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ORIGINAL PAPER

The phenolic composition, aroma compounds, physicochemical and antimicrobial properties of *Nigella sativa* L. (black cumin) honey

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Abstract

Honey is an important bee product with its nutritional and medicinal properties. Characterization of different monofloral honey has importance for focusing on honey's biocomponents and the potential use of its medicinal applications. Although Black cumin (*Nigella sativa* L.) is a valuable monofloral honey, there are less study about its both chemical and biochemical study in literature. In this study, some characteristic properties of black cumin honey (*Nigella sativa* L.) were evaluated. The mean total polyphenol content (TPC), total flavonoid content (TFC), and total antioxidant capacity based on the ferric reducing/antioxidant power (FRAP) and 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity were 55.23 ± 1.27 mg GAE/100 g, 1.18 ± 0.11 mg QUE/100 g, 219.50 µmol $FeSO_4.7H_2O$/g, 219.50 ± 8.67 µM$FeSO_4$/g, and 38.40 ± 2.26 mg/mL, respectively. Phenolic composition was measured by means of high-performance liquid chromatography-photodiode-array detection (HPLC–PDA) using 25 standards. Ellagic acid and pinocembrin were identified as the major components. Antimicrobial activities were tested on *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella enteric* subsp. enterica, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes* and *Candida albicans*. The results showed that *Nigella sativa* honeys were especially effective against *L. monocytogenes*, S. *aureus*, and *E. faecalis*. The results show that N. sativa honey possesses high apitherapeutic potential, although further research is now needed.

Keywords Black cumin · *Nigella sativa* · Honey · Antimicrobial · Volatile compounds

Introduction

Honey is a natural product that has been used for nutritional and medicinal purposes throughout the course of history. It exhibits a wide range of biological active properties such as antioxidant, antimicrobial, antitumoral, anti-inflammatory, and anti-dispersant activities, and is also used in apitherapeutic applications [1–3]. It is particularly consumed for apitherapeutic purposes, these functions being largely determined by the production method and flora involved. Dark-colored honeys, such as chestnut, oak, heather, and Manuka honey, have been proved to be rich in higher biological active compounds [4–6].

Turkey is known for its rich floral sources and plant diversity deriving from its geographical location and is the world's third-largest honey producer. It produces a wide variety of unifloral and heterofloral blossom and dew honeys. Chestnut, thyme, heather, blackthorn, rhododendron, lime, clover and astragalus and sunflower, naringin, and cotton honeys are among the most intensively researched unifloral blossom honeys. Pine, oak and cedar are the most cultivated dew honeys [2, 7–9]. Many other kinds of blossom honeys are also produced in relatively small quantities, but these have not been investigated to date [2].

Black cumin (*Nigella sativa* L.) is an herbaceous plant frequently used in the food industry, especially bakery, and one that is also employed in folk medicine for the treatment or prevention of several diseases, such as asthma, diarrhea, and dyslipidemia [10, 11]. The seeds of black cumin contain

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essential oils, alkaloids, saponins, polyphenols, and proteins [12, 13]. Many clinical studies conducted with the seed blended with honey or other natural products have observed high antibacterial, antiviral, and anti-inflammatory effects [11, 14–18]. The flowers of the plant, which is cultivated only for its seed, are used in honey production since they are abundant in nectar. While several studies have been conducted using black cumin seed mixed with honey, few have investigated black cumin honey. This monofloral honey is largely produced in the southern regions of Turkey, due to the very short flower harvest time, from June to July. This honey is also produced in Middle Eastern and Arabian Peninsula countries such as Saudi Arabia and Yemen, Sudan, and Bangladesh. The honey is dark colored and consumed as a health-preserving agent [11, 13, 19].

Due to the limited number of studies of black cumin honey produced in Turkey, the present research examined its physicochemical, bioactive compounds and in vitro antioxidant and antimicrobial properties, for the first time in the current literature.

Materials and methods

Samples and botanical properties

Black cumin honey samples were obtained from experienced beekeepers in the regions of Burdur ($n=3$), Kayseri ($n=3$) and Kilis ($n=3$) following the 2021 harvest. Authentic properties of the honey samples were determined by means of palynological analysis [20]. Honey with a major or dominant pollen ratio higher than 45% is defined as monofloral or unifloral [20]. Briefly, 5 g of honey sample and 10 mL of distilled water were mixed, placed into a water bath at approximately 45 °C for 30–45 min, and shaken. Following centrifugation at 4000 rpm for 45 min, the supernatant was removed. The precipitate at the bottom of the tube was mixed with basic fuchsine, glycerin-gelatin was added, and transferred to glass slides. These were heated at 30–40 °C to dissolve basic fuchsine, and glycerin gelatin was added. The freshly prepared slide was then examined using light microscopy (Nikon Eclipse E100, Germany).

Physicochemical analysis

Brix (%), moisture (%), electrical conductivity (mS/cm), and Hunter color (Lab) values and optical rotations were determined as physical parameters of the honey samples in agreement with the European honey codex [21, 22]. The samples' conductivity values were measured directly with a conductivity meter (Hanna HI 2030–02 edge®, Romania). After the honey samples had been diluted with 1:10 distilled water, both values were read at 25 °C. The color of the honey samples was measured using Hunter (Lab) tritium color (Konica, Minolta, and CM-5, Minolta, Osaka, Japan). The colors of the honeys were measured in terms of (L) for darkness/lightness (0 black, 100 white), a (− a greenness, + a redness), and b (− b blueness, + b yellowness. Calibrations were first performed for black and white, after which the colors were read. For this purpose, the honey samples were heated in a 50 °C water bath for 45 min to dissolve the sugar crystals [23]. The optic rotation of the samples was measured with a polarimeter (Beta PPP7, Cambridge, UK) using Carrez's reagent for the precipitation of the honey proteins. The filtrate solution was inserted into the polarimeter, and the results were read in angular on a 200 mmol basis [22].

Sugar analysis of the samples was carried out using high-performance liquid chromatography (HPLC-RID) with a refractive index detector. The mobile phase used was 80% acetonitrile (Merck, KGaA, Darmstadt, Germany): water. A 20 µL sample was injected into Shodex NH2P-50 4 E column (5 µm, 250 × 4.6 mm). Measurement conditions were a 30 °C column temperature and a flow rate of 1.3 mL/min. For the preparation of the honey samples, 1 g sample was weighed into a flask, to which was added 50 ml of ultrapure water. The mixture was then filtered through Millipore 0.45 µm PVDF filter and injected. The diastase activity of the samples was measured by means of a spectrophotometric method as described by [21] using an insoluble blue-dyed cross-linked type of starch as the substrate. The results were expressed as Diastase Units (DU). Proline contents were measured using the spectrophotometric method, based on the color reaction of proline with ninhydrin reagent. The absorbance was read at 510 nm, and the results were expressed as mg/kg [24].

Aroma compounds

The aroma compounds of the honey samples were measured on a GC–MS (Shimadzu QP2010 Ultra) device with a 30 m 5 Ms column. Solid phase micro-extraction (SPME) fibers were used to isolate volatile compounds contain in divinylbenzene/carboxin/polydimethylsiloxane. The aroma components were analyzed by combining the honeys from each region. For this process, 2 g of the honey sample was mixed with 7 mL of bidistilled water and added to 1.0 g NaCI in a 40 mL capacity vial. After this mixture had been vortexed, the aromatic components were allowed to adhere to the fiber by stirring continuously for 30 min at 50 °C in a hot water bath with a stirrer at 200 rpm without fiber [25]. The analyses were performed on a GC–MS device with a 30 m 5 Ms column. Qualitative analysis was performed using the NIST and Wiley libraries integrated with the device.

Total phenolic contents (TPC)

Methanol extracts from the honey samples were prepared. For this purpose, 3 g of honey was dissolved in 30 ml of methanol (99%), stirred for 24 h, and then filtered, first with a coarse and then with a fine filter paper. The filtrate was made up to 30 mL with the methanol. The methanol extract was used in total polyphenol, total flavonoid and total antioxidant analysis and phenolic composition studies [26].

The total phenolic content (TPC) of the honey samples was measured using Folin-Ciocalteu's assay [27]. For this analysis, 20 µL of the methanol extract and 400 µL 0.2 N Folin–Ciocalteu's reagent were mixed and diluted to 680 µL with distilled water. Following 3-min incubation, 400 mL of Na_2CO_3 (7.5%) was added, and the mixture was incubated for a further 2 h at room temperature. After incubation, the absorbance was read at 760 nm on a spectrophotometer (Thermo Scientific Evolution TM 201, UV–VIS Spectrophotometer, USA). The TPC of the samples was calculated as mg gallic acid equivalents (GAE)/ 100 g sample using a standard curve ($y = 1.627x + 0.0456$, R^2: 0.994) with gallic acid standards from 0.032 to 1.0 mg GAE/mL.

Total flavonoid contents (TFC)

The total flavonoid content (TFC) of the honey samples was measured according to the method described by [28]. Briefly, to 25 µL of the methanol extract was added 50 µL of 10% Al $(NO_3)_3$ and 50 µL of 1.0 M $NH_4.CH_3COO$. The mixture was then diluted to 3.0 mL with methanol (99%) and incubated at 25 °C for 45 min, after which the absorbance was measured at 415 nm. TFC was expressed as mg quercetin equivalent (QUE)/ 100 g sample using a standard curve. Quercetin standards from 0.031 to 0.5 mg QUE/ mL were used for the preparation of the standard curve ($y = 2.2414x + 0.017$; R^2: 0.9981).

Ferric-reducing antioxidant power (FRAP)

The total antioxidant capacity of the honey samples was measured using the ferric-reducing antioxidant power assay (FRAP) method [29]. Freshly prepared FRAP reagent (ferric tripyridyltriazine (Fe-III-TPTZ)), $FeCl_3$, and acetate buffer solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution were prepared in a 20 mL test tube. The FRAP reagent (3 mL) and 100 µL sample were mixed and incubated for 4 min at 37 ºC, after which the absorbance was read at 595 nm. Different concentrations of $FeSO_4.7H_2O$ (from 1000 to 31.25 µmol/g) were used to prepare the standard curve ($y = 0.0006x + 0.0049$; R^2: 0.9904). The results were expressed as µmol $FeSO_4.7H_2O$ equivalents/100 g sample using the curve.

2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity

Free radical scavenging activity was measured using the method described by [30]. Briefly, 750 µL extract of the sample was mixed with 750 µL of DPPH radical solution. This mixture was then kept in the dark for 45 min at 25 °C, absorbance being read at 517 nm. The result was calculated as SC_{50}, the sample concentration value causing a 50% reduction in the concentration of DPPH• radicals, lower SC_{50} values indicating higher radical scavenging activity.

Determination of phenolic profiles

Twenty-five phenolic standards of gallic acid, protocatechuic acid, chlorogenic acid, *p*-OH benzoic acid, *m*-OH benzoic acid, ferulic acid, ellagic acid, caffeic acid, *p*-coumaric acid, syringic acid, epicatechin, rutin, daidzein, myricetin, luteolin, quercetin, resveratrol, apigenin, hesperidin, rhamnetin, chrysin, curcumin, pinocembrin, caffeic acid phenyl ester(CAPE), and *t*-cinnamic acid of were subjected to HPLC–PDA (Elite LaChrom Hitachi, Japan) in a UV–VIS detector, with a C18 column (150 mm × 4.6 mm, 5 mm; Fortis). The mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile: water (70:30). The sample and standard injection volumes were 25 mL, with a column temperature of 30 °C and flow rate of 1.5 mL/min. The programmed solvent used began with a linear gradient held at 95% A for 3 min, decreasing to 80% A at 10 min, 60% A at 20 min, 20% A at 30 min, and finally 95% A at 50 min [31]. For quantitative determination, all phenolic component calibration curves were between 0.998 and 1.000.

Bacterial strains and growth conditions

The antimicrobial activity of honey samples was studied using seven bacteria as *Escherichia coli ATCC®25,922, Enterococcus faecalis* ATCC® *29,121, Staphylococcus aureus* ATCC 6538, *Salmonella enteric subsp. enterica* ATCC *14,028/363–154, Bacillus subtilis* B*209, Bacillus cereus, Listeria monocytogenes* ATCC®*7677*, Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck), and a fungi, *Candida albicans* ATCC®10,231) and Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and fungal cells, respectively.

Disc diffusion assay

To determine antibacterial and antifungal activity disk diffusion plate's method was used. (Ronald, 1990). Bacterial strains were grown in MHA for 24 h at 37 °C, and fungal strains were grown in SDA at 27 °C for 48 h. Overnight cultures were diluted with 0.9% w/v saline solution and turbidities of bacterial and fungal cell solutions were adjusted to 0.5 McFarland, respectively. 100 µL of each diluted suspension was placed over agar in petri dishes and dispersed. Then sterile paper discs (Oxoid, CT09988, 6 mm diameter) were placed on agar and 30 µl of the sample (2 g/ml) was loaded each well. As a positive control, nystatin for fungi and amoxicillin and cephazolin for bacteria were used. Alcohol was also used as a negative control. Inhibition zones that formed on the medium were measured in millimeter (mm) after incubation for 24 h at 37 °C and 27 °C for antibacterial and antifungal activities, respectively. All tests were made in triplicate.

Minimum inhibition concentration (MIC) analysis

The MIC values were determined through the micro-well dilution method. The molecules were dissolved in dimethyl sulfoxide and then the dilution series were prepared in a 96-well plate (Corning). A Tris buffer (Amresco 0826-500G) mixture (1:4) was mixed at 30 ◦C with an equal amount of broth solution (Sabouraud Dextrose Agar (Oxoid) for fungi and Mueller Hinton broth (Merck) for bacteria. Each sample was tested at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 µg/mL. Inoculants were obtained from an overnight broth culture of the test organism. The broth culture was incubated at 35 ◦C until it achieved the turbidity of the 0.5 McFarland standards (usually 24–48 h hours). The inoculum of each bacterium was prepared, and the suspensions were adjusted to 10^8 CFU/ mL for bacteria and 10^7 CFU/mL for fungi. After solubilization, each well was inoculated with 5 µL of freshly prepared bacterial suspension of 1×10^8 bacteria, 1×10^7 fungus/mL and incubated at 37 ◦C for 24 h. Then, 30 µL of 3-(4, 5- dimethyl-thiazol-2-yl)-2.5-diphenyl-tetrazolium bromide (MTT) at a final concentration of 0.5 mg/mL freshly prepared in water was added to each well and incubated for 30 min. The change to red color indicated that the bacteria were biologically active. The MIC was taken to the well, where no change of color in MTT was observed and the MIC values were given as the mean of triplicate analysis [32, 33].

Statistical analyses

All measurements were performed in triplicate, the results being expressed as mean plus/minus standard deviation ($X \pm SD$). The non-parametric Mann–Whitney test was used to determine whether differences between the groups were significant ($p < 0.05$).

Results

Table 1 shows the melissopalynological analyses of the honey samples from the three different regions. The major pollen, N. sativa, was detected in samples from all three regions, although the proportions differed. The most monofloral honey was that from the Burdur region with 62% N. sativa pollen, followed by Kayseri and Kilis. Microscopic examination revealed that different secondary and minor pollens in the three regions.

The physicochemical properties of black cumin honeys from the three study regions are summarized in Table 2. The color values of the honeys were measured using the Hunter lab method. The *L* values (darkness and lightness) of the honeys varied between 22.05 and 40.04, the values (greenness and redness) between 15.80 and 36.30, and the b values (blueness and yellowness) between 52.20 and 73.30. The lowest mean *L* value, indicating the darkest honey, was in the Burdur region honey.

The mean Brix% value was 83.43 ± 0.92, ranging between 82.40 and 84.00. Another physical parameter of the honeys was the optical rotation value, which was found to be negative. The values varied between -1.20 and -2.40, meaning that the honeys were blossom in character. The mean electrical conductivity value was 0.57 ± 0.03 mS/cm, ranging between 0.52 and 0.68 mS/cm, with Burdur region honey exhibiting higher conductivity.

The proline values of the honey samples varied between 790.85 and 1404.25 mg/kg, with a mean of 1044.86 ± 85.36 mg/kg. Burdur region honey exhibited a higher proline value than the others. The diastase enzyme activities of the honey were quite high, ranging between 24.08 and 30.05 DU. Only three sugar types were examined in this study, the region with the highest fructose value being Burdur, with a value of 38.93 ± 1.67 g/100 g. The mean glucose value in the honey was 24.94 ± 0.97 mg/100 g. The F + G values differed in all three honeys, but the mean value was 63.87 ± 2.64 g/100 g. Sucrose was not detected in the samples.

TPC ranged between 44.22 and 64.25 mg GAE /100 g, Burdur region honeys exhibiting higher values than those from the other regions. Flavonoid, a major subunit of polyphenol, ranged between 0.56 and 2.03 mg QUE/100 g, the highest average value being found in the honeys from the Burdur region, similarly to TPC. The total antioxidant values of the honeys were tested using the FRAP method. The FRAP values ranged between 190.53 and 254.60 µg $FeSO_4.7H_2O$ /100 g, the highest value being found in the honeys from the Burdur region. DPPH radical scavenging

Table 1 Melissopalynological analyses of the honey samples

Region	Predominant pollen (> 45%)	Secondary pollen (16–45%)	Important Minor Pollens (3–15%)
Burdur region*	*Nigella* spp.(Ranunculaceae) 62%	Rosaceae 23.21	Brassicaceae Lamiaceae Cistaceae Poaceae Apiaceae Fabaceae Globularaincee
Kayseri*	*Nigella* spp.(Ranunculaceae) 54%	Brassicaceae 24.48	Fabaceae: Cistaceae: Rosaceae: Asteraceae: Lamiaceae Salicaceae Dipsacacaae
Kilis*	*Nigella* spp. (Ranunculaceae) 48%	*Salix* spp. 32.66	Fabaceae Asteraceae Brassicaceae Rhamnaceae Rosaceae Asteraceae Boraginaceae

*Honey samples were pooled according to the region

activity was calculated in terms of SC_{50} values, the lowest average SC_{50} value, 34.88 mg/mL being observed in Burdur region honeys, followed by those from Kayseri and Kilis.

The phenolic compositions of the black cumin honey collected from the three regions were analyzed using HPLC–UV. The honey collected from each region was mixed prior to the assay. The components obtained, using 25 standards, are summarized in Table 3. In the phenolic component analysis performed by HPLC, the presence of nine substances in 25 standard phenolic substances was determined, and ellagic acid detected as the major component. Pinocembrin, quercetin, myricetin and chrysin were the second most abundant polyphenols in all samples. Cinnamic acid was only detected in honey from Kayseri, while gallic and coumaric acids were found in all samples.

The analyses of the volatile components of the honey samples using GC–MS are given in Table 4. All three honeys were observed to possess many similar and dissimilar rigging components. However, linalool, sabinene, pinene, carene, limonene, pelargol, cymene, capryalcohol, carene, terpinene, carvacrol, thymoquinone, and aromadendrene were detected as common aroma components in different quantities of honey.

Antimicrobial activities of honey samples dissolved in ethanol were determined. Seven human pathogenic bacteria as Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Salmonella enteric subsp. enterica, Bacillus subtilis, Bacillus cereus, Listeria monocytogenes and a fungus (Candida albican) with disk diffusion plates in the agar method. The results did not show appreciable antibacterial and antifungal activity against all strains tested but proved that honey samples had good antimicrobial activity against S. aureus and B. subtilis bacteria, but significant antifungal activity. Extracts of all three honey samples were more effective against Gram-negative bacteria than Gram-positive ones. Honey did not show significant activity for gram-negative bacteria. Likewise, the same result is valid for fungi. Therefore, we cannot evaluate them as high-potential antimicrobial and antifungal agents for 3 honey samples. (Table 5). We also evaluated the Minimum Inhibition Concentrations (MIC) for the honey samples. The results showed that honey samples were active on Gram-positive bacteria and fungi but not active on Gram-negative bacteria. MIC values of honey extracts range from $100 \leq \mu g/mL$ to $12.5 \leq \mu g/mL$ and when we compare honey samples among themselves, it has been seen that they generally give similar results as average.

Discussions

Black cumin honey is produced in limited amounts in Turkey and its monofloral characteristic is very important for consumers. Palynological (i.e., melissopalynological) analyses of the samples showed that the honey samples exhibited unifloral properties, but that these were highest in the Burdur

Table 2 Physico-chemical and biochemical parameters of *Nigella sativa* honey

		Honey samples	Min	Max	Mean	Mean
Color (Hunter)	L	Burdur	22.05	30.20	$26.08 \pm 4.08^{a.b.c}$	32.22 ± 2.70
		Kayseri	30.26	34.10	$32.22 \pm 1.92^{a.b.c}$	
		Kilis	36.00	40.04	$38.35 \pm 2.10^{a.b.c}$	
	a	Burdur	30.15	35.20	$32.48 \pm 2.08^{a.b.c}$	28.78 ± 2.15
		Kayseri	33.40	36.30	$34.70 \pm 1.20^{b.c}$	
		Kilis	15.80	23.40	$18.92 \pm 3.45^{a.b.c}$	
	b	Burdur	54.20	62.50	$57.92 \pm 3.49^{a.b.c}$	60.76 ± 3.43
		Kayseri	65.30	73.30	$68.55 \pm 3.44^{a.b.c}$	
		Kilis	52.30	60.35	$55.82 \pm 3.36^{b.c}$	
Brix		Burdur	82.40	84.05	83 ± 0.76^{a}	83.48 ± 0.92
		Kayseri	82.05	84.05	83 ± 0.87^{a}	
		Kilis	82.00	84.05	83 ± 1.14^{a}	
Conductivity (mS/cm)		Burdur	0.62	0.68	$0.62 \pm 0.03^{b,c}$	0.57 ± 0.03
		Kayseri	0.52	0.56	0.54 ± 0.02^{a}	
		Kilis	0.48	0.54	0.51 ± 0.03^{a}	
Optic Rotation $[\alpha]_{20}$		Burdur	− 1.20	− 1.60	$-1.59 \pm 0.39^{a.b.c}$	1.92 ± 0.43
		Kayseri	− 1.56	− 2.05	$-1.97 \pm 0.37^{a.b.c}$	
		Kilis	− 1.60	− 2.40	$-2.18 \pm 0.51^{a.b.c}$	
Prolin (mg/kg)		Burdur	1258.45	1404.25	1326.23 ± 73.44^{a}	1045.86 ± 85.36
		Kayseri	980.60	1050.00	949.63 ± 118.92^{a}	
		Kilis	790.85	914.23	$861.72 \pm 63.70^{a.c}$	
Diastase (DU)		Burdur	28.50	30.05	$29.18 \pm 0.65^{a.b.c}$	27.33 ± 0.85
		Kayseri	25.40	27.80	$26.73 \pm 1.00^{a.b}$	
		Kilis	24.08	28.30	$26.13 \pm 1.73^{a.b}$	
Fructose (g/100 g)		Burdur	40.10	44.20	$41.6 \pm 2.24^{a.c}$	38.93 ± 1.67
		Kayseri	37.45	39.90	$38.82 \pm 1.25^{a.c}$	
		Kilis	35.00	38.00	$36.33 \pm 1.53^{a.b.c}$	
Glucose (g/100 g)		Burdur	26.30	28.66	27.39 ± 1.19^{a}	24.94 ± 0.97
		Kayseri	22.10	23.42	23.04 ± 0.82^{a}	
		Kilis	23.60	25.36	24.42 ± 0.89^{a}	
Sucrose (g/100 g)		Burdur	n.d	n.d	n.d	n.d
		Kayseri	n.d	n.d	n.d	
		Kilis	n.d	n.d	n.d	
F + G		Burdur	66.40	72.86	69.02 ± 3.43^{a}	63.87 ± 2.64
		Kayseri	59.55	63.32	61.86 ± 2.07^{a}	
		Kilis	58.35	61.85	59.383 ± 2.17^{a}	
Total phenolic contents (mg GAE/100 g)		Burdur	60.39	64.25	$62.36 \pm 1.58^{a.b.c}$	55.23 ± 1.27
		Kayseri	56.36	58.00	$57.23 \pm 0.67^{a.b.c}$	
		Kilis	44.22	48.0	$46.09 \pm 1.54^{a.b.c}$	
Total Flavanoid contents mg (QUE/100 g)		Burdur	1.56	2.03	$1.86 \pm 0.21^{a.b.c}$	1.18 ± 0.11
		Kayseri	1.00	1.20	$1.08 \pm 0.09^{a.b.c}$	
		Kilis	0.56	0.64	$0.60 \pm 0.030^{a.b.c}$	
FRAP µM ($FeSO_4.7H_2O$ /100 g)		Burdur	227.10	254.60	$240.5 \pm 11.23^{a.b.c}$	219.50 ± 8.67
		Kayseri	210.05	228.00	$219.02 \pm 7.33^{a.b.c}$	
		Kilis	190.53	208.60	$198.91 \pm 7.43^{a.b.c}$	
DPPH (SC_{50} mg/mL)		Burdur	37.20	33.02	$34.76 \pm 1.76^{a.b.c}$	38.40 ± 2.26
		Kayseri	42.10	35.05	$38.05 \pm 2.97^{a.b.c}$	
		Kilis	45.08	42.00	$42.41 \pm 2.04^{a.b.c}$	

n.d not detected; a, b, c, d Letters within the same column show significant differences between methods ($p < 0.05$)

Table 3 Phenolic composition of the *Nigella sativa* honeys

Phenolic compounds µg/100 g	Burdur	Kayseri	Kilis
Gallic acid	682	148	128
p-hydroxy benzoic acid	640	480	586
Ellagic acid	13,748	8564	75,860
p-Coumaric acid	324	349	154
t-Cinnamic acid	n.d	472	n.d
Myricetin	2670	124	108
Quercetin	1426	480	135
Chrysin	508	248	243
Pinosembrin	2050	10,240	560

region honey. In the most general definition, a honey with a dominant pollen level of 45% or more is regarded as monofloral, but this value is much higher in some honeys, such as 80% in chestnut honey, and much lower in others, such as lavender honey [20, 34]. Studies of N. sativa honey are very limited, and no detailed investigation of major pollen species has been performed. One study reported a figure of 20% for N. sativa pollen in Yemeni honey [35].

The physicochemical properties of the honey samples were studied according to their color, Brix, conductivity, and optic rotation values. When the color values measured according to the Hunter Lab method were examined, the L values indicating the dark and lightness values of honey were different in the third region. In the Hunter Lab method, low L (< 50) values indicate a dark honey color, and a high L value indicates light-colored honey [23]. The color measurement values show that the L values of the honeys from the three regions were from the dark-colored honeys class, although there were significant color differences between them. However, the average L values showed that the darkest-colored honeys were from the Burdur region, followed by the Kayseri and Kilis region honeys. A direct correlation was observed between the honey color and its monofloral properties. It was determined that black cumin nectar content and Hunter L value of honey changed in direct proportion. Chestnut, oak and heather honeys are generally dark colored, with L values between 42 and 47 [2]. The honey was found to belong to the dark-colored class. The color of honey is determined by various polyphenolic compounds, mineral substances, and various pigments in its structure. In general, dark-colored honeys are among those containing high polyphenols, such as chestnut honey and oak honey [6, 36].

There was no significant difference between the Brix values of the honeys, Brix indicates the opposite of the humidity of honey. The average moisture value being 17%. According to the honey codex, the maximum honey humidity was 21%, and the samples' moisture levels were low. High humidity in honey is undesirable, since it increases the water activity and accelerates fermentation [21, 37, 38].

Table 4 Aroma compounds of *Nigella sativa* honeys

Aromatic compounds	RI	Burdur	Kayseri	Kilis
Butenol	725	1.32	0.13	–
Oxybenzene	737	1.17	–	–
Ethane	740	–	1.60	–
Pyrazole	760	–	1.33	–
Acetylbutyryl	784	–	0.70	–
Lactate	776	2.04	–	–
Amyl methyl carbinol	786	4.07	–	–
Caprylalcohol	790	0.60	0.30	0.55
Lactate	797	5.04	0.18	0.60
Nonane	801	2.45	1.88	3.96
Furfural	832	18,88	8.30	5.74
Furfuryl alcohol	859	–	0.53	–
n-Hexanol	873	–	13.91	16.64
Anisate	912	2.05	–	–
Furan	915	2.04	0.64	0.98
Pinene	936	–	0.3	1.31
Enanthic acid	993	–	0.77	–
Carene	1004	1.46	0.99	3.85
Caprylaldehyde	1008	–	–	1.71
Cymene	1029	2.40	0.74	1.65
Limonene	1033	1.30	0.44	1.29
Hexanol	1036	–	–	1.44
Cyclopentanone	1045	–	–	0.79
Isobutyrate	1042	3.14	–	1.51
Benzyl alcohol	1044	–	0.60	1.51
Thymoquinone	1046	3.52	1.20	0.90
Phenylacetaldehyde	1048	14.70	8.63	1.87
Terpinene	1058	2.20	3.10	0.61
Benzaldehyde	1077	1.14	1.78	4.73
Linalool oxide	1078	4.37	3.16	0.82
Pelargonaldehyde	1111	–	–	14.00
Hex-3(Z)-enyl butyrate	1112	–	35.51	–
Phenethyl alcohol	1120	2.96	2.47	2.33
Isophorone	1126	2.64	–	–
Hydrocinnamaldehyde	1137	–	0.52	–
Oxophorone	1150	2.40		–
Sabinene hydrate	1158	2.06	2.40	0.40
Linalool	1173	1.01	1.30	
Pelargol	1180	0.30	0.43	0.92
Terpinen-4-ol	1185	0,30	0,30	2.08
Terpineol	1199	0.20	2.24	0.75
Salicylate	1201	–	–	2.24
Capraldehyde	1213	–	0.39	1.88
Neodene	1243	1.27		
Carvacrol	1315	0.60	0.38	1.30
Pentylallyl butyrate	1353	–	0.49	–
Aromadendrene	1432	2.20	3.26	4.20
Ethylene brassylate	1648	0.56	–	–
Heptadecane	1712	2.27	–	–
Octadecane	1813	2.33	–	–

Table 4 (continued)

Aromatic compounds	RI	Burdur	Kayseri	Kilis
Salicylate	1848	1.69	–	–
Mercaptan	1930	–	–	11.04
Non-2(E)-enoic acid < methyl- > ester	2117	0.67	–	–
Total		97.35	98.12	93.30

Low humidity also extends the shelf life of honey. The Brix value of honey depends on the time of harvest. The moisture value of immature honey in the hives is high. A study involving Bangladeshi black cumin honey reported a moisture content of 14% [39]. Another study of Bangladeshi black seed honey reported a Brix value of 77.50% with 19.50% of moisture [13]. The samples' conductivity values, an important physical parameter of honey, varied between 0.48 and 0.68 mS/cm. Apart from such exceptions as chestnut and heather honey, conductivity values of some flower honeys range between 0.30 and 0.80 mS/cm, approaching 0.3 mS/cm in light-colored flower honeys [2, 37]. The conductivity of honey derives from mineral substances such as Na, K, Mg, Fe, Cu, and various organic acids [36]. The optical rotation value is the ability to convert polarized light, which is generally negative in flower honey and positive in secretory honey [36, 40]. It shows that the optical rotation values of the black cumin honey were negative, meaning that all samples were blossom honeys [40]. Honey contains all amino acids, the major amino acid being proline. In general, a high proline value indicates the quality of the honey, and the minimum proline value is 150 mg/Kg according to the honey codex [37, 38]. All the black cumin honeys had high proline values, the highest being determined in those from the Burdur region. Proline values as high as those in the present study have been reported in black cumin honey from Morocco [19].

Diastase is one of the few important enzymes found in honey, and enzyme activity is considered an important freshness parameter [37, 38, 41]. According to the honey codex, the minimum diastase activity value is 8, and this decreases in honey that has been subjected to heat treatment and has exceeded its shelf life [21, 37]. High diastase activities in the black cumin honey samples from the three regions indicated that the honeys were very fresh.

Polyphenols are the main agents responsible for the biological activity of honey [24, 42]. The total polyphenol content of black cumin honey in this study varied between 44 and 64 mg GAE/100 g. It is thought that the higher the monofloral value of black cumin honey, the darker the color of the honey and this is due to the polyphenols in its structure [43]. A study from Morocco reported that black cumin honey is one of the dark, amber-colored honeys, with high polyphenol content [19]. Polyphenols are secondary metabolites with very large subunits and numerous members, and flavonoids are the most effective natural molecules, constituting the largest class [42–45]. The flavonoid contents of the honeys in the present study varied between 0.56 mg QUE/g and 1.56 mg QUE/g, with the highest being observed in honeys from the Burdur region. Polyphenols are agents responsible for biological activity, and from which most of the antioxidant properties of honey derive [19, 42, 44].

The antioxidant capacities of the honey samples were investigated using two methods, FRAP and DPPH. These are the most frequently used techniques for measuring the antioxidant properties of bee products [1, 31, 46]. In this study, in which a high FRAP value revealed high antioxidant capacity, all honeys were antioxidant-rich, with those from the Burdur region exhibiting the highest antioxidant value. This is mainly due to the honey's high polyphenol content. DPPH activity was determined in terms of SC_{50} values, defined as the amount of honey in mg/ml that scavenges 50% of the radicals. In contrast to the FRAP value, a low DPPH value indicates high antioxidant capacity, and honeys from the Burbur region were also found to exhibit high radical scavenging activity, with the lowest SC_{50} values. In a study, the physicochemical and biochemical properties of different monofloral honey samples were characterized. It was found that SC_{50} value of honey samples ranged between 13.20 and 110.57 mg/mL and FRAP activity ranged from 0.54 to 5.19 µmolTrolox/g [47]. In another study, the SC_{50} value of different honey samples changed between 9.650 and 50.169 mg AAE/100 g honey and the FRAP value ranged between 52.386 to 82.529 mg TE/100 g honey [6]. In a study, the antioxidant and phenolic properties of oak honey were determined. It was reported that the FRAP value of oak honey ranged between 410 and 973 µmol $FeSO_4 \cdot 7H_2O$/100 g and SC_{50} value were ranged between 7.67 and 27.89 mg/mL [48]. When the literature examined, it was clear that N. sativa honey samples had good antioxidant activity and they had major phenolics of honey samples reported in literature [6, 47, 48].

Components responsible for the aroma and odor of the honey samples were analyzed using headspace analysis and GC–MS. The GC–MS volatile components analysis performed by combining honeys from the three regions revealed that all three samples contained both common and different components. Aromadendrene, phenylacetaldehyde, furfural, thymoquinone, pelargol, carvacrol, cymene, limonene, carene, caprylalcohol, terpinene and phenethyl alcohol were the common volatile compounds in the samples. A previous study of N. sativa seeds reported that thymoquinone, oxygenated monoterpenes, represented 77% of the content, with lower levels of terpinen-4-ol, methyl chavicol, and trans-sabynil acetate, while no carvacrol was detected [47, 49]. Similarly to our results,

Table 5 Antimicrobial screening results of *Nigella sativa* honey by agar disc diffusion method (inhibition zone in mm) and MIC assay results expressed as µg /mL of to inhibit 100% of the microbial growth in vitro

Microorganism	Burdur		Kayseri		Kilis		Ampisilin	Cephazolin	Nystatin	Ethanol	Average of Activity on Bacteria Most Affected By Honey Samples
	IZ (nm)	MIC (µg/mL)	IZ (nm)	MIC (µg/mL)	IZ (nm)	MIC (µg/mL)					
Escherichia coli	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	20.00 ± 0.30	18.56 ± 0.00	–	6.00 ± 0.00	6.00 ± 0.00
Enterococcus faecalis	9.99 ± 5.80	50 ≤	11.73 ± 0.90	50 ≤	10.30 ± 0.50	50 ≤	32.50 ± 0.80	22.23 ± 0.70	–	6.00 ± 0.00	10.66 ± 0.32
Staphylococcus aureus	15.19 ± 0.70	12.5 ≤	14.49 ± 0.70	12.5 ≤	12.48 ± 0.30	12.5 ≤	11.66 ± 0.90	6.00 ± 0.00	–	6.00 ± 0.00	14.33 ± 0.80
Salmonella enteric subsp. enterica	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	33.94 ± 0 90	35.21 ± 0.40	–	6.00 ± 0.00	6.00 ± 0.00
Bacillus subtilis	11.68 ± 0.90	25 ≤	11.40 ± 0.50	25 ≤	14.02 ± 0.60	12.5 ≤	30.46 ± 0.70	32.57 ± 0.90	–	6.00 ± 0.00	12.42 ± 0.10
Bacillus cereus	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	23.58 ± 0.050	26.43 ± 0.50	–	6.00 ± 0.00	6.00 ± 0.00
Listeria monocytogenes	12.37 ± 0.50	12.5 ≤	11.83 ± 0.90	50 ≤	12.46 ± 1.00	12.5 ≤	27.34 ± 0.50	30.34 ± 1.00	–	6.00 ± 0.00	12.22 ± 0.34
Candida albicans	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	6.00 ± 0.00	100 ≤	–	–	17.80 ± 0.30	6.00 ± 0.00	6.00 ± 0.00
The average of the most effective honey sample on bacteria	9.625 ± 084		9.145 ± 0.63		9.250 ± 0.45						

(–) Not tested, zone diameter (mm) 6–10 average inhibition, 11–18 good inhibition, > 18 very good inhibition, *Escherichia coli ATCC®25,922, Enterococcus faecalis* ATCC® *29,121, Staphylococcus aureus* ATCC 6538, *Salmonella enteric subsp. enterica* ATCC *14,028/363–154, Bacillus subtilis* B*209, Bacillus cereus, Listeria monocytogenes* ATCC®*7677 and Candida albicans* ATCC®*10,231*

terpineol derivatives such as terpinen-4-ol, terpineol and terpinene, and thymoquinone were detected in differing amounts. Since we encountered no previous studies involving volatile compound contents of N. sativa honey, no comparison was possible. However, many studies with volatile compounds of N. sativa seeds have identified thymol, thymoquinone, limonene, and cymene as major components [48–51]. Studies show that the aroma components of honey vary according to its floral properties, collection time, and storage conditions. However, many aroma components are also present as common components [45].

N. sativa honey, most of which is produced in the Middle East region, is also produced in limited quantities in Turkey [3]. Although this honey has an interesting smell, aroma, and taste, it is also used for medicinal purposes as an antimicrobial agent. The antimicrobial activity of black cumin honey was tested against seven bacteria and one fungus, and activities were determined against each microorganism at varying inhibition values. The highest activity was obtained against *S. aureus* and *B. subtilis* B209. The main four factors affecting the antimicrobial activity of honey ate viscosity, pH, glucose oxidase-derived hydrogen peroxide, and its secondary metabolites. The most important secondary metabolites of honey are polyphenols, honeys with higher polyphenol levels being reported to possess higher antimicrobial values [3, 52]. One previous study reported that N. sativa honey and Manuka honey exhibited similar inhibition values against *Pseudomonas aeruginosa* ATCC 27,853, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* [3]. In a study, it was reported that different honey samples with different sources had different antimicrobial activity [48]. It was reported that honey samples inhibited Gram (+) bacterias and there were a good correlation between antimicrobial activity and the total phenolic content [48].

Conclusion

N. sativa seeds are obtained from an aromatic plant that is widely consumed for medicinal purposes, and N. sativa honey is also regarded as a highly valuable product. This study investigated the chemical composition, and antioxidant and antimicrobial properties of N. sativa honey harvested in Turkey. The honey was found to be rich in ellagic acid and pinocembrin, with a high biological active value and suitability for apitherapy applications. Further study could be carried out for revealing the apitherapeutic value of N. sativa honey with an animal model.

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Declarations

Conflicts of interest All the authors declare there is no conflict of interest.

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

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