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Impact of Growth medium on Yield and Biochemical Characteristics of *Rheum rhabarbarum* L.

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ABSTRACT

Rhubarb (*Rheum rhabarbarum* L.) is a widely consumed vegetable appreciated for its vibrant red stalks and tart flavor. This study examines the effects of hydroponic systems on the growth, yield, and biochemical composition of rhubarb. Plants cultivated in hydroponic media—specifically volcanic slag and a mixture of volcanic slag with gravel—exhibited significantly greater fresh and dry biomass, with yields 2.6 to 6.0 times higher than those grown in traditional soil, particularly during June and July. Hydroponically grown rhubarb also produced 1.6 to 1.8 times more petioles per plant, with each petiole having 1.6 times the fresh weight and 1.1 times the dry weight of those from soil-grown plants. Furthermore, the hydroponic media enhanced the levels of plant extracts (1.2–3.2 times), total flavonoids (1.4–3.7 times), and total phenols (1.2–4.0 times) compared to soil-grown counterparts. Antioxidant activity, assessed via DPPH assay, was also higher in hydroponically grown plants, particularly in the volcanic slag and gravel mixture, which exhibited the strongest antioxidant capacity. However, FRAP analysis revealed that soil-grown plants recovered more iron, followed by those grown in pure volcanic slag. Additionally, hydroponically grown rhubarb in volcanic slag displayed significantly higher concentrations of chlorogenic acid, rutin, quercetin, and ferulic acid than plants grown in the slag-gravel mixture. Notably, quercetin content was comparable between soil-grown and volcanic slag-grown plants. These findings suggest that hydroponic cultivation, especially using volcanic slag as a medium, offers a promising approach for producing rhubarb with enhanced yields and elevated levels of health-promoting compounds.

Abbreviations: Dry weight (DW), Ferric reducing antioxidant power (FRAP), Fresh weight (FW), Soil (S), Trifluoroacetic acid (TFA), Volcanic Slag (VS), Mixture of Volcanic Slag and Gravel (VS+ G), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

Introduction

Rhubarb (*Rheum rhabarbarum* L.), known for its vibrant red stalks and tart flavor, is a popular springtime vegetable. Beyond its culinary appeal, rhubarb possesses a rich biochemical profile containing numerous health-promoting compounds. This article explores the major biochemical constituents contributing to rhubarb's unique characteristics, including total flavonoids, phenols, tannins, sugars, fiber, and its antioxidant potential (Kalisz et al., 2020). Among these, phenolic

compounds—particularly flavonoids—play a central role in both the plant's astringent taste and its antioxidant activity (Clapa et al., 2020).

Flavonoids are naturally occurring antioxidants responsible for rhubarb's pigmentation and associated health benefits. Several flavonoid subtypes, such as catechin, epicatechin, and rutin, have been identified in rhubarb. These compounds are believed to protect cells from oxidative damage caused by free radicals, potentially reducing the risk of chronic diseases (Cojocaru et al., 2020). Additionally, rhubarb contains tannins—another class of phenolic compounds—that contribute to its characteristic puckering sensation while also exhibiting antioxidant properties (Kalisz et al., 2020; Mosleh et al., 2023). Despite its notable tartness, rhubarb contains moderate amounts of natural sugars, primarily fructose and glucose, albeit in lower quantities than most fruits. Its relatively low sugar content, combined with its dietary fiber, makes rhubarb a suitable option for individuals managing their sugar intake (Góraj-Koniarska et al., 2018; Mezeyová, 2021). Antioxidant activity in rhubarb has been confirmed through standard assays such as DPPH and FRAP, both of which demonstrate the plant's ability to scavenge free radicals, underscoring its potential health benefits (Won Jang, 2018; Lee et al., 2022; Mehrabani et al., 2023).

Hydroponics—a soilless cultivation method—has gained attention as a sustainable agricultural approach, especially in regions facing water scarcity or unsuitable soil conditions (Vardanyan et al., 2021). Compared to traditional soil-based farming, hydroponics significantly reduces water usage and allows for precise nutrient management, resulting in higher and more consistent crop quality with elevated levels of nutrients and bioactive compounds (Barbosa et al., 2015; Chaudhuri and Balasubramanian, 2025; Roosta and Hamidpour, 2011; Tadevosyan et al., 2024). Previous studies have demonstrated the superior growth, photosynthetic performance, and antioxidant activity of hydroponically grown crops such as spinach (Gaikwad et al., 2024) and *Moringa oleifera* (Tadevosyan et al., 2024). The flexibility of hydroponic systems to accommodate diverse substrate types enables the selection of optimal growing media tailored to specific crops and their biological needs (Spencer et al., 2024; Wu et al., 2025). For instance, Komorowcka et al. (2023) investigated the use of sheep wool in hydroponic cucumber cultivation, while Rosli et al. (2023) found that a mixture of palm kernel biochar and hydroton enhanced the growth, color, and nutritional quality of red lettuce (*Lactuca sativa* L.). Despite growing interest in hydroponics, scientific data on hydroponic rhubarb cultivation remain limited. Duke et al. (1999) demonstrated rhubarb's potential in phytoremediation when grown hydroponically. In our earlier study, we highlighted the feasibility of hydroponics in maximizing rhubarb yield and influencing key quality parameters such as vitamin C content and acidity throughout the growing season (Daryadar et al., 2024).

Given rhubarb's unique biochemical profile—rich in flavonoids, phenols, tannins, and antioxidants—and the increasing demand for sustainable, high-yield cultivation methods, the present study was designed to evaluate how different hydroponic growing media influence the biochemical composition and productivity of *Rheum rhabarbarum* L. Building on the scarcity of scientific data on hydroponic rhubarb cultivation, and informed by prior findings that suggested hydroponics may enhance yield and modify quality traits such as vitamin C content and acidity, this research specifically compares the performance of rhubarb grown in two hydroponic substrates—volcanic slag alone and a mixture of volcanic slag with gravel—with that of soil-grown plants. The study investigates key agronomic and biochemical parameters, including fresh and dry biomass, number and weight of petioles, antioxidant activity (DPPH and FRAP assays), and concentrations of bioactive compounds such as total phenols, total flavonoids, chlorogenic acid, rutin, quercetin, and ferulic acid. By doing so, it aims to determine whether hydroponic cultivation, particularly using volcanic substrates, can be a viable strategy for producing rhubarb with enhanced nutritional and functional properties, especially in regions where traditional soil-based agriculture is constrained by land degradation or water scarcity.

Materials and Methods

Plant growth and sample collection

An experiment was conducted in 2022-2023 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 -26.1-32.6 °C, while summers are hot, with temperatures between 37.5 and 42.6 °C (Margaryan and Mkhitaryan, 2018).

The plants were planted in the last 10 d 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, the EBB and Flow system was used (Wortman, 2015). The experiment was set up with three treatments: 1. hydroponic with growth medium of volcanic slag (VS); 2. hydroponic with growth medium of the mixture of volcanic slag and gravel in a 1:1 ratio (VS+G) and 3. soil (S) as the growth medium. Volcanic slag and gravel were sourced from mines in Armenia. Each hydroponic substrate had particles of 3 to 15 mm in diameter. Soil total porosity was 61.9%, bulk density - 1.18 g cm^{-3}, EC - 1.7 dS^{-1}, pH - 7.86, and humus content -

1.5-2.5% (Tadevosyan et al., 2024). Standard agricultural practices were followed for the control group. Each treatment group had 6 replicates.

In the hydroponic system, plants were nourished once or twice daily with a specific nutrient solution developed by Davtyan (Ghalachyan et al., 2023). This solution, with a pH range of 5.5–7.0 (Tadevosyan et al., 2022), is well-suited for rhubarb cultivation. In contrast, soil-grown rhubarb was irrigated every three to four days. Petiole harvests were conducted at physiological maturity. The harvesting period spanned from May to October for hydroponic systems and from June to September for soil-based cultivation. In total, six harvests were performed for hydroponically grown rhubarb and five for soil-grown plants, with intervals of approximately one month between each harvest. The first harvest in the hydroponic group was carried out on 19 May, whereas soil-grown plants were not harvest-ready until 26 June.

Following each harvest, the aerial parts of the plants were transported to the laboratory, where leaves were separated from the petioles. The fresh weight (FW) of the petioles was recorded, after which both leaves and petioles were dried in a well-ventilated, shaded room to determine their dry weight (DW). As the petioles represent the most economically and pharmacologically valuable part of the plant, particular emphasis was placed on their analysis. For each plant, growth rate, developmental stage, and both FW and DW of the petioles were measured. Additionally, during the growing season, several morphological characteristics of the petioles were evaluated: length (measured from the substrate or soil surface to the leaf base at full maturity), thickness (measured using an electronic caliper at the petiole base), and number per plant. Biopharmaceutical and chemical analyses were also performed throughout the experiment using six randomly selected plants from each treatment group (Fig. 1).

(a) (b) (c)

Fig. 1. Rhubarb plants in different growth medium. (**a**) VS+G-mixture of volcanic slag and gravel; (**b**) VS-Volcanic slag; (**c**) S-Soil.

Determination of total flavonoids and total phenols

Throughout the experiment, the total flavonoid and phenol contents were measured in dried herb samples. To begin, 1 g of finely ground plant material—sieved through a 2.0 mm mesh—was accurately weighed and transferred to a 250 mL flask. Then, 100 mL of 70% ethanol was added, and the mixture was heated in a water bath at reflux for 60 minutes using a reflux condenser. After cooling, the extract was filtered through filter paper into a 100 mL volumetric flask. The flask was subsequently filled to volume with 70% ethanol to produce Solution A. From this, 2 mL of Solution A was transferred into a 25 mL volumetric flask and diluted to the mark with 96% ethanol. The absorbance of this final solution was measured using an Agilent Cary 60 UV-Vis spectrophotometer with a 10 mm quartz cuvette. Total flavonoid content was determined by measuring absorbance at 370 nm, while total phenol content was assessed at 277 nm. In all cases, 96% ethanol served as the blank reference solution (Vardanyan et al., 2023).

Determination of tannins

Tannin content was determined using a titrimetric method. A 2 g sample of crushed, dried plant material was extracted with 50 mL of boiling distilled water in a conical flask. The mixture was heated in a water bath for 30 minutes with frequent agitation. This extraction procedure was repeated five times, each time filtering the extract through cotton wool into a 250 mL volumetric flask until no tannins remained detectable. The final combined

extract was then brought to volume with distilled water. To quantify tannins, 25 mL of the prepared extract was transferred to a 1 L conical flask. Then, 750 mL of distilled water and 25 mL of indigo sulfonic acid solution were added. The resulting solution was titrated with 0.1 N potassium permanganate solution under constant stirring until the color changed to golden yellow. A control titration was also performed using 25 mL of indigo sulfonic acid in 750 mL of distilled water (without plant extract). The tannin content was calculated based on the difference in the volume of potassium permanganate used between the sample and control titrations.

$$X = \frac{(V\,1 - V2) \times D \times V \times 100}{m \times V3}$$

where V1 is the volume of 0.1 N $KMnO_4$ taken for titration, mL; V2-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; V3-volume of extract taken for titration, mL (Atanassova and Christova-Bagdassarian, 2009).

Determination of crude fiber

Crude fiber content was determined using a 1 g sample of dried, ground plant material. The sample was placed in a 1 L beaker, and to prevent evaporation during boiling, a round-bottomed flask filled with cold water was positioned over the beaker as a condenser. The sample was boiled under reflux for 30 min with 250 mL of 1.25% sulfuric acid solution. Following acid hydrolysis, the mixture was quickly filtered through a Whatman No. 42 ashless filter paper placed in a Buchner funnel, and the residue was thoroughly washed with distilled water to remove residual acid. The acid-free residue was then subjected to alkaline digestion under reflux with 200 mL of 1.25% sodium hydroxide solution for 30 minutes, maintaining constant volume. After filtration and thorough washing to eliminate alkali, the residue was transferred to a pre-weighed crucible and dried in an oven at 100 ± 5 °C until a constant weight was achieved. The crucible was cooled in a desiccator and weighed. Subsequently, it was ignited in a muffle furnace at 600 °C for 4 hours. After cooling, the final weight was recorded. Crude fiber content was calculated based on the weight loss after ashing (Matevosyan et al., 2023).

Sugar quantification

For sugar quantification, 5 g 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 in a water bath for 1 hour with periodic shaking. After cooling, a 50 mL aliquot of the extract was hydrolyzed by adding 5 mL of concentrated hydrochloric acid and heating at 68–70 °C for 8 minutes. The hydrolysate was neutralized using 20% sodium hydroxide and diluted to 100 mL with distilled water.

Reducing sugar content was then determined using Fehling's method. A 10 mL aliquot of the hydrolyzed solution was treated with Fehling's reagent to precipitate cuprous oxide, which was filtered, washed, and dissolved in an iron (II) sulfate solution. The resulting iron (III) ions were titrated with potassium permanganate, and sugar content was calculated accordingly (Matevosyan et al., 2023).

Antioxidant capacity by DPPH

The free radical scavenging capacity of the extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, as described by Brand-Williams et al. (1995). A stock solution of 10^{-3} M DPPH in ethanol was prepared and diluted to achieve an absorbance of 1.00 ± 0.200 at 517 nm. For the assay, 3 mL of the DPPH working solution was mixed with 0.5 mL of the sample extract and incubated in the dark for 30 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer, with ethanol serving as the blank. Antioxidant activity was expressed as the percentage reduction in absorbance relative to the control. All samples were analyzed in triplicate (Selseleh et al., 2020).

FRAP antioxidant assay

FRAP was measured according to the method of Birasuren et al. (2013). The FRAP reagent was freshly prepared by combining acetate buffer (300 mM, pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) solution in HCl (10 mM), and $FeCl_3 \cdot 6H_2O$ (20 mM), and then pre-warmed to 37 °C. Diluted extracts (100 µg mL^{-1}) were incubated with the FRAP reagent for 10 min at 37 °C in the dark. Absorbance was then measured at 593 nm against a reagent blank. Antioxidant capacity was calculated based on a standard calibration curve prepared using ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and expressed in µmol Fe^{2+} equivalents. The FRAP value of ascorbic acid was determined under identical conditions for comparison.

HPLC analysis of phenolic compounds

Phenolic compound analysis was performed using high-performance liquid chromatography (HPLC). For extraction, 100 mg of finely ground plant powder was mixed with 10 mL of methanol and homogenized for 4 minutes. The mixture was then centrifuged at 4400 rpm for 10 minutes, and the resulting supernatant was collected for chromatographic analysis.

HPLC was carried out using a Waters liquid chromatography system equipped with a Waters 2695 Separations Module and a Waters 996 Photodiode Array (PDA) Detector. Data acquisition and integration were conducted using Millennium32 software, with an auto-sampler injector used for automated sample loading.

Chromatographic separation was achieved using a Eurospher 100-5 C18 analytical column (25 cm × 4.6 mm, 5 µm particle size), fitted with a pre-column. A gradient elution system was employed, consisting of methanol containing 0.02% trifluoroacetic acid (TFA) as the organic phase (solvent A) and distilled water containing 0.02% TFA as the aqueous phase (solvent B). The mobile phase was delivered at a flow rate of 0.5 mL min^{-1}. The injection volume was 20 µL, and the column temperature was maintained at 25 °C. Phenolic compounds were monitored across a wavelength range of 200–600 nm (Ahadi et al., 2023).

Statistical analysis

All experimental data were analyzed using GraphPad Prism 8 and Microsoft Excel 2016. Statistical comparisons between means were performed using Student's *t*-test and analysis of variance (ANOVA). Duncan's multiple range test was applied to determine significant differences among treatment means. Random sampling was used for all experimental groups. Differences were considered statistically significant at $P \leq 0.05$.

Results

Plant growth and yield in vegetation periods

The results of the research showed that the growth medium significantly affected the yield of rhubarb petioles throughout the growth season. In all hydroponic substrates, petiole fresh and dry mass increased in June and in soil in July (Figs. 2 and 3).

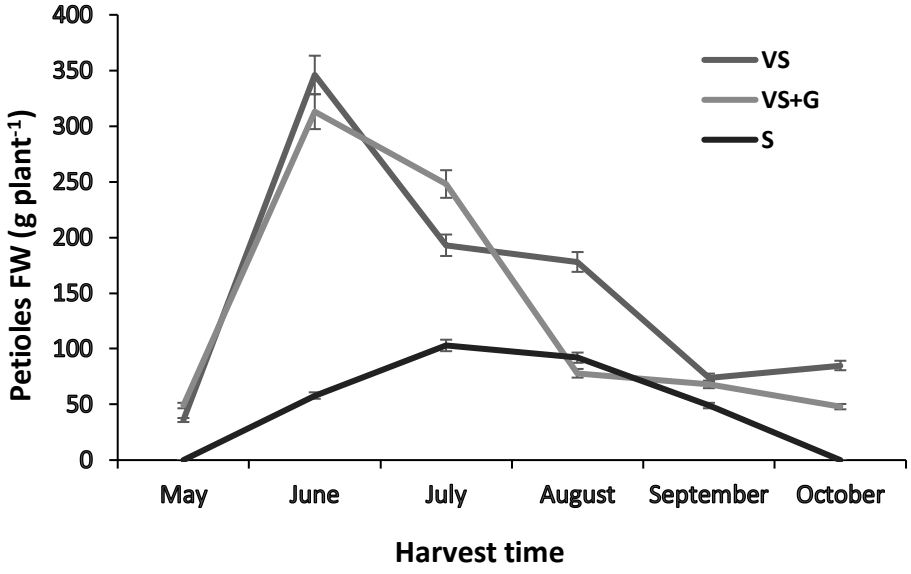


Fig. 2. Rhubarb petiole FW harvested during the vegetation period (May, June, July, August, September and October). Growth medium: VS-volcanic slag, VS+G-mixture of volcanic slag and gravel, S-Soil. Bars are standard errors.

During the vegetation period, hydroponically grown plants surpassed soil-grown (S) plants in petiole fresh weight (FW) and dry weight (DW) by 2.6–6.0 and 2.9–4.8 times, respectively, in June, and by 1.5 and 2.4 times, respectively, in July. However, this trend reversed in August: S plants outperformed plants grown on the VS+G substrate in FW and DW by 1.2 and 1.9 times, respectively, but were outperformed by those grown on the VS substrate by 1.9 and 1.2 times, respectively. In September, the FW of S plants was 1.4–1.5 times lower than that of the hydroponic treatment groups (Fig. 2), while no significant difference was observed in DW (Fig. 3).

According to Table 1, different hydroponic substrates did not significantly influence the total petiole FW per plant, the number of petioles per plant, or the FW and DW per individual petiole. However, S plants exhibited significantly lower petiole FW (2.7–3.0 times) and DW (1.8–2.0 times) compared to hydroponic plants. Additionally, the number of petioles formed in hydroponically grown rhubarb was 1.6–1.8 times greater than in soil-grown plants. The FW per petiole in soilless systems was 1.6 times higher than in S plants, although no significant difference was detected in DW.

Hydroponic substrate type had a significant effect on petiole length. Petioles of plants grown in the VS substrate were relatively thick and short, whereas those grown in the VS+G substrate were longer. Biometric analysis of petioles revealed that significant differences in petiole width were observed only between VS substrate and S plants, with VS petioles being 1.2 times wider. Moreover, petioles from the VS substrate were 2.6 cm shorter than those from the VS+G substrate. The FW/DW ratio of petioles in hydroponically grown plants ranged from 13.5 to 13.7, while in S plants it was 9.1, indicating that the water content in hydroponic plants was approximately 1.5 times higher than in soil-grown counterparts.

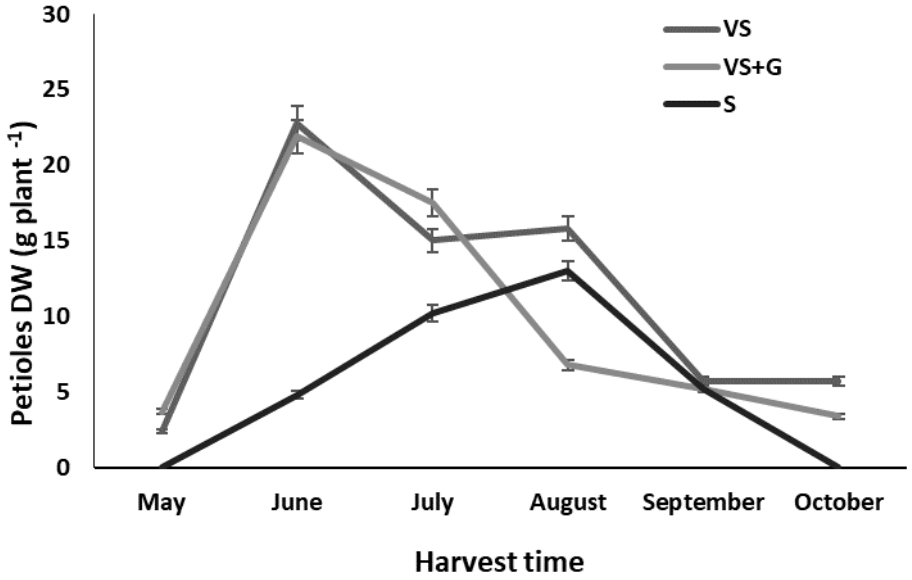


Fig. 3. Rhubarb petiole DW harvested during the vegetation period (May, June, July, August, September and October). Growth medium: VS-Volcanic slag, VS+G-mixture of volcanic slag and gravel, S-Soil. Bars are standard errors.

Table 1. Effect of the different growth medium (VS, VS+G, S) on Rhubarb petioles fresh and dry yield and biometric parameters.

Growth medium		Petioles (g plant^{-1})		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		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}

VS-Volcanic slag, VS+G-mixture of volcanic slag and gravel, S-Soil. Values are mean values ± SE of six replicat es. Mean values marked with the same letter in columns do not differ significantly based on Duncan's Multiple R ange Test ($P \leq 0.05$).

Plants grown in the VS substrate (Fig. 4) exhibited the highest percentage of yield in June (38%), which gradually declined over the course of the growing season—reaching 20–21% in July and August, and 8–9% in September–October. Similarly, the VS+G substrate produced the highest yield percentage in June (39%), followed by a decrease to 31% in July, and a threefold reduction in August. In the final months of the growing season, yield percentages continued to decline. For soil-grown (S) plants, the highest yield percentage was observed in July (34%), after which it decreased. Notably, during the hottest month of the vegetation period (August), S plants exhibited a higher yield percentage than the

hydroponic treatment groups. Among the hydroponic systems, the presence of gravel in the substrate had a significant impact on yield during August, as overheating of the gravel particles led to a threefold reduction in yield percentage.

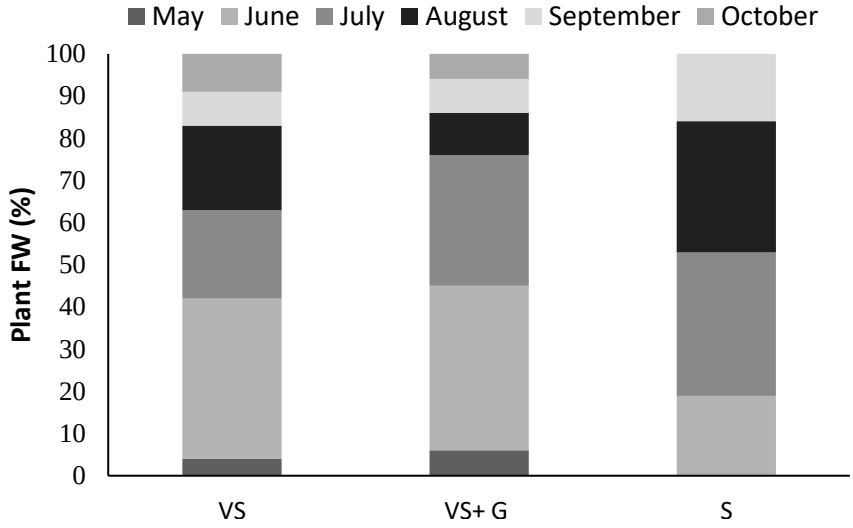


Fig. 4. Rhubarb petioles FW (%) harvested during the vegetation period (May, June, July, August, September and October). Growth medium: VS-Volcanic slag, VS+G-mixture of volcanic slag and gravel, S-Soil.

Biochemical analyses during the vegetation period

Biochemical analyses of rhubarb petioles were conducted throughout the growing season to assess the influence of the growth medium on the biosynthesis of key metabolites. The results indicated that the VS substrate, compared to the VS+G substrate, had a positive effect on the accumulation of plant extracts (1.2 times higher), total flavonoids (1.4 times higher), and tannins (1.2 times higher) in the petioles. The hydroponic growth medium did not significantly affect the contents of total phenols, crude fiber, or sugars in the petioles.

The lowest concentrations of extractives, total flavonoids, and total phenols were observed in petioles of soil-grown (S) plants, which were 1.3–1.6, 1.3–1.7, and 1.9–2.0 times lower, respectively, than those in the hydroponic treatments. However, S plants exhibited a significantly higher tannin content—2.9–3.4 times greater than that found in the hydroponic treatment groups. The growing medium did not have a significant effect on the levels of crude fiber and sugars, which remained relatively consistent across all treatments, ranging from 13.1–15.3% and 6.2–6.6%, respectively (Table 2).

Table 2. Effect of the different growth medium (VS, VS+G, S) on the content and yield of biochemical indicators of rhubarb dry petioles.

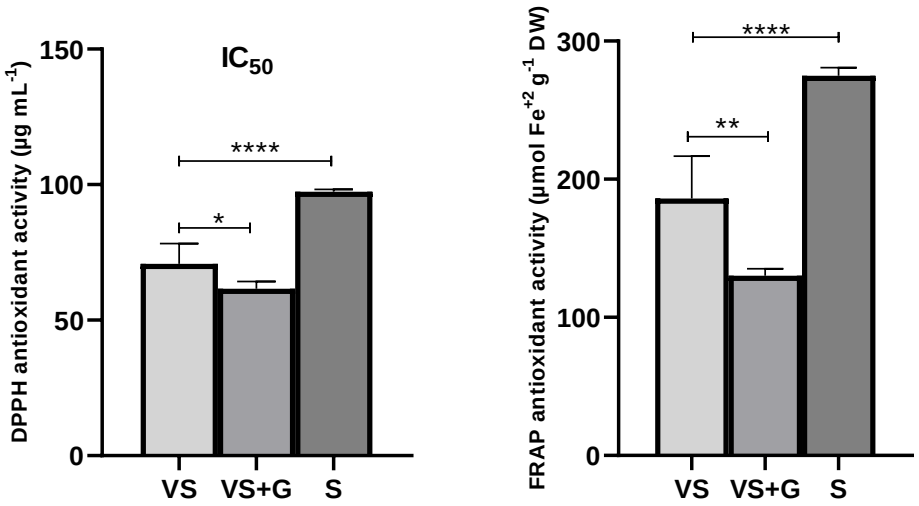
Growth medium	Plant extracts		Total flavonoids		Total phenols		Tannins		Crude fiber		Sugars	
	%	g plant^{-1}	%	mg plant^{-1}	%	mg plant^{-1}	%	g plant^{-1}	%	g plant^{-1}	%	g plant^{-1}
VS	34.2±2.7^{a}	23.10	0.33±0.05^{a}	222	0.18±0.01^{a}	121	1.75±0.12^{c}	1.18	14.6±1.2^{a}	9.84	6.2±0.4^{a}	4.18
VS+G	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	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

VS-Volcanic slag, VS+G - mixture of volcanic slag and gravel, S-Soil. Values are mean values ± SE of six replica tes. Mean values marked with the same letter in columns do not differ significantly based on Duncan's Multiple R ange Test ($P \leq 0.05$).

Plants grown in the VS substrate exhibited higher yields of key biochemical compounds compared to the VS+G hydroponic treatment group, with increases of 1.3 times in plant extracts, 1.6 times in total flavonoids, 1.2 times in total phenols, and 1.3 times in tannins. The hydroponic growth medium had no significant effect on the yields of crude fiber and sugars. Soil-grown (S) plants were markedly inferior to hydroponic treatment groups in the yield of plant extracts (2.3–3.2 times lower), total flavonoids (2.3–3.7 times lower), total phenols (3.3–4.0 times lower), crude fiber (2.1–2.3 times lower), and sugars (1.8–2.3 times lower). However, S plants surpassed the hydroponic treatment groups in tannin accumulation, with yields 1.4–1.9 times higher.

DPPH and FRAP antioxidant activity

The potential antioxidant activity of the plant extracts obtained from the dry rhubarb petioles was evaluated by DPPH and FRAP methods (Fig. 5). Experiments were performed at different concentrations to determine the IC50. The results showed that VS+G plants had high antioxidant activity (61.58 ± 2.46 µg mL^{-1}). Plants of the VS treatment group were slightly inferior to them (70.77 ± 6.82 µg mL^{-1}). The lowest antioxidant activity was recorded in the S treatment group (97.35 ± 0.79 µg mL^{-1}). According to the data of FRAP analysis, 1 g of plant recovered more iron in the S treatment group (274.92 ± 5.44 µmol Fe^{+2} g^{-1} DW, followed by the VS substrate (186.02 ± 27.96 µmol Fe^{+2} g^{-1} DW). The smallest value was observed for the VS+G substrate (130.21 ± 4.45 µmol Fe^{+2} g^{-1} DW).



(a) (b)

Fig. 5. (**a**) The DPPH; and (**b**) FRAP antioxidant activity in different growth mediums. VS-Volcanic slag, VS+G-mixture of volcanic slag and gravel, S-Soil. Data are mean values ± SD of 6 replicates, significant * $P < 0.05$, significant ** $P < 0.01$, significant **** $P < 0.0001$.

The presence of 16 phenolic compounds was investigated in the dried raw material of rhubarb petioles, including: gallic acid, 3,4-dihydroxyphthalic acid, chlorogenic acid, vanillic acid, caffeic acid, 2,5-dihydroxybenzoic acid, syringic acid, ferulic acid, coumaric acid, rutin, rosmarinic acid, salicylic acid, cinnamic acid, quercetin, kaempferol, and apigenin. Of these, only four compounds—chlorogenic acid, ferulic acid, rutin, and quercetin—were detected (Figs. 6–9). The levels of chlorogenic acid, ferulic acid, and rutin varied significantly across the different treatment groups. The lowest concentrations were recorded in plants grown in the VS+G substrate, while the highest were observed in soil-grown plants. Notably, soil-grown plants exhibited the highest rutin content, with a remarkable increase of 174–195% compared to hydroponic plants. Rutin concentrations ranged from 0.883 to 0.950 mg g^{-1} in hydroponic treatments and reached 2.602 mg g^{-1} in soil-grown plants.

Ferulic acid content averaged 0.913 mg g^{-1} in soil-grown plants, while it ranged from 0.669 to 0.821 mg g^{-1} in hydroponic variants. The highest chlorogenic acid content (0.225 mg g^{-1}) was also found in the soil group, while hydroponic treatments showed a broader range from 0.072 to 0.225 mg g^{-1}. Quercetin levels were similar in the soil and VS treatments (0.368 mg g^{-1}), while the VS+G group exhibited a

slightly lower value of 0.354 mg g^{-1}. Among hydroponic systems, plants grown in the VS substrate significantly outperformed those in the VS+G substrate in terms of chlorogenic acid, rutin, and ferulic acid content. However, no significant difference in quercetin content was observed between soil and VS-grown plants.

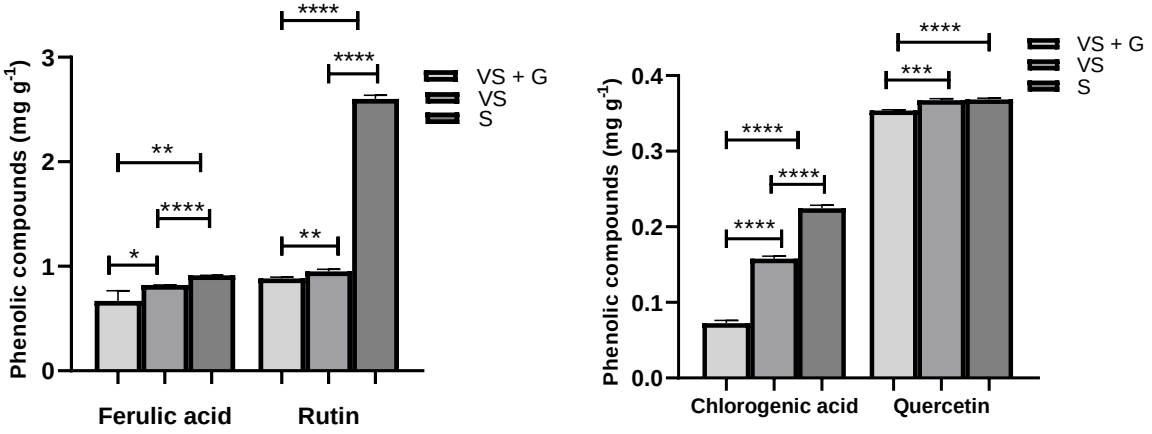


Fig. 6. Effect of different growth medium on the phenolic compounds (chlorogenic acid, ferulic acid, rutin and quercetin) of rhubarb plants. VS-Volcanic slag; VS+G-mixture of volcanic slag and gravel; S-Soil. Data are mean values ± SD of 6 replicates * Significant ($P < 0.05$), ** Significant ($P < 0.01$), *** Significant $P < 0.001$, **** Significant $P < 0.0001$.

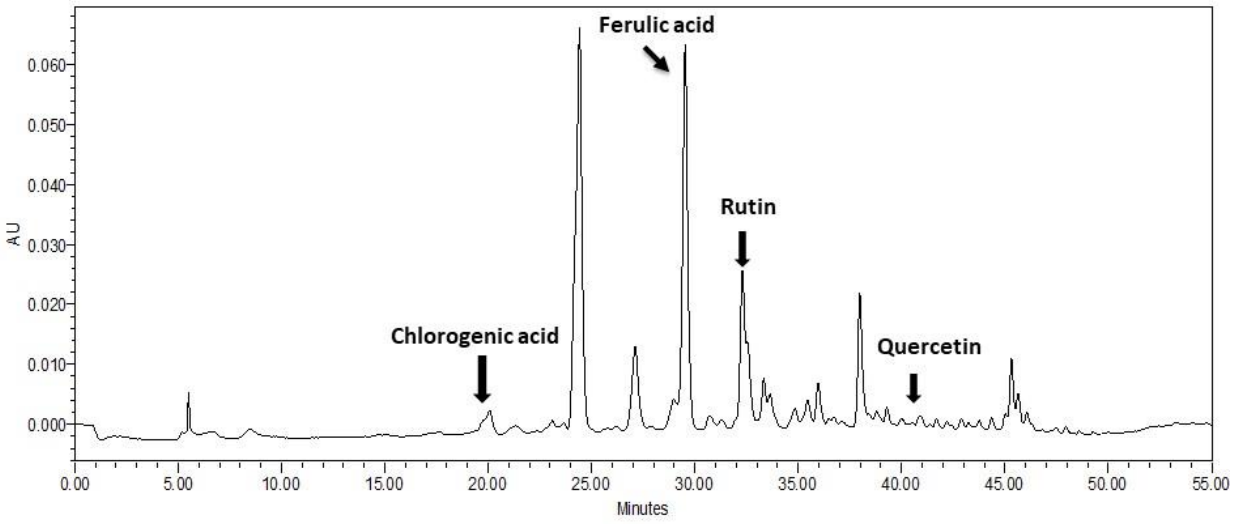


Fig. 7. HPLC chromatogram obtained from a methanolic extract of rhubarb petioles of the VS treatment group: Rutin and quercetin showed peaks at 355 nm, chlorogenic acid and ferulic acid showed peaks at 326 and 327 nm. Retention time for rutin was 32.5 min, for quercetin it was 40.45 min, for chlorogenic acid it was 19.8 min, and for ferulic acid, it was 29.7 min.

Discussion

According to literature data, rhubarb plants grown in the Moscow region at a similar planting density yielded between 722.3 and 2273.3 g of petiole biomass per 6-year-old plant (Kharchenko et al., 2014). In our study, soil-grown plants produced lower petiole biomass, whereas hydroponic variants achieved yields comparable to those reported by Kharchenko et al. (2023), suggesting that hydroponic cultivation is a more favorable method for rhubarb production.

It is also noteworthy that the dry biomass of rhubarb grown under soil conditions in our study accounted for 11% of the fresh biomass, exceeding the 6.4–8.5% range reported by Kharchenko et al. This indicates a higher dry matter accumulation in our soil-grown plants. However, there is no published data on rhubarb cultivation under hydroponic conditions, making our findings particularly novel.

Our results show that the fresh-to-dry biomass ratio of rhubarb petioles was approximately 1.5 times higher in hydroponic treatments than in soil-grown controls, indicating higher water content in hydroponically grown tissues. Additionally, hydroponic cultivation extended the harvesting period, allowing six harvests from May to October, compared to four harvests from June to September under soil conditions. The highest biomass yield was obtained in June from plants grown in the VS substrate. Overall, our findings indicate that the VS substrate is the most effective medium for maximizing total rhubarb biomass over the growing season.

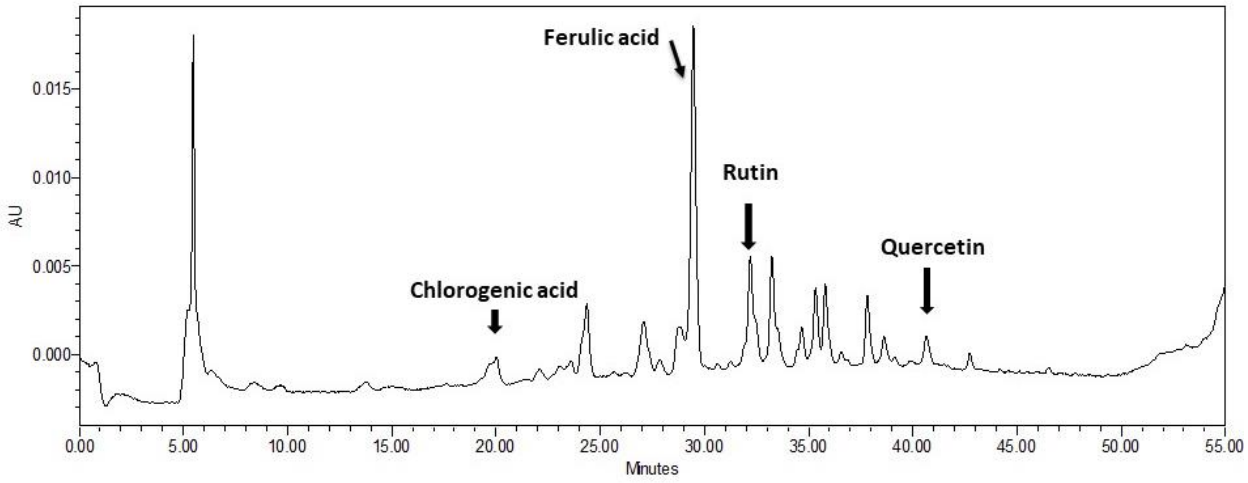


Fig. 8. HPLC chromatogram obtained from a methanolic extract of rhubarb petioles of the VS+G treatment group: Rutin and quercetin showed peaks at 355 nm, chlorogenic acid, and ferulic acid showed peaks at 326 and 327 nm. Retention time for rutin was 32.5 min, for quercetin it was 40.45 min, for chlorogenic acid it was 19.8 min, and for ferulic acid, it was 29.7 min.

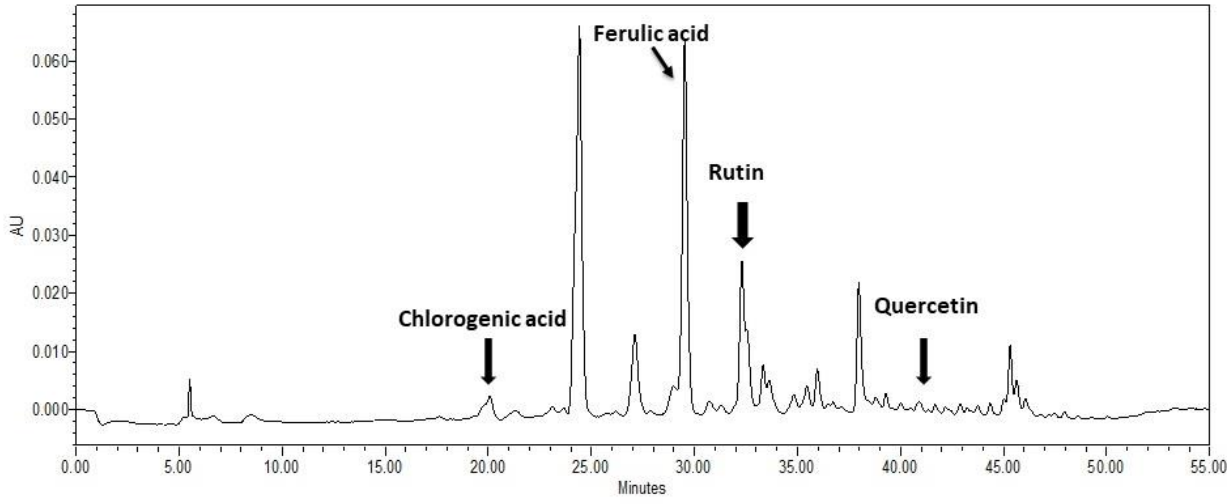


Fig. 9. HPLC chromatogram obtained from a methanolic extract of rhubarb plant petioles of the Soil treatment group: Rutin and quercetin showed peaks at 355 nm, chlorogenic acid, and ferulic acid showed peaks at 326 and 327 nm. Retention time for rutin was 32.5 min, for quercetin it was 40.45 min, for chlorogenic acid it was 19.8 min, and for ferulic acid, it was 29.7 min.

A study by Clapa et al. (2020) reported total phenolic and flavonoid contents in rhubarb stalks as 375.4 µg g^{-1} (0.038%) and 385.6 µg g^{-1} (0.039%), respectively—both values lower than those observed in our study. In particular, the volcanic slag (VS) treatment group exhibited the highest accumulation of both total phenols and flavonoids. These compounds are known antioxidants that help neutralize free radicals—unstable molecules that can damage cells and contribute to the development of

chronic diseases. By counteracting oxidative stress, phenols and flavonoids may help reduce the risk of conditions such as cardiovascular disease, cancer, Alzheimer's disease, and Parkinson's disease (Oluwole et al., 2022; Tiwari et al., 2017).

Given that hydroponic cultivation enhances the accumulation of these bioactive compounds in rhubarb, it represents a more effective approach not only for agricultural productivity but also from a medicinal and nutraceutical perspective.

Data from research conducted in Romania indicate that the ferulic acid content in the dry mass of rhubarb petioles varies from 15.18 to 48.56 μg g^{-1}, with an average of 27.06 μg g^{-1} across ten harvests, depending on the season. These values were obtained using a 70% ethanol extraction method (Cojocaru et al., 2020). In contrast, our study revealed substantially higher ferulic acid concentrations in rhubarb petioles, ranging from 669 to 913 μg g^{-1} DW, depending on the growth medium.

A study conducted in Poland reported the absence of quercetin in rhubarb petioles (Liudvytska et al., 2023). However, our analysis detected its presence in small but consistent quantities, ranging from 0.354 to 0.368 mg g^{-1} DW. Similarly, Cojocaru et al. (2020) reported that chlorogenic acid content in the methanolic extract of dried rhubarb petioles was 6.1 μg g^{-1}. Our results significantly exceeded this value, with concentrations ranging from 72 to 225 μg g^{-1}. Moreover, while the Romanian study found no detectable rutin in dried rhubarb petioles, our findings revealed rutin levels ranging from 883 to 2602 μg g^{-1} DW, indicating a substantial accumulation in our experimental conditions.

The total tannin content in rhubarb can vary depending on factors such as variety, environmental conditions, and plant part analyzed. A review of the scientific literature revealed no specific data on the tannin content of *Rheum rhabarbarum* L. However, Wang et al. (2011) reported total tannin contents of 6.12% in raw material and 19.20% in total extract for *R. palmatum*. In our study, soil-grown rhubarb plants exhibited a 33% higher tannin content compared to those grown hydroponically.

According to data from Germany, the total sugar content in rhubarb juice exceeds the total acid content and ranges between 16.31 and 17.49 g L^{-1} (Will et al., 2013). In a separate study on *Rheum tanguticum*, crude fiber was identified as the most abundant macronutrient in the inflorescence stem, with a concentration of 89.14% ± 0.75 (Osei et al., 2021). In our research, hydroponically cultivated rhubarb plants yielded 51% more sugars and 44% more crude fiber than soil-grown counterparts.

Cojocaru et al. (2020) demonstrated that applying chemical, organic, or biological fertilizers can enhance the antioxidant activity of rhubarb plants grown in soil. Furthermore, according to Zhou and Yu (2006), rhubarb exhibits the highest antioxidant capacity among various vegetables, ranking above green beans, tomatoes, potatoes, kale, spinach, and broccoli. In our study, rhubarb plants cultivated in the VS+G substrate displayed higher antioxidant activity than those grown in VS substrate alone.

In terms of ferric reducing antioxidant power (FRAP), Jargalsaikhan et al. (2021) reported FRAP values of 4.66 mM g^{-1} in ethanol extract of *Rheum undulatum* L. and 1.98 mM g^{-1} in *Rumex crispus* L. In our study, FRAP values in rhubarb petioles ranged from 130.22 to 274.92 μmol g^{-1}, depending on the growth medium used.

Conclusion

Hydroponically grown rhubarb generally outperformed soil-grown plants in terms of fresh weight and dry weight of petioles throughout most of the growing season, except in August. Despite these advantages, there were no statistically significant differences in total petiole yield per plant or average individual petiole weight between the two hydroponic substrates. Plants cultivated in the VS substrate produced thicker and shorter petioles, whereas those grown in the VS+G substrate developed longer and narrower petioles. Notably, the VS+G substrate negatively impacted yield in August, likely due to elevated temperatures causing overheating of gravel particles. Rhubarb cultivated hydroponically exhibited a higher water content compared to soil-grown plants. Yield distribution varied over the season, with hydroponically grown plants showing peak yields in June (38% for VS and 39% for VS+G), followed by a gradual decline. The VS+G treatment group experienced a sharper reduction in yield in August, attributed to thermal stress. In contrast, soil-grown plants reached their maximum yield in July (34%) and interestingly maintained relatively high productivity even in the hottest month (August), unlike their hydroponic counterparts.

In terms of biochemical composition, hydroponically cultivated rhubarb accumulated higher levels of plant extracts, total flavonoids, and total phenols compared to soil-grown plants. However, soil-grown plants exhibited significantly higher tannin concentrations. The content of sugars and crude fiber remained unaffected by the cultivation method. Among the hydroponic substrates, the VS medium was more effective than VS+G in promoting the accumulation of plant extracts, total flavonoids, total phenols, and tannins. In contrast, the VS+G substrate induced the highest antioxidant activity, indicating a substrate-dependent modulation of bioactive compound profiles. Overall, rhubarb cultivated under hydroponic conditions demonstrated superior antioxidant capacity relative to soil-grown plants, with the VS+G treatment showing the most pronounced effect. These findings highlight the

potential of hydroponic systems for enhancing the nutritional and medicinal value of rhubarb. However, the choice of substrate significantly influences the phytochemical composition. Interestingly, soil-grown plants had the highest concentrations of chlorogenic acid, rutin, and ferulic acid, with rutin levels being particularly elevated compared to hydroponically grown rhubarb. Among hydroponic treatments, the VS substrate significantly outperformed the VS+G substrate in terms of chlorogenic acid, rutin, quercetin, and ferulic acid contents. No significant differences in quercetin levels were observed between the soil and VS-grown plants.

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Author contributions
Methodology, AT; investigation, MD; resources, AM and AGh; data curation, AS; writing—original draft preparation, MD and AT; writing—review and editing, HRR and MGh; supervision, AT. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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