total phenolic content and reducing ferric reducing antioxidant power (FRAP) of 70% ethanolic extracts from analyzed bee products

Samples	TPC mg GAE/g	FRAP ($\mu\text{molFeSO}_4 \cdot 7\text{H}_2\text{O/g}$)
Honey	0.70 ± 0.03	6.92 ± 0.08
Bee bread	15.49 ± 0.14	110.70 ± 0.50
Propolis	151.00 ± 2.02	1033.37 ± 8.06

Results and Discussion

Determination of Total Phenolic Content (TPC) and Antioxidant Activity FRAP

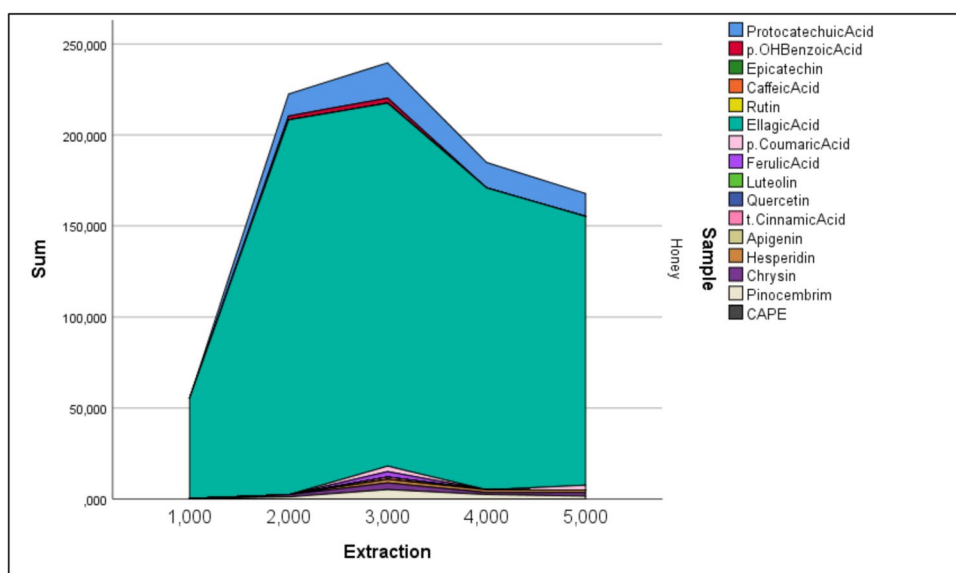
The Folin–Ciocalteu (FC) method is commonly used to assess the total phenolic content (TPC) in various food items, plant materials, beverages, honey, and other natural substances. The total phenolic content (Table 1) of the 70% ethanolic extracts of the analyzed bee products (honey, bee bread, and propolis) was found to be 0.70, 15.49, and 151.00 mg GAE/g.

The FRAP assay is a colorimetric method based on the $\text{Fe}^{3+}/\text{Fe}^{2+}$ reduction capacity and is one of the most widely used techniques in the literature for determining the antioxidant activity of natural products. The FRAP results were determined to be 6.92, 110.70, and 1033.37 ($\mu\text{mol Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O/g}$ sample) for the honey, bee bread, and propolis samples, respectively. Biluca et al. (2021) mention TPC values between 11.0 and 38.9 mg GAE/100 g for the analyzed Brazilian honey. The elevated TPC value observed in our honey sample can be attributed to the distinct botanical origin of the sample. Saral (2023) reported the total

phenolic content (TPC) of Turkish Chestnut honeys 72.79 mg GAE/100 g. In the present study, the TPC of the analyzed honey sample was determined to be 0.696 mg GAE/g. The FRAP value of the honey samples were determined to be $6.92 \pm 0.08 \mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$. Akgün et al. (2021) reported that the FRAP values of various honey types ranged between 0.48 and 1.49 mg TE/g. In our previous study, the total phenolic content (TPC) of bee bread sample collected from the Çanakkale region was determined to be 14.917 mg GAE/g (Can et al. 2024a). It can be concluded that this result is in agreement with the TPC values obtained in the present study. Similarly, it is observed that our FRAP results are consistent with those reported in the same study. In our previous study, the total phenolic content (TPC) of propolis sample was determined to be 52.9 to 203.5 mg GAE/g (Can et al. 2024b). It can be concluded that this result is in agreement with the TPC values obtained in the present study. Similarly, it is observed that our FRAP results are consistent with those reported in the same study.

Effect of Different Extraction Conditions on the Phenolic Content of Honey, Bee Bread, and Propolis

In order to determine the phenolic profiles of honey, bee bread, and propolis samples, five distinct enrichment methods were studied. These methods comprised an ethanolic extract prepared without any prior treatment: 10 mL of pure water at pH 2 combined with 20 mL diethyl ether, 10 mL of pure water at pH 2 combined with 20 mL ethyl acetate, 10 mL of pure water at pH 2 combined with 10 mL diethyl ether and 10 mL ethyl acetate, and 10 mL of pure water at pH 7 combined with 10 mL diethyl ether and 10 mL ethyl

Fig. 2 Phenolic content of honey sample according to different extraction conditions

acetate. In the enrichment method used for determining the phenolic profile, the most suitable solvent for honey samples was identified as 10 mL pure water at pH 2 combined with 20 mL ethyl acetate.

As illustrated in Fig. 2 the phenolic composition of the honey sample varied considerably across different solvent conditions (Table 2). Notably, the application of ethyl acetate under acidic conditions (pH:2) resulted in a substantially higher of phenolic compounds compared to other extraction methods. This result can be attributed to the increased solubility of phenolic acids particularly cinnamic and hydroxybenzoic acid derivatives—in moderately polar solvents under acidic environments.

Among the identified compounds, caffeic acid emerged as the high phenolic constituent, followed by protocatechuic acid, ferulic acid, and smaller quantities of quercetin and luteolin. These findings suggest that the choice of solvent and pH plays a critical role in modulating the phenolic profile extracted from honey samples. Paulo et al. (2024) reported the presence of gallic acid, quercetin, and ferulic acid in honey samples, while Wang et al. (2023) found that extraction using 0.01 mol/L HCl aqueous solution and ethyl acetate yielded a broader phenolic spectrum. Therefore, the use of ethyl acetate at pH 2 may be considered a highly effective extraction method when targeting phenolic acid enrichment in honey.

Table 2 Phenolic content of honey sample

Phenolic standards (µg/g)		1	2	3	4	5
Phenolic acids	Hydroxybenzoic acids					
	p-OH Benzoic acid	-	2.150	2.761	-	-
	Protocatechuic acid	-	12.117	19.379	13.974	12.630
	Gallic acid	-	-	-	-	-
	Chlorogenic acid	-	-	-	-	-
	Syringic acid	-	-	-	-	-
	Ellagic acid	55.137	205.917	199.394	165.828	147.137
	m-OH Benzoic acid	-	-	-	-	-
	Curcumin	-	-	-	-	-
	Hydroxycinnamic acids					
	t-cinnamic acid	-	-	1.270	-	-
	Ferulic acid	-	-	2.980	-	-
	p-Coumaric acid	-	-	3.005	-	2.634
	Caffeic acid	-	-	-	-	-
	Caffeic acid phenethyl ester (CAPE)	-	-	-	-	-
	Total phenolic acid	55.137	220.184	228.789	179.802	162.401
Flavanoids	Flavonol					
	Rhamnetin	-	-	-	-	-
	Quercetin	-	-	-	-	-
	Rutin	-	-	-	-	-
	Myricetin	-	-	-	-	-
	Flavan-3-ols					
	Epicatechin	-	-	-	-	-
	Flavones					
	Chrysin	0.146	1.124	3.713	1.124	1.979
	Daidzein	-	-	-	-	-
	Apigenin	-	-	-	-	-
	Luteolin	-	-	-	-	-
	Flavanones					
	Pinocembrin	0.146	1.197	5.155	2.467	1.588
	Hesperidin	-	-	1.954	1.441	1.368
	Total flavonoid	0.292	2.321	10.823	5.032	4.935
	Stilbenes					
	Resveratrol	-	-	-	-	-

not detected (1: Non-liquid-liquid extraction honey, 2: pH:2 20 mL diethyl ether honey, 3: pH:2 20 mL ethyl acetate honey, 4: pH 2 combined with 10 mL diethyl ether and 10 mL ethyl acetate honey, 5: 7 combined with 10 mL diethyl ether and 10 mL ethyl honey); acetate

In addition to quantitative comparisons, principal component analysis (PCA) was conducted to explore the underlying patterns and relationships among the phenolic profiles of honey samples extracted with different solvent systems. The PCA revealed a clear separation between extraction methods based on their phenolic composition, particularly distinguishing the pH 2 and ethyl acetate method (solvent 3) from the others. This clustering indicates that solvent 3 resulted not only in a higher concentration of phenolic compounds but also in a more diverse phenolic profile.

The first two principal components accounted for a significant proportion of the total variance in the dataset, suggesting that a limited number of variables effectively explain most of the variation observed across extraction methods. Compounds such as caffeic acid, ferulic acid, and protocatechuic acid showed strong loadings on the principal components, further supporting their influential role in characterizing the phenolic signature of the honey extracts.

Overall, PCA provided valuable insights into the multivariate relationships between extraction conditions and phenolic content, reinforcing the finding that acidic ethyl acetate extraction yields a distinct and phenolic-rich profile compared to other methods.

In this study, the effect of different solvent conditions on the diversity of phenolic compounds in bee bread was investigated (Fig. 3, Table 3), and this effect was further evaluated

in detail using principal component analysis (PCA). The results obtained showed that extractions using solvent 2 (pH: 2, 20 mL diethyl ether) and solvent 4 (pH: 2, 10 mL diethyl ether, 10 mL ethyl acetate) were more effective in terms of phenolic content compared to other solvents.

PCA analysis provided a clearer understanding of the impact of solvents on the diversity of phenolic compounds. The first two principal components (PC1 and PC2) explained 85% of the total variance, summarizing the relationship between phenolic compounds and solvents. Notably, the extractions with solvent 4 and solvent 2 were located close to each other on the PCA plot, indicating a high concentration of compounds such as *p*-coumaric acid and chrysin. This suggests that these solvents are more efficient in extracting phenolic compounds.

Solvent 4, in light of PCA analysis, emerged as the most prominent solvent in terms of flavonoid diversity. This solvent allowed for the extraction of high levels of compounds such as apigenin, kaempferol, and *p*-coumaric acid, thus enhancing the richness of the phenolic profile. Furthermore, PCA analysis revealed that solvent 4 has a broader solvent capacity and selective solubility for different phenolic classes, contributing to its effectiveness.

The PCA results obtained in this study also indicated that differences in the polarity of solvents and pH conditions significantly alter the solubility of phenolic compounds,

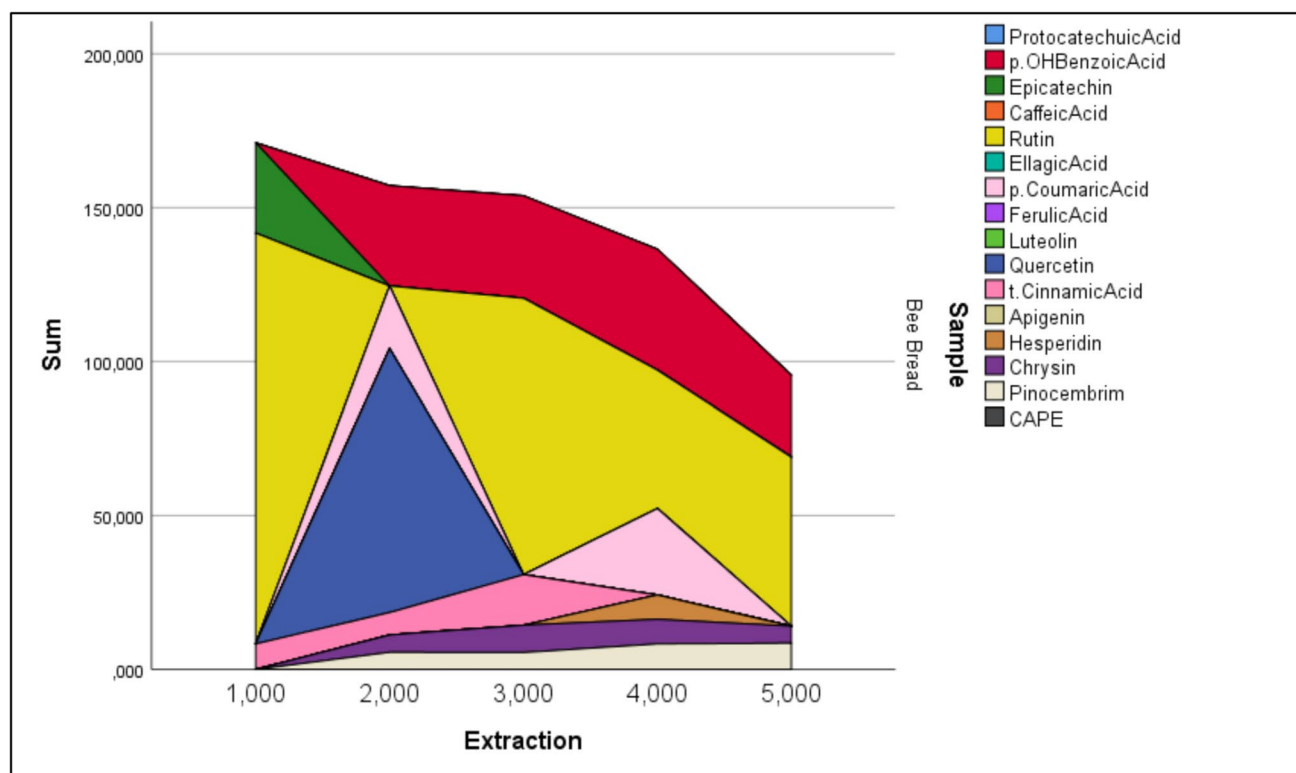


Fig. 3 Phenolic content of bee bread sample according to different extraction conditions

Table 3 Phenolic content of bee bread sample

Phenolic standards (µg/g)		1	2	3	4	5
Phenolic acids	Hydroxybenzoic acids					
	p-OH Benzoic acid	-	32.598	33.295	39.272	26.571
	Protocatechuic acid	-	-	-	-	-
	Gallic acid	-	-	-	-	-
	Chlorogenic acid	-	-	-	-	-
	Syringic acid	-	-	-	-	-
	Ellagic acid	-	-	-	-	-
	m-OH Benzoic acid	-	-	-	-	-
	Curcumin	-	-	-	-	-
	Hydroxycinnamic acids					
	t-Cinnamic acid	8.293	7.272	16.411	-	-
	Ferulic acid	-	-	-	-	-
	p-Coumaric acid	-	20.371	-	28.115	-
	Caffeic acid	-	-	-	-	-
	Caffeic acid phenethyl ester (CAPE)	-	-	-	-	-
	Total phenolic acid	8.293	60.241	49.706	67.387	26.571
Flavanoids	Flavonol					
	Rhamnetin	-	-	-	-	-
	Quercetin	-	85.691	-	-	-
	Rutin	133.429	-	89.775	44.899	54.961
	Myricetin	-	-	-	-	-
	Flavan-3-ols					
	Epicatechin	29.361	-	-	-	-
	Flavones	-	-	-	-	-
	Chrysin	-	5.653	8.890	7.994	5.504
	Daidzein	-	-	-	-	-
	Apigenin	-	-	-	-	-
	Luteolin	-	-	-	-	-
	Flavanones					
	Pinocembrin	-	5.553	5.479	8.268	8.542
	Hesperidin	-	-	-	7.919	-
	Total flavonoid	162.79	96.897	104.144	69.08	69.007
	Stilbenes					
	Resveratrol	-	-	-	-	-

not detected (1: Non-liquid-liquid extraction bee bread, 2: pH:2 20 mL diethyl ether bee bread, 3: pH:2 20 mL ethyl acetate bee bread, 4: pH 2 combined with 10 mL diethyl ether and 10 mL ethyl acetate bee bread, 5: 7 combined with 10 mL diethyl ether and 10 mL ethyl bee bread)

thereby affecting extraction efficiency. PCA analysis has provided an important visual representation of how solvents differentiate the spectrum of phenolic components, helping to classify the solvents' extraction efficiencies more clearly.

Various studies in the literature have shown that bee bread contains significant levels of phenolic compounds, which are an important source of biological activity, rich in different phenolic acids and flavonoid compounds. Isidorov et al. (2009) reported that phenolic compounds such as kaempferol, *p*-coumaric acid, and isorhamnetin dominate bee bread. Similarly, Markiewicz et al. (2013) and Sobral et al. (2017) found high concentrations of flavonoid and phenolic compounds, including apigenin, chrysin, naringenin, caffeic

acid, ferulic acid, gallic acid, and quercetin in bee bread. Kaškonienė et al. (2008) indicated that the predominant phenolic compounds in bee bread samples from Lithuania were *p*-coumaric acid, kaempferol, apigenin, and chrysin, which formed the main components of the phenolic profile. In other study was reported that gallic acid, caffeic acid, quercetin, and kaempferol were the major phenolic constituents in both monofloral and multifloral bee bread samples collected in Turkey (Çobanoğlu et al. (2023)). In conclusion, the solvent selection and pH conditions play a crucial role in the extraction of phenolic compounds, directly influencing the diversity of phenolic components and their potential biological activities.

In this study, the influence of various solvent conditions on the phenolic composition of propolis was systematically evaluated. As presented in Fig. 4, among the different solvent systems evaluated, solvent 2 (pH: 2, 20 mL diethyl ether) and solvent 3 (pH: 2, 20 mL ethyl acetate) were found to be more effective in facilitating the extraction of a broader range and higher concentrations of phenolic compounds, as also supported by the data in Table 4. In particular, ethyl acetate exhibited notable selectivity and efficiency in the extraction of both flavonoids and phenolic acids, which may be attributed to its intermediate polarity and reduced affinity for non-target matrix constituents.

The PCA (principal component analysis) was employed to further investigate the variability among the solvent systems and their influence on phenolic compound profiles. The first two principal components (PC1 and PC2) cumulatively explained approximately 82% of the total variance, indicating a robust differentiation power. PCA biplot visualization revealed that extractions performed with solvent 3 clustered distinctly, demonstrating strong associations with compounds such as *p*-coumaric acid, CAPE (caffeic acid phenethyl ester), ferulic acid, and pinocembrin key bioactive constituents in propolis known for their antioxidant, antimicrobial, and anti-inflammatory properties. Solvent 2 also showed a close proximity to solvent 3 in the PCA space, indicating a similar phenolic profile, particularly in the concentration of quercetin, hesperidin, and chrysin. These findings insight that both solvents are effective in targeting phenolic subclasses that contribute significantly to the bioactivity of propolis.

From a bioanalytical perspective, the observed clustering patterns suggest that solvent polarity and pH conditions are decisive parameters in modulating phenolic solubility and thus extraction efficiency. Ethyl acetate, in particular, stands

out as a solvent that combines efficiency with low toxicity, a characteristic that has been validated in previous literature (Yang et al. 2011; Niyomtham et al. 2021). In conclusion, the integration of PCA analysis has provided valuable insights into the extraction performance of different solvents. Solvent 3 (ethyl acetate) emerges as the optimal choice for extracting a broad spectrum of phenolic compounds from propolis, enhancing the potential for therapeutic use. These results underscore the importance of solvent selection in analytical extractions and bioactive profiling.

Principal Component Analysis (PCA)

Principal component analysis (PCA) is an analysis that can be used to reveal the variance structure of the original variable p with a smaller number of new variables that are linear components of the variables. Principal components for phenolic content of bee bread, honey, and propolis samples determined using five different solvents were determined using PCA. Eigenvalues and variance percentages of PCA analysis are shown in Table 5 and the graph is shown in Fig. 5.

The PCA analysis showed that variance explanation ratios greater than 70% were adequate (Larrigaudiere et al. 2004). For PCA, PC1, PC2, and PC3 explained 85.02% of the total variation, while PC1 alone elucidated 59.297% of the variance, indicating its dominant role in capturing the primary source of variability among the samples. PC2 contributed an additional 15.378%, while PC3 accounted for 10.345%, together highlighting the cumulative effect of these components in representing the data structure.

According to Kaiser rules, eigenvalues of greater than 1.0 are accepted as the principal component and descriptor of the variance (Kaiser 1960). In PCA, eigen values of PC1

Fig. 4 Phenolic content of propolis sample according to different extraction conditions

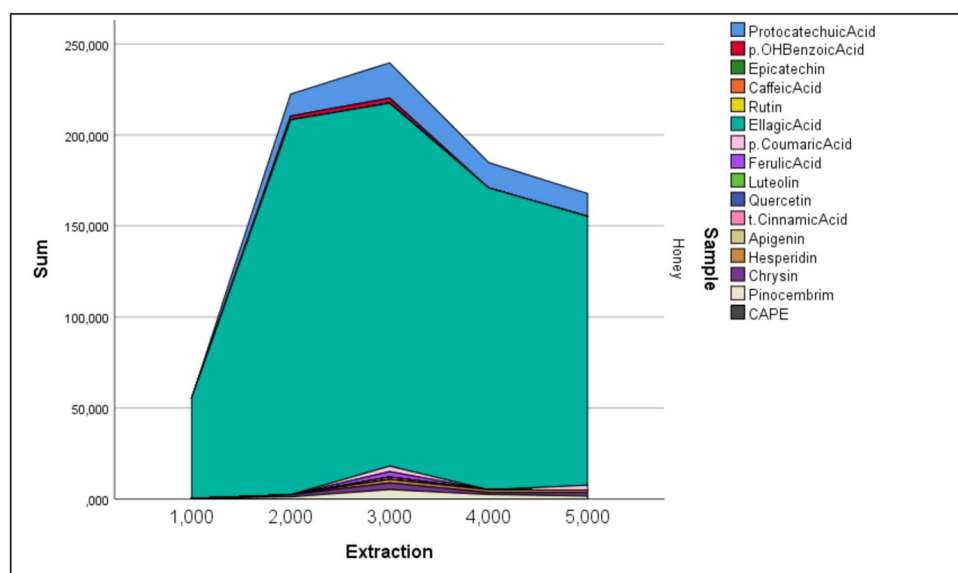


Table 4 Phenolic content of propolis sample

Phenolic standards (µg/g)		1	2	3	4	5
Phenolic acids	Hydroxybenzoic acids					
	p-OH Benzoic acid	-	-	-	-	-
	Protocatechuic acid	-	-	-	-	-
	Gallic acid	-	-	-	-	-
	Chlorogenic acid	-	-	-	-	-
	Syringic acid	-	-	-	-	-
	Ellagic acid	-	-	-	-	-
	m-OH Benzoic acid	-	-	-	-	-
	Curcumin	-	-	-	-	-
	Hydroxycinnamic acids					
	t-Cinnamic acid	282.028	728.716	800.165	766.409	959.376
	Ferulic acid	3376.711	8912.472	9285.294	8834.542	10,598.887
	p-Coumaric acid	1752.599	4497.469	4664.398	4589.709	5406.669
	Caffeic acid	773.315	1897.340	1903.946	1868.895	2341.557
	Caffeic acid phenethyl ester (CAPE)	1137.561	3117.368	2618.603	3165.906	3826.561
	Total phenolic acid	7322.21	19,153.4	19,272.4	19,225.5	23133.1
Flavanoids	Flanonol					
	Rhamnetin	-	-	-	-	-
	Quercetin	-	198.414	195.448	207.489	235.791
	Rutin	-	-	-	-	-
	Myricetin	-	-	-	-	-
	Flavan-3-ols					
	Epicatechin	-	-	-	-	-
	Flavones	-	-	-	-	-
	Chrysin	1006.631	5190.786	4779.074	4997.307	4276.576
	Daidzein	-	-	-	-	-
	Apigenin	-	502.082	510.508	484.631	480.015
	Luteolin	-	14.908	13.512	-	-
	Flavanones					
	Pinocembrin	1244.808	4642.410	4559.918	4637.100	4390.881
	Hesperidin	-	-	-	-	-
	Total flavonoid	2251.4	10,548.6	10,058.5	10,326.5	9383.3
	Stilbenes					
	Resveratrol	-	-	-	-	-

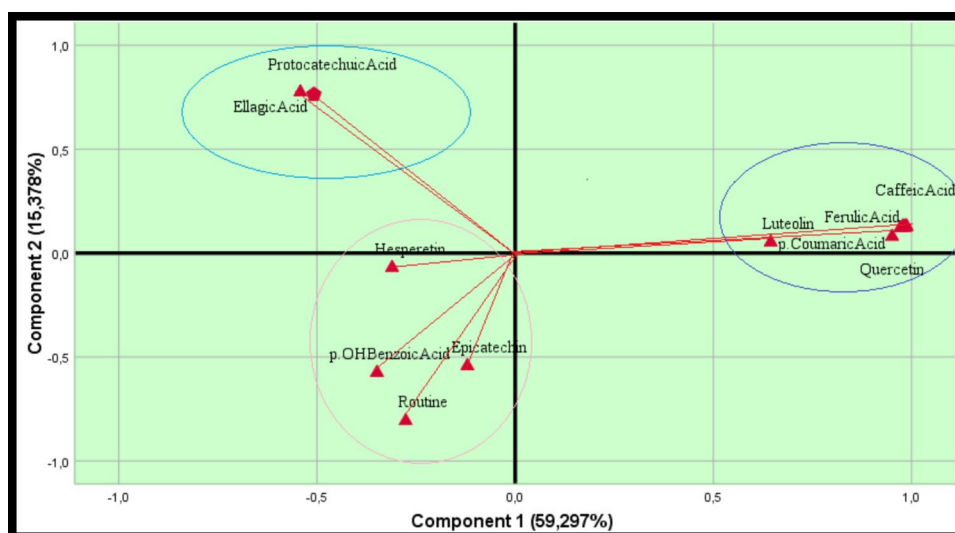
not detected (1: Non-liquid–liquid extraction propolis, 2: pH:2 20 mL diethyl ether propolis, 3: pH:2 20 mL ethyl acetate propolis, 4: pH 2 combined with 10 mL diethyl ether and 10 mL ethyl acetate propolis, 5: 7 combined with 10 mL diethyl ether and 10 mL ethyl propolis)

Table 5 Eigenvalues and percentage of variance for investigated parameters of PCA analysis

	Eigenvalue	Variability (%)	Cumulative (%)
PC1	10.673	59.297	59.297
PC2	2.768	15.378	74.675
PC3	1.862	10.345	85.020

(10.673), PC2 (2.768), and PC3 (1.862) were greater than 1.0. This result further emphasizes the importance of these components in the data representation, with PC1 being particularly influential. The high eigenvalue of PC1 suggests it captures the most critical variation in the dataset, while PC2 and PC3 serve as additional axes that explain further, albeit lesser, dimensions of variability.

Fig. 5 PCA score plot bases on the phenolic content of bee bread, honey, and propolis samples determined using different solvents



Conclusions

In this study, the phenolic profiles of various bee products honey, bee bread, and propolis were systematically analyzed following an enrichment procedure involving different organic solvents. A total of 25 phenolic compounds were identified using RP-HPLC–PDA analysis. Principal component analysis (PCA) effectively revealed the impact of different solvent systems on the extraction efficiency and compositional diversity of phenolics. The findings demonstrated that solvents 2 and 3 yielded higher phenolic diversity across all sample types, while solvent-specific efficiency varied by matrix: solvent 3 was most effective for honey, solvents 2 and 4 for bee bread, and solvents 2 and 5 for propolis. The PCA further emphasized the solvent-dependent clustering of phenolic compounds and highlighted the distinct chemical characteristics inherent to each bee product. Overall, these results underscore the critical role of solvent selection in maximizing phenolic recovery and suggest that targeted optimization of extraction protocols can significantly enhance the analytical and functional evaluation of apicultural matrices. This study provides a valuable framework for future research in food chemistry, natural product extraction, and bioactivity-oriented profiling.

Author Contributions CB antioxidant activity and phenolic compound analysis. YK phenolic compounds analysis. GB statistical analysis. SK designed the experimental plans. ZC Conceptualization, Methodology, Data curation, Formal analysis, Writing-Original draft preparation, and Editing. All authors have read and approved the final manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

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