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Biological active compounds and biological activities of the foam used in the traditional *kerebiç* dessert

*Savcı, A.

Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Mus Alparslan University, 49250 Mus, Turkey

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Abstract

In the present work, foam and water extracts were obtained from *Gypsophila* root, which forms the foam part of the dessert known as *kerebiç*. The bioactive compounds of samples were analysed with Fourier transform infrared (FT-IR) and high-performance liquid chromatography (HPLC). To determine their antioxidant properties, ferric-reducing antioxidant power (FRAP) method, cupric-reducing antioxidant capacity (CUPRAC) method, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) method, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, and total antioxidant activity method were performed. The pUC18 plasmid DNA was used to investigate the effect of the samples on the deoxyribonucleic acid (DNA). In addition, the inhibitory properties of the samples against key enzymes (acetyl and butyryl-cholinesterases) involved in the pathogenesis of diseases were investigated. Based on the results of FT-IR analysis, terpenes and sugars were detected in the extracts. Based on the HPLC results, glucose, fructose, maleic acid, acetic acid, acetoin, and 2,3 butanediol were detected in the extracts. Antioxidant results showed that extracts had similar and moderate activities as compared to the standard antioxidants. It was observed that the extracts preserved the stable structure of pUC18 plasmid DNA, but could not remove the scavenging effect of peroxide. In addition, it was determined that the extracts showed a stronger inhibitory effect on acetylcholinesterase (AChE) and butyryl-cholinesterases (BChE) than galantamine, which was used as the control.

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Introduction

Gypsophila spp. belonging to the Caryophyllaceae family are important plants used in various areas in Asia and Europe (Zheleva-Dimitrova *et al.*, 2018). The family Caryophyllaceae is widely distributed with more than 2,000 species. There are approximately 126 species of the Caryophyllaceae family and 56 species of *Gypsophila* in Turkey (Korkmaz and Ozçelik, 2014). *Gypsophila* spp. are traditionally used for therapeutic purposes because of their anti-inflammatory, antifungal, antibacterial, antiparasitic, anticancer, antioxidant, anticholesterol, and antidiabetic properties (Gevrenova *et al.*, 2010). They are commercially used in various products including drugs, detergents, excipients, and cosmetics (Özçelik and Yıldırım, 2011).

Gypsophila spp., which are known as *coven otu* in Turkey, contain a large number of saponins, sugars, and organic acids (Korkmaz and Ozçelik, 2014). Saponins have anticarcinogenic, anti-inflammatory, antioxidant, and antimicrobial effects. It was also reported that saponins have positive effects on the immune system, and are effective in repairing DNA damage (Yazici and Özmen, 2018). *Gypsophila* spp. which are used in various industrial areas in Turkey play an important role in the food industry. *Gypsophila* saponins are used in food products such as ice creams, Turkish delights, *halvas*, breads, and cheeses (Altay *et al.*, 2019). In addition, *Gypsophila* root is used in the foam of the *kerebiç* dessert, which belongs to the Mersin region in the south of Turkey.

Plants containing phenolics and organic acids have an important place among dietary antioxidants used to prevent oxidative damage caused by free radicals (Altay *et al.*, 2019; Zhang *et al.*, 2020). Several previous studies observed the antioxidant property of *Gypsophila* spp. (Sekmen *et al.*, 2012;

*Corresponding author.
Email: a.savci@alparslan.edu.tr

Simeonova *et al.,* 2016). DNA is one of the target molecules used in the development of many drugs. There is an increasing interest in studies on organic and inorganic substances that can bind or scavenge DNA. Recent studies have shown that butyryl-cholinesterases (BChE) play a role in various diseases such as Parkinson's disease, Alzheimer's disease, diabetes, and cardiovascular diseases (Özbey *et al.,* 2016; Özgeriş *et al.,* 2016; Eruygur *et al.,* 2019; Ma *et al.,* 2020). The inhibitory effect of acetylcholinesterase (AChE) has attracted great interest from researchers recently, and it has been reported that excessive consumption of acetylcholine in the human body by acetylcholinesterase likely causes nervous system dysfunction (Peng *et al.,* 2021).

The contents and various biological activities of the root part of the *Gypsophila* that constitutes the foam of the *kerebiç* dessert were investigated in the present work. In addition, the water extract (GRE) of the root part of the *Gypsophila* was prepared, and the biological activity results were compared with the results of the foam extract. The saponin content of the samples was determined by FT-IR, and the sugar and organic acid contents were determined by HPLC. The total antioxidant method, ferric and cupric reduction methods, DPPH, and ABTS radical scavenging methods were used for antioxidant studies. pUC18 was used to examine the effects of samples on the DNA. In addition, the inhibitory properties of the samples against key enzymes (acetyl and butyryl-cholinesterases) involved in the pathogenesis of diseases were also investigated.

Materials and methods

Sample preparation

Gypsophila root was purchased from the Köryusuflar firm in Mersin, Turkey. Foam production was prepared as it is consumed in the *kerebiç* dessert. Approximately, 250 g of *Gypsophila* root was cut in a spiral shape and placed in distilled water, and approximately 50 g of sugar was added. It was boiled several times. After boiling, the mixture was filtered, and the liquid part was whisked thoroughly to obtain foam. The resulting foam was dried in a vacuum oven, and stored as a stock for subsequent experiments. For water extract, 250 g of *Gypsophila* root was boiled in distilled water, and filtered. The filtrate was dried in a vacuum oven, and stored as a stock for subsequent experiments.

Determination of sugars and organic acids

For the determination of sugar and organic acid contents by HPLC, standards such as glucose, fructose and rhamnose, maleic acid, citric acid, tartaric acid, pyruvic acid, malic acid, succinic acid, acetoin, fumaric acid, 2,3-butanediol, and acetic acid were used. Stock standards were prepared by weighing the final concentrations of the standards to be 1 mg/mL and dissolving them with 0.03 M sulphuric acid (H_2SO_4) in 10-mL Falcon tubes. The prepared stock standards were prepared in eight different dilutions (5, 10, 25, 50, 100, 200, 300, and 400 ppm), loaded into the HPLC (Agilent Technologies 1260 Infinity II), and the calibration curve was constructed (Ball and Lloyd, 2011).

For HPLC analysis, 0.2 mL sample was vortexed in 1.8 mL of 0.03 M H_2SO_4. The mixture was then centrifuged at 2,000 rpm for 5 min, 100 µL was then taken from the supernatant, and 0.9 µL was then mixed in 0.03 M H_2SO_4. The mixture was passed through filters with a pore diameter of 0.45 µm, and approximately 0.5 mL was transferred into vials. To determine the sugar and organic acids in the resulting mixture, the samples were loaded into the HPLC system, and 0.03 M H_2SO_4 was used as the mobile phase.

Antioxidant activity assays

Total antioxidant activity determination according to ferric thiocyanate method

The total antioxidant activity determination of the samples and standard antioxidants (BHA, BHT, and AA) were performed with small modification of the thiocyanate method (Gülçin, 2012; 2020; Cetin Cakmak and Gülçin, 2019; Kilic *et al.,* 2020). Based on this method, the stock solution corresponding to the desired quantities in the concentrations was pipetted with automatic pipettes into Eppendorf tubes, and the volume was completed up to 500 µL with buffer solution. Then, 500 µL of linoleic acid was added. As the control, 500 µL of buffer solution and 500 µL of linoleic acid emulsion were used. The incubation was performed at 37°C; 20 µL of the samples were taken every 8 h, and placed in Eppendorf tubes containing 470 µL of ethanol. Then, 20 µL of Fe^{2+} solution and 20 µL of SCN^- solution were added. The solution that was prepared by adding 20 µL of Fe^{2+} and 20 µL of SCN^- to the test tube that had 480 µL of ethanol was used as the blank. The absorbance of the samples was read at 500 nm against the blank using a spectrophotometer. The incubation

was terminated when the control reached maximum absorbance. The percentage of samples in eliminating lipid peroxides was calculated using Eq. 1.

$$\text{Lipid peroxidation inhibition (\%)} = 100 - \left(\frac{As}{Ac} \times 100\right) \quad \text{(Eq. 1)}$$

where, As = absorbance value of samples, and Ac = absorbance value of control.

Determination of Fe^{+3} reduction power (FRAP)

The determination of the Fe^{+3} reduction power of the samples and the standard antioxidants was performed according to Oyaizu (1986) with slight modification; 10, 20, and 30 µg/mL were taken from the stock solution as samples, and were transferred to 2-mL test tubes, completing the volume up to 200 µL with distilled water. Then, after 500 µL of 0.2 M phosphate buffer (pH: 6.6) and 500 µL of 1% potassium ferrocyanide [$K_3Fe(CN)_6$] were added to the tubes, the mixture was incubated at 50°C for 20 min. Next, 500 µL of 10% trichloracetic acid (TCA) was added to the reaction mixture. Then, 500 µL of upper phase of the solution was taken, 500 µL of distilled water and 100 µL of 0.1% $FeCl_3$ were added, and absorbance was read against the blank at 700 nm using a spectrophotometer.

Determination of Cu^{+2} reduction power (CUPRAC)

The cupric ion (Cu^{2+}) reducing capacities of the samples were determined according to Apak (2006). For this, 0.01 M 0.25 mL $CuCl_2$ solution was added to the test tubes. Then, 0.25 mL of 7.5×10^{-3} methanolic neocuproine solution and 1 M ammonium acetate buffer were transferred. After the solution was mixed, the samples and standards were added at different concentrations (10, 20, and 30 µg/mL). After half an hour of incubation, the absorbance was read at 450 nm using a spectrophotometer. The increased absorption of the reaction mixture indicates the cupric ion (Cu^{2+}) reduction capacity.

$DPPH^{\bullet}$ radical scavenging

The activity of $DPPH^{\bullet}$ free radical removal activity was performed according to Blois (1958) with slight modifications; 1 mM $DPPH^{\bullet}$ solution was used as a free radical. Stock solutions were transferred to the test tubes to obtain solutions at 10, 20, and 30 µg/µL concentrations, respectively, and the total volumes were completed up to 600 µL with distilled ethanol. Then, 200 µL of stock $DPPH^{\bullet}$ solution was added to each sample tube. After incubation for 30 min at room temperature in the dark, the absorbance was read against the blank which consisted of ethanol at 517 nm using a microvolume spectrophotometer, and 600 µL of ethanol was used along with 200 µL of $DPPH^{\bullet}$ as the control. The radical scavenging activity of the samples was calculated using Eq. 2:

$$\text{DPPH radical scavenging activity (\%)} = \left[\left(\frac{Ac-As}{Ac}\right) \times 100\right] \quad \text{(Eq. 2)}$$

where, Ac = absorbance value of control, and As = absorbance value of samples.

$ABTS^{\bullet+}$ radical scavenging

The $ABTS^{\bullet}$ radical scavenging activity of the compounds was determined according to Re *et al.* (1999). Firstly, 2 mM $ABTS^{\bullet}$ solution was prepared. $ABTS^{\bullet+}$ was obtained by adding 2.45 mM potassium persulfate solution to this solution. Before measuring the absorbance value of the compounds, the absorbance of $ABTS^{\bullet+}$ solution at 734 nm was adjusted to 0.700 ± 0.025 nm with phosphate buffer. After the volumes of the samples of different concentrations (10, 20, and 30 µg/mL) were filled to 50 mL with distilled water, 1 mL of $ABTS^{\bullet+}$ solution was added to them. After 30 min of incubation, their absorbance at 737 nm was read using a spectrophotometer. The $ABTS^{\bullet+}$ radical scavenging activity of the samples was calculated using Eq. 3:

$$\text{ABTS radical scavenging activity (\%)} = \left[\left(\frac{Ac-As}{Ac}\right) \times 100\right] \quad \text{(Eq. 3)}$$

where, Ac = absorbance value of control, and As = absorbance value of samples.

Cholinesterase inhibition assay

Acetylcholinesterase from *Electrophorus electricus* (electric eel) and butyryl-cholinesterases from equine serum were purchased from Sigma-Aldrich (St. Louise, MO). The inhibitory effects of samples obtained from *Gypsophila* against AChE and BChE were measured using a slight modification of Ellman *et al.* (1961) spectrophotometric method, using commercially available galantamine as the reference compound. The acetylthiocholine iodide (AChI) and butyrylcholine iodide (BChI) that were used as the substrate in the reaction, and the

5′dithiobis-2-nitrobenzoic acid (DTNB) that was used as Ellman's reagent were also purchased from Sigma-Aldrich (St. Louis, MO).

The absorbance of the reaction mixture was read at 412 nm, three times within 5 min of the start of the reaction, on a Thermo Fisher Scientific Multiskan GO, and the results were reported as mean ± standard deviation. Activity (%) was plotted to determine the inhibitory effects of samples on AChE and BChE. IC_{50} values were obtained by activity (%) versus compound plots (Huseynova *et al.*, 2018; Burmaoglu *et al.*, 2019; Bingol *et al.*, 2021).

DNA protective activity

The effect of extracts on DNA was determined by agarose gel electrophoresis using pUC18 plasmid DNA (Siddall *et al.*, 2002). Stock solutions of 200 mg/mL were prepared from the extracts dissolved in dimethyl sulfoxide (DMSO). For the study, stock solutions were diluted to 25, 50, and 100 mg/mL. Samples and reagents were pipetted into 16 PCR tubes at the following concentrations: 15 µL of pUC18 DNA (200 ng), 5 µL of hydrogen peroxide (H_2O_2), 10 µL of DMSO, 5 µL of distilled water, and 5 µL of extract. The total volume was adjusted to 30 µL. The samples were incubated in the dark. After incubation, 5 µL of the extracted DNA mixture was mixed with the loading buffer. Electrophoresis was performed in TBE buffer at 40 V, free current, for 2 h after loading on 1% agarose gel. After electrophoresis, the gels were stained with ethidium bromide and photographed on the imaging system (BioRad).

Results and discussion

FT-IR analysis results

FT-IR (Perkin Elmer 65) was used for the quantitative analysis of the saponin in the samples. The distinctive IR spectral peaks obtained are shown in Figures 1 and 2. The peaks were interpreted based on previous studies.

According to the study of Elzey *et al*. (2016) on pure lemon essential oil, the expected characteristic in FT-IR spectra (C=H (~2900 cm^{-1}), CO (~1700 cm^{-1}), wide OH (~3400 cm^{-1}), and CO stretch bands (~1100 cm^{-1})) showed the presence of terpenoid compounds. They also reported that the differences in the composition of the essential oil may vary depending on the chemical properties of the soil in which it is planted. In the present work, several characteristic bands were extracted from the FT-IR spectra of the sample. The OH stretch (~3419 cm^{-1}), CH stretch (~2922 cm^{-1}), amide (1639), and COC stretch (1717 cm^{-1}) observed in the samples showed the main characteristic bands of terpenes (864, 827 cm^{-1}), and pyranosides showed the ring stretching vibration. Also, as shown in Figures 1 and 2, peaks at < 800 cm^{-1} in the respective compound structure indicated complex vibrations corresponding to the pyranose ring in the glucose unit (Elzey *et al.*, 2016; Hasani *et al.*, 2018).

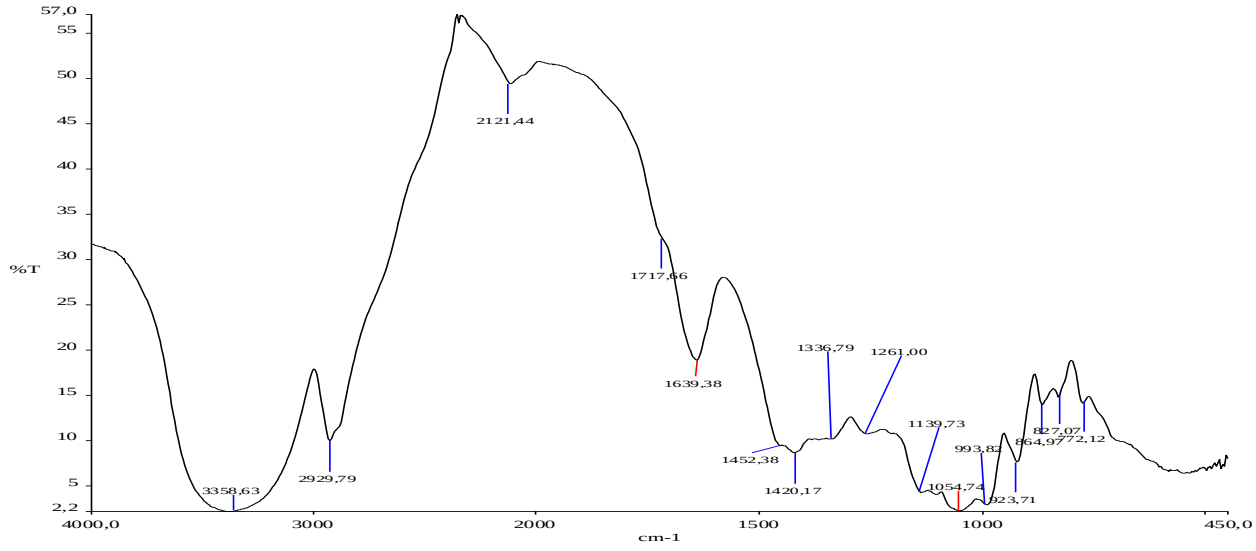

Figure 1. FTIR result of foam extract from *Gypsophila* root.

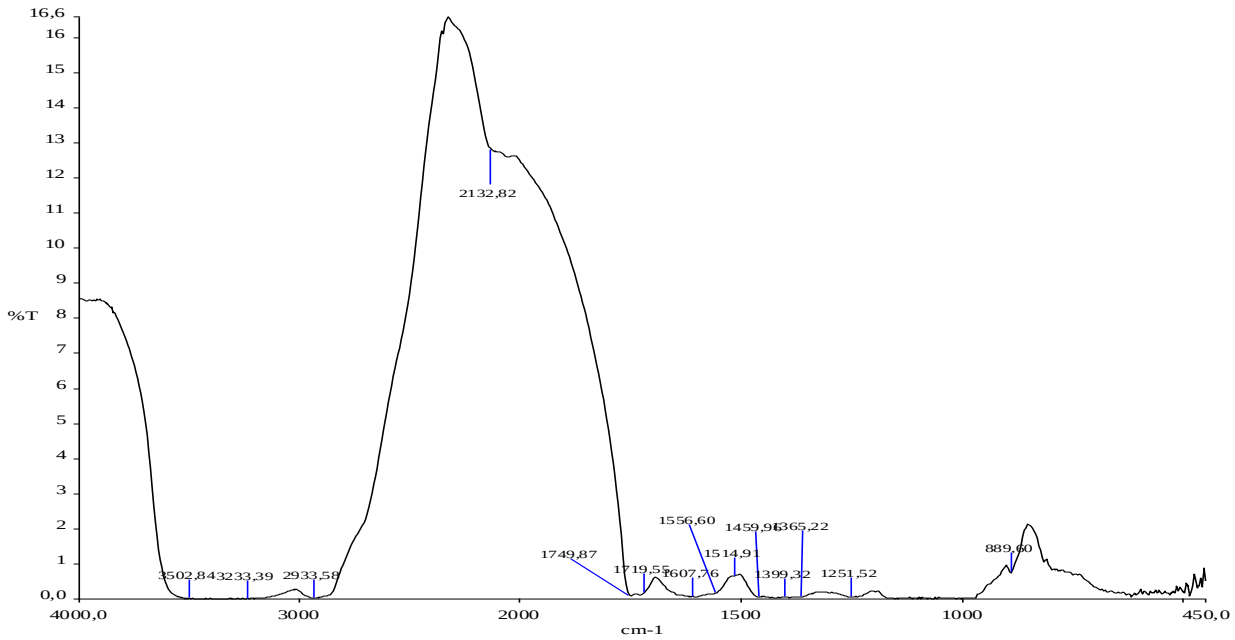

Figure 2. FTIR result of distilled water extract from *Gypsophila* root.

Determination of organic acids and sugars by HPLC

The importance of organic acids and sugars for living organisms has been demonstrated by various researchers. Organic acids are important bioactive compounds, and have an active role in antioxidant defence (Angonese *et al.*, 2021). It has been reported that these compounds have antioxidant, antimicrobial, and protective effects on DNA, and are also effective in enzyme inhibition (Zheleva-Dimitrova *et al.*, 2018; Khan *et al.*, 2020).

Based on Table 1, it was determined that the extracts contained high amounts of glucose and fructose. The presence of rhamnose could not be detected. It was determined that the GRF structure contained 621.43 µg/mL glucose and 300.19 µg/mL fructose. In the GRE structure, the amount of glucose was found to be 638.33 µg/mL, and the amount of fructose was 312.13 µg/mL. Based on the results of organic acid analysis, the amount of maleic acid in the GRF structure was 99.51 µg/mL, and the amount of acetic acid was 64.16 µg/mL. In the GRE structure, only maleic acid (94.49 µg/mL) was determined. Other organic acids could not be detected in the samples.

In addition to organic acids, some secondary metabolites were also found in the structure of the samples. It is known that secondary metabolites have important biological activities in the structure of organisms. Based on the HPLC results, 33.24 µg/mL acetoin and 76.10 µg/mL 2,3-butanediol was detected in the GRF structure. Similarly, 61.03 ng/mL acetoin and 64.66 µg/mL 2,3-butanediol were found in the GRE structure. In previous studies, bioactive components of different *Gypsophila* species were analysed by HPLC. Previous studies observed that these species are quite rich in phenolic contents (Yazici and Özmen, 2018; Altay *et al.*, 2019). However, studies determining the organic acid and sugar contents could not be found.

Table 1. Bioactive compounds of extracts obtained from *Gypsophila* root.

Sugar	GRF (µg/mL)	GRE (µg/mL)
Glucose	621.43	638.33
Fructose	300.19	312.13
Organic acid and metabolite		
Maleic acid	99.51	94.49
Acetic acid	64.16	ND
Acetoin	33.24	61.03
2,3-butanediol	76.10	64.66

GRF: *Gypsophila* root foam, GRE: *Gypsophila* root extract, ND: not detected.

Antioxidant activity

Cu^{+2} and Fe^{+3} reduction methods (CUPRAC and FRAP), DPPH and ABTS radical removal methods, and total antioxidant activity methods were

performed to determine the antioxidant activities of the extracts. CUPRAC and FRAP results were measured in Trolox equivalents (µg TE/mL), total antioxidant results in % inhibition of lipid peroxides, and radical removal in IC_{50}. The results are shown in Table 2.

Table 2. Comparison of the antioxidant activity results of the extracts with the results of standard antioxidants.

Sample	FRAP (µg TE/mL)	CUPRAC (µg TE/mL)	DPPH (IC_{50})	ABTS (IC_{50})	Lipid peroxidation (%)
RF	11.53 ± 0.23	1.17 ± 0.01	26.69 ± 1.11	67.54 ± 4.66	29.6 ± 0.38
RE	17.82 ± 4.37	1.35 ± 0.03	24.01 ± 0.19	92.02 ± 8.48	34.6 ± 0.09
BHA	112.37 ± 0.25	5.47 ± 0.02	14.24 ± 0.11	14.38 ± 0.37	55.2 ± 0.09
BHT	86.24 ± 1.66	4.38 ± 0.02	14.91 ± 0.02	14.35 ± 0.23	52.9 ± 0.02
AA	199.54 ± 1.83	2.29 ± 0.23	12.66 ± 0.06	13.59 ± 0.09	43.6 ± 0.22

TE: Trolox equivalent.

Based on the results, it was found that the standards (BHA, BHT, and AA) generally showed better activity than the extracts. In addition, it was found that the water extract generally performed better than the foam extract; however, the activities of the extracts were close to each other. Inhibition of ferric and cupric ions by samples is very important in terms of preventing oxidative damage. Free ferric and cupric ions are transformed into highly reactive superoxide and hydroxyl radicals due to Fenton and Haber-Weiss reactions. It is known that these radicals attack various tissues in the body, and cause oxidative damage that leads to diseases (Bayarsaikhan *et al.*, 2019). Based on the results, it was determined that both extracts were effective in reducing ferric and cupric ions. Based on the CUPRAC results, it was observed that the extracts showed activity similar to the standards. In previous studies using different *Gypsophila* species, the extracts were reported to be potent reducing agents based on the results of CUPRAC and FRAP assays (Zheleva-Dimitrova *et al.*, 2018; Altay *et al.*, 2019). Although the results of previous work are similar to those reported in the present work, it is thought that the activities varied due to the use of different species.

Peroxidation, which occurs as a result of the reaction of free radicals with lipids, leads to various diseases (Park *et al.*, 2021). In the present work, the ferric thiocyanate method was used to measure the inhibition of lipid peroxides. The percentages of inhibition of lipid peroxides by the extracts and standard antioxidants at the highest concentration (30 mg/mL) were as follows: BHA (55.2%) > BHT (52.9%) > AA (43.6%) > GRE (34.6%) > GRF (29.6%); the extracts exhibited more moderate activity than the standards.

DPPH and ABTS radical removal are among the most preferred methods among radical scavenging methods (Aboagye *et al.*, 2021). DPPH analysis is widely used because it takes a relatively short time. The DPPH free radical is very stable, reacts with compounds capable of donating hydrogen atoms, and reaches its maximum absorbance value at about 515 nm (Gülçin, 2006a; 2006b; Polat Köse *et al.*, 2015; Tohma *et al.*, 2017). The method is based on the scavenging of DPPH by antioxidants and measures the ability of antioxidants to reduce DPPH radicals (Škrovánková *et al.*, 2012).

Based on the DPPH radical removal results, it was found that the extracts were close to each other and had strong scavenging effects. The % radical scavenging activities of samples and standards at the highest concentration were ranked as follows: AA (89.68%) > BHA (81.71%) > BHT (76.40%) > GRE (47.49%) > GRF (43.07%). There are several studies in the literature reporting that *Gypsophila* species are potent DPPH radical scavengers (Zheleva-Dimitrova *et al.*, 2018; Altay *et al.*, 2019).

ABTS radical removal results were also observed to be similar to DPPH results. It was found that the antiradical activity of the extracts was moderate but effective as compared to the standards. It was observed that the activities increased with increasing concentration. The % ABTS radical removal activities of samples and standards at the highest concentration were as follows: AA (85.71%) > BHT (80.95%) > BHA (79.05%) > GRF (20.00%) > GRE (15.71%). We were able to find very limited

studies on the antioxidant properties of *Gypsophila* species in the literature. Altay *et al.* (2019) investigated the biological activities of water and methanolic extracts of different *Gypsophila* species. They reported that the extracts had more moderate activities than standard antioxidants (BHA, BHT, α-tocopherol, and Trolox). It can be said that the results obtained are parallel to our study and support our results.

Anticholinergics activity

Today, the inhibition of cholinesterases has emerged as an encouraging approach to alleviate the symptoms of Alzheimer's Disease (AD). Therefore, the inhibition of acetylcholinesterase (AChE) and butyryl-cholinesterases (BChE) enzymes is one of the main goals of researchers. Various cholinesterase inhibitors such as galantamine, tacrine, and rivastigmine are produced synthetically (Gülçin *et al.*, 2016; Taslimi *et al.*, 2017; Biçer *et al.*, 2019). However, these drugs have side effects such as nausea, diarrhoea, dizziness, and vomiting (Martins and Ferreira, 2017). For these reasons, the discovery of natural and safe enzyme inhibitors has become inevitable. The enzyme inhibition properties of *Gypsophila* extracts tested against AChE and BChE enzymes were examined by comparing them with galantamine. GRE exhibited strong acetylcholinesterase and butyryl-cholinesterase inhibition activity at 500 µg/mL with 0.013 ± 0.001 and 0.037 ± 0.002, respectively (Table 3). In addition, this extract showed a better AChE and BChE inhibitory effect than galantamine, a cholinesterase inhibitor used as a therapeutic agent in the treatment of AD. Galantamine which is used as standard AChE and BChE inhibitors was found to have IC_{50} values of 0.066 ± 0.009 and 0.179 ± 0.010 µmol/L, respectively, against these enzymes. GRF also showed a stronger inhibitory effect compared to galantamine. As shown in the table, the two *Gypsophila* extracts tested appeared to effectively inhibit AChE and BChE enzymes.

Studies on enzyme inhibition of different *Gypsophila* species in previous studies are in parallel with the results of the present work. However, the results may not support each other due to the differences in the chemical structure of the extracts. Zheleva-Dimitrova *et al.* (2018) reported that different *Gypsophila* species strongly inhibited the AChE enzyme, but could not be effective on the BChE enzyme. In another study, it was reported that water and methanolic extracts of various *Gypsophila* species were very effective inhibitors of AChE and BChE enzymes (Altay *et al.*, 2019).

Table 3. Anticholinesterase activity of *Gypsophila* extracts.

Sample	AChE (IC_{50} value)	BChE (IC_{50} value)
GRF	0.014 ± 0.002	0.044 ± 0.005
GRE	0.013 ± 0.001	0.037 ± 0.002
*Galantamine	0.066 ± 0.009	0.179 ± 0.010

GRF: *Gypsophila* root foam, GRE: *Gypsophila* root extract. *Galantamine was used as positive control for AChE and BChE enzymes, and expressed as µM levels. Cholinesterase inhibitory activity of the extracts was tested against acetylcholinesterase and butyrylcholinesterase at 500 µg/mL concentration.

DNA protective activity

Changes that occur as a result of the interaction of any compound with DNA can be analysed by looking at the transformations in different forms of DNA. Form I, where DNA is most stable, shows its superhelical structure. When a DNA strand in Form I structure is broken, the less stable Form II structure occurs. The changes in the structure of DNA are followed by the bands that occur in the gel images, giving information about the effect of the compounds on the DNA (Zhang *et al.*, 2001).

In the present work, the gel electrophoresis method was used to examine the effect of the samples on the DNA. The DNA protective activity of the samples was tested using pUC18 plasmid DNA. Based on this method, the ability of the samples to prevent DNA damage was evaluated in the presence of H_2O_2 and DMSO, which are the factors that cause damage to DNA. The gel image obtained as a result of the study is shown in Figure 3.

When the gel image was examined, it was found that H_2O_2 and DMSO had a scavenging effect on DNA. It was observed that GRF and GRE kept the DNA stable, but could not remove the scavenging effect of H_2O_2 and DMSO. In addition, it was determined that GRF could not protect Form I and Form II structures of DNA at high concentrations (1 mg/mL), while it helped to stabilise DNA at low concentrations (0.25 and 0.5 mg/mL). It was observed that GRE showed good activity at all concentrations, and protected Form I and Form II structures of DNA.

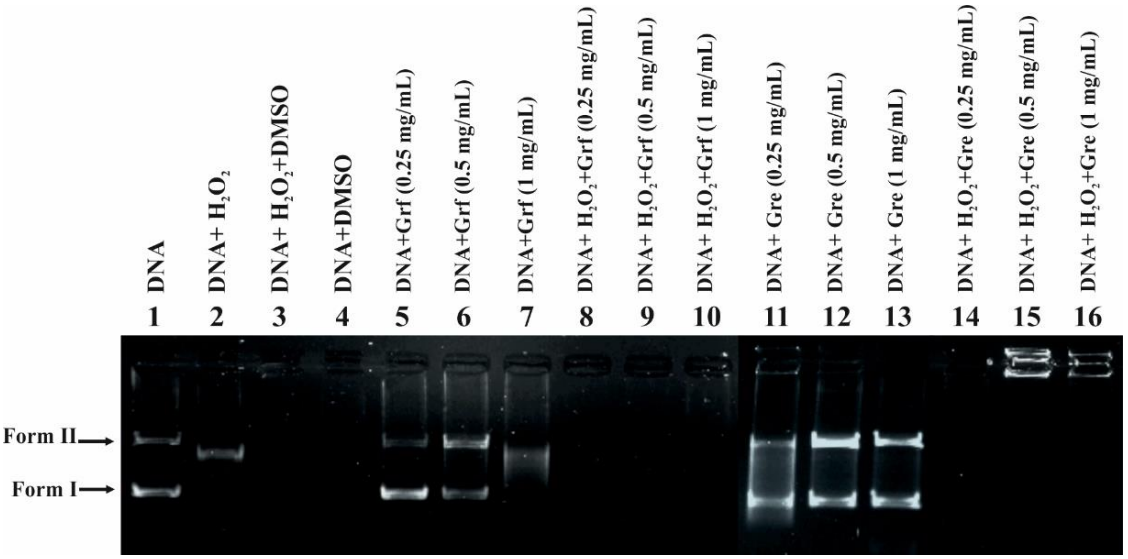

Figure 3. Gel image of DNA protective activity of extracts obtained from *Gypsophila* root.

The development of new-generation drugs by *in vivo* and *in vitro* experiments, and studies on DNA in the evaluation of the biological activities of consumed foods attract the attention of researchers (Wang *et al.*, 2021). No DNA studies related to *Gypsophila* were found in the literature search. For this purpose, the effect of samples on DNA was investigated in the present work. However, various studies are reporting that plants have a protective effect on DNA thanks to the bioactive compounds in them to ensure that the DNA remains stable (Wang *et al.*, 2021).

Conclusion

The foam part of the dessert which is popularly called *kerebiç* is obtained from the root of *Gypsophila*. To the best of our knowledge, the studies on *kerebiç* are very limited. No study was found on the foam obtained from the root of *Gypsophila*. For this purpose, foam and water extracts were obtained from the root of *Gypsophila*. Saponin, organic acid, and sugar contents of the extracts were then analysed. Next, the *in vitro* biological activities of the extracts were investigated. FTIR results confirmed the terpene and sugar contents of the extracts, and HPLC results confirmed the sugar and organic acid contents. Based on the antioxidant results, the extracts showed similar and more stable activity than standard antioxidants. DNA studies showed that the extracts had protective activity on plasmid pUC18 DNA. Finally, it was determined that the extracts inhibited AChE and BChE enzymes more than the control. The results reported herein could fill an important gap in the literature. However, it is still necessary to further elucidate the structures of the extracts, and to determine their biological activities with more *in vitro* and *in vivo* studies.

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