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Comparison of Chemical Contents of Extracts in Different Solvents of Propolis Samples Produced in Duzce Province

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Abstract

Objective: Although the volatile components in the propolis composition are in very low concentration, they are extremely important for the characterization of propolis due to their aroma-giving properties and various biological activities. Since propolis is a product obtained from plants, its chemical composition depends on the local plant flora and the geographical and climatic characteristics of the region where the sample is collected. Therefore, different propolis samples can differ completely in terms of their chemistry and biological activities. Propolis extracts obtained by using different solvents have different contents. For this reason, the content of antioxidants varies, which causes differences in phenolic and flavonoid amounts. In the study, it was aimed to determine the efficacy of different propolis extracts produced as a result of beekeeping activities in Düzce province by comparing the chemical content.

Material-Method: The extracts of propolis collected from Düzce province were prepared using ethanol, water and PEG400 – water solvents. In the study carried out, the volatile components of three different extracts of propolis samples obtained from hives belonging to Yığılca Region were examined with LC-MS/MS, GC-MS UV. The determination of the total phenolic component (TPC) level was carried out with the Folin-Ciocalteu reagent, and the total flavonoid content (TFC) level with the AlCl3 based method.

Results: Major volatile constituents of Ethanolic extract; Diphenyl-1,2,5-oxadiazole (86.11%) and Benzenepropanoic acid ethyl ester (6.3%), Major volatile components of PEG400-water (50% - 50%) extract; 4-vinyl-2-methoxy-phenol (40.40%), Benzyl benzoate (17.16%), Methyl benzyl ketone (16.87%) ve Ethyl 3-methylnaphtho[1,2-c]pyrrole-1-carboxylate (14.32%), major volatile components of water extract; Benzyl Alcohol (79.91%) ve 4-vinyl-2-methoxy-phenol (8.86%). The highest TPC level was in ethanolic extract with 23,192.45 ± 396.54 mgGAE/100 g. Similarly, the highest TFC was found in ethanolic extract (7,190.12 ± 203.85 mgQE/100g). The water extract had the lowest levels at both TPC and TFC levels.

Conclusion: A It has been concluded that the highest phenolic content of propolis, which has recently begun to find an important area of use in the food and health sector, is obtained by ethanol extraction. When evaluated in terms of the obtained results from all methods, it is listed as Etanolic extract> PEG400> water extract. Further studies should be done using different solvents in order to extract as much of the components from propolis as possible.

Keywords: Propolis Extraction, LC-MS/MS, GC-MS, UV

INTRODUCTION

Apitherapy is among the traditional and complementary medicine practices and defined as "the way that bee and bee products are used as a protective and complementary application method in the treatment of some diseases" by the Ministry of Health [1]. One of the bee products used in apitherapy is propolis. Propolis has been used in traditional medicine both internally and externally since the early ages of humanity. It is one of them the products used as a food supplement in different parts of the world such as America, Europe, Brazil, Taiwan and Japan to support health and prevent

diseases such as aging, inflammation, heart diseases, diabetes and cancer [2–5].

In addition, there are many studies showing the antibacterial, antifungal, antiviral, local anesthetic, anti-inflammatory, antioxidant, hepatoprotective, immunostimulant and cytostatic activity of propolis [6].

Propolis is widely used in traditional medicine in many countries from Europe to East Asia due to all these features. This natural product, which is becoming increasingly important nowadays, also attracts a great deal of attention in the pharmaceutical, cosmetic and food industry [4,7,8].

The raw form of propolis cannot be used in the food and pharmaceutical industry. So, there are many studies such that the most effective content of propolis was investigated using different solvents. One of the most common solvents for propolis extraction is ethanol, because it contains more phenolic acid and polar compounds than water extract [9]. It is quite easy to extract the lipophilic components in propolis with ethanol. However, in the another study, it has also been reported that aqueous extract of propolis due its caffeeoilquinic acids to have a higher antioxidant activity and a high inhibitor/activator effect against some enzymes [10]. In addition, various solvents such as glycerin, propylene glycol and polyethylene glycol are also used in propolis extraction for pharmaceutical and cosmetic applications [11–14].

LC-MS/MS makes it possible to quantify the substance even at very low concentrations and provides a high sensitivity and precision for quantitative applications. GC-MS is also an excellent technique for identifying volatile substances [15,16]. GC provides a perfect separation, but to make flavonoids suitable for analysis, an extra derivatization step is often required prior to analysis [17]. Among all these methods, HPLC and accompanying MS, UV, DAD or PDA systems are undoubtedly still the most valid and reliable analytical technique for characterization of polyphenolic compounds [17–19].

In this study, it was aimed to determine the qualitative and quantitative content of ethanolic, aqueous and polyethylene glycol extracts of propolis produced in Düzce province using LC-MS/MS, GC-MS and spectrophotometric methods.

MATERIALS AND METHODS

The propolis samples used in the study were supplied from beekeepeers in Düzce province. The solvents used were provided in pharmacological purity. The extract preparation parts of our study were carried out at Düzce University, Traditional and Complementary Medicine Application and Research Center Laboratory, LC-MS/MS analyzes at Düzce University, Faculty of Medicine, Department of Pharmacology Research Laboratory, GC-MS and TPC and TFC analyzes were carried out in Düzce University Scientific and Technological Research Application and Research Center Laboratory.

Preparation of ethanolic extract

100 mL of 70% ethanol (70 ml ethanol+30ml water) was added to 10 grams of propolis sample. It was then left to stir for one week at 1100 rpm and room temperature in the dark. At the end of the period, the insoluble propolis sample was filtered using qualitative filter paper and filtrates were kept in the dark until analysis time.

Preparation of the water extract

100 mL of distilled water was added to 10 grams of propolis sample. It was then left to stir for one week at 1100 rpm and room temperature in the dark. At the end of the period, the insoluble propolis sample was filtered using qualitative filter paper and filtrates were kept in the dark until analysis time.

Preparation of the PEG400 - water extract

100 mL of 70% ethanol (70 ml ethanol+30ml water) was added to 10 grams of propolis sample. It was then left to stir for one week at 1100 rpm and room temperature in the dark. At the end of the period, the insoluble propolis sample was filtered using qualitative filter paper and filtrates were kept in the dark until analysis time.

The filtrate was evaporated to dryness. The obtained dry propolis extract was dissolved in 100 mL of 50% Polyethylene glycol 400 (PEG400)-water solvent mixture. The mixture was re-filtered through black band filter paper to remove insoluble. The filtrates were kept in the dark until analysis time.

LC-MS/MS analysis

Chemicals and instruments

Standards were supplied and MS grade methanol and formic acid solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). High quality ultra-pure water was supplied by Human Zeneer Navi Power I Integrate (Human Corporation, Korea). The chemical content and composition of propolis were determined by using LC-ESI-MS/MS (Shimadzu, Kyoto, Japan).

Preparation of samples

The extract of samples (1 ml) were taken and added dilution solvent (9 ml) for each individual extract. The mixtures were vortexed for a minute and then diluted with the same solvents to 1 to 10. They were vortexed for a minute and filtered with 0.45 µm filters. Filtered solution was used for injection.

Analysis method

We used linear gradient LC-MS/MS method for all propolis analysis. Analysis was performed 100-mm x 4.6-mm, 5-mm particle C18 column.

Column oven set to 40 °C. Mobile phases (A) Ammonium formate (50 mM) +0.1% formic acid and (B) methanol. 0.3 ml/min flow and starting conditions with mobile phases %80/%20 respectively. From start to 5 min B was used %80; from 5 to 8 min B linear gradient remains %80; thereafter, a linear gradient back to %20 for 4 min to equilibrate column for next injection. Injection volume was 5 µl.

ESI-MS/MS analysis was performed using multiple reaction monitoring (MRM) to detect the major product ions from the protonated molecules of some phenolic and flavonoid contents (Table 1). The MS conditions were: nebulizer gas 2 ml/min and heat block temperature 450°C.

GC-MS analysis

The study was carried out on an Agilent 7890A GC System coupled to an Agilent 5975C inert MSD with Triple Axis Detector. Agilent HP5-MS (30 m × 0.25 mm × 0.25 µm) column was used as GC column.

The oven temperature was held at 40 ºC for 5 min., then ramped at 5 ºC / min. to 100 ºC for 5 min., then ramped at 20 ºC / min. to 225 ºC and held at this temperature for 8 min. The total run time was 33.25 min. The injector temperature was fixed at 200 ºC and splitless mode was used with helium carrier gas. The ion source was electron ionization and the MS source temperature was set at 230 ºC. The injection volume was 1.0 µL.

Total phenolic content (TPC)

The TPC of extracts was determined according to the Folin-Ciocalteau method with slight modifications [20]. All extracts were analyzed in triplicate. Briefly, the extracts were diluted with methanol (1:4). 800 µl 0.5 N Folin-Ciocalteau reagent was mixed with 40 µl extract solution and allowed to react for 5 min at room temperature in darkness. Then 800 µl of Na_2CO_3 (10%) was added and the volume of mixture was brought up to 3.0 ml with distillated water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 760 nm using spectrophotometer (Shimadzu, UV-1800). Gallic acid solution was used as a standard for constructing the calibration curve. TPC was expressed as mg of gallic acid equivalents (GAE) per g of propolis samples [21,22].

Total flavonoid content (TFC)

The TFC of extracts was measured by the colorimetric $AlCl_3$ method with few modifications [22]. Briefly, the extracts were diluted with methanol (1:4). 0.5 ml extract solution was mixed with equal volume of 2% $AlCl_3$ and 3.0 ml distillated water. The mixture incubated at room temperature in darkness for 1 h. Then the absorbance was measured at 415 nm with spectrophotometer (Shimadzu, UV-1800). Quercetin solution was used as a standard for constructing the calibration curve. TFC was expressed as mg of quercetin equivalents (QE) per g of propolis samples. All extracts were analyzed in triplicate [20,21,23,24].

Statistical analysis

Data were expressed as average ± standard error of the mean (SEM). The test results of TPC and TFC data were subjected to statistical analysis using SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA) for the analysis of variance (ANOVA) with comparison of means by Tukey test ($P < 0.05$).

RESULTS

In LC-MS/MS MRM (Multi Reaction Monitoring) mode was used for analysis. As seen in Table 2, the peaks of the generally expected phenolic and flavonoid groups are found in separate extractions. Depending on the type of solvent used in the extraction, the types of extractable substances differ. According to these results, it was observed that the highest component was in the ethanolic extract of propolis, and the lowest was in the aqueous extract of propolis.

GC-MS anaylsis

The volatile components of the extracts obtained from the propolis samples using three different extraction methods and the percentage distributions among these components are given in Table 3-5.

It was determined that the extract containing the most volatile component among the extracts was prepared with the ethanol solvent system. While the benzaldehyde compound is not included in the PEG400-water extract, it is included in the other two extracts. Although Methyl benzyl ketone compound is not present in water extract, it is included in the composition of the other two extracts and is one of the main volatile components of the PEG400-water extract with a ratio of 16.871%. While Ethyl benzoate compound is not found in PEG400-water extract, it is included in Ethanolic extract with a rate of 0.47% and in Water extract with a rate of 2.70%. While 4-vinyl-2-methoxy-phenol compound is the main volatile component of PEG400-water extract with a ratio of 40.40%, this ratio is reduced by 8.86% in water extract and none in Ethanolic extract. While the Benzyl Alcohol compound is the main volatile component of the water extract, it is not included in the other two extracts. Diphenyl-1,2,5-oxadiazole compound is the main volatile compound of Ethanolic extract.

Table 1. MRM parameters of compounds

	Compound	m/z	Ref. Ions	Ret. Time	Scan
1	Coumarin	147.30>91.10	147.30>103.10	6.902	(+)
2	Quercetin-3-o-rutinoside-7-o-glucoside	773.20>773.20	773.20>465.20-773.20>105.00-773.20>400.00-773.20>773.20	4.610	(+)
3	Robinin	741.20>287.20	741.20>595.20-741.20>433.20-741.20>257.30-741.20>741.20	5.988	(+)
4	Caffein	195.00>138.10	195.00>110.10	5.136	(+)
5	Chlorogenic acid	353.10>191.10		4.408	(-)
6	Cryptochlorogenic acide	353.20>173.20	353.20>135.20-353.20>179.10	4.282	(-)
7	EPGC	457.10>169.10	457.10>125.00-457.10>305.20	4.599	(-)
8	Vanillic acid	167.10>108.20	167.10>152.20	5.885	(-)
9	Catechin hydrate	288.90>109.10	288.90>245.10-288.90>271.20	4.302	(-)
10	Ellagic acid	301.10>301.10	301.10>145.10	6.673	(-)
11	Fisetin	284.90>135.10	284.90>121.00	6.850	(-)
12	Gallic acid	169.20>125.10	169.10>97.00	3.049	(-)
13	Kaemferol 3-glucoside	447.20>255.20	447.20>284.00	6.598	(-)
14	Procynadin B2	577.20>289.20	577.30>425.10	4.196	(-)
15	Quinic acid	190.90>173.20	190.90>127.00	1.755	(-)
16	Rosmarinic acid	359.10>161.10	359.10>179.00	5.883	(-)
17	Trans-ferulic acid	193.10>178.20	192.90>149.10-192.90>134.10	6.074	(-)
18	Quercetin-3-Galactoside	463.20>300.10	463.20>255.20	6.309	(-)
19	Cinamic acid	147.30>103.10	147.30>147.20	7.491	(-)
20	Quercetin-3-glucoside	463.00>463.00	463.00>300.00	6.374	(-)
21	Caffeic acid	179.00>135.00	179.00>179.00	5.374	(-)

Table 2. Contents of different extractions.

Compounds	Ethanolic extract	PEG400-water extract	Water extract
Coumarin	+		
Quercetin-3-o-rutinoside-7-o-glucoside	+	+	+
Robinin	+	+	+
Caffein			
Chlorogenic acid			
Cryptochlorogenic acide			
EPGC			
Vanillic acid			
Catechin hydrate			
Ellagic acid	+	+	+
Fisetin	+		
Gallic acid			
Kaemferol 3-glucoside	+		
Procynadin B2			
Quinic acid			
Rosmarinic acid			
Trans-ferulic acid	+	+	
Quercetin-3-Galactoside	+		
Cinamic acid	+	+	
Quercetin-3-glucoside	+	+	
Caffeic acid	+	+	

Total phenolic content and total flavonoid content analysis

Total phenolic and flavonoid content, which are known to be important factors that play a part in the antioxidant activities, were measured and results of propolis extracts obtained with three different solvents are given in Figure 1. Our results showed that the ethanolic extract has the highest total phenolic content (TPC, 23,192.45 ± 396.54 mgGAE/100g). PEG400-water extract followed it and water extract showed the lowest TPC value (6.191,03 ± 162.39 mgGAE/100g). Total flavonoid results (TFC) were also parallel to TPC results. Ethanolic extract showed the highest TFC value with 7190.12 ± 203.85 mgQE/100g. PEG400-water and water extracts followed respectively.

Table 3. Volatile components of ethanolic extract as determined by GC-MS

Compound	RT (min)	Compound Name	Distribution Ratio (%)
1	8.141	Benzaldehyde	0.64
2	8.457	Silicic acid tetraethyl ester	3.27
3	9.894	Methyl benzyl ketone	0.62
4	10.252	Benzoic acid ethyl ester (Ethyl benzoate)	0.47
5	10.346	[1'-(phenylsulfinyl)prop-2'-enyl]cyclohex-2-en-1-ol	0.34
6	11.472	2-Methoxy-4-vinylphenol (p-Vinylguaiacol)	6.39
7	11.638	Benzenepropanoic acid ethyl ester (Ethyl 3-phenylpropionate)	0.50
8	11.731	4-Phenyl-3-buten-2-one (Benzalacetone)	0.65
9	12.463	3-phenyl-ethyl ester -2-Propenoic acid (Ethyl cinnamate)	1.00
10	25.689	Diphenyl-1,2,5-oxadiazole (3,4-Diphenylfurazan)	86.12

Table 4. Volatile components of PEG400-water (50 % - 50 %) extract as determined by GC-MS

Compound	RT (min)	Compound Name	Distribution Ratio (%)
1	9.894	Methyl benzyl ketone	16.871
2	11.093	2H-1-benzopyran (1,2-Chromene)	5.254
3	11.555	4-vinyl-2-methoxy-phenol	40.401
4	11.736	4-Phenyl-3-buten-2-one (Benzalacetone)	5.978
5	15.166	Benzyl benzoate (Ascabin)	17.167
6	17.983	Ethyl 3-methylnaphtho[1,2-c]pyrrole-1-carboxylate	14.329

Table 5. Volatile components of water extract as determined by GC-MS

Compound	RT (min)	Compound Name	Distribution Ratio (%)
1	8.089	Benzaldehyde	3.10
2	9.147	Benzyl Alcohol	79.92
3	10.258	Benzoic acid ethyl ester (Ethyl benzoate)	2.70
4	10.564	N-3-chloro-2-methylpropyl)-N,N-Dimethylammoniumchloride	1.73
5	11.415	4-vinyl-2-methoxy-phenol	8.87
6	11.742	Tolpropamine	1.50
7	13.521	*o*-(2-(Methylamino)propyl)phenol	2.20

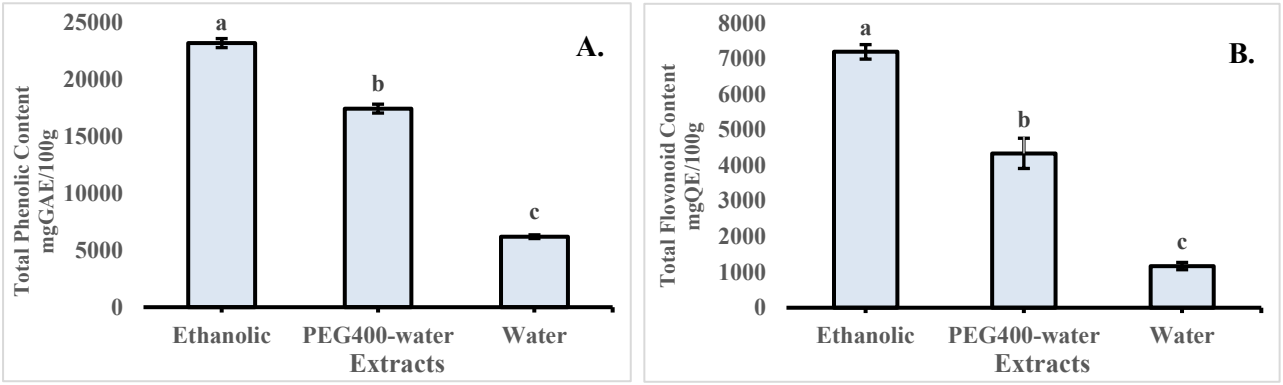

Figure 1. Total phenolics (A) and flavonoids (B) content of Düzce Propolis extracts. Different superscript letters (a–c) by each extraction method indicate significant differences according to Tukey's test at significance level $P < 0.05$.

DISCUSSION

There are many studies showing that the active ingredients of propolis easily dissolve in ethanol [25–32]. Despite the rich content of ethanolic extract, dissolution of the ethanolic extract in many products, especially in the pharmaceutical and food industry is a problem. The propolis aqueous extract cannot show the expected efficacy since the active ingredient content is very weak compared to the content of the ethanolic extract. Therefore, investigating the contents of propolis in different solvents is still an important issue that attracts the attention of many researchers. One of these solvents is polyethylene glycol (PEG). Water PEG solutions can also be used as suspending agents in topical ointments or to adjust the viscosity of other suspending agents [33]. PEG400 is known to be non-toxic if not taken in very high doses in one go [34]. Therefore, PEG400 is suitable for use in the pharmaceutical, food and cosmetic industries to increase the solubility of oil-based additives in water-based products. In addition, since PEG400 can be used as a thickener in pharmaceutical products, another advantage is that there is no need for extra thickening additives in products produced with the use of this extract.

There are many studies about the chemical composition of Turkish Propolis using different methods. However, the number of studies on propolis produced in Düzce is limited. Rasgele and Kekecoglu [35] investigated the ethanolic extraction of propolis produced in Yığılca district Düzce province by using another method (HPLC-DAD). But there are GC-MS studies made with propolis produced in the Black Sea Region. For example; Çelemli [36] reported that "benzoic acid" and "17-pentatriacontene" are determinant for the propolis samples collected from the Black Sea Region. Gençay and Salih [37] stated that propolis collected

from Rize, Bartın, Trabzon and Gümüşhane were rich in flavonoids and that, benzene,1-(1,1-dimethyl ethyl) and 28-norolean-17-en-3-one were the major component in Rize propolis. Popova et al. [38] indicated that Artvin propolis contained phenolic glicorides according to their GC-MS results. Sorkun et al. [39] determined that major flavonones in propolis were 43.55% and 50.55% Trabzon and Gümüşhane, respectively. Kocabas et al. [40] conducted with propolis samples collected from the Eastern Black Sea Region, volatile components of propolis samples were determined as; phenyl ethyl alcohol (7.7%), benzyl alcohol (7.4%), decanal (6.7%), ethyl benzoate (6.5%), nonanal (5%) and cedrol (4.1%). These results reveal that geographical location, vegetation and climatic conditions have an important role on the volatile components of propolis when compared to the study carried out [40].

In this study, it was aimed to compare the contents of propolis in different solvents using different methods. According to obtained GC-MS results, major components were diphenyl-1,2,5-oxadiazole (3,4-diphenylfurazan) for ethanolic extracts, 4-vinyl-2-methoxy-phenol for PEG400-water and benzyl alcohol for water extracts of propolis. The 3,4-Diphenylfurazan derivatives, the major component of propolis ethanolic extract, have been found to be potent indolamine 2,3-dioxygenase inhibitors and are useful for the treatment of cancer cells. They are also useful as a new class of SENP2 inhibitors and can be used in the development of new therapeutic agents for various diseases targeting SENPs. Studies have revealed that 4-Amino-1,2,5-oxadiazole-2-oxide-3-carboxylic acid and azo derivatives, which are 3,4-diphenylfurazan derivatives, have vasodilating properties. The 3,4-Diphenylfurazan derivative 1,2,5-Oxadiazole-2-oxide and benzo [c] [1,2,5] oxadiazole-N-oxide derivatives show herbicidal activity [41].

2-Methoxy-4-vinylphenol, the major component of propolis PEG400-water extract, is an aromatic substance used as a sweetener and one of the compounds responsible for the natural aroma of buckwheat [42].

Benzyl alcohol, which is the major component of propolis water extract, is used as a bacteriostatic preservative in intravenous drugs, cosmetics and topical drugs [43]. In addition, the US Food and Drug Administration (FDA) permitted the use of a 5% solution of benzyl alcohol for head lice treatment in people six months and older in 2009 [44].

Total phenolic compounds and flavonoids have an important role in antioxidant activity by neutralizing free radicals. According to obtained UV results, total phenolic component values vary among different solvents in the amount of 6,191.03 ± 162.39 and 23,192.45 ± 396.54. In terms of total phenolic component, the highest amount was obtained from ethanol extract and the lowest amount was obtained from water extract. Asem et al.[45] stated that flavonoids from propolis exhibited a stronger antioxidant effect than vitamins C and E. Kubiliene et al.[14] used different solvents such as PEG400-water mixture, PEG400, olive oil, olive oil-water mixture and ethanol extract in their researches. They reported that total phenolic activity of nonethanolic solvents does not differ significantly from the concentration found in ethanolic extract and they have radical scavenging and antimicrobial activity. Similarly, the highest amount in terms of total flavonoid content was obtained with ethanol solvent. These results showed that the solvent with the highest amount of both phenolic compounds and flavonoids was ethanol. Although PEG400-water solvent was determined to be more successful than water, it was able to extract less phenolic compounds than ethanol. Our results have been found to be compatible with some previous studies. Mouhoubi et al.[46] stated that the optimum extraction solvent was 85% ethanol. It has been stated that 75% ethanol extract has wider polarity than water extract, making it a better solvent for propolis extraction. Fikri et al.[24] reported that the water extract can contain non-phenolic ingredients such as carbohydrates and terpenes. According to the results, extracts of propolis showed a phenolic composition significantly richer in phenolic compounds than flavonoids. Ozdal et al.[20] indicated that the TPC of the ethanolic extract was higher

than a previous study, which investigated propolis samples from different region of Turkey. The amount of flavonoid in the Düzce sample included in the same study was found approximately 3 times higher than our results. In addition, it was emphasized that the highest TPC and antioxidant capacity was obtained from the Marmara region, Kırklareli and Düzce samples [20]. It has been stated that the amount of polyphenols in propolis may vary depending on its geographical origin, and factors such as climatic conditions, vegetation around the hive, and harvest period affect the chemical composition of propolis [47]. In the light of this information, although the polyphenol content of propolis varies depending on various factors, ethanolic extract of Düzce propolis has been found to have higher phenolic and flavonoid content than the data in many studies in the literature [7,20,23,48–50]. According to the obtained LC-MS/MS results, the fact that the components expected to be found in the extracts are mostly in ethanol is a situation predicted from the literature. PEG was observed to be more effective than water in extracting these bioactive components. Since there are only so many MRMs in our method, it is not correct to make a complete generalization, but seeing the expected main phenolic components or derivatives can be considered sufficient [51,52]. Chong and Chua [53] reported the chemical composition of aqueous, PEG+aqueous, ethanolic and PEG+ethanol extracts of propolis using GC–MS and LC–MS/MS. In their study, they found that ethanolic extracts of propolis had higher yields than water extracts at different pH values without significant difference. Kubiliene et al.[14] analyzed the chemical content of aqueous, PEG-aqueous and ethanolic extracts of propolis using HPLC and UV. They reported that the ethanolic extract was 10-17 times higher in terms of phenolic component than PEG-aqueous and aqueous extracts, respectively. All these findings are in agreement with our studies which investigated chemical composition of propolis in different solvents.

CONCLUSION

As a result, according to the results obtained from our study in which three different extracts of propolis were analyzed using three different methods, the most suitable solvent is ethanolic extract. When evaluated in terms of the obtained results from all methods, it is listed as Etanolic extract> PEG400> water extract. PEG can be used as an alternative for sections that are sensitive to the use and consumption of alcoholic extract. However, it may not be considered sufficient since it is not close to the variety of substances that pass to ethanol. Further studies should be done using different solvents in order to extract as much of the components from propolis as possible.

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