

# Comparative Study of Some Commercial Propolis Extract with New Prepared Ethanolic Propolis Extract

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## Abstract

In this study, quality parameters of some commercially sold in Turkey propolis extracts were compared with freshly prepared ethanolic propolis extract (70%). The pH, total phenolic substance, total flavonoid and total antioxidant capacity values of 11 different commercial samples, including aqueous, ethanolic, water-based and vegetable oil, were compared. As a result, it was determined that ethanolic samples had the highest activity and the activity of propolis with olive oil was very low. It was found that products with high total polyphenol content (TP) had high total antioxidant values. It was also determined that a standard method was not used for the preparation of commercial propolis samples and that the TP values differ greatly.

## Introduction

Honey, pollen, propolis and royal jelly are the most used bee product in apitherapeutic applications (Sahin & Kemal, 2020). Propolis is a natural paste as a result of the accumulation of resinous substances collected from the bark, leaves and stems of the honey bees in the hives. This complex mixture, which is used to protect the hive for multiple purposes, is an indispensable natural bee product of apitherapy (Akçay, Birinci, Birinci, & Kolaylı, 2020; Bankova et al., 2019). It is known that the best solvent for raw propolis is 70% ethanol, but some glycol derivatives and vegetable oils are also used in the preparation of extracts (González-Búrquez et al., 2018; Oroian, Ursachi, & Dranca, 2020). The apitherapy active ingredients of propolis are a family of polyphenols. It has subclasses such as polyphenols, phenolic acids, flavonoids, anthocyanins and tannins, which have a

wide range of compounds. Phenolic acids are more hydrophilic, that is, bimolecular dissolving in aqueous environments and flavonoids in hydrophobic environments. For this reason, flavonoids are insoluble in aqueous media, and phenolic acids such as gallic acid in apolar solvents (Galeotti, Maccari, Fachini, & Volpi, 2018). Propolis is one of the natural products with the highest biological activity and is a moist agent of traditional and complementary medicine with its high antioxidant, antimicrobial, anti-inflammatory and antitumoral properties (Kolaylı & Keskin, 2020; El Adaouia Taleb, Djebli, Chenini, Sahin, & Kolayli, 2020). Until now, an ideal solvent for the raw propolis has not been found exactly. However, since ethanol is more apolar than water and more polar than oils, it has the ability to dissolve a large number of both polar and apolar compounds.

The aim of this planned is to investigate whether commercial propolis extracts are in the order of total polyphenol and other quality.

## Materials and Methods

### Samples

11 commercial propolis samples were purchased from various markets and herbalists. A codename data for each sample was not used in stealing the trade name. The crude propolis specimen was made in Turkey, named also Anatolia propolis. Fifteen different regions of propolis sample (10 g) of Anatolia were collected obtained a pool. 10 g of powder sample was taken the pool and extracted in 70% ethanol (1:10 w/v). It was protected in the refrigerator at + 4 °C.

### Extraction

For the extraction, it was first extracted in an ultrasonic bath for 1 hour and then in a shaker (Heidolph Promax 2020, Schwabach, Germany), for 24 hours. Then it was filtered and used in the study. Water-based and olive oil samples were prepared by extracting them in 70% ethanol for 24 hours.

### Total Phenolic Content

The total amount of phenolic substance was measured by Folin Ciocalteu method (Singleton & Rossi, 1965). This method contains all the polyphenols in solution and reflects their total values. 10 mL of different concentrations of standard and 10 mL ethanolic samples (1 mg/mL), 200 mL of 0.2 N Folin–Ciocalteu's reagents and 680 mL of distilled water were mixed. Following 4 min incubation, 400 mL of Na<sub>2</sub>CO<sub>3</sub> (10%) was added. The mixture was incubated for 2 h then absorbance was read at 760 nm. The calibration curves were occurred using each individual standard at six different concentrations (0.5; 0.25; 0.125; 0.0625, 0.03125 and 0.015 mg/mL) and all analysis were carried out in triplicate. Results were expressed as milligrams of gallic acid equivalents per 100 mL sample (mg GAE 100 g/mL) by using a standard graph.

### Total Flavonoid Determination

Determination of total flavonoid substance was used according to Fukumoto and Mazza (2000). It was used quercetin in different concentrations (QU) for standard. Total flavonoid amount as quercetin equivalent as mg quercetin (KE)/100 g honey. The total amount of flavonoid substance was found by measuring the absorbance of the coloured product formed as a result of the redox reaction between flavonoids and aluminium (III) at a wavelength of 415 nm. Working solutions of 0.500, 0.250, 0.125, 0.062, 0.031 and 0.015 mg/mL were prepared from this stock by serial dilution

and the absorbance values were read according to the method. A standard calibration graph was obtained by drawing a graph of absorbance against quercetin (1 mg/mL) concentration. Using the standard calibration graph, the total amount of flavonoids in the sample was calculated and the results were given as mg Quercetin equivalent per gram sample (mg QE/mL).

### Total Antioxidant Activity

The ferric reducing antioxidant power (FRAP) was used to measure the total antioxidant power of the samples (Benzie & Strain, 1996). Firstly, FRAP reagent was prepared with 25 mL of 0.30 M acetate buffer (pH 3.6), 2.5 mM TPTZ solution in 40 mM hydrochloric acid, and 2.5 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution. Briefly, 100 mL of standard or samples solution was added to 3 mL of freshly prepared FRAP reagent and the reaction mixture was incubated at 37 °C for 4 min. Then, the absorbance was measured at 593 nm against distilled water as blank by using the spectrophotometer. Trolox was prepared at different concentrations (31.5, 62.5, 125.0, 250.0, 500.0 and 1000 mM), using as a standard antioxidant compound. The results were expressed in micromole Trolox equivalents per gram.

## Results and Discussion

Table I summarizes the quality parameters of some trade propolis samples from Turkey. And also, the pH values of all propolis extracts were compared. It was determined that the pH values varied between 4.0 and 5.0 except for only 2 samples. The pH values of ethanol and glycol derived samples were found to be around 4-5 acidic. The pH was 9 in one aqueous sample and 10 in the other. It is normally crude phenolic acids, and when dissolved in water or alcohol, it is below pH 6. The pH of the various phenolic acids found in propolis is below 7. Interestingly, it was considered quite surprising that the pH of the two samples was well above 7. The exact reason for this is unknown, but it is thought that it is not from crude propolis and that a basic solvent is used to dissolve the propolis. While the stomach pH varies between 1.5-2.0, the pH of the foods we consume is around 4-5. Direct consumption of propolis extract at such high pH can cause stomach damage. Therefore, the pH should be taken into account when standardizing propolis extracts (González-Búrquez et al., 2018; Galeotti et al., 2018; Bankova et al. 2019).

The most important feature of propolis extracts are polyphenols in their structure. Total polyphenol content (TP) is the most important determinant for propolis, and high polyphenol value indicates high biological activity. Table I gives the total polyphenol values. Accordingly, to the result, the total polyphenol values of ethanolic, aqueous and glycol extracts differ. It was determined that the highest TP value was in ethanolic samples, and the water and olive oil samples had the lowest activity.

**Table 1.** Comparative study of some commercial propolis extracts of Turkey.

Code	Used Solvent	pH	Total phenolic	Total Flavanoid	FRAP
Anatolia Propolis					
P	Ethanol	5.30	5105±120	2040±23	285±32
Ethanol commercial extracts					
P1	Ethanol	9.0	1093±34	752±21	266±17
P3	Ethanol	5.12	5931±93	1945±12	858±74
P4	Ethanol	5.10	3287±21	2114±39	553±22
P5	Ethanol	4.80	2605±34	1044±24	687±11
Water-Soluble commercial extracts (glycol derivates)					
P6	Glycol	5.20	2283±42	2116±22	363±85
P7	Glycol	5.30	3090±23	2040±21	320±53
Water commercial extracts					
P8	Water	10.00	645±23	449±37	58±80
P9	Water	5.40	102±4	17±1	33±4
Olive oil commercial extracts					
P10	Olive oil	4.00	492±7	260±8	98±1
P11	Olive oil	4.20	642±16	310±22	246±9

Total phenolic: mg GAE/100 mL,

Total Flavanoid: mg QE/100 mL,

FRAP: mmol FeSO<sub>4</sub>·7H<sub>2</sub>O/mL

It is seen that the TP value of ethanolic extracts varied between 1093 and 5931 mg GAE/100 g. Samples dissolved in glycol were found to have lower TP than ethanol, but significantly higher than aqueous and olive oil samples. While there are significant differences between the two water-soluble samples and the differences is too high. The reason why the water-based P8 sample, which is soluble in water at any ratio and has a very dark red colour, contains approximately 10 times higher than the P9 sample. However, when this sample is evaluated together with the high pH value, it is thought that this may be due to the solution used. It was found that oil-based propolis extracts had values close to each other. Comparing the TP values of commercial propolis samples with Anatolian propolis that we prepared in the laboratory, only one ethanolic sample contained higher polyphenols and the others were found to be lower.

When the total flavanoid amount (TF) was examined, it was found that ethanolic samples had the highest flavanoid value. In the study, it was found that TF values increased or decreased in parallel with TP values and the lowest TF values were in aqueous samples.

Total antioxidant capacity (TAC) values were calculated in terms of the reducing ability of the Fe (III) complex in the study. While high FRAP value indicates high capacity, it was determined that the highest FRAP value was in the commercial ethanolic sample and it was followed by Anatolian propolis. Olive oil samples and water samples were found to have very low TAC values.

When studies on propolis are scanned in the literature, studies with mostly raw propolis draw attention. Since there were not many studies on commercial propolis, there was no possibility of comparison or discussion.

## Conclusion

As a result, the amount of solvent and solvent used in the extraction of raw propolis is very important. However, the most important criteria to be sought in commercially purchased propolis are the use of solvents that will not harm human health and that products with high polyphenol content are more valuable.

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