Determination of antioxidant activity and phenolic compounds for basic standardization of Turkish propolis

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Abstract
This study aimed to determine the standard amount of antioxidant content and compounds of the propolis for the standardization of propolis. For this purpose, the total flavonoids, total phenolic, CUPRAC antioxidant capacity content and the diversity of phenolic and flavonoid components of these propolis samples were found by HPLC determined at the 23 propolis samples which were collected different regions of Turkey. Beside that, the similarities and differences of these 23 provinces to each other according to their antioxidant capacities were investigated by multidimensional scaling analysis. The total flavonoid content in the propolis samples were determined between 21.28 and 152.56 mg CE/g. The total phenolic content in the propolis samples was found between 34.53 mg and 259.4 mg GAE/g. CUPRAC antioxidant capacity of the propolis samples and antioxidant range was found from 95.35 to 710.43 mg TE/g. Also, 4 flavonoid [Quercetin (min.1.12–max.4.14 mg/g), Galangin (min.0.72–max.40.79 mg/g), Apigenin (min.1.07–max.17.35 mg/g), Pinocembrin (min.1.32–max.39.92 mg/g) and 6 phenolic acid [Caffeic acid (min.1.20–max.7.6 mg/g), p-Coumaric acid (min.1.26–max.4.47 mg/g), trans-Ferulic acid (min.1.28–max.4.92 mg/g), Protocatechuic acid (1.78 mg/g), trans-Cinnamic acid (min.1.05–max.3.83 mg/g), Caffeic Acid Phenethyl Ester (CAPE) (min.1.41–max.30.15 mg/g)] components were detected as mg/g, in different ratios in propolis samples collected from different regions. The feature of this study, so far, is to have the maximum number of samples representing the Turkish propolis, and so is thought to help to national and international propolis standard workings.

Keywords: CUPRAC antioxidant capacity, HPLC, Propolis, Total flavonoid, Total phenolic

Introduction
Propolis or bee glue is a substance containing a mixture of wax and resin collected by honeybees (Apis mellifera L.) from different parts (tree and flower buds, sap flows, mucilage, latex, resin etc.) of plants [1–5]. Honeybees, collect propolis from protective resins of flowers and trees buds with their lower jaws and carry them to the hive in the pollen sacs on their hind legs. They also add substances from their bodies during the resin collection and modeling phase. The collected propolis ensures that the hive is protected from all kinds of diseases and prevents the entrance of insects and animals by closing the small openings in the hive [6, 7].

Propolis, generally consists of 50% balsam, 30% wax, 10% essential oils and 5% pollen. Since the 1950s, scientists have started to isolate important components in propolis with the help of new analytical methods and have shown people that they have many benefits [7]. Propolis and its many compounds show a wide variety of biological and pharmacological activities [8]. It is a supplementary and supportive food that has become popular all over the world in 2000s, thanks to its antimicrobial [9–11], antioxidant [12], anticancer [13], antiulcer [14], antidiabetic [15], anti-inflammatory [16], antigenotoxic [17] and antiviral [18–22] activities. Propolis, which is...
also used in traditional and complementary medicine, is
an important bee product and it is used in “Apitherapy”,
which is a treatment method with bee products [23–26].
In particular, it has been determined by many scientists
that it is effective on the corona virus in the COVID-19
pandemic in 2020 [18–22]. Therefore, people’s interest in
propolis has increased more.

The content of propolis varies according to the plant
source and when it is collected [27]. In addition, the vari-
ety in the content of beeswax affects the chemical com-
position of the raw propolis [28]. More than 300 different
compounds have been detected to date in the propolis
[29–31]. The majority of these components are phenolic
acids and flavonoids [32].

Plant species in a geographic region determine the
amount and type of compounds found in the propolis.
In a study in New Zealand, dihydroflavonoids, pinobank-
sin and pinocembrin accounted for approximately 70% of
the flavonoids in the analyzed samples. However, in a
similar study conducted in Brazil, Uruguay and China,
the dihydroflavonoid in the samples was 10% less than the
samples in New Zealand [7]. Moreover, it has been
found to vary the amount of flavonoids and phenolic con-
cents in propolis samples collected from different regions
of Turkey [33–35]. The most important pharmacological
activity elements in propolis are flavones, flavanols and
flavanones, which are common names flavonoids, and
various phenolics and aromatics [7].

Propolis contains hundreds of different substances with
antimicrobial properties, about 80 of which are flavonoids
[1, 36–39]. Phenolic compounds in propolis are found in
large quantities at about 1 in 3, while flavonoids are only
up to 10% (w/w) of the concentrated form of propolis
[40, 41]. Among them, pinocembrin and galangin pro-
vide antibacterial activity. It also has pinocembrin, fun-
gicidal and local anesthetic properties [36]. Cinnamyl
alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic
acid, caffeic acid, coumaric acid and ferulic acid are some
phenolics found in propolis [7]. In recent years, studies
on propolis have found that pinocembrin, pinobanksin,
quercetin, chrysin and galangin flavonoids and caffeic
acid and coumaric acid phenolic acids are the most com-
mon components in propolis [9, 42–44].

In this study, total flavonoids and total phenolic com-
ounds content and total antioxidant capacity was deter-
mined at the 23 propolis samples which were collected
different regions of Turkey. In addition, the diversity of
phenolic and flavonoid components of these propolis
samples was found by HPLC and compared with other
studies. Beside that, the similarities and differences of
these 23 provinces to each other according to their anti-
oxidant capacities were investigated by multidimensional
scaling analysis. On the other hand, there are marketing
difficulties for propolis because of the lack of the national
or international propolis standard. For this reason,
national and international standard studies will progress
more easily thanks to studies that reflect the general
characteristics of country propolis, such as this study,
and this will solve marketing problems.

Material and methods
Collecting of propolis samples
Propolis samples were collected from 23 different cities
in Turkey in 2019. Propolis traps placed in the hives in
spring season were harvested end of the summer. Traps
were kept in the freezer and were removed from the
freezer while preparing propolis extracts (Fig. 1).

Preparation of extracts from raw propolis
About 30 mL of 70% ethanol solution is added to the
powdered 1 g of raw propolis sample, shaken for 24 h at
room temperature in shaker. The upper part is filtered
through coarse filter paper and transferred to 100 mL
flask. The process is repeated by adding 30 mL of 70%
ethanol solution to the remaining solid part. The super-
natant is added to the flask in which the first extract is
collected, completed to 100 mL with 70% ethanol solu-
tion [45].

Total phenolic analysis
Total phenolic content was found by modifying the Meda
et al. [46], method. According to this method, the work-
ing curve was prepared using varying concentrations
(0.25–0.13–0.06–0.03–0.02) of the Gallic acid (GAE)
standard (0.5 mg/mL) for calibration. The dilution appro-
priate for the sample was done with extraction solution
and 200 µL of diluted sample was put into the tubes for
analysis. For the blank, 200 µL extraction solution was
substituted for the sample. For the working curve, 200 µL
tubes of varying concentrations of gallic acid were placed
in tubes. Then, 1.5 mL of 0.2 N Folin solution was added
to the tubes and left for 5 min. The tubes were then vor-
texed by adding 1.2 mL of NaCO₃ (7.5%) solution. It was
incubated in the dark at room temperature for 90 min.
Finally, the UV-spectrophotometer was read against the
curve at a wavelength of 765 nm.

Total flavonoid analysis
Total flavonoid content was found by modifying the
Dewanto et al. [47], method. According to this method,
the working curve was prepared using varying concen-
trations (0.1–0.08–0.05–0.02–0.01) of the catechin (CE)
standard (1 mg/mL) for calibration. The dilution appro-
priate for the sample was done with extraction solution
and was placed in tubes from 1 mL of diluted sample for
analysis. For the blank, 1 mL of extraction solution was


substituted for the sample. For the working curve, 1 mL tubes of varying concentrations of catechin were placed in each tube. The timing is started with the stopwatch and 300 µL of 5% NaNO₂ (at t = 0 time), 300 µL of 10% AlCl₃ (at t = 5 time), 2 mL of 1 M NaOH (at t = 6 time) and finally 2.4 mL distilled water was added and vortexed. Without delay, the UV-spectrophotometer was read against the curve at a wavelength of 510 nm.

**CUPRAC antioxidant capacity analysis**

CUPRAC antioxidant capacity was detected according to the Apak et al. [48], method. According to this method, the working curve was prepared using varying concentrations (0.5–0.25–0.13–0.06–0.03) of the trolox standard (1 mg/mL) for calibration. Dilution appropriate to the sample was done with extraction solution and 100 µL of diluted sample was put into the tubes for analysis. For the blank, 100 µL extraction solution was substituted for the sample. For the working curve, 100 µL of the varying concentrations of trolox were put into the tubes. Then 1 mL of CuCl₂, 1 mL of neocuproin, 1 mL of NH₄CH₃COO and 1 mL of pure water were added and vortexed, respectively. Incubated for 1 h at room temperature in the dark. Finally, a 450 nm wavelength reading was made on the UV-spectrophotometer.

**HPLC component analysis**

Modified Aliyazıcıoglu et al. [33], method was used for propolis HPLC component analysis. Powdered 1 g raw propolis sample is weighed into a 50 mL falcon tube. Add 30 mL of 70% ethanol solution, shake for 24 h on a shaker. After centrifugation, the upper phase is transferred to a 100 mL volumetric flask. The shaking process is repeated once more. The upper phase is added to the volumetric flask where the first extract is collected, and complete to 100 mL with 70% ethanol solution. The solution is filtered through a PVDF syringe filter and transferred to the vial and 20 µL is injected to the HPLC device. VWR Hitachi HLC-UV Detector (UV 280 nm) and Supelcosil LC-18 25 cm × 4.6 mm, 5 µm column is used in the HPLC. Mobile phase A: 99% Ultra pure water: 1% Acetic Acid and Mobile phase B: 100% Methanol is used and flow was 0.9 mL/min, a linear gradient was applied by increasing the B mobile phase from 10 to 90%.

**Statistical analysis**

Multidimensional scaling (MDS) is a way to visualize the level of similarity between binary distances between a series of n objects or units. With multi-dimensional scaling analysis, objects are displayed in a k-dimensional (k>p) space based on the distance determined by p variable between n observations or units [49]. In this study, the similarities between the provinces according to the variables phenolic, flavonoid, and CUPRAC parameters were investigated using the multidimensional scaling analysis with Euclidean distance model. The similarity matrix obtained based on the variables in question was used to show the proximity and distance of the provinces to each other. The differences between provinces in relation to flavonoid was researched with One-Way analysis of variance (ANOVA). The Kruskall Wallis test was used for phenolic and CUPRAC because of normality.
and for Brazilia, China and Australia; 33–53 mg QE/g, Socha et al. [54] in Poland; 35.64–62.04 mg QE/g and Bonvehi and Gutierrez [55] in Spain; 72–161 mg QE/g. These results are compatible with our study.

**Total phenolic content**

The total phenolic content in the propolis samples was found between 34.53 mg GAE/g and 259.4 mg GAE/g. Kirklareli (259.4), Giresun (208.2), Ankara (191.55), Ordu (190.92) provinces have the highest values, while Kahramanmaraş (34.53), Adana (62.92), Düzce (69.3), Rize (78.06) provinces have the lowest values determined. The average of all provinces were found to be 150.09 mg GAE/g value and statistically Adana, Kirklareli and Maraş were found statistically different from each other (p < 0.05) (Table 1, Fig. 2). There are many studies on total phenolic compound in propolis. Some of them are as follows: Zarate et al. [50] in Mexico; 68–500 mg GAE/g, Ozdal et al. [51] in Turkey; 314.36 mg GAE/g, Narimane et al. [52] in Algeria; 0.57–3.53 mg GAE/g, Wang et al. [53] in South Korea; 21–50 mg QE/g and

| Provinces | N | Flavonoid mg Catechin/g Mean ± sd error | Phenolic mg Gallic Acid/g Mean ± sd deviation | CUPRAC mg TE/g Mean ± sd deviation | p value |
|-----------|---|---------------------------------------|-------------------------------------------|-----------------------------------|---------|
| Adana     | 3 | 32.20 ± 0.21 n                        | 62.92 ± 0.75                              | 150.63 ± 0.65                    | < 0.001 |
| Ankara    | 3 | 125.6 ± 0.31 o                         | 191.55 ± 0.79                             | 402.65 ± 0.50                    | < 0.001 |
| Amasya    | 3 | 96.6 ± 2.54 ph                         | 175.10 ± 2.70                             | 495.87 ± 3.33                    | < 0.001 |
| Bursa     | 3 | 115.2 ± 2.09 a                         | 177.7 ± 3.85                              | 506.2 ± 3.81                     | < 0.001 |
| Bolu      | 3 | 104.49 ± 0.34 a                        | 158.59 ± 0.95                             | 430.99 ± 1.75                    | < 0.001 |
| Balıkesir | 3 | 99.54 ± 0.31 g                         | 186.7 ± 1.55                              | 427.86 ± 0.85                    | < 0.001 |
| Düzce     | 3 | 28.35 ± 0.74 a                         | 69.3 ± 0.32                               | 182.7 ± 0.84                     | < 0.001 |
| Giresun   | 3 | 146.35 ± 0.49 a                        | 208.2 ± 2.64                              | 580.9 ± 3.02                     | < 0.001 |
| Isparta   | 3 | 71.40 ± 0.86 a                         | 108.13 ± 1.98                             | 282.3 ± 1.93                     | < 0.001 |
| İstanbul  | 3 | 83.52 ± 0.37 i                         | 161.77 ± 1.86                             | 384.07 ± 2.25                    | < 0.001 |
| İzmir     | 3 | 42.10 ± 2.14 m                         | 107.20 ± 0.46                             | 144.7 ± 2.06                     | < 0.001 |
| Konya     | 3 | 115.50 ± 2.17 f                        | 187.9 ± 2.18                              | 441.53 ± 0.26                    | < 0.001 |
| Karaman   | 3 | 57.67 ± 0.72 l                         | 129.07 ± 2.80                             | 245.3 ± 3.51                     | < 0.001 |
| Kastamonu | 3 | 100.18 ± 0.64 d                        | 160.9 ± 0.56                              | 424.0 ± 2.61                     | < 0.001 |
| Kocaeli   | 3 | 91.10 ± 0.13 i                         | 167.2 ± 0.40                              | 443.9 ± 0.15                     | < 0.001 |
| Kirklairel| 3 | 152.57 ± 1.47 a                        | 259.40 ± 1.73                             | 710.4 ± 2.01                     | < 0.001 |
| Marş      | 3 | 21.28 ± 1.02 p                         | 34.53 ± 2.10                              | 95.3 ± 2.83                      | < 0.001 |
| Muğla     | 3 | 82.95 ± 0.09 j                         | 139.28 ± 0.67                             | 342.56 ± 0.74                    | < 0.001 |
| Ordu      | 3 | 80.55 ± 0.49 i                         | 190.92 ± 0.88                             | 368.53 ± 2.50                    | < 0.001 |
| Rize      | 3 | 44.04 ± 0.96 m                         | 78.06 ± 0.86                              | 191.13 ± 0.40                    | < 0.001 |
| Sakarya   | 3 | 73.93 ± 0.10 k                         | 182.48 ± 2.69                             | 369.57 ± 0.93                    | < 0.001 |
| Tekirdağ  | 3 | 93.39 ± 0.53 h                         | 155.2 ± 0.10                              | 386.6 ± 1.16                     | < 0.001 |
| Zonguldak | 3 | 91.20 ± 0.70 j                         | 159.8 ± 0.96                              | 307 ± 1.78                      | < 0.001 |

Different letters in the same columns show statistically differences between means (p < 0.05)
for Brazilia, China and Australia; 127–142 mg GAE/g, Socha et al. [54] in Poland; 150.05–197.14 mg GAE/g, Aliyazıcıoglu et al. [33] in Turkey; 115–210 mg GAE/g and Bonvehi and Gutierrez [55] in Spain; 200–340 mgGAE/g. All results are consistent with our study.

**Antioxidant capacity (CUPRAC)**

CUPRAC method gives information about reductive capabilities of propolis extracts and based on reduction of Cu$^{+2}$ to Cu$^{+}$ by antioxidants [52]. Table 1 and Fig. 2 show the antioxidant capacity of the propolis samples and antioxidant range was found from 95.35 to 710.43 mg TE/g. Kırklareli (710.43), Giresun (580.93), Bursa (506.26) provinces have the highest values, while Maraş (95.35), Adana (150.63), Düzce (182.7), provinces have the lowest values were determined. The average of all provinces was found to be 365.46 mg TE/g value and İzmir, Kırklareli, and Maraş provinces were found statistically different from each other (p < 0.05) (Table 1, Fig. 2). Researchers found the CUPRAC value in propolis in different countries respectively: Bayram et al. [56] in Turkey; 282.8 mg TE/g, Ozdal et al. [51] in Turkey; 1184.94 mg TE/g, Nirmame et al. [52] in Algeria 8 µM TE/g, Daraban et al. [57] in Romania; 12404–35721 µM TE/100 mL. These results are similar to our study results.

**MDS analysis**

The results of the examination according to the similarities and differences of all provinces according to Flavonoid, Phenolic and CUPRAC antioxidant capacity contents are given in Fig. 3. After several dimensional scaling analysis, two-dimensional (k = 2) scaling was determined the best because of giving the lowest The Kruskal’s stress value and higher the coefficient of determination (R$^2$), as 0.004 and 0.99, respectively. Therefore, the results were given, and comments were made on two-dimension scaling.

The stimulus coordinates of provinces and configurations of provinces showed that Muğla, Isparta, Düzce, Rize, Adana and Maraş were found similar, Tekirdağ, Karaman, Kocaeli, Bolu, Ankara, Balyesir, Giresun and Kastamonu were found similar; Zonguldak, İstanbul, Kırklareli, Bursa, Sakarya, Amasya and Ordu were found similar and Konya and İzmir were found similar among each other. Optimal two-dimensional configuration of provinces based on stimulus coordinates was illustrated in Fig. 3.

**HPLC component analysis**

Propolis has many biological and pharmacological activities thanks to its large number of phenolic and flavonoid components [33]. For the HPLC method validation of the study, the repeatability, reproducibility, recovery, linearity, limit of detection limit (LOD) and limit of quantitation (LOQ) validation parameters were examined and presented in Table 2.

In this study, 4 flavonoid [quercetin (min.1.12–max.4.14 mg/g), galangin (min.0.72–max.40.79 mg/g),
apigenin (min.1.07–max.17.35 mg/g), pinocembrin (min.1.32–max.39.92 mg/g) and 6 phenolic acid [caffeic acid (min.1.20–max.7.6 mg/g), p-coumaric acid (min.1.26–max.4.47 mg/g), trans-ferulic acid (min.1.28–max.4.92 mg/g), protocatechuic acid (1.78 mg/g), trans-cinnamic acid (min.1.05–max.3.83 mg/g), caffeic acid phenethyl ester (CAPE) (min.1.41–max.30.15 mg/g)] components were detected as mg/g, in different ratios in propolis samples collected from different regions of Turkey (Table 3, Fig. 4).

Cunha et al. [58] found caffeic acid, p-coumaric acid and ferulic acid in all of the extracts prepared in their study using different solvents to determine the phenolic content of Brazilian propolis. Also, Choi et al., [59] stated that in many propolis samples obtained from Korea contains caffeic acid (min.1.0–8.7 mg/g), p-coumaric acid (min.1.2–7.1 mg/g), ferulic acid (min.0.5–1.9 mg/g), apigenin (min.0.6–2.4 mg/g), pinocembrin (min.1.5–87.8 mg/g) and galangin (min.4.9–max.26.3 mg/g). These results are in line with our results. On the other hand, Lagouri et al., [60]
Table 3 Propolis component analysis results by HPLC

| Provinces   | Quercetin (mg/g) | Galangin (mg/g) | Apigenin (mg/g) | Pinocembrin (mg/g) | Caffeic acid (mg/g) | p-Coumaric acid (mg/g) | Trans-ferulic acid (mg/g) | Protocatechuic acid (mg/g) | Trans-cinnamic acid (mg/g) | CAPE (mg/g) |
|-------------|------------------|----------------|----------------|-------------------|---------------------|------------------------|--------------------------|---------------------------|-----------------------------|-------------|
| Adana       | N.D              | 1.07 ±0.39     | 1.96 ±0.05     | 2.14 ±0.88        | 4.03 ±0.2           | 2.33 ±0.23             | N.D                      | ND                        | 2.26 ±0.5                   | 2.04±0.83   |
| Ankara      | N.D              | 19.24 ±0.42    | 1.75 ±0.04     | 7.93 ±0.48        | 2.01 ±0.8           | 1.26 ±0.03             | N.D                      | ND                        | 1.20 ±0.07                  | 19.26±0.66  |
| Amasya      | N.D              | 12.86 ±0.7     | 1.81 ±0.4      | 6.65 ±0.45        | 1.55 ±0.02          | N.D                    | N.D                      | ND                        | 1.33 ±0.02                  | 19.32±0.19  |
| Bursa       | N.D              | 10.87 ±0.09    | N.D            | 7.25 ±0.04        | 4.74 ±0.07          | N.D                    | N.D                      | N.D                       | N.D                        | 16.08±0.06  |
| Bolu        | 4.14 ±0.4        | 10.42 ±0.04    | 1.87 ±0.3      | 15.39 ±1.54       | 1.32 ±0.02          | 2.00 ±0.05             | 2.26 ±0.38               | 1.78 ±0.04                | 1.05 ±0.02                  | 26.99±0.87  |
| Balikesir   | 2.58 ±0.16       | 9.64 ±1.92     | 1.20 ±0.05     | 11.93 ±3.06       | 2.83 ±1.1           | N.D                    | N.D                      | N.D                       | 1.31 ±0.07                  | 8.87±0.03   |
| Düzce       | 1.58 ±0.28       | 3.98 ±0.68     | N.D            | 7.39 ±0.88        | N.D                 | 1.28 ±0.07             | N.D                      | N.D                       | N.D                        | 3.65±0.25   |
| Giresun     | 2.46 ±0.04       | 12.52 ±0.45    | N.D            | 5.42 ±0.05        | 4.82 ±1.4           | 4.47 ±0.09             | 4.92 ±0.05               | N.D                       | 1.41±0.04                   |             |
| Isparta     | N.D              | 1.58 ±0.04     | 1.19 ±0.07     | 3.09 ±0.05        | 1.20 ±0.03          | N.D                    | N.D                      | N.D                       | N.D                        |             |
| İstanbul    | N.D              | 7.69 ±0.4      | 17.35±0.53     | 11.07 ±0.55       | ND                  | ND                     | ND                       | ND                        | 1.80 ±0.03                  | 3.30±0.38   |
| İzmir       | N.D              | 2.50 ±0.48     | N.D            | 5.97 ±0.36        | ND                  | ND                     | ND                       | ND                        | N.D                        | 1.58±0.05   |
| Konya       | N.D              | 6.12 ±0.44     | N.D            | 10.41 ±0.6        | 2.51 ±0.6           | 2.59 ±0.45             | ND                       | ND                       | 1.12 ±0.04                  | 9.79±0.65   |
| Karaman     | N.D              | 0.97 ±0.03     | N.D            | 13.2 ±0.06        | ND                  | ND                     | ND                       | ND                       | N.D                        | 4.03±0.08   |
| Kastamonu   | 1.18 ±0.05       | 2.77 ±0.26     | 1.07 ±0.06     | 5.32 ±0.63        | 76 ±0.06            | 2.69 ±0.09             | ND                       | ND                       | 2.13 ±0.03                  | 19.53±0.04  |
| Kocaeli     | 1.46 ±0.28       | 10.89 ±5.90    | 1.19 ±0.20     | 29.17 ±5.08       | 2.07 ±0.75          | 3.47 ±0.79             | 2.50 ±0.43               | ND                       | 2.25 ±0.08                  | 25.41±4.41  |
| Kırklareli  | 2.10 ±0.04       | 40.79 ±0.04    | 4.08 ±0.06     | 39.92 ±0.04       | 5.77 ±0.04          | 3.40 ±0.26             | ND                       | ND                       | 3.47 ±0.04                  | 30.15±0.04  |
| Marag       | N.D              | 0.72 ±0.06     | N.D            | 1.65 ±0.06        | ND                  | ND                     | N.D                      | N.D                       | N.D                        | 3.18±0.18   |
| Muğla       | 1.14 ±0.04       | 6.71 ±3.73     | 1.46 ±0.4      | 16.32 ±0.52       | 1.59 ±0.07          | ND                     | N.D                      | ND                        | 2.16 ±1.02                  | 2.13±0.33   |
| Ordu        | N.D              | 4.83 ±0.32     | 2.67 ±0.23     | 20.22 ±0.25       | 1.96 ±0.7           | 1.39 ±0.05             | ND                       | ND                       | 12.54±3.15                 |             |
| Rize        | N.D              | 3.73 ±0.05     | 3.77 ±0.07     | 17.42 ±0.07       | N.D                 | ND                     | ND                       | ND                       | 2.01 ±0.05                  | 4.58±0.08   |
| Sakarya     | 3.89 ±0.04       | 16.49 ±0.03    | 2.98 ±0.02     | 34.82 ±0.07       | 2.06 ±0.04          | 3.25 ±0.04             | 3.26 ±0.09               | ND                       | 1.15 ±0.03                  | 15.14±0.05  |
| Tekirdağ    | 1.12 ±0.02       | 11.77 ±1.56    | 1.67 ±0.42     | 5.88 ±1.88        | 5.03 ±0.02          | 1.53 ±0.05             | 1.32 ±0.15               | ND                       | 3.83 ±0.03                  | 21.79±0.35  |
| Zonguldak   | N.D              | 6.95 ±0.04     | N.D            | 2.90 ±0.02        | ND                  | ND                     | ND                       | ND                       | 2.55 ±0.08                  | ND          |
identified that caffeic acid (min.0.64–max.4.17 mg/g), caffeic acid phenyl ester (min.0.36–max.2.04 mg/g), ferulic acid (min.0.53–max.1.41 mg/g), p-coumaric acid (min.0.83–max.3.00 mg/g), apigenin (min.0.48–max.2.74 mg/g) and galangin (min.1.32–max.8.55) components at the lower amounts according to our study at the Greek propolis samples. Also, Keskin and Kolayli [34] found caffeic acid (min.0.40–max.7.33 mg/g), ferulic acid (min.0.52–max.9.83 mg/g), coumaric acid (min.0.71–max.4.30 mg/g) phenolic compounds amounts similar to our results at the Turkish propolis samples. On the other hand, Aliyazıcıoglu et al. [33] determined similar results for Turkish propolis samples with our results. They found caffeic acid (1446.8–4658.1 µg/g), p-coumaric acid (381.7–4579.8 µg/g) and ferulic acid (223.3–7126.9 µg/g). Ristivojevic et al. [35] also, found phenolic and flavonoid compounds at the another Turkish propolis study. They revealed caffeic acid (min.3.96–max.34.78 mg/mL), ferulic acid (min.1.00–max.19.42 mg/mL), coumaric acid (min.0.19–max.4.91 mg/mL), protocatechuic acid (min.0.45 mg/mL–max.1.69 mg/mL), trans-cinnamic acid (min.3.00–max.5.28 mg/mL), quercetin (min.1.11–max.4.33 mg/mL), galangin (min.0.96–max.2.70 mg/mL), apigenin (min.0.54–max.1.56 mg/mL), pinocembrin (min.0.94–max.2.81 mg/mL). Their results were similar to our results. Beside that, Pavlovic et al., [61] determined caffeic acid (min.4.21–max.4.37 mg/g), p-coumaric acid (min.1.40–max.6.97 mg/g), ferulic acid (min.1.64–max.7.41 mg/g), pinocembrin (min.17.90–max.19.06 mg/g) at the Italian propolis samples. Also, these results close to our study results. As a result, in this study, we found caffeic acid, caffeic acid phenethyl ester (CAPE), galangin and pinocembrin as major components for the Turkish propolis. Because these components have been detected in almost all provinces and these components can be used for quality determination and standardization of Turkish propolis. As a similar, Sorucu and Oruc [62] determined pinocembrin, CAPE, caffeic acid highest amounts at the propolis samples from the northwest of Turkey.

As a result, the content of raw propolis varies according to the botanical origin of the region where it is obtained. Turkey, where they grow different plant species, is a country with rich botanical resources. Because, there are three phytogeographical regions in Turkey (Euro & Siberian, Mediterranean, Irano & Turanian) and the plant diversity varies from region to region [63]. So, there are about 12,000 plant species in Turkey and 3000 of them are endemic. About 500 plant species are nectar plants and are preferred by honeybees [64]. Since propolis is in very different phytogeographic regions in the 23 cities studied, the botanic origin varies. For this reason, propolis contents also differ greatly and it is very important to making content analysis for propolis standardization.
In this study, total phenolics, total flavonoids and anti-
oxidant capacity amounts were determined and com-
pared statistically at the propolis samples, which were
collected from different regions of Turkey. By illumi-
ning the phenolic and flavonoid components contained in
propolis samples, components [caffeic acid, caffeic acid
phenethyl ester (CAPE), galangin and pinocembrin] that
could be markers for Turkish propolis were determined.
Thus, for the basic standardization of Turkish propo-
lis, the range in which the total phenolic substance and
flavonoid substance amounts should be and the compo-
nents it should contain were determined. These com-
ounds can be used in the marketing quality control of
Turkish propolis.

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EYO, MK, AO analyzed data and wrote the manuscript and CT made the statis-
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read and approved the final manuscript.

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All data analysed during this study are included in this published article.

Declarations

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

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