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Bioactive properties, antibacterial activity, and color features of *Polygonum cognatum*: The effects of frozen storage and cooking process

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Abstract

In this study, the effects of frozen storage (for six months at -30 °C) and cooking on total phenolic, antioxidant activity and color characteristics of *Polygonum cognatum* Meissn. were determined. In addition, the antibacterial activity of the ethanol extract of the plant against *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Bacillus subtilis*, and *B. cereus* was investigated. Both frozen storage and cooking have been found to cause significant decreases in the total phenolics. During the storage, differences among DPPH radical scavenging activities were statistically insignificant. However, the DPPH activity has been found to increase significantly with cooking. Copper-reducing antioxidant capacity has started to decrease considerably after four months of storage. Cooking process slightly increased the copper-reducing capacity. Various changes were detected in the color properties of both frozen stored and cooked samples. The ethanol extract of the plant was effective against all the tested bacteria at different ratios.

Keywords: *Polygonum cognatum* Meissn.; madimak; frozen storage; cooking; bioactivity; color characteristics.

Practical Application: Since *Polygonum cognatum* is fresh for only a few months of the year, it is usually stored frozen and consumed after cooking. In this study, frozen storage and cooking effects on the bioactivity of *P. cognatum* were studied. As a result of the study, although it has some negative effects, frozen storage has been found to be useful for the storage of *P. cognatum*.

1 Introduction

Polygonum cognatum Meissn., locally known as "madimak" in Turkey, is a wild edible plant belonging to the family Polygonaceae and grows naturally between 720 and 3000 m altitudes above sea level on the edges of fields, roadsides, pastures, slopes and cliffs (Kibar & Kibar, 2017). *P. cognatum* is a 15-30 cm long, pinkish flower and small green leafy perennial plant lying on the ground (Baytop, 1999; Sayili et al., 2013; Kibar & Kibar, 2017). As *P. cognatum* is resistant to different climatic conditions, it is a plant that grows in various parts of Anatolia, especially in Tokat, Sivas, and Erzurum provinces of Turkey. Meals made using the leaves and young shoots of *P. cognatum* plant can be considered as regional dishes of these cities (Kibar & Kibar, 2017). Dishes such as salad, meal, patty, and even omelets made using this plant are consumed lovingly by the regional people. Although there are few scientific studies about the plant, the local people traditionally believe that the plant has a diuretic effect and balances blood sugar (Gürağaç-Dereli et al., 2019).

The plant is harvested in spring (from April to June) and sold in local markets. Since the plant is only fresh for a few months, locals freeze the plant in deep-freezer and generally consume it cooked throughout the winter. As it is well known, the freezing method is one of the most common methods used to maintain the quality of fruits and vegetables for a long time (Sahari et al., 2004). By the frozen storage method, the chemical, biochemical and microbiological reactions in the food products are minimized and the color, flavor and nutritional values of the products are preserved (Ancos et al., 2000).

Free radicals are molecules or molecule fragments that contain one or more unpaired electrons in atomic or molecular orbitals that are not stable and are highly reactive. In order to be stable, these molecules try to trap electrons from other molecules and as a result of this, oxidative stress may occur in living organisms (Halliwell & Gutteridge, 1999; Chauhan & Chauhan, 2006; Valko et al., 2007; Carocho & Ferreira, 2013). Living organisms have antioxidative protection systems to cope with the aforementioned problems. However, in some cases the antioxidative protection system is insufficient and free radicals can cause cellular damage. Therefore, supplementary medicines or nutrients are needed to combat oxidative stress (Niki, 2010; Vieitez et al., 2018). Antioxidant molecules neutralize free radicals that damage the body. As a result of neutralizing free radicals, many biomolecules work properly in the body. Thus, the risk of encountering pathological conditions is reduced (Halliwell, 1997; Sindhi et al., 2013). Previous studies have shown that many plant extracts have strong antioxidant and antimicrobial activity due to their bioactive compounds (Parejo et al., 2003; Gupta & Sharma, 2006; Taghvaei et al., 2014; Xu et al., 2017).

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There are a few studies which examine the bioactive compounds of *P. cognatum*. In one of these studies, the total phenolic content, antioxidant and antimicrobial activity of *P. cognatum* extract was investigated by Yildirim et al. (2003). However, there is no study investigating the effect of frozen storage and cooking on the color and bioactivity of *P. cognatum*.

The aim of this study was to determine the bimonthly changes in antioxidant activities, total phenolic content, and color characteristics of *P. cognatum* samples during the six months of frozen storage period and to compare them with the fresh-dried sample. In addition, the analyses were carried out on the samples that were frozen and stored for six months to determine the effect of cooking on color characteristics, antioxidant activities and total phenolic content. Moreover, the antibacterial activity of fresh-dried plant extract against certain foodborne pathogen bacteria has been examined.

2 Materials and methods

2.1 Plant samples

P. cognatum samples were purchased from the local market of Tokat, Turkey in May and used two days later. The leaves and young shoots of the plant were used, and plant samples were separated for analysis of fresh-dried, frozen and cooked samples.

2.2 Preparation of fresh-dried samples

The fresh sample was dried on Whatman filter paper at 50 °C in the oven for 24h.

2.3 Preparation of frozen samples

The samples were separately packaged in sealed bags similar to the conventional condition for the frozen samples analysis. Packaged samples were stored in deep freezer at -30 °C for 60, 120 and 180 days. Bimonthly, the frozen samples were dried on Whatman filter paper at 50 °C in the oven for 24 h after being thawed at room temperature.

2.4 Preparation of cooked samples

The cooking process was carried out with slight modification in the method applied by Turkmen et al. (2005). The frozen plant sample for six months was thawed at room temperature before cooking. 150 mL of distilled water was added to 100 g plant sample that had just reached the boiling point in a stainless steel pan and cooked by constantly stirring with a spoon until reaching a temperature of 105 °C for 10 min. The temperature was monitored using a thermometer. After cooling at room temperature, the samples were dried just as fresh-dried and frozen samples.

2.5 Determination of color

The surface color of the plant samples (fresh-dried, frozen and cooked samples) were measured by using a chromameter (Lovibond RT Series Reflectance Tintometer, UK) calibrated against a standard white plate. According to CIELAB color notation system, L*, a*, b* designated lightness (L*; black = 0, white = 100), redness (+a* = red, -a* = green) and yellowness (+b* = yellow, -b* = blue) respectively. Plant samples were homogenized and placed in a Petri dish for the evaluation.

2.6 Preparation of the extracts

Dried samples (fresh-dried, frozen and cooked samples) were ground to a fine powder with a grinder. Then, 15 g powdered material was mixed with 150 mL of ethanol:water mixture (80:20 v/v, Merck, Germany) in glass bottle with lid on an orbital shaker at 150 rpm and room temperature in the dark. Following overnight extraction, the supernatant was separated from the pulp by using Whatman filter and total phenolic content, DPPH radical scavenging activity and copper-reducing antioxidant capacity analyses were performed.

2.7 Quantification of total phenolic content

Folin-Ciocalteu method was used to determine the total phenolic content of plant extracts (Singleton et al., 1999). The plant extracts were diluted at 1:20 with distilled water and 0.5 mL of diluted extracts were placed in a reaction tube. 2.5 mL Folin-Ciocalteu phenol reagent (0.2 N) was added into the tube. After 3 min, 2 mL of 2% (w/v) Na_2CO_3 solution was added into prepared mixture. After keeping the mixture for 30 min in a dark place at room temperature, the absorbance of the samples was detected at 760 nm using a spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Japan) against distilled water. The results were expressed as gallic acid equivalents (GAE) in milligrams per 100 g of sample weight using the calibration curve obtained from gallic acid.

2.8 Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The radical scavenging activity of the plant extracts against DPPH was specified spectrophotometrically using the method described by Singh et al. (2002) with slight modifications. The plant extracts (0.1 mL) were mixed with 4.9 mL ethanolic DPPH solution (0,1 mM), vortexed and incubated for 20 min in a dark place at 27 °C. After the incubation period, the absorbance of the samples was measured with a spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Japan) at 517 nm against ethanol. The control was prepared as above without the extract. The percentage of DPPH inhibition values of the samples were calculated using the following formula (Equation 1):

$$\%\ \text{DPPH radical scavenging activity} = 100\ \text{x}\left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right) \quad (1)$$

2.9 Measurement of antioxidant activity by copper-reducing method (CUPRAC)

The copper-reducing capacity of the plant extracts was determined using the method described by Apak et al. (2004) with slight modifications. 1 mL of each of copper (II) chloride (0.01 M), neocuproine (7.5 mM), 1 M ammonium acetate buffer (pH 7.0) solutions and distilled water were mixed in a reaction tube. The plant extracts were diluted at 1:25 with distilled water and 0.1 mL of the diluted extracts were put into a reaction tube.

The total volume was adjusted with micropipette to 4.1 mL in each reaction tube. All samples were incubated for 60 min in the dark place at room temperature before absorption was measured. At the end of 60 min, the absorbance values of the samples were measured at 450 nm using a spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Japan) against a reagent blank. The results of copper-reducing antioxidant capacity were expressed as mg TE (Trolox equivalent) per g sample.

2.10 Procedure for antibacterial activity

Only the antibacterial activity of the fresh-dried plant extract was tested. Five bacterial strains were used as test bacteria: *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *B. cereus* ATCC 11778. These bacteria were obtained from the microbial culture collection of Yıldız Technical University, Food Engineering Department (Istanbul, Turkey). Antibacterial activity of the fresh-dried plant extract was determined using the agar diffusion method (Sagdic et al., 2008). The bacteria cultures were grown in nutrient broth (Merck-Darmstadt, Germany) at 37 °C for 22-24 h. Suspensions (100 µL) of the bacteria, adjusted to 10^6-10^7 cfu/mL final cell concentration, were added to flasks containing 25 mL sterile nutrient agar (Merck-Darmstadt, Germany) and poured into Petri dishes (9 cm diameter). Then the agars were allowed to solidify at 4 °C for 60 min. Four equidistant holes were made in the agar using sterile cork borers (Ø = 6 mm). An aliquot of fresh-dried extract sample (0.5 g) was dissolved in 4.5 mL of pure ethanol (Merck-Darmstadt, Germany). Plant extracts (20 µL) were prepared in pure ethanol and applied to the holes using a micropipette. It was used as a control because pure ethanol had no inhibitory effect. The bacteria were grown on the agar at 37 °C for 24 h and the inhibition zones were measured in millimeters (mm) at the end of the period.

2.11 Statistical evaluation of the results

Statistical analysis was performed by using IBM SPSS Statistic (Version 24), all experiments were carried out in triplicate and the data were expressed as means ± standard deviations. The results of all assays were subjected to one-way variance analysis (ANOVA) and post hoc comparisons were made using the least significant difference (LSD) and Games-Howell tests.

Since the variances of the total phenolic content, copper-reducing antioxidant capacity (CUPRAC) and color values were homogeneous, the LSD test was applied to determine the difference between the means, and the Games-Howell test was applied to determine the difference between the means since the variances of the DPPH radical scavenging activity values were not homogeneous. Differences between values at the 95% and 99% ($P \leq 0.05$ and $P \leq 0.01$) confidence levels were considered statistically significant.

3 Results and discussion

3.1 Total phenolic content

The total phenolic content of fresh-dried, frozen and cooked *P. cognatum* extracts are summarized in Table 1.

The total amount of phenolic content in the fresh-dried plant sample was measured as 124.85 mg GAE/100 g. This total phenolic value found was higher than the ones reported in the previous studies (Yildirim et al., 2003; Samancioglu et al., 2016). This difference could be attributed to the growing conditions and experimental procedure.

During the study, the total phenolic content decreased to 67.10 mg GAE/100 g ($P \leq 0.05$) after storage at -30 °C for two months, this decline continued in a regular manner, and decreased to 27.93 mg GAE/100 g ($P \leq 0.05$) at the end of six months. In the literature, there is no study about the effects of frozen storage on the content of bioactive compounds and color scores of *P. cognatum*. Similar to our results, Turkben et al. (2010) found that the amounts of total phenolic content of various raspberry samples were significantly decreased ($P \leq 0.01$) as a result of just-frozen and frozen storage (six months at -22 °C) storage compared to the fresh sample. In addition, Michalczyk & Macura (2010) have determined that total phenolic compounds of shadblow serviceberry are decreased after frozen storage for six months at -23 °C. The reason for decreasing the amount of phenolic compounds in frozen storage could be the result of the degradation of the phenolic compounds by the polyphenol oxidase enzyme (PPO) which is released by the broken cells (Michalczyk & Macura, 2010).

The findings reveal that after cooking the plant sample, the total phenolic content decreased to 25.88 mg GAE/100 g, which was statistically significant ($P \leq 0.05$) when compared to the fresh-dried and uncooked samples stored for six months. Similar decreases in the amount of phenolic substances due to boiling were observed in squash, pea and leek samples (Turkmen et al., 2005), and carrot, cauliflower and spinach samples ($P \leq 0.05$) (Mazzeo et al., 2011).

Table 1. Variations in total phenolic content, DPPH radical scavenging, and copper-reducing antioxidant activities of fresh-dried, frozen and cooked *Polygonum cognatum* ethanol extracts.

	Storage time (day) at -30 °C				
	Fresh-Dried	60	120	180	Cooked
Total phenolic content (mg GAE/100 g)	124.85 ± 0.18^{a*}	67.10 ± 0.09^{b}	46.74 ± 0.45^{c}	27.93 ± 0.45^{d}	25.88 ± 0.23^{e}
DPPH radical scavenging inhibition percent (%)	67.46 ± 3.61^{a}	66.00 ± 4.62^{a}	64.77 ± 0.90^{a}	62.14 ± 0.31^{a}	90.22 ± 1.66^{b}
Copper-reducing antioxidant capacity (mg TE/g)	6.04 ± 0.19^{a}	5.71 ± 0.55^{a}	3.54 ± 0.23^{b}	3.28 ± 0.37^{b}	3.52 ± 0.18^{b}

*Values are mean ± standard deviations of three parallel measurements. a-e; means different letters within the same rows are statistically significant ($P \leq 0.05$).

Phenolic compounds are generally known as heat-sensitive components. However, recent studies have shown that it is not possible to say definitively that heat treatment affects the number of phenolic compounds positively or negatively. Complex structures of phenolic compounds affect their behavior during heat treatment. Some phenolic compounds become inactive after heat treatment while some phenolic compounds can be released (Meral, 2016). Our study has shown that heat-treatment significantly decreased ($P \leq 0.05$) phenolic compounds. This decrease in phenolic compounds could be due to the breakdown of the phenolic compounds during the cooking process (Crozier et al., 1997; Turkmen et al., 2005) but more study needs to be done to clarify the nature of this phenomenon.

3.2 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity of the fresh-dried plant extract was determined to be 67.46%. After two months of storage at -30 °C, this value decreased slightly to 66.00%, but this change was not statistically significant ($P > 0.05$). This statistically insignificant decline continued regularly during the 4th and 6th months ($P > 0.05$) (Table 1). In the case of the cooked plant sample, it was determined that the radical scavenging activity value significantly increased compared to the uncooked sample stored for six months and the fresh-dried sample ($P \leq 0.05$). The observed increase in DPPH radical scavenging activity after cooking operation may be due to different reasons. Some researchers claim that pro-oxidants and antioxidant compounds may be formed depending on the degree of heat treatment applied. These newly formed antioxidant compounds may be the end products of the Maillard reaction (Meral, 2016). Besides, the increase in antioxidant activity may also be the result of the inactivation of oxidative enzymes by heat treatment (Pinelo et al., 2005; Meral, 2016). In a study in which the antioxidant activity of pepper, green beans, broccoli, and spinach samples was determined by DPPH radical scavenging method, it was determined that cooking of boiling, steaming and microwave significantly increased ($P \leq 0.05$) antioxidant activity (Turkmen et al., 2005). The authors of this study thought that the reason for this increase might be the inhibition of peroxidase enzymes due to the cooking process (Gazzani et al., 1998; Turkmen et al., 2005).

P. cognatum was reported to have the highest DPPH radical scavenging activity among the 21 wild edible plants grown in Turkey (Samancioglu et al., 2016). Yildirim et al. (2003) found that DPPH radical scavenging activity of *P. cognatum* changed depending on the concentration of ethanol extract used. They found that radical scavenging activity was 4% when the dried ethanol extract was used at a concentration of 25 μg mL^{-1}, while it was found to be 12% when used at 100 μg mL^{-1} concentration. The variation in the values obtained in the present study may be due to the difference in the concentration, growing condition of the plant and the experimental methodology adopted for the study.

3.3 Copper-reducing antioxidant capacity (CUPRAC)

The copper-reducing antioxidant capacity of the fresh-dried plant extract was measured 6.04 mg TE/g. This value decreased to 5.71 mg TE/g at the end of the two months storage period at -30 °C of the plant sample ($P > 0.05$), decreased considerably after four months of storage ($P \leq 0.05$) and further to 3.28 mg TE/g at the end of six months ($P > 0.05$), whereas increased to 3.52 mg TE/g after the cooking process ($P > 0.05$) (Table 1). In the light of these results, it can be claimed that the antioxidant activity results obtained by the CUPRAC method show similar behavior as the results obtained by the DPPH radical scavenging method. Similar results were reported by the research of Chaovanalikit & Wrolstad (2004) which indicated that frozen storage of cherry samples at -23 °C led to a steady decrease in antioxidant activity as measured by ORAC and FRAP methods.

In our study, there was a significant decrease ($P \leq 0.05$) in the amount of total phenolic content by cooking, whereas showed a significant increase ($P \leq 0.05$) in DPPH antioxidant activity and an insignificant increase ($P > 0.05$) in CUPRAC antioxidant activity compared to frozen uncooked sample stored for six months. In line with this, Turkmen et al. (2005) reported that the total phenolic content of the squash sample significantly decreased ($P < 0.05$) by boiling, while antioxidant activity showed an insignificant increase ($P > 0.05$). Murakami et al. (2004) determined that radical scavenging activity was more stable to heat treatments than the content of the original polyphenols. They showed that some heat-treated products deteriorated and thus increased antioxidant activity. On the other hand, Mazzeo et al. (2011) observed a significant decrease ($P \leq 0.05$) both in total phenolic content and antioxidant activities in cauliflower and spinach samples after boiling compared to uncooked ones. While it is widely accepted that there is a correlation between antioxidant activity and phenolic compounds, it is difficult to say exactly what effects heat treatments have on phenolic and antioxidant activity. Further investigations are needed to better understand this issue.

3.4 Color

L*, a* and b* color values obtained from fresh-dried, frozen and cooked plant samples are summarized in Table 2.

Table 2. Color parameters for fresh-dried, frozen and cooked *Polygonum cognatum* samples.

	Storage time (day) at -30 °C				
	Fresh-Dried	60	120	180	Cooked
L*	47.32 ± 1.58^{a*}	36.90 ± 3.46^{b}	39.32 ± 1.57^{bc}	37.75 ± 2.45^{b}	42.92 ± 0.95^{c}
a*	-1.79 ± 0.75^{a}	1.16 ± 0.74^{b}	0.67 ± 0.31^{b}	-0.32 ± 0.20^{c}	-2.34 ± 0.5^{a}
b*	19.75 ± 0.74^{a}	8.25 ± 1.10b^{c}	6.77 ± 1.27^{c}	9.04 ± 1.45^{b}	9.88 ± 0.31^{b}

*Values are mean ± standard deviations of three parallel measurements. a-c; means different letters within the same rows are statistically significant ($P \leq 0.05$).

The color of the external surface of the fresh-dried plant sample had a lightness of 47.32 ± 1.58 (L*), greenness of -1.79 ± 0.75 (-a*), and yellowness of 19.75 ± 0.74 (b*). L* and b* values significantly decreased ($P \leq 0.05$) after storage at -30 ˚C for two months while a* value significantly increased ($P \leq 0.05$) after storage at -30 ˚C for two months.

Regarding the brightness (L*) of the plant samples, irregular insignificant increases and decreases were observed during the frozen storage. It was determined that the brightness value (L*) decreased to 37.75 ± 2.45 at the end of six months storage and this value is statistically significant compared to the fresh-dried sample ($P \leq 0.05$). The brightness value (L*) of the cooked plant sample was found to be 42.92 ± 0.95. This value which is also statistically significant compared to the fresh-dried sample and the uncooked sample stored for six months ($P \leq 0.05$). Compatible with our results, Pellegrini et al. (2010) reported that the L* value of the frozen broccoli samples increased after being cooked ($P \leq 0.05$).

The redness value (a*) started to decline after the increase in the 2nd-month and decreased to -0.32 ± 0.20 at the end of the 6th-month. It was found that the greenness value (-a*) increased after the plant sample was cooked and this increase was statistically significant ($P \leq 0.05$) compared to the uncooked sample stored for six months. Moreover, it was determined that the greenness value (-a*) of the cooked sample slightly increased compared to the fresh-dried one ($P > 0.05$). In agreement to these results, Turkmen et al. (2006) determined that the greenness value (-a*) increased in the cooked spinach and pea samples. It was thought that this change might be the result of the destruction of the heat-treated cell membrane and the penetration of air into the cell cavities. Furthermore, Aktas & Bakkalbasi (2016) determined that the greenness value (-a*) increased after cooking in a study on the surface color of white cabbage.

Fluctuations were monitored in the yellowness (b*) value of the plant samples during the frozen storage period. It was recorded that the yellowness value (b*) was 9.04 ± 1.45 at the end of six months storage and that this value was statistically significant when compared with the fresh-dried sample ($P \leq 0.05$). The yellowness value (b*) of the cooked plant sample was found to be 9.88 ± 0.31. While this value is statistically significant ($P \leq 0.05$) compared to the fresh-dried plant sample, it was found to be statistically insignificant ($P > 0.05$) compared to the uncooked plant sample stored for six months. In accordance with our findings, Pellegrini et al. (2010) found that the yellowness value (b*) of frozen boiled cauliflower was slightly higher than frozen raw cauliflower, but this difference was not statistically significant ($P > 0.05$).

3.5 Antibacterial activity

The antibacterial activity of *P. cognatum* extract is demonstrated in Table 3. Pure ethanol (control) had no inhibitory effects on the five types of bacteria tested *in vitro*. The *P. cognatum* extract (extract/pure ethanol, 0.5 g/4.5 mL) showed antibacterial activities against all tested bacteria. The results of *in vitro* antibacterial activity assay revealed that the plant extract possessed broad antibacterial activity against the bacteria tested. The plant extract caused different inhibition zones on the tested bacteria. The most sensitive bacteria were *B. subtilis* and *B. cereus* while the most resistant bacteria was *S. aureus*. Various phenolic compounds such as phenolic acids are known to have antibacterial properties against some bacteria (Liu et al., 2020). In our study, it was determined that the total phenolic content of the *P. cognatum* plant was rich, and this situation is thought to contribute to the antibacterial activity of the plant.

Table 3. Antibacterial activities of ethanol extract of *Polygonum cognatum*.

Bacteria	Inhibition zones (cm)
Escherichia coli ATCC 25922	1.33 ± 0.32*
Salmonella Typhimurium ATCC 14028	1.30 ± 0.17
Staphylococcus aureus ATCC 25923	0.70 ± 0.10
Bacillus subtilis ATCC 6633	1.55 ± 0.15
Bacillus cereus ATCC 11778	1.40 ± 0.20

*Values are mean ± standard deviations of three parallel measurements.

There are very limited data on the antibacterial activity of *P. cognatum* grown in Turkey. In a study, Yildirim et al. (2003) investigated the antimicrobial effect of *P. cognatum* extract (extract/ethanol, 50 mg/50 mL) on *S. aureus*, *B. subtilis*, *E. coli*, *Candida albicans* and *Pseudomonas aeruginosa* by disk diffusion and determined that the plant extract had an antibacterial effect on *S. aureus* and *B. subtilis* as in this study. Contrarily to our outcomes, they determined that the plant had no antibacterial effect against *E. coli*. Although it is difficult to compare the results of different studies because of the different methods used for evaluation of antibacterial activities, further research is needed on the antibacterial activities of *P. cognatum*.

4 Conclusions

This study has shown that fresh-dried *P. cognatum* plant is rich in total phenolic compounds. In addition, high level of antioxidant and antibacterial activities of fresh-dried *P. cognatum* plant have been determined. The ethanol extract of the plant was found to be effective against the tested *B. subtilis*, *B. cereus*, *E. coli*, *S.* Typhimurium and *S. aureus* bacteria. As a result of the frozen storage of the plant samples, it has been determined that there is a significant decrease in the total phenolic compounds regularly throughout the storage period. At the end of six months, it was observed that there was a further decrease in the amount of phenolic content of the plant sample by cooking. Despite the decrease in the total amount of phenolic compound, there was no significant change in the DPPH radical scavenging activity of the plant samples during all storage periods. Interestingly, the DPPH radical scavenging activity significantly increased with the cooking process. Copper-reducing antioxidant capacities of plant samples were also investigated and the findings reveal that there was a further decrease than DPPH radical scavenging activity during storage. The cooking process resulted in an insignificant increase in the copper-reducing antioxidant capacity of the plant. Despite the decrease in the total phenolic content by cooking, the observed increase in the antioxidant activity has been interesting for us. Further research is needed to determine the cause of this condition. As a result of our study, it is possible to

say that although the frozen storage method has some negative effects, is useful in the storage of foods.

Conflict of interest

The authors declare that they have no conflicts of interest resulting from the contents of this article.

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