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Antioxidant activity and mineral nutrient composition of *Polygonum cognatum* - a potential wild edible plant

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Wild edible plants are of considerable interest to scientists, governments, and local people living in rural regions, since they are the potential source of novel nutraceuticals and may contribute to food security. Thus, they are increasingly important to understand their nutritional values. *Polygonum cognatum* is among the widely used wild edible plants in Turkey and worldwide. However, the available literature on the mineral contents and antioxidant properties of this plant is limited. In this study, the *P. cognatum* samples collected from six different areas in Erzurum, Turkey, were investigated for antioxidant activity (β-carotene Bleaching, DPPH and FRAP), total phenolic content, and presence of macro minerals (P, K, Ca Mg and Na), micro minerals (Fe, Cu, Mn, Zn, B) and heavy metals (Ni and Pb). According to the present research results, total phenolic content and antioxidant potential of *P. cognatum* were determined to be high, with significant differences according to sampling areas. Our results also showed that *P.cognatum* contained considerable amounts of magnesium, calcium, potassium, iron, manganese, zinc and low amounts of sodium, boron, nickel, and lead. Therefore, based on the current data, it could be used as a new source of nutraceutical foods and enhance the diversity in a modern functional diet.

Keywords: Antioxidant, Edible plant, Mineral nutrient, *Polygonum cognatum*, Total phenolics

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Recently, there has been an increase in consumer interest in using nutraceutical and functional foods worldwide. According to the published reports, the global nutraceutical market value is estimated to reach $ 241.1 billion by 2019[1]. These foods provide extra benefits for human physiology and metabolic functions, and they possess antioxidant effects, which play an important role in preventing diseases and the promotion of a healthier life. Antioxidants are mostly provided from food products such as cereals, fruits, vegetables, mushrooms, flowers, medicinal and aromatic plants. These natural antioxidants derived from plant materials are also important in protecting food and improving the body's defense system[2]. Many plant species have been examined for antioxidative activity. For this reason, it is critical to explore new sources of inexpensive and safe natural antioxidants.

The *Polygonum* genus, also known as knotweed or bistort, is one of the significant genera of the family *Polygonaceae*. There are about 300 species worldwide[3] and in Turkey, 37 *Polygonum* species are available[4]. *P. cognatum* grows on elevations of between 760–5600 m on dry riverbeds and gravelly mountain slopes. Apart from Turkey, it is widely distributed in Iran, Central to Western Asia, Caucasia, Siberia, Mongolia, Pakistan and Afghanistan[5-7]. *P. cognatum*, locally known as "Madimak", is commonly used in Turkey, especially in Central and Eastern Anatolia region as a wild edible vegetable (cooked vegetable dish, leaves eaten in salads) and has been used in traditional medicine in the treatment of diabetes mellitus[8]. Available literature has shown that various *Polygonum* species such as *P. chinense, P. muricatum, P. nepalense, P. orientale*[9], *P. aviculare, P. bistorta, P. hydropiper, P. viviparum*[10], *P. perfoliatum*[11], *P. capitatum*[12] are used as a wild edible plant. However, limited literature is available on the mineral contents of *P. cognatum*[13,14]. Mineral contents of wild edible plants vary with plant genotype, ecological conditions, and soil properties.

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Therefore, it would be beneficial to determine the mineral content of *P. cognatum* for food scientists and consumers. On the other hand, antioxidant properties of *P. cognatum* also remain poorly understood and there have only been two studies[8,15] that have assessed its antioxidant activity. Hence, the aim of this research was to appoint the antioxidant activity, total phenolic content, and mineral contents of *P. cognatum*, which is commonly consumed in Turkey and many parts of the world.

Methodology

Plant collection

Polygonum cognatum samples were collected from the six different sites in Erzurum province, Turkey, in 2015 (Fig. 1). Each sample of *P. cognatum* was represented by a circle in Figure 1. The aerial parts of plants were collected, since in this region the shoots of *P. cognatum* plants are consumed by local people. The plant samples were cleaned and homogenized in a blender, and then homogenates were used for analysis.

Extract preparation

The dried and milled samples were extracted with 200 mL of methanol (MeOH) using a Soxhlet apparatus for 6 h. The extracts were filtered using filter paper (Whatman No. 1) and then concentrated by evaporator to dryness. The residues obtained were stored at 4°C with nitrogen until tested.

Total phenolic content (TPC)

The total phenolic compounds of *P. cognatum* samples were determined using the Folin-Ciocalteu reagent assay[16]. The results obtained were given as mg GA/g DW sample.

β-carotene bleaching assay

β-Carotene/Linoleic acid mixture was prepared as below: 1 mg β–Carotene was dissolved with 2 mL chloroform, by adding 25 µL linoleic acid and 200 mg Tween 40 in it emulsion was obtained. Chloroform was removed on rotary evaporator and then 100 mL of water added. By taking 2500 µL from prepared β-Carotene/Linoleic stock solution tubes were transferred and the extract of 350 µL added. The mixture was vortexed and incubated. The absorbance of the mixtures was read at 490 nm[17].

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) scavenging activity

This spectrophotometric method uses the stable radical DPPH as a reagent[18]. About 50 µL sample extracts and 5 mL DPPH solution were transferred in test tube. The mixture incubated at 20^{o}C then the absorbance was read at 517 nm using a UV–VIS spectrophotometer. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as inhibition percentage against extract concentration.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay followed procedures reported by Benzie and Strain[19]. Plant extracts (100 µL) were allowed to react with 2.750 µL of the FRAP reagent and mixed with 150 µL water and kept for 30 min in the dark. Absorbance of the colored product was measured at 593 nm. Iron sulfate ($FeSO_4$) was used as the standard (r^2 = 0.999).Results were expressed in µmol $FeSO_4$/g dry matter.

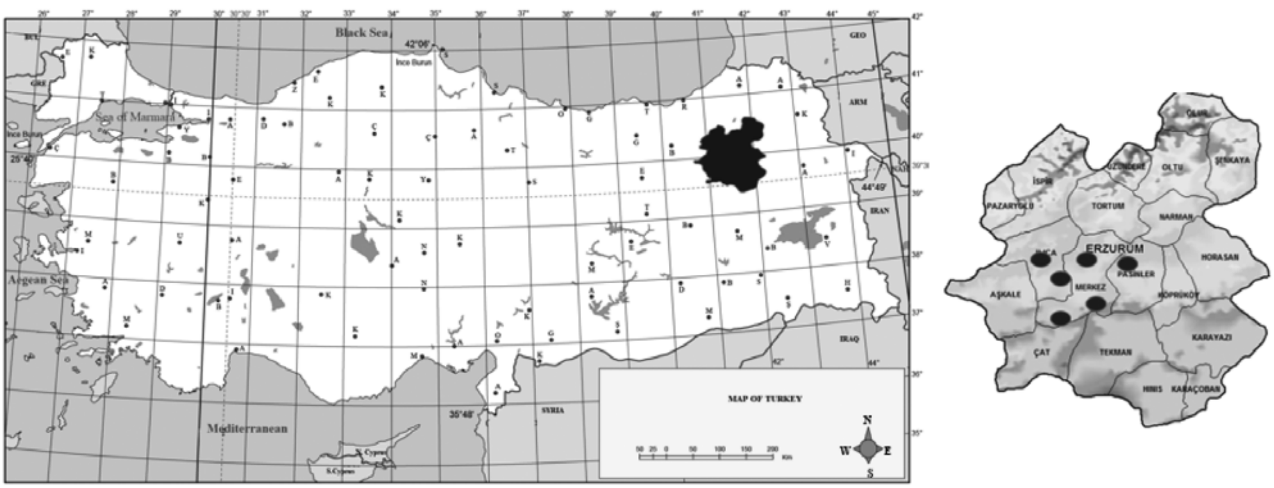

Fig. 1 — Map showing the collection sites of the samples of *Polygonum cognatum* at Erzurum province, Turkey. Each sample is represented by a circle

Determination of mineral nutrients

For determination of macro and microelements with heavy metals content, all plant samples were oven-dried at 68°C for 48 h. The nitrogen contents of the plant samples were detected using the Kjeldahl method[20]. Macro (P, K, Mg, Ca and Na) and microelements (Fe, Mn, Cu, Zn, and B) and heavy metals (Ni and Pb) were determined after wet digestion of dried and ground samples. The ground *P. cognatum* samples were burned in microwave (Bergh of Speed wave Microwave Digestion Equipment MWS-2) using the procedure described by Mertens and co-workers[21]. The contents of macro and microelements with heavy metals in tissue were detected using Inductively Couple Plasma spectrophotometer (ICP) (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA)[21].

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range test at p<0.01 significant level. Standard deviation (SD) values were shown as ± the mean value (n = 3). Correlations were determined using Pearson's correlation coefficient (r).

Result and Discussion

Total phenolic content

The total phenolic contents of *P. cognatum* samples were shown in Fig. 2. The differences among different *P. cognatum* samples in total phenolic content were statistically significant (p<0.01, Fig. 2). As shown in Figure 2, the total phenolic content of *P. cognatum* plants varied from 181,97 to 334,75 mg GAE/g, suggesting that there was a wide variation among these samples. This variation could be explained by soil and climatic differences. The total phenolic contents of our *P. cognatum* samples were consistent with those mentioned by Yildirim *et al.*[8] and Samancioglu *et al.*[15].

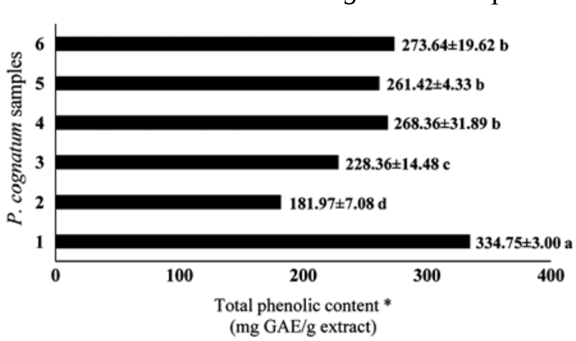

Fig. 2 — Total phenolic contents of *Polygonum cognatum* samples. * Values are means ± S.D. of three replicates. Different letters indicate differences in the tested *P. cognatum* samples (p<0.05).

The differences in total phenolic contents of *P. cognatum* may be associated with redox properties[22]. Moreover, it may be due to variations in soil properties, ecological conditions, the used solvents and methods. Indeed, some researchers reported the total phenolic contents in other *Polygonum* species extract to be 45,24 mg GAE/g for *P. multiflorum*[2], 165,3 mg GAE/g for *P. minus*[23], 13,88 mg GAE/g for *P. minus*[24], 13,88 mg GAE/g for *P. hyrcanicum*[25], indicating that the total phenolic contents in our *P. cognatum* samples were generally higher than those in other *Polygonum* species.

Antioxidant activity

The antioxidant activities of *P. cognatum* samples were shown in Figure 3. The antioxidant activity

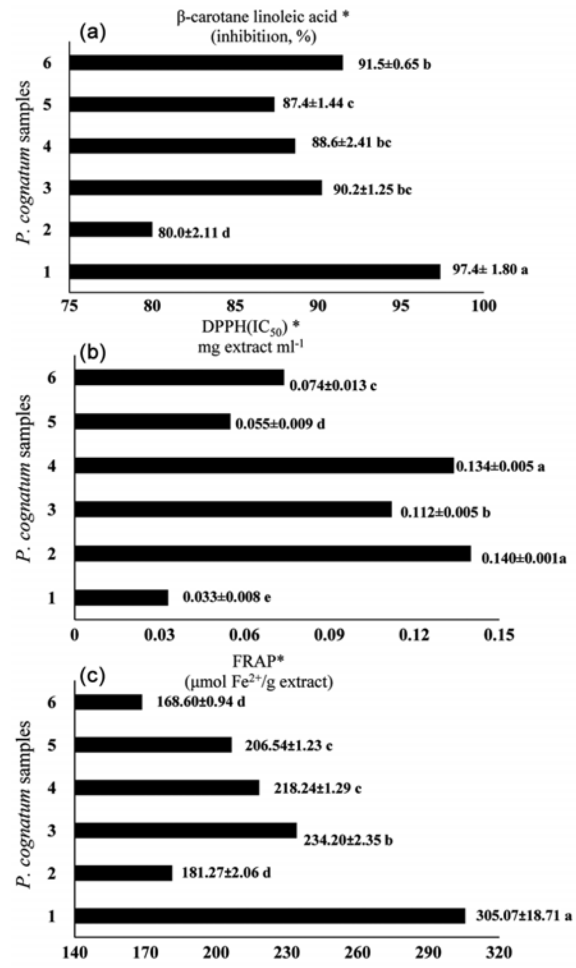

Fig. 3 — Antioxidant activity a) β-carotane linoleic acid (inhibitiıon, %) b) DPPH, IC_{50} (mg extract ml^{-1}) c) FRAP (μmol Fe^{2+}/g extract) of *Polygonum cognatum*. * Values are means ± S.D. of three replicates. Different letters indicate differences in the tested P. cognatum samples (p<0.05).

assay of β-carotene bleaching, DPPH and FRAP ranged from 80 to 97.4%, 0.033 to 0.140 mg extract ml^{-1} and 168,59 to 305,06 µmol Fe^{2+}/g extract, respectively. Significant differences ($p<0.01$) were determined among the samples examined in all three assays.

If there is not enough antioxidant substance, β-carotene undergoes oxidation and its color changes over time. The antioxidants can neutralize free radicals by donating a hydrogen atom and hinder the extent of β-carotene-bleaching[26]. The most effective inhibition of linoleic acid was achieved by sample 1 (97.4%). The lowest inhibition was determined in sample 2 (80%) (Fig. 3a). Inhibition by BHT (96.7%), which was used as a standard antioxidant, was lower than the other samples except for sample 1. The correlation between total phenolics content (TPC) and β-carotene bleaching assay ($r = 0.913$) was positive and quite high (Fig. 4a). The antioxidant activity of our samples can be mainly caused by phenolic compounds. In fact, in an earlier study on different vegetable extracts[27], a positive and significant correlation ($r = 0.6578$, $p<0.05$) between TPC and β-carotene bleaching assay was found. Vitamin C, a reducing agent, contributes to the increase of antioxidant activity and also carotenoids possess antioxidant activity due to their chain-breaking properties. Considering this information, the present study suggests that vitamin C and carotenoids, as well as phenolics, can be responsible for the antioxidant activity of *P. cognatum*[8].

DPPH is the easiest, simple and cheapest method. Therefore, it is frequently used in determining the antioxidant activity of the samples. The IC_{50} is defined as the efficient sample concentration required to reduce the first DPPH concentration by 50%. IC_{50} is similar to the EC_{50} in biological measurements[28]. The IC_{50} (EC_{50}) expresses the sample concentration required to reduce the radical sweeping activity by 50%. The lower the IC_{50}, the higher the antioxidant capacity of the sample[29]. In DPPH scavenging activity test, the IC_{50} values of *P. cognatum* plants ranged from 0.033 to 0.140 mg/mL extract and the best IC_{50} values were obtained from sample 1 as 0.033 mg/mL extract (Fig. 3b). The correlation between total phenolic content and DPPH assay of *P. cognatum* extracts was shown in Figure 4b. The correlation was negative and high (r= 0,783) (Fig. 4b). This inverse relationship is expected because, as the total amount of phenolic compounds increases, the concentration of the extracted corresponding to DPPH (IC_{50}) decreases. The high correlation coefficient supports this situation. Yildirim *et al.*[8] compared the DPPH radical scavenging activities of ethanol, ether, and water extracts of *P. cognatum* from Turkey and the authors found that *P.cognatum* extracts showed antioxidant activity since they possibly possessed both polar and apolar antioxidant compounds. The highest DPPH radical-scavenging activity was

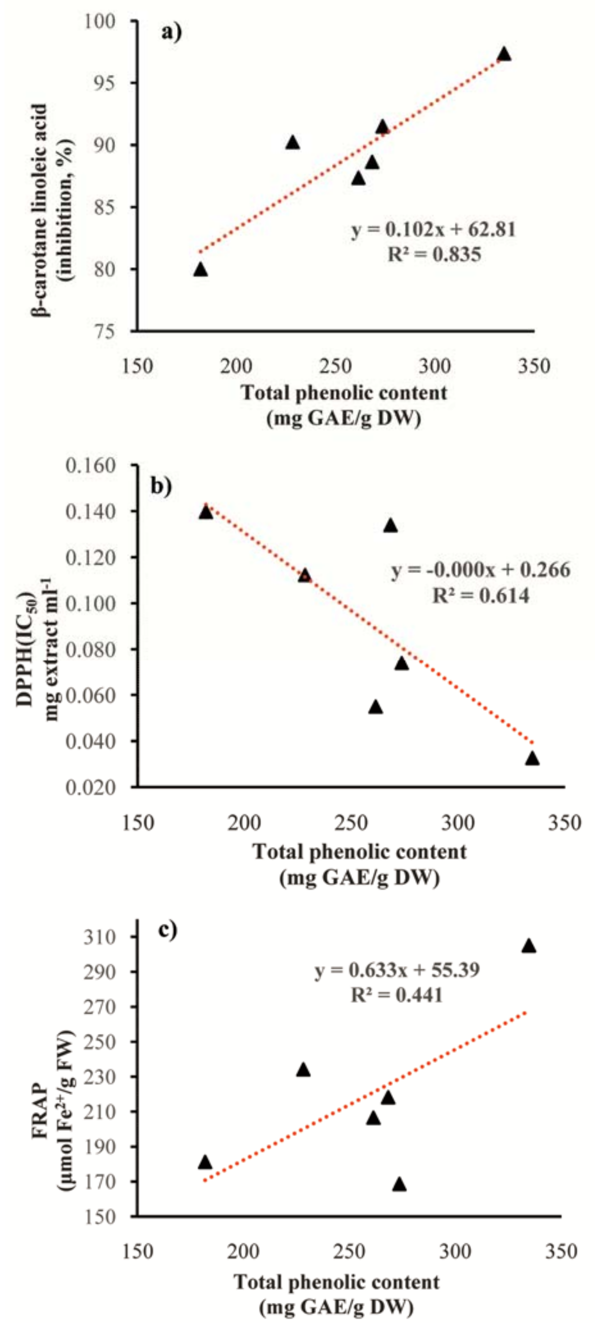

Fig. 4 — The correlation between total phenolic content and β-Carotene (a), DPPH (b) and FRAP (c) assays of *Polygonum cognatum*.

observed in the water extract by 50% DPPH radical scavenging, 100 mg/mL extract concentration.

FRAP assay is widely used to investigate the antioxidant activity of foods. The FRAP antioxidant capacity of *P. cognatum* extracts is based on the reduction of iron (III) in the FRAP reagent to iron (II) due to the antioxidants present in these samples[19]. FRAP activity ranged from 168.59 to 305.06 mg FeSO4 µmol Fe^{2+} / g extract (Fig. 3c). In our study, *P. cognatum* extracts a positive correlation coefficient (r = 0,664) was found between the total amount of phenolic compounds and FRAP assay (Fig. 4c). Our results regarding the FRAP assay were in general consistent with the data obtained from the DPPH test and β-carotene/linoleic acid model system. In the current study, the antioxidant activities of our sample extracts increased due to the total amount of phenolic compounds and this result was also confirmed by Maizura *et al.*[21]. On the other hand, we also noted that antioxidant capacities of *P. cognatum* samples were generally higher than those previously reported by Sreeramulu and Raghunath[30] and Deng *et al.*[31] for the commonly consumed vegetables.

Mineral nutrients

We also determined the mineral composition of *P. cognatum*, as it is considered to contribute significantly to maintenance of health and nutrition. The study focused on the following elements: Mg, Ca, K, Na, Cu, Zn, Mn, P, Fe, B, Ni, and Pb. Comparing metal contents in *P. cognatum* leads to useful information for nutritional science.

The minimum and maximum values of the minerals that are important for human nutrition (i.e., Mg, Ca, K and Na) are reported in Figure 5. In general, the average Mg, Ca, K and Na values of the samples were 345.02, 840.5, 1452.3 and 10.0 mg 100 g^{-1}, respectively. Magnesium and calcium are important minerals for humans since they play a substantial role in bone formation[32] and cardiovascular system[33]. Dairy products provide the majority of human dietary Mg and Ca requirements. However, some vegetables can also provide significant quantities of these minerals[34]. In the present investigation, the magnesium and calcium levels were higher than those reported by Turan *et al.*[13] and Kibar and Kibar[35]in *P. cognatum*. These differences might be due to soil properties, climatic and geographical variations, and *P. cognatum* populations. In our study, results showed that magnesium and calcium were higher than those in some vegetables. Roe *et al.*[36] reported that mineral contents of various vegetables varied from 7.00 to 80.00 mg/100 g for magnesium and from 7.00 to 119.00 for calcium, respectively. In comparison with values by Roe *et al.*[39] report, the level of calcium in *P. cognatum* (840.5 mg/100 g) was 7 times higher than that of spinach (119.00 mg/100 g) and about 16 times higher than that of fresh bean (52 mg/100 g).

The differences among different *P. cognatum* samples in potassium content were statistically significant ($p<0.01$, Fig. 5c). Our *P. cognatum* samples showed potassium levels ranging from 1101.5to 1924.5 mg 100 g^{-1}. The average potassium level of *P. cognatum* samples was 1452.3 mg 100 g^{-1}, which was higher than that previously reported in *P. cognatum*. Turan *et al.*[13] and Kibar and Kibar[35] reported a potassium value of 610.0 and 1359.34 mg 100 g^{-1} for *P. cognatum* plants. In many other wild edible plants and commercially cultivated vegetables, potassium content was found in the range of 138 to 4700 mg/100 g[13,32,37-40]. The proposed intake of potassium for adolescents and adults is 4700 mg/day[41]. Therefore, *P. cognatum* can be seen as a good source of potassium. In the present study, sodium (Na) was found in very low amounts in *P. cognatum* plants with values ranging from 7.1 to 16.4 mg 100 g^{-1} (Fig. 5d). Sodium content (10.00 mg 100 g^{-1}) was similar to that reported in *P. cognatum* by Turan *et al.*[13]. The previous research indicates that a diet high in sodium and low in potassium can be

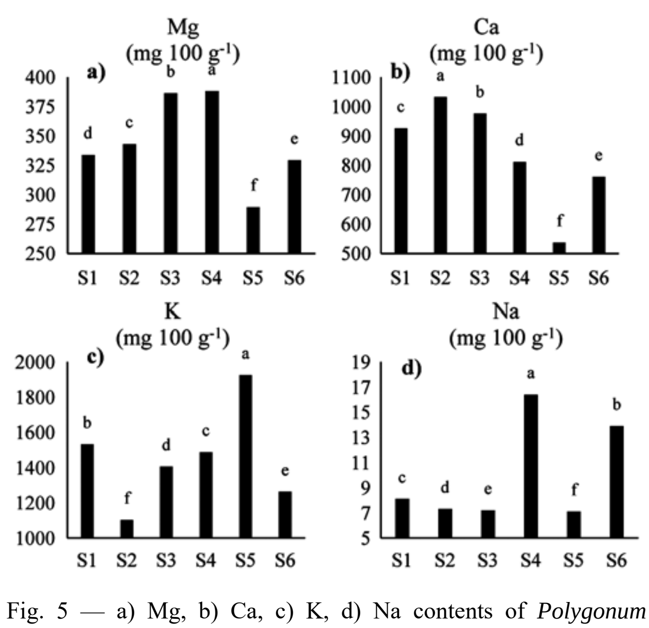

Fig. 5 — a) Mg, b) Ca, c) K, d) Na contents of *Polygonum cognatum* samples. Values were expressed in mg/100 g dry weight basis.

related to an increased risk of high blood pressure, stroke, cardiovascular disease, and early death[42]. Consequently, potassium was greatly higher than sodium, suggesting that *P. cognatum* can be a good choice for diets poor in Na.

Copper is an essential mineral that cannot be produced by the human body, so it must be supplied by dietary sources every day as indispensable part of red blood cells, many oxidation and reduction processes in animals and humans[43]. According to the report of the WHO[44], 1-3 milligrams per day of copper are necessary to prevent deficiency symptoms. Also, in our study, the total copper content of *P. cognatum* samples ranged from 0.62 to 0.84 mg 100 $g^{-1}$and was statistically significant ($p<0.01$, Fig. 6a). The available literature indicates that copper content ranges between 0.90 and 10.6 mg 100 g^{-1}in the studies conducted on wild edible plants and commercially cultivated vegetables in China[45], India[46], Turkey[47], Spain[48] and Nigeria[49].

The average Zn, Mn and P values of our *P. cognatum* samples were 12.4, 4.27 and 3.5 mg 100 g^{-1}, respectively (Fig. 6). Zinc is implicated in the functioning of over 300 different enzymes and plays a critical role in numerous biological processes[38]. Zinc content varies depending on plant species. In our *P. cognatum* samples, zinc content (12.4 mg/100 g) (Fig. 6b) was much higher than that reported by Turan *et al.*[13]. The differences in the zinc and copper content of this plant may be due to soil properties, genetic factors and growth stage of the plant. *P. cognatum* Mn content was found twofold higher than daily intake suggested by Trumbo *et al.*[50]. Phosphorus content varied significantly among *Polygonum cognatum* samples and was statistically significant ($p<0.01$, Fig. 6d). Phosphorus is very important for the human body since it is a part of DNA materials and they take part in energy distribution. Phosphorus is a dietary requirement; the recommended intake is 800 mg/day[50]. *Polygonum cognatum* as an edible plant can be included in diet due to its low Na content and adequate P content. However, it is necessary that all values be taken into consideration when using.

As shown in Figure 7a, *P. cognatum* samples showed iron levels ranging from 31.5 to 173.1 mg 100 g^{-1} and statistically significant difference ($p<0.01$) was observed among our plant samples. The average Fe content of our *P. cognatum* samples was 70.95 mg 100 g^{-1}, which had higher values compared to *P. cognatum* (0.20 mg 100 g^{-1})[13] and commercially cultivated vegetables such as spinach (2.71 mg 100 g^{-1}), parsley (8.78 mg 100 g^{-1}), leek (9.88 mg 100 g^{-1}) and wild edible plant such as *Taraxacum obovatum* (3.57 mg 100 g^{-1}), *Chondrilla juncea* (3.97 mg 100 g^{-1}), *Malva sylvestris* (22.48 mg 100 g^{-1}), so our study indicated that the *Polygonum cognatum* plants collected in Erzurum, eastern Anatolia region, could be good source of iron[13,32,48,51]. *P. cognatum* samples showed boron content ranging from 0.9 to 1.9 mg 100 g^{-1} (Fig. 7b). The average boron content of *P. cognatum* samples was 1.4 mg 100 g^{-1}. The value is about equivalent to those suggested for carrot,

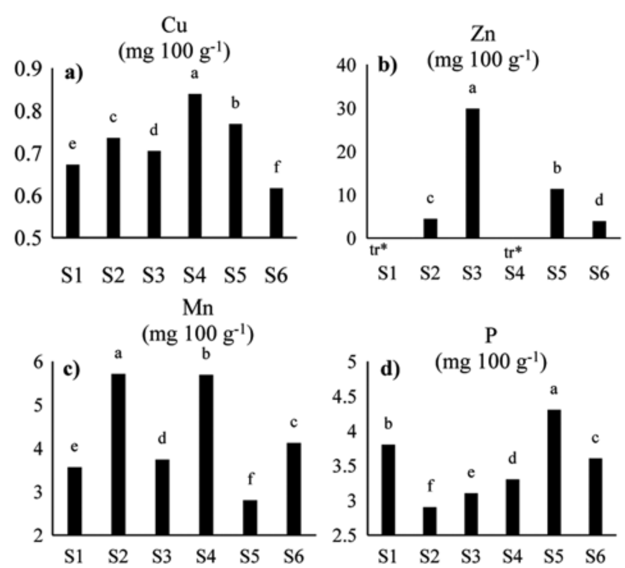

Fig. 6 — a) Cu, b) Zn, c) Mn, d) P contents of *Polygonum cognatum* samples. Values were expressed in mg/100 g dry weight basis. *Trace amount

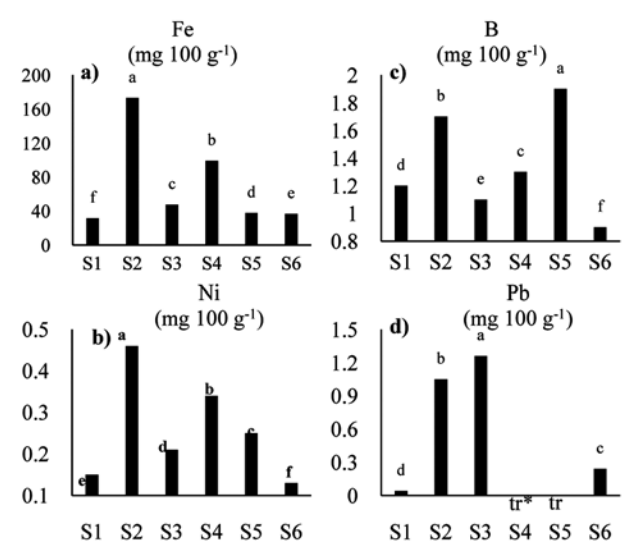

Fig. 7 — a) Fe, b) B, c) Ni, d) Pb composition of *Polygonum cognatum* samples. Values were expressed in mg/100 g dry weight basis. *Trace amount

broccoli, carrot (0.46, 1.85 and 0.75 mg 100 g^{-1}, respectively) by Hunt *et al.*[52]. Nickel is a ubiquitous metal, which contacts with nickel compounds can cause a variety of adverse effects on human health[53]. The total nickel contents of our *P. cognatum* samples ranged from 0.13 to 0.46 mg 100 g^{-1} (Fig. 7c), which had similar values compared to vegetables[54].

Lead is regarded as one of the environmental pollution resources, due to its toxic effects. The amount of lead taken by the oral route is less than 100 µg in the USA and less than 30 µg in Europe[55]. In our research, *P. cognatum* samples showed the lead levels ranging from 0.04 to 1.26 mg 100 g^{-1} (Fig. 7d), implying that lead content showed high variation. These levels of Pb were similar to found in spinach leaves[56] and *Brassica pekinensis*[45]. This can be attributed to the fact that Pb content may be influenced by environmental conditions. Based on The Scientific Committee on Food reports[57], it can be said that *P. cognatum* may not constitute a health hazard for consumers because the values are far below the purposed daily intake of nickel and lead.

Conclusions

Increased consumption of fruits and vegetables can be useful in providing health benefits due to their effects on reducing the risk of many diseases. These health benefits are in part attributed to their phytochemical constituents. The present study enhanced our understanding regarding the total phenolics, antioxidant properties, and macro and micronutrients of *P. cognatum*, wild edible plant and showed that consumption of *P. cognatum* could be beneficial for improving human nutrition and health due to their high antioxidant properties and total phenolic contents. Also, another important aspect of *P. cognatum* is that it is rich in macro and micro minerals and is low in heavy metals such as Ni and Pb. To summarize, *P. cognatum,* which has long been used as wild edible plant worldwide, can play an important role as a vegetable for healthy nutrition and contribute to food security since it grows at a wide range of soil and climate conditions. The information from this study can be useful to consumers, agronomists and the nutritionists examining rich therapeutic diets. However, further research is needed to fully evaluate and quantify the other nutritional properties of *P. cognatum* as edible functional food.

Conflict of Interest

The authors do not have any conflicts of interest to declare.

Author Contributions

FC: Writing, Editing, Data curation, Visualization, Investigation, MT: Writing, Editing, Investigation, Data curation, HO: Conceptualization, Supervision, Writing, Editing, Methodology, Investigation, AG: Investigation, Data curation, Writing, EO: Methodology, Data curation, EA: Writing, Data curation, TP: Methodology, Data curation.

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