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Research Article

Investigation of The Biochemical Content of Some Plant Microgreens from The *Asteraceae* Family

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Abstract: Microgreens strengthen the immune system with their intense vitamin, mineral, and antioxidant values; Scientific studies have proven that they are very effective in solving important health problems such as cancer, cardiovascular diseases, and cholesterol. In this study, the changes in photosynthetic pigment, antioxidant capacity, phenolic and flavonoid content, and ascorbic acid (vit C) contents of microgreens of some medicinal plant species (*Echinacea purpurea*, *Calendula officinalis,* and *Silybum marianum*) were investigated. At the same time, the accumulation of Ca, K, Mg, and Na, which have a direct impact on human health, was examined. The trial was designed according to the Randomized Plot Trial Design, in which the growth medium consisting of a mixture of peat, cocopeat, and perlite was used in a fully controlled climate cabin. In the results of working; While the best results in terms of photosynthetic pigment, total antioxidant substance, and flavonoid substance amount were obtained from the echinacea plant, it was determined that the phenolic substance content was higher in the thistle plant and there was no significant difference between the echinacea and thistle plant in terms of ascorbic acid content. In the study, Ca and Mg accumulation was determined to be higher in thistle, K in echinacea, and Na in calendula.

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1. Introduction

The food industry, which is in constant change and innovation, creates different trends every day. Today, vegetarian cuisine, raw nutrition, molecular cuisine, fusion cuisine, slow food, surf&turf, edible insects, and flowers are some of these trends. Raw nutrition, which is a diet based on the consumption of unrefined, natural, and raw foods, has attracted a lot of attention recently. In raw nutrition, plant seeds such as barley, oats, wheat, buckwheat, rye, rice, quinoa, soy, and lentils are consumed by sprouting. Microgreens, which have recently been offered for sale, especially in luxury restaurants and markets, have the potential to be a solution to this search. Today, consumers are focusing on healthy diets that can provide a variety of benefits. In this context, it is suggested that phytochemicals in fruits and vegetables reduce the risks of chronic diseases such as cancer, cardiovascular diseases, and some degenerative diseases (Padalia et al., 2010; Wakeham 2013; Xiao and Bai, 2019).

According to the studies conducted, the mature versions of many microgreen species contain high amounts of carotenoids (lutein, β-carotene, zeaxanthin), vitamins (E, C, and K), and mineral substances (Fe, Zn, Ca, Mg, Mn, Mo and Se), especially vitamin C, which has an antioxidant function in the human body. It is stated that it is rich in minerals such as copper and zinc and phytochemicals such as phenolic compounds, and contains low amounts of nitrate (Xiao et al., 2012; Weber, 2016; Choe et al., 2018; Kyriacou et al. 2019). One of the key features of microgreens is that they are more sustainable than mature ones; because more nutritious products can be obtained in a shorter time by using less land and less water. As antioxidants, carotenoids, phenolic compounds, and some minerals in microgreens scavenge free radicals and protect against diseases triggered by high oxidative stress.

The cotyledon leaves of microgreens are the first leaves of the plant that emerge above the ground and provide the energy required for the plant to grow by photosynthesis. As the plant grows and develops, the nutrients stored in the cotyledon leaves are transferred to other parts of the plant and the nutritional value of the cotyledon leaves decreases. However, since microgreens are consumed before this transfer occurs and all parts of them, including the cotyledon leaves, are edible, the nutritional value of microgreens is quite high. Some studies have shown that microgreens are 20% to 600% more nutritious than the mature form of the plant (Xiao et al., 2012; Weber, 2016; Choe et al., 2018; Kyriacou et al. 2019). However, this nutritional value varies depending on the soil where the plants are grown and the time of harvest.

Throughout history, medicinal plants have consistently served as an effective form of treatment, and research indicates that plants have been a primary source of medicine for thousands of years. Currently, the World Health Organization states that more than 80 percent of people still depend on traditional medicine, including plant-based remedies (Rahimi et al., 2019). This study was carried out to determine some biochemical parameters and macronutrient contents of some plants belonging to the *Asteraceae* family, which are widely used as medicinal and aromatic plants. Since there is no previous study on the subject and it is the first of its kind, it is anticipated that it will contribute to the literature and alternative products with high nutritional value can be offered to the food industry.

2. Material and Methods

The research was conducted in 2021 in the fully controlled plant growth room of Van Yuzuncu Yil University, Faculty of Agriculture, Department of Field Crops. The seeds used in the study were obtained from the Van Yuzuncu Yil University Medicinal and Aromatic Plants Garden. In the study, the potential of the species *Echinacea purpurea*, *Calendula officinalis,* and *Silybum marianum*) to be used as microfils was investigated. The experiment was designed according to the Randomized Parcel Trial Design with 4 replications. The growth medium, which consists of a mixture of sterile peat, coconut shell (cocopeat), and perlite, obtained from a certified commercial company, is placed by pressing lightly to cover 3/10 of the 500 cc plastic chalets, and the seeds are spread on it, although it varies depending on the type, in rows and the ovens. It was placed in the form of planting. The seeds were covered with soil, twice the diameter of the seed, and lightly pressed. Watering was done by spraying and they were placed in a fully controlled climate cabinet with a 16/8 (light/dark) period. Irrigation was done with distilled water and sprayed every day. The microgreens, whose growth was completed, were harvested with the help of sterile scissors from the connection part where the soil and the stem part meet. Fresh plant samples required for some pigment analyses were taken and kept in a deep freezer at -20 ºC. For the analyses to be performed on dry samples, plant samples were dried in an oven at 40 ºC and stored under appropriate conditions. In the obtained plant samples; total antioxidant activity amount (µmol TE g^{-1}), total phenolic substance amount (mg GAE g^{-1}), total flavonoid substance amount (mg QE $100g^{-1}$), ascorbic acid amount (mg LAA 100 g^{-1}), chlorophyll a amount (µg g^{-1} TA), chlorophyll b amount (µg g^{-1} TA), total chlorophyll amount (µg g^{-1} TA), total carotenoid amount (µg g^{-1} TA) and macronutrient contents (Ca, K, Mg and Na) were determined.

In the study, the total amount of antioxidant activity was determined by Lutz et al. (2011). The total phenolic substance amount was determined by Obanda and Owuor (1997). The total amount of flavonoid substances was determined by Quettier et al. (2000). The amount of ascorbic acid was determined according to the method developed by AOAC (1990) and pigment analysis by Lichtentaler and Welburn (1985).

For ion analyses, 200 mg of each leaf sample stored in the deep freezer was weighed, 10 ml of 0.1 N HNO (Nitric acid) was added to it, and the samples were kept in a dark environment at room temperature in lidded plastic boxes for a week. At the end of this period, they were shaken in a shaker for 24 hours. K^+ and Ca^{+2} ions in the prepared extracts were determined by the flame photometric method (Eppendorf flame photometer), and the amount of ions in the fresh leaf sample was determined as g/kg fresh weight (Taleisnik et al., 1997). Na and Mg elemental analyses were prepared by wet combustion method and the readings were determined with the help of an atomic absorption spectrometer device. After the samples were washed and dehumidified, they were placed in paper bags, dried in an oven at 65 °C, and then ground (Kacar and İnal, 2008). 200 mg ground samples, filters prepared according to the wet digestion method, were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) and Atomic absorption spectrophotometer (AAS) device at Van YYU Science Application and Research Center Laboratories.

The data obtained as a result of the research were subjected to variance analysis according to the Randomized Parcel Trial Design. Statistical calculations were made using the computer analysis program COSTAT (Version 6.3). Differences between means were determined according to the LSD test at the 0.05 significance level.

3. Results

In the study, significant differences were detected between species at the 5% level in terms of total chlorophyll, chlorophyll a, and chlorophyll b content, while no statistically significant differences were determined between species in terms of carotenoid content. Some biochemical content results are given in Tables 1 and 2. At the same time, among the parameters examined; It was determined that there were significant differences at the 1% level between species in terms of total antioxidant capacity, phenolic, flavonoid, and ascorbic acid contents. It can be seen in Table 3 that the contents of macro elements calcium, magnesium, potassium, and sodium vary by 1% among species. As a result of the research; While the highest total chlorophyll (23.22 µg g^{-1}), chlorophyll a (15.13 µg g^{-1} TA), and chlorophyll b (6.75 µg g^{-1} TA) contents were determined from echinacea, the lowest values were determined from thistle plant. However, no statistical difference was detected between calendula and thistle in terms of total chlorophyll and chlorophyll b contents. Carotenoid content between species was recorded between 3.31-3.78 µg g^{-1} TA. In spinach microgreens, where partially similar results were obtained with our study in terms of chlorophyll content; It was determined as 44.0 µg g^{-1} (Petropoulos et al., 2021), 290.0 µg g^{-1} in carrots (Paradiso et al., 2018), 26.5 µg g^{-1} in leek and 522.75 µg g^{-1} in green peas (Wojdylo et al., 2020), 13.36 mg kg^{-1} Fw in coriander (Kyriacou et al., 2020). Also; Choe et al. (2018), in their study, compared the phytochemical concentration between microgreen and adult growth stages of red cabbage (*Brassica oleracea* L. var. capitata); They found that microgreens contained higher amounts of carotene 11.5 mg 100 g^{-1} FW. It has been reported that carotenoids have antioxidant activity and play an important physiological role in the human body, and especially brightly colored vegetables are the main food source of carotenoids (Khoo et al., 2011). As a result of their study, Niroula et al., (2019) examined the carotenoid profile of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) microgreens; They reported that the carotenoid content in the microgreen phase was higher than that in the seed phase. Approximately 2 weeks after planting, the carotenoid content of wheat increased from 0.42 mg 100 g^{-1} DW (dry weight) to 53.36 mg 100 g^{-1} DW, while the carotenoid content of barley increased from 0.78 mg 100 g^{-1} DW (dry weight) to 56.08 mg 100 g^{-1} DW. They found that it increased either (Khoo et al., 2011). According to the results of the study conducted by Rani et al., (2024), all nine types of microgreens exhibited sufficient levels of β-carotene, with mung bean boasting the highest concentration and buckwheat the lowest. As a fat-soluble antioxidant, β-carotene, or pro-vitamin A, plays a crucial role in shielding cell membranes against oxidative stress by neutralizing free radicals. According to Xiao et al. (2012), radish microgreens contained 8.14 mg of β-carotene per 100 g of fresh microgreens, while peas contained 7.30 mg per 100 g. However, when normalized to a per 100 g basis, radish microgreens displayed a concentration range of 5.4–6.3 mg per 100 g FW, whereas peas exhibited 8.2 mg per 100 g. Despite the absolute variances, their β-carotene concentrations proved comparable when adjusted for weight. Carotenoids are lipophilic compounds with low molecular weight in chloroplasts and protect plants against oxidative stress. Carotenoids use the xanthophyll cycle to consume oxygen and protect chlorophyll from photo-oxidation. In addition to their structural role, carotenoids act as photosystem protectors against singlet oxygen radicals that absorb light and cause free radical reactions (Akhzari et al., 2018).

Table 1. Photosynthetic pigment contents of some plant microgreens from the Asteraceae family

PLANTS	Total Chlorophyll (µg g^{-1} TA)	Chlorophyll a (µg g^{-1} TA)	Chlorophyll b (µg g^{-1} TA)	Carotenoid (µg g^{-1} TA)
Echinacea purpurea L.	23.22±2.47 a	15.13 ±0.23 a	6.75±1.35 a	3.31±0.70
Calendula officinalis L.	19.24±0.31 b	14.51 ±0.24 ab	4.73±0.24 b	3.57±0.40
Silybum marianum L.	18.27±0.58 b	13.98±0.58 c	3.95±0.58 b	3.78±0.58
CV	6.51*	2.80*	16.51*	15.10

* Significant at P<0.05 level, ** Significant at P<0.01 level, and there is no statistical difference between the means indicated with letters.

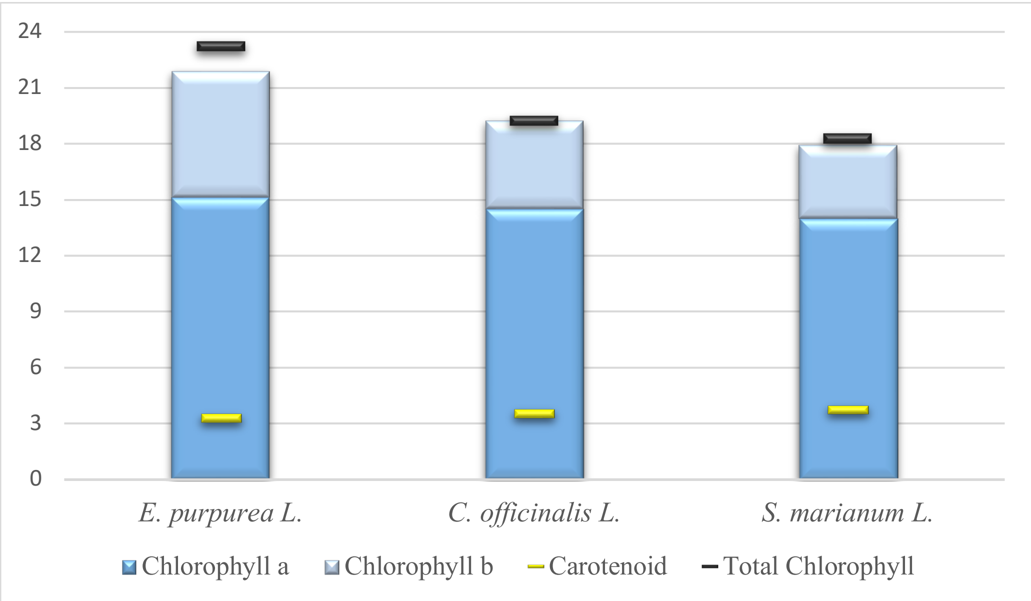

Figure 1. Photosynthetic pigment contents of some plant microgreens from the Asteraceae family.

On the other hand; While the highest amount of total antioxidant capacity (134.1 µmol TE g^{-1}) and amount of flavonoid substance (23.83 mg QE 100g^{-1}) were detected from echinacea, the highest amount of phenolic substance (211.3 mg GAE g^{-1}) and ascorbic acid content (66.53 mg LAA 100g^{-1}) was obtained from thistle. The lowest phenolic substance content (115.9 mg GAE g^{-1}) and Asa content (47.34 mg LAA 100g^{-1}) were determined from calendula. In the study, no statistical difference was noted between echinacea and thistle plants in terms of ascorbic acid content. According to Rani et al. (2024), radish microgreens boasted the highest concentration of Ascorbic Acid (AsA), while buckwheat exhibited the lowest. Specifically, the vitamin C content per 100 g of fresh radish microgreens was recorded at 83.21 mg, aligning closely with the 88.5 mg reported by Ghoora et al. (2020a) and surpassing figures from Kowitcharoen et al. (2021). These findings underscore the antioxidant potential of microgreens, as they possess more intricate polyphenol profiles and higher content compared to their mature plant counterparts (Cartea et al., 2010). Phenolic antioxidants are secondary metabolites found in microgreens that help promote metabolic activity, inhibit free radical oxidation, and reduce inflammation. The total phenolic content in microgreens was found to be between 10.71-11.88 mg g^{-1}, especially in broccoli, and this value was 10 times higher than mature counterparts and sprouts Niroula et al., (2019). Phenolic contents in microgreens are compounds responsible for various metabolic reactions in the body.

Table 2. Biochemical contents of some plant microgreens from the Asteraceae family

PLANTS	Amount of antioxidant capacity (µmol TE g^{-1})	Phenolic Substance Content (mg GAE g^{-1})	Flavonoid Substance Content (mg QE 100g^{-1})	Amount of Ascorbic Acid (mg LAA 100g^{-1})
Echinacea purpurea L.	134.1 ±11.6 a	199.3 ±3.12 b	23.83 ±1.33 a	66.11 ±2.31 a
Calendula officinalis L.	16.38 ±2.50 c	115.9 ±5.15 c	12.68 ±0.64 b	47.34 ±3.54 b
Silybum marianum L.	87.94±2.08 b	211.3±0.79 a	9.46±0.53 c	66.53±2.26 a
CV	6.76 **	2.41**	2.84**	1.20**

* Significant at P<0.05 level, ** Significant at P<0.01 level, and there is no statistical difference between the means indicated with letters.

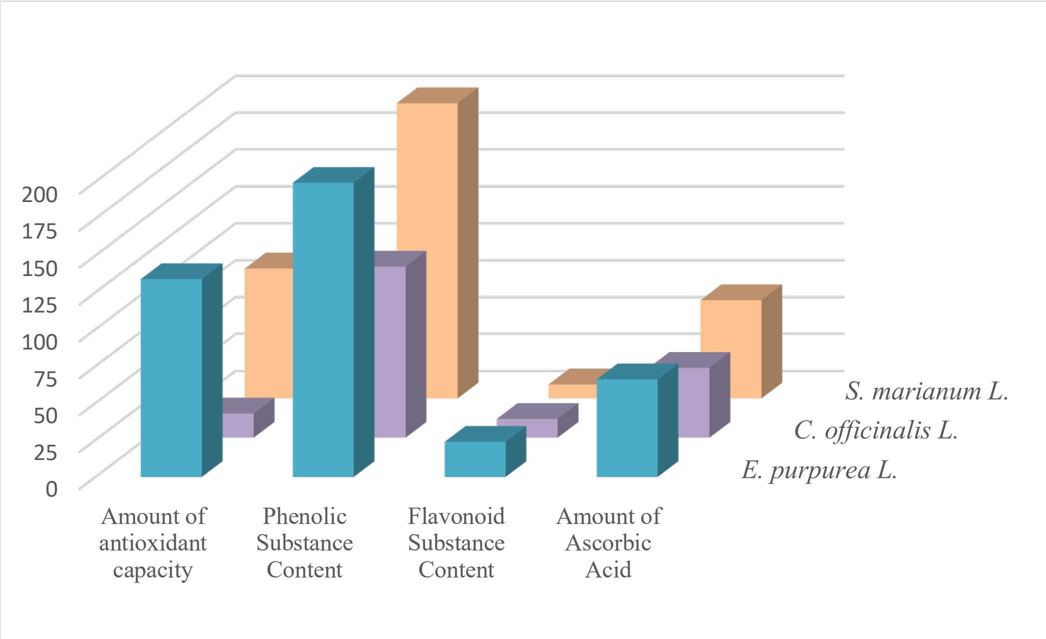

Figure 2. Biochemical contents of some plant microgreens from the Asteraceae family.

While the accumulation of macro elements Ca (23.28 g kg^{-1}) and Mg (8.82 g kg^{-1}) was highest in thistle, the lowest values were obtained from calendula. The highest K value (46.12 g kg^{-1}) was recorded from echinacea, and the highest Na value (9.24 g/kg) was recorded from calendula. In the study, the least potassium accumulation was determined from calendula, and the least sodium accumulation was determined from echinacea. Potassium (K), which is needed for skeletal and cardiac muscle activity, works together with sodium in the body to ensure fluid balance and continuity of intracellular fluid, and calcium (Ca), which is responsible for the functioning of muscles and nerves, strong bone and tooth structure and blood clotting, is responsible for the functions of the nervous system and muscle function. Nutritional elements such as magnesium (Mg), which plays a role in many basic processes from energy production to protein synthesis, and sodium (Na), which helps keep the blood volume and blood pressure in the human body under control and is needed for the normal functions of muscles and nerves, are higher in microgreens than in ripe vegetables. It has been reported that microgreens have significant advantages in terms of being high in content, being able to be produced according to consumer needs, and even being able to replace medical drugs used for deficient minerals. (Paradiso et al., 2018). Additionally, Pinto et al., (2015), Brassica microgreens, reported that they are good sources of K, Ca, Fe, and Zn, and lettuce microgreen has more mineral content (Ca, Mg, Fe, Mn, Zn, Se, and Mo) than its mature counterpart (10 weeks old). In their study, Rani et al. (2024) observed variations in the concentration of elements across nine different microgreens, including radish, cabbage, broccoli, beetroot, peas, mung beans, sunflower, garden orach, and buckwheat. Among these, potassium (K), magnesium (Mg), and calcium (Ca) were the most abundant macro-elements. Beetroot displayed the highest levels of Ca and Na, while mung beans exhibited the highest concentrations of K and Mg, suggesting their potential as rich sources of these essential elements. Conversely, cabbage microgreens showed the lowest concentration of K. These findings align with previous literature, such as studies by Xiao et al. (2012), Pinto et al. (2015), Weber et al. (2017), and Ghoora et al. (2020b), which have also reported variations in element content among different microgreens. For instance, the Ca (57.5 mg), Na (57 mg), K (272.5 mg), and Mg (54.19 mg) content per 100 g of fresh radish white microgreens closely resembled values reported in previous studies. Similarly, the nutritional composition of broccoli microgreens, with Ca at 54.9 mg and Mg at 43.83 mg per 100 g of fresh weight, was consistent with values reported by Weber et al. (2017) and Xiao et al. (2012). The variability in nutrient content among microgreens can be attributed to factors such as varietal differences and the composition of the growing substrate. External factors like growing conditions (temperature, light, and relative humidity) and substrate composition also influence the accumulation of various element concentrations in microgreens. It also varies according to genotype and maturity stage (number of days in the case of microgreens). In previous studies; The K content in micro sprouts of amaranthus, gourd, cucumber, jute, zucchini, radish, and water spinach is higher than in mature vegetables (Yadav et al., 2019), basil and chard microsprouts are good sources of K and Mg, green basil and coriander greens are good sources of K and Mg. It has been reported that they are good sources, especially of beta-carotene and total polyphenols (Kyriacou et al., 2019).

Table 3. Macronutrient Contents of some plant microgreens from the Asteraceae family

PLANTS	Ca (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Na (g kg^{-1})
Echinacea purpurea L.	16.07±0.06 b	46.12±0.06 a	6.09±0.06 b	3.12±0.39 c
Calendula officinalis L.	8.84± 0.06 c	28.88±0.06 c	3.94±0.06 c	9.24±0.35 a
Silybum marianum L.	23.28±0.07 a	33.24±0.42 b	8.82±0.13 a	5.50±0.55 b
CV	0.03**	0.69**	1.51**	6.15**

* Significant at P<0.05 level, ** Significant at P<0.01 level, and there is no statistical difference between the means indicated with letters.

Conclusion

The highest phytochemicals found in microgreens are; chlorophyll, phenolic compounds, anthocyanins, and glucosinolates. In this study; It was determined that the species with the highest content among the examined species in terms of total chlorophyll, photosynthetic pigments such as chlorophyll a and chlorophyll b and carotenoids and antioxidant capacity, flavonoid substance and ascorbic acid content was *Echinacea purpurea*, and the best phenolic substance content was the thistle species. It has been noted that among the macro elements, the highest accumulation of calcium (Ca) and magnesium (Mg) is from *Silybum marianum*, the best source of potassium (K) is *Echinacea purpurea*, and the best source of sodium (Na) is *Calendula officinalis.* This study revealed the potential of these three medicinal plant species to be used as microgreens. Contribution to the literature can be made by investigating the potential of many medicinal and aromatic plants that can be consumed as functional foods, especially those containing various secondary metabolites, such as microgreens.

Ethical Statement

Ethical approval is not required.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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Author Contributions

Authors contributed equally.

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