The effect of different drying techniques on some bioactive compounds and antibacterial properties of *Polygonum sivasicum*

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ABSTRACT Turkey has several endemic edible plant species having health benefits. *Polygonum sivasicum* is widely consumed as fresh or dried throughout the year by rural people in Sivas province. This study aimed to reveal the health benefits of *P. sivasicum* and to assess the effects of different drying methods on nutritional, phytochemical, and biological activities. *Polygonum sivasicum* leaves were collected from Sivas province and dried freeze, oven, microwave, and shade treatments. For each sample, total phenolic content and profile, antioxidant activity, mineral analysis and antibacterial properties were investigated. The highest phenolic content and antioxidant activity were observed in freeze-dried samples ($P < 0.05$). Rutin was detected as a major phenolic compound, followed by chlorogenic acid, kaempferol-3-O-glucoside, iso-rhamnetin-3-O-glucoside, ferulic acid, luteolin, and caffeic acid, respectively. The *P. sivasicum* leaves seem to be rich in potassium, phosphorus, and magnessium. *P. sivasicum* extracts showed antimicrobial activity against *S. aureus* and *L. innocua* depending on the extraction solvent. Freeze-drying method can be used as a convenient drying method due to preserve the total phenolic content and individual phenolic compounds.

KEYWORDS *Polygonum sivasicum*; Madimak; Phenolic profile; Antioxidant activity; Antibacterial activity; Drying techniques

1. Introduction

Polygonum cognatum called by the local people 'madimak' is a perennial herbaceous plant grown mainly in Central Anatolia, which belongs to Polygonaceae family. It has a thin stalk, 15 - 30 cm long in height. The leaves are rectangular-elliptical in shape, the flowers are pinkish and have a thin mucosal layer (Onen et al., 2011; Yildirim et al., 2003). The best-known endemic species of Polygonum genus are *Polygonum sivasicum*, *P. samsunicum*, *P. afyonicum*, *P. istanbulicum*, and *P. cappadocium* (Onen et al., 2011; Yildirim et al., 2003). *Polygonum sivasicum* was first described by Tan and Yıldız (1989) and named as Polygonum sivasicum Kit Tan & Yıldız. It differs from the other endemic *Polygonum cognatum* species by means of having minutely scabrous indumentum, equal to or longer ocrea than the node, very tiny pedicel and strongly revolute leaves (Keskin, 2009). The young shoots with leaves are collected in spring and sold in the local markets to be consumed raw as salads or cooked vegetable dishes in Sivas or are dried to be consumed throughout the year. It contributes significantly to human nutrition, especially in rural areas thanks to having rich phenolic content, antioxidant activity and mineral composition (Kibar and Kibar, 2017; Unlu et al., 2017). Therefore, determination of the nutritional properties and their preservation are important on revealing comparable or superior endemic species to cultivated vegetables.

Fruits and vegetables are highly perishable due to high water activity, limiting their shelf-life. In order to prolong the shelf-life, different drying techniques are applied, such as hot-air drying, shade drying, freeze-drying, and microwave drying (Hasan et al., 2019). Drying also enables storage stability and reduces the transportation weight (Sagar and Suresh Kumar, 2010). The choice of a suitable drying method is, therefore, significant. It dramatically affects the rehydration capacity, color, and nutritional properties. Madimak is dried conventionally using hot-air or shade drying techniques by the local people. The long drying period with a high temperature seriously affects the texture, color, and nutritional properties of madimak (Koca et al., 2018). Alternatively, freeze-drying is one of the most effective drying methods for fruits and vegetables on maintaining the physical, chemical, and nutritional properties; however, it is an expensive method due to requiring much energy consumption and requires a much longer drying time in comparison to microwave-drying. In this case, the absence of convection accelerates moisture removal in microwave drying by applying energy (Fan et al., 2019).

To date, some studies revealed the phenolic profiles, antioxidant activities, minerals compositions, and antibacterial activities of the *Polygonum cognatum* species collected from different provinces of Turkey (Yildirim et al., 2003; Guzelsoy et al., 2017; Kibar and Kibar, 2017; Unlu et al., 2017). The geographical differences play vital roles on both morphological characteristics and the biological activities of *P. cognatum* species (Keskin, 2009).

To the best of our knowledge, Eruygur et al. (2020) studied some biological activities of *P. cognatum* collected

Copyright © 2024 Latin American Applied Research
DOI: 10.52292/j.laar.2024.1968
Received: November 23, 2022.
Accepted: March 19, 2023.
Recommended by Diego Lomonaco.
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in Sivas province. Since the antioxidant, antimicrobial, cytotoxic, and enzyme inhibition activities are promising, there is still a need for further analyses and assessing a suitable drying method to preserve these biological activities. Therefore, this study aimed to determine some bioactive compounds and the antimicrobial activities of endemic *P. sivasicum* collected from Sivas province and to determine the effect of different drying methods on some nutritional, phytochemical and biological activities.

2. Methods

2.1 Collection of samples and drying experiment

Polygonum sivasicum young shoots with leaves were harvested in July 2017 from Suşehri in Sivas and the collection area was located on 40° 07' N and 37° 46' E. The collected plant materials were cleaned and brought to the laboratory with cold chain. They were stored at a temperature of 4±0.5 °C until drying process. Taxonomic identification of the species was carried out according to Tan and Yıldız (1989). Total phenolic content and profile, antioxidant activity, major and minor minerals, were determined for the fresh plants before drying process.

Drying of the samples were conducted using four different methods, which are freeze-drying, shade-drying, oven-drying and microwave-drying. For freeze-drying, 1 kg plant was kept at -18 °C in deep-freezer for 24 h. Lyophilization of samples was carried out in a freeze-dryer (Labogene, ScanVac Coolsafe 110-4, Lynge, Denmark). Throughout the drying operation, vacuum chamber pressure and condenser temperature were 2.0 mbar and -110 °C, respectively. For drying in the shade, 1 kg plant was laid on a tray at room temperature for 72 hours. For microwave oven drying method, the plant material was dried in a microwave oven (Arcelik, MD 564) at 600 W for 90 seconds. Lastly the same amount of the plant was spread on a tray and dried in an oven (Nuve, Turkey) at 50 °C for 24 h.

2.2 Extraction procedure

One g of ground dried plant was extracted by using a homogenizer (WiseMixTM HG-150; Daihan Scientific, Korea) in 50 mL methanol:water (Merck, Darmstadt, Germany) (80:20) mixture. The extract was centrifuged (NF 800R, Nuve, Turkey) at 9000 x g for 10 min. The supernatant was removed and put into a glass jar. The residue was re-extracted with the solvent mixture. Supernatants from two extractions were poured in a jar glass and stored at -18 °C.

2.3 Analysis of Total Phenolic Content and Profile

The total phenolic contents of fresh and dried *P. sivasicum* plant were determined by Folin–Ciocalteu procedure (Singleton and Rossi, 1965). The methanolic extract (0.5 mL) was mixed with 2.5 mL Folin–Ciocaleu's reagent (0.2 N) and 2.0 mL sodium carbonate (Merck, Darmstadt, Germany) (75 g·L^{-1}). Absorbance of the reaction mixtures was measured against the blank at 765 nm after 120 min using a spectrophotometer (U-1800, Hitachi, Japan). Results were expressed as mg gallic acid equivalents (GAE) g^{-1} DW.

The analysis of phenolic compounds in the extract was carried out by Agilent 1260 Infinity Series HPLC system equipped with diode array detector. Separation was achieved by a reverse phase C18 column (5 µm, 250×4.6 mm i.d.). Mobil phase consisted of acetic acid: water and water:acetonitrile:acetic acid, and the flow rate was 0.75 mL·min^{-1}. The detector was set at 280, 320 and 360 nm for phenolics. The identification of phenolics was confirmed by comparing their retention times and UV spectra. The data analysis was performed by ChemStation software (Agilent, Waldbronn, Germany).

2.4 Antioxidant Activity Analyses

For DPPH assay, 2,2-Diphenyl-1-picrylhydrazyl antioxidant activities of the plants were determined according to Brand-Williams et al. (1995). Extract (0.1 mL) was added to 3.9 mL of DPPH (6·10^{-5} M) methanolic solution. After 30 min incubation at room temperature in the dark, the absorbance was measured at 515 nm by using a spectrophotometer. Results were expressed as mmol Trolox Equivalent (TE) kg^{-1} DW.

For ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, the protocol, described by Re et al. (1999) was followed. The extract (10 µL) was added to 990 µL ABTS solution, generated with potassium persulfate (2.45 mm) and ABTS solutions (7 mm). The absorbance at 734 nm was measured after 6 min and the reduction in the absorbance was determined. Results were reported as mmol trolox equivalent (TE) kg^{-1} DW.

2.5 Mineral analysis

Fresh and dried samples (0.2 g) were digested in a microwave oven (MARS 5) at 200 °C and 1600 W with 10 mL of nitric acid (65 %) and diluted to 25 mL with ultrapure water (Millipore, USA). Major and minor minerals in the samples were determine by using inductively coupled plasma atomic emission spectrophotometer (ICP-AES) (Varian Vista Model; Vista AX, Varian, Victoria, Australia). Working conditions of ICP-AES was as follows: RF power, 0.7 - 1.5 kW (1.2 - 1.3 kW for axial); plasma gas flow rate (Ar), 10.5 - 15.0 L/min (radial) and 15 L/min (axial); auxiliary gas flow rate (Ar), 1.5 L/min; viewing height, 5-12 mm; copy and reading time, 1 - 5 s (maximum of 60 s); copy time, 3 s (maximum of 100 s) (Akbulut et al., 2009).

2.6 Antibacterial Activity

The antibacterial activity of methanol and acetone extracts was carried using the disc diffusion method. *Staphylococcus aureus* ATCC 43300, *Listeria monocytogenes* ATCC 13932, *Escherichia coli* ATCC 25922, *Listeria innocua* ATCC 33090 and *Enterecoccus faecalis* ATCC 51559 strains were used. Ten mL of Mueller-Hilton Agar (Merck, Darmstadt, Germany) was poured into sterile Petri dishes (as a basal layer) followed with 15 mL of seeded medium previously inoculated with bacterial suspension (100 mL of medium/1 mL of 10^7 CFU) to attain 10^5 CFU/mL of medium. The discs loaded with plant extract concentration of (15 uL/disc) were placed on the top of Mueller-Hilton agar plates. The acetone and methanol treated discs were used as controls. The plates were kept in the fridge at 5 °C for 2 h to permit plant extracts diffusion and then incubated at 35 °C for 24 h. The presence of inhibition zones was measured by a caliper (Insize, China), recorded and considered as indication for antibacterial activity. The diameter of the clear zones around each well was measured. The strains with clear zones less than 11 mm, 11 - 16 mm, 17 - 22 mm and more than 23 mm, were grouped as negative (-), mild (+), strong (++), and very strong (+++) inhibitor, respectively (Davoodabadi et al., 2015).

2.7 Statistical Analyses

Results were presented as means of ± standard deviations (SD), and subjected to one-way analysis of variance (ANOVA), at a confidence level of 95 %, to determine the effect of drying methods on total and individual phenolic compounds, antioxidant activity and minerals. Duncan multiple range test were used in order to compare differences between the means. Pearson's correlation coefficients were calculated to estimate the correlation between total phenolic content and antioxidant activities. Statistical analyses were performed by using SPSS 21.0 software.

3. Results

3.1 Total phenolic content of fresh and dried samples

The total phenolic contents of different *P. sivasicum* samples were shown in Table 1. The TPC contents were lower for shade, oven, and microwave dried samples comparing to fresh version with no significant difference ($P > 0.05$). However, the TPC value increased significantly in freeze dried samples ($P > 0.05$) compared to other drying methods, which is in line with the results obtained by Adetoro et al. (2020) and Shishehgarha et al. (2002) for pomegranate and strawberry, respectively. The increase in TPC content could be attributed to stronger cell destruction during freeze-drying and ice sublimation, enhancing the extraction of biochemical components (Asami et al., 2003).

3.2 Individual phenolic compounds

Chlorogenic acid, caffeic acid, ferulic acid, rutin, kaempferol-3-O-glucoside, isorhamnetin-3-O-glucoside, and luteolin were identified in fresh *P. sivasicum* (Table 2). Rutin was detected as major phenolic compound with the amount of 4.60±0.05 mg/kg DW and followed by chlorogenic acid, kaempferol-3-O-glucoside, isorhamnetin-3-O-glucoside, ferulic acid, luteolin, and caffeic acid in a decreasing order. Similar to results of this study, Erol et al. (2022) have detected the rutin, and chlorogenic acid as main phenolic compounds in P. sivasicum Kit Tan & Yıldız. Methyl gallate, (+)-catechin, quercetin 3-O-β-D-glucuronide, tetrahydroxyflavonol derivative, and kaempferol sulfate in Polygonum odoratum (Pawłowska et al., 2020), quinic, gallic, and protocatechuic acids, quercetin-3-O-galactoside, catechin, and epicatechin in Polygonum equisetiforme, hyperoside and kaempferol, myricetin, and quercetin glycosides, luteolin, rutin, and chlorogenic acid in Polygonum persicaria (Gudkova et al., 2020) have been reported in previous studies.

Table 1: Effect of different drying methods on total phenolic content and antioxidant activity of *Polygonum sivasicum* extracts.

Drying type	TPC* (mg/g DW)	ABTS (mmol TE/g DW)	DPPH (mmol TE/g DW)
Fresh	34.05 ± 0.56^{b}	0.178 ± 0.019^{ab}	0.213 ± 0.037^{ab}
FD	35.88 ± 0.27^{a}	0.186 ± 0.021^{a}	0.238 ± 0.011^{a}
SD	33.97 ± 0.61^{b}	0.168 ± 0.004^{abc}	0.217 ± 0.018^{ab}
OD	33.39 ± 0.30^{b}	0.152 ± 0.030^{c}	0.196 ± 0.016^{b}
MD	32.97 ± 1.15^{b}	0.159 ± 0.002^{bc}	0.194 ± 0.007^{b}

*TPC: Total phenolic contents, DW: Dry weight, TE: Trolox equivalent. Different letters indicate statistically significant differences ($P < 0.05$).

Drying processes used in this study had significant effects on nearly all phenolic compounds of *P. sivasicum*. Dried samples in all methods contained lower amounts of chlorogenic acid, rutin and kaempferol-3-O-glucoside than that of fresh *P. sivasicum*. Nearly 36 % of the kaempferol-3-O-glucoside degraded after drying in the freeze dryer, shade and microwave oven. Shade drying was the most detrimental method for caffeic acid, ferulic acid and rutin. The lowest chlorogenic acid content (2.40±0.23 mg/kg DW) was found in microwave dried sample. Luteolin content of other samples dried in shade, oven and microwave was equal to each other but lower than that of fresh sample. On the other hand, freeze drying had no effect on the compound. If consider the all individual polyphenols, it can be said that the most protective method was freeze drying followed by oven drying method.

According to the results of Ma et al. (2021), individual phenolic compounds of apple peel have affected from both drying method and drying temperature. The most effective methods were freeze drying for chlorogenic acid, and heat pump drying for quercitrin, phlorizin, rutin, epicatechin. All drying methods had the same and destructive effects on caffeic acid and hyperoside.

Drying might cause losses in individual phenolic compounds by the effect of oxidation and/or heat degradation or increases due to the releasing bond phenolic from food matrix (Ma et al., 2021). It is a well-known phenomenon that drying modifies the food structure and it causes increases or decreases in the extractability of each phenolic compound. If a sample has a higher porous matrix, this accelerates the diffusion of solvent to the tissue and it increases the extraction efficiency (Joardder et al., 2015). In most cases, freeze-dried materials have more porous matrix than the samples dried in different techniques. This leads observing higher amounts of polyphenols in freeze dried sample even higher than raw material (Marques et al., 2009).

3.3 Antioxidant activity

Antioxidant activity results were provided in Table 1 as ABTS and DPPH (mmol TE/g DW) values. Freeze drying of the fresh samples led to a significant increase in both ABTS and DPPH values similar to TPC. Oven drying method caused a significant decrease in ABTS value

Table 2: Effect of different drying methods on phenolics acid profiles of *Polygonum sivasicum* extracts.

Sample	Chlorogenic acid	Caffeic acid	Ferulic acid	Rutin	Kaempferol-3-O-glucoside	Isorhamnetin-3- O-glucoside	Luteolin
FS	3.43±0.22[a]	0.08±0.00[a]	0.27±0.08[b]	4.60±0.05[a]	0.42±0.04[a]	0.33±0.04[a]	0.21±0.01[a]
FD	3.05±0.20[ab]	0.08±0.00[a]	0.39±0.02[a]	4.46±0.27[b]	0.27±0.02[b]	0.55±0.01[a]	0.21±0.01[a]
SD	2.93±0.16[b]	0.06±0.00[b]	0.12±0.01[c]	3.88±0.07[c]	0.27±0.00[b]	0.13±0.00[b]	0.04±0.00[b]
OD	3.03±0.15[ab]	0.08±0.00[a]	0.35±0.00[ab]	4.37±0.04[ab]	0.30±0.04[b]	0.17±0.08[b]	0.07±0.01[b]
MD	2.40±0.23[c]	0.08±0.00[a]	0.28±0.03[ab]	4.14±0.35[ab]	0.27±0.01[b]	0.33±0.03[a]	0.04±0.01[b]

Table 3: Effect of different drying methods on mineral contents and profiles of *Polygonum sivasicum* extracts.

	Drying methods				
	Fresh	FD	SD	OD	MD
Ca	14.68±1.37[a]	14.34±1.18[a]	14.14±1.71[a]	14.38±1.81[a]	13.32±1.67[b]
Cr	0.03±0.00[a]	0.03±0.00[a]	0.03±0.00[a]	0.03±0.00[a]	0.03±0.00[a]
Cu	0.40±0.04[a]	0.36±0.04[ab]	0.37±0.01[ab]	0.40±0.01[a]	0.32±0.01[b]
Fe	9.37±0.67[a]	9.42±0.33[a]	9.33±0.28[a]	9.43±0.27[a]	9.28±0.23[a]
K	368.45±21.15[a]	359.56±19.05[a]	361.97±11.59[a]	363.09±13.57[a]	357.66±7.37[a]
Mg	43.75±0.95[a]	42.90±0.24[a]	42.98±1.43[a]	42.98±0.41[a]	42.90±0.11[a]
Mn	0.58±0.03[a]	0.56±0.01[a]	0.55±0.01[a]	0.56±0.03[a]	0.55±0.02[a]
Na	2.60±0.37[ab]	2.94±0.01[a]	2.02±0.03[c]	2.19±0.01[bc]	2.20±0.03[bc]
P	57.31±1.77[a]	51.78±1.97[b]	57.18±0.72[a]	58.60±0.47[a]	52.75±1.12[b]
Zn	6.80±0.04[a]	6.30±0.04[ab]	6.60±0.05[a]	6.80±0.02[a]	5.60±0.03[b]

similar to results obtained by Coklar et al. (2018). The retention of both values was better in freeze-drying method. Yet, shade drying method was also efficient in the retention of both values and could be used as an alternative drying method for freeze-drying. Similarly, the highest DPPH value was obtained for freeze-dried samples (0.238±0.011), followed by shade dried (0.217± 0.018), oven dried (0.196±0.016), and microwave dried (0.194±0.007) samples. The results indicate that drying method has a strong effect on the retention and the preservation of the antioxidant activity of *P. sivasium*. Considering all drying methods, freeze drying seems to be the most convenient drying method for the preservation of antioxidant activities and total phenolic content, as a strong correlation were found between the TPC and antioxidant activities. Similar findings were also reported by other researchers for different vegetables and fruits (Samoticha et al., 2016; Shofian et al., 2011).

3.4 Mineral composition

The amount of the elements in fresh and dried *P. sivasicum* samples are given in Table 3. According to results, the madimak plant seems to be rich in potassium (K; 368.45±21.15 mg·100^{-1}), phosphorus (P; 57.31±1.77 mg 100 g^{-1}), and magnessium (Mg; 43.75±0.95 mg 100 g^{-1}) elements. It also has considerable amounts of calcium (Ca; 14.68±1.37 mg 100 g^{-1}) and iron (Fe; 9.37 mg 100 g^{-1}). The results are highly consistent with those reported by Turan et al. (2003) who used *P. cognatum* from a very close province to Sivas in Eastern Anatolia region. Sivas is the border of Eastern Anatolia and Central Anatolia regions. The climate and the soil type is very similar to those of both regions. On the other hand, Çoban et al. (2021), Kibar and Kibar (2017) and Altay et al. (2013) reported higher amounts of the same elements in other endemic *Polygonum cognatum* plants collected from different regions of Turkey. The results indicate that low sodium and high potassium levels support a diet poor in Na content. In addition, relatively lower Mg and Ca contents than Çoban et al. (2021) suggest that *P. sivasicum* could be a good choice for those who are at high risk of high blood pressure, stroke, and cardiovascular disease.

The fresh *P. sivasicum* also contains minor elements (Zn, Cr, Cu, and Mn) at considerable amounts that regulates some specific functions in human. Copper is an essential element for human. It helps form red blood cells together with iron and contribute to maintain healthy bones, blood vessels and immune function (White and Broadley, 2009). In our study, the copper amount was found 0.38±0.04, which is comparable to those obtained by Çoban et al. (2021).

Zinc is required for DNA synthesis, a good immune function, and growth. Healthy people need approximately 10 mg of zinc. The findings of this study reveal that *P. sivasicum* contain 6.80±0.04 mg 100 g^{-1} zinc, which is higher than those reported by Turan et al. (2003). It could be used in diets as a good zinc source, as well. The soil type may have caused this difference together with the genetic factors.

Different drying methods did not significantly change the Cr, Fe, K, mg, and Mn compositions ($P > 0.05$). The highest mineral values were obtained for oven dried samples along with free-drying method. On the other hand, microwave drying generally resulted in lower mineral content comparing to other samples dried using other techniques. The energy type affects the solubility of the elements. The convective energy type and the wave strength of the oven drying may have caused an increase in the solubility of the elements. Similarly, Özcan et al. (2005) and Arslan and Musa Özcan (2010) reported that the oven drying method was better on the retention of the mineral composition in onion and basil.

3.5 Antibacterial activity

Polygonum sivasicum extracts exhibited mild and strong antimicrobial activity against *S. aureus* and *L. innocua* depending on the extraction solvent. Extraction solvents used as controls had no inhibitory effects on the five bacteria tested (data not shown). Some of the phenolic compounds related to a stronger antimicrobial activity are more soluble in acetone, which is also supported by our findings (Negi et al., 2005). Yet, a further study is required regarding the phenolic composition of solvent extracts with aqueous solvent for a comprehensive assessment of individual compounds possessing the antimicrobial activity. Yildirim et al. (2003) reported that *P. cognatum* exhibited mild to strong antibacterial activities against *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633. Similar findings were also reported by the other researchers (Eruygur et al., 2020; Özbay and Alim, 2009; Pekdemir et al., 2021). The antibacterial activities obtained by different drying techniques were not found statistically significant ($P > 0.05$).

4. Conclusions

P. sivasicum, locally known as madimak, is an important source of phenolic compounds in terms of both phenolic profile diversity and concentrations individually. The obtained results suggest that phenolic components and antioxidant capacity have been preserved in freeze-dried method better. On the other hand, shade, oven, and microwave drying methods caused a significant decrease in phenolic compounds, which lead to decrease in antioxidant capacity of fresh madimak plant. In the mineral composition, although other methods did not cause a significant change, it was revealed that the microwave dried method caused significant decreases. To sum up, it can be said that the concentrations of phenolic compounds and antioxidant capacity can be preserved better in the storage of the madimak plant using the freeze-dried process.

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