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Chemical Analysis and Antioxidant Activity of Four Propolis Samples Collected from Different Regions of Lebanon

Lübnan'ın Farklı Bölgelerinden Toplanan Dört Propolis Örneğinin Kimyasal Analizi ve Antioksidan Aktivitesi

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Abstract

Propolis samples were collected from four different regions of Lebanon characterized by high biodiversity and high honey production. The samples were analyzed for their total phenolic contents (TPC), total flavonoid contents (TFC), chemical profiles, and antioxidant activity. The TPC were determined using Folin-Ciocalteu method while the TFC using the $AlCl_3$ method. The antioxidant activity of propolis was examined by two different methods, namely free radical scavenging assay and reducing ability. The chemical profiles of the samples were assessed by thin-layer

Özet

Propolis örnekleri yüksek miktarlarda bal üretimi ve biyolojik çeşitliliği ile karakterize edilen Lübnan'ın 4 farklı bölgesinden toplanmıştır. Örneklerin toplam fenolik içeriği, toplam flavonoid içeriği, kimyasal profilleri ve antioksidan aktiviteleri analiz edilmiştir. Fenolik madde içeriği Folin-Ciocalteu ve flavonoid içeriği $AlCl_3$ metodu ile belirlenmiştir. Propolisin antioksidan aktivitesi serbest radikal süpürme aktivitesi ve indirgeme gücü olarak incelenmiştir. Örneklerin kimyasal profilleri ince tabaka kromatografisi, UV-Vis ve gaz kromatografisi/kütle

chromatography (TLC), UV-Vis, and gas chromatography/mass spectrometry GC-MS analysis. Total phenolic content ranged from 53.35 ± 7.09 to 148.27 ± 15.08 mg gallic acid equivalents per gram (mg GAE/g), total flavonoid content ranged from 45.73 ± 2.8 to 134.5 ± 8.46 mg rutin equivalents per gram (mg RUE/g). GC/MS analysis revealed the presence of 9-octadecene and tetradecene as major compounds that have been previously reported to demonstrate antioxidant activity. In addition, Berqayel propolis sample showed high content of phenolic compounds and high antioxidant activity, while samples from Wadi Faara recorded poor chromatograms with the absence of most of the compounds present in Berqayel samples. The majority of propolis samples showed relatively interesting antioxidant activity, which was also correlated with TPC and TFC. These findings highlighted the effect of the beehive locations on the quality of Lebanese propolis in terms of chemical constituents and biological activities.

Keywords: Propolis, Antioxidant, Total phenolic content, Total flavonoid content, GC-MS analysis

spektrometrisi ile belirlenmiştir. Toplam fenolik madde içeriği g başına 53.35 ± 7.09 ile 148.27 ± 15.08 mg gallik asit eşdeğeri (mg GAE/g), flavonoid içeriği ise g başına 45.73 ± 2.8 ile 134.5 ± 8.46 mg rutin eşdeğeri arasında değişmektedir. GC/MS analizinde antioksidan aktiviteye sahip olduğu bilinen 9-oktadesen ve tetradesen bileşenlerinin varlığı ortaya konulmuştur. Ek olarak, Berqayel propolisinin yüksek fenolik madde içerdiği ve yüksek antioksidan aktiviteye sahip olduğu belirlenmiş, Wadi Faara'dan alınan numunelerin ise kromatogramları zayıf bulunmuş, Berqayel propolisinde bulunan birçok bileşeni içermediği görülmüştür. Propolis örneklerinin çoğu toplam fenolik ve flavonoid madde içeriğiyle korelasyon gösteren yüksek antioksidan aktiviteye sahiptir. Bu bulgular, kimyasal bileşenler ve biyolojik aktiviteler açısından kovan lokasyonlarının Lübnan propolisinin kalitesi üzerindeki etkisini ortaya koymaktadır.

Anahtar kelimeler: Propolis, Antioksidan, Toplam fenolik içeriği, Toplam flavonoid içeriği, GC-MS analizi

Abbreviations: EEP, ethanolic extract of propolis; TPC, Total phenolic content; TFC, Total flavonoid content; TLC, Thin layer chromatography; GC-MS, Gas chromatography/mass spectrometry.

1. INTRODUCTION

Propolis is a natural resinous product assembled by honey bees (*Apis mellifera* L.) from different sources of plant. It's used to make the protective shield at the entrance of beehives and its human use dates back to ancient times, when the product was employed in embalming bodies in Egypt (Soltani et al., 2017). In addition to antimicrobial activity of propolis, other biological and pharmacological properties have been demonstrated including antitumor, antibacterial, antioxidant, antifungal and other activities (Anjum et al., 2019).

Propolis is generally composed of 50 % resin and balm (including phenolic compounds), 30 % wax and fatty acids, 10 % essential oils, 5 % pollen and 5 % various organic and inorganic

compounds. The specific composition of propolis depends on the vegetation at the site of collection (Boisard, 2014).

Propolis showed the most potent antioxidant of all the bee products (Nakajima et al., 2009). Antioxidant activity of propolis was originated from their polyphenolic substances (Isla et al., 2005; Wang et al., 2016). Thus, propolis can be used for prevention and treatment of diseases related to the increase of oxidative stress such as cancer, aging, and cardiovascular diseases (Kocot et al., 2018).

In the present study, propolis extracts from four different Lebanese locations were prepared by maceration using ethanol/water, considered as green solvents. The effect of geographical origin on the phytochemical contents was assessed and results were compared for the antioxidant capacity in terms of free radical scavenging assay and reducing power assay. Moreover, the volatile compounds profile in the Berqayel sample was characterized using gas chromatography/mass spectrometry (GC-MS).

2. MATERIALS AND METHODS

2.1. Chemicals and Instrumentation

All chemicals used were of analytical grade. Methanol (MeOH), Ethanol (EtOH), chloroform, Folin-Ciocalteu, ascorbic acid, aluminum chloride hexahydrate ($AlCl_3.6H_2O$) and rutin were purchased from BDH (England). 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium carbonate anhydrous (Na_2CO_3), potassium ferricyanide, trichloroacetic acid and iron (III) chloride ($FeCl_3$), were purchased from Sigma Aldrich (USA). Samples were weighed using a RADWAG XA 82/220/2X laboratory balance. The absorbance values were measured using a VWR UV-6300PC double beam spectrophotometer and extracts were concentrated using HEIDOLPH (Germany) rotavapor apparatus.

2.2. Sample Collection

In this study, four Lebanese propolis samples were collected from four apiaries located in different Lebanese regions (Figure 1), more specifically from Berqayel (34°28′38.6″ N, 36°2′1.54″ E, 350 m MSL), Debaal (33°15′02″ N, 35°20′56″ E, 280 m MSL), Fakeha (34°14′44″ N, 36°24′21″ E, 900 m MSL) and Wadi-Faara (34°17'22.0" N 36°18'15.8" E, 2100 m MSL). The average annual temperature (AAT) in Berqayel and Debaal reaches 20 ^oC, and the average precipitation (AP) ranges between 700 and 1,000 mm. whereas, the AAT in Fakeha is lower than that and reaches 16 ^oC while the average precipitation drops to 400 mm in this

region. However, Wadi Faara experiences an ◌AAT less than 10 ^{o}C and the AP between 1,500 and 2,000 mm. The main process to collect the raw propolis samples was started by the initial preparation to separate it from extraneous macro impurities if present. The obtained samples were frozen at - 20 ^{o}C until analysis.

2.3. Obtaining the Ethanolic Extract of Propolis (EEP)

Each frozen brown to yellow propolis sample was chopped into small pieces and immediately homogenously pulverized. Then, one gram of each sample was macerated in 100 mL of ethanol 80 % for 48 hours at room temperature under magnetic stirring. The mixture was then filtered, and the filtrate was evaporated under reduced pressure to

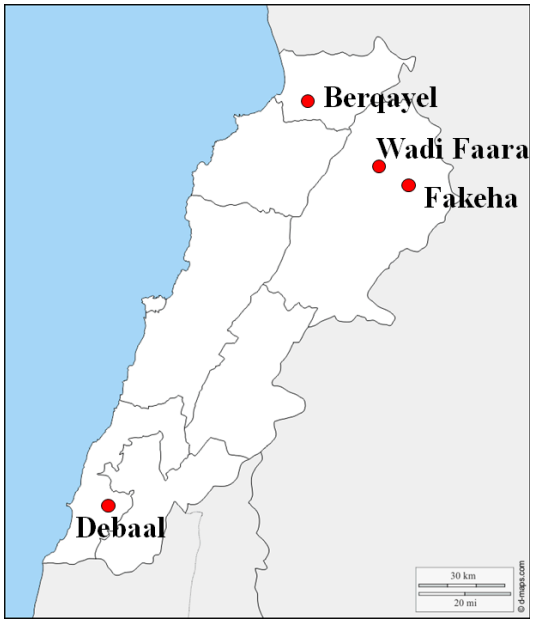

Figure 1. Approximate geographical location of the samples of the propolis evaluated.

produce the ethanolic extract of propolis (EEP). Finally, the EEP was weighed and stored at +4 °C for further use.

2.4. Phytochemical Screening

The phytochemical screening was carried out by means of qualitative phytochemical tests based on color or precipitation reactions with the extract.

2.4.1. Test for Alkaloids

Extracts were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Jaber et al., 2019).

2.4.2. Test for Phenols

A few drops of ferric chloride solution were added to 2 mL of the extract in a watch glass; the appearance of bluish green color indicated the presence of phenol (Yadav & Agarwala, 2011).

2.4.3. Test for Terpenoids (Salkowski test)

1 mL of each extract was mixed in 2 mL of chloroform, and concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids (Iqbal et al., 2015).

2.4.4. Test for Hydrolysable Tannins

A few drops of 0.1 % ferric chloride were added and observed for brownish green or a blue-black coloration (Jaber et al., 2019).

2.4.5. Test for Quinons

1 mL of concentrated hydrochloric acid (HCl) was added to one mL of EEP. The presence of quinons is confirmed by the appearance of yellow color (Yadav & Agarwala, 2011).

2.4.6. Test for Flavonoids

1 mL of KOH is added to 1 mL of each extract. The color shifting to yellow indicates the presence of flavonoid (Morsy, 2014).

2.4.7. Test for Saponins

1 mL of extract was shaken with 2 mL of water. The persisting of foam for ten minutes indicates the absence of saponins (Jaber et al., 2019).

2.5. Determinatıon of Total Phenolic Content (TPC)

The total polyphenol content in propolis extract was determined by using Folin-Ciocalteu method (Singleton et al., 1999) with some modifications. Briefly, 100 µL of the EEP extract was taken and mixed with 500 µL of aqueous Folin-Ciocalteu solution (10 %). After 5 min, 2 mL of sodium carbonate (7.5 %) were added. The obtained mixture was allowed to stand for 30 min in the dark. After which the absorbance was read at 760 nm in a spectrophotometer. The TPC in the extract was extrapolated from the calibration curve derived by repeating the same procedure for different concentrations of methanolic solutions of gallic acid (30-270 $\mu g.mL^{-1}$), and results were expressed in mg of gallic acid equivalents per g of propolis (mg GAE/g).

2.6. Determinatıon of Total Flavonoid Content (TFC)

Total flavonoid content was determined by aluminium chloride spectrophotometric assay (Barreca et al., 2016). Briefly, 1 mL of each EEP was mixed with 1 mL of 2 % aluminium chloride ($AlCl_3$) methanolic solution. After incubation for 30 min at room temperature, absorbance was measured at 410 nm. Rutin was used as the standard and a curve was constructed by preparing different dilutions (0 - 50 µg.mL^{-1}) using the same procedure for EEP. The blank sample consisted of 1 mL of extract solution in 1 mL methanol without $AlCl_3$. The amount of total flavonoids in the extracts was expressed as rutin equivalents (mg RUE/g).

2.7. *In Vitro* Antioxidant Capacity

In order to evaluate the *in vitro* antioxidant activity, two spectrophotometric methods were used: DPPH free radical scavenging assay and reducing power assay.

2.7.1. DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the extracts was evaluated with the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method developed by Blois (1958) (Kedare & Singh, 2011). The DPPH methanolic solution (0.032 mg.mL^{-1}) was prepared freshly. To 1 mL of aliquot of extract solution, 1 mL of DPPH• methanolic solution was added. The mixture was vortexed and then left to stand at room temperature for 30 min in the dark at room temperature. The absorbance was read at 520 nm using an UV-Vis spectrophotometer. Different concentrations of ascorbic acid (0.81 - 4.05 µg.mL^{-1}) have been produced for use as the positive control. A similar procedure was used for the blank, where the extract sample was replaced with methanol. The free radical scavenging capacity was expressed as the percentage inhibition of the radical oxidation and calculated using the following equation:

$$\% \text{ scavenging activity} = \frac{\text{Abs Control} - \text{Abs Extract}}{Abs\ control} \text{x}100$$

The results were expressed in IC_{50} (The half-maximal inhibitory concentration) as the amount of antioxidant required to decrease the initial DPPH concentration by 50 %.

2.7.2. Reducing Ability

The reducing ability of EEP was determined according to the method reported by Oyaizu (Oyaizu, 1986). This assay is normally based on the blue coloration that develops due to the reduction of ferric iron to the ferrous. A serial dilution of extract solutions and ascorbic acid (0.7 - 0.05 g.mL^{-1}), were prepared in water. 200 µL of each extract solution was mixed with

200 µL of 0.2 M phosphate buffer (pH 6.6) and 200 µL of potassium ferricyanide (1 %). Reaction mixture was incubated at 50º C for 20 min. After cooling, 200 µL of trichloroacetic acid (10 %) were added and mixture was centrifuged at 1000 rpm for 8 min. The upper layer (800 µL) was mixed with 800 µL of distilled water and 160 µL of ferric chloride (0.1 %). After a 10 min reaction time, the spectrometric absorbance was recorded at 700 nm and compared with ascorbic acid as positive control. The absorbance values were plotted against the concentration, and a linear regression analysis was carried out. Higher absorbance readings indicate higher reducing power.

2.8. Measurement of the Absorption Spectra of Extracts

The UV- vis spectra of the propolis extract solutions were recorded from a mixture of 25 µL of the extracts with 1750 µL of ethanol (80 %). All the samples have the same concentration in mg solid propolis/mL. The obtained solutions were scanned at wavelengths range between 190 and 600 nm by UV-vis spectrophotometer. Quartz cuvettes of 1 cm optical path were used and the absorbance being recorded against an ethanol 80 % blank.

2.9. TLC and GC-MS Analysis

TLC analyses of EEP were performed on thin layer chromatographic (TLC) plates, composed of Silica gel 60 GF 254. The plate was developed in chamber previously saturated by eluent. Two systems of mobile phase were used: Petroleum ether / ethyl acetate 7:3 and cyclohexane / ethyl acetate 8:2. After drying, spots were investigated; visually and under UV 254/366 light.
One microliter of EEP samples (Berqayel and Wadi Faara) was diluted (1:100) with hexane and injected into the Gas chromatography–mass spectrometry (GC-MS) system. GC SHIMADZU QP2010 system was used to analyze the volatile compounds in the propolis extracts (without derivatization). DB-5MS (5 % Diphenyl / 95 % Dimethylpolysiloxan) capillary column having (30 m length, 0.25 i.d., film thickness 0.28 µm) and helium as carrier gas was used for compound separation. The oven temperature was programmed from 65 ^{o}C (2 min initial time) increased to 300 ^{o}C at 10 ^{o}C/min (isothermal for the final time) and the MS was operated in the electron impact mode at 70 eV ion source energy. Injection volume was 1 µL and total run of one hour is performed. Data receipt and processing were performed using Shimadzu GC-MS solution software. The compounds were identified based on a comparison of their mass spectra with data in NIST (National Institute of Standards and Technologies, Mass Spectra Libraries).

2.10. Statistical Analysis

All determinations were conducted in triplicates (n = 3); the correlation coefficients (R^2) and the statistical mean ± SD were calculated using Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

3. RESULTS and DISCUSSION

3.1. Phytochemical Screening

Table 1 shows the results of the phytochemical screening for different propolis samples. Different chemical profiles are observed between propolis samples from distinct geographic locations. The samples from Berqayel and Debaal were the richest in terms of potentially active constituents (phenols, alkaloids, hydrolysable tannins, flavonoids and terpenoids) while those from Wadi-Faara were the poorest.

Table 1. Phytochemical Screening of EEP.

Phytochemical Compounds	Debaal	Wadi-Faara	Fakeha	Berqayel
Flavonoid	+	+	+	+
Alkaloids	+	+	+	+
Hydrolysable Tannins	+	-	-	+
Terpenoids	+	-	+	+
Quinons	+	-	-	+
Phenols	+	+	+	+
Saponins	-	-	-	-

Note. +: detected, -: No detected

On the other hand, saponins were absent in all samples. Although, the later observation contradicts the study of Chamandi et al. (Chamandi et al., 2015) done on Lebanese propolis samples, it is in agreement with another study reported by Labyad et al. (Labyad et al., 2016). It is worth to point out here that the phytochemical composition (and subsequent biological activity) of propolis is highly dependent on the plant cover diversity as well as biotic and abiotic factors (Bueno-Silva et al., 2017; do Nascimento et al., 2019; Huang et al., 2014; Salatino et al., 2011; Savickas et al., 2005).

The phytochemicals detected have previously been shown to exhibit biological activity, such as antibacterial, antitumor and antihelmintic activity (Cowan, 1999; Rosli et al., 2016; Zeitoun et al., 2019).

3.2. Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

Phenolic compounds are known as powerful chain breaking antioxidants, which may contribute directly to antioxidative activity. These compounds are very important constituents of plants and their radical scavenging ability due to their hydroxyl groups (Labiad et al., 2017). The propolis from Berqayel registered the greatest TPC (148.27 mg GAE/g) and TFC values (134.5 mg RUE/g) (Figure 2). These TPC and TFC values were about 3-fold of the lowest TPC and TFC values detected in Debaal region (53.35 mg GAE/g and 50.99 mg RUE/g, respectively), indicating the significant variations of TPC and TFC in propolis samples between the four Lebanese regions.

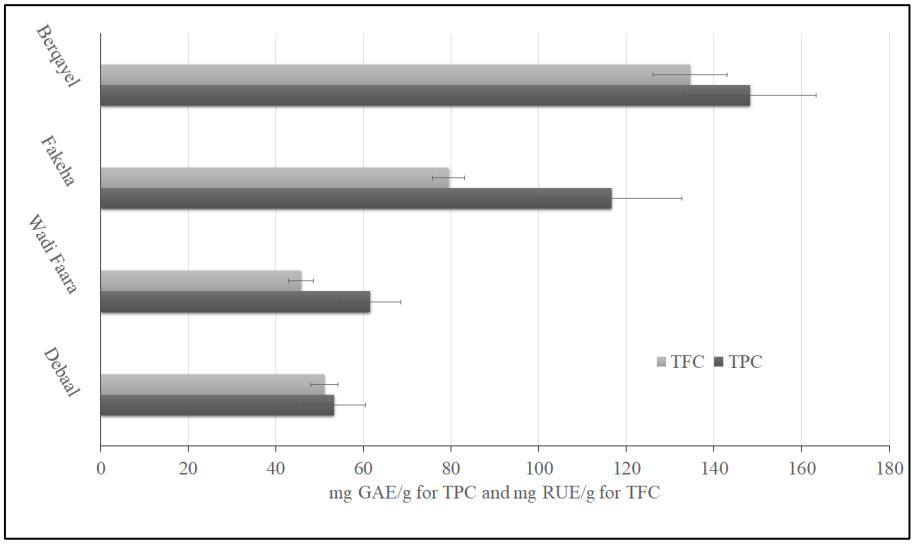

Figure 2. TPC and TFC of the four EEP. Values are the mean ± SD of three replicates.

The TPC range (53.35–148.27 mg GAE/g) was comparable to that of Uruguayan propolis (Silva et al., 2011) and South African (Kumazawa et al., 2004); and, lower than others such as Brazilian propolis (277.81- 398.11 mg GAE/g) (Reis et al., 2019). The TFC contents of 50.9–134.5 mg RUE/g recorded in this study are in the same order with those reported in other propolis origins, using quercetin as standard in the colorimetric method instead of rutin. For instance, the TFC ranges of some Chinese propolis were 52.11-173.90 mg QE/g (Shi et al., 2012) and 08.3-188 mg QE/g **(**Ahn et al., 2007). Moreover, the TFC ranges of Brazilian and Canadian propolis were 42.00-108.02 mg QE/g (Reis et al., 2019) and 01.58-137.06 mg QE/g (Cottica et al.,2015), respectively. However, the values in this study are lower than those reported in Indonesia (Pratami et al., 2017).

3.3. Antioxidant Activity of Propolis Extracts

The presence of different secondary metabolites in EEP is an indication that the extract studied might have antioxidant capacity.

3.3.1. DPPH Assay

Free radicals produced in living systems and encountered exogenously, lead to various disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing (Singh & Singh, 2008). The antioxidant activity was determined to be potent via two different assays, the DPPH assay and the reducing power assay. Antioxidant potency is usually associated with the content of phenolic compounds due to their extensive conjugated π-electron systems that facilitate the donation of electrons from the hydroxyl moieties to oxidizing radical species (Bittencourt et al., 2015). DPPH radical-scavenging activity has been widely used in propolis studies (Kumazawa et al., 2004).

Results in Figure 3 showed that *propolis from Berqayel and Fakeha* presented the highest free radical scavenging activity compared with vitamin C. The obtained results for DPPH are in agreement with the phenolics and flavonoids contents determined for each sample. Moreover, the found IC_{50} values are in good agreement with many previous works (Pratami et al., 2017; da Silva et al., 2019; Touzani et al., 2018). Nevertheless, the antioxidant activities of different Lebanese propolis are lower than those of Egyptian propolis (Ezzat et al., 2019).

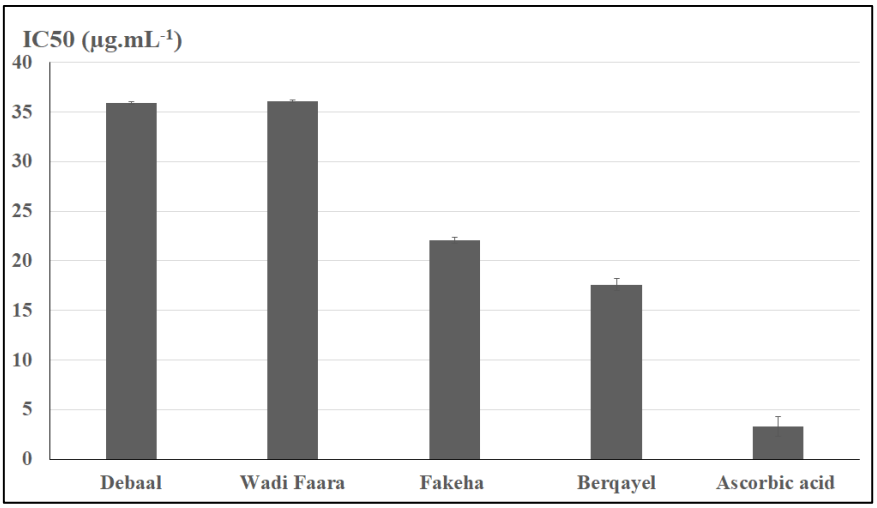

Figure 3. Comparison of 50 % radical inhibition by the different propolis extracts
(Values are the mean ± SD of three experiments)

3.3.2. Reducing Ability

The transformation ability of compounds from Fe^{3+}/ ferricyanide complex to Fe^{2+} /ferrous form acts as a potential indicator for the antioxidant activity of the extract (Do et al., 2014). In the FRAP assay, the yellow color test solution changes to green and blue depending on the reduction capacity of extracts. The complexing of metal ions by phenols typically induces a bathochromic displacement of their absorption bands in the UV-Visible range (Ghedadba et al., 2015). In Figure 4, and similarly to the radical scavenging activity, all the EEP showed concentration-dependent reducing power. The greatest reducing antioxidant power was recorded for propolis collected in Berqayel, while the lowest was found in the Wadi Faara extract. This is interpreted by the richness of Berqayel extract in phenolic compounds proved by the TPC and TFC results. Thus, it can be deduced from this test that polyphenols, especially flavonoids, play a very important role in the chelation of transition metals.

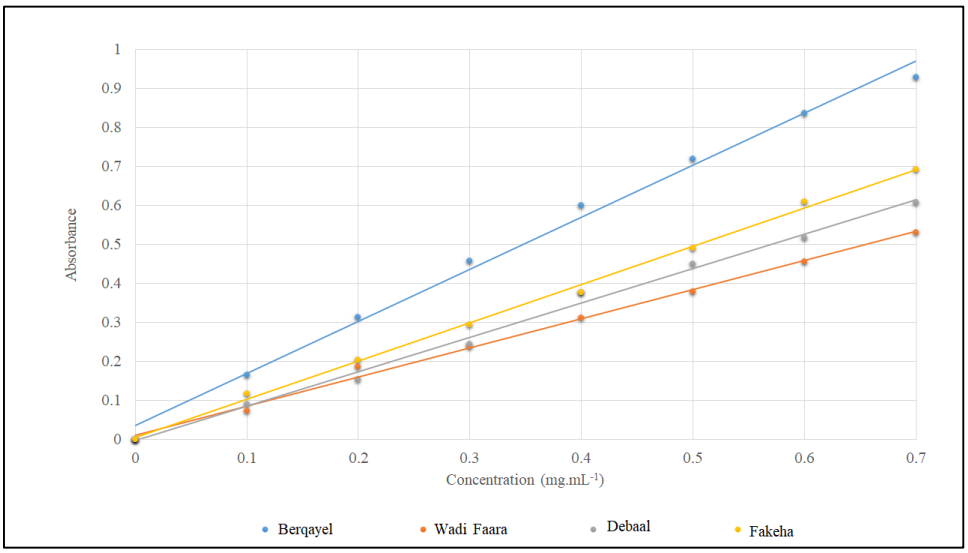

Figure 4. Reducing power assay of all extracts of expressed as absorbance at 700 nm (n = 3).

3.3.3. Correlation Between Antioxidant Activities, Phenolics and Flavonoids Contents of EEP

Several studies (Karou et al., 2005; Verzelloni et al., 2007) have shown the presence of good correlation between the total phenolic content and the antioxidant activity of the extracts, suggesting that phenolic compounds, particularly flavonoids are responsible for the antioxidant activity of the extracts. The results (Table 2) show a strong positive correlation between phenolics, flavonoids and antioxidant activity, this suggests that polyphenols, especially flavonoids may be responsible of the antioxidant activity.

Table 2. Correlation coefficient (R2) among TPC, TFC and antioxidant activity.

	DPPH method	FRAP method
TPC	0.9843	0.7744
TFC	0.8521	0.9675

Moreover, these correlations are consolidated by many previous works (Galeotti et al., 2018; Kumazawa et al., 2004; Wang et al., 2016) which showed that the high polyphenol and flavonoid contents are responsible for the highest antioxidant activity of propolis.

3.4. UV Spectrograms

The UV-vis spectra of EEP obtained from the four regions are illustrated in Figure 5. Two absorption bands were observed, the first between 190–250 nm, while the second is a broad band centered around 280–300 nm with a shoulder around 330 nm. They are similar to typical polyphenol spectra (Paganotti et al., 2014), indicating that the used extraction solution (EtOH: water, 80: 20, v/v) was able to recover the phenolic compounds.

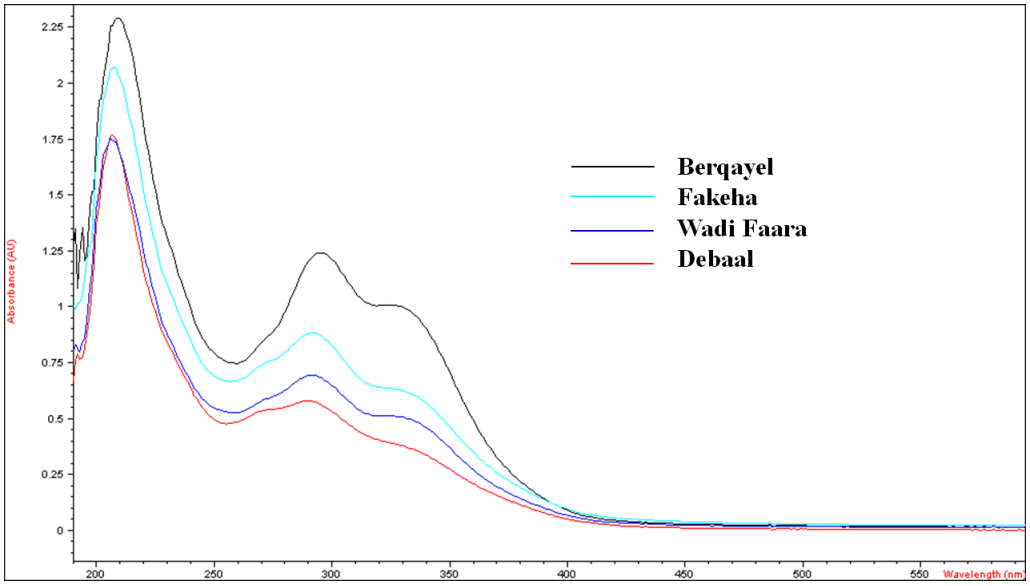

Figure 5. UV absorption spectra of Lebanese propolis extracts.

Furthermore, the spectral profiles were identical, suggesting a homogeneous chemical composition among the samples, despite their collecting regions. The Argentine Food Code (CAA) establishes in its physical and chemical requirements that the UV–Vis spectrogram of propolis should have a maximum absorption between 270 nm and 315 nm, regardless of the profile obtained (Isla et al., 2005; Maldonado et al., 2020). In this study, the CAA requirement was met in all propolis samples from different locations. By comparison with reported studies, UV-Vis spectra profile was similar to the Argentinian (Maldonado et al., 2020), Romanian (Moţ

et al., 2011), and Brazilian propolis with a slightly bathochromic shifting (Tomazzoli et al., 2015).

3.5. TLC and GC-MS Analysis

The current study displays the presence of different compounds as TLC experiment separates numbers of spots. TLC analysis of the EEP samples from the four regions showed common chromatographic plates with slight differences. Some compounds such as pinocembrin, pinostrobin and phenylethyl cafeate can be identified by comparing their retention factors with those of previous works (Boisard, 2014). The obtained results are in agreement with those obtained by UV-Vis analysis, i.e. the main compounds are present in all samples and differences are mainly quantitative not qualitative.

The EEP were analyzed by GC–MS (Figure 6) to detect volatile, small and non-thermolabile metabolites. The analysis was done for the two samples the one who give the high antioxidant effect (Berqayel) and the low effect (Wadi Faara). The EEP from Berqayel was found to have small metabolites, mostly hydrocarbons. Compounds that were identified to be present in large amounts include; 1-tetradecene (23.84 %), 9-octadecene (32.57 %) and isodecene (14.9 %) (Table 3). GC-MS analysis of Wadi Faara extract revealed a poor chromatogram in term of peaks and the absence of most of the compounds founded in Berqayel sample.

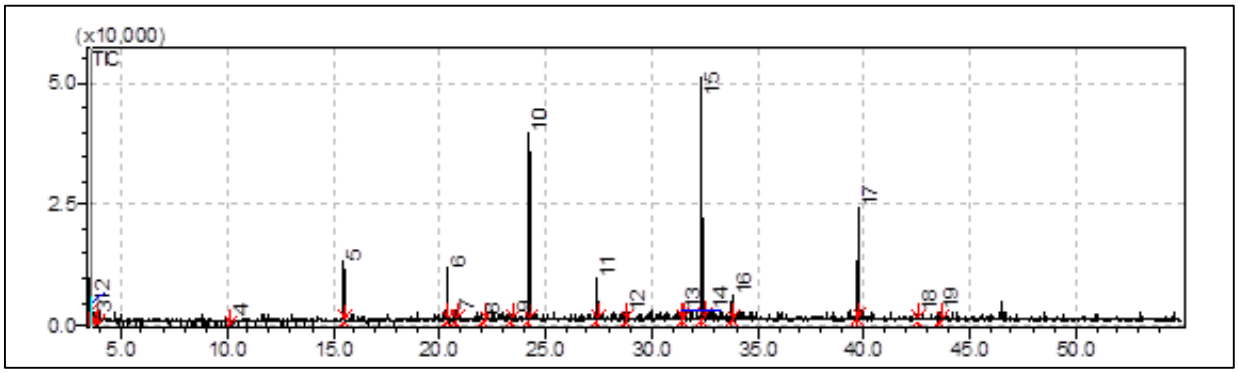

Figure 6. GC-MS profile of propolis ethanolic extract from Berqayel.

Table 3. GC/MS analysis of main constituents of EEP.

Compound	R.Time	M.W.	Molecular formula	Area(%)
anhydride-propanoic acid	10.067	130	$(CH_3CH_2CO)_2O$	1.31
2-Ethylhexanol	15.508	130	$C_8H_{18}O$	5.87
Dodecamethylcylohexasiloxane	20.383	444	$C_{12}H_{36}O_6Si_6$	6.58
5-hexen-3-one	20.808	98	$C_6H_{10}O$	0.47
tert-pentane	23.417	72	C_5H_{12}	0.38
1-tetradecene	24.217	196	$C_{14}H_{28}$	23.84
Unknown	27.433	--	--	5.24
Isocyanomethane	28.817	40	C_2H_3N	0.56

propane,1-3,diamine	31.425	74	$C_3H_{10}N_2$	0.36
9-octadecene	32.358	252	$C_{18}H_{36}$	32.57
Unknown	33.833	--	--	2.69
Isodecene	39.758	140	$C_{10}H_{20}$	14.92

According to literature, the compound, octadecene has been reported to have anticancer, anti-inflammatory and antioxidant activities (Gautam et al., 2018). Moreover, tetradecene is another compound reported to possess antioxidant abilities (Manoj et al., 2012; Tiloke et al., 2018). Thus, the presence of these compounds can explain the antioxidant capacity of EEP from Berqayel propolis observed in this study.

4. CONCLUSION

In this work, we focused on the contents and the levels of TPC, TFC and antioxidant for EEP obtained by maceration of propolis from different geographical origins in Lebanon. Phytochemical screening shows the existence of different secondary metabolites: flavonoids, polyphenols, alkaloids, tannins, quinons and terpenes, while saponins are absent. The quantitative determination of polyphenol and flavonoids clearly shows the richness of propolis by these compounds. Moreover, it has shown that the propolis from Berqayel had the greatest TPC of 148.27 mg GAE/g and TFC value of 134.5 mg RU/g propolis. On the other hand, the study of the antioxidant activity by evaluating their anti-free radical and reducing power revealed the good potential of Berqayel and Fakeha extracts to scavenge the DPPH radical and to reduce iron from the Fe^{3+} to the Fe^{2+} form. In addition, the antioxidant activity was also correlated with the total polyphenol and flavonoid content. Results of UV-VIS and TLC showed, similar profiles in comparison to previous works with no small differences among them. Finally, GC-MS was employed to identify the volatile compound in Wadi Faara and Berqayel samples. The chromatogram from Berqayel contain mostly hydrocarbons e.g. 1-tetradecene (23.84 %), 9-octadecene (32.57 %) and isodecene (14.9 %). While, the chromatogram obtained from Wadi Faara extract revealed the absence of most of these compounds.

Therefore, our results demonstrated that propolis samples from different Lebanese locations have different biological activity. However, further studies are needed to support our results, to evaluate another biological potential of these propolis extracts and to identify the extract biomolecules.

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The Importance of Propolis in Combating COVID-19

COVID-19 ile Mücadelede Propolisin Önemi

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Abstract

The Coronavirus Disease 2019 (COVID-19) pandemic has been going on since November 2019 in the World with different variants of SARS-CoV-2. Effective vaccine and drug investigations for COVID-19 are still ongoing. For decreasing the mortality rate of COVID-19 keeping social distance, using a mask, washing hands, and improving immune systems are important. Propolis is a natural bee product that contains various bioactive substrates such as polyphenolic acids, flavonoids, vitamins, minerals. Propolis via antiviral, anti-inflammatory, antioxidant, and antithrombotic activities could be used as prophylactic or adjuvant COVID-19 treatment.

Keywords: COVID-19, SARS-CoV-2, Propolis

Özet

Koronavirüs Hastalığı 2019 (COVID-19) salgını Dünya'da Kasım 2019'dan beri SARS-CoV-2'nin farklı varyantlarıyla devam etmektedir. COVID-19 için etkili aşı ve ilaç araştırmaları devam etmektedir. COVID-19'un ölüm oranını azaltmak için sosyal mesafeyi korumak, maske kullanmak, elleri yıkamak ve bağışıklık sistemlerini geliştirmek önemlidir. Propolis, polifenolik asitler, flavonoidler, vitaminler, mineraller gibi çeşitli biyoaktif substratları yapısında içeren doğal bir arı ürünüdür. Antiviral, antiinflamatuar, antioksidan ve antitrombotik aktiviteleri aracılığıyla propolis, profilaktik veya COVID-19 tedavisine yardımcı olarak kullanılabilir.

Anahtar kelimeler: COVID-19, SARS-CoV-2, Propolis

Abbreviations: COVID-19, Coronavirus disease 2019, WHO, World Health Organization, SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus-2, CoV, Coronaviruses, ACE-2, Angiotensin-Converting Enzyme 2, TMPRSS2, Transmembrane Serine Protease 2, PAK 1, RAC/CDC42-activated kinase 1, IL, Interleukin**,** TNF-α, Tumor necrosis factor-alpha, IFNγ, Interferon-gamma, G-CSF, Granulocyte colony-stimulating factor RdRp, RNA-dependent RNA polymerase, PL-pro, Papain like protease, M-pro, Coronavirus main proteinase, CAPE, Caffeic acid phenethyl ester, HIV, Human Immunodeficiency Virus, NF-κB, Nuclear factor kappa-light-chain-enhancer, 3CL-pro, 3C-like proteinase, COX, Cyclooxygenase, iNOS, inducible nitric oxide synthase, NO, Nitric oxide, PAI-1, Plasminogen activator inhibitor-1, BGP, Brazillian Green Propolis, EPP-AF®, The Standardized Propolis Extract

1. INTRODUCTION

As of April 26th, 2021, the world is still laboring to overcome coronavirus disease 2019 (COVID-19) in 223 countries, over 146 841 882 cases, and 3 104 743 deaths have been reported by World Health Organization (WHO) (WHO, 2021a). COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) which was firstly identified in Wuhan, China, in December 2019. COVID-19 may have various symptoms, such as fever, headache, exhaustion, sputum, hypogeusia, painful throat, dyspnea, cough, diarrhea, anorexia, dizziness, rhinorrhea, nasal congestion, hyposmia, myalgia (Kim et al., 2020; Singhal, 2020; Vipul et al., 2020). Most infected people will develop mild to moderate illness and recover without hospitalization. While most common symptoms are fever, dry cough, and tiredness, serious symptoms are difficulty breathing or shortness of breath, chest pain or pressure, loss of speech or movement. Otherwise, some patients haven't any symptoms too during the illness. When someone is contaminated or infected with the SARS-CoV-2 it can take 5-6 days or take up to 14 days for getting symptoms (WHO, 2021b). Polymerase Chain Reaction (PCR), Real Time-Polymerase Chain Reaction (RT-PCR), Complete Blood Count and other laboratory tests, X-ray, and CT scans are used to diagnose COVID-19. CT imaging is more sensitive and specific for diagnosing COVID-19. WHO is suggested using all symptoms, RT-PCR and other laboratory tests, and CT imaging for diagnosing infection and emphasis that only PCR test is not enough for diagnosis COVID-19 (Feng et al., 2020; Singhal, 2020; Zitek, 2020). Infection is transmitted human to human among asymptomatic and symptomatic patients by coughing, sneezing, or touching contaminated surfaces (Singhal, 2020). People who are 65 or >65 years old or who have a chronic disease such as diabetes mellitus, chronic liver disease, chronic lung disease, chronic renal failure, chronic cardiovascular disease, hematological malignancy, and receiving chemotherapy or immunosuppressive agents are in the high-risk groups for COVID-19 (Kim et al., 2020). Rapid detection, treatment, and prevention of COVID-19 are very important for saving lives in the world urgently. Social distancing, lockdown of cities, hygienic products have been used to control the COVID-19 Pandemic (Lima et al., 2020). Scientists are still developing more effective vaccines and drugs for COVID-19 (Al Naggar et al., 2021). Medical plants, some compounds that are isolated from plants, and bee products such as propolis, honey which have antiviral activity are used by people for preventing COVID-19 and supporting immune systems and treatment (Berretta et al., 2020; Ripari et al., 2021). This review summarizes the information on COVID-19 disease, the anti-viral activity of propolis, and its effects on SARS-CoV-2.

2. SARS-COV-2

Coronaviruses (CoV) are a group of positive-sense single-stranded RNA viruses. SARS-CoV-2 uses spike glycoproteins for attaching Angiotensin-Converting Enzyme 2 (ACE 2) and Transmembrane Serine Protease 2 (TMPRSS2) of the host cell than resulting membrane fusing (Dalan et al., 2021; Elmahallawy et al., 2021; Harisna et al., 2021). Figure 1 shows the structure of SARS-CoV-2 (Elmahallawy et al., 2021). SARS-CoV-2 includes 4 structural proteins (Spike, Envelope, Membrane, and Nucleocapsid proteins), Cysteine proteinase, RNA polymerase, and nonstructural proteins (Elmahallawy et al., 2021; Sahlan et al., 2021).

ACE-2 is a receptor not only for Angiotensin II but also SARS-CoV-2 too. ACE-2 is expressed in the ciliated airway epithelium of the lungs, enterocytes of the small intestine, arterial and venous endothelial cells, arterial smooth muscle cells in the heart, kidneys, adrenal glands, pancreas, skeletal muscle, and adipose tissues (Dalan et al., 2020). Because of the wide expression of ACE-2, using inhibitors of TMPRSS2 is more useful for preventing enter of SARS-CoV-2 into the host cell (Hoffmann et al., 2020).

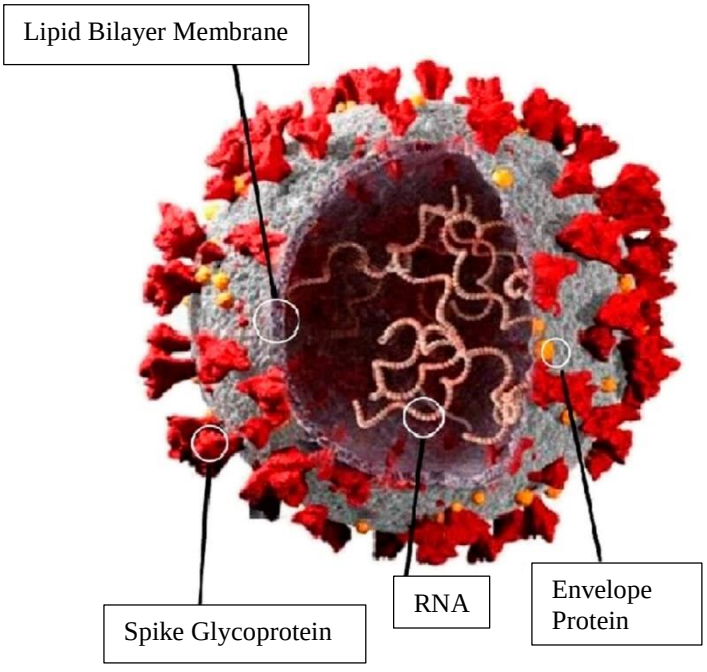

Figure 1. Structure of SARS-CoV-2.

Due to the imbalance of ACE-2 pathways patients with hypertension, type II diabetes, or cardiovascular disease belong to high-risk groups for respiratory failure and morality in COVID-19. As there is not enough evidence that shows the harmful or beneficial effects of using ACE-inhibitors or Angiotensin 2 Tip 1 receptor blockers for preventing COVID-19 in

high-risk group patients, all patients should continue to take their medicine as before (Dalan et al., 2020). Figure 2 shows host cell and SARS-CoV-2 (Coronavirus (SARS-CoV-2) Viral Proteins, Sigma-Aldrich, 2021). Besides ACE-2 and TMPRSS2, RAC/CDC42-activated kinase 1 (PAK 1) is also a target for the scientist to prevent COVID-19. After SARS-CoV-2 enters the host cell PAK 1 upregulation has occurred which causes lung inflammation, lung fibrosis, and other mortality factors (Dalan et al. 2020). Activation of PAK 1 that also known as pathogenic kinase is related to various diseases/disorders such as cancers, malaria, inflammation, and viral infection including Human Immunodeficiency Virus (HIV), influenza, and COVID-19 (Maruta & He, 2020). Increased PAK 1 in a host cell induces replication of SARS-CoV-2 and inhibits immune response too (Dalan et al., 2020). The other problem in COVID-19 patients is cytokine storm that occurred after increased production of proinflammatory cytokines such as Interleukins (IL) (IL2, IL-6, IL7, IL-10), Tumor necrosis factor-alpha (TNFα), Interferon-gamma (IFNγ), Granulocyte colony-stimulating factor (G-CSF). Cytokine storms may cause multi-organ system failure that is a life-threatening disorder (Elmahallawy et al., 2021). The other mechanism for fighting against SARS-CoV-2 is suppressing its replication by different agents. RNA-dependent RNA polymerase (RdRp) that catalyzes the synthesis of coronavirus RNA, is so important for coronaviral replication/transcription machinery complex. Also, Papain like protease (PL-pro) and coronavirus main proteinase (M-pro) that has a role to replicate and generate new RNA are therapeutic targets for developing pharmacy too (Huang et al., 2020).

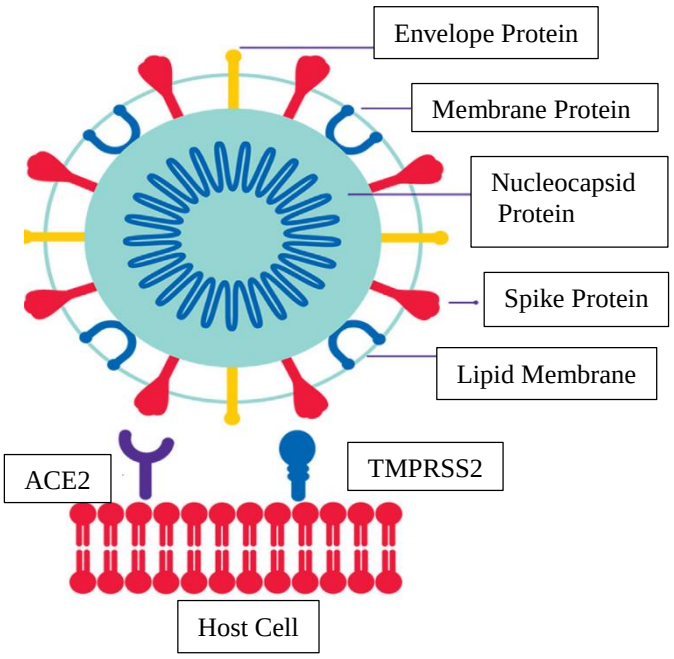

Figure 2. Host cell and SARS-CoV-2

Several drugs were investigated to treatment COVID-19 such as nevilnafir, as a RdRp inhibitor remdesivir, ribavirin, favipiravir, as a protease and proteinase inhibitor lopinavir and darunavir, as a PAK 1 blocker melatonin, ciclesonide, ivermection, ketorolac, chloroquine, and hydrochloroquine and propolis (Harisna et al., 2021; Huang et al., 2020; Maruta & He, 2020). Also, several reports suggest that chloroquine and hydrochloquine caused serious arrhythmia, kidney injuries, liver problems, blood and lymph system disorders, and failure in patients with COVID-19 (United States Food and Drug Administration, 2020).

Some SARS-CoV-2 mutations repressive vaccine developments, affect the affinity to ACE-2 receptor and infectiousness. and immune response (Conti et al., 2021; Huang et al., 2020; Volz et al., 2021; Zhang et al., 2020). Different mutants form of SARS-CoV-2 found in the United Kingdom, Brazil, and Sound Africa and recently double mutant SARS-CoV-2 found in India. Scientists need time to be sure this variant is more deadly or more transmissible (https://www.bbc.com/news/world-asia-india-56507988). On the other hand, various supplements, herbal and apitherapy products such as ginseng extracts, garlic extracts, echinacea, curcumin extracts, propolis, honey, royal jelly, bee wax, bee venom, bee pollen, quercetin, Vitamin C, Vitamin D, Vitamin E, zinc, selenium used to support treatment of COVID-19 by antiviral, anti-inflammatory, antioxidant and immunomodulatory activities (Ali & Kunugi, 2021; Al Naggar et al., 2021; Jin et al., 2020; Keflie et al., 2021).

3. PROPOLIS

Propolis is a bee product that occurred by molding resinous balsam of plants and trees with bee wax and saliva. Propolis is also known as bee glue originates from Greek and occurred Pro- (in meaning for or defense) and -polis (in meaning city) word parts that are a defense of city or hive. Bees use propolis as a detoxification agent and fixing material for their hives to maintain homeostasis, to promote a beneficial microbiome, and protect from insects and animals (Burdock, 1998; Zulhendri et al., 2021). Propolis is also used by Egyptians, Greeks, Romans, and Incas for wound healing, corpse embalming, and antipyretic. It was used in Europe in the 17^{th} and 20^{th} centuries as an antibacterial agent and during the Second World War due to the antimicrobial and anti-inflammatory activity (Santos et al., 2019). Propolis compositions and colors can change depending on the geographic area, climate. Generally, propolis colors are dark brown, dark green, and dark yellow. Some propolis samples were shown in Figure 3 (Çolak, 2009).

Propolis consists of 50% resins, 30% bee wax, 10% aromatic and essential oils, 5% bee pollen, 5% multiple organic compounds, vitamins, and minerals (Ali & Kunugi, 2021). It includes more than 300 different compounds such as flavonoids, phenolic acids, phenolic acid esters, terpenoids, xanthones, fatty acids, volatile fatty acids, ketones, lactones, steroids, pollens, various minerals, vitamins. Some phenolic compounds and flavonoids found in propolis are shown in Table 1 (Çolak, 2009; Duca et al., 2019; Santos et al., 2019;).

Figure 3. Propolis samples

Propolis can solve with ethanol, methanol, diethyl ether, acetone, toluene, trichloroethylene, oils, water, and others with different ratios and compositions (Burdock, 1998; Ripari et al., 2021). Commercial many kinds of extracts with different concentrations may find in markets and pharmacies in capsule, liquid, pastilles or supplement form alone or with the other herbals, vitamins or minerals. As for its antibacterial and antimicrobial activities, it is used for the production of toothpaste and mouthwash solutions. (Burdock, 1998; Santoset al., 2019; Zulhendri et al., 2021). Propolis is also used by the food industry and cosmetic industry especially its antibacterial, antioxidant, and antiaging properties, and recently it is famous and important in veterinary medicine too (Santos et al. 2019).

Table 1. Some flavonoids and phenolic compounds found in propolis

Flavonoids	**Phenolic Compounds**
Apigenin	1,1-dimethylallylcaffeate
Chrysin	2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran
Formononetin	3-(4-hydroxy-3-(oxo-butenyl)-phenylacrylic acid
Hesperetin	3,5-diphenyl-4-hydroxycinnamic acid derivate
Kaempferol	*p*-Coumaric acid
Medicarpin	Artepillin C
Naringenin	Caffeic acid
Neovestiol	Caffeic acid phenethyl ester (CAPE)
Pinocembrin	Resveratrol
Quercetin	Epicatechin
Vestitol	Feruric acid
Galangin	Isoliquiritigenin
Luteolin	Mono/Dicaffeoylquinic acids

Propolis has various biological activities for humans such as antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral, antimutagenic, antitumoral, anticancer, cytotoxic, anti-proliferative, anti-angiogenic, immunomodulatory (Braakhuis, 2019; Burdock, 1998; Zulhendri et al., 2021). Figure 4 summarise the use of propolis with its properties for bees and humans (Zulhendri et al., 2021).

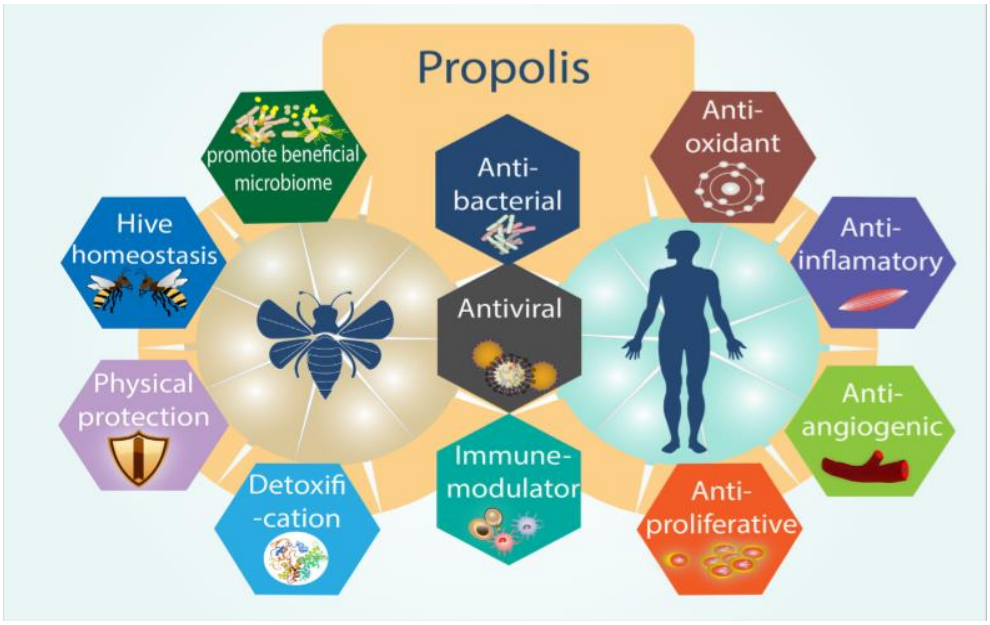

Figure 4. The use of propolis by bees and humans

4. USE OF PROPOLIS IN WAR WITH CORONAVIRUS

As mentioned before propolis and propolis-derived compounds such as CAPE, benzoic acid, resveratrol, p-cumaric acid, quercetin, chrysin, pinocembrin, and galangin have antiviral, anti-inflammatory, antioxidant, and antithrombotic activity. In previous studies, it was shown that propolis and its extracts have antiviral activity against both DNA and RNA virus such as Herpes Simplex Virus Type 1 and Type 2, Adenovirus Type 2, Vesicular Stomatitis Virus, Poliovirus Type 2, Varicella zoster virus, HIV, Influenza. (Burdock, 1998; Governa et al., 2019; Haris et al., 1997; Labska et al., 2018; Yildirim et al., 2016). It was reported that various propolis fractions affected the replication of Vaccinia Virus, Newcastle Disease Virus, and Influenza Viruses A and B (Burdock, 1998). Debiagi et al. (1990) reported that kaempferol and chrysine were reduced the replication of several Herpes Viruses, Adenoviruses, and a Rotavirus concentration-dependently and quercetin was reduced infectivity and intracellular replications of viruses in high concentrations. Also, Erdemli et al. (2015) suggested that CAPE inhibits the HIV-1 infection, nuclear factor kappa-light-chain-enhancer (NF-κB) production, and Hepatitis C virus replication too. Singh et al. (2020) showed that hesperidin has a higher binding activity to RdRp of SARS-CoV-2 than remdesivir, and many polyphenols such as myricetin, epigallocatechin gallate, theaflavin, theaflavin-3'-O-gallate, theaflavin-3'-gallate, theaflavin 3,3'-digallate, quercetagetin, and myricetin strongly bind to the active site of RdRp and other polyphenols such as quercetin, curcumin, kaempferol, epicatechin can bind to RdRp with lower binding energy than remdesivir. It was concluded that some natural polyphenols can be used as an inhibitor of RdRp of SARS-CoV-2.

On the other hand, Maruta & He (2020) suggested that caffeic acids, CAPE, Artepillin C, nymphaeols inhibit PAK1 activity, act like PAK1 inhibitors, and could be useful for inhibiting or preventing COVID-19-induced lung fibrosis and stimulating the immune system. Also, 3C-like proteinase (3CLpro) is an important enzyme that has a role replication of the virus. It was shown that 2',4'-dihydroxychalcone and 2',4'-dihyroxy-3'-methoxychalcone that also found in propolis have potential repressing properties against 3CLpro. As amyrin (Triterpenes), procyanidin and proanthocyanidin influence the activity of 2'-o-ribose methyltransferase, propolis compounds have potential restrictive properties against methyltransferase too (Elmahallawy et al., 2021). Ali & Kunugi (2021) reviewed that rutin, nicotiflorin, luteolin, CAPE, and Artepillin C inhibit viral replication and inflammatory reactions by affecting 3CLpro/Mpro, PLpro, RdRp, and B56 regulatory unit of phosphatase 2A. Sahlan et al. (2021) and Kumarb et al. (2020) showed that propolis components glyasperin

A, broussoflavonol F, and CAPE have binding affinity to SARS-CoV-2 main protease and have therapeutic value for COVID-19.

Also, Khayrani et al. (2021) detected that propolis components glyasperin A, broussoflavonol F, sulabiroins A, isorhamnetin, and (25)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone have the potential to inhibit the binding of ACE-2 and SARS-CoV-2. As phenolic compounds of propolis such as galangin, p-coumaric acid, quercetin, chrysin, and kaempferol could block or reduce the adsorption and entrance of the virus into the host cells, propolis consumption might be useful for protecting COVID-19 and supporting adaptive immune response (Lima et al., 2020). In previous in vitro and in vivo studies, it was shown that flavonoids could inhibit the activity of ACE. Recently Guler et al. (2021) used ten flavonoids (Caffeic acid, CAPE, chrysin, galangin, myricetin, rutin, hesperetin, pinocembrin, luteolin, and quercetin) for detecting their binding ability to ACE-2 receptors and it was shown that rutin has the best inhibition potentials for ACE-2 receptors and then followed by myricetin, CAPE, hesperetin, and pinocembrin. It was concluded that flavonoids in ethanolic propolis extracts have a high potential for COVID-19 treatment by inhibition of ACE-2 receptors and preventing entry of virus to host cells (Guler et al., 2021). Also, Refaat et al. (2021) and Vijayakumar et al. (2020) established that rutin, luteolin, and CAPE inhibit ACE 2 receptors too. Kumara et al. (2020), showed that CAPE inhibits the TMPRSS2 and block the entry of SARS-CoV-2 into the cell. Refaat et al. (2020) and Jain et al. (2021) detected that naringin, rutin, and quercetin have the binding activity to S protein and inhibit viral fusion in the host cell membrane. Harisna et al. (2021) suggested that propolis components genistin, methylophopogonone A and 3'-methoxydaidzin inhibit main protease and spike protein and these compounds could be used as antiviral agents.

The other mechanism of antiviral activity of propolis may be related to its zinc content. Propolis has variable amounts of zinc such as 21 mg/kg or 9326 mg/kg (Cvek et al., 2008; Tosic et al., 2017). Zinc ions inhibit viral enzymes that are important for the replication of the virus in the host cells (te Velthuis et al., 2010). Kaushik et al. (2017) reported that zinc salts block hepatitis E virus replication by inhibiting of RdRp. Also, zinc has the potential to threaten COVID-19 by antioxidant, anti-inflammatory, and immunomodulatory properties. Zinc can suppress the expressing of various chemokines, acute phase proteins such as fibrinogen and C-reactive protein, proinflammatory cytokines, and some factors that have a role in inflammatory responses such as inhibition of NFκB and modulation of T cell functions that cause cytokine storms in COVID-19 (Keflie et al., 2021).

Another trace element in propolis is selenium that has a role in maintaining adaptive immune systems, inhibiting proinflammatory cytokines, chemokines, and production of free oxygen radicals. Experimentally it was shown that a selenium-deficient diet in mice is related to developing lung injury in post-influenza virus infections (Keflie et al., 2021; Suhupharani et al., 2019). Suhupharani et al. (2019) biosynthesized selenium nanoparticles from ethanolic extracts of propolis for human health due to the antimicrobial and antioxidant activity of selenium.

Also, it was established that Vitamin A, Vitamin B12, Vitamin C, Vitamin D, Folate, Pyridoxine, Nicotinamide, and Retinoic acid have a protective role against COVID-19 by antioxidant, antiviral, anti-inflammatory activities and affecting the immune response (Keflie et al., 2021). It is also known that propolis has many kinds of vitamins and micronutritions and it could support immune systems too (Burdock, 1998; Marcucci, 1995).

Additionally, the anti-inflammatory activity of propolis is related to its components such as phenolic acids and their esters, flavonoids, steroids, terpenoids, and amino acids. The basic mechanisms of anti-inflammatory activity of propolis are the inhibition of cyclooxygenase (COX) and prostaglandin biosynthesis, antioxidant activity, inhibition of nitric oxide (NO) synthesis, reducing the level of cytokines, and immunosuppressive activity (Braakhuis, 2019). In many studies it was reported that CAPE, quercetin, naringenin, pinocembrin, Artepillin C, terpenoids showed anti-inflammatory activity by inhibiting COX-2, suppressing the production of prostaglandins and leukotrienes, reducing the expression of inflammatory mediators such as IL-10, IL-1β, inducible nitric oxide synthase (iNOS) and inhibiting the production of TNF-α, IL-1β, IL-6, NF-κB, NO (Braakhuis, 2019; Santos et al., 2019; Zulhendri et al., 2021).

Tromboembolism, thrombosis, and microthrombosis are common in COVID-19 patients and associated with high mortality rates of COVID-19. Generally, anticoagulants use to reduce mortality. In a previous study antithrombotic effect of propolis was also established by decreasing platelet aggregation, other thrombosis-related parameters and suppressing lipopolysaccharide-induced increases in plasminogen activator inhibitor-1 (PAI-1) in mice (Berreta et al., 2020). Quercetin might use for thrombotic disease treatment as a thrombin inhibitor. Quercetin may utilize blood clotting dysregulation conduced by viral infection (Berreta et al., 2020; Shi et al., 2012). In an in vivo study, it was detected that CAPE inhibits collagen-induced platelet aggregation via downregulating Tromboksane B2, COX-1, and 5-hydroxytryptamine and increasing NO and cyclic guanosine monophosphate activity. In addition, it was shown that CAPE, galangin, pinostropin inhibit platelet aggregation and

propolis components including CAPE have the potential to threaten thrombotic disease (Ohkura et al., 2020).

Duarte Silveria et al. (2021) used nan-alcoholic preparation of Brazillian Green Propolis (BGP) The Standardized Propolis Extract (EPP-AF®) at two concentrations (400 mg and 800 mg) for 82 hospitalized adult COVID-19 patients and evaluated the patient's length of hospital stay, dependence on oxygen therapy, development of acute kidney injury, intensive care unit admission and use of vasoactive drugs. 400 mg BGP EPP-AF® has 21.2 total flavonoids such as quercetin and 54 mg of total phenolics, such as gallic acid. It was shown that BPG treatment decreased the length of hospital stay (6 days for 400 mg BGP EPP-AF®, 7 days for 800 mg BGP EPP-AF®, and 12 days for the control group (n=42)) and renal injury significantly, but didn't have any effect on the need for oxygen therapy and didn't observe any side effect of propolis. In a case report BGP (EPP-AF®) confirmed a COVID-19 patient who was 52 years old in a dose of 45 drops/3 times/day for 2 weeks. Patients viral clearance occurred within 12 days of treatment (Fiorini et al., 2021) Kosari et al. (2021) used a syrup that contains 1.6 mg *Hyoscyamus niger* L. extract and 450 mg propolis per 10 mL, in 25 COVID-19 patients aged between 17-85 and 25 patients also classified as placebo group in the investigation. In this study, 10 mL syrup was administered during the 6 days three times a day. It was shown that syrup reduced the clinical symptoms of COVID-19 such as dry cough, shortness of breath, sore throat, chest pain, headache, dizziness, fever, abdominal pain, and diarrhea but didn't have any effect on nausea and vomiting. The dose of propolis at 500 mg/day is approximately equal to 30 drops of propolis extract (11% w/v of dry matter). Berretta et al. (2020) claim that 30 drops/day or one capsule might be used for preventing purposes of propolis but Soroy et al. (2014) suggested that 1200 mg/day water extract of propolis capsule (Propoelix™) could decrease the level of TNF-α and length of hospitalized day and increase platelet count in 31 patients with dengue hemorrhagic fever that is caused by the mosquito-borne dengue viruses.

5. CONCLUSIONS

While production and developing SARS-CoV-2 vaccines and drugs are continued individually people should protect themselves by having potent immunity as well as using masc, antiseptics, keeping social distance, and washing hands against COVID-19. Natural products such as propolis could be useful for improving immune response by immunomodulatory activity, protecting binding, entry, and colonization of SARS-CoV-2 in the host cells of people by antiviral activity, preventing cytokine storms and thrombosis, various tissue injuries such as

lung, kidney by the antioxidant, anti-inflammatory and antithrombotic activity in COVID-19 patients. Also, it is important to increase the number of randomized and controlled clinical trials to assess the benefits and therapeutic potential of propolis in COVID-19. But people who haven't any allergies to propolis might use propolis for protecting themselves against SARS-CoV-2 and COVID-19.

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Spread in the flora of the Nakhchivan Autonomous Republic of Azerbaijan Satureja L. (wild grandmother) Biomorphological Characteristics, Results of Phytochemical Analysis and Perspectives of Use

Azerbaycan Nahçıvan Özerk Cumhuriyeti Florasında Yayılış Gösteren Satureja L.'nin Biyomorfolojik Özellikleri, Fitokimyasal Analiz Sonuçları ve Kullanım Perspektifleri

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Abstract

The article deals with the genus *Satureja* L. *of Lamiaceae* Lindl. Biomorphology of the species of garden mint, ecogeographic characteristics, results of phytochemical analysis, useful properties, with detailed comments on the possibilities of its use in scientific medicine, in the World and in Azerbaijan and detailed comments have been given on its distribution in the territories of the Nakhchivan Autonomous Republic. A thin layer of ethanol extract of the leaves of the species was chromatographed and Rf-ed. The ingredients were determined based on the result of spectral and chromatographic analysis, the compounds in the extracts have identified. In thin-layer chromatography, the purely separated part was dissolved in ethanol and the substances spectra at a wavelength of 200-700 nm via spectrophotometer. Flavonoids (28.8%) of the spectra obtained at 200.0-294.5

Özet

Bu makale *Lamiaceae* Lindl. familyasına ait Satureja L. cinsi ile ilgilidir. Nahçıvan Özerk Cumhuriyeti florasında yayılan bağ çöl nanesi türünün biyomorfolojini, ekocoğrafik karakteristik özelliklerini, fitokimyasal analiz sonuçlarını, faydalı özelliklerini ve bilimsel kullanım olanakları hakkında detaylı yorumlarını, ve ayrıca dünyada, Azerbaycanda ve Nahçıvan Özerk Cumhuriyeti topraklarında dagılımının ayrıntılı açıklamasını içermektedir. *Satureja hortesis* L. yapraklarının etanol özünün ince tabaka kromatografisi gerçekleştirilmiş ve Rf. değerleri belirlenmiştir. Spektral ve kromatografik testler sonucunda ekstraktlardaki bileşikler belirlendi. İnce tabaka kromotografisinde, saflaştırılan kısım etanolde çözülmüş ve bileşenlerin spektrumları spektrofotometre ile 200-700 nm dalga boyunda kaydedilmiştir. 200.0-294.5 (MeOH λmak. nm) dalga boyunda

wavelengths (MeOH λmax. nm), 340.5-378.5 nm wavelength (26.8%) flavonols, flavanones, chalcone 556.5-630.5 nm wavelength (44.3%) was found to be characteristic of anthocyanins.

spektrumların flavonoidleri (28,8%) , 340.5-378.5 nm dalga boyunda flavoioller, flavononlar, kalkonlar ve 556.5-630.5 nm dalga boyunda (%44,3) antosiyaninler için karakteristik olduğu gösterilmiştir.

Keywords: Chromatography, flavonols, spectrum, halcyons, flavonones.

Anahtar kelimeler: Kromatografi, flavonoller, spektrum, halyonlar, flavononlar

Abbreviations: UV-VIS, ultraviolet–visible spectrophotometry

1. INTRODUCTION

Research of natural resources in the development of the economy of the Republic of Azerbaijan, its use, restoration and protection are important issues of State importance and Natural resources. One of the important conditions is to protect, restore and direct properly. Azerbaijan National Strategy for the Conservation and Sustainable Use of Biodiversity by the Republic and the action plan has been approved. The chapter is modernized to address issues in the direction to study the situation, to determine the ecological and anthropogenic transformations that are taking place, and its importance both theoretically and practically (Alakbarov 2013; 2015).

Looking at the chronology of the historical study in the flora of the Nakhchivan Autonomous Republic; it appears that Lamiaceae Lindl. (Dalamazkimis, Lipsticks) is a comprehensive chapter not studied, thus the biomorphology, ecology, distribution patterns of this chapter taking in, account's for the relevance of the phytochemical composition, treatment directions and prospects for use and the great need to learn more. For this purpose, in the territory of the Autonomous Republic; research work has been started and is underway distributed in Nakhchivan Autonomous Republic and included in the chapter of collection, drying, botanical, ecobiomorphological, therapeutic, properties, distribution of species, phytochemical composition, pharmacological effect and it's possibilities of use in scientific and folk medicine (Ibadullayeva & Alakbarov, 2013).

In addition to the sexual botanical properties of Satureja L. its medicinal value is also important. It is important to study widely. The flowers of the species included in this chapter are 4-15 mm long, bluish-white, light bluish or pink, consisting of 3-7 flower buds, bowl bell-shaped, two-lipped, five-toothed, lower teeth not deep, straight hairs on top the upper lip is straight, the lower lip is curved and triangular. The male is 4 pieces, top shorter than or equal to the lip (Akhundov et al., 1983; Mustafayeva et al., 2015). Unity of shrubs or shrubs of the leaves have

metal-like vesicles on them, the leaves are mostly entirely short-stemmed. Thirty species of this genus are found in Mediterranean countries. Based on the composition of the species; contains biologically active substances with antibacterial and anthelmintic properties. In folk medicine it is used during respiratory infections to cure tachycardia, gastrointestinal diseases, cystitis, flatulence, headache, dizziness, rhinitis, its aqueous extract has insecticidal activity. Essential oil from the products are mainly used in gastric diseases and aromatherapy, Containing carvacrol flavonoid which is antifungal and has also antioxidant effect. It also contains fat acids that reduces the risk of oncological diseases in the brain. Currently used in combination with beans in cooking, peas and other legumes (Kuliev & Ibadullaeva, 2009; Mehdiyeva, 2011).

Family name: Lamiaceae Lindl. –

Genus: Satureja L. - Wild mint

Satureja horteisis L. - Garden mint

This plant is called savory in England, sarriette in France, savouree, bohnenkraut in Germany, pfefferkraut, weinkraut, santoreggia in Italy, ajedrea comun in Spain, segurelha in Portugal. balver plant is united; it is mainly found in dry stony-gravel places, on rocks, in melons gardens and in algae, the body is straight, short-haired, branched, 15-30 cm is high, the root is thin, straight, almost cylindrical and 10-15 cm long, the leaves consisting of narrow or linear scales, sharp, few dots in the form of vesicles is 1.5-2.5 cm. The inflorescence consists of 1-3 flowers and is located in the axils of the upper leaves.

Bowl bell-shaped plant is 3-4 mm long, narrow scissor or linear, ciliated, teeth is the same size. Flower crown is 6-8 mm long, purplish, pink or light blue, the mouthparts are darkly spotted. The lower lip is long and triangular, and the upper lip is short and bipolar.

Fruit 1 mm; consisting of hazelnuts, brown ovate-trilingual. in July-August it blooms and bears fruit in September-November around the Kura of Azerbaijan, from the low mountain belts of the Nakhchivan Autonomous Republic, the stony-gravelly land of the middle mountains to areas, especially in scatterings and bushes, it is a good balverina plant that produces many vaccines (Anonimous, 1989; Wolfe & Malaeev, 1969). In addition to it’s essential oils, it also produces mucus and resic, there are also ingredients. The essential oil is light yellow in color and has a pungent mint odor. Plants very dark aroma (Guliyeva et al., 2016) are found with the composition of carvacrol (30-42%), o-chimol (20%) and triterpene carbohydrates (40%).

As it is spicy, it’s freshly dried leaves are used to salt cucumbers and tomatoes in cooking used when laying boiled mushrooms, meat, potato salads, along with green and white peas used in the preparation of fish and poultry, In perfumery and cosmetics because it is an essential oil, it

is especially used in medicine in the preparation of therapeutic teas and tinctures, Besides it is prescribed in bath and it is used for useflatulence, astringent, deworming and strengthening in gastrointestinal diseases (spasms). In Bulgarian medicine it is used as anesthetic, diuretic, laxative, prescribed for tachycardia and migraine, as well as for vomiting, bactericidal, antispasmodic and antitussive, it is considered an indispensable plant in the medicine of European countries (Helnz Rechinqer, 1697), Central and Southern parts of Europe, North America, Portugal, Northern Italy, South France, the Balkans, South Ukraine, the Caucasus, Iran, India, Asia Minor, South Africa and It's widespread in the mountainous areas of Ceylon. It was first introduced to science from Georgia. Spicy, and for the first time as an ornamental plant in Western Europe, the Mediterranean countries, Central Asia and America cultivated it. The plant Contains 0.1-3.2% essential oil, flavanoids: carvacrol, thymol, p-simol, 6.8-35.8%, α-z pinene 0.6-1.5%, sabinen 0.2%, kamfen 0.1%, mirsen 1-2.3%, 1.8-sineol 0.1-0.3%, limonene 0.1- 0.4%, μ terpine 6.3-32.3%, α-terpineol 0.1-3.4%, eugenol 0.1%, apomadendren 0.3%, humulene 0.1-0.3%, p-fellandrene 0.1%, α-tuyen 0.4-1.9%, linalool 0.1-0.8%, citronellol, saponins, C, E, vitamins, triterpenoids in the trunk: 0.17% ursolic acid, 0.4% ursolic acid in the leaves, phenolic acid and its derivatives: rosemary, chlorogen (3-caffeine), 0.012%, tartaric acid, 0.074-0.49% caffeine, 0.0032% L-coumarin, 0.0005-0.011% gentianin, 0.0002-0.0058% salicyl, 0.0003-0.0073% vanillin, 0.0005-0.006% protokatexin, fatty oils in seeds and its hydrolyzate acids: palmitic, 4% stearin, 12% olein, 18% linoleic and 62% linoleic.

In Nakhchivan Autonomous Republic Ordubad district; Gilanchay, Bilav, Shahbuz district; Nursu, Badamli, Julfa district; Goynuk, Arafsa, Khoshkeshin the plants are especiallyin dry areas of the former Paradash spreads in sandy, stony-gravelly and rocky places. It is used in rheumatism as antiperspirant, anthelmintic, neurological diseases, gastralgia and bath, has lactogenic properties. The extract has antibacterial and antifungal activity, are included in the official medicinal plants of France, it is used as in flatulence in Indian medicine, in folk medicine; it is prescribed as an appetite suppressant, cough suppressant, anthelmintic for ore tachycardia, dizziness, gastrointestinal tract, urinary tract, flatulence, rhinitis and used in acute respiratory infections. It is however used as spices for canned food, sausages and other products used in the preparation. Aqueous extract has insecticidal activity as it is an essential oil for stomach cramps and includes spicy foods. It has antibacterial and antifungal properties, Especially carvacrol has antifungal effect, cucumbers and culinary, as the leaves spice is used in marinating tomatoes, it's fatty oils can replace the composition of flax.

1.1. *Satureja macrantha* C.A. Meyer - Large Mint

It is a perennial plant, its body is numerous, slender, rod-like, hard as a tree, simple and weak, 30-50 cm high and branched. The leaves are numerous, linear or oblong-oblong, blunt. Bowled 5 mm long, tubular-bell-shaped, two-lipped, short, scattered hairy, bizarre teeth, 3 times shorter than the tube. Flower crown 12-15 mm long,

Figure 1. *Satureja macrantha* C.A. Meyer

It is pink, the tube is narrow and long from the flower crown, the stamen column from the crown is long. Fruits are 1.5 mm long, brown, ovate, consisting of colored hazelnuts. It blooms in May-July and bears fruit in July-September. Fruits are used as a spice due to its aromatic essential oil content when freshly collected, however the presence of phenolic compounds (toxic) prevents the use of this plant as a spice, therefore, it is advisable to use less of this plant as a spice. It is found In the woods, It is moderately widespread in bushes, rocky slopes and areas up to the middle mountain belts (Anonymous, 2013; Davis, 1969).

It is widely spread in the East and South Caucasus and northern Iran. For the first time from the South Caucasus it was included in science (Kasumov et al., 1977). it can be foundin the Nakhchivan Autonomous Republic Ordubad district; Bilav, Kalaki, Pazmari, Kotam Julfa district; Nahajir (“Intermediate” territory), Goynuk, Arafsa, Kazanchi (Berdikdag), Shahbuz district; South Qishlag, and It is distributed in dry sandy, stony, gravelly and rocky areas of coal areas, Contains 0.34-2.33% essential oil: 27.5% thymol, 20.1% μ-terpine, α-pinene, p-pinene, α-tuyen, amphen 2.8% p-mirsen, limonene, p-fellandren, 8% methyl carvacrol, 4% p-karyophylline, 3.6% Contains kalakoren, apomadendron, nerol, heranilol, camphor, vitamins C, E and flavanoids. As it contains essential oil, it is used in perfumery, in diseases due to its antibacterial properties and the stems are used to flavor cheese. Balveren is a plant. It’s Biological activity in the flora of the Autonomous Republic are traced with the substances like (flavonoids,

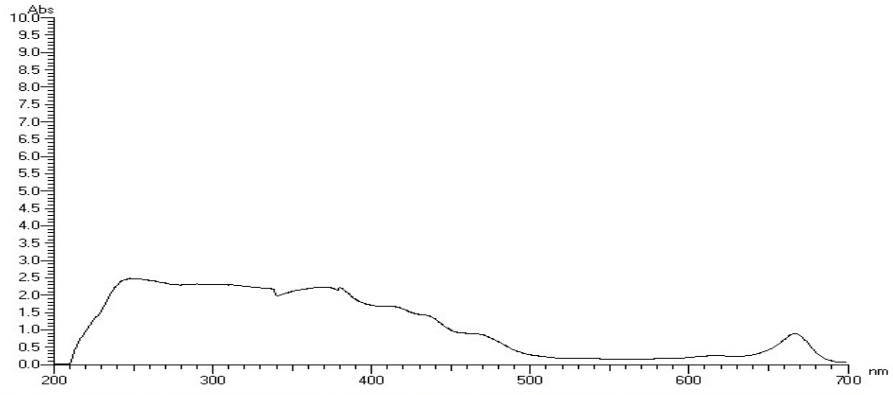
alkaloids, coumarins, polycarbohydrates, glucosides, etc.), as well as folk ; confectionery, soft drinks, pharmaceuticals and other in different areas of the economy. There is a great need for it's use in those fields. For this purpose, *Satureja horterisis L.* - Garden field mint is dried by standard method, analyzed by extract preparation.

2. MATERIALS AND METHODS

The details of the biologically active substances contained in the garden mint species studies research depicts the future use of its constituent substances mainly in the direction of scientific medicine as considered quite relevant. It is from this point of view that this type of E.A. apply the Wolf method (Wolfe & Malaeev, 1969); learned by passing Garden mint for 3 hours through hexane and ethanol, which are solvents of different polarity using Hitachi U-2900 UV-VIS spectrophotometer DC-fertigfolien ALUGRAM SIL G / UV 254, while chromatographic analysis are carried out through the layer through Solvent system for thin-layer chromatography: butanol: vinegar acid: water 4: 1: 5 and petroleum ether: acetone: chloroform 3: 1: 1 by volume, analyzed according to the methodology (Anonymous,1983).

3. RESULT AND DISCUSSION

Satureja horterisis L. - ethanol extract from the leaves of garden mint is performed through thin layer chromatography and the ingredients were determined based on Rf values (Fig.). The composition of Simultaneous spectral and chromatographic analysis of leaf and stem extracts was determined on the basis of the resulting prices Figure 2. *S.horterisis* L. - UV spectrum of ethanol extract from the body of garden wild mint, the Hitachi U-2900 was captured at a wavelength of 200-700 nm using a UV-VIS spectrophotometer. Flavonoids of the spectra (28.8%) obtained at a wavelength of 200.0-294.5 nm (MeOH λ max. nm) - baikalein, apigenin, flavanols - galangin, flavonones - pinosembrin, chalcones - dihydroxyxalkon, 340.5-378.5 nm wavelength (26.8%) flavonols-guerchetin, formononetine, genistein, ramnetin, isoramnetin, galangin, kempferol, herbasetin orobol, flavanones - dihydrocempferol, dihydroquercetin, pinosembrin, naringenin, hesperetin to chalcans -dihydroxyxalkon, 556.5-630.5 wavelength (44.3%) was found to be characteristic of anthocyanins.



Peak #	Start (nm)	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)	Valley (nm)	Valley (Abs)
1	700.0	667.0	630.5	0.885	28.252	630.5	0.234
2	630.5	616.5	556.5	0.248	14.243	556.5	0.140
3	556.5	380.0	378.5	2.216	160.333	378.5	2.135
4	378.5	369.5	340.5	2.233	82.782	340.5	1.964
5	340.5	299.0	294.5	2.310	104.535	294.5	2.292
6	294.5	290.0	279.5	2.317	35.636	279.5	2.280
7	279.5	248.0	200.0	2.485	119.208	200.0	-3.064

Figure 2. *Satureja horterısis* L. - UV spectrum of ethanol extract of garden field mint's stem.

It was captured by the Hitachi U-2900 spectrophotometer at a wavelength of 200-700 nm. (28.8%) spectrum of 200.0-294.5 nm (MeOH λ_{max}. nm) determined for flavonoids - baikalein, apigenin, flavanols - galangin, flavonones - pinosembrin, chalcones – dihydroxyxalkon; the wavelength of 340.5-378.5 nm with (26.8%) was determined for flavonols-guerchetin, formononetin, genistein, ramnetin, isoramnetin, galangin, kempferol, herbasetin orobol, flavanones - dihydrocampferol, dihydroquercetin, pinosembrin, naringenin, hesperetin chalcones – dihydroxyxalkon; and (44.3%) of 556.5-630.5 nm wavelength determined for anthocyanins.

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Determination of Color Parameters, Phenolic Status and Antioxidant Potential in Some Industrial and Traditional Bread Varieties

Bazı Endüstriyel ve Geleneksel Ekmek Çeşitlerinde Renk Parametreleri, Fenolik Durum ve Antioksidan Potansiyelinin Belirlenmesi

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Abstract

Bread and especially wheat bread as the basic food of society is one of indispensable food sources of human nutrition. The golden brown color caused by baking bread dough is the result of non-enzymatic chemical reactions and usually Maillard reaction (MR) and caramelization affect to this. Amino gropes and reducing sugars contained in bakery products can perform caramelization and MR simultaneously with effect of temperature. Processing conditions of bakery products also have a very important effect on the antioxidant activity. As bread types, francala bread, grissini, rusk and traditional oven-dry (Şebinkarahisar region) bread were examined. Antioxidant substances in bread crust have an important place in preventing colon cancer for terms of health. In this study, types of bread made from wheat flour; samples were taken from crust of francala bread, grissini, rusk and traditional (Şebinkarahisar region) breads, and reactions occurring with effect of heat were measured with tone angle Hue (°), Chroma (C) and Browning Index (BI). Amount of antioxidant substance formed was determined by 2,2-

Özet

Toplumun temel besinlerinden biri olan ekmek ve özellikle buğday ekmeği, insan beslenmesinin vazgeçilmez besin kaynaklarından biridir. Ekmek hamurunun pişirilmesi ile oluşan altın sarısı renk kahverengileşmesi, enzimatik olmayan kimyasal reaksiyonların sonucudur ve Maillard Reaksiyonu (MR) ve karamelizasyonun etkili olduğu bilinmektedir. Unlu mamüllerin içerdiği amino gropları ve indirgen şekerler sıcaklıklığın etkisi ile karamelizasyon ve MR'yi aynı anda gerçekleştirebilir. Unlu mamullerin işleme koşulları antioksidan aktivitesi üzerinde de oldukça önemli etkiye sahiptir. Ekmek kabuğunda meydana gelen antioksidan maddeler sağlık açısından kolon kanseri önlemede önemli bir yere sahiptir. Bu çalışmada, buğday unundan yapılan ekmek tipleri; francala ekmek, galeta, etimek ve geleneksel fırın kurusu (Şebinkarahisar yöresi) ekmeklerinin kabuk kısımlarından örnekler alınarak ısı etkisi ile oluşan reaksiyonlar sonucu meydana gelen ton açısı Hue (°), Chroma (C) ve Browning Indeksi (BI) değerleri ölçülmüştür. Oluşan

diphenylpicrilhydrazil (DPPH) and total phenolic content (TPC) methods. TPC value to bread crusts is 122,76±2,62 mg GAE/100g in francala bread, 91,30±21,83 mg GAE/100g in rusk, 54,49±16,76 mg GAE/100g in oven-dry bread and 85,60±16,39 mg GAE/100g in grissini bread. %DPPH values are 45,62±0,43 in francala bread, 21,57±14,84 in rusk, 17,30±0,53 in grissini bread and 12,59±8,14 in oven-dry bread, respectively. H, C, BI, DPPH and TPC results were analyzed by Pearson Correlation Test and one-way analysis of variance (ANOVA).

Keywords: Antioxidant, Bread types, DPPH, TPC, Traditional bread

antioksidan madde miktarı 2,2-difenilpikrilhidrazil (DPPH) ve toplam fenolik madde içeriği (TFM) yöntemleri ile belirlenmiştir. Ekmek kabuklarına TFM değeri francala ekmeğinde 122,76±2,62 mg GAE/100g, peksimette 91,30±21,83 mg GAE/100g, fırın kurusu ekmekte 54,49±16,76 mg GAE/100g ve grisini ekmeğinde 85,60±16,39 mg GAE/100g'dır. %DPPH değerleri sırasıyla francala ekmeğinde 45,62±0,43, peksimette 21,57±14,84, grisini ekmeğinde 17,30±0,53 ve fırın-kuru ekmekte 12,59±8,14'tür. H, C, BI, DPPH ve TFM sonuçları Pearson Korelasyon Testi ve tek yönlü varyans analizi (ANOVA) ile analiz edildi.

Anahtar kelimeler: Antioksidan, Ekmek çeşitleri, DPPH, TFM, Geleneksel ekmek

Abbreviations: TPC, Total phenolic content; GAE, gallic acid equivalent

1. INTRODUCTION

Cereal products, fruits and vegetables contain phytochemicals that have nutritional complementary effects. The phenolic compound classes in cereals are benzoic and cinnamic acid derivatives, anthocyanidins, quinones, flavonols, chalones, flavones and amino phenolic compounds (Liyana-Pathirana & Shahidi, 2005). Antioxidants have been focus of attention in recent years with their ability to scavenge / remove free radicals. There are many defense mechanisms to prevent the formation of reactive oxygen species and damage they cause. These mechanisms are known as antioxidant defense systems or simply antioxidants (Altınışık, 2000).

Bread and bread products have an important role in human nutrition. In general, wheat bread is considered a good source of energy and indispensable food for the human body (Różyło, 2014). While white bread is preferred by most consumers, bioactive compounds, especially antioxidants (phenolic compounds) found in cereal, are especially concentrated in bran and aleurone layer (Mateo Anson et al., 2011). In particular, whole grain bread is a rich source of fiber and bioactive compounds such as oligosaccharides, fatty acids, sulfur amino acids, minerals, B vitamins, phytosterols, and antioxidants (Gani et al., 2012). Chemical reactions occurring in bread production; enzymatic production of sugars (30-70°C), starch gelatinization

(50-65°C), caramelization (50-65°C), protein denaturation and coagulation (60-70°C), yeast inactivation (45-50°C) and enzymes (60-80°C), MR (230-250°C) play an important role especially in determining the quality characteristics of bread (Hui et al., 2008).

The yellow-gold color, which is browning, colored compounds occur during the baking of the dough, which is caused by non-enzymatic chemical reactions, especially MR and caramelization. MR products (melanoidins) appear in bread crusts where reducing sugars and amino acids, proteins and/or other nitrogen-containing compounds are heat treated together. Caramelization is a complex reaction due to direct heating of carbohydrates, especially sucrose and reducing sugars. (Fennema & Tannenbaum 1996). The suitable temperature for estimating crust browning in bread crust using dried and ground breadcrumbs is 250°C (Zanoni et al., 1995). In addition, browning development can be effectively evaluated by measuring color during baking and is exponentially related to baking time. (Ramírez-Jiménez et al., 2000). Recently, a strongly correlated brownness model has been developed between concentration of melanoidin resulting from MR and color development in bread with baking (Purlis, 2010). MR can be associated with the formation of toxic and mutagenic compounds, as well as the formation of antioxidative products (Martins et al., 2000). Caramelization and MR can occur simultaneously (Villota et al., 2019); Both reactions depend on temperature, water activity and pH (Zanoni et al., 1995). Therefore, bread baking is a process in which heat and mass transfer occur at the same time (Purlis & Salvadori, 2009a).

Product formulation and processing conditions of bakery products also affect hydroxymethyl furfural color properties and antioxidant activity (Ertop & Sarikaya, 2017).

Different biological activities of melanoidids formed in the last stages of MR are known, including prebiotic, metal chelating, antihypertensive and antioxidant capacity (Rufian-Henares & Morales, 2007; Patrignani et al., 2016). MR products protect biological tissues from oxidation and this is associated with cancer, cardiovascular and neurological diseases that adversely affect human health (Pérez-Burillo et al., 2018; Pastoriza & Rufián-Henares, 2014).

Effect of baking conditions and dough supplements on amount of antioxidant and Phase II-Enzyme modulated, protein-bound 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonyl-pentyl) in bakery products 3-oxo- 2H-pyrrole (pronyl-L-lysine) has been studied in quantitative studies. These studies revealed that pronyl-L-lysine antioxidant is high in bread crust, low in crumbs and absent in unprocessed flours. Pronyl-L-lysine amounts were found to be strongly influenced by intensity of heat treatment, increasing cooking time from 70 to 210 minutes or increasing the cooking temperature from 220 to 260°C resulted in a 3 to 5-

fold increase in these antioxidant concentrations in shell, respectively (Lindenmeier & Hofmann, 2004). Pronyl-L-lysine, an important antioxidant substance in bread crust, has been shown to have a beneficial effect against chemically induced colonic preneoplastic progression in rats (Selvam et al., 2008).

In this study, industrial bread varieties with baking temperatures between 230-250°C; oven bread (225-230°C) (Elgün, 1981); In order to evaluate the relationship between the color parameters of grisini bread (175-250°C) (Garipoğlu, 2019), rusks and traditional Giresun Şebinkarahisar oven dry bread (230-250°C) (Anonymous, 2015) with the total phenolic substance and antioxidant content of the same samples; Hue (°) angle (H), chroma (C) and Browning index (BI) values were measured. Oven-dried berad, which is a traditional food according to others, is kept in a quenched oven for 3 days at 40°C after cooking process is finished in a stone oven, and its shelf life is cool and dry for approximately 2 years due to its low water activity (Anonymus, 2018).

Samples were taken from outer crust parts of these breads with advanced browning reaction and color parameters formed here were analyzed according to Hunter color system. Using obtained *L**, *a**, *b** data, H, C and BI values were calculated. Amount of antioxidant substance formed was determined by 2,2-diphenylpicrilhydrazil (DPPH) and total phenolic substance determination (TPC) methods. The results obtained were evaluated by ANOVA and Pearson Correlation statistical tests.

2. MATERIALS and METHODS

2.1. Materials

In this study, a total of 4 types of bread were used. Breads purchased commercially from Giresun city; grissini bread, rusk, francala bread and Giresun Şebinkarahisar traditional oven-dry bread. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin & Ciocalteu phenol reagent purchased from Sigma-Aldrich. Sodium carbonate and 98% ethyl alcohol were purchased from Merck Company.

2.2. Methods

2.2.1. Extraction of Antioxidant Compounds

In the extraction of antioxidant compounds, firstly, 10 mg bread crusts were crushed in a ceramic mortar and then 20 ml of 60% ethyl alcohol solution was added. It was shaken for 2

hours on a horizontal shaker and filtered through coarse filter paper and extract was made up to 50 ml with distilled water.

2.2.2. Determination of Total Phenolic Substance

300 µL sample extract was placed into test tubes and 400 µL of Folin & Ciocalteu's phenol and 1.5 ml sodium carbonate (20%) were added. Then, in same way, tubes containing 20, 40, 60 and 80 µg gallic acid were prepared, 400 µL of Folin & Ciocalteu's phenol and 1.5 ml of sodium carbonate (20%) were added and 2 minutes later, tubes were completed to 5 ml with distilled water.

The prepared mixtures were kept at room temperature for 45 minutes and measured at 765 nm with Shimadzu UV-1700 Double Beam Scanning UV-Vis brand Spectrophotometer. The total phenolic concentration was calculated from calibration graph created with gallic acid and results were expressed as gallic acid equivalent (GAE) (Bae & Suh, 2007).

2.2.3. DPPH Radical Scavenging Property

415 µL of sample extracts containing antioxidant substance were taken into a test tube and final volume was completed to 1 ml with ethanol. 0.4 ml of 2, 2-diphenylpicrylhydrazil (DPPH) solution at a concentration of 0.004% prepared daily in ethanol was added to samples and shaken in vortex. Then test tubes were incubated for 30 minutes in a dark environment and readings were made at 517 nm wavelength in the spectrophotometer. The radical scavenging property of each sample was calculated using following equation and the %inhibition (1) corresponding to sample amounts was determined (Brand-Williams et al., 1995).

% Inhibition = (A control-A example) / Acontrol (1)

A Control: Absorbance of DPPH solution and sample containing ethanol

A Example: Absorbance of DPPH solution and sample containing sample

2.2.4. Color Parameters

Color measurement of bread crusts were done with a Hunter colorimeter (3NH Technology Co. Ltd. NR10QC Colorimeter). L^* (brightness), a^* (greenness / redness), and b^* (blueness / yellowness) values were determined in each sample. From L^*, a^* and b^* values obtained at result of analysis, hue (H), chroma (C) and browning index (BI) values were calculated with following equations. Hue angle is defined as a color circle and red-purple colors take angle values of 0°-360°. The chroma value indicates saturation of color. Chroma values decrease in dull colors and chroma values increase in vivid colors. BI value increases depending on the

amount of caramelization and MR products. The equations (2), (3), (4) used in obtaining C, H and BI values are given below (Askari et al., 2008; Mutlu & Ergüneş, 2008).

$$Chroma = \sqrt{a^2 + b^2} \quad (2)$$

$$BI = \frac{100X[\frac{(a+1.75XL)}{(5.645XL+a-3.012Xb)} - 0.31]}{0.17} \quad (3)$$

$$H = \arctan^{b/a} \quad (4)$$

2.2.5. Statistical Analysis

Statistical analyses were performed with SPSS (version 25 for Windows, SPSS Inc.) software. Number, arithmetic mean and standard deviation were evaluated using one-way analysis of variance (ANOVA). Tukey test, one of the Post Hoc Tests, was used to determine between which groups the difference was and relationship between DPPH, TFM and Hue was compared with the Pearson Correlation test. The significance level of analyzes is 0.05 (p-value). All data were presented as mean ± standart deviation.

3. RESULTS and DISCUSSION

In the study, H, C and BI values were calculated by considering the *L**, *a**, *b** values of the crust samples taken from bread types also antioxidant amounts and TPC values were measured Average Hue value for oven-dry bread type bread calculated with *L**, *a** and *b** values in the samples taken from outer crust parts of bread types was 64.94±2.55; it is 57.69±1.53 for bakery bread and BI value was the lowest with 105.32±22.23 for oven-dry bread and 71.78±19.79 for grissini bread (Table 1). According to the Hue angle color scale, oven-dry bread, rusk and grissini bread are light yellow but francala bread reddening was observed (Figure 1). Francala bread crust %DPPH value is 45.62±0.43 and oven dry bread is 12.59±8.14, and chroma values are close to each other for all bread types.

According to ANOVA analysis, there was no statistically significant difference between BI and C, but there was a significant difference in Hue (°) due to 1>4 (1 different 4) and 3>4 (3 different 4) (Table 3). The comparison of the phenolic/antioxidant activity properties of bread types with the Pearson Correlation Test is given in Table 4. While there is a strong and significant positive correlation (r=0.895, p<0.01) between DPPH and TPC values, there is a negative strong and significant (r=-0.804, r=-0.875.p) relationship between Hue and DPPH and TPC values. <0.01) relationship was detected (Table 4).

Table 1. Color parameters in bread types.

Bread Types		*L**	*a**	*b**	Hue (°)	Chroma	Browning Index
	N	X̄±ss	X̄±ss	X̄±ss	X̄±ss	X̄±ss	X̄±ss
Oven-dry Bread	3	53,30±6,78	14,50±1,81	30,94±0,90	64,94±2,55	34,19±1,33	105,32±22,23
Rusk	3	60,76±2,66	16,06±0,66	28,82±1,01	60,87±0,17	33,00±1,21	92,25±5,87
Grissini Bread	3	66,91±6,94	14,43±2,00	28,34±2,85	63,07±0,89	31,81±3,44	71,78±19,80
Francala Bread	3	49,02±8,84	16,17±0,65	25,63±2,24	57,69±1,53	30,31±2,19	98,01±17,17
Total	12	57,51±9,16	15,29±1,49	28,44±2,57	61,64±3,11	32,33±2,42	89,34±20,22

X̄: mean, ss: standard deviation

Table 2. DPPH activity and total phenolic content in bread types.

Bread Types	N	% DPPH	Total Phenolic Content (mg GAE/100g)
Oven-dry Bread	3	12,59±8,14	54,49±16,76
Rusk	3	21,57±14,84	91,30±21,83
Grissini Bread	3	17,30±0,53	85,60±16,39
Francala Bread	3	45,62±0,43	122,76±2,62
Total	12	24,27±15,13	88,64±28,77

X̄: mean, ss: standard deviation

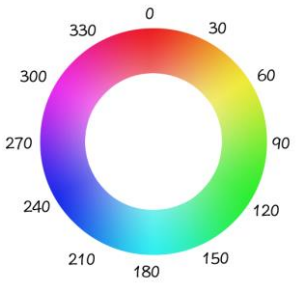

Figure 1. Hue (°) angle color scale (Anonymous. 2000)

Table 3. Hue (°), Chroma and BI mean values One Way ANOVA analyses.

	Groups	**N**	**X̄±ss**	**F**	**p**
H (°)	Oven-Dry Bread(1)	3	64,90±2,55[a]	12,020	**0,002**
	Rusk (2)	3	60,87±0,16[a]		
	Grissini Bread(3)	3	63,07±0,89[bc]		
	Francala Bread(4)	3	57,70±1,53[ac]		
	Total	12	61,64±3,11		
C	Oven-Dry Bread(1)	3	34,19±1,32[a]	1,655	0,253
	Rusk (2)	3	33,00±1,21[a]		
	Grissini Bread(3)	3	31,81±3,44[a]		
	Francala Bread(4)	3	30,31±2,19[a]		
	Total	12	32,33±2,42		
BI	Oven-Dry Bread(1)	3	105,32±22,23[a]	2,268	0,158
	Rusk (2)	3	82,25±5,87[a]		
	Grissini Bread(3)	3	71,78±19,79[a]		
	Francala Bread(4)	3	98,01±17,16[a]		
	Total	12	89,34±20,22		

N: Number of samples, X̄: mean, ss: standard deviation, [a, b, c,] Values with the same superscript letters in the same line are non-significant at $p < 0.05$.

Table 4. TPC and DPPH Pearson Correlation Test.

		% DPPH	TPC	Hue (°)
% DPPH	r	1	0,895**	-0,804**
	p		0,000	0,000
TPC	r		1	-0,875**
	p			0,000
H (°)	r			1
	p			

*p<0,01

4. CONCLUSION

The bread making process generally has a positive effect on phenolic profile and antioxidant activities of whole wheat products (Tian et al., 2021). In the study, in the samples taken from the crust parts of traditional and industrial bread varieties that were heat-treated at 175-250°C, BI value of oven-dry bread produced by traditional method was 105.32±22.23 and H was 64.94°±2.55 %DPPH radical scavenging value in bread made from wheat flour is 48.3±0.02 (Msaddak et al., 2017). The TPC value of loaf bread known as francala bread is 579.27° ± 3.93 (Tian et al., 2021). TPC values were 122.76±2.62 mgGAE/100g and antioxidant activity %DPPH 45.62±0.43 in francala bread and this shows that TPC value is lower in shell part and antioxidant activity is approximately same. In the oven-dry bread produced by traditional method TPC value is 54.49±16.76 and DPPH 12.59±8.14%. According to the results of the statistical analysis, there is a strong positive correlation between the phenolic/antioxidant activity values of the bread types examined and significant relationship between Hue value and DPPH and TPC values, there was a strong and significant negative relationship.

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The Use of St. John's Wort (Hypericum perforatum) Extract in Drinking Yoghurt Production and Determination of Changes Occuring During Storage

Ayran Üretiminde St. John's Wort (Hypericum perforatum) ekstraktının kullanımı ve depolama boyunca meydana gelen değişikliklerin belirlenmesi

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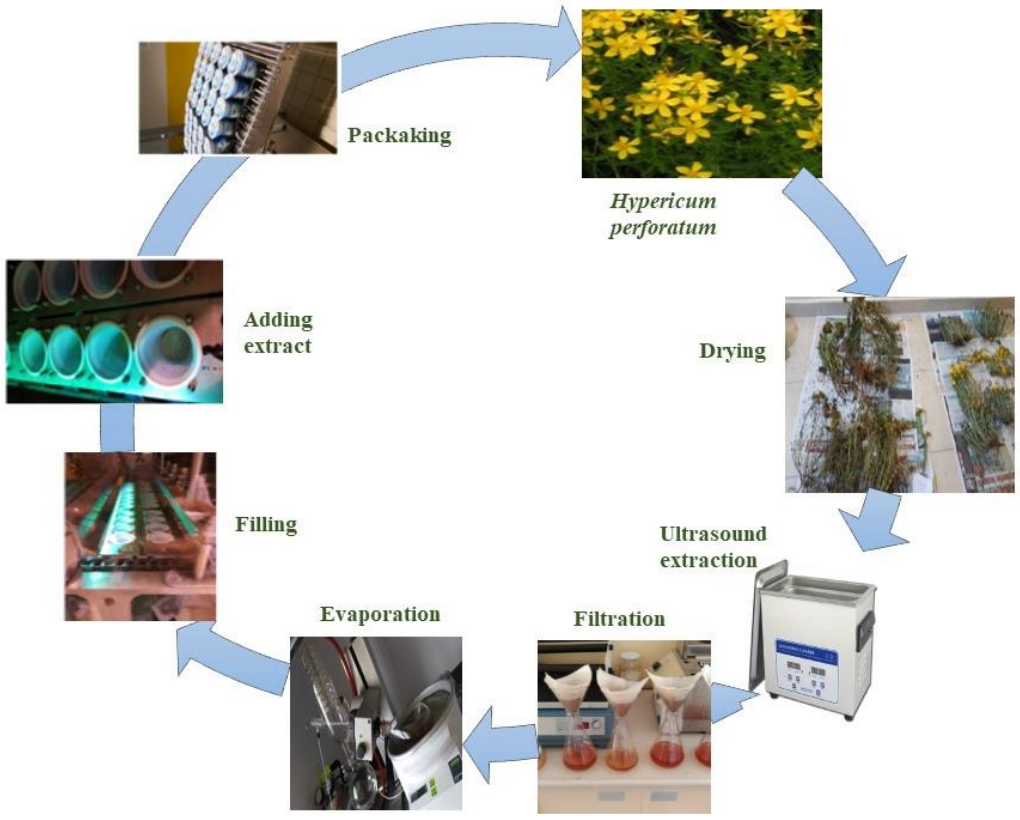
Graphical Abstract



Abstract

Medicinal and aromatic plants and extracts, essential oils obtained from these plants are widely used in many sectors as well as meeting the nutritional needs of people. Hypericum species have many biological activities such as antidepressant, antiviral, antibacterial, antioxidant and antinflammatory and are used as folk remedies in many areas such as sleep enhancement, rheumatic pain treatment, wound healing, skin diseases treatment among the public. One of the Hypericum species, St. John's wort (*Hypericum perforatum*) is found in almost all regions of our country and is one of the most studied plants. In our study, this plant, which has a very common medical use, was extracted and added to different amounts of drinking yoghurt. A mixture of 70% ethanol + 30% water was used as solvent during the extraction. In the study, three types of drinking yoghurt containing 1%, 2% and 3% St. John's wort extract were obtained. The control group and the drinking yoghurt samples obtained were stored for 28 days and some physicochemical and microbiological analyzes were performed on the 1st, 7th, 14th, 21st, and 28th days of the storage. According to the findings obtained; During the storage period, it was observed that the ash and salt values of the samples did not change, the dry substance, water activity and pH values decreased and the acidity values increased. In the microbiological* analyzes performed during the storage period, the number of mesophyl bacteria groups increased statistically, while yeast – mold and coliform groups were not observed. Sensory analyzes were applied to the panelist group consisting of 10 people and when the taste – smell and general appreciation points were considered, samples containing 2% St. John's wort extract were the most liked. General appreciation scores increased statistically during storage. With this study, a functional product has been produced by adding this plant, which is not widely used alone, to a product that we consume a lot in

Özet

Tıbbi ve aromatik bitkiler ve bu bitkilerden elde edilen ekstraktlar, uçucu yağlar insanların besin ihtiyaçlarını karşılamanın yanında birçok sektörde de yaygın olarak kullanılmaktadır. Hypericum türleri antidepresan, antiviral, antibakteriyel, antioksidan, antienflamatuvar gibi birçok biyolojik aktiviteye sahip olup, halk arasında uyku güçlendirme, romatizmal ağrı tedavisi, yara iyileştirme, cilt hastalıkları tedavisi gibi birçok alanda da halk ilacı olarak kullanılmaktadır. Hypericum türlerinden sarı kantaron (*Hypericum perforatum*) ülkemizde hemen hemen tüm bölgelerde bulunmakta ve üzerinde en çok çalışılan bitkilerdendir. Çalışmamızda tıbbi kullanımı oldukça yaygın olan bu bitkinin ekstraksiyonu gerçekleştirilmiş ve farklı miktarda ayrana ilave edilmiştir. Ekstraksiyon sırasında çözücü olarak %70 etanol + %30 su karışımı kullanılmıştır. Çalışmada %1, %2 ve %3 oranında sarı kantaron ekstraktı içeren üç çeşit ayran elde edilmiştir. Kontrol grubu ve elde edilen ayranlar 28 gün boyunca depolanmış ve depolamanın 1., 7., 14., 21. ve 28. günlerinde bazı fizikokimyasal ve mikrobiyolojik analizleri gerçekleştirilmiştir. Elde edilen bulgulara göre; depolama süresi boyunca örneklerin kül ve tuz değerlerinin değişmediği, kuru madde, su aktivitesi ve pH değerlerinin azaldığı, asitlik değerlerinin ise arttığı gözlemlenmiştir. Depolama süresi boyunca yapılan mikrobiyolojik analizlerde, toplam mezofil bakteri grubu sayısı istatistiksel olarak artış gösterirken, maya-küf ve koliform grup gözlemlenmemiştir. 10'ar kişiden oluşan panelist grubuna duyusal analizler uygulanmış ve tat- koku ve genel beğeni puanlarına bakıldığında en fazla beğeniyi %2 oranında sarı kantaron ekstraktı içeren örnekler almıştır. Depolama süresince genel beğeni puanları istatistiksel olarak artış göstermiştir. Bu çalışmayla tek başına kullanımı yaygın olmayan bu bitkinin günlük hayatta çok tükettiğimiz bir ürüne ilave edilmesiyle fonksiyonel bir ürün üretimi gerçekleştirilmiş ve kullanımı daha yaygın hale getirilmeye çalışılmıştır.

daily life, and its use has been tried to be made more common.

Key Words: St. John's wort (*Hypericum perforatum*), extraction, drinking yoghurt, storage

Anahtar kelimeler: Sarı kantaron (*Hypericum perforatum*), ekstraksiyon, ayran, depolama.

Abbreviations:

1. INTRODUCTION

Studies conducted for a longer life span aim to increase the quality of life as well as increase the importance of a healthy and balanced diet (Anonym, 2014). Due to the wrong nutritional rules that are known as correct, people have turned to the search for healthy and reliable food. In this context, the tendency to foods whose effects on health are scientifically proven and approved is increasing day by day (Sevilmiş, 2013). For this reason, studies in recent years have focused on food production, which is rich in nutrients and has positive effects on human health (Seçkin & Baladura, 2011). Considering the role of nutrition in health and preventing diseases, the importance of studies in this field increases even more. In the world and in our country, medicinal and aromatic plants and products produced from these plants have gained value each passing day by gaining the appreciation of the consumers. In the future, it is expected that the production of medicinal and aromatic plants, the production of the plant extracts obtained from these plants and the industrial production that processes them will increase and will be standardized in order to meet the demand of the consumer and to obtain a product of standard quality (Bayram et al., 2010).

Represented by 350 – 400 species in the world, Hypericum genus has 84 species in our country (Wichtl, 1986; Zeybek & Zeybek, 1994). Although H. perforatum L., the most common in Hypericum species, is used as a 'Natural Antidepressant' without side effects in European countries (Chrea et al., 2014), it also plays an active role in the treatment of symptoms such as insomnia and anxiety (Özgür Devrim, 2009). While cardiac and anticholinergic side effects were observed in patients using antidepressant drugs, no side effects were observed in studies using Hypericum extract (Cass, 1997; Ernst, 2003; Francis, 2005; Linde & Knüppel, 2005). Antidepressant drugs, which are becoming more common today, are widely used to prevent alcoholism and various types of addiction (Buonopane, 2005). In the studies conducted, H. perforatum extracts were given to experimental animals and as a result, serious decreases were observed in the alcohol intake of the experimental animals (Rezvani et al., 1999; Xu et al., 2005). Extracts of H. perforatum plant reduce alcohol addiction and withdrawal symptoms

caused by addiction (Abstinens Syndrome), (Coskun et al., 2006), as well as prevent withdrawal symptoms observed as a result of addictions such as nicotine (Uzbay et al., 2005; Uzbay et al., 2007) and caffeine (Uzbay et al., 2005; Uzbay et al., 2006). It has been determined that the antidepressant property of the plant is due to the secondary metabolite content of the plant, especially the hypericin molecule, and this molecule provides a significant increase in the transmission of nerve impulses in the brain (Uzbay, 2008).

Apart from the antidepressant properties of H. perforatum plant, it is used in the treatment of cancer, diabetes, chronic rheumatism, liver – biliary diseases, stomach ulcer and gastrointestinal diseases; In addition, it is known to be used in many areas such as jaundice, bronchitis, diarrhea and dysentery (Duke, 2002), throat infection (Tümen & Sekendiz, 1989) and the treatment of colds (Duke, 2002; Baytop, 1999). As a result of the studies, it has been determined that the compounds contributing to the pharmacological activity of H. perforatum are hypericin, pseudohypericin, hyperforin, flavonoids (rutin, hyperoside, and quersitrin), xanthones and tannins (Barnes et al., 2001; Kaçar & Azkan, 2004). Studies have shown that the bioactive components of Hypericum species, hypericin and pseudohypericin, are highly effective against viruses. In studies conducted in in vitro environment; It has been found to be effective against Type A and B influenza, Herpessimplex, Hepatisis C viruses, Vesicular Stomatitis virus, Epstein-Barrvirus. (Cass, 1997; Mazza, 1998; Mills & Bone, 2000). It has been established that the bioactive compound hypericin exerts an antiviral effect by inactivating the HIV virus and protecting the membranes of healthy cells from virus attack (Cass, 1997; D'Hallewin et al., 2002). It has been supported by studies that the hypericin compound also shows anticancer activity in many different types of cancer (Ali et al., 2001; Blank et al., 2003; Colasanti et al., 2000; Linde et al., 1996; Martarelli et al., 2004; Mills & Bone, 2000). Hyperforin, another important bioactive ingredient, has been determined to have more antibacterial and antidepressant effects (Hölzl & Petersen, 2003). Hyperforin compound shows cytotoxic activity by inducing apoptosis in various cancer cells (Martarelli et al., 2004). This plant, which has many health benefits, has been accepted by the Council of Europe as a natural source for sweetening foods. Its aroma and fragrance has increased its use in the liquor industry (Anonym, 2000). It is used as a herbal additive in instant soups, breakfast cereals, chocolate, cakes, desserts and fruit – flavored beverages because it contains many bioactive ingredients that are beneficial for health (Anonym, 2000; Anonym, 2014).

Milk, which contains water, sugar (lactose), lipids and proteins as well as trace amounts of minerals, enzymes, vitamins, hormones and various compounds in its structure, is a very

complex liquid (Tilki, 2008). Milk is of great importance in food groups as it contains water, carbonhydrates, fat, lipids, vitamins, macro and micro elements and trace elements that are necessary for our metabolism and has a high nutritional value (Souza et al., 2018). According to the Food and Agriculture Organization (FAO), global milk consumption per capita is expected to increase by 12.5% by 2025. It is known that milk and its products have an important role in preventing or treating cardiovascular disease, metabolic syndrome, osteoporosis, digestive disorders and cognitive decline (Coutinho et al., 2018).

Being in the low viscosity yoghurt class in many countries around the world and known as 'drinkiable yoghurt' or 'lactic drink', drinking yoghurt is accepted as one of the most important consumptions forms of yoghurt or a yoghurt derivative product that has settled in our culture (Anonym, 2009; Bölükbaşı, 2007). Drinking yoghurt is expressed as a fermented milk product created by adding water to yoghurt or adding yoghurt cultures to milk whose dry matter value is adjusted in the Turkish Food Codex Fermented Milks Communiqué (Anonym, 2009). Drinking yoghurt, with its high nutritional value, is an important alternative to carbonated beverages in our country and takes its place in the market as a food with a wide range of consumers from children to adults (Kuş, 2010; Taş, 2005). Hypericum species, which have many biological activities such as antidepressant, antiviral, antibacterial, antioxidant, anti-inflammatory, are popularly used as folk remedies in many areas such as sleep enhancement, rheumatic pain treatment, wound healing skin diseases treatment. Studies on the use of this plant, which has a very common medical use, in the food field are very few and insufficient. In the literature, various additions were made to yogurt and drinking yoghurt, but no studies were found on the use of Hypericum species in milk and its products. Thanks to the bioactive compounds it contains, *Hypericum perforatum*, which has a wide area of use in the treatment of many diseases in the medical field, has been added to our traditional drink drinking yoghurt, which plays an important role in daily nutrition; thus, a healthy, natural and useful functional product has been obtained and the deficiency in the literature has been tried to be overcome.

2. MATERIAL AND METHODS

2.1. Material

The *Hypericum perforatum* plant to be used in the study was collected from Amasya region in 2018 between June and August. Ayran samples to be used were produced in OTAT Provisions Industry and Trade LLC, Samsun/Havza according to the process of the factory and supplied from the factory.

Production was carried out by going to the Otat Provisions Industry and Trade LLC. The factory made production the ayran samples according to its own process. *Hypericum perforatum* extracts were added to ayran samples immediately after filling and before capping process. Production was carried out by adjusting the dry matter amount of the milk. The composition of the produced control group ayrans was calculated as (%): dry matter content: 7.9, fat content: 1.5, salt content: 0.5, protein content: 2.

Table 1. Composition of milk used in ayran production

Properties	Fat milk	Skimmed milk
Fat (%)	3.7	0.1
Dry matter (%)	11.25	10.5
Sh	6.8	7
pH	6.78	6.72
Briks	9.8	10

The solvent used in the extraction process and all chemicals used during the analysis were of analytical purity and were obtained from Merck (Darmstadt, Germany).

2.2. Preparation of Extract and Drinking Yoghurt Samples

The aerial parts of the *Hypericum perforatum* plant were dried for 3 weeks in the Suluova Vocational School of Amasya University in a cool place and in the shade. Dried plant samples were grinded in a grinder and cut into small pieces. Later, they were sealed and placed in boxes for use in the study. Ultrasonic wave assisted extraction technique was used to isolate the bioactive components of the *Hypericum perforatum* plant. The most suitable solvent to be used in ultrasonic wave assisted extraction, the temperature and time to be applied have been tried to be determined by previous preliminary tests (Seyrekoğlu & Temiz, 2019). Considering the studies carried out, the most worked temperature, time and solvent were used in Hypericum species. Optimum conditions found as a result of preliminary tests; 70% ethanol + 30% water as a solvent, the optimum temperature and time were found to be 30 ^{o}C and 40 minutes. The *Hypericum perforatum* plant was extracted under these conditions in the ultrasonic wave assisted bath (Çalışkan Lab. Ult 4010, Turkey) Then the ethanol remaining in the extract was removed in Rotary evaporator (Buchi). The obtained *Hypericum perforatum* extract was added to drinking yoghurt in three different proportions (1%, 2%, 3%). Drinking yoghurt samples containing *Hypericum perforatum* extract were stored in (+4) ^{o}C. Physical, chemical, microbiological and sensory analyses were performed on the 1st, 7th, 14th, 21st and 28th days of the storage.

Ayran samples production were carried out according to Otat Food Industry operating standards. According to the factory's own production process, the content of dry matter of milk was adjusted and ayran samples were produced. Then *Hypericum perforatum* extracts were added to ayran samples after filling process. The ayran production process was carried out as follows; dry matter content of milk was adjusted by mixing fat milk, skimmed milk and water and then standardization process was carried out. After evaporation and homogenization, pasteurization was applied at 92±2 ºC at 5±1min. Then culture was added to the milk, which was cooled to 42-43 ºC temperature. After the fermentation was completed, the clot was broken and sent to the filters. *Hypericum perforatum* extract in determined proportions added to ayran samples at this stage and then were filling to the 200 mL pet cups in the filling machine. At the last stage lid closure, dating and packaging processes were carried out. The ayran samples were stored at 4 °C until analysis.

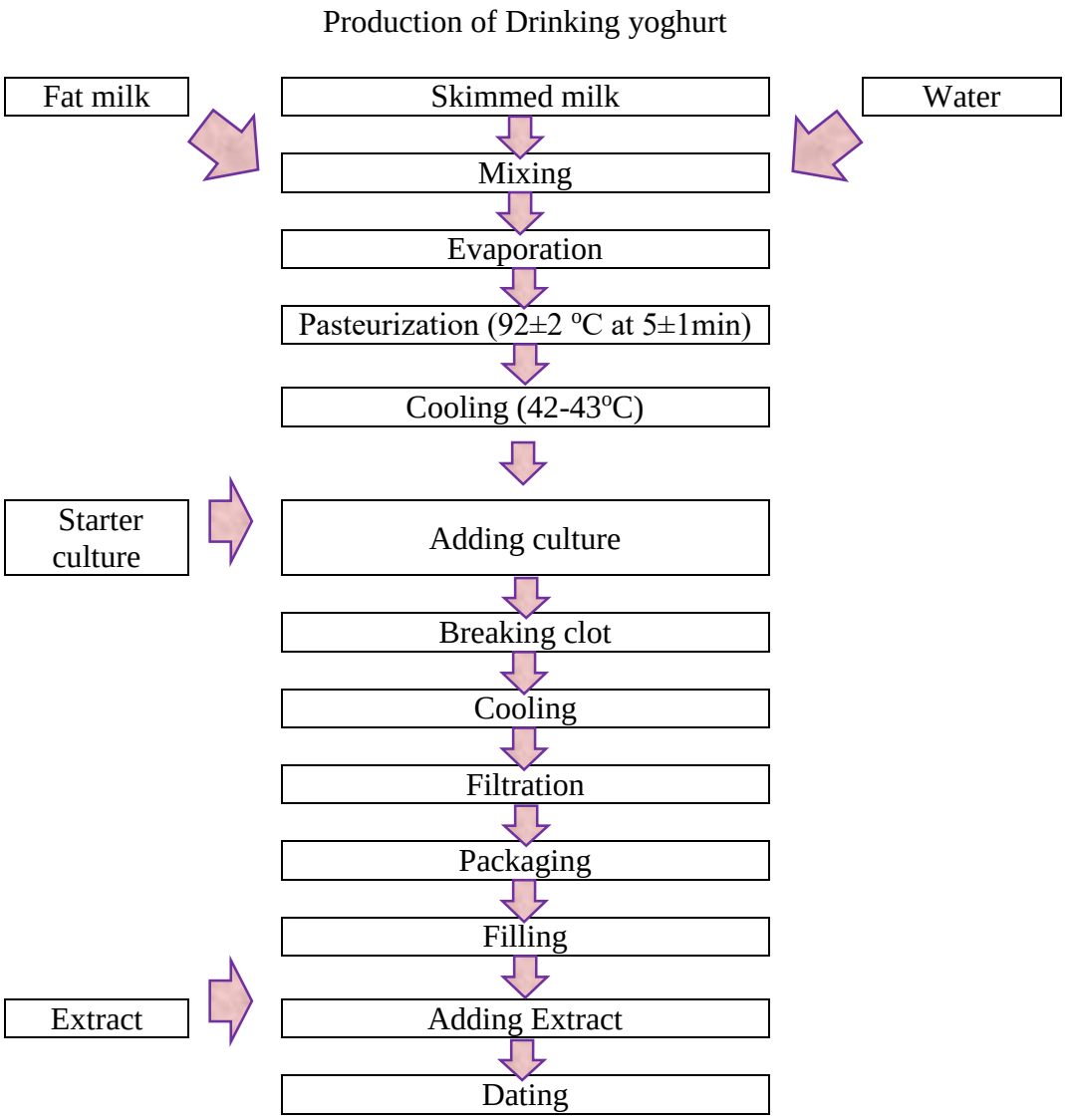


Figure 1. Production of drinking yoghurt

Figure 2. *Hypericum perforatum*

2.3. Physico-Chemical Analyses

The pH values of drinking yoghurt samples (at 25 ± 1 °C) to which *Hypericum perforatum* extract was added were determined with an inoLab (Wellheim, Germany) brand pH meter, and the water activity amount was determined with a water activity analyzer (Novasina, LabSwift-aw). The acidity of samples was made according to TS 1018 (Anonym, 2000). Dry matter values of drinking yoghurt samples were calculated based on the method proposed by TS 1018 in the determination of dry matter (Anonym, 2000). The method made by Kezer (2013) was used in the determination of ash (Kezer, 2013). The amount of salt (%) in drinking yoghurt samples was made according to the Mohr method (Şeker & Patır, 2011).

2.4. Microbial Analyses

Dilutions up to 10^{-6} were prepared from drinking yoghurt samples for microbiological analysis. Total mesophilic aerobice bacteria count was made according to Harrigan (1998), yeast-mold count was made according to the method specified in TS ISO 6611 (Anonym, 1996). The coliform group bacteria count in the samples was made according to the method made by Al-Kadamany et al. (2002).

2.5. Sensory Analyses

Sensory analyses were carried out on the basis of color and appearance, texture and consistency, taste and aroma and general taste characteristics, on the 1st, 7th, 14th, 21st and 28th days of storage with a panelist group of ten people. Before the sensory analysis, the panelists were informed about the product and the sensory analysis they would apply. Ayran samples were presented in 200 mL pet glasses with their covers closed. They were stored at 4 ^{o}C until analysis. By giving codes on the samples and was asked to fill in the characteristics corresponding to

those codes in the sensory analysis form. Drinking yoghurt samples added with *Hypericum perforatum* extract were compared among themselves and during storage. The evaluation was done in a range of points from 1 (quite bad) to 5 (quite nice) and their likelihood was checked (Anonym, 1982).

2.6. Statistical Analyses

All the analyzes applied to the samples in the study were made with at least two replications and their mean standard deviations were calculated. The data, analysis of variance and comparisons between groups obtained as a result of the study were made using the SPSS 16.0 package program (Tukey Test). The significance levels of the groups were evaluated at the $p < 0.05$ level (SPSS, 2011).

3. RESULTS AND DISCUSSION

3.1. Physicochemical Analyses

The results in physicochemical analyses of our drinking yoghurt samples were indicated in the Table 2.

When the physical and chemical analyses of the samples were examined, it was found that the added extracts did not cause a significant change on the physicochemical analysis (Table 2). Similarly, Şanlı et al. (2011), found that the physicochemical properties were not affected in the study in which transglutaminase was added to drinking yoghurt, and Dilek et al. (2018), similarly found that there was no difference between the control group and samples in their study where they added black carrot powder to drinking yoghurt.

Table 2. Change in physicochemical properties of drinking yoghurt samples during storage.

		Samples			
Properties	**Storage days**	**K**	**P1**	**P2**	**P3**
pH	**1st**	3.93^{Ac} ±0.04	3.93^{Ab} ±0.02	3.93^{Ab} ±0.01	3.94^{Ab} ±0.02
	7th	3.96^{Abc} ±0.04	3.93^{ABb} ±0.03	3.92^{ABb} ±0.01	3.91^{Bb} ±0.02
	14th	3.83^{ABd} ±0.01	3.83^{Bc} ±0.02	3.86^{Ac} ±0.02	3.82^{Bc} ±0.01
	21st	3.73^{ABab} ±0.02	3.78^{Ca} ±0.01	3.76^{ABCa} ±0.00	3.80^{Ca} ±0.00
	28th	3.73^{Aa} ±0.02	3.76^{BCb} ±0.01	3.66^{ABCb} ±0.01	3.72^{Bab} ±0.01
Acidity (% lactic acid)	**1st**	0.66^{ABCb} ±0.01	0.64^{BCa} ±0.05	0.63^{Cb} ±0.00	0.66^{ABCa} ±0.01
	7th	0.68^{ABab} ±0.01	0.61^{Ba} ±0.02	0.67^{ABab} ±0.04	0.67^{ABa} ±0.11
	14th	0.72^{Aa} ±0.04	0.65^{Ba} ±0.05	0.67^{ABab} ±0.01	0.65^{Ba} ±0.00
	21st	0.65^{Cb} ±0.00	0.69^{Ba} ±0.00	0.67^{Ba} ±0.01	0.71^{Aa} ±0.00
	28th	0.71^{ABa} ±0.02	0.65^{Ca} ±0.01	0.68^{BCa} ±0.00	0.68^{BCa} ±0.00
a_w	**1st**	0.96^{Ab} ±0.00	0.97^{Aa} ±0.00	0.97^{Ab} ±0.00	0.96^{Ab} ±0.00
	7th	0.97^{Aa} ±0.00	0.97^{ABa} ±0.00	0.97^{Aab} ±0.00	0.97^{Aab} ±0.00
	14th	0.97^{ABab} ±0.00	0.97^{ABa} ±0.00	0.96^{Bb} ±0.00	0.97^{ABab} ±0.00
	21st	0.97^{Aa} ±0.00	0.97^{ABa} ±0.00	0.97^{Aa} ±0.00	0.97^{Aa} ±0.00
	28th	0.96^{Ac} ±0.00	0.96^{Ab} ±0.00	0.96^{Ac} ±0.00	0.96^{Ac} ±0.00
Dry matter (%)	**1st**	7.96^{Aa} ±0.46	7.98^{Aa} ±0.01	8.08^{Aa} ±0.27	8.11^{Aa} ±0.02
	7th	7.56^{Cc} ±0.03	7.80^{Bb} ±0.05	7.83^{Ba} ±0.26	7.98^{ABab} ±0.07
	14th	7.88^{Bd} ±0.22	7.64^{Cc} ±0.01	7.86^{Ba} ±0.01	8.21^{Aa} ±0.01
	21st	7.64^{ABc} ±0.06	7.79^{ABb} ±0.10	7.73^{ABa} ±0.46	7.75^{ABbc} ±0.03
	28th	7.84^{Ab} ±0.05	6.61^{Cd} ±0.13	7.26^{ABb} ±0.44	7.61^{Ac} ±0.41
Ash (%)	**1st**	1.20^{Aa} ±0.07	1.20^{Aa} ±0.46	1.09^{Ab} ±0.02	1.11^{Ab} ±0.01
	7th	1.18^{Aa} ±0.01	1.18^{Aa} ±0.01	1.14^{Aab} ±0.09	1.15^{Aa} ±0.02
	14th	1.11^{Aa} ±0.05	1.12^{Aa} ±0.01	1.10^{Ab} ±0.03	1.067^{ABc} ±0.01
	21st	1.18^{Aa} ±0.00	1.17^{ABa} ±0.01	1.18^{Aa} ±0.01	1.14^{BCab} ±0.00
	28th	1.06^{Ba} ±0.00	1.11^{Aa} ±0.01	1.10^{Ab} ±0.00	1.06^{Bc} ±0.01
Salt (%)	**1st**	0.54^{Aa} ±0.06	0.58^{Aa} ±0.00	0.54^{Aa} ±0.06	0.58^{Aa} ±0.11
	7th	0.58^{Aa} ±0.17	0.59^{Aa} ±0.067	0.58^{Aa} ±0.13	0.54^{Aa} ±0.06
	14th	0.51^{Aa} ±0.00	0.52^{Aa} ±0.06	0.53^{Aa} ±0.06	0.53^{Aa} ±0.00
	21st	0.51^{Aa} ±0.17	0.57^{Aa} ±0.13	0.53^{Aa} ±0.00	0.51^{Aa} ±0.00
	28th	0.50^{Aa} ±0.11	0.50^{Aa} ±0.06	0.51^{Aa} ±0.06	0.50^{Aa} ±0.13

*: mean standard ± deviation. A-C: For drinking yoghurt samples, the capital letters on the same line are comparable and the same letters show no statistical difference between the samples. (P> 0.05).
a-c: The lower case letters in the same column are the comparison of the storage times and the same letters show that there is no statistical difference between the samples. (P> 0.05).

K: Control drinking yoghurt, P1: Drinking yoghurt produced by adding 1 % H. perforatum extract, P2: Drinking yoghurt produced by adding 2 % H. perforatum extract, P3: Drinking yoghurt produced by adding 3 % H. perforatum extract.

3.2. pH and acidity of drinking yoghurt samples

When the physicochemical properties of the samples are examined; While the pH values decreased with the storage, the amount of lactic acid (%) increased in accordance with this. pH values are quite close to each other. Lactic acid that formed as a result of the activities of lactic acid bacteria during storage, increased the acidity values and caused the pH values to decrease. Similar to our samples, pH values decreased with storage in the production of drinking yoghurt with pepper made by Akçay (2016), and drinking yoghurt samples with quinoa flour made by Temen (2018). In our examples, the amount of acidity is 0.61–0.72%, and this amount is quite close with the amount of 0.55–0.61% found in the study made by Avsar et al. (2001), and the amount of 0.58–0.67% in the study on the use of pectin in the production of durable drinking yoghurt by Atamer et al. (1999).

3.3. Dry matter content

While the dry matter values of the samples decreased with storage, the water activity, salt and ash values did not change. While the dry matter value in our study was between 6.61% and 8.21%; Patır et al. (2006), found the dry matter amounts of 3.80%- 8.70 % in packaged drinking yoghurt samples, and Saltoğlu (2014), between 8.61 and 8.83% in fruit drinking yoghurt samples, and they are very similar to our results.

3.4. Water activity

The values of the water activity of our samples wasn't observed change during the storage importantly. Since the amount of added extract was not high the amount of water activity did not change, and in paralel with the amount of water activity, the microbiological analysis of the samples were also similar. When we look at the literature, there are no studies on the amount of water activity of drinking yoghurt.

3.5. Ash content

While the ash values of the drinking yoghurt with the addition of hot pepper and the control group made by Akçay (2016), varied between 0.55% and 0.61%, the amount of ash in the samples used in our study was between 1.06 – 1.20%. The difference between the ash values in our study and the values reported in the literature is due to the raw material, production method

and the added extract. Physicochemical analysis results of all samples showed similar results when compared with the literature. The differences arise from the raw materials used, auxiliary substances, initial culture, fermentation conditions and the different components and extracts added into it.

When the literature is examined, it has not been found that the plant *Hypericum perforatum* is added to drinking yoghurt. For this reason, while making comparisons, studies in which different ingredients are added to make the drinking yoghurt more functional have been looked at and the analyzes made were compared in this way. The number of studies on *Hypericum perforatum* is quite high, but the use of plant in foods is very low. *Hypericum perforatum* extract was added to ice cream and some of its properties were studied. Addition of St. John's wort extract to ice cream caused a decrease in pH values and an increase in acidity and dry matter values (Aydemir, 2015). Similarly, in our samples, it caused a increase in acidity values and decrease pH values. The fact that the extract added was the same plant provided similar results. When *Hypericum perforatum* extract was added to drinking yoghurt, it did not cause major changes on physical and chemical properties and the product preserved its unique properties.

3.6. Microbial Analyses

The results in microbial analyses of our drinking yoghurt samples were indicated in the Table 3. As seen in Table 3 the total mesophylic aerobic bacteria group showed a linear increase with storage. Yeast-mold counts of the samples were similar to the control during storage. Yeast-mold was not observed in any of the samples. On the first day of storage, P3 sample (drinking yoghurt containing 3% *Hypericum perforatum* extract) had the lowest bacteria count with 4.07 log cfu / mL, while C (Control) was showed the highest number of bacteria with 4.67 log cfu / mL. On the 7th day of storage, the control sample contained the highest total mesophilic bacteria group with 4.68 log cfu, while the P3 sample contained the least bacteria with 4.13 log cfu / mL. Control and other samples were showed statistically similarity on other days of storage. *Hypericum perforatum* is showed antimicrobial effect in normally but everything could changed this antimicrobial effect. The reason for this change may be the year the plant was collected, the region where it was collected, the part used, the drying process applied before the extraction and the parameters used in the extraction.

Table 3. Change in microbial properties of drinking yoghurt samples during storage.

		Samples			
Microbiological Analysis	**Storage days**	**K**	**P1**	**P2**	**P3**
Total Mesophyl Bacteria (log cfu / mL)	**1st**	$4.67^{Ca} \pm 0.02$	$4.20^{Ba} \pm 0.01$	$4.11^{Aa} \pm 0.01$	$4.07^{Aa} \pm 0.02$
	7th	$4.68^{Ba} \pm 0.02$	$4.23^{Aa} \pm 0.09$	$4.14^{Aa} \pm 0.12$	$4.13^{Aa} \pm 0.06$
	14th	$4.73^{Ba} \pm 0.04$	$4.42^{Ab} \pm 0.06$	$4.69^{Bb} \pm 0.01$	$4.73^{Bb} \pm 0.14$
	21st	$4.87^{Ab} \pm 0.08$	$4.87^{Ac} \pm 0.03$	$4.89^{Ac} \pm 0.04$	$4.85^{Ab} \pm 0.06$
	28th	$4.94^{Ab} \pm 0.08$	$4.95^{Ac} \pm 0.03$	$4.97^{Ac} \pm 0.04$	$4.96^{Ab} \pm 0.06$
Coliform Group (log cfu / mL)	**1st**	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	7th	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	14th	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	21st	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	28th	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
Yeast and Mold (log cfu / mL)	**1st**	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	7th	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	14th	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	21st	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$
	28th	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$	$<1^{Aa}$

*: mean standard ± deviation.
A-C: For drinking yoghurt samples, the capital letters on the same line are comparable and the same letters show no statistical difference between the samples. (P> 0.05)
a-c: The lower case letters in the same column are the comparison of the storage times and the same letters show that there is no statistical difference between the samples. (P> 0.05)
K: Control drinking yoghurt, P1: Drinking yoghurt produced by adding 1 % H. perforatum extract, P2: Drinking yoghurt produced by adding 2 % H. perforatum extract, P3: Drinking yoghurt produced by adding 3 % H. perforatum extract

Phenolic compounds contribute directly to the antioxidative effect and it has inhibitory effects on mutagenesis and carcinogenesis in humans (Barış et al., 2011). Also *Hypericum perforatum* extract could be affect from envoirement conditions undesirable reactions such as temperature, oxygen and heat during processing and storage. Everything of this situations afffect antimicrobial charecteristic of plant. The antimicrobial effect of *Hypericum perforatum* was investigated by Düzgüner & Erbil (2020), and it was concluded that methanol extract was effective on all test bacteria. Likewise, Orhan et al. (2013), found that the plant has antibacterial activity. Essential oils in the structure of the plant have antimicrobial, antiproliferative and antioxidant effects (Schepetkin et al., 2020). Hyperforin, one of its bioactive components, was found to be responsible for its antibacterial effect (Hölzl & Petersen, 2003). Aydemir (2015), determined the total number of aerobic mesophilic bacteria as 8 log cfu / g in the samples he

obtained by using saffron and St. John's wort in ice cream production, and found quite higher values than our results. The difference here may be due to the plant extract used, its composition, the amount of bioactive ingredient it contains, especially the amount of hyperforin. In addition, the year, location, climatic conditions, altitude difference, the parts of the plant used for extraction and extraction methods affect the antimicrobial properties of the plant. In addition, if examined on a product basis, ayran is an acidic product and structurally, it is completely different from ice cream. The amount of secondary components used in ice cream production is quite high and this causes an increase in microbial load. Coliform group and yeast development could not be observed in any of the samples used in our study. This is an indication that the raw material, extract, production and storage conditions used are completely hygienic and a healthy functional dairy product is produced.

Ekici (1998), in a study examining the microbiological and chemical quality of ayran, found the number of yeast and mold between log 4 - 7.14 log cfu/ mL, and Aydemir (2015), found the average number of yeast and mold to log 5 cfu / g. Yeast and mold development was not observed in the samples used in our study, both at the beginning and at the end of storage, and our samples are quite healthy and microbiologically safe products within the limits given in the Microbiological Criteria Communiqué. Adding *Hypericum perforatum* to our drinking yoghurt samples has reduced the bacterial load and it is very advantageous for safe food production.

3.7. Sensory Analyses

The results in sensory analyses of our drinking yoghurt samples were indicated in the Table 4.

In the sensory analysis (Table 4), when looking at the color and appearance, texture – consistency, taste – aroma scores, the control group received the highest scores, followed by P1 (Drinking yoghurt containing 1% *Hypericum perforatum* extract) sample. As the amount of extract added increases, sensory scores decrease. After the control, the most admired P1 sample got higher scores at the end of the storage in terms of taste – aroma and general taste compared to the beginning of the storage. At the end of the 28 days of storage, while the P1 sample got the same score as the control group, according to their taste and aroma scores; it was preferred by the consumers by getting higher scores than control in structure – consistency and general taste scores. With storage, the interaction of drinking yoghurt, which is a fermented dairy product and extract has increased, and at the end of the storage, a popular product has been obtained for the consumer. However, the increase in the amount of extract caused the scores to decrease, as it was out of the traditional habits of the consumer in general.

Table 4. Change in sensory properties of drinking yoghurt samples during storage.

		Samples			
Sensory analyses	**Storage days**	**K**	**P1**	**P2**	**P3**
	1st	$4.71^{Aab} \pm 0.48$	$3.71^{Bb} \pm 0.75$	$3.28^{Bb} \pm 0.75$	$3.00^{Bbc} \pm 0.57$
	7th	$5.00^{Aa} \pm 0.00$	$4.14^{Bab} \pm 0.37$	$3.28^{Cb} \pm 0.75$	$2.71^{Cc} \pm 0.75$
Color and appearance	**14th**	$5.00^{Aa} \pm 0.00$	$4.57^{ABa} \pm 0.53$	$3.85^{BCab} \pm 0.69$	$3.28^{Cabc} \pm 1.11$
	21st	$4.42^{ABbc} \pm 0.53$	$4.71^{Aa} \pm 0.48$	$4.00^{Bab} \pm 0.57$	$3.85^{Ba} \pm 0.37$
	28th	$4.00^{Ac} \pm 0.57$	$3.85^{Ab} 7 \pm 0.37$	$4.14^{Aa} \pm 0.37$	$3.71^{Aab} \pm 0.48$
	1st	$4.85^{Aa} 7 \pm 0.37$	$4.42^{ABa} \pm 0.78$	$3.71^{Ba} \pm 1.11$	$3.42^{Ba} \pm 1.13$
	7th	$5.00^{Aa} \pm 0.00$	$4.28^{ABa} \pm 0.75$	$4.00^{Ba} \pm 0.81$	$3.85^{Ba} \pm 1.06$
Texture – consistency	**14th**	$4.85^{Aa} \pm 0.37$	$4.42^{ABa} \pm 0.53$	$4.42^{ABa} \pm 0.78$	$3.85^{Ba} \pm 1.06$
	21st	$4.28^{Ab} \pm 0.48$	$4.14^{Aa} \pm 0.89$	$4.14^{Aa} \pm 1.06$	$4.14^{Aa} \pm 0.89$
	28th	$3.85^{Ab} \pm 0.69$	$4.14^{Aa} \pm 0.69$	$4.00^{Aa} \pm 0.81$	$3.71^{Aa} \pm 0.95$
	1st	$4.57^{Aab} \pm 0.78$	$3.28^{Ba} \pm 1.25$	$2.71^{Bb} \pm 1.11$	$3.00^{Ba} \pm 1.15$
	7th	$4.85^{Aa} \pm 0.37$	$3.85^{ABa} \pm 1.21$	$3.14^{Bab} \pm 1.06$	$3.28^{Ba} \pm 1.13$
Taste – aroma	**14th**	$4.28^{Aabc} \pm 0.48$	$3.85^{ABa} \pm 0.69$	$3.28^{BCab} \pm 0.75$	$2.57^{Ca} \pm 0.97$
	21st	$3.85^{Ac} \pm 0.69$	$3.71^{Aa} \pm 0.48$	$3.28^{Aab} \pm 0.95$	$3.28^{Aa} \pm 0.75$
	28th	$4.00^{Abc} \pm 0.57$	$4.00^{Aa} \pm 0.00$	$3.85^{Aa} \pm 0.37$	$3.14^{Ba} \pm 0.69$
	1st	$4.57^{Aa} \pm 0.53$	$3.57^{Ba} \pm 0.53$	$3.28^{Ba} \pm 0.48$	$2.71^{Ca} \pm 0.48$
	7th	$4.71^{Aa} \pm 0.48$	$4.00^{ABa} \pm 1.00$	$3.57^{ABa} \pm 1.27$	$3.28^{Ba} \pm 1.38$
General taste	**14th**	$4.42^{Aa} \pm 0.53$	$4.14^{Aa} \pm 0.69$	$3.28^{Ba} \pm 0.75$	$2.85^{Ba} \pm 0.89$
	21st	$4.28^{Aab} \pm 0.48$	$3.71^{ABa} \pm 0.75$	$3.71^{ABa} \pm 1.11$	$3.14^{Ba} \pm 0.69$
	28th	$3.71^{ABb} \pm 0.75$	$4.14^{Aa} \pm 0.37$	$3.14^{BCa} \pm 0.37$	$2.85^{Ca} \pm 0.69$

*: mean standard ± deviation.
A-C: For drinking yoghurt samples, the capital letters on the same line are comparable and the same letters show no statistical difference between the samples. (P> 0.05)
a-c: The lower case letters in the same column are the comparison of the storage times and the same letters show that there is no statistical difference between the samples. (P> 0.05)
K: Control drinking yoghurt, P1: Drinking yoghurt produced by adding 1 % H. scabrum extract, P2: Drinking yoghurt produced by adding 2 % H. scabrum extract, P3: Drinking yoghurt produced by adding 3 % H. scabrum extract

4. CONCLUSION

In our study, *Hypericum perforatum* extract was added to drinking yoghurt, thus an alternative and healthy functional product was obtained. Whether *Hypericum perforatum*, which is not widely used in foods, is suitable for use in ayran has been demonstrated with scientific data. The drinking yoghurt samples obtained were evaluated in terms of physicochemical and

microbiology and presented to the consumer. In addition, the sensory properties of *Hypericum perforatum* were investigated and in case such a product was produced, a demand for the product was tried to be determined. The effects of the plant on the product and shelf life during the storage period were determined and evaluated by storing longer than the normal storage period. As a result, a product that is microbiologically safe and received high scores from consumer appreciate at the end of storage was obtained. Considering the antimicrobial properties of the plant, the fact that it can be used especially in products that have a microbial risk and have a long storage time becomes clear. This plant, which is very valuable in terms of its components, grows spontaneously in nature and can be used in industrial products without any cost in order to benefit from its antimicrobial properties. This kind of herbs can be used not only as tea but also as natural additives in food formulations.

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CONFLICT OF INTEREST

The authors of the article declare that there is no conflict of interest between them.

AUTHOR CONTRIBUTION

The authors declare that they have contributed equally to the article.

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