

Simultaneous determination of twenty-five polyphenols in multifloral and cactus honeys using solid-phase extraction and high-performance liquid chromatography with photodiode array detection

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Abstract Simple and accurate high-performance liquid chromatography–photodiode array detection method has been proposed for simultaneous separation and determination of 25 polyphenol compounds, including 10 phenolic acids, nine flavonoids, and six other phenols in 17 varieties of multifloral and cactus honey of Yemen. Multiwalled carbon nanotubes were used as solid-phase adsorbent for extraction of the polyphenols from honey samples. The total amount of phenolic acids, flavonoids, and other phenols from all 17 analyzed honeys were found in the range of 328–3448, 122–5482, and 2.4–677.6 µg/100 g of honey, respectively. The 4-hydroxybenzoic acid was found in major quantity (10.642 mg/100 g honey) among all analyzed honeys, while chrysin (5.029 mg/100 g honey) was found to be the main detected flavonoid and cinnamic acid the main other phenolic compound (2.330 mg/100 g honey). Among 25 analyzed compounds, 24 were detected (except luteolin) and determined quantitatively in honey samples. The regeneration of solid-phase adsorbent and recovery results showed potential usefulness of the proposed method for the routine analysis of polyphenols in honey extracts.

Keywords HPLC · Multiwalled carbon nanotubes · Honey · Polyphenols · Ultraviolet detection

Introduction

Honey is yellowish or brownish viscid fluid produced by bees from the nectar of flowers or from the secretion obtained from the living parts of plants. It is produced naturally, but the synthetic forms are also available commercially where the former is considered healthier [1]. Since the ancient times, it has been used as medicinal food and preservative having multiple tastes and flavors [2]. The honey can be made from a variety of different flowers, and its flavor, texture, and chemical composition depend on the floral source from which it was collected [3]. Hence, to check the authenticity and quality control of honey, it is necessary to establish a simple and accurate method to perform extensive honey compositional analysis which will help to identify its most characteristic constituents [4].

Although sugar and water are the major components of honey, there are many kinds of polyphenol compounds that are found in it including flavonoids [5]. The findings from recent research studies have discovered that phenolic compounds present in honey can be used as indicators of floral origins and the quality of honey [6, 7]. The health implications also warrant further knowledge of flavonoid contents of the food supply such as honey [8]. Many authors have reported that flavonoids and phenolic acids of honey are responsible for significant antioxidant capacity [9–13], and other beneficial pharmacologic properties of honey include wound healing, anti-inflammatory, anti-mutagenic and anti-tumoural [14, 15], protection of skin cells and tissues from oxidative damage [16], and food preservation [17, 18]. Therefore, it is highly demanding to

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analyze honey and find out which polyphenols are present and in what amount.

Many analytical methods have been reported in the literature for the analysis of polyphenol contents in honey [19–23]. The reported liquid chromatographic techniques for the analysis of organic acids in honey have proven to be very useful in determining the authenticity of the sample from the floral and geographical origin of honey [7, 19, 22, 24, 25]. Therefore, the aim of the present work was the separation and simultaneous identification and quantitation of phenolic acids, flavonoids, and other phenols of various multifloral and cactus Yemeni honeys by HPLC using UV absorbance detection. Multiwalled carbon nanotubes (MWCNTs) have been used as solid-phase extraction adsorbent [26] for extraction of the polyphenols from honey samples. The unique physical, mechanical, chemical, and thermal properties of MWCNTs provide exceptionally better sorption ability and high stability to the extraction methodology [26–28]. These nanomaterials have also shown excellent recovery and regeneration properties. On addition, the developed HPLC–UV technique was easy to apply, sensitive for routine analysis, and a potential step forward in the analysis of various honey samples.

Materials and methods

Chemicals and reagents

HPLC-grade solvents were used throughout the experiment. Carvacrol, 4-hydroxybenzoic acid, maleic acid, chlorogenic acid, sinapic acid, and naringenin were bought from Aldrich Chemicals Co. (Milwaukee, WI, USA). Galangin, gallic acid, apigenin, kaempferol, luteolin, caffeic acid, quercetin, and p-coumaric acid were obtained from Sigma Chemicals Co. (St Louis, MO, USA). Acetonitrile, acetic acid, ethanol, formic acid, diethyl ether, methanol, hydrochloric acid, and anhydrous sodium carbonate were purchased from BDH Chemicals Co. (England). Naringin, syringic acid, thymol, 4-hydroxyphenyl acetic acid, chrysin, myricetin, and vanillic acid were purchased from Acros Organics (New Jersey, USA). Potassium acetate was bought from Riedel-de-Haen Co. (Seelze, Germany), while benzoic acid was obtained from Winlab, cinnamic acid from SAFC, ferulic acid from Fluka (St. Louis, MO, USA), phenol from Merck (NJ, USA), sodium sulfate from Koch-Light Lab. Ltd. (Haverhill, Suffolk, UK), and MWCNTs from Timesnano (Chengdu Organic Chemicals Co. Ltd., China). Ultrapure water used for HPLC mobile phase and sample preparation was obtained using a Milli-Q water purification instrument from Millipore (Bedford, MA, USA).

Sample collection

The honey samples were collected from beehives fed either with multifloral species or with cactus trees. Mainly, nine types of multifloral and eight types of cactus honey from various Yemeni regions were collected in different harvesting seasons, in order to study the influence of geographical origin on the profile of polyphenols in honey composition. The samples were numbered from 1 to 17. All these samples were preserved at a temperature below 0 °C inside the refrigerator. The production year and region of collection of these analyzed honey samples are listed in Table 1.

Extraction procedure

In the proposed method, solid-phase extraction procedure was used for the extraction of polyphenols from honey. The extractions of polyphenol species were accomplished by following the procedure described in our previously published paper [29]. In brief, the procedure can be explained as follows. Each honey sample of 200 g was dissolved thoroughly in three parts using distilled water (200 mL each), while the solution pH was maintained at 2 by addition of HCl. Then, 1 g of MWCNTs which act as solid-phase adsorbent was added to this acidified honey solution. The resultant solution was then magnetically stirred for 20–30 mins, and the extreme large surface area and the unique tubular structure of MWCNTs [30, 31] allow the adsorption of honey phenolics onto them [32]. The vacuum filtration on a cellulose nitrate membrane filter (47 mm

Table 1 Honey samples collection region and respective year of cultivar

Floral source	Sample code	Yemeni region	Harvesting year
Multifloral	1	Hadramout	2008
	2	Hadramout	2010
	3	Hadramout	2009
	4	Hadramout	2011
	5	Hadramout	2010
	6	Abaien	2010
	7	Shanwa	2010
	8	Hadja	2010
	9	Scotry	2010
Cactus	10	Hadramout	2009
	11	Hadramout	2011
	12	Hadramout	2010
	13	Hadramout	2010
	14	Hadramout	2009
	15	Hadja	2011
	16	Mantuka	2010
	17	Abh	2010

diameter, 0.45 μm porosity) was performed to separate the MWCNTs from honey samples. After that, the separated MWCNTs were washed with 100 mL of acidified water of pH 2.0, followed by washing with 300 mL of ultrapure water to remove sugars and other polar constituents [33]. After that the phenolic compounds which were retained onto the surface of MWCNTs were eluted with methanol (300 mL) and then concentrated under reduced pressure and at around 40 °C temperature in a rotary evaporator (Buchi, Model V-850, Switzerland). The residue was then mixed with 6 mL of distilled water and extracted with diethyl ether (5 mL \times 3). Then, re-concentration of the extraction solution was carried out by removing diethyl ether using nitrogen flushing. Again, this concentrated residue was dissolved in 2 mL of methanol and filtered with 0.45- μm membrane filter before injecting into the HPLC system.

Apparatus

Finnigan Surveyor Plus HPLC system from Thermo Scientific (FL, USA) was used for the analyses of phenolic acids, flavonoids, and other phenols contents of Yemeni honey. The system is equipped with a quaternary LC Pump Plus, a photodiode array detector (PDA Plus), and an Auto sampler Plus.

Chromatography conditions

The chromatographic separation of all the polyphenols was achieved using a reversed-phase Betasil C₁₈ column (Thermo Scientific, FL, USA) of 150 mm length and 4.6 mm I.D and packed with 3- μm size particles. Mobile phases of various compositions of water, acetonitrile, methanol, and mixtures of 0.1 % (V/V) formic acid with methanol or acetonitrile were tested at different flow rates (in the range 0.05–1 mL/min) in both isocratic and gradient elution modes to achieve the best separations of the polyphenols. The studied PDA detection wavelengths were in the range of 200 to 600 nm [31]. Column oven temperature was also checked from 30 to 60 °C to see the effect of column temperature. The standards and honey extracts were injected to the system with an auto-injector, and the target polyphenol compounds were detected using PDA detector. A Welch Duo-Seal vacuum pump (Model No.1400, USA) was used for sample filtration. A shaker (Kjanke & Kunkel Ika Labor Technik Ks501D) was used for mixing the solutions, while a pH meter (Metrohm 6.0228.000) was used for pH measurements of the samples.

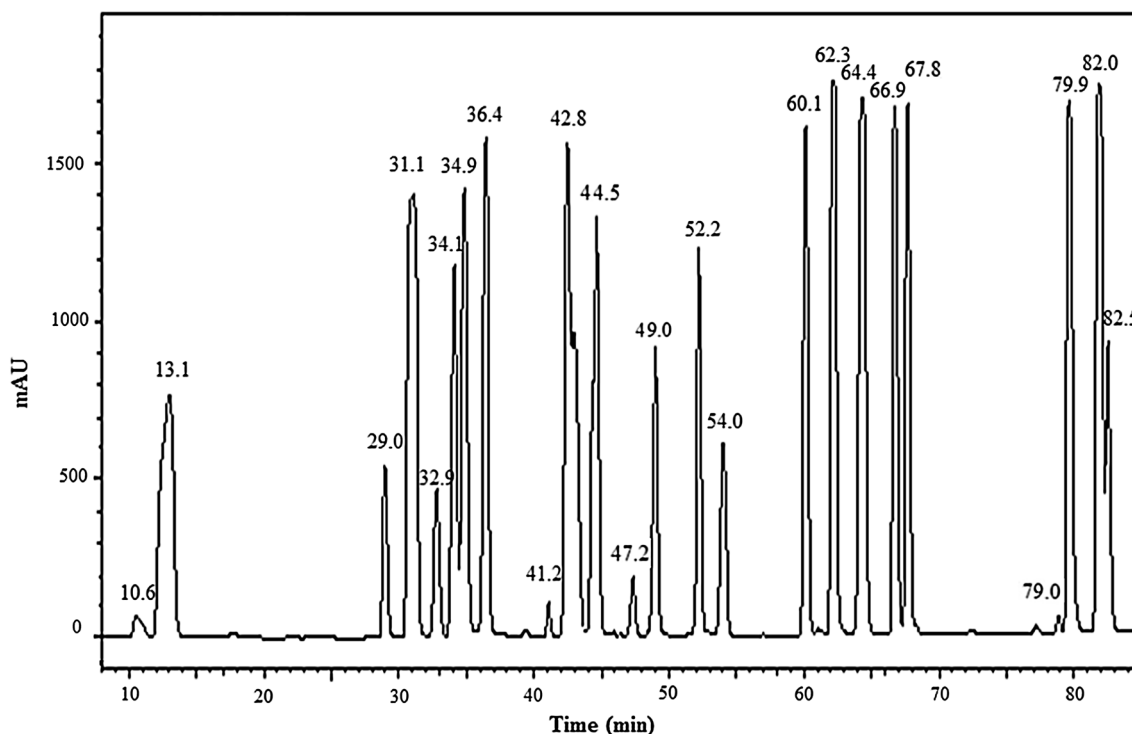


Fig. 1 HPLC–PDA chromatogram of mixture of all standard polyphenols at λ_{max} 270 nm, where retention times for Mala, 10.6; Gala, 13.1; Cloa, 29.0; 4Hba, 31.1; 4Hpa, 32.9; Cafa, 34.1; Vana, 34.9;

Syra, 36.4; Pcoa, 41.2; Phe, 42.8; Fera, 44.5; Sina, 47.2; Nar, 49.0; Myr, 52.2; Bena, 54.2; Quer, 60.1; Narn, 62.3; Cina, 64.4; Kaem, 66.8; Api, 67.8; Thy, 79.0; Gal, 79.9; Chr, 82.0 and Car, 82.5 min

Table 2 UV wavelength of each polyphenol component and their corresponding abbreviations and retention times

Abbreviations	Analyte names	Retention time (min)	UV wavelength (nm)
Mala	Maleic acid	10.6	245
Gala	Gallic acid	13.1	275
Cloa	Chlorogenic acid	29.0	249–325
4Hba	4-Hydroxybenzoic acid	31.1	259
4Hpa	4-Hydroxyphenyl acetic acid	32.9	240–279
Cafa	Caffeic acid	34.1	320
Vana	Vanillic acid	34.9	251–283
Syra	Syringic acid	36.4	278
Pcoa	p-Coumaric acid	41.2	246–316
Phe	Phenol	42.8	275
Fera	Ferulic acid	44.5	320
Sina	Sinapic acid	47.2	240–321
Nar	Naringin	49.0	287–332
Lut	Luteolin	nd	260–337
Myr	Myricetin	52.2	249–379
Bena	Benzoic acid	54.2	247–279
Quer	Quercetin	60.1	250–376
Narn	Naringenin	62.3	280–325
Cina	Cinnamic acid	64.4	273
Kaem	Kaempferol	66.8	260–356
Api	Apigenin	67.8	260–327
Thy	Thymol	79.0	279
Chr	Chrysin	79.9	257–325
Gal	Galangin	82.0	245–340
Car	Carvacrol	82.5	275

nd not detected

Results and discussion

Optimization of separation conditions

The preliminary analyses were carried out on a standard mixture of 25 polyphenol compounds. The optimization of chromatographic conditions was performed to obtain the best chromatograms with good resolution of adjacent peaks and to avoid peak tailing. To establish the optimum separation conditions for all analyzed compounds, the different chromatographic parameters such as columns, mobile-phase composition, flow rate, and detection wavelength were investigated. Various types of reversed-phase chromatographic columns such as Zorbax SB-C₁₈ column, Hypersil ODS column, Betasil C₈ column, and Betasil C₁₈ column were tested. Betasil C₁₈ column showed better results than other columns. The mobile-phase optimization was performed by checking the different solvents. To obtain the proper mobile phase, methanol–water, acetonitrile–water, formic acid–methanol, and combination of tetrabutyl ammonium hydroxide–methanol/acetonitrile were tried, and it was found that the methanol–water combination gave good results, but tailing was observed.

However, when the aqueous formic acid (0.1 %) was used along with the methanol, there was a considerable improvement in the peak shape and its symmetry was noticed. But to achieve the optimum separation, both the isocratic and gradient elution modes with a series of different solvent programs were tested. Best separation was achieved in linear gradient elution profile with a binary mobile-phase mixture of aqueous 0.1 % formic acid (A) and methanol (B) at a constant flow rate of 0.2 mL/min with shortest analysis time. Initially, the composition of A was 80 % and it was decreased linearly to 15 % during the time interval of 70 min; then, it remains constant up to final analysis time (90 min). The column oven temperature was kept fixed at 50 °C.

The sample injections were carried out with an autosampler, and the target compounds were detected using PDA. The chromatograms of all standard samples were monitored in the ultraviolet range of 200–600 nm, but 270 nm was chosen as optimum, since the majority of the honey flavonoids and phenolic acids show their UV absorption maxima around this wavelength (Fig. 1). The results show good agreement with the reported results obtained by Martos et al. [34]. The volume for each sample injection was

Table 3 Validation parameters of the studied method (HPLC–PDA)

Samples type	Samples	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	Correlation coefficient (r^2)	Precision (RSD %) ^a	
					Inter-day	Intra-day
Phenolic acids	4Hba	0.031	0.111	0.998	1.6	2.2
	4Hpa	0.081	0.271	0.999	1.9	2.1
	Cafa	0.027	0.086	0.999	2.1	2.4
	Cloa	0.016	0.051	0.998	1.8	2.4
	Fera	0.024	0.075	0.999	1.9	2.5
	Gala	0.033	0.122	0.998	2.0	2.1
	Pcoa	0.019	0.058	0.999	2.1	2.5
	Sina	0.030	0.095	0.999	1.9	2.2
	Syra	0.029	0.100	0.997	1.8	2.0
	Vana	0.020	0.054	0.999	2.0	2.4
Flavonoids	Api	0.029	0.097	0.998	1.8	2.1
	Chr	0.036	0.120	0.999	1.9	2.1
	Gal	0.018	0.063	0.997	2.1	2.5
	Kaem	0.041	0.140	0.998	1.7	2.1
	Lut	0.029	0.095	0.999	1.9	1.9
	Myr	0.036	0.121	0.999	1.8	2.2
	Nar	0.14	0.461	0.999	2.1	2.1
	Narn	0.027	0.089	0.997	1.9	2.3
	Quer	0.029	0.096	0.999	1.7	2.1
	Other phenols	Bena	0.037	0.122	0.998	1.8
Car		0.018	0.062	0.998	1.8	2.2
Cina		0.034	0.110	0.999	2.0	2.1
Mala		0.400	1.350	0.998	2.0	2.3
Phe		0.037	0.120	0.999	1.9	2.2
Thy		0.038	0.131	0.998	2.2	2.4

^a Relative standard deviation ($n = 5$)

10 μL . The obtained UV wavelength corresponding to the absorbance maximum of each individual component with its retention time is listed in Table 2. In this table, the list of abbreviations of each analyte is also provided.

Performance of the proposed method

Calibration and linearity

0.05, 0.1, 0.5, 1, 5, 10, 50, 100, and 200 $\mu\text{g/mL}$ calibration standards of each polyphenol were prepared in methanol. Linear relationship of the proposed method was established under the optimal experimental conditions by investigating the detection signal as a function of analyte concentration, with the aid of a linear regression equation by the method of least squares. The calibration curve was plotted for each standard of phenolic acids, flavonoids, and other phenols which was linear over the range of 0.05–200 $\mu\text{g/mL}$. The correlation coefficient (r^2) was found to be >0.996 (Table 3) for each regression equation.

Determination of the limit of detection and quantification

The limit of detection (LOD) (at a signal-to-noise ratio 3:1) and limit of quantification (LOQ) (at a signal-to-noise ratio 10:1) values of the proposed method were separately determined by analyzing three replicates of a blank sample (Milli-Q water) spiked with each standard at low concentration levels [35]. The LOD values were found between 0.015 and 0.041 $\mu\text{g/mL}$, while the LOQ values were between 0.051 and 1.35 $\mu\text{g/mL}$ for each standard compound (Table 3).

Accuracy and precision

The precisions (intra-day and inter-day) of the proposed HPLC–PDA method were tested by injecting five replicates of the standard mixture (100 $\mu\text{g/mL}$) on the same day and 15 replicates over three consecutive days (five replicates each day), respectively [36]. High intra- and inter-day precisions were achieved with relative standard deviation (RSD) values lower than 2.5 % for all analyzed

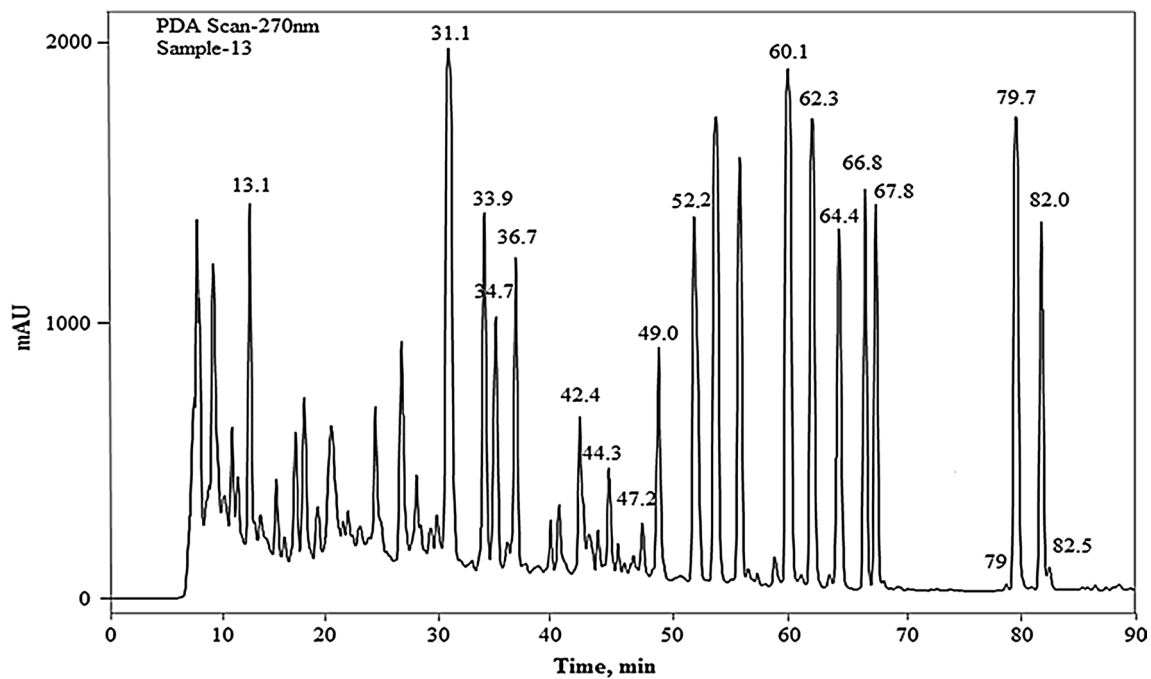


Fig. 2 HPLC–PDA chromatogram of the analyzed polyphenol compounds in extracts of sample 13 at λ_{\max} 270 nm (Gala, 13.1; 4Hba, 31.1; Cafa, 33.9; Vana, 34.9; Syra, 36.4; Phe, 42.4; Fera, 44.3; Sina,

47.2; Nar, 49.0; Myr, 52.2; Quer, 60.1; Narn, 62.3; Cina, 64.4; Kaem, 66.8; Api, 67.8; Chr, 79.0; Gal, 79.7; Thy, 82.0 min)

compounds (Table 3). The low values of precision (<2.5 % RSD) confirm that the proposed analytical method can be successfully applied in the routine analysis of phenolic acids, flavonoids, and other phenols present in various natural samples such as honey. The recovery assay was performed to assess the applicability of the proposed method for determination of phenolic acids, flavonoids, and other phenol compounds. Recoveries of all the polyphenol compounds were established by adding the standards at three different concentration levels to each honey sample prior to performing the extraction procedure. The results of spiking honey with each individual polyphenol showed the average recoveries range from 90.0 to 101.0 % with relative standard deviations (RSDs) between 1.3 and 1.9 %.

Honey sample analysis

The solid-phase extraction technique coupled with HPLC–PDA was applied for the simultaneous determination of polyphenol compounds in Yemeni multifloral and cactus honey samples. All the solution obtained using solid-phase extraction technique from each sample of multifloral and cactus honeys of Yemen (10 μ L) was injected into the HPLC–PDA instrument, and the peaks in the chromatograms obtained were identified by comparison with retention times and on-line UV spectra of the standards recorded in the same conditions. Therefore, the constituents in the

honey extracts were recognized and determined by comparison with their retention times and UV spectra with those of the standard compounds [7, 22, 34]. In the proposed method, the UV spectral data and their corresponding retention times were used as library data for identification and quantitation of the analyzed compounds. As an example, Fig. 2 shows the HPLC–PDA chromatogram of sample 13. The chromatogram shows well-separated peaks, and no interferences from any other components were observed since there were no detectable matrix peaks eluted in the retention time of the analyzed compounds [37]. Also, the absorption spectra of the corresponding analyte were found at the same wavelength as those obtained from analysis of standard mixture solution (Fig. 1). Therefore, the compounds were quantitated by their absorbance in the HPLC chromatograms against the standards at their corresponding wavelength provided in Table 2.

Phenolic acids in multifloral and cactus Yemeni honeys

The results of analyzed samples of multifloral and cactus Yemeni honey show that the averaged content of total phenolic acids was 1.646 mg/100 g honey which is smaller than in jujube honey [29]. It was also found that 4-hydroxybenzoic acid (58.02 %) was the main component (10.642 mg/100 g honey) (Table 4). On the other hand, gallic acid (15.69 %) was the secondary phenolic

Table 4 Quantitative determination of phenolic acids, flavonoids, and other phenols in multifloral and cactus honeys ± SD (µg/100 g of honey) (n = 3) (R = recovery rates, %)

Sample	Phenolic acids											Total
	Gal(R)	Cloa(R)	4Hba(R)	4Hpa(R)	Cati(R)	Vana(R)	Syra(R)	Pcoa(R)	Fera(R)	Sina(R)		
1	72 ± 1.0(94)	nd(96)	366 ± 1.1(98)	77 ± 0.2(91)	nd(97)	nd(96)	nd(99)	nd(96)	nd(99)	nd(96)	nd(96)	515 ± 0.9
2	137 ± 1.1(96)	nd(95)	272 ± 2.9(97)	nd(96)	nd(96)	nd(99)	nd(97)	10 ± 0.3(91)	nd(96)	34 ± 2.5(93)	nd(96)	453 ± 1.6
3	nd(94)	nd(96)	322 ± 1.9(98)	nd(98)	nd(93)	nd(96)	nd(94)	nd(96)	45 ± 0.5(93)	nd(99)	nd(99)	367 ± 1.1
4	284 ± 1.2(97)	149 ± 1.0(96)	456 ± 1.5(98)	nd(96)	31 ± 1.1(91)	nd(97)	nd(96)	15 ± 0.1(93)	nd(98)	nd(95)	nd(95)	935 ± 0.9
5	204 ± 2.4(96)	nd(96)	524 ± 2.5(99)	130 ± 1.0(92)	99 ± 1.6(95)	nd(95)	306 ± 1.9(98)	43 ± 1.1(92)	nd(90)	nd(96)	nd(96)	1306 ± 1.9
6	48 ± 0.9(94)	nd(93)	448 ± 3.1(99)	nd(98)	nd(93)	17 ± 0.1(92)	nd(94)	26 ± 0.2(R)	nd(96)	nd(92)	nd(92)	539 ± 1.4
7	250 ± 1.1(97)	53 ± 0.5(92)	1160 ± 1.8(100)	268 ± 0.8(95)	nd(95)	86 ± 0.5(95)	nd(96)	nd(95)	38 ± 0.5(93)	29 ± 0.9(98)	nd(96)	1880 ± 0.9
8	312 ± 1.7(98)	21 ± 0.5(91)	1190 ± 1.9(100)	nd(94)	35 ± 0.4(92)	34 ± 1.4(95)	326 ± 1.9(98)	07 ± 0.5(93)	106 ± 0.5(95)	nd(96)	nd(96)	2030 ± 1.2
9	97 ± 0.2(94)	nd(90)	1020 ± 1.8(100)	nd(96)	17 ± 0.6(94)	nd(94)	122 ± 0.5(99)	12 ± 0.1(95)	nd(92)	53(93)	nd(92)	1321 ± 0.9
10	173 ± 0.3(96)	nd(95)	312 ± 1.4(96)	nd(95)	12 ± 0.5(93)	nd(94)	35 ± 0.3(92)	18 ± 0.2(92)	nd(96)	nd(99)	nd(99)	549 ± 0.5
11	258 ± 0.5(97)	234 ± 0.3(96)	608 ± 1.8(97)	nd(96)	nd(96)	124 ± 1.1(96)	104 ± 0.7(95)	nd(96)	nd(91)	nd(98)	nd(98)	328 ± 0.9
12	nd(96)	nd(96)	426 ± 1.6(97)	348 ± 0.5(95)	09 ± 0.8(95)	nd(97)	nd(96)	55 ± 0.6(95)	67 ± 0.1(95)	nd(96)	nd(96)	904 ± 0.6
13	614 ± 0.3(99)	nd(98)	1550 ± 1.4(101)	nd(94)	406 ± 0.5(98)	458 ± 0.2(99)	152 ± 0.2(98)	nd(95)	167 ± 0.2(98)	136 ± 0.3(R)	nd(96)	3448 ± 0.5
14	318 ± 0.5(98)	178 ± 0.3(95)	670 ± 1.8(99)	nd(95)	61 ± 1.1(92)	nd(96)	153 ± 1.1(99)	166 ± 0.7(96)	nd(96)	nd(96)	nd(96)	1546 ± 0.9
15	72 ± 0.1(95)	218 ± 0.6(95)	272 ± 1.6(95)	186 ± 0.3(93)	35 ± 0.8(91)	nd(99)	58 ± 0.5(90)	18 ± 0.6(94)	nd(91)	nd(95)	nd(95)	859 ± 0.6
16	24 ± 0.5(91)	nd(98)	490 ± 1.8(96)	17 ± 1.1(91)	nd(95)	nd(97)	140 ± 0.3(98)	21 ± 0.7(92)	nd(97)	43 ± 0.8(96)	nd(97)	735 ± 0.9
17	16 ± 0.1(92)	02 ± 0.3(91)	552 ± 1.6(97)	nd(95)	16 ± 0.8(92)	nd(96)	27 ± 0.3(92)	13 ± 0.6(93)	nd(93)	nd(96)	nd(96)	626 ± 0.6
Total	2879 ± 0.8	855 ± 0.3	10642 ± 1.8	1026 ± 0.7	721 ± 0.9	719 ± 1.5	1423 ± 1.6	404 ± 0.4	423 ± 1.0	295 ± 0.5	295 ± 0.5	18341 ± 1.2
Average	191.93	122.14	626	171	72.1	143.8	142.3	33.67	84.6	59	59	1646.54 ^a
% ^b	15.69	4.66	58.02	5.59	3.93	3.92	7.76	2.20	2.30	1.60	1.60	
Sample	Flavonoids											Total
	Nar(R)	Myr(R)	Lut(R)	Quer(R)	Nam(R)	Kaem(R)	Api(R)	Chr(R)		Gal(R)		
1	nd(92)	nd(92)	nd(93)	63 ± 1.3(91)	nd(91)	46 ± 0.2(96)	nd(95)	nd	13 ± 0.2(91)	122 ± 0.8		
2	nd(96)	15 ± 1.9(92)	nd(96)	72 ± 2.6(92)	nd(90)	8 ± 2.5(90)	2 ± 0.5(91)	183 ± 2.9(97)	nd(91)	279 ± 1.8		
3	nd(93)	nd(90)	nd(94)	3 ± 1.5(90)	272 ± 0.5(99)	nd(91)	nd(94)	nd	nd(90)	275 ± 1.3		
4	31 ± 0.8(93)	43 ± 0.8(90)	nd(98)	65 ± 1.2(94)	nd(92)	30 ± 2.1(91)	7 ± 1.0(91)	171 ± 2.1(96)	nd(91)	275 ± 1.3		
5	99 ± 1.2(99)	77 ± 2.1(91)	nd(91)	123 ± 2.5(99)	nd(93)	41 ± 1.5(93)	89 ± 1.2(96)	nd	81 ± 0.8(96)	347 ± 1.7		
6	46 ± 0.4(96)	nd(92)	nd(90)	nd(90)	nd(93)	nd(93)	43 ± 0.4(95)	131 ± 2.5(95)	13 ± 0.1(92)	510 ± 1.0		
7	nd(93)	28 ± 1.2(91)	nd(96)	25 ± 1.2(95)	462 ± 1.2(99)	25 ± 1.9(92)	nd(90)	330 ± 2.2(99)	57 ± 0.9(95)	927 ± 1.4		
8	23 ± 0.2(93)	13 ± 1.9(90)	nd(92)	17 ± 2.1(96)	776 ± 2.1(100)	nd(91)	75 ± 0.2(92)	586 ± 2.8(100)	45 ± 1.2(95)	1535 ± 1.4		
9	5 ± 0.5(90)	nd(90)	nd(92)	37 ± 2.2(95)	103 ± 1.5(96)	nd(92)	24 ± 0.2(93)	426 ± 2.6(100)	9 ± 1.5(91)	652 ± 1.6		
10	nd(96)	nd(96)	nd(94)	13 ± 2.2(90)	nd(90)	5 ± 2.2(91)	3 ± 2.2(91)	159 ± 2.2(97)	11 ± 2.2(92)	191 ± 1.8		
11	93 ± 2.2(98)	nd(93)	nd(91)	226 ± 2.2(99)	nd(91)	54 ± 1.5(92)	nd(90)	292 ± 2.6(99)	nd(91)	663 ± 1.6		
12	nd(96)	nd(92)	nd(96)	33 ± 2.0(95)	nd(90)	nd(92)	nd(96)	197 ± 2.9(98)	13 ± 2.6(92)	243 ± 1.7		
13	236 ± 2.6(99)	450 ± 1.5(101)	nd(94)	1340 ± 2.6(101)	1060 ± 2.5(99)	554 ± 2.6(99)	444 ± 2.6(99)	1140 ± 2.9(101)	258 ± 1.6(99)	5482 ± 2.0		

Table 4 continued

Sample	Flavonoids											Total
	Nar(R)	Myr(R)	Lut(R)	Quer(R)	Narn(R)	Kaem(R)	Api(R)	Chr(R)	Gal(R)	Total		
14	36 ± 1.1(R)	232 ± 1.6(100)	nd(94)	248 ± 2.2(99)	422 ± 1.4(98)	43 ± 1.0(95)	25 ± 1.0(93)	494 ± 2.2(100)	230 ± 1(99)	1730 ± 1.8		
15	nd(93)	nd(94)	nd(91)	91 ± 2.2(95)	nd(92)	nd(94)	6 ± 0.2(92)	272 ± 2.6(99)	39 ± 1.5(94)	408 ± 1.6		
16	nd(94)	nd(95)	nd(96)	nd(91)	172 ± 2.6(R)	73 ± 1.5(95)	nd(90)	324 ± 2.9(100)	65 ± 0.8(93)	634 ± 1.7		
17	nd(96)	27 ± 0.8(95)	nd(91)	36 ± 2.0(91)	nd(93)	nd(93)	nd(96)	324 ± 2.9(99)	65 ± 0.8(95)	387 ± 1.7		
Total	569 ± 1.1	885 ± 1.3	nd(91)	2392 ± 2.1	3267 ± 2.5	879 ± 1.5	718 ± 1.0	5029 ± 1.3	899 ± 1.1	14660 ± 1.7		
Average	71.12	110.63		159.46	466.71	87.9	71.8	359.21	69.15	1395.98 ^e		
% ^d	3.88	6.03		16.32	22.28	5.99	4.90	34.30	6.13			
Sample	Other phenols											Total
	Mal(R)	Phe(R)	Bena(R)	Cina(R)	Thy(R)	Car(R)	Total					
1	nd(94)	nd(94)	35.0 ± 0.2(94)	25.6 ± 2.6(94)	nd(92)	nd(91)	60.6 ± 1.3					
2	nd(90)	nd(91)	55.0 ± 1.5(95)	nd(96)	5.2 ± 1.8(92)	4.6 ± 0.3(99)	64.8 ± 1.6					
3	nd(92)	nd(92)	17.0 ± 1.3(94)	nd(94)	nd(91)	nd(90)	17 ± 1.3					
4	nd(92)	nd(91)	33.0 ± 0.1(95)	63.0 ± 2.6(98)	4.8 ± 0.9(93)	nd(91)	100.8 ± 1.2					
5	nd(91)	nd(93)	nd(92)	118.0 ± 2.7(98)	12.4 ± 1.5(94)	5.6 ± 0.4(93)	136.0 ± 2.1					
6	nd(93)	nd(92)	7.0 ± 0.3(92)	47.2 ± 0.9(97)	nd(92)	nd(90)	54.2 ± 0.9					
7	0.4 ± 0.1(91)	0.2 ± 0.2(90)	nd(93)	57.0 ± 1.2(97)	nd(96)	6.8 ± 0.7(91)	64.4 ± 1.2					
8	nd(95)	0.6 ± 0.1(91)	3.0 ± 0.4(93)	113.8 ± 2.8(96)	nd(91)	nd(90)	117.4 ± 1.4					
9	nd(94)	nd(93)	5.2 ± 0.1(95)	nd(90)	10.0 ± 1.9(97)	nd(92)	15.2 ± 1.1					
10	nd(96)	0.4 ± 0.1(92)	nd(92)	139 ± 1.5(95)	3.4 ± 0.1(94)	2.4 ± 0.1(91)	145.2 ± 1.5					
11	nd(95)	nd(92)	nd(92)	206.0 ± 2.6(99)	6.2 ± 1.1(93)	nd(91)	212.2 ± 1.6					
12	nd(98)	0.8 ± 0.1(91)	1 ± 0.9(91)	226.0 ± 1.2(100)	nd(92)	nd(96)	227.8 ± 1.6					
13	nd(92)	0.4 ± 0.2(92)	nd(92)	670.0 ± 2.8(101)	7.2 ± 2.1(94)	12.2 ± 2.1(91)	689.2 ± 1.4					
14	nd(91)	0.2 ± 0.1(91)	nd(93)	522.0 ± 1.3(101)	6.4 ± 1.9(94)	nd(92)	528.6 ± 1.1					
15	0.4 ± 0.2(90)	nd(92)	20.6 ± 1.1(95)	142.4 ± 1.5(99)	6.6 ± 0.4(93)	nd(93)	170.0 ± 1.5					
16	nd(92)	nd(91)	nd(92)	nd(96)	5.0 ± 1.1(94)	nd(94)	5.0 ± 1.1					
17	nd(90)	nd(91)	nd(93)	nd(92)	2.4 ± 1.1(93)	nd(93)	2.4 ± 1.6					
Total	0.8 ± 0.1	2.6 ± 0.1	176.8 ± 0.5	2330 ± 1.1	69.6 ± 0.9	31.6 ± 0.2	2599.2 ± 1.5					
Average	0.4	0.43	19.64	194.16	6.32	6.30	225.8 ^e					
% ^f	0.03	0.10	6.80	89.64	2.68	0.74						

nd not detected

^a Total phenolic acids averaged^b Percentage of each individual phenolic acid in the total phenolic acids^c Total flavonoids averaged^d Percentage of each individual flavonoid in the total flavonoids^e Total other phenols averaged^f Percentage of each individual other phenol in the total other phenols

acid (2.879 mg/100 g honey) in all the analyzed multifloral and cactus honeys. The other phenolic acids were found in relatively lower proportion ranging from 1.60 % (sinapic acid) to 7.76 % (syringic acid) of total phenolic acids, 18.341 mg/100 g honey (Table 4). However, the amounts of phenolic acids were found to be different in each analyzed honey samples, and among them sample 13 which was obtained from cactus has shown much higher concentration of 4-hydroxybenzoic acid (1.550 mg/100 g honey) than other honey samples. More interestingly, 4-hydroxybenzoic acid was detected in all analyzed honey samples (Table 4).

Flavonoids in multifloral and cactus Yemeni honeys

The HPLC–PDA data of flavonoid compounds in Yemeni multifloral and cactus honeys showed that chrysin was the main flavonoid component (5.029 mg/100 g honey, 34.3 %) present in most of the samples analyzed (Table 4). The experimental results also prove that among the nine investigated flavonoids, eight were detected (except luteolin) in these honeys, and most of them occurred in small amounts except chrysin, naringenin, and quercetin. The total flavonoid content in all analyzed honeys was found to be 14.66 mg/100 g honey (Table 4). In addition, naringenin (3.267 mg/100 g honey) and quercetin (2.392 mg/100 g honey) were found as the secondary main constituents representing 22.28 and 16.32 % of the total flavonoids, respectively. The other flavonoids were found in lower proportion, ranging from 3.88 to 6.13 % of the total flavonoids. However, most of the honey samples showed a common flavonoid profile, comprising chrysin, quercetin, kaempferol, apigenin, and galanin, suggesting that they could be used as characteristic floral markers for Yemeni multifloral and cactus honeys [33].

Other phenols in multifloral and cactus Yemeni honeys

The total other phenolic compounds in all cactus and multifloral honey samples were found to be 2.599 mg/100 g honey, where the cinnamic acid (89.64 %) was the main component (2.330 mg/100 g honey) (Table 4). The other phenolic constituents present in the honey samples were found in relatively lower proportion ranging 0.03 % (maleic acid) to 6.80 % (benzoic acid) of total phenols. The amounts of phenolic components were different in each analyzed honey sample, while the concentration of cinnamic acid was found much more than other phenols almost in all honey samples. The maximum cinnamic acid concentration was found to be 2.330 mg/100 g honey in sample 13. In less than 50 % samples, maleic acid (samples 7 and 15), carvacrol (samples 2, 5, 7, and 10), and phenol (samples 7, 8, 10, 12, 13, and 10) were found in very low amount (Table 4). From the quantitative results (Table 4), it

can be concluded that there is no clear correlation between the geographical origin of the honey and its chemical composition as the concentration of each polyphenol varies in wide range for samples collected from same region.

Conclusions

A simple method has been described for the simultaneous separation and determination of 25 polyphenol compounds including 4Hba–4-hydroxybenzoic acid, 4Hpa–4-hydroxyphenyl acetic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, p-coumaric acid, sinapic acid, syringic acid, vanillic acid, nine flavonoid (apigenin, chrysin, galangin, kaempferol, luteolin, myricetin, naringin, naringenin, and quercetin), and six other phenols (benzoic acid, carvacrol, cinnamic acid, maleic acid, phenol, and thymol) in Yemen multifloral and cactus honey by HPLC–PDA. A total of 17 multifloral and cactus honey samples were analyzed, and the total content of phenolic acids, flavonoids, and phenolic components was found in the range of 328–3448, 122–5482, and 2.4–677.6 µg/100 g (Table 4) of honey samples, respectively. The 4-hydroxybenzoic acid was found in major quantity (10.642 mg/100 g honey) in all analyzed honeys, while chrysin (5.029 mg/100 g honey) was the main detected flavonoid, and cinnamic acid the other main phenolic compound (2.330 mg/100 g honey). Among the 25 investigated compounds, maleic acid was found only in two samples, while luteolin was not detected at all. Also, a common flavonoid profile corresponding to chrysin, quercetin, kaempferol, apigenin, and galangin was established in all honey samples. In addition, the values of the quality parameters achieved with this proposed analysis method as well as the results obtained in the analysis of honey samples confirmed the application of the methodology for the analysis of polyphenols in natural honeys.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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