

Quantification of Biochemical and Antioxidant Properties of Contrasting Common Purslane Populations

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ABSTRACT

Plants play a crucial role in human nutrition and health, yet many species with high nutritional and antioxidant potential remain underutilized. Common purslane (Portulaca oleracea), a fast-growing and easily cultivable plant, is a rich source of phytoconstituents and bioactive compounds, making it a promising candidate for functional food development. This research aimed to explore common purslane's viability as a unique Mediterranean vegetable. We collected seeds from 25 distinct populations across Turkey, addressing a significant research gap in their biochemical and antioxidant properties. Among these 25 populations, lycopene and lutein levels ranged from 52.59 to 10.52 and 37.33 to 12.63 mg g⁻¹ fresh weight (FW), respectively. Ferric Reducing Antioxidant Power (FRAP) values ranged from 8.23 to 3 mg g⁻¹ FW, Cupric Reducing Antioxidant Capacity (CUPRAC) from 123.44 to 24.17 mg g⁻¹ FW, and Trolox Equivalent Antioxidant Capacity (TEAC) from 135.33 to 66.25 mg g^{-1} FW. In conclusion, our study not only provides an innovative approach for expanding unexploited markets but also highlights the potential for developing valuable functional foods.

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INTRODUCTION

The Mediterranean region is rich in both wild and cultivated plant species that have served as a crucial source of food, feed, and medicine for humans over hundreds of years (Ceccanti et al., 2018). Common purslane (*Portulaca oleracea* L.) is one among such wild plants and is often deemed a weed in many summer crops. This plant belongs to the Portulacaceae family, which has around 258 plant species as its members (Petropoulos et al., 2018, Ocampo et al., 2018, WFO, 2024). Common purslane has a previous history of being used as a vegetable or as a part of fresh salads. The plant exhibits glabrous, unifacial stems with sessile leaves arranged in an alternate phyllotaxis, displaying a variegation of green and yellow (Butnariu, 2018).

Scientific evidence suggests that the use of common purslane as a nutraceutical and pharmaceutical source dates back to ancient Egyptian times, with records appearing as early as the pharaonic period (Mohamed and Hussein, 1994). Common purslane shoots emerge as a nutritional powerhouse, surpassing many other plants considering their content of health-promoting compounds. They are particularly rich in omega-3 fatty acids that are essential for brain function and heart health. Additionally, they boast significant levels of α tocopherol (vitamin E), a potent antioxidant, ascorbic acid (vitamin C) crucial for immune function, β carotene, and a precursor to vitamin A important for vision, and glutathione, a detoxifying molecule. Beyond its impressive omega-3 content, purslane shoots boast a comprehensive vitamin profile, encompassing vitamins A, and B complex (including B1, B2, B3, B6, and B9), and vitamin E. They are further enriched with essential minerals like potassium (K), calcium (Ca), iron (Fe), magnesium (Mg), sodium (Na), phosphorus (P), copper (Cu), and manganese (Mn) (Butnariu, 2018; Unsal et al., 2014). Pharmaceutical research on common purslane suggests potential health benefits of this plant. In vitro studies have demonstrated its ability to inhibit cancer cell growth, combat bacterial infections, promote skeletal muscle relaxation, and exert anti-inflammatory effects (Yan et al., 2012). Further, biologically active molecules within common purslane have demonstrated their ability to suppress the acetylcholinesterase (ACHE) enzyme, a pivotal target in Alzheimer's disease therapy (Montoya-García et al., 2018, Chen et al., 2016). Such health advantages have led the World Health Organization to recognize common purslane as one of the most widely employed medicinal plants (Uddin et al., 2014). Common purslane extends its utility beyond a nutritional powerhouse. Extracts from its roots and leaves exhibit a robust allelopathic capacity, suggesting potential applications in organic and sustainable agriculture. Common purslane has substantial allelopathic effects, impacting the growth and development of nearby plants by releasing bioactive substances into the environment (Hamad, 2021). These allelochemicals, consisting of phenolic acids and flavonoids, are released to the environment by roots, leaf leachates, and plant decomposition (Moh et al., 2024). Additionally, depending on the species they target, they can either hinder or promote the emergence and several other physiological processes in other plants, including seed germination, root elongation, and growth (Moh et al., 2024). The allelopathic properties of common purslane are especially pertinent to agricultural settings, where they can play a role in suppressing weeds naturally or as a component of sustainable crop management techniques (Li et al., 2019). These extracts could function as natural growth regulators and organic herbicides (El-Shora et al., 2015).

Despite its long history of use and native status in the Mediterranean region, common purslane remains a relatively understudied wild species. Some limited studies include (Keser et al., 2021) examining common purslane from Sivrice/Elazig (Turkey), Osma et al. (2014) from Istanbul (Turkey), Binici and Binici (2021) from Mersin and Antalya (Turkey), and Gul et al. (2017) from Diyarbakir (Turkey). There has been a scarcity of data on biochemical and antioxidant attributes of various common purslane populations found across diverse Turkish regions. Consequently, this is the first study to investigate the biochemical makeup of common purslane from across Turkey. This study aimed to fill this gap in knowledge by looking at the different biochemical compounds found in common purslane from various regions of Turkey. This information could be useful for developing new healthy foods and supplements. Furthermore, this research helps farmers choose the most beneficial common purslane plants. By selecting those with high levels of healthy compounds, they can create new types of leafy vegetables that are both nutritious and profitable.

MATERIALS and METHODS

Plant Material and Growth Conditions

Seeds from 25 different geographical locations in Turkey were obtained that represented the country's biodiversity. These populations, totalling 25 in number, including Agr Tohum, Agri, Aktarix, Arzuman, Assem, Asya, Balikesik, BT Altinkulak, Dogal, Duzce, ERO-I, Ero-II, Gungurler, Mercan, Noroz Nazlisi, Pasa, Rita, Sari Ferik, Simagro, Sun Agri, Tunc, Yagmur, Yerli, Yesim-I, and Yesim-II. These populations were studied under greenhouse conditions for their comparative production of biochemical compounds and antioxidants. The study was conducted according to a completely randomized design (CRD) with four replications. Pots measuring 22.5 cm in depth and 16.5 cm in diameter were filled using a mixture having a 2:1 compost-perlite combination. Each pot witnessed the immediate planting of seeds corresponding to a specific population. Four seeds were planted carefully in each pot, and the sowing depth was about 2-2.5 cm. After germination, the pots were irrigated regularly based on the plant's needs, and the pots were subjected to a rouging procedure to maintain a pair of common purslane plants per pot. The plants received a split application of nitrogen fertilizer at a total rate of 46 kg ha⁻¹. The fertilizer was applied twice in equal amounts, on days 10 and 20 after planting. Importantly, no pesticides or other chemicals (agrochemicals) were used at any point while growing the common purslane.

Experimental Site

This study was conducted at the Department of Plant Productions and Technologies, Nigde Omer Halisdemir University, Turkey, between 2021 and 2022. The plants were grown in a semi-controlled greenhouse that mimicked natural conditions, without using artificial lights. The greenhouse replicated a natural day-night cycle with temperatures set at 25°C during the day and 15°C at night. Humidity in the greenhouse was around 50%.

The experiment was conducted twice during the summer season, employing two independent repeats following identical methods for each. Harvesting was done 30 days after germination, corresponding to the onset of the flowering stage.

Lycopene contents

The method of Davis et al. (2003) was used to determine the lycopene in common purslane. A 0.5 g plant sample was used for the determination of lycopene in common purslane plants. Then, 5 ml of 80% acetone was added and the sample tubes were placed on the shaker (200 rpm) for 2 h. After this, the samples were centrifuged at 13000 rpm for 5 min and the supernatant was collected, and the volume was raised to 25 mL by adding 80% acetone. Then, 10 mL of extract was taken, and 10 mL of hexane was added. Finally, the upper phase was taken in a separate tube and measured on a spectrophotometer at 503 nm using hexane as a blank.

Lutein contents

Spectrophotometric analysis was used to assess the lutein content of the leaves of common purslane, as previously described by Bulda et al. (2008). A frozen sample of liquid nitrogen was pulverized in a mortar and pestle using an acetone-to-ethanol ratio of 1:1. By adding freshly made KOH (1 g m L^{-1}) to the extract, the saponification procedure was carried out to remove the chlorophylls and lipids from the extract. The sample was allowed to settle after the mixture was incubated for 5 min at 45°C and cooled on ice. For analysis, the uppermost colored percent was chosen. The final lutein content in common purslane leaves was calculated using the following equation after samples were read on a spectrophotometer at 480 and 495 nm.

Formula = $11.51_{A480} - 20.61_{A495}$.

Total antioxidant activity

Total antioxidant activity was calculated by using four different methods i.e., cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and TEAC (Total Equivalent Antioxidant Capacity).

Ferric Reducing Antioxidant Power (FRAP)

A leaf sample (1 g) was obtained and combined with a 10 mL solution of methanol and HCl (99:1 v/v). A 1mL of sample material was obtained and the FRAP protocol outlined by Benzie and Strain (1996) was followed. The buffers were made by combining 0.1 mol L^{-1} acetate (pH 3.6), 10 mmol L^{-1} 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and 20 mmol L^{-1} ferric chloride solutions (10:1:1). Before analysis, an aliquot of the material was combined with FRAP reagent. After 30 min, the absorbance of the mixture was measured using a spectrophotometer at 593 nm.

Cupric Reducing Antioxidant Capacity (CUPRAC)

The CUPRAC technique (Apak et al. 2004) is based on antioxidants reducing copper I to copper II. A 10 M Cu (II) solution was made, and test tubes were filled with 1mL Cu (II), Nc, and NH4Ac (pH: 7) buffer solution. After 30 minutes of incubation, the absorbance at 450 nm (A450) was measured against a blank reagent.

Trolox Equivalent Antioxidant Capacity (TEAC)

The sample was made by adding 15 mL of a 99:1 v/v solution of methanol and HCl to 0.5 g of the sample material. Following the TEAC technique as detailed by Ozgen and Sekerci (2003), 1 mL of the sample was obtained. Potassium bisulfate was combined with 2,2-aniso-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), which was then mixed and incubated with aliquots of the sample. After 10 min, 2 mL of the prepared buffer was added to 1 mL of the sample extract, followed by measurement at a wavelength of 734 nm in the spectrophotometer.

2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH scavenging assay was carried out using the Berwal et al. (2021) technique. Ethanolic extracts of common purslane leaves (100 mL) were permitted to interact with 2.9 mL of 0.006% ethanolic DPPH for 10 min in the dark. In addition, instead of extract, 100 mL of distilled water was used as a control. At 517 nm, the absorbance was measured.

Reducing power

The Oyaizu (1996) technique was used to estimate the reducing power of the samples in the study. A 25 μ L of common purslane sample was taken and 25 μ L ethanol was added, mixed with 2500 μ L of 0.2 M phosphate buffer (pH 6.6), then 2500 μ L of 1% potassium ferricyanide solution was added and incubated at 50°C for 20 min. After the reaction mixture had been incubated, 2500 μ L of TCA 10% was added, and it was vortexed. Samples were centrifuged for 10 min at 4000 rpm. A 2500 μ L supernatant was collected, combined with 2500 μ L distilled water, and 500 μ L FeCl₃ was introduced. The samples were measured at 700 nm.

Total soluble protein contents

It was carried out according to Manual (1998). A working reagent albumin (BSA) standard and bicinchoninic acid (BCA) were prepared. A volume of 1.6 mL of working reagent and 0.1 mL of each standard and leaf extract sample were mixed well in separate, labeled microcentrifuge tubes. After that, the tubes were kept for 30 minutes at 37°C. The samples were then measured at 562 nm.

Glycine betaine

A technique published by Grieve and Grattan (1983) was used to measure the glycine betaine content. H_2SO_4 (1.5 mL 2N) was mixed with 1 mg lyophilized cells, and the samples were heated in an Eppendorf for 10 min at 60°C. Samples were centrifuged at 14000 rpm at room temperature. The tubes were kept at 4°C for 16 h before being centrifuged at 14,000 rpm for 30 min. Only crystals of glycine betaine remained adhered to the walls and bottom of the tubes after the supernatant was carefully removed. A spectrophotometer of 550 nm was used to measure absorbance.

Statistical analysis

Following data collection, all measurements were subjected to rigorous analysis using Analysis of Variance (ANOVA) to identify and statistically validate any significant variations among the samples. The Least Significant Difference (LSD) test was employed to statistically differentiate between treatment groups.

Further, a statistical tool called the Pearson correlation coefficient was used to assess connections between various aspects measured in the purslane samples. In pursuit of dimensional reduction and pattern recognition, Principal Component Analysis (PCA) was harnessed. Principal Component Analysis (PCA) was used to analyze the data and uncover hidden patterns using the software Origin 2021 (Origin Pro, Version 2021. Origin Lab Corporation, Northampton, MA, USA).

RESULTS

Lycopene content exhibited significant variation (p<0.05) across the different common purslane populations (Figure 1). Among the populations, "Assem" displayed the highest lycopene concentration, reaching 52.95 mg g⁻¹ of fresh weight (FW). Following closely were the "Balikesik" and "Pasa" populations. Meanwhile, the "Arzuman" and "Gungurler" populations showcased statistical similarities with the "Balikesik" and "Pasa" variants. Conversely, the "Yagmur" population displayed the lowest lycopene concentration (10.52 mg g⁻¹ FW), statistically similar to the "Sari Farik" population (Figure 1). Looking into lutein content, population "Assem" again took the lead with a substantial 37.33 mg g⁻¹ of FW, as shown in Figure 3. Similar to lycopene content, the "Agr Tohum" and "Yesim-I" populations exhibited lutein concentrations statistically close to "Assem" (Figure 2). Conversely, the "Arzuman" population displayed the lowest lutein content, measured at 12.63 mg g⁻¹ FW (Figure 2).



Figure 1. Quantitative assessment of lycopene concentration in common purslane populations Şekil 1. Semizotu popülasyonlarında likopen konsantrasyonunun kantitatif değerlendirmesi



Figure 2. Assessment of Lutein in common purslane populations Şekil 2. Semizotu popülasyonlarında Lutein konsantrasyonunun değerlendirmesi

This exploration extended to determining the antioxidant activities exhibited by the collected common purslane populations. The Ferric Reducing Antioxidant Power (FRAP) values exhibited significant variation among the common purslane populations (Figure 3). Among the populations, "BT Altinkulak" displayed the highest FRAP value (8.23 mg g⁻¹ FW), while "Rita" exhibited the lowest (3.0 mg g⁻¹ FW) (Figure 3). Notably, the FRAP value of "BT Altinkulak" was statistically similar to those of the "Yesim-I" and "Sari Farik" populations (Figure 3). The Cupric Reducing Antioxidant Capacity (CUPRAC) assay showed similar patterns, indicating notable differences among the different common purslane populations, as depicted in Figure 4. Interestingly, "BT Altinkulak" notably achieved the highest CUPRAC value, measuring at 123.44 mg per g of FW, while "Tunc" recorded the lowest value of 24.17 mg per g of FW.

The DPPH assay results (Figure 5) revealed the "Sun Agri" population possessed the highest antioxidant activity, exhibiting 0.79% DPPH radical scavenging capacity. Conversely, the "Agri" population displayed the lowest DPPH radical scavenging capacity (0.39%) (Figure 5). Interestingly, statistical analysis exposed a high degree of similarity in antioxidant activity among most of the populations.

Furthermore, this investigation encapsulated the Total Equivalent Antioxidant Capacity (TEAC) values, with "Yagmur" boasting the highest concentration at 135.33 mg per g of FW, and "Duzce" revealing the lowest at 66.25 mg per g of FW (Fig. 6).

The study also examined the reducing power of the purslane samples, and clear differences were found between the populations (Figure 7). The reducing power ranged from a high of 0.701 to a low of 0.316 Trolox mg per g of fresh weight. Among the populations, "Ero-I" exhibited the highest reducing power (Figure 7), suggesting strong antioxidant capabilities. Conversely, the "Sari Farik" population displayed the lowest reducing power (Figure 7), indicating weaker antioxidant activity.

The concentration of Total Soluble Protein (TSP) exhibited significant variation (p < 0.05) across the common purslane populations, ranging from 14.00 to 8.20 mg per g of fresh weight (FW) (Figure 8). The "Pasa" population exhibited the highest TSP concentration, whereas the "Agri" population displayed the lowest (Figure 8).

The Glycine Betaine (GB) concentration exhibited significant variation (p < 0.05) across the common purslane populations, ranging from 4.80 to 1.30 mg per g of fresh weight (FW) (Figure 9).



Figure 3. FRAP analysis of common purslane plant: Assessing antioxidant capacity in common purslane populations

Şekil 3. Semizotunun bitkisinin FRAP analizi: Semizot popülasyonlarında antioksidan kapasitesinin değerlendirilmesi



Figure 4. CUPRAC assessment of antioxidant activity in common purslane populations Şekil 4. Semizotu popülasyonlarında antioksidan aktivitenin CUPRAC değerlendirmesi



Figure 5. DPPH assay: Common purslane's antioxidant capacity Şekil 5. DPPH testi: Semizotunun antioksidan kapasitesi



Figure 6. Quantifying antioxidant capacity in common purslane populations via TEAC analysis Şekil 6. TEAC analizi yoluyla semizotu popülasyonlarında antioksidan kapasitesinin ölçülmesi



Figure 7. Quantitative analysis of reducing power in common purslane populations Şekil 7. Semizotu popülasyonlarında indirgeyici gücün nicel analizi



Figure 8. Analysis of total soluble protein content in common purslane populations Şekil 8. Semizotu popülasyonlarındaki toplam çözünür protein içeriğinin analizi



Figure 9. Exploring glycine betaine levels in common purslane populations Şekil 9. Semizotu popülasyonlarında glisin betain düzeylerinin incelenmesi

DISCUSSION

The results showed that the plant populations studied were significantly different from each other in their antioxidant and biochemical profiles. The results of this confirmed the high antioxidant attributes and presence of other important biochemical components in common purslane plants. Common purslane, despite its often-labeled status as a weed, stands as a pivotal herb that is found ubiquitously, in gardens and lawns.

Plant antioxidants are complex mixtures, and a single test may not be enough to capture all their antioxidant abilities (Chu et al., 2000, Yurt et al., 2024). Because of this, scientists often use several in vitro tests to assess the overall antioxidant power of plants, especially for leafy vegetables. Remarkable diversity in antioxidant activity was found among the collected common purslane populations during this analysis. Many plants contain natural chemicals that can fight oxidative stress in the body. These chemicals might therefore help treat various diseases (Ketnawa et al., 2022; Kario et al., 2022; Çelik et al., 2024). Increasing fruit and vegetable consumption is a widely recognized approach to preventing or treating chronic diseases (İzol and Turhan, 2024)..

Lycopene and lutein are carotenoids, natural pigments found in many plants and foods. These pigments have received a lot of interest because of their potential health benefits (Mapelli-Brahm et al., 2020). Lycopene, a key carotenoid pigment found in many plants, has gained attention for its potential health benefits, particularly in reducing the risk of various diseases (Khan et al., 2021; İzol et al., 2024). Studies suggest that taking lycopene supplements in reasonable amounts appears safe (Hedayati et al., 2021). Furthermore, lycopene is being explored as a potential nutraceutical due to its promise in improving blood vessel function and lowering blood pressure. Notably, the study shows the presence of substantial lycopene content in the collected common purslane populations, consistent with prior findings such as Sakil et al. (2018).

Common purslane, being a green leafy vegetable, is unsurprisingly rich in lutein, a type of carotenoid pigment also found in vegetables like kale, spinach, and broccoli (Mitra et al., 2021). Lutein offers several potential health benefits, including protecting against brain and eye problems, as well as skin irritation (Vijay et al., 2018; Yılmaz et al., 2023). A study by Ejoh et al. (2021) found similar levels of lutein in *Basella alba*. Another study by Mrowicka et al. (2022) compared lutein content in various green leafy vegetables, and common purslane stood out for its significantly higher nutritional value compared to other plants.

Measuring antioxidant activity involves using various techniques, each revealing different aspects of how effective the plant is at fighting off harmful molecules. In this study, we employed four methods—FRAP, CUPRAC, TEAC, and DPPH radical scavenging—to ascertain the antioxidant prowess of collected common purslane. "BT Altinkulak" emerged with the highest FRAP and CUPRAC values, while "Sun Agri" and "Yagmur" showed superior

TEAC and DPPH radical scavenging activities, respectively. Notably, a positive correlation was evident among FRAP, CUPRAC, TEAC, and DPPH radical scavenging activity, as depicted in Figure 10.



Figure 10. Correlation analysis of biochemical traits in common purslane: unveiling interconnections and associations

Şekil 10. Semizotunda biyokimyasal özelliklerin korelasyon analizi: bağlantıların ve ilişkiler

Note. *Significant (p<0.05). LYCO, lycopene; LC, Lutein; FRAP; TEAC; CUPRAC; GB, glycine betaine; TSP; total soluble protein

The FRAP test measures hydrophilic antioxidants, which explains why it shows lower values for common purslane since its main antioxidants are likely fat-soluble, and this aligns with previous findings (Apak et al., 2004; Uddin et al., 2014; Alam et al., 2014; İzol et al., 2024). Similarly, the CUPRAC assay is a common method used to assess the effectiveness of bioactive compounds against oxidative damage and overall antioxidant activity in biological samples or food (Uzunboy et al., 2017; Bayarsaikhan et al., 2019; Inci et al., 2023). These findings are supported by the results presented by Banerjee et al. (2013). The DPPH scavenging activity outcomes partially align with Uddin et al. (2014) and Alam et al. (2014) also established similar TEAC values. The resemblance between the carried results and those of Gunathilake and Ranaweera (2016) demonstrates consistency. Since plant extracts contain many different antioxidant compounds, scientists need to use several methods to measure their overall antioxidant capacity (Ketnawa et al., 2022; Izol et al., 2021).

Plant proteins are becoming increasingly popular as alternatives to animal proteins in human diets. This study supports previous research by Sarkar et al. (2020) on protein content in *Marsilea quadrifolia* L. The leaves of common purslane were found to be high in protein, making them a promising source of protein for our diet. Additionally, the presence of glycine betaine in the plants aligns with findings by Xu et al. (2018) and Li et al. (2019) who stated that the GB helps plants maintain photosynthesis during stressful conditions.

CONCLUSIONS

In this study, the common purslane seeds were collected from various regions of Turkey, and were grown under greenhouse conditions to determine their biochemical contents. Significant variations were observed among the collected populations, highlighting the wide range of antioxidant and biochemical properties inherent to common purslane. These differences may be attributed to the genetic variability of the seeds rather than external factors such as climate, soil composition, and sunlight exposure.

TurkeyInterestingly, some populations exhibited higher antioxidant potential, suggesting they could be valuable resources for medicine. Future research can explore how to cultivate these high-antioxidant purslane varieties and determine their effectiveness in treating specific health conditions.

Understanding these differences can unlock the full potential of common purslane as a medicinal plant. Common purslane is easy to grow and thrives in many environments, making it a potentially sustainable source of bioactive compounds. Additionally, its historical use in traditional medicine suggests promise for further investigation. By adding to this research, we can explore the potential of common purslane as a safe and natural alternative to conventional medications.

Conflict of Interest

The authors declare no conflict of interest.

Contribution of authors

KJ and MYN conceived the idea. MYN conducted the study and prepared the initial draft. KJ approved the final draft.

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Ethics approval and consent to participate

This article lacks any study related to human or animal participants performed by any of the authors.

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