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An alternative plant for sustainable future: some biochemical properties of watercress (*Nasturtium officinale* R.Br.)

by

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Abstract

Watercress, a semi-aquatic plant with high nutritional value, is a member of the Brassicaceae family. It is widely distributed in wetlands in Turkey and grows in or around water. This study aimed to determine some biochemical properties of *Nasturtium officinale* grown in eastern Turkey, such as fatty acids, lipophilic vitamins, and phytosterols. In this context, plant collected from a predetermined area were separated into roots, stems, and leaves after the washing process in the autumn of 2023. Chemical characterisation was determined by techniques such as gas chromatography for fatty acids and HPLC for vitamins and sterols. The importance of the components contained in the plant was revealed by evaluating the results obtained in the light of the literature. These results show that linolenic acid (C18:n3 LNA) was found to be present in the root at a rate of 22.71% and in the leaf at a rate of 42.45%. Palmitic acid (C16:0) was found in the stem at a rate of 17.73%. The lipophilic vitamin analysis of watercress revealed the presence of vitamins K1, K2, D2, D3, δ-tocopherol, α-tocopherol, ergosterol, sterol, β-sitosterol, retinol, and retinol acetate. It was determined that ergosterol had the highest value in the leaf part (416.5 $\mu g/g^{-1}$).

Key words: *Nasturtium officinale*, fatty acid, lipophilic vitamin, phytosterol, Türkiye

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1. Introduction

Watercress (*Nasturtium officinale* R.Br.) is a plant with valuable biological properties. It has recently gained attention as a potential alternative source in many fields and has become the subject of numerous studies. The need for alternative sources of consumption is evident in almost every area of today's world, and the search for healthy and quality living has increased. At this point, research has increased to discover the beneficial properties of plants to increase various alternative products that will protect human health, prevent diseases, and benefit humanity without depleting nature. Watercress is also considered a sustainable crop in many different various sectors, such as food, medicine, pharmacology, biotechnology, and many others. In traditional medicine, *N. officinale* is used to treat hyperglycaemia, hypertension, asthma, and cough (Suroowan & Mahomoodally, 2016; Teixidor-Toneu et al., 2016).

Some studies focus on the pharmacological effects of *N. officinale* plant extracts. Proven effects include anticancer, antioxidant, antibacterial, anti-inflammatory, anti-psoriatic, and cardioprotective properties (Alshahrani, 2020; Bahramikia & Yazdanparast, 2008; Yehuda et al., 2012; Zafar et al., 2017). In Germany, *N. officinale* is officially listed for use in phytotherapy and holds an important position in the German Commission Monographs on homeopathic medicines (Monographie, 1989). *N. officinale* is also recognised as a safe edible plant by the European Food Safety Authority. It is included in monographs on leafy vegetables, medicinal plants, and edible flowers (URL 1). In addition, *N. officinale* is recognised as a valuable plant for the production of cosmetics in the European Commission's Cosmetic Ingredient Database (URL 2). The pharmacological potential of *N. officinale* is due to its rich chemical composition. In addition to other compounds such as isothiocyanates, polyphenols, vitamins, and carotenoids, the most important group of secondary metabolites in the plant is glucosinolates. These valuable chemical components are the reason for the important position of *N. officinale* in the food and cosmetic industries (Jeon et al., 2017). In addition, the *N. officinale* plant has recently gained interest as an alternative food source.

Watercress (*N. officinale* R.Br.) plant contains many useful components. The most important among these components is fatty acids, which are essential for the human body. Vegetable fatty acids are important sources of essential oils. Fats are not only used as a source of energy in the body (Santos et al., 2017; Wassell et al., 2010).

In particular, linoleic acid (C18:2n6) is an 18-carbon, long-chain polyunsaturated fatty acid (PUFA). It is called omega-6 fatty acid because of the position of two double bonds in its structure. It is an essential fatty acid for cell membrane synthesis and brain development. Omega-6 fatty acids also have a positive effect on lowering blood cholesterol. Linoleic acid is found in vegetable oils and dark green leafy vegetables. Nowadays, depending on the eating habits of people, the consumption of liquid oil in the direction of margarine and frying oils has led to an increase in linoleic acid (C18:2n6) intake. Linoleic acid (omega (ω)-6 group) and α-linolenic acid (omega (ω)-3 group) are two of the most important fatty acids among PUFAs. These two fatty acids belong to the very long-chain PUFA group. The consumption of linolenic acid (C18:3n3), one of the essential fatty acids, is decreasing. Such long-chain fatty acids have many important effects such as early intelligence development, resistance to diseases, child birth weight, and prevention of heart diseases (Leskanıch & Noble, 1997). Since the body lacks the desaturase enzymes necessary for the production of these fatty acids, humans must obtain them through diet. Both fatty acids play an important role in human growth and development and in the prevention and treatment of coronary heart disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune diseases, and cancer (Erzen, 2014).

The essential fatty acids, α-linolenic acid and linoleic acid, are required for the synthesis of various molecules that affect vital functions (Das, 2006). In addition to many benefits, deficiency of essential oils may also have negative effects. It is therefore important to study the fatty acids present in plants. It is necessary to quantify them and reveal their valuable aspects. *N. officinale* is a valuable plant whose health benefits are supported by research. Moreover, due to this feature, its water absorption capacity is quite high. Watercress (*N. officinale*) is a candidate species to be used in many biotechnological materials.

N. officinale contains not only fatty acids but also lipophilic vitamins (A and E) and minerals (iron and copper), and it is a good source of bioactive substances called polyphenols and glucosinolates (Bahramikia & Yazdanparast, 2008). Watercress (*N. officinale* R.Br.) contains a rich source of protein, minerals (calcium, potassium, phosphorus, sodium, magnesium, iron, and zinc), and vitamins (such

as vitamins A, K, C, B, E, thiamine, niacin, and riboflavin). Therefore, it is necessary to determine the nutritional content of wild plants that grow naturally and are edible and to support their effects on human health with scientific studies. This study was carried out to determine the biochemical properties of *N. officinale*, an edible wild plant collected from Elazığ province and its surroundings, which grows in the eastern region of Turkey. It is important to investigate, for the first time, the fatty acid, vitamin, and sterol content of different parts (root, stem, and leaf) of watercress (*N. officinale* R.Br.), a semi-aquatic plant belonging to the Brassicaceae family, using solvents such as water and ethanol. The specific biochemical values of watercress may vary depending on growing conditions, soil properties, and climatic conditions. This reason increases the originality of the study.

2. Materials and methods

2.1. Collection of plant materials

N. officinale R.Br. species were collected from the wetlands in and around Elazığ Province during the autumn season of 2023. The collected plant materials were identified to species. After washing the collected plant materials, the root, stem, and leaf parts were separated and grouped to make extracts.

2.2. Extraction of plant materials

The aboveground parts of the dried plant samples were extracted using ethanol as a solvent using a Soxhlet device (Khan et al., 1998). The extraction process continued until the last extract was colourless. The ethanol content of the extracts obtained from the Soxhlet device was removed using an evaporator, and the dry extracts (mg · mL) were stored at +4°C.

2.3. Determination of lipophilic vitamin content and sterols

2.3.1. Analysis of A, D, E, K vitamins and sterols by HPLC method: Five millilitres of supernatant was taken into 25 mL capped tubes, 5% KOH solution was added, and the mixture was kept at 57°C for 2 hr. The tubes were removed, cooled to room temperature, and 5 mL distilled water was added and mixed. The unsaponifiable lipophilic molecules were extracted with 2 × 5 mL hexane, and the solvent was evaporated under nitrogen gas. Then, they were dissolved in 1 mL (50% + 50%, v/v) acetonitrile/methyl alcohol mixture, transferred into autosampler vials, and analysed. A PDA-UV detector was used for the analysis, and a Nucleodur C18 (15 cm × 4.6 cm, 5 µm; Sigma, USA) column was used as the column. At 202 nm, lipophilic vitamins and phytosterols were analysed (Karpińska et al., 2006; Katsanidis & Addis, 1999; López-Cervantes et al., 2006).

2.4. Extraction of lipids from biological samples

Lipids were extracted from plant samples using the method of Hara and Radin (1978) using a 3:2 (v/v) hexane-isopropanol mixture. For this, 1 g of plant sample was homogenised in 5 mL of a hexane-isopropanol mixture in a ratio of 3:2 (v/v) for 30 s. The homogenisation vessel was washed with 2 mL of digestion solution, which was then transferred into centrifuge tubes. Then, the supernatant portion of the tissue samples centrifuged at 4500 rpm for 10 min was collected and placed in sealed test tubes.

2.5. Preparation of fatty acid methyl esters

To perform gas chromatographic analysis of the fatty acids present in lipids, they need to be converted into derivatives, such as methyl esters, which are non-polar, volatile, and stable in structure (Christie, 1992). To prepare methyl esters, the lipid extract in hexane/isopropanol phase was transferred to 30 mL non-leaking test tubes. Then, 5 mL of 2% methanolic sulphuric acid was added, and the mixture was thoroughly vortexed. The mixture was left to methylate in an oven at 50°C for 15 hr. After removing the tubes from the oven, they were cooled to room temperature, and 5 mL of 5% NaCl was added and mixed thoroughly. The resulting fatty acid methyl esters in the tubes were extracted with 5 mL of hexane, and the hexane phase was pipetted and treated with 5 mL of 2% $KHCO_3$. The phases were allowed to separate for 4 hr. The mixture containing methyl esters was then dried at 45°C under a nitrogen stream. After drying, it was dissolved in 1 mL of hexane and analysed by gas chromatography in 2 mL autosampler vials with sealed caps.

2.6. Gas chromatographic analysis of fatty acid methyl esters

Fatty acids in the lipid extract were converted into methyl esters and analysed using SHIMADZU GC 17 Ver. 3, GC Solution 2.3 programme. For this analysis, a Machery-Nagel (Germany) and Sigma capillary column

with a length of 25 m, an inner diameter of 0.25 mm and a PERMABOND film thickness of 25 microns was used. During the analysis, the column temperature was maintained at 120°C–220°C, injection temperature at 240°C, and detector temperature at 280°C. The column temperature programme was set from 120°C to 220°C. The temperature increase was set at 5°C · min^{-1} up to 200°C and 4°C · min^{-1} from 200°C to 220°C. It was kept at 220°C for 8 min and the total time was determined as 35 min. Nitrogen gas was used as a carrier gas. Before the analysis of fatty acid methyl esters of the samples, mixtures of standard fatty acid methyl esters were injected and the retention times of each fatty acid were determined. After this process, the necessary programming was performed, and the fatty acid methyl ester mixtures of the samples were analysed. The results were determined as a percentage of the total fatty acids for each fatty acid. Calculations were carried out using the GC Solution 2.3 programme.

3. Results

This paper investigates the fatty acid composition, fat-soluble vitamin content, and phytosterol content of watercress (*N. officinale* R.Br.) collected from Elazig province and its surroundings. Tables and graphs are presented below.

The *N. officinale* plant contains various fatty acids, such as capric (C10:0), lauric (C12:0), myristic (C14:0), pentadecenoic (C15:1), palmitic (C16:0), palmitoleic (C16:1, n-7), margaric (C17:0), heptadecenoic (C17:01), stearic (C18: 0), oleic (C18:1, n-9c), vaccenic (C18:1 n11), linoleic (C18:2, n-6c), linolelaidic (C18:2 n-6t), and linolenic (C18:3, n-3 LNA). When examining the variation of these fatty acids between the root, stem, and leaf sections, the highest levels of fatty acids were found in the root section, with 22.71% being the unsaturated fatty acid linolenic acid (C18:n3 LNA). In the stem section, 17.73% of the fatty acids were saturated palmitic acid (C16:0), while in the leaf section, 42.45% of the fatty acids were polyunsaturated linolenic acid (C18:n3 LNA) (Table 1).

The highest value of linolenic acid (C18:3, n-3) was found to be 22.71% in the root part of *N. officinale*, followed by palmitic acid (C16:0) with 20.50%, and the lowest value of 0.43% was for heptadecanoic acid (C17:01). When the chromatogram graphs of the stem part of *N. officinale* were analysed, it was determined that palmitic (C16:0) acid had the highest value of 17.73%, followed by linolenic (C18:3, n-3) acid with 17.37%, and the lowest value of 0.43% was for margaric acid (C17:0). According to the chromatogram graphs of the leaf part of *N. officinale*, it was determined that linolenic (C18:3, n-3) acid had the highest value with 42.45%, followed by palmitic (C16:0) acid with 18.49% and heptadecanoic (C17:01) acid with 0.14% (Figs 1–3).

Table 1

Fatty acid composition in the root, stem, and leaf parts of watercress (*N. officinale* R.Br.) (%).

Fatty acids	Root	Stem	Leaf
C10:0	6.37	5.05	1.76
C12:0	6.68	5.24	1.86
C14:0	4.50	4.31	5.43
C15:01	5.18	7.22	3.00
C16:0	20.50	17.73	18.49
C16:1 n-7	2.49	0.78	1.04
C17:0	0.57	0.40	0.24
C17:01	0.43	0.58	0.14
C18:0	5.95	6.84	12.09
C18:1 n9 c	2.99	7.33	1.23
C18:1 n11	1.28	-	0.59
C18:2 n6 t	8.34	7.90	5.67
C18:2 n-6 c	11.96	16.19	5.94
C18: n3 LNA	22.71	17.37	42.45

3.1. Lipid soluble vitamin and phytosterol contents (µg · g^{-1})

The lipid-soluble vitamin contents and sterol contents of *N. officinale* are shown in Table 2. In general, it appears to have low vitamin content. It has been determined that *N. officinale* is very rich in ergosterol content in the root, stem, and leaf parts, and the highest value is in the leaf part (416.5 µg · g^{-1}).

When the *N. officinale* used in the study was evaluated in terms of vitamins A, D, E, K, and sterols, it was seen that the vitamin K2 level was significantly higher in the leaf part than in the root and stem. It was observed that the γ-tocopherol level was also higher in the leaf than in the root and stem. D2 and D3 values were also found to be high in the leaf part. It was observed that α-tocopherol and ergosterol were at higher levels in the leaf part than in other parts. Vitamin K1 and sterol levels were found to be highest in the stem part compared to the root and leaf parts. It was found that the level of β-sitosterol in the stem part was higher than in the root and leaf, retinol was at an equal value in the root and leaf, and retinol acetate was at a higher level in the leaf than in the root and stem (Fig. 4).

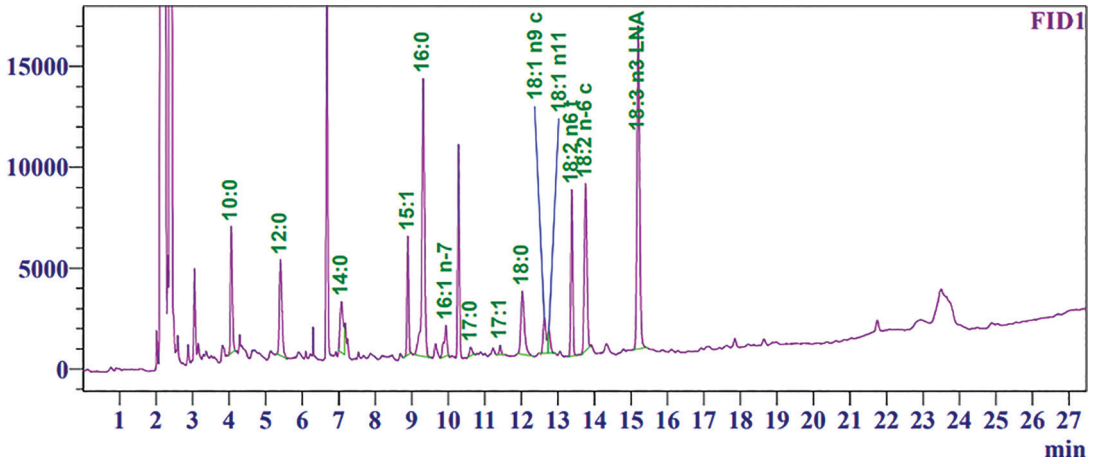

Figure 1

Fatty acid chromatogram of the root of watercress (*N. officinale* R.Br.).

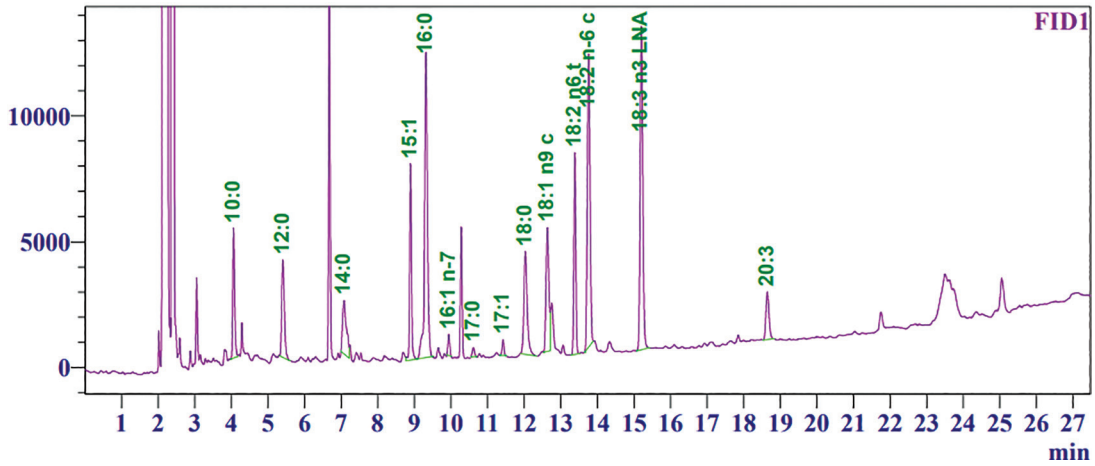

Figure 2

Fatty acid chromatogram of the stem of watercress (*N. officinale* R.Br.).

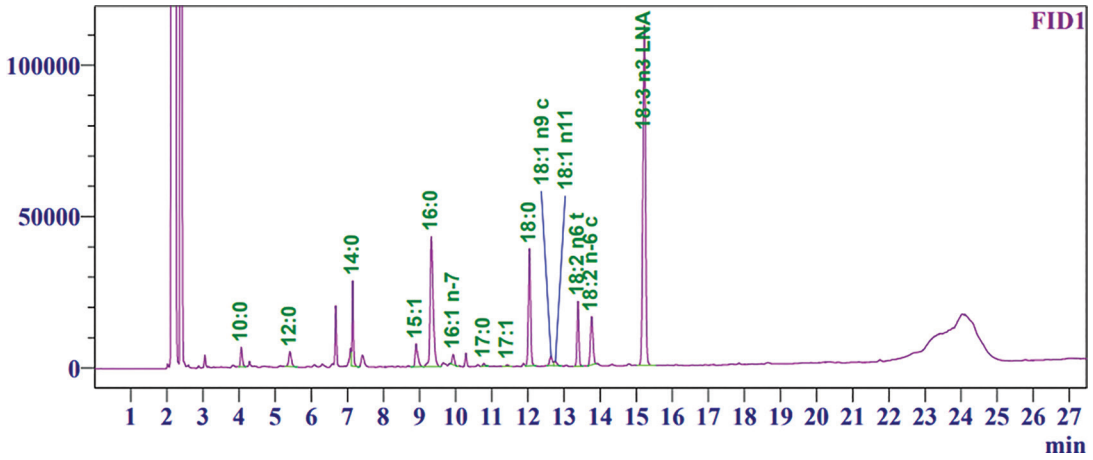

Figure 3

Fatty acid chromatogram of the leaf of watercress (*N. officinale* R.Br.).

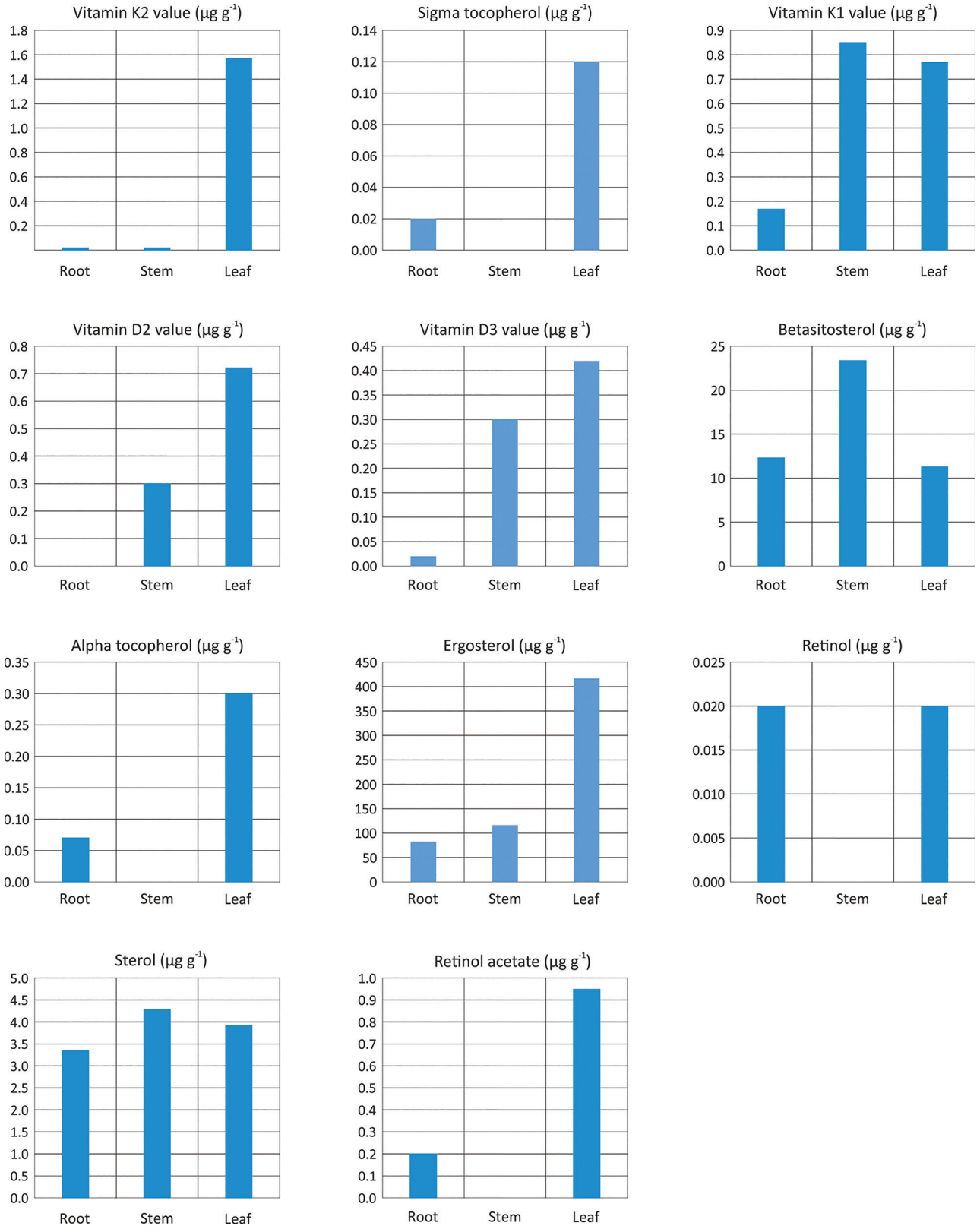

Figure 4

Vitamin and sterol values (µg · g^{-1}).

Table 2

Vitamin and sterol contents in root, stem, and leaf parts of watercress (*N. officinale* R.Br.) ($\mu g \cdot g^{-1}$).

Vitamin and sterol	Root	Stem	Leaf
K2	0.02	0.02	1.57
δ-tocopherol	0.02	-	0.12
D2	-	0.3	0.72
D3	0.02	0.3	0.42
α-tocopherol	0.07	-	0.3
Ergosterol	82.57	116.32	416.5
K1	0.17	0.85	0.77
Sterol	3.35	4.3	3.92
β-sitosterol	12.25	23.4	11.25
Retinol	0.02	-	0.02
Retinol acetate	0.2	-	0.95

4. Discussion

Watercress is a high-value plant species (Qian et al., 2022). The research findings indicate that both the edible parts (leaves) and the inedible parts (roots) of watercress may have significant nutritional value. Analysis has shown that *N. officinale* grown in the eastern region of Turkey has significant nutritional value in terms of its fatty acids, lipophilic vitamins, and phytosterol content.

In this context, it has been determined that the *N. officinale* plant has a significant content, especially in terms of unsaturated fatty acids. When the variation of these fatty acids between root, stem, and leaf parts was analysed, the highest fatty acid values were found as linolenic acid (C18:n3 LNA) from unsaturated fatty acids with 22.71% in the root part, palmitic acid (C16:0) from saturated fatty acids with 17.73% in the stem part, and linolenic acid (C18:n3 LNA) from polyunsaturated fatty acids with 42.45% in the leaf part. These results are consistent with previous studies (Moser et al., 2009). Some research have shown that watercress is rich in α-linolenic acid (omega-3), an essential fatty acid that is important for the human body and may be an important nutritional component of the daily diet. Oleic, linoleic, and linolenic acids have various beneficial effects on human health, and some of them have been approved by the Food and Drug Administration (FDA) (Bartella et al., 2023). Plants are valuable sources of nutrition due to their biochemical content. It has been found that *N. officinale* has particularly high levels of linoleic and linolenic fatty acids. The most common saturated fatty acids are palmitic acid (C16) and stearic acid (C18), while oleic acid, an unsaturated fatty acid, is also present. In highly organised plants and animals living in low temperatures, the amount of unsaturated fatty acids is higher compared to saturated fatty acids (Gözükara, 2001; Harold et al., 2007). In this study, the significant presence of stearic, palmitic, and oleic acids in the root, stem, and leaf parts of *N. officinale* makes the plant valuable.

Essential fatty acids such as linoleic acid and linolenic acid are only synthesised by plants. The plant enzyme Δ12 desaturase synthesises linoleic acid (18:1 n-9) from oleic acid and Δ15 desaturase synthesises linolenic acid from linoleic acid. These enzymes are not present in human and animal organisms; therefore, they cannot synthesise these fatty acids (Leonard et al., 2004). To maintain a healthy diet, it is necessary to obtain these fatty acids through plant-based foods. In this study, the presence of linolenic acid at 42.45% in the leaf part is another valuable feature. Omega 3 and omega 6 fatty acids, which have positive effects on health, are included in this group (Turan et al., 2013).

Thus, the root, stem, and leaf parts of *N. officinale* provide an alternative source of linolenic acid. Ercan (2021) reported the presence of unsaturated fatty acids, including elaidic, α-linolenic, and linolenic acids. The author identified the saturated fatty acids, palmitic and arachidic acids. In this study, the unsaturated fatty acids such as palmitoleic, heptadecenoic, oleic, linoleic, linolelaidic, and linolenic acids are reported. Palmitic acid, which was identified as a saturated fatty acid in Ercan's (2021) study, is also found in this study.

The secondary important group of vitamins in plants is lipophilic vitamins. In this study, along with the analysis of *N. officinale* plant extracts, lipophilic vitamins were also detected. According to the results of the vitamin analysis, K1, K2, D2, D3, δ-tocopherol, α-tocopherol, ergosterol, sterol, β-sitosterol, retinol, and retinol acetate were found in *N. officinale*. Tocopherols are essential components of the human diet due to their critical functions, including the removal and elimination of various reactive oxygen species (ROS) and free radicals, as well as the protection of PUFA from lipid peroxidation (Morales et al., 2012). This study suggests that the high concentration of tocopherols, particularly in the leaf part, indicates the edibility of the plant. The results indicate that α-tocopherol, which is a fat-soluble vitamin in *N. officinale*, can be used as a source of vitamin E and can prevent lipid peroxidation. Therefore, it can be used to prevent lipid peroxidation in food. Panayotova and Stancheva (2013) found that linoleic acid (C18:2 n-6) and eicosapentaenoic acid (EPA) (C20:5 n-3) were rich in fatty acids and fat-soluble vitamins, despite the low total lipid content. They

also determined that the α-tocopherol content was associated with high unsaturated fatty acid content and protected polyunsaturated fatty acid (PUFA) oxidation in tissues. This study also obtained parallel results in terms of fatty acid content.

In addition to fatty acids and vitamins, sterols are also an important group found in *N. officinale* Upon examination of its sterol content, it was observed that it is richer in ergosterol (416.5 $\mu g \cdot g^{-1}$) compared to other types. Sterols are essential components for cell membranes and are produced by both animals and plants. They are naturally found in vegetable oils, oily seeds, plant seeds, grains, and cereals. The most common sterols found in plants are beta-sitosterol (constituting 80%), stigmasterol, and ergosterol (a yeast sterol) (Liu, 2003). Our analysis of the root, stem, and leaf parts of *N. officinale* revealed that the phytosterol content consisted of ergosterol, sterol, and β-sitosterol. Among the phytosterols, ergosterol was found to be the most abundant. It has been observed that ergosterol has the highest level among these phytosterols in the leaf part. Caf (2021) reported that β-sitosterol, stigmasterol, and ergosterol were observed.

The biochemical properties of watercress grown in Elazığ may differ from those grown in other provinces depending on the climate, soil structure, water quality, and environmental factors of the place. Plants such as watercress (*N. officinale*) are very sensitive to environmental conditions, and these conditions may affect their biochemical content.

In conclusion, we predict that the nutritional content of this plant species, which is rich in fatty acids and lipophilic molecules, may have a phytotherapeutic effect and be of interest to health-conscious consumers and researchers.

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Authors' contributions

Sample collection and laboratory work: SMY, HB. -Article writing and evaluation of data: H.B

Conflict of interest

All authors declare no conflict of interest.

Availability of data and material

The data are part of the second author's master's thesis work, and the data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No humans or animals were used in this study.

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