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Impact of growing media on yield, biochemical, and physiological characteristics of *Rheum rhabarbarum* L.

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Abstract

Rhubarb (*Rheum rhabarbarum* L.) is a popular springtime vegetable known for its vibrant red stalks and tart flavor. This study explores the intriguing biochemical profile of rhubarb and highlights its potential health benefits. Hydroponic systems (volcanic slag and volcanic slag with gravel) significantly increased rhubarb yield (both fresh and dry weight) by 2.6-6.0 times compared to traditional soil, especially in June and July. Rhubarb grown hydroponically also produced 1.6-1.8 times more petioles per plant, each with 1.6 times the fresh weight and 1.1 times the dry weight of soil-grown petioles. Compared to soil, hydroponics significantly increased the levels of extractive substances (1.2-3.2 times), flavonoids (1.4-3.7 times), and phenols (1.2-4.0 times). The research evaluated antioxidant activity, with hydroponically grown rhubarb demonstrating superior performance, particularly in the volcanic slag + gravel plants, which exhibited the strongest antioxidant activity. In contrast, the soil-grown plants displayed the lowest antioxidant activity. According to the FRAP analysis, 1 gram of plant dry mass recovered more iron in the soil-grown variant, followed by the volcanic slag variant. These findings suggest that hydroponic systems, especially those using the volcanic slag substrate, hold promise for cultivating rhubarb enriched with health-promoting compounds in the Ararat Valley.

Keywords Antioxidant activity. Fiber. Sugars. Flavonoids. Phenols. Tannins

1 Introduction

Rhubarb (*Rheum rhabarbarum* L.), with its vibrant red stalks and tart flavor, is a popular springtime vegetable. Aside from its culinary uses, rhubarb has a rich biochemical profile containing health-promoting compounds. This article explores the key components that contribute to rhubarb's unique characteristics, including flavonoids, phenolics, tannins, sugars, fiber, and its potent antioxidant activity [1]. Phenolic compounds, a category that encompasses flavonoids, are significant contributors to rhubarb's biochemical profile. These compounds give the plant its astringent taste and play a crucial role in its antioxidant activity [2]. Flavonoids, which are among the most essential compounds of rhubarb, are naturally occurring antioxidants responsible for the plant's vibrant colors and potential health benefits. Research has identified various flavonoid subtypes in rhubarb, such as catechin, epicatechin, and rutin. These flavonoids are believed to protect cells from damage caused by free radicals, potentially reducing the risk of chronic diseases [3]. Rhubarb contains tannins, a type of phenolic compound, which give it a puckering sensation. Despite this, tannins also have antioxidant properties, offering protection against free radical damage [1,4]. While rhubarb is known for its tartness, it also contains fructose and glucose, albeit in lower quantities compared to most fruits. The balanced sugar content and dietary fiber make rhubarb an appealing option for those managing sugar intake [5,6]. Antioxidants are crucial for protecting the body from free radicals. Rhubarb has strong antioxidant activity, as seen in tests like DPPH and FRAP. These tests demonstrate rhubarb's ability to scavenge free radicals, indicating potential health benefits from consuming rhubarb [7,8,9].

The parameters Fv/Fm and PI are derived from chlorophyll fluorescence measurements and provide important information about a plant's photosynthetic efficiency. Fv/Fm represents the maximum quantum efficiency of Photosystem II (PSII), the stage where light energy is captured and used to excite electrons. A value close to 0.8 indicates a healthy plant, while lower values suggest potential damage or stress affecting PSII's ability to use light energy efficiently [10]. PI reflects the overall efficiency of electron transport through both PSII and Photosystem I (PSI) involved in photosynthesis. It's often recommended to measure both Fv/Fm and PI for a more complete understanding of plant health. These parameters can be used to detect stresses impacting photosynthesis, such as drought, nutrient deficiency, or pest damage. Early detection allows for timely intervention to minimize harm to the plant. Additionally, Fv/Fm and PI can be used in breeding programs to select plants with superior photosynthetic efficiency, potentially leading to higher crop yields [11].

Hydroponics, a method of growing plants without soil, offers several potential benefits for agriculture in the Ararat Valley of Armenia. The region faces challenges with water scarcity, especially during dry summers [12]. Compared to traditional soil-based agriculture, hydroponics uses significantly less water. Hydroponic systems enable precise control over nutrient delivery, resulting in more consistent product quality with higher levels of desired nutrients and potentially reducing bitterness in crops like rhubarb [13]. Due to the unique biochemical characteristics of rhubarb, we have decided to explore the possibility of growing this plant for the first time in the Ararat Valley of Armenia. The research aims to investigate the impact of growing media on the biochemical composition of rhubarb.

2 Materials and Methods

2.1. Plant growth and sample collection

An experiment was conducted in 2021-2022 in the Ararat Valley of Armenia to compare plant growth in two conditions: outdoor hydroponics and soil. The average annual rainfall in the valley is 325 mm, and it sits 800-1000 meters above sea level. Winter temperatures range from -26.1 to 32.6 °C, while summers are hot, with temperatures between 37.5 and 42.6 °C [14].

The plants were planted in the last ten days of April. The rhizomes of 3-year-old mother plants were divided into 4 parts and planted in 25 L hydroponic pots and soil. In hydroponics EBB and Flow system was used [15]. The experiment was set up with three treatments: 1. hydroponic with growing medium volcanic slag (VS); 2. hydroponic with growing medium volcanic slag and gravel in a 1:1 ratio (VS+G), 3. soil (S) which served as the control group. Volcanic slag and gravel were mined in Armenia. Each hydroponic substrate had particles of 3 to 15 millimeters in diameter [16]. Standard agricultural practices were followed for control variant. Each variant had 6 replicates.

In hydroponics plants were nourished with a specific nutrient solution developed by Davtyan [17] to 1-2 times daily. This solution has a pH range of 5.5-7.0 [18], making it suitable for rhubarb. In contrast, the rhubarb grown in soil was irrigated every three-four day. The harvest of petioles was done on the stage of their maturity. Harvesting of plants was done according to months, in hydroponics - from May to October, and in soil - from June to September. The experiment involved six harvests of rhubarb petioles from the hydroponic systems and five harvests from the soil-grown plants. Harvests for both groups were spaced out by about a month. Hydroponically grown rhubarb was ready for its first harvest in 19 May, while the soil-grown rhubarb wasn't ready until 26 June. After harvesting, the aerial part of the plant was transported to the laboratory, the leaves were separated from the petioles and the fresh weight of petioles (FW) was measured. Then leaves and petioles were dried in a well-ventilated, shade room and the dry weight of petioles (DW) was measured. Considering the fact that petioles are the most useful parts of the plant, that is why emphasis was placed on the study of petioles. The growth rate, development stage and weight of both fresh and dried petioles for each plant was measured. During the growing season, studies were carried out on a number of morphological characteristics of petioles obtained per plant: length (measured at maturity stage from the hydroponic substrate or soil surface to the base of the leaf), thickness (was measured by electronic caliper as a diameter at the base of petiole), quantity. Additionally, bio-pharmaceutical and chemical analyses were conducted throughout the experiment using six randomly chosen plants from each group.

2.2 Statistical Analysis

All data were analyzed using statistical programs, GraphPad Prism 8 and Microsoft Excel 2016. t-test analysis of variance (ANOVA) determined statistical significance and comparison of mean values. Sample selections of the studied groups were random, and the values were assessed for significant differences ($P \leq 0.05$).

2.3 Determination of total flavonoids and total phenols

The experiment measured the total flavonoid and phenol content of dry herbs. First, one gram of finely ground herb powder, sieved through a mesh with 2.0 mm openings, was weighed and placed in a 250 mL flask. Then, 100 mL of 70 % ethanol was added, and the mixture was heated in a water bath for 60 minutes with a reflux condenser attached. After cooling, the extract was filtered through paper into a 100 mL volumetric flask. The remaining volume in the flask was then filled to the mark with 70 % ethanol, creating solution A. 2 mL of solution A was transferred to a separate 25 mL volumetric flask. This solution was then topped up to the mark with 96 % ethanol. To measure the total flavonoid content, the absorbance of this final solution was recorded at a wavelength of 370 nm using an Agilent Cary 60 UV-Vis spectrophotometer with a 10 mm cuvette. Similarly, the absorbance for total phenols was measured at 277 nm. For all measurements, 96 % ethanol was used as the reference solution [19].

2.4 Determination of tannins

The tannin content in 2 g of crushed dry material was determined using a titrimetric method. The material was first added to a conical flask with 50 mL of boiling water and heated in a water bath for 30 minutes with frequent stirring. This extraction process was repeated five times, filtering the extract through cotton wool into a 250 mL volumetric flask each time, until no tannins were detected. The final volume in the flask was then adjusted to the mark with water. To quantify the tannins, 25 mL of the combined extract was transferred to a 1-Liter conical flask. Distilled water (750 mL) and 25 mL of indigo sulfonic acid solution were added. The solution was then titrated with 0.1 N potassium permanganate solution with constant stirring until the color changed to a golden yellow. This process was repeated with a control solution containing only 25 mL of indigo sulfonic acid in 750 mL of water. The tannin content was then calculated using a specific formula based on the difference in potassium permanganate solution used between the sample and control titrations.

$$X = ((V_1 - V_2) \times D \times V \times 100) / (m \times V_3)$$

where V_1 is the volume of 0.1 N KMnO_4 taken for titration, mL; V_2 – volume of 0.1 N KMnO_4 sample taken for titration, mL; D – conversion factor to tannin: for hydrolysable tannins 0.004157, for condensed 0.00582, V – total volume of extract, mL; m – sample mass, g; V_3 - volume of extract taken for titration, mL [20].

2.5 Determination of crude fiber

This experiment measured the crude fiber content in a one-gram sample. The dried and ground sample was placed in a 1-Liter beaker. To prevent evaporation during boiling, a round-bottomed flask filled with cold water was used as a condenser on top of the beaker. The sample was then boiled for 30 minutes with 250 mL of 1.25 % sulfuric acid solution under reflux conditions. After boiling, the mixture was quickly filtered through a Whatman No. 42 ashless paper filter placed in a Buchner funnel. The residue was washed thoroughly to remove all acid. Next, the acid-free residue was refluxed again for 30 minutes with 200 mL of 1.25 % sodium hydroxide solution, maintaining a constant volume throughout. The mixture was then filtered and washed to remove any remaining alkali: The residue was carefully transferred to a crucible and dried in an oven at $100^\circ\text{C} \pm 5^\circ\text{C}$ until its weight remained

constant. The crucible was then cooled in a desiccator and the weight of the dried residue was recorded. Finally, the crucible was placed in a muffle furnace and ignited at 600 °C for four hours. After cooling again, the final weight was measured. The amount of crude fiber in the sample was determined by the weight loss after ignition [21].

2.6 Sugar Quantification

A 5-gram sample of finely ground plant material was extracted with 70-200 mL of hot water in a 100 mL volumetric flask. The mixture was maintained at 80-90 °C for an hour in a water bath with periodic shaking. The cooled filtrate was then subjected to hydrolysis by adding 5 mL of concentrated hydrochloric acid to a 50 mL aliquot and heating at 68-70 °C for 8 minutes. After neutralization with 20% sodium hydroxide and dilution to 100 mL, the reducing sugars in a 10 mL aliquot were determined by the Fehling's method. The cuprous oxide precipitate was filtered, washed, and dissolved in iron(II) sulfate solution. The resulting iron(III) ions were titrated with potassium permanganate to quantify the sugar content [21].

2.7 Antioxidant Capacity by DPPH

A stock solution of 10^{-3} M DPPH radicals in ethanol or methanol was freshly prepared and diluted to an absorbance of 1.00 ± 0.200 . To determine antioxidant activity, 3 mL of this DPPH working solution was mixed with 0.5 mL of the sample extract and incubated in the dark for 30 minutes. The decrease in absorbance at 517 nm, compared to a solvent-only control, indicated the antioxidant capacity of the sample. All measurements were performed in triplicate [22, 23].

2.8 FRAP Antioxidant Assay

The ferric reducing antioxidant power (FRAP) of extracts and isolated compounds was determined according to Birasuren et al. [24]. A FRAP reagent, prepared by mixing acetate buffer, TPTZ (2,4,6-tripyridyl-s-triazine), and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and heating to 37 °C, was incubated with diluted samples (100 $\mu\text{g/mL}$) for 10 minutes at 37 °C in the dark. Absorbance at 593 nm was measured against a blank. The results were expressed as aqueous solution of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), and derived from a calibration curve of the standards. Likewise, the FRAP value of ascorbic acid was obtained by the same procedure.

2.9 Chlorophyll fluorescence

A Pocket PEA model Chlorophyll Fluorimeter was used to measure chlorophyll fluorescence. This device measures fluorescence recorded chlorophyll based on F_v / F_m and PI parameters. The method used was that two leaves were selected from each pot and placed in special clamps to create dark conditions for 15 minutes, and after this, the amount of chlorophyll fluorescence was recorded. Chlorophyll a fluorescence transient data was used to calculate the parameters. The F_0 level was measured as the fluorescence at 50 μs ($F_{50 \mu\text{s}}$). Maximum quantum efficiency of PSII photochemistry (F_v / F_m) was calculated according to the equation [25].

$$F_v / F_m = (F_m - F_0) / F_m$$

Performance index (PI) was calculated as:

$$\text{PI}_{\text{abs}} = (1 - (F_0 / F_m)) / (m_0 / V_J) \times (F_m - F_0) / F_0 \times (1 - V_J) / V_J$$

where F_0 means fluorescence intensity at 50 μ s, F_m represents maximal fluorescence intensity, V_J is relative variable fluorescence at 2 ms (J-step), calculated as:

$$V_J = (F_{2ms} - F_0) / (F_m - F_0)$$

M_0 represents the initial slope of fluorescence kinetics, which can be derived as:

$$M_0 = 4 (F_{300\mu s} - F_0) / (F_m - F_0)$$

3 Results

3.1 Plant growth and yield in vegetation periods

The results of the research showed that the growing medium significantly affected the yield of rhubarb petioles throughout the growing season. In all hydroponic substrates, petiole fresh and dry mass increased in June and in soil in July (Fig. 1, 2).

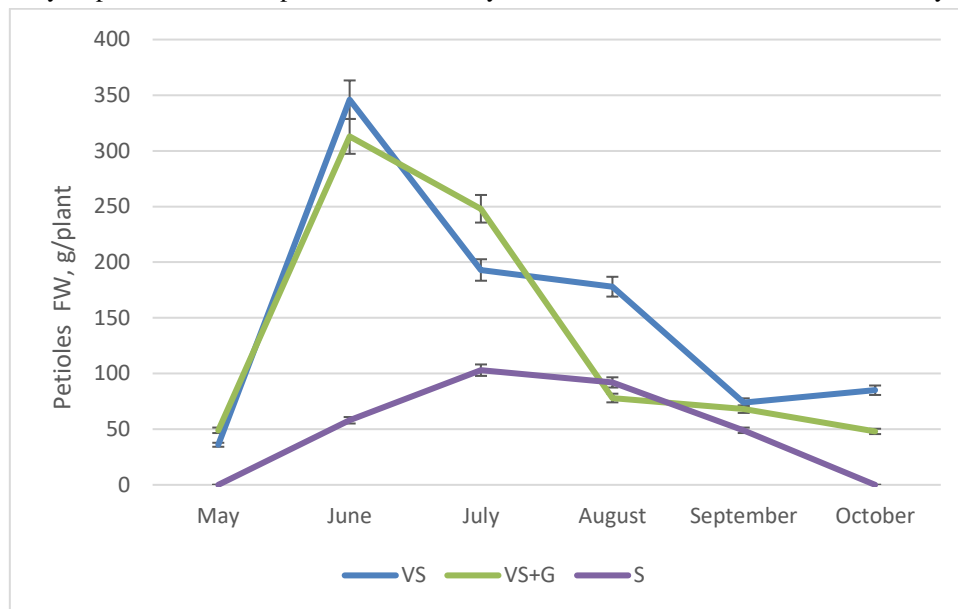


Fig. 1 The dynamics of accumulation of fresh rhubarb petioles in the vegetation period

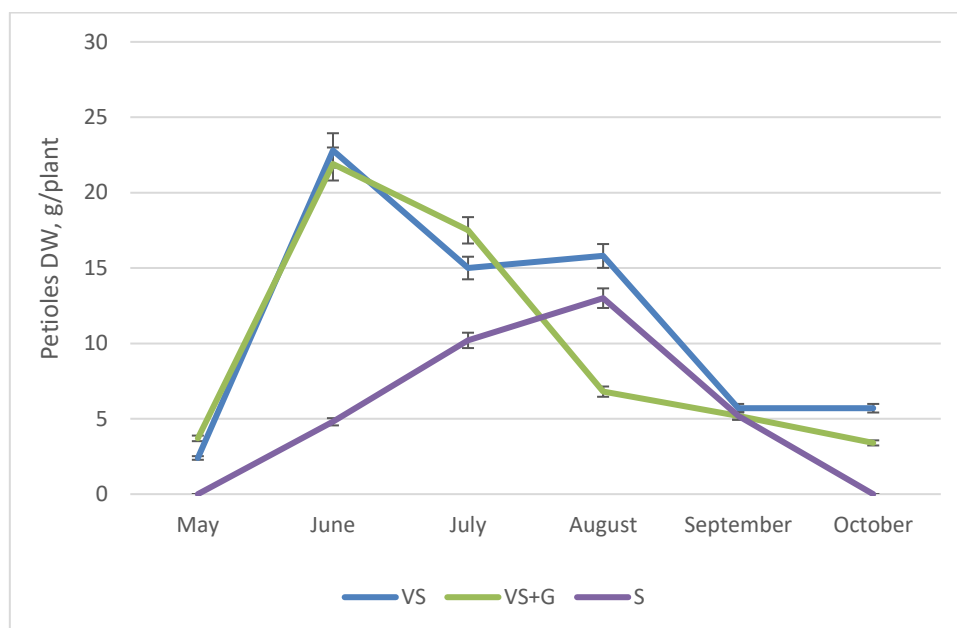


Fig. 2 The dynamics of accumulation of dry rhubarb petioles in the vegetation period

During the vegetation period, hydroponic plants exceeded S plants in petiole FW and DW by 2.6-6.0 and 2.9-4.8 times in June and 1.5 and 2.4 times in July, respectively. The tendency changed in August: soil plants outperformed plants of VS+G substrate in FW and DW by 1.2 and 1.9 times, respectively, but were inferior to VS substrate grown plants by 1.9 and 1.2 times, respectively. In September, soil plants were 1.4-1.5 times lower in FW than hydroponic versions (Fig. 1), but no significant difference was observed in DW (Fig. 2).

Table 1 Rhubarb petioles yield and biometric parameters according to growing medium

Growing medium		Petioles (g/plant)		Number of petioles	Per petiole (g)		Width of petioles (mm)	Length of petioles (cm)
		FW	DW		FW	DW		
Hydroponic substrates	VS	913.0±59.3 ^a	67.4±2.2 ^a	74±12.6 ^a	12.3±1.3 ^a	0.91±0.06 ^a	10.6±0.4 ^a	14.2±0.7 ^b
	VS+G	804.0±83.5 ^a	58.5±4.2 ^b	66±7.7 ^a	12.2±0.5 ^a	0.89±0.07 ^a	9.7±0.5 ^{ab}	16.8±0.9 ^a
S (control)		302.0±26.5 ^b	33.2±3.3 ^c	41±5.1 ^b	7.4±0.7 ^b	0.81±0.07 ^a	9.0±0.3 ^b	15.9±1.9 ^a

Different letters indicate significant differences, $P < 0.05$

From the data in Table 1, it can be seen that the different hydroponic substrates had no significant influence on FW of the total petiole yield, the number of petioles obtained from per plant, and FW and DW of per petiole. At the same time, the S plants were inferior to the hydroponic versions in FW (2.7-3.0 time) and DW (1.8-2.0 time) of petioles. In the hydroponic versions, 1.6-1.8 times more petioles of rhubarb were formed than in the soil one. The FW of per petiole of the plants grown in soilless conditions was 1.6 times higher than those of the soil plants. But in DW there is no significant difference. It was found that the hydroponic substrate significantly affected the length of petioles. Thus, petioles of VS substrate grown plants were relatively thick and short, while VS+G substrate grown plants formed relatively long petioles. From the analysis of petiole biometric data, it is clear that only VS substrate and S plants differed significantly in petiole width (1.2 times). At the same time, VS substrate petioles were 2.6 cm shorter than those in VS+G substrate. FW/DW ratio of petioles in hydroponics was 13.5-13.7, while it was 9.1 in S plant. Thus, the water content in hydroponic plants was about 1.5 times higher than in S plants.

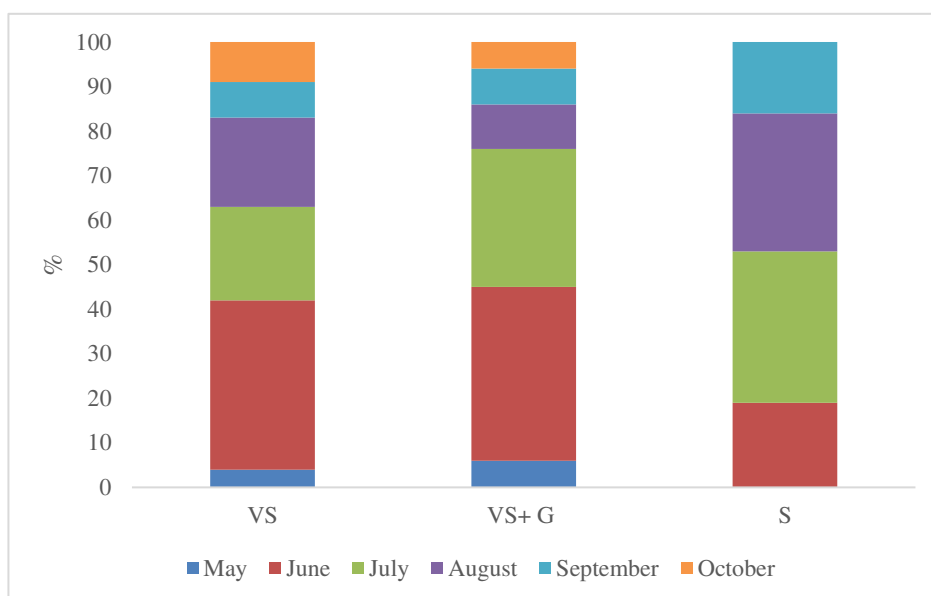


Fig. 3 Rhubarb petioles FW ratio by month during the growing season, %

The plants grown in the VS substrate (Figure 3) showed the highest percentage of yield (38 %) in June, then it gradually decreased during the growing season, the percentage of yield was 20-21 % in July and August, and 8-9 % in September-October. VS+G substrate showed the highest percentage of yield in June (39 %), in July the yield percentage decreased to 31 %, then it decreased in August by 3 times, In the last months of the growing season, the yield gradually decreased. The maximum percentage of yield of S plants was obtained in July (34 %), and then it decreased. It should be emphasized that in the hottest month (August) of vegetation period, S plants formed the highest percentage of yield compared to hydroponic versions. In hydroponics, in different substrates, the percentage of the yield during the August was significantly affected by the presence of the gravel substrate, which led to a decrease in yield percentage trice due to overheating of substrate particles.

3.2. Biochemical analyses during the vegetation period

Biochemical analyzes of rhubarb petioles were performed during the growing season to reveal the influence of the growing medium on the biosynthesis of key substances. Experiments showed that VS substrate, compared to VS+G one, had a positive effect on the content of extractives (1.2 times), flavonoids (1.4 times) and tannins (1.2 times) in petioles. The hydroponic growing medium did not affect the content of phenols, crude fiber and sugars in the plant petioles. The minimum content of extractives, flavonoids, and total phenols was observed in petioles of S plants, which were 1.3-1.6, 1.3-1.7, and 1.9-2.0 times lower than the hydroponic versions, respectively. However, the S plants exceeded the hydroponic versions in tannins content by 2.9-3.4 times. The growing environment did not have a significant effect on the content of crude fiber and sugars. In all versions, they varied between 13.1-15.3 and 6.2-6.6 %, respectively.

Table 2 Values of biochemical indicators of rhubarb petioles depending on the growing medium

Growing medium	Extractives		Flavonoids		Total phenols		Tannins		Crude fiber		Sugars	
	%	g/plant	%	mg/plant	%	mg/plant	%	g/plant	%	g/plant	%	g/plant
VS	34.2±2.7 ^a	23.10	0.33±0.05 ^a	222	0.18±0.01 ^a	121	1.75±0.12 ^c	1.18	14.6±1.2 ^a	9.84	6.2±0.4 ^a	4.18
VS+G	29.3±2.1 ^b	17.14	0.24 ±0.02 ^b	140	0.17±0.03 ^a	99	1.50±0.1 ^b	0.88	15.3±1.3 ^a	8.95	6.6±0.4 ^a	3.86
S (control)	22.0±1.6 ^c	7.30	0.19 ±0.02 ^c	63	0.09±0.01 ^b	3	5.08±0.29 ^a	1.69	13.1±1.4 ^a	4.35	6.4±0.5 ^a	2.12

Different letters indicate significant differences, $P < 0.05$

Plants grown in the VS substrate provided an increase in the yield of extractives by 1.3 times, flavonoids by 1.6 times, total phenols by 1.2 times and tannins by 1.3 times compared to the other hydroponic version. Crude fiber and sugars yields were not affected by the hydroponic growing medium. Soil plants were inferior to the hydroponic versions in yield of extractives by 2.3-3.2 times, flavonoids by 2.3-3.7 times, phenols by 3.3-4.0 times, crude fiber by 2.1-2.3 times and sugars by 1.8-2.3 times, but they exceeded the accumulation of tannins by 1.4-1.9 times.

3.3 DPPH and FRAP antioxidant activity

The potential antioxidant activity of the extracts obtained from the dry rhubarb petioles was evaluated by DPPH and FRAP methods (Figure 4). Experiments were performed at different concentrations to determine the IC_{50} . The results showed that VS+G plants have high antioxidant activity ($61.58 \pm 2.46 \mu\text{g/mL}$). Plants of the VS variant is slightly inferior to them ($70.77 \pm 6.82 \mu\text{g/mL}$). The lowest antioxidant activity was recorded in the S variant ($97.35 \pm 0.79 \mu\text{g/mL}$). According to the data of FRAP analysis, 1 gram of plant recovered more iron in the S variant ($274.92 \pm 5.44 \mu\text{mol Fe}^{+2}/\text{g DW}$), followed by the VS substrate ($186.02 \pm 27.96 \mu\text{mol Fe}^{+2}/\text{g DW}$). The smallest value was observed for the VS+G substrate ($130.21 \pm 4.45 \mu\text{mol Fe}^{+2}/\text{g DW}$).

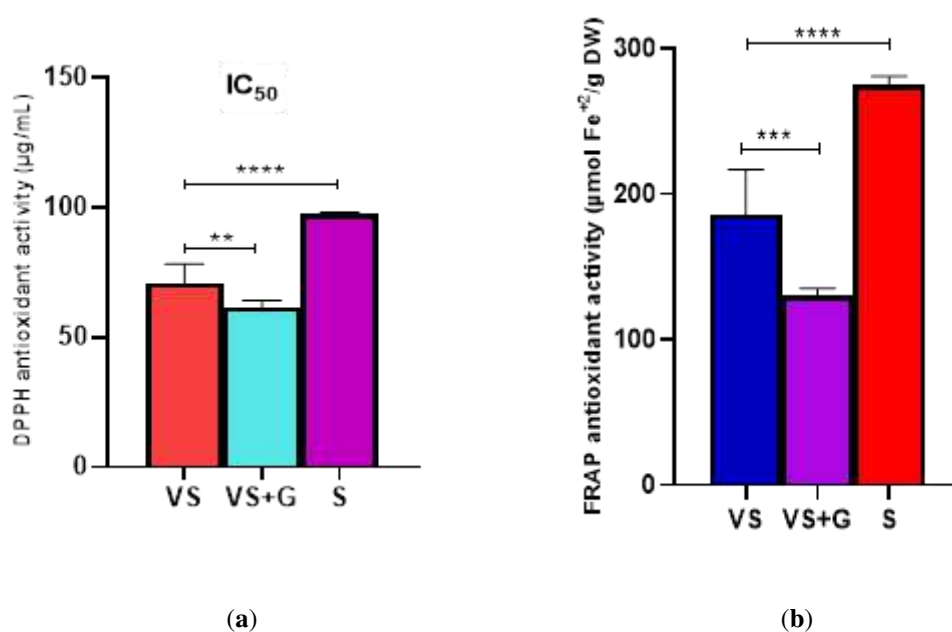


Fig. 4 The DPPH (a) and FRAP (b) antioxidant activity in different growing medium. VS, VS+G, and S. Data are mean values $\pm$ SD of three replicates, $P < 0.01$

3.4 Chlorophyll fluorescence

During the vegetation period from July to August, the value of F_v / F_m did not exceed the norm (0.8) in all variants. The PI data showed that the hydroponic variants reached peak viability in June and the S plants in July. General senescence was observed in August for all variants.

Table 3 Chlorophyll fluorescence indicators under hydroponics and soil

Growing medium	Fv / Fm			PI		
	June	July	August	June	July	August
VS	0.818	0.823	0.814	8.358	7.584	4.821
VS+G	0.802	0.821	0.802	9.289	8.943	3.635
S (control)	0.820	0.795	0.702	3.761	5.876	3.163

4 Discussion

The literature data shows that 722.3-2273.3 grams of petiole were obtained from each 6-year-old plant, which was planted with the same density in the Moscow region [26]. In our experiment, the plants grown in soil produced a lower biomass of petiole, whereas the hydroponic versions were close to the data obtained by Kharchenko et al [26]. This indicates that it is more preferable to cultivate this plant in hydroponic conditions in the Ararat Valley. Additionally, it's worth noting that the dry biomass of rhubarb grown in the soil conditions of the Ararat Valley is 11 % of the fresh biomass, which exceeds the data obtained by Kharchenko et al. (6.4-8.5 %). There is no information on the hydroponic cultivation of rhubarb in the literature. Our study results indicate that the ratio of fresh to dry leaf petiole mass was approximately 1.5 times higher in hydroponic substrates compared to soil controls. Furthermore, our research shows that hydroponic conditions led to an extended harvest season (6 harvests from May to October in hydroponics compared to 4 harvests from June to September in soil). Additionally, the maximum biomass was obtained in the VS substrate in June. According to our data, the VS substrate is the most effective for providing the maximum total rhubarb biomass per growing season.

Study analysis by Clapa et al. [2] focused on the presence of different phenols and flavonoids in rhubarb stalks. The total phenol content was measured at 375.4 $\mu\text{g/g}$, which is 0.038%, and the total flavonoid content was 385.6 $\mu\text{g/g}$, which is 0.039 %. These findings were lower than those obtained in our study. In our research, we found that the volcanic slag variant had the highest accumulation of flavonoids and phenols. Both flavonoids and phenols act as antioxidants, helping to neutralize free radicals and unstable molecules that can harm cells and contribute to chronic diseases. By combating these free radicals, they may help prevent various health issues such as cardiovascular disease, cancer, Alzheimer's, and Parkinson's disease [27-28]. Considering that hydroponic cultivation yields higher amounts of these bioactive compounds in rhubarb, it is a more effective cultivation method from a medical standpoint.

The total tannin content in rhubarb can vary based on factors such as the specific variety, growing conditions, and the part of the plant being measured. Research indicates that rhubarb does contain tannins. Wang et al. [29] found that the total tannin content in the raw material and the total extract of rhubarb were 6.12 % and 19.20 %, respectively. In our study, soil-grown plants exhibited a 33 % higher tannin content compared to hydroponically grown versions. Literature from Germany indicates that the total sugar

values in rhubarb juice surpassed the total acid content, ranging from 16.31–17.49 g/L [30]. A study on *Rheum tanguticum* revealed that crude fiber (89.14 % $\pm$ 0.75) was the most abundant macronutrient in its inflorescence stem [31]. Based on our research, hydroponically grown plants outperformed soil-grown plants in sugars output by 51 % and crude fiber by 44 %.

Cojocaru et al. [32] found that the use of chemical, organic, or biological fertilizers increases the antioxidant activity of rhubarb plants in soil. According to Zhou and Yu [33], the antioxidant capacity of studied vegetables ranks as follows: rhubarb > green bean > tomato > potato > kale > spinach > broccoli. In our study, plants in VS+G substrate showed higher antioxidant activity than plants grown in VS substrate, with rhubarb plants exhibiting the highest antioxidant activity. When comparing FRAP levels, Jargalsaikhan et al. [34] discovered that in ethanol solution, the level of FRAP was 4.66 mM/g in *Rheum undulatum* L. and 1.98 mM/g in *Rumex crispus* L. variety. In our study, the FRAP level ranged between 130.22–274.92 μ mol/g in the rhubarb variety, depending on the growth medium. Chlorophyll fluorescence is a technique used to assess the efficiency of photosynthesis in plants. Limited research on chlorophyll fluorescence in rhubarb indicates that this issue has not been widely investigated. Our data indicates that the chlorophyll fluorescence value for the rhubarb plant is close to 0.8, suggesting that the plant is healthy [35]. According to the PI value, the aging process of rhubarb begins in August across all versions.

5 Conclusions

Rhubarb grown hydroponically generally performed better than soil-grown plants in terms of FW and DW of petioles during most of the growing season, except for August. However, there were no significant differences in total petiole yield per plant or individual petiole weight between different hydroponic substrates. VS substrate grown plants produced thicker and shorter petioles, while VS+G substrate resulted in narrower and longer petioles. It is important to note that VS+G substrate negatively affected yield in August due to high temperatures. Hydroponically grown rhubarb had a higher water content compared to soil-grown plants. The highest percentage of yield of hydroponically grown rhubarb occurred in June (38 % for VS, 39 % for VS+G) and gradually decreased throughout the season. VS+G substrate showed a sharper yield decline in August due to overheating of gravel particles. The maximum percentage of yield of soil-grown rhubarb occurred in July (34 %) and then decreased. Interestingly, soil plants produced the highest yield percentage in the hottest month (August).

Rhubarb grown hydroponically had higher levels of extractable substances, flavonoids, and phenols compared to soil-grown plants. However, soil-grown plants had significantly higher tannin content. Sugars and crude fiber content were not affected by the growing environment. Within hydroponics, the VS substrate promoted a greater accumulation of beneficial compounds (extractives, flavonoids, phenols, tannins) compared to VS+G one. However, VS+G substrate showed the highest antioxidant activity. Overall, hydroponically grown rhubarb exhibited higher antioxidant activity compared to soil-grown plants, with VS+G substrate showing the strongest effect. These results suggest that hydroponics can be a valuable method for producing rhubarb with enhanced levels of beneficial compounds and antioxidant activity. However, the choice of substrate can influence the specific profile of these compounds.

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Declarations

Ethics approval and consent to participate

Seeds of *Rheum rhabarbarum* L were collected from the mountains located nearby in the Shahrebabak region of Kerman in Iran. It was determined and authenticated by Prof. Dr. Mansour Ghorbanpour (Arak University, Iran). The voucher specimen (code: AMH1008) was deposited at the Herbarium of the Department of Medicinal Plants, Arak University, Iran.

Statement on experimental research and field studies on plants

All methods performed in this study including the collection of plant materials were in compliance with the relevant institutional, national, and international guidelines and legislation.

Competing interests The authors indicate no conflict of interest for this work.

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