Journal of Food Composition and Analysis 146 (2025) 107958

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Nutritional profile, phytonutrient composition and biocidal properties of *Portulaca oleracea* L. regarding the ammonium to total nitrogen ratios in hydroponics

Beatriz H. Paschoalinotto [a], Antonios Chrysargyris [b], Mikel Añibarro-Ortega [a], Tânia C.S.P. Pires [a], Filipa Mandim [a], Maria Inês Dias [a], Nikolaos Tzortzakis [b,*], Lillian Barros [a,*]

[a] CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300- 253, Portugal
[b] Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol 3603, Cyprus

ARTICLE INFO

Keywords:
Ammonium:total nitrogen ratio
Cytotoxicity
Purslane
Nutritional value

ABSTRACT

The contribution of the ammonium:total nitrogen ratio (Nr0.01–0.15) in the nutrient solution on chemical profile and bioactive characteristics of *Portulaca oleracea*, investigated. Increased Nr levels resulted in increased proteins, total fats, carbohydrates and oxalic acid in leaves and stems, but decreased yield. The only detected tocopherol isoforms were α- and β-tocopherol, with the former to reveal increased values at Nr0.10. The most abundant sugars found were fructose, glucose, sucrose, and trehalose, while total sugars were positively affected by the increased Nr in stems. The higher Nr ratio had a favourable effect on the fatty acid profile, while Oleracein A was the main phenolic component detected in higher levels at Nr0.05 (leaves) and Nr0.10 (stems). The increased Nr levels increased antimicrobial activity, while antioxidant activity was varied in different Nr levels. The Nr ratio for *P. oleracea* may stimulate health-promoting components, while reducing antinutrients content, hence improving overall product quality.

1. Introduction

Contemporary consumer preferences prioritize the consumption of food products with high nutritional value, produced by sustainable methods that ensure safety and high quality (Murteira et al., 2022). This recent shift in consumer behaviour has increased the popularity of wild edible and medicinal/aromatic plants, as "functional" foods and material for pharmaceutical and medicinal purposes, driven by their inherent nutraceutical properties and bioactive compounds (Ceccanti et al., 2020). This increasing demand for these plants has attracted considerable interest from the pharmaceutical and agro-industrial sectors, who are actively exploring the potential for their utilization and commercialization (Maggini et al., 2022). The increased interest for these plants, however, poses a significant challenge due to overharvesting and inconsistent quality (Bafort and Jijakli, 2024). Subsequently, the introduction of functional and medicinal plants in commercial cultivation has been growing rapidly recently, driving research efforts for optimizing their production and adapting them in intensive cultivation systems (Chrysargyris and Tzortzakis, 2023; Sugier et al., 2022).

Wild edible species and medicinal plants have the potential to enhance human health, demonstrating physiological benefits and protection against chronic illnesses. These are attributed to the increased content of compounds known as secondary metabolites, including phenolics and alkaloids, and glycosides, which have shown antioxidant, anti-cancer, anti-diabetic, anti-inflammatory, and antimicrobial characteristics (Atherton and Li, 2023; Gul et al., 2016). Besides these secondary metabolites, medicinal plants are rich in primary metabolites, including sugars, organic acids and tocopherols (Roriz et al., 2014). In addition, organic acids, including ascorbic, malic and citric acid, which are integral to both primary and secondary plant metabolism, have a substantial influence on the organoleptic properties of plants, including flavour, colour, and aroma (Mihaylova et al., 2018; Pereira et al., 2013). Tocopherols encompass diverse vitamin E (α-, β-, γ-, and δ-tocopherol) types, which are potent antioxidants, demonstrating anticancer, nephroprotective, neuroprotective, antihypertensive, and anti-inflammatory activities (Saini and Keum, 2016; Sianipar et al., 2024). In addition,

* Corresponding authors.
E-mail addresses: nikolaos.tzortzakis@cut.ac.cy (N. Tzortzakis), lillian@ipb.pt (L. Barros).

https://doi.org/10.1016/j.jfca.2025.107958
Received 1 February 2025; Received in revised form 4 June 2025; Accepted 24 June 2025
Available online 24 June 2025

natural products derived from various medicinal products have exhibited anticancer properties in clinical evaluations, preventing the proliferation of tumour cells due to the anticancer ability of metabolites including flavonoids and sesquiterpene lactones. In addition, these compounds have also been associated with significant antibacterial properties, influencing *Listeria monocytogenes*, *Enterobacter cloacae* and *Escherichia coli* (Petropoulos et al., 2020; Sugier et al., 2022). Finally, the limited availability of dietary sources rich in omega-3 has prompted significant research into the examination of medicinal and wild edible plants as sources of these essential fatty acids (Simopoulos, 2004; Uddin et al., 2014).

Purslane (*Portulaca oleracea* L.) is a widely recognised edible plant belonging to the *Portulacaeae* family (Fernández-Poyatos et al., 2021). Although considered a weed, it is consumed in various countries, including Italy, Greece, Cyprus, United Kingdom, Spain, China, Brazil and Mexico, finding extensive use in folklore medicine (Fernández-Poyatos et al., 2021; Montoya-García et al., 2023). This is due to its rich nutritional composition, attributed to the increased content of fatty acids, carotenoids, vitamins, proteins and minerals. In addition, purslane presents a diverse phytochemical profile, containing bioactive components, like alkaloids, polysaccharides, and flavonoids (Iranshahy et al., 2017). Apart from the presence of antioxidants, the high levels of omega-3 fatty acids in purslane, demonstrate high potential in strengthening the human immune system, and contribute to the prevention of various chronic diseases (Hosseinzadeh et al., 2020). In fact, purslane presents various modern pharmacological properties, including anticancer anti-hyperglycemic efficacy elicited by polysaccharides derived from purslane (Hu et al., 2018), anti-inflammatory and antioxidant activities in diabetes by suppressing pro-inflammatory cytokines (Nkhumeleni et al., 2024), as well as hepatoprotective effects of ethanolic extracts preserving the histological integrity of liver cells (Eidi et al., 2015), which is related to the purslane rich nutritional profile. In addition, purslane plants have diverse use cases; the plants' extracts employed for to extend the shelf-life of Greek yoghurt, due to its antimicrobial activity towards *Escherichia coli* and *Salmonella typhimurium* (El-Sayed et al., 2019), and for the phytoremediation and biomonitoring of contaminated environments (Subpiramaniyam, 2021).

The health-promoting compounds of medicinal plants are impacted by several factors, including agronomic practices, irrigation, fertilization and harvest times, as well as external environmental factors. More significantly, fertilization has a pivotal role in the balance between yield and quality of plants (Yang et al., 2017). To improve quality, precise fertilizer application methods may be applied to stimulate the production of secondary metabolites (Khammar et al., 2021). For purslane specifically, research has shown that fertilization can influence its nutraceutical content, with optimum fertilizer applications influencing the qualitative traits of the plant, including total phenolics content, antioxidant capacity, fatty acids content, and carotenoids (Hosseinzadeh et al., 2020; Montoya-García et al., 2018). While the influence of various minerals on plant growth is well-documented, the specific nutrient requirements for specialty and underutilized crops remain relatively understudied. Consequently, each species necessitates tailored nutrient management (Chrysargyris and Tzortzakis, 2021). Soilless cultivation presents it-self is an efficient mean of sustaining the market demand for specialty crops, allowing for the complete control of the their nutrition, while eliminating hindrances related to the soil characteristics (e.g. poor soil fertility, salinity, soil-borne diseases) (Maggini et al., 2022). In addition, soilless culture gives a total control over the plants' nutrition through the nutrient solution, providing a valuable tool or improving product yield and quality (Tzortzakis et al., 2020). Due to these benefits, soilless culture systems are particularly well-suited for the cultivation of medicinal and wild edible plants (Maggini et al., 2022), as it enables researchers and growers to optimize the supply of nutrients and to monitor plant responses, yielding reproducible results regarding plant growth, nutrient status and quality (Chrysargyris and Tzortzakis, 2021).

Nitrogen (N) is an important element for plants, influencing the primary and secondary metabolite production of functional and medicinal crops (Khammar et al., 2021). After carbon, plants require most N, as N is regulating the yield and quality, and is an important ingredient of amino acids, proteins, chlorophyll, co-enzymes, phytohormones and secondary metabolites, influencing the production of several bioactive compounds. This includes the production flavonoids, glucosinate, carotenoids, etc., as N application in soilless cultivation directly affect the production of these constituents, impacting the quality of the final product (Khammar et al., 2021). The main sources of N taken up by plants include nitrate (NO_3^-) and ammonium (NH_4^+) (Hawkesford et al., 2011). These predominant forms of N affect various biochemical processes in plants, influencing their physiology, growth and development, particularly yield and biomass production, root and leaf growth, flowering time, and senescence (Hameed et al., 2022; Zhang et al., 2019). In soilless cultivation, the adjustment of the NH_4^+:NO_3^- ratio is conducted to optimize the pH of the solution. Hence, the NH_4^+-N concentration should be limited to no more than 25 % of total nitrogen output (Sonneveld and Voogt, 2009). However, leafy vegetables are susceptible to nitrate accumulation, which is a concern due to its association with various human ailments, including gastric cancer and methemoglobinemia (Bian et al., 2020). Consequently, the ammonium to total nitrogen ratio (Nr= NH_4^+/total N) has attracted the attention of researchers (Chrysargyris and Tzortzakis, 2023). Previous studies have demonstrated this, especially with the application diverse N levels and Nr ratios, impacting the morphological and biochemical attributes of commonly cultivated crops, including lettuce (*Lactuca sativa* L.), sweet basil (*Ocimum basilicum* L.) and spinach (*Spinacia oleracea* L.) (Hameed et al., 2022; Kolega et al., 2020; Machado et al., 2020).

Proper application of nitrogen, utilizing management strategies and technologies to increase the nitrogen use efficacy, is required to optimize the plants' growth and development, while limiting the negative impacts of N leaching and nitrate accumulation in plant tissues (Cebani et al., 2024). The cultivation of purslane has been researched in several studies (Hajisolomou et al., 2024; Petropoulos et al., 2023). In addition, studies have also explored the impacts of N fertilization and Nr on the plant's development, growth and physiology (Camalle et al., 2020; Chrysargyris et al., 2024, 2023; Hosseinzadeh et al., 2020), primarily focused on optimizing the cultivation and fertilization practices for purslane, to enhance its agricultural viability and yield, placing less emphasis on examining the impact of these parameters on the nutraceutical properties of the plant. Other studies had partially addressed some quality attributes of purslane exposed to different Nr levels (Fontana et al., 2006; Palaniswamy et al., 2000). Thus, this study was commissioned to examine the impact of the NH_4^+/total N ratio on purslane's centesimal composition, chemical profile, antioxidant, cytotoxic, anti-inflammatory, antimicrobial, and hepatotoxic potential, and providing better understanding on the correlation of Nr with various nutritional components.

2. Material and methods

2.1. Experimental set up and growing conditions

Portulaca oleracea (L.) seedlings were subjected to the different N sources following the principles of NFT (Nutrient-Film-Technique), whereas nutrient solutions were recirculated continuously. The detailed experimental set up and the greenhouse conditions have been previously described (Chrysargyris and Tzortzakis, 2023). In brief, 12 independent NFT channels were connected with 60-L catchments tanks to set up 12 hydroponic units. The nutrient solution received by the plants was restored by automated water filling from the replenishment tanks and the daily adjustments on the nutrient solution by utilizing stock nutrient solutions, as previously described (Chrysargyris and Tzortzakis, 2023). The desired electrical conductivity and pH for the nutrient solution were 5.8 and 2.3 mS/cm, respectively. Every hydroponic system housed 12 plants, resulting in a total plant density of 2500 plants per stremma.

The effects of four Nr ratios (ammonium to total nitrogen ratio: Nr) were tested (Nr ratios: 0.01–0.05–0.10–0.15), whereas the N, potassium-K and phosphorus-P levels were kept consistent at 200 mg/L, 350 mg/L and 70 mg/L, respectively, according to preliminary research and prior findings (Tzortzakis et al., 2022). The meteorological parameters during the study were recorded and the air temperature ranged between 18.12 and 31.28 °C during autumn. Plants were grown for 21 days under the four tested Nr nutrient solutions. After the experimental completion, plants were sampled and lyophilized for further analysis.

2.2. Plant yield and nitrogen levels in plant tissue

Nine individual plants from each Nr treatment were marked for plant growth measurements. The total plant fresh weight was determined, and plant yield was computed. At the experiment's completion, leaf and stems samples collected from the same plants, for the determination of the N content, in four replicates per treatment. Nitrogen was determined by the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjeldahl K-360, Flawil, Switzerland). Data were presented in g/kg of dry weight.

2.3. Nutritional profile: proximate composition, free sugars, tocopherols and fatty acids contents

The proximate composition in terms of crude protein, total fat, ash, total fibre dietary of the purslane leaves and stems were performed by gravimetric methods attendant the standard analysis (AOAC, 2016), the carbohydrate amount was determined by carbohydrate = (100 – (crude protein + total fat + ash + total fibre dietary)). All analysis was established in triplicate, apart from total fibre dietary analysis, which was performed in quadruplicate. As described in AOAC nº 991.02 the crude protein was determined with some adjustments. Each sample with catalyst tablets and sulfuric acid in Kjeldahl tubes were digested at 400 ºC/1h10min, after cooling, distilled water was added. Kjeldahl analyser was used to measure the quantity of nitrogen, then the crude protein amount was determined by N multiplied by 6.25. The total fat was settled following the AOAC nº 989.05 through constant cycles of extraction with petroleum ether for 6 h.

The total fibre dietary amount was performed succeeding a combination of enzymatic and gravimetric methods based on AOAC nº 985.29 using the Total dietary fiber assay kit TDF-100A (Sigma-Aldrich). Briefly, the extraction procedure was based on 3 enzymes (α-amylase, protease and amyloglucosidase) in a pH and temperature ranges of 4–7.5 and 60 ºC to 100 ºC, respectively, to optimise enzyme action. The precipitation of total fibre dietary was performed employing ethanol. The residue was washed with different percentages of ethanol and acetone. Thus, some of residue was used to determine ash and the remainder to perform crude protein. On the other hand, the amount of ash was determined over AOAC nº 935.42 in a muffle at 550 ºC. The energy value, in kcal per 100 g of dw, was calculated following the Regulation of European Union nº1169 from 2011 (Regulation (EU) No 1169/2011, 2011), which 1 g of crude protein and C provides 4 kcal, while 1 g of total fat and total fibre dietary provides 9 and 2 kcal, respectively.

The free sugars, tocopherols and fatty acids contents were performed over chromatographic methods, and different extraction methodologies were applied depending on the target compounds. In the extraction of free sugars, the purslane samples (leaves and stems) with an ethanol solution 80 % were placed at 80 ºC/90 min. The organic fraction was washed three times with diethyl ether, then made up to 5 mL in a volumetric flask with distilled water and filtered into vials for injection in HPLC-RI. The melezitose (25 mg/mL) was employed as an internal standard (Alonso-Esteban et al., 2022).

The extraction of tocopherols was performed following Liberal et al. (2024), the purslane samples (leaves and stems) with a solution of butylated hydroxytoluene, tocol (internal standard), and methanol were mixed. After it added hexane and vortexed once more, then added sodium chloride solution and further vortexed. The supernatant was collected into a tube with anhydrous sodium sulphate. The procedure was performed two more times without methanol. A nitrogen flow was used to dry the collected supernatant, then redissolved in hexane for injection in HPLC-FL. The results of organic acids and free sugars contents were expressed in grams per 100 g of dry weight (dw) while tocopherols were described in milligrams per 100 g of dw.

The total fat obtained from the AOAC analysis in the proximate composition was then subjected to agitation (50 ºC/12 h) with a methanol:toluene:sulphuric acid solution to obtain fatty acid methyl esters as previously described by Liberal et al. (2024). The injection was conducted via split/splitless (1:80) into gas chromatography with flame ionisation detection (260 °C). The identification of the samples was conducted through a comparative analysis of the retention times of the purslane leaves and stems samples with those of the standard containing 37 fatty acid methyl esters.

2.4. Phytonutrient composition

The organic acids were extracted in accordance with Alonso-Esteban et al. (2022). The purslane samples (leaves and stems) were placed under stirring with metaphosphoric acid covered with aluminium for 20 min at 20 ºC. The extract was filtered into a vial for subsequent injection in UFLC-DAD. Calibration curves were employed to determine the concentration of oxalic, succinic and fumaric acids in the samples.

The purslane samples (leaves and stems) were subject to a conventional maceration without temperature as described before (Polyzos et al., 2024) to evaluate phenolic compounds and biocidal activities. In brief, each sample with ethanol solution 80 % was placed under stirring for 1 h, filtered, and the solid phase was extracted once more under the same conditions. The hydroethanolic extract was evaporated, lyophilized (-55 ºC, 0.020 bar) and stored until further analysis, such as phenolic compounds and biocidal activities.

The extracts of *P. oleracea* from the conventional maceration were solubilised at 10 mg/mL with a 20 % ethanol solution to evaluate the phenolic compounds. HPLC (Dionex Ultimate 3000 UPLC; Thermo Scientific, San Jose, CA, USA) was employed, following the methodology reported by Mandim et al. (2021). Detection was performed using a dual online approach: a diode array detector (DAD) set at 280 and 370 nm, and a mass spectrometer coupled to the HPLC system downstream of the DAD cell. Compound identification was carried out through Xcalibur® software, based on ultraviolet and mass spectral data, retention times, and comparison with authentic standards when available. For quantification, a calibration curve with five concentration levels was established through injections of standard compounds.

2.5. Biocidal activities

The antimicrobial activities of leaves and stems of *P. oleracea* were performed in accordance to Polyzos et al. (2024) for ten Gram- and six Gram+ strains and two fungus. The strains tested were ATCC 8610 (*Yersinia enterocolitica*), ATCC 9027 (*Pseudomonas aeruginosa*), ATCC 13076 (*Salmonella enterocolitis*), ATCC 25922 (*Eschericia coli*) and ATCC 49741 (*Enterobacter cloacae*), ATCC 19111 (*Listeria monocytogenes*), ATCC 11778 (*Bacillus cereus*), ATCC 11632 (*Staphylococcus aureus*), clinically derived isolated strains (methicillin-resistant *S. aureus* – MRSA, *Listeria monocytogenes, Enterococcus faecalis, Pseudomonas aeruginosa E. coli, Morganella morganii, Klebsiella pneumoniae and Proteus mirabilis*), ATTC 16404 (*Aspergillus brasiliensis*) and ATCC 204305 (*Aspergillus fumigatus*) as described in Table S1. For antioxidant activities, two *in vitro* antioxidant activities assays (OxHLIA and TBARS, trough the oxidative haemolysis and substances reactive to thiobarbituric acid, respectively) were implemented as described earlier (Mandim et al., 2021; Pires et al., 2021), while the cytotoxic and hepatotoxic (Pereira et al., 2023) and and anti-inflammatory activities (Pires et al., 2021) were also evaluated (Table S2).

2.6. Statistical methods

The one-way analysis of variance (ANOVA) was used for the analysis of data, while significant effects were recorded means were compared with the Tukey HSD-test at $p = 0.05$ through IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, New York, NY, USA). The results of centesimal composition, chemical characterization, phenolic compounds and antioxidant activities (OxHLIA and TBARS) are presented as average values ± standard error. The correlation coefficients between Nr ratios with individual parameters tested in leaves and stems by Pearson's correlation test.

3. Results and Discussion

3.1. Plant yield and nitrogen accumulation

Purslane yield decreased when plants were grown in nutrient solution with Nr≥ 0.10 (Fig. 1**A**). Nitrogen content in leaves and stems did not change in regard to the Nr ratios and averaged in 54.03 g/kg and 40.51 g/kg, respectively (Fig. 1**B**), and this was most possible related to the short growing period in the present research, that could affect the N uptake in plant organs. However, higher Nr (Nr>0.4) levels in purslane grown in floated trays, resulted in increased N content in leaves (Fontana et al., 2006). The observed yield in the current research was similar to those obtained in earlier studies (D'Imperio et al., 2022). Previous reports indicated that increased Nr resulted in decreased plant fresh weight (Chrysargyris et al., 2023) whereas other studies with higher Nr levels results in similar purslane fresh weight (Fontana et al., 2006).

3.2. Nutritional profile: proximate composition, free sugars, tocopherols and fatty acids contents

The proximate composition of purslane grown in various ammonium to total N ratios was researched, which indicated significant changes among the examined Nr levels (Table 1). In leaves, the highest total fat content (4.4 g/100 g dw), carbohydrates content (12.2 g/100 g dw) and energy (274 kcal/100 g dw), were found in purslane grown in Nr0.10. The highest protein content (32 g/100 g dw) and ash content (23.2 g/100 g dw) were found in leaves in Nr0.15 treatment. Total fiber dietary content was found increased (39.1 g/100 g dw) in leaves with the Nr0.01 applications. In stems, total fat content (averaged in 2.88 g/100 g dw) was increased in Nr0.01, Nr0.10 and Nr0.15 applications. The highest protein content (21.1 g/100 g dw) in stems was found in Nr0.10 treatment, while the highest ash content (23.4 g/100 g dw) was observed in stems in Nr0.01 treatment. Total fiber dietary content was found increased (43.2 g/100 g dw) in stems with the Nr0.05 applications. The highest carbohydrates content (18.9 g/100 g dw) and energy (262 kcal/100 g dw) were found in stems of purslane grown in Nr0.15.

The leaves and stems of the purslane were analysed for their proximate composition, since these different parts of the plant are eaten as a single dish or combined in typical dishes in various areas of the world (Fernández-Poyatos et al., 2021), and the accumulation of macro and micronutrients is contingent on the function performed by each part of the plant during its life cycle (Cosgrove and Jarvis, 2012). However, there remains a lacuna in the extant literature concerning the nutritional and therapeutic potential of different parts of purslane, with most studies focusing on the differences between wild and cultivated whole plants and/or different genotypes (Camalle et al., 2020; Oliveira et al., 2009; Petropoulos et al., 2015; Santiago-Saenz et al., 2018). Petropoulos et al. (2019) distinguished the proximate composition of purslane leaves and stems at varying stages of the harvesting process, cultivated in soil. Regarding total fat content, the leaves exhibited higher levels in comparison to their respective stems, a finding that aligns with the conclusions of the present study. The purslane in the present study was harvested after 21 days from a hydroponic system, while the authors evaluated the harvest after 29 days from soil cultivation. However, the fat contents in the leaves and stems observed under different Nr ratios were at most 2.5 and 3 times higher, respectively, than those reported by the authors in the same harvest period. In contrast, Oliveira et al. (2009) conducted a study on the fat content of wild purslane, collected from four distinct regions in northern Portugal. Their findings revealed levels ranging from 4.53 to 5.48 g/100 g of dry weight in the leaves and from 1.2 to 5.9 g/100 g of dry weight in the stems. These levels were found to exceed those observed in the present study, underscoring the significance of controlled cultivation and the distinction of fat accumulation in purslane leaves and stems.

The accumulation of crude protein in plant matrices has become a salient issue in the context of the emergence and increasing popularity of vegetarian and vegan diets over the years (Carbajal, 2013). It is evident that plants possess the capacity to absorb nitrogen in the form of nitrates and nitrites. However, elevated levels of nitrates, in comparison to total nitrogen, have been observed to induce toxicity in crops (Fontana et al., 2006). Consequently, the NH_4^+ to total nitrogen ratios in the present study were constrained to 0.15. A similar study by Fontana et al. (2006) evaluated the impact of different nitrate: ammonium (NO_3^-:NH_4^+) ratios and N concentrations in hydroponic cultivation on the crude protein content of whole purslane. The authors observed an increase in the crude protein content of the total dry matter in two experiments with increasing nitrogen levels, with the NO_3^-:NH_4^+ ratio fixed at 40:60. In the

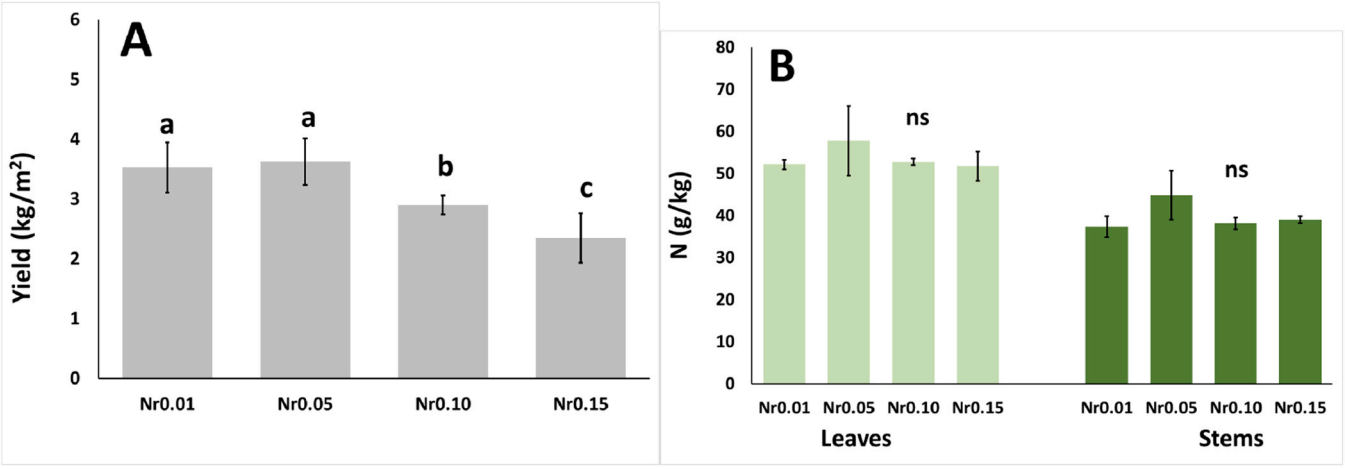

Fig. 1. Effect of ammonium to total nitrogen ratio (NH_4^+/Total N = Nr 0.01–0.05–0.10–0.15) on purslane yield (kg/m^2) and nitrogen (N) content (g/kg) in leaves and stems, when plants grown hydroponically. Significant changes ($p < 0.05$) among Nr ratios are highlighted by different letters. ns: indicate no significance differences.

Table 1
Nutritional profile and energy value of leaves and stems of purslane.

	Leaves				Stems			
	L_Nr0.01	L_Nr0.05	L_Nr0.10	L_Nr0.15	S_Nr0.01	S_Nr0.05	S_Nr0.10	S_Nr0.15
				Nutritional profile (g/100 g dw)				
Total fat	3.6 ± 0.1^{c}	3.9 ± 0.1^{b}	4.4 ± 0.1^{a}	3.6 ± 0.1^{c}	2.85 ± 0.01^{a}	2.69 ± 0.09^{b}	2.9 ± 0.1^{a}	2.9 ± 0.1^{a}
Crude protein	28 ± 1^{c}	29 ± 1^{b}	29 ± 1^{b}	32 ± 1^{a}	19.1 ± 0.4^{c}	18.613 ± 0.002^{d}	21.1 ± 0.5^{a}	20.1 ± 0.1^{b}
Ash	21.8 ± 0.6^{b}	21.5 ± 0.3^{b}	20.02 ± 0.68^{c}	23.2 ± 0.4^{a}	23.4 ± 0.1^{a}	19.9 ± 0.3^{b}	19.09 ± 0.28^{c}	18.2 ± 0.1^{d}
Total fiber dietary	39.1 ± 0.5^{a}	38.09 ± 0.08^{b}	34.1 ± 0.8^{c}	32.6 ± 0.4^{d}	41.2 ± 0.7^{b}	43.2 ± 0.6^{a}	40.4 ± 0.6^{c}	39.9 ± 1.1^{c}
Carbohydrates	7.8 ± 0.5^{c}	7.07 ± 0.52^{d}	12.2 ± 0.2^{a}	8.6 ± 0.9^{b}	13.5 ± 0.7^{d}	15.6 ± 0.7^{c}	16.6 ± 0.4^{b}	18.9 ± 0.6^{a}
Energy (kcal/100 g dw)	253 ± 2^{d}	257 ± 1^{c}	274 ± 3^{a}	259.9 ± 0.1^{b}	238.5 ± 0.5^{d}	248 ± 1^{c}	257 ± 1^{b}	262 ± 2^{a}

Values in the same row with different Latin letters indicate significant differences at $p < 0.05$, based on Tukey's HSD test for leaves and stems.

present study, it was observed that the content of this macronutrient increased with the increase of NH_4^+ in relation to the total nitrogen of the nutrient solution (Nr0.15) in the leaves (32 ± 1 g for every 100 g of dry weight). Conversely, this behavior was not observed in the stems, and the ratio that favored this parameter was NH_4^+ under total nitrogen of 0.10. The accumulation of this macronutrient was found to be proportional to the increase in NH_4^+ in the NO_3^-:NH_4^+ ratio in the nutrient solution, as reported in the study conducted by Camalle et al. (2020), on two genotypes of *P. oleracea*.

Despite the application of different methodologies in the quantification of crude protein in purslane leaves by the authors and in the present study, it was still possible to suggest that the availability of NH_4^+ in purslane cultivation positively impacts the crude protein content.

Dietary fibres act as a functional nutrient in the modulation of diseases due to their favorable effects, such as metabolic improvement, regulation of the intestinal microbiota and immune strengthening (Li and Ma, 2024). To date, no scientific reports have been found that have assessed the total dietary fiber content exclusively in purslane stems, so this approach becomes relevant in the present study, as the stems have been shown to be promising sources of this macronutrient. However, in contrast to the observations made in the crude protein content, the total dietary fiber content was found to be enhanced when lower NH_4^+ ratios were utilized during cultivation. The leaves exhibited the highest fiber content when cultivated with Nr0.01 (39.1 ± 0.5 g/100 g dry weight), while for the stems, the cultivation Nr0.05 was more conducive (43.2 ± 0.6 g/100 g dry weight).

On the other hand, Santiago-Saenz et al., (2018), studied wild purslane leaves, reporting a total dietary fiber content of 41.14 g per 100 g of dry leaf. This value is notably higher than that observed in leaves cultivated with Nr 0.01. Whilst it is not possible to make a direct comparison with the present study, Fukalova et al. (2022) found contents of 20.58 and 15.58 g per 100 g of dry weight of total dietary fiber in the leaves of wild purslane and those cultivated in organic agriculture, respectively. These are lower than even the lowest content in the present study. Therefore, both parts evaluated under different Nr are an interesting source of this nutrient.

The leaves of Nr0.10 presented the highest energy value (274 ± 3 kcal/100 g of dry weight) due to the high total fat content, since it is the macronutrient with the highest energy value (9 kcal for every 1 g consumed). The stems of Nr0.15, on the other hand, exhibited an energy contribution of 262 kcal for every 100 g of dry weight, most likely due to the greater amount of carbohydrates and fibres.

The nutritional quality of a food is determined by the ratio of nutrient density to energy density, which is a key factor in its selection. As demonstrated in the following references, foods rich in proteins and fibers have been shown to promote greater satiety, favor the accumulation and recovery of skeletal muscle mass, regulate glycemic levels in the body and promote the health of the intestinal microbiota (Monteiro et al., 2010; WHO, 2020).

Four free sugars, fructose, glucose, sucrose and trehalose, were identified in stems, and only the first two ones (fructose, glucose) were evidenced in leaves (Table 2). Free sugars in stems were 11.5 times more than in leaves with trehalose to be the main sugar component. Trehalose greatest content (15.79 g/100 g) was observed in stems in plant grown in Nr0.01, while the highest sucrose content (0.54 g/100 g) was found in stems with the Nr0.10 application. The highest fructose (16.7 g/100 g) and glucose (10.2 g/100 g) content were found in purslane stems under the Nr0.15 treatment, which mirrored the highest total free sugars content (18.9 g/100 g). Purslane leaves revealed the highest fructose and glucose content in plants grown in Nr0.01. It has been reported that free sugars content increased at late purslane growth stages while the lower sucrose content in comparison to fructose and glucose was related to the utilization of carbon from the sucrose for the biosynthesis of the latter's sugars (Petropoulos et al., 2019). Furthermore, Petropoulos et al. (2015) reported the accumulation of fructose, glucose, sucrose and trehalose in the leaves of six purslane genotypes, with fructose being the

Table 2
Organic acids, free sugars and tocopherols content of leaves and stems of purslane.

	Leaves				Stems			
	L_Nr0.01	L_Nr0.05	L_Nr0.10	L_Nr0.15	S_Nr0.01	S_Nr0.05	S_Nr0.10	S_Nr0.15
				Organic acids (mg/100 g dw				
Oxalic acid	12.03 ± 0.36^{c}	12.7 ± 0.3^{b}	11.3 ± 0.6^{d}	26.6 ± 0.9^{a}	20 ± 0.2^{a}	18.5 ± 0.2^{c}	19.3 ± 0.3^{b}	20.1 ± 0.5^{a}
Succinic acid	22.4 ± 0.9^{b}	22.4 ± 0.2^{b}	19 ± 0.6^{c}	28.2 ± 0.6^{a}	44 ± 0.2^{b}	43.9 ± 0.6^{b}	46.36 ± 0.41^{a}	33.46 ± 0.23^{c}
Fumaric acid	tr	tr	tr	tr	tr	tr	tr	tr
Total	34.39 ± 1.28^{b}	35.1 ± 0.5^{b}	30.32 ± 1.16^{c}	54.8 ± 1.4^{a}	63.96 ± 0.01^{b}	62.4 ± 0.7^{c}	65.65 ± 0.11^{a}	53.6 ± 0.7^{d}
				Free sugars (g/100 g dw)				
Fructose	1.7 ± 0.1^{a}	0.31 ± 0.01^{d}	0.61 ± 0.01^{b}	0.49 ± 0.01^{c}	2.15 ± 0.03^{c}	1.96 ± 0.03^{d}	2.4 ± 0.04^{b}	3.57 ± 0.04^{a}
Glucose	0.66 ± 0.01^{a}	0.59 ± 0.01^{c}	0.54 ± 0.03^{d}	0.59 ± 0.01^{b}	0.094 ± 0.004^{d}	0.28 ± 0.01^{c}	0.56 ± 0.01^{b}	0.97 ± 0.03^{a}
Sucrose	nd	nd	nd	nd	0.39 ± 0.02^{c}	0.24 ± 0.01^{d}	0.54 ± 0.01^{a}	0.53 ± 0.02^{b}
Trehalose	nd	nd	nd	nd	15.79 ± 0.04^{a}	12.6 ± 0.2^{d}	13.27 ± 0.03^{c}	13.83 ± 0.04^{b}
Sum	2.4 ± 0.1^{a}	0.896 ± 0.024^{d}	1.14 ± 0.04^{b}	1.09 ± 0.02^{c}	18.42 ± 0.02^{b}	15.1 ± 0.3^{d}	16.78 ± 0.01^{c}	18.9 ± 0.05^{a}
				Tocopherols (mg/100 g dw)				
α-tocopherol	3.93 ± 0.03^{d}	3.99 ± 0.03^{c}	4.32 ± 0.07^{a}	4.148 ± 0.004^{b}	2.58 ± 0.04^{bc}	2.57 ± 0.01^{c}	3.195 ± 0.021^{a}	2.61 ± 0.08^{b}
β-tocopherol	0.108 ± 0.004^{a}	0.072 ± 0.003^{b}	0.05 ± 0.002^{c}	0.034 ± 0.001^{d}	0.07 ± 0.01^{b}	0.069 ± 0.003^{b}	0.07 ± 0.003^{b}	0.08 ± 0.01^{a}
Total	4.04 ± 0.03^{c}	4.06 ± 0.03^{c}	4.37 ± 0.07^{a}	4.182 ± 0.003^{b}	2.64 ± 0.03^{c}	2.64 ± 0.01^{c}	3.27 ± 0.02^{a}	2.68 ± 0.07^{b}

Values in the same row with different Latin letters indicate significant differences at $p < 0.05$, based on Tukey's HSD test for leaves and stems. tr – traces; nd – not detected.

most abundant. The content of free sugars in different parts of purslane is still poorly reported but could be an approach to explore in commercial cultivation, as they influence organoleptic characteristics that are essentially related to consumer acceptability.

Two tocopherol isoforms were identified for both leaves and stems, α-tocopherol and β-tocopherol with the former to contribute most to the total tocopherol profile of purslane (Table 2). In oppose to organic acids and sugars which were found in increased levels in stems, tocopherols were detected in higher levels in leaves. The highest α-tocopherol content (4.32 mg/100 g) was found in leaves of purslane grown in Nr0.10 and this was mirrored the increased total tocopherols content observed in the same treatment (4.37 mg/100 g). The β-tocopherol was increased in leaves of purslane grown in Nr0.01. In stems, plant gown in Nr0.10 obtained the highest α-tocopherol content (3.195 mg/100 g) which reflected the increased total tocopherol content. Similar to the present study, α-tocopherol was the main identified isoform in both purslane leaves and stems (Petropoulos et al., 2019). Also, Simopoulos (2004) and Nemzer et al. (2020) observed higher α-tocopherol levels in purslane leaves from commercial cultivation compared to wild ones. Szalai et al. (2010) reported that increasing the nitrate in the nitrate:ammonim ratio favoured the accumulation of α- and γ-tocopherols in the leaves of *P. oleracea* microspecies.

Sixteen different fatty acids were identified in stems, of which only twelve were identified in leaves (Table 3). In leaves, the main fatty acids were linolenic acid (averaged in 58.93 %), followed by linoleic acid (averaged in 13.74 %) and palmitic acid (averaged in 13.31 %). The highest linolenic acid content was found in leaves for plants grown in Nr0.10 (59.91 %). In stems, the main acids were linoleic acid (averaged in 38.15 %), followed by linolenic acid (averaged in 23.78 %) and palmitic acid (averaged in 22.06 %). The highest linoleic acid was found in stems after the Nr0.10 and Nr0.15 applications (38.6 %). Indeed, palmitic acid was found in highest levels in stems following lower Nr applications (Nr0.01 and Nr0.05). Purslane is abandon in fatty acids, especially the ω3 (linolenic acid) (D'Imperio et al., 2022; Petropoulos et al., 2019). The three main fatty acids observed in the present study were consistent with earlier investigations (D'Imperio et al., 2022). The α-linolenic acid has been documented to pose anti-inflammatory effects *in vitro* (Ghorani et al., 2023). Vital fatty acids, both ω6 (linoleic acid) and ω3 (linolenic acid) are important for human wellbeing, while increased levels of them were found at Nr0.10. Indeed, different purslane ecotypes were affected differently by the Nr levels (Camalle et al., 2020). Moreover, increased N levels in soil, resulted in purslane with increased ω6 (linoleic acid) and ω3 (linolenic acid) fatty acids but decreased palmitic acid and stearic acid (Montoya-García et al., 2018).

It has been suggested that the ω6/ω3 ratio should vary optimally from 4/1–1/1 for decreasing the possibilities for several chronic diseases (Simopoulos, 2004), and a lower ω6/ω3 ratio is more desirable to protect human health against many chronic diseases. In our purslane the ω6/ω3 ratio ranged 1/4.28 in leaves to 1/0.64 in stems, indicating a high nutritional quality of the produced purslane. Similar increased ω6/ω3 ratio were found in purslane subjected to zinc biofortification (D'Imperio et al., 2022). Interestingly, the ω6/ω3 ratio decreased as the Nr ratio was increased for both leaves and stems, which actually shows that NH_4-N can act as a stimuli to quality attributes of purslane. Similar to the present findings, palmitic acid dropped when the Nr ratio was raised in the nutrient solution for both leaves and stems, being in agreement with previous reports in purslane when greater Nr ratios used (Nr 0.4–0.6) (Fontana et al., 2006).

Polyunsaturated fatty acids were the majority at the composition of fatty acids and represented an average of 72.7 % and 61.9 % of the fatty acids for leaves and stems, respectively. The greatest polyunsaturated fatty acids (PUFA) levels were found in leaves for plants grown in Nr0.10 (74.5 %) treatment, and in stems for plants grown in Nr0.10 and Nr0.15 (63.5 %) treatment. However, in stems it was found increased saturated fatty acids and monounsaturated fatty acids in plant grown in Nr0.05 (33.41 %) and Nr0.01 (7.01 %), respectively. In leaves, both saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were increased in plants grown under Nr0.01 applications. Increased NH_4-N in the nutrient solution stimulated the fatty acid content in canola (Bybordi, 2012), being in consistent with the current findings as PUFA was found increased in higher Nr ratios. The PUFA/SFA ratio was averaged at 3.6 in leaves and 1.93 in stems, while this ratio was increased as the Nr levels were raised in the nutrient solution. Therefore, PUFA/SFA ratio was 3.13 and 1.87 at Nr0.01 and 4.01 and 2.02 at Nr0.15 in leaves and stems, respectively. Such increased ratios were also evidenced previously (Petropoulos et al., 2019), which were largely exceeding the suggested values of PUFA/SFA ratio above 0.4–0.5 for a balanced diet (FAO, 1994). Therefore, increased Nr ratios can be used to

Table 3
Fatty acids expressed as a relative percentage of leaves and stems of purslane.

	Leaves				Stems			
	L_Nr0.01	**L_Nr0.05**	**L_Nr0.10**	**L_Nr0.15**	**S_Nr0.01**	**S_Nr0.05**	**S_Nr0.10**	**S_Nr0.15**
			Fatty acids (%)					
C12:0	0.213 ± 0.004^{c}	0.236 ± 0.004^{a}	0.224 ± 0.003^{b}	0.124 ± 0.003^{d}	0.149 ± 0.001^{b}	0.169 ± 0.007^{a}	0.12 ± 0.01^{c}	0.12 ± 0.01^{c}
C13:0	0.95 ± 0.01^{a}	0.88 ± 0.01^{b}	0.754 ± 0.004^{c}	0.654 ± 0.004^{d}	0.254 ± 0.003^{b}	0.34 ± 0.01^{a}	0.18 ± 0.01^{c}	0.18 ± 0.01^{c}
C14:0	0.238 ± 0.005^{c}	0.252 ± 0.003^{b}	0.278 ± 0.002^{a}	0.208 ± 0.002^{d}	0.275 ± 0.005^{b}	0.346 ± 0.03^{a}	0.23 ± 0.01^{c}	0.231 ± 0.01^{c}
C14:1	0.487 ± 0.007^{a}	0.43 ± 0.05^{c}	0.37 ± 0.01^{d}	0.45 ± 0.02^{b}	0.104 ± 0.004^{b}	0.21 ± 0.01^{a}	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}
C15:0	nd	nd	nd	nd	0.168 ± 0.001^{b}	0.206 ± 0.006^{a}	0.132 ± 0.005^{c}	0.131 ± 0.006^{c}
C16:0	14.7 ± 0.1^{a}	14.1 ± 0.2^{b}	12.4 ± 0.1^{c}	12.03 ± 0.02^{d}	22.4 ± 0.2^{a}	22.3 ± 0.6^{a}	21.774 ± 0.002^{b}	21.783 ± 0.003^{b}
C16:1	2.55 ± 0.07^{a}	2.12 ± 0.01^{b}	1.85 ± 0.01^{d}	1.95 ± 0.01^{c}	0.27 ± 0.01^{a}	0.28 ± 0.02^{a}	0.25 ± 0.01^{b}	0.25 ± 0.01^{b}
C17:0	nd	nd	nd	nd	0.32 ± 0.014^{b}	0.34 ± 0.001^{a}	0.25 ± 0.006^{c}	0.25 ± 0.006^{c}
C18:0	2.989 ± 0.004^{a}	2.676 ± 0.005^{b}	2.34 ± 0.11^{c}	2.196 ± 0.008^{d}	4.179 ± 0.05^{b}	4.798 ± 0.08^{a}	3.713 ± 0.1^{c}	3.715 ± 0.1^{c}
C18:1n9c	4.74 ± 0.06^{b}	4.87 ± 0.09^{a}	3.96 ± 0.19^{c}	4.9 ± 0.1^{a}	6.401 ± 0.006^{a}	5.917 ± 0.002^{b}	4.55 ± 0.07^{c}	4.56 ± 0.07^{c}
C18:2n6c	12.79 ± 0.01^{d}	13.1 ± 0.2^{c}	14.6 ± 0.1^{a}	14.5 ± 0.1^{b}	38.1 ± 0.2^{b}	37.3 ± 0.2^{c}	38.6 ± 0.3^{a}	38.6 ± 0.3^{a}
C18:3n3	57.14 ± 0.09^{d}	59.01 ± 0.08^{c}	59.91 ± 0.05^{a}	59.66 ± 0.02^{b}	22.5 ± 0.1^{c}	22.8 ± 0.2^{b}	24.9 ± 0.1^{a}	24.9 ± 0.1^{a}
C20:0	0.896 ± 0.005^{c}	0.768 ± 0.004^{d}	1.22 ± 0.02^{a}	1.14 ± 0.01^{b}	1.6 ± 0.1^{c}	1.8 ± 0.1^{b}	2.1 ± 0.1^{a}	2.1 ± 0.1^{a}
C20:1	nd	nd	nd	nd	0.227 ± 0.001^{a}	0.156 ± 0.004^{c}	0.21 ± 0.02^{b}	0.21 ± 0.02^{b}
C22:0	2.24 ± 0.08^{a}	1.62 ± 0.08^{c}	2.16 ± 0.1^{b}	2.16 ± 0.05^{b}	2.8 ± 0.1^{a}	2.8 ± 0.1^{a}	2.64 ± 0.07^{b}	2.64 ± 0.07^{b}
C23:0	nd	nd	nd	nd	0.334 ± 0.001^{c}	0.36 ± 0.03^{b}	0.4 ± 0.1^{a}	0.31 ± 0.01^{d}
SFA	22.3 ± 0.2^{a}	20.5 ± 0.3^{b}	19.4 ± 0.3^{c}	18.5 ± 0.1^{d}	32.4 ± 0.5^{b}	33 ± 1^{a}	31.4 ± 0.3^{c}	31.3 ± 0.3^{c}
MUFA	7.78 ± 0.08^{a}	7.41 ± 0.06^{b}	6.18 ± 0.03^{d}	7.31 ± 0.03^{c}	7.01 ± 0.02^{a}	6.55 ± 0.04^{b}	5.11 ± 0.03^{c}	5.12 ± 0.03^{c}
PUFA	69.9 ± 0.2^{d}	72.13 ± 0.34^{c}	74.5 ± 0.4^{a}	74.2 ± 0.3^{b}	60.598 ± 0.232^{b}	60.04 ± 0.39^{c}	63.5 ± 0.5^{a}	63.5 ± 0.5^{a}

Values in the same row with different Latin letters indicate significant differences at $p < 0.05$, based on Tukey's HSD test for leaves and stems. C12:0 – lauric acid, C13:0 – tridecanoic acid, C14:0 – myristic acid, C14:1 – myristoleic acid, C15:0 – pentadecanoic acid, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C17:0 – heptadecanoic acid, C18:0 – stearic acid, C18:1n9c – oleic acid, C18:2n6c – linoleic acid, C18:3n3 – linolenic acid, C20:0 – arachidic acid, C20:1 – eicosenoic acid, C22:0 – behenic acid; C23:0 – tricosanoic acid. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; nd – not detected.

boost the nutritional value of purslane by increasing the content of ω3 fatty acids and the PUFA/SFA ratio.

3.3. Phytonutrient composition

The organic acid composition of purslane leaves and stems when subjected to different Nr ratios is illustrated in Table 2. Three organic acids were identified for both leaves and stems, namely oxalic and succinic acid while fumaric acid was identified in traces. Organic acids were mainly concentrated in purslane stems rather than in leaves, with plants grown in Nr0.10 to have the highest total organic acids content in stems (65.65 mg/100 g), followed by Nr0.01, Nr0.05 and finally by Nr0.15 treatments. Interesting, even though total organic acids content was the least in stems for the Nr0.15, it was the highest in case of leaves (54.8 mg/100 g), compared to the other Nr treatments in leaves. Succinic acid was the most abundant one, which revealed the highest content under Nr0.10 in stems (46.36 mg/100 g). However, the greatest oxalic acid content was found in stems for plant grown in Nr 0.15 and Nr0.01 (averaged in 20.1 mg/100 g). In leaves, both oxalic and succinic acids were profound at the higher Nr ratio examined (Nr0.15). The synthesis and concentration of different organic acids in purslane can vary depending on the genotype, growth stage, parts of the plant and type of nitrogen fertilizer (Fontana et al., 2006; Oliveira et al., 2009; Palaniswamy et al., 2004; Petropoulos et al., 2019). As additional organic acid components in purslane leaves and stems in previous studies, quinic, malic and citric acids have been identified in field-grown purslane in Greece (Petropoulos et al., 2019) and aconitic in wild purslane from Northeast Portugal (Oliveira et al., 2009), while significant levels of ascorbic acid have also been identified in the leaves of three members of the *P. oleracea* aggregate in Israel (Szalai et al., 2010).

Oxalic acid may have a protective and detoxification effects on plants while increased oxalic acid levels may have adverse effects on human health, especial for people with low plasma levels of iron and calcium (Fontana et al., 2006). Hence, cultivation systems that promote a reduction in oxalic acid levels are essential to encourage the safe consumption of purslane leaves and stems. Therefore, the decreased levels of oxalic acid were evidenced in the lower Nr levels in purslane grown in hydroponics in the current research, being in agreement with prior reports in purslane when subjected to high Nr levels (Fontana et al., 2006). However, in *in vitro* studies with Murashige–Skoog medium, Camalle et al. (2020) reported opposed results when two purslane ecotypes were studied. In both parts of *P. oleracea*, the highest accumulation of oxalic acid was observed at the highest nitrogen levels (Nr0.15), even though these were significantly lower levels than previously reported (Petropoulos et al., 2019) and Oliveira et al. (2009), who observed levels between 72.63 and 13878.5 mg/100 g and 65.03 and 21662.8 mg/100 g dry weight in the leaves and stems, respectively.

Phenolic compounds in the extracts were identified by comparing their characteristics (such as retention times, λmax, pseudomolecular ions, and major MS^2 fragment ions) with chemical standards, as shown in Table 4.

Eight compounds were tentatively identified in the leaves and stems of purslane grown at different Nr. Of these compounds, four were described as cyclo-dopa amide alkaloids belonging to the oleracein group (compounds 4, 5, 7 and 8), relevant to *P. oleracea* (Farag and Shakour, 2019; Jiao et al., 2014; Xiang et al., 2005) plants, representing 50 % of the characterisation in the current study. Compounds 4 ($[M - H]^-$ at *m/z* 710 and fragments *m/z* 664, 502) and 8 ($[M - H]^-$ at *m/z* 502 followed by breaks 340, 296, 268, 202) relate to the structure of oleracein C, while compound 7 showed the ion $[M - H]^-$ at 502 and fragmentation *m/z* 340 and 296 (MS^2) characteristic of oleracein A (Fernández-Poyatos et al., 2021; Petropoulos et al., 2019). Regarding the compound 5, Voynikov et al. (2021) have indicated that it is oleracein, on account of its glucosyl-indoline core-caffeoyl-glucosyl (GIAG) structure. The molecular ion $[M - H]^-$ at *m/z* 680 initially cleaves any of the glucosyl, causing the MS^2 transition at *m/z* 518, which can fragment another glucosyl (*m/z* 356). However, it does not have a corresponding structure that is popularly known (as is the case with oleraceins A-Y).

The chemical structures of oleraceins A and C are similar (Fig. 2) and differ in one radical (Fernández-Poyatos et al., 2021). Radical 1 (R^1) corresponds to a glucoside molecule, while radicals 2, 3, 4 and 6 correspond to hydrogen. The distinction between these oleraceins is attributable to R^5, which corresponds to a hydroxyl molecule in oleracein A, while in oleracein C, it represents an oxygen molecule linked to a glucoside.

These structures were determined through NMR spectroscopy by Xiang et al. (2005) as 5-hydroxy-1-*p*-coumaric acyl-2,3-dihydro-1H-indole-2-carboxylic acid-6-O-beta-D-glucopyranoside (oleracein A) and 5-hydroxy-1-(*p*-coumaric acyl-7′-O-beta-D-glucopyranose)-2,3-dihydro-1H-indole-2-carboxylic acid-6-O-beta-D-glucopyranoside (oleracein C), so the quantification of the oleraceins (compounds 4, 5, 7 and 8) tentatively characterised in the leaves and stems of the purslane in this study was based on the *p*-coumaric acid standard, since this compound showed the best correspondence with the standards available for quantification.

Compounds 1, 2 and 3 were grouped together as other compounds identified using chromatographic methodology. Compounds 1 and 2 correspond respectively to isocitric and citric acids, which were previously identified through analytical standards by High Performance Liquid Chromatography-quadrupole-time-of-flight mass spectrometry (HPLC-Q-TOF-MS) in purslane (Fernández-Poyatos et al., 2021). In view of the unavailability of the citric acid standard in HPLC-DAD-MS/ESI in the present study, its quantification was instead carried out using the

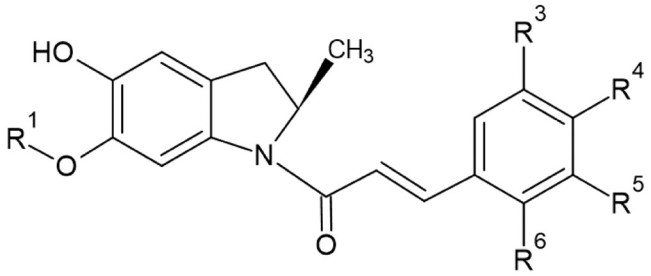

Fig. 2. Basic chemical structure of oleraceins A and C.

Table 4
Analytical parameters for phenolic compounds in hydroethanolic extracts of purslane samples include retention time (Rt), maximum absorption wavelength (λmax), deprotonated ion ($[M-H]^-$), main MS^2 fragments, and tentative compound identification.

Peak	Rt (min)	λmax	$[M-H]^-$ (*m/z*)	MS^2	Tentative identification
1	2.87	275	191	173 (41), 111 (100)	Isocitric acid
2	3.14	263	191	173 (80), 111 (100)	Citric acid
3	4.22	280	315	153 (100), 135 (52)	Hydroxytyrosol hexoside
4	7.18	347	710	664(100), 502 (37)	Oleraceic C
5	8.34	336	680	518 (26), 356 (90), 246 (66)	GIAG
6	12.75	334	613	519 (100), 425 (11), 331(41)	Unknown
7	16.03	333	502	340 (45), 296 (17), 268 (3), 202 (2)	Oleracein A
8	17.93	333	502	340 (86), 296 (30), 268 (11), 202 (2)	Oleracein C

gallic acid calibration curve. Although not structurally identical, this was selected because it exhibited similar chromatographic and spectral characteristics in terms of polarity and DAD-MS response. The fragmentation of compound 6 has previously been reported by Farag and Shakour (2019), but it could not be identified in the methodologies applied by the authors and in the present study. Consequently, no calibration line was applied for its quantification.

In *Portulaca* plants grown in different ammonium to total N ratios, the major compound was Oleracein A followed by the Hydroxytyrosol hexoside, while both of them were differentiated in leaves and in stems and predominated in the former (Table 5). The highest Oleracein A content in leaves was observed Nr0.05 (7.61 mg/g), followed by Nr0.15 (5.36 mg/g), Nr0.10 (2.976 mg/g) and finally by Nr0.01 (1.32 mg/g), while in stems, the highest Oleracein A content was observed in Nr0.10 (0.1961 mg/g). The highest Hydroxytyrosol hexoside content in leaves was observed Nr0.05 (3.85 mg/g), followed by Nr0.01 (3.41 mg/g), Nr0.15 (3.34 mg/g) and finally by Nr0.10 (2.3 mg/g), while in stems, the highest Hydroxytyrosol hexoside content was observed in Nr0.10 (1.27 mg/g). The greatest total phenolic compounds (TPC) in leaves was found in Nr0.05, while in stems, the greatest TPC was found in Nr0.10.

Previous reports indicated five oleraceins in dried purslane plants (Xiang et al., 2005) that can be used as a criterion for different *Portulaca* taxa classification (Petropoulos et al., 2019). In contrast, Voynikov et al. (2021) identified 51 oleraceins in the aerial parts (leaves and stems) of purslane, while Nemzer et al. (2020) identified 184 compounds in two genotypes. Other groups of phenolic compounds have also been reported, such as flavonoids (derivatives of kaempferol, quercetin and apigenin) in purslane (Al-Quwaie et al., 2023; Rahimi et al., 2019), phenolic acids, flavanols, and anthocyanins (derivatives of pelargonidin and cyanidin) in the leaves, stems and flowers of the plant (Silva and Carvalho, 2014).

The observed diversity among the phenolic compounds identified in the present study in comparison to those presented in other studies is attributable to a range of factors. These include differences in cultivation systems, harvesting stages, genotypes and the selection of plant parts studied, the different methodologies applied to these compounds, as well as different extraction protocols (Barroso et al., 2024; Fernández-Poyatos et al., 2021; Petropoulos et al., 2015; 2019).

Nonetheless, the levels of oleraceins A and C were measured in the hydroethanolic extracts (in the same ratio as in the present study) in the leaves and stems of purslane in the study conducted by Petropoulos et al. (2019) in soil. About the leaves, the authors reported a variation in the concentration of oleracein A from 0.08 ± 0.01 to 1.03 ± 0.02 mg/g of extract, while in the present study it ranged from 1.32 ± 0.01 to 7.61 ± 0.01 mg/g of extract. These concentrations are significantly higher, suggesting that the different Nr ratios favour the accumulation of this compound. In contrast, Oleracein A was not detected by the authors in the extracts of stems from the harvest period like the present study. The present study demonstrated that the content of oleracein C was elevated in both parts of the plants.

As previously observed by Oliveira et al. (2009), leaves had a greater concentration of total phenolic compounds than stems. This observation was supported by the suggestion that the synthesis of secondary metabolites is more pronounced in the leaves, while primary metabolism plays a more significant role in the stems.

3.4. Biocidal properties

For Gram^{+} bacteria, *E. cloacae, E. coli, P. aeruginosa,* and *S. enterocolitica* showed significant inhibition for all the tested Nr levels in leaves, especially to the Nr ≥ 0.05, while in stems, the plant extracts revealed strong inhibition for *E. coli* (Table S1). For Gram^{-} bacteria, *B. cereus, L. monocytogenes* and *S. aureus* revealed great inhibition in all tested Nr levels in leaves, with greater effects to be profound as the Nr was increased in the nutrient solution. In stems, *S aureus* was inhibited the most compared to the *B. cereus* and *L. monocytogenes.* Previous studies indicated the antibacterial effects of purslane extracts derived from roots (Ojah et al., 2021), and this is of interest, as plants grown in NFT system, the roots are borne in the nutrient solution, allowing the successful root harvesting if needed. Results revealed that leaf extracts had greater antibacterial activity on the clinical bacteria examined, compared to stem extracts (Table S1). Both microfungi examined revealed great inhibition in both leaf and stem extracts used, while the antibacterial and antifungal properties of purslane have been previously reported (Zhou et al., 2015). Chowdhary et al. (2013) have reported efficacy against bacteria, including *S. aureus*, *Bacillus cereus* and *Klebsiella pneumoniae*, as well as fungi such as *A. fumigatus* and *Neurospora crassa* of the chloroform and ethanol extracts of *Portulaca oleracea*.

Leaf and stem extracts from purslane grown in different Nr ratios revealed antioxidant activity, whereas previous studies demonstrated that purslane mature plants revealed higher total phenolics and antioxidant capacity than the immature plants (Uddin et al., 2012), indicating the greater antioxidant capacity could be evidenced after multiple harvestings. The antioxidant potential of extracts from various parts of

Table 5
Phenolic compounds quantification (mg/g extract) in hydroethanolic purslane extracts.

Tentative identification	Leaves				Stems			
	L_Nr0.01	L_Nr0.05	L_Nr0.10	L_Nr0.15	S_Nr0.01	S_Nr0.05	S_Nr0.10	S_Nr0.15
Isocitric acid	0.71 ± 0.04^{c}	1.66 ± 0.06^{a}	0.59 ± 0.02^{d}	1.34 ± 0.04^{b}	1.05 ± 0.04^{b}	0.843 ± 0.004^{d}	0.99 ± 0.003^{a}	1.07 ± 0.02^{c}
Citric acid	2.05 ± 0.01^{c}	2.28 ± 0.02^{a}	0.48 ± 0.01^{d}	2.1 ± 0.1^{b}	0.98 ± 0.02^{a}	0.6053 ± 0.0004^{d}	0.81 ± 0.03^{c}	0.92 ± 0.02^{b}
Hydroxytyrosol hexoside	3.41 ± 0.02^{b}	3.85 ± 0.1^{a}	2.3 ± 0.01^{d}	3.34 ± 0.02^{c}	0.84 ± 0.002^{c}	0.658 ± 0.005^{d}	1.27 ± 0.01^{a}	0.92 ± 0.02^{b}
Oleraceic C	0.28 ± 0.01^{d}	0.81 ± 0.03^{a}	0.402 ± 0.003^{c}	0.56 ± 0.02^{b}	0.18 ± 0.01^{b}	0.093 ± 0.001^{d}	0.27 ± 0.01^{a}	0.169 ± 0.002^{c}
GIAG	0.175 ± 0.004^{d}	0.66 ± 0.02^{a}	0.26 ± 0.01^{c}	0.45 ± 0.01^{b}	0.96 ± 0.02^{b}	0.37 ± 0.01^{d}	1.055 ± 0.004^{a}	0.527 ± 0.001^{c}
Unknown	nq	nq	nq	nq	nq	nq	nq	nq
Oleracein A	1.32 ± 0.01^{d}	7.61 ± 0.01^{a}	2.976 ± 0.002^{c}	5.36 ± 0.01^{b}	0.0855 ± 0.0003^{b}	0.0348 ± 0.0001^{d}	0.1961 ± 0.0006^{a}	0.052 ± 0.001^{c}
Oleracein C	0.0127 ± 0.0004^{d}	0.463 ± 0.002^{a}	0.199 ± 0.001^{c}	0.33 ± 0.01^{b}	0.009 ± 0.001^{b}	nq	0.013 ± 0.001^{a}	nq
TPC	7.8 ± 0.1^{c}	16.7 ± 0.1^{a}	6.95 ± 0.04^{d}	13.04 ± 0.01^{b}	3.1 ± 0.1^{c}	2.23 ± 0.01^{d}	3.55 ± 0.03^{a}	3.13 ± 0.03^{b}

Values in the same row followed by different Latin letters are significantly different ($p < 0.05$), according to Tukey's HSD test for leaves and stems. nq - not quantifiable.
Calibration curves used for quantification: Chlorogenic acid (y = 168823x – 161172, R^2 = 0.9999, LOD = 0.47 μg/mL; LOQ = 1.42 μg/mL, peaks 1 and 2); Hydroxityrosol (y = 124154x + 17393, R^2 = 0.9999, LOD = 1.22 μg/mL, LOQ = 3.68 μg/mL, peak 3) and *p*-Coumaric acid (y = 301950x + 6966.7, R^2 = 0.9999, LOD = 0.62 μg/mL; LOQ = 1.89 μg/mL, peaks 4, 5, 7 and 8).

purslane—predominantly the leaves—across diverse cultivation systems has been extensively investigated in the literature; however, these studies primarily evaluate antioxidant capacity through different chemical assays (Oliveira et al., 2009; Petropoulos et al., 2015, 2019; Santiago-Saenz et al., 2018). Therefore, more refined assays such as the in vitro assays used in the present study (OxHLIA and TBARS) are essential to enhance antioxidant properties and promote the valorization of plant matrices.

The antioxidant activity of purslane is related to the different plant components such as gallotannins, ω3 fatty acids, ascorbic acid, α-tocopherols, kaempferol, quercetin and apigenin, as reviewed by Zhou et al. (2015) and Rahimi et al. (2019). Given the higher levels of bioactive compounds, including tocopherols, oleracein derivatives and polyunsaturated fatty acids (PUFA), detected in the leaves, an elevated antioxidant capacity was anticipated, as demonstrated in Table S2. It has been demonstrated that lower IC50 values are indicative of greater antioxidant capacity, since a lower concentration of the extract is required to promote 50 % protection. The leaves exhibiting lower Nr ratios demonstrated a superior capacity to impede the oxidation pathways of both assays. Despite the Nr0.01 leaves not exhibiting the highest levels of tocopherols, PUFA or oleraceins (Tables 2, 3 and 5), they demonstrated a superior capacity to inhibit oxidative hemolysis (32 ± 1 μg/mL), exhibiting a capacity analogous to that of the control (trolox). This finding underscores their significant application potential. Conversely, the leaves Nr0.05 (higher oleracein content) exhibited superior performance in the lipid peroxidation inhibition assay (54 ± 1 μg/mL).

Neither the Nr ratios (Nr0.01-Nr0.15) nor the plant organs (leaves, stems) extracts revealed any cytotoxic activity in the tested tumor cell lines, or any hepatotoxic and anti-inflammatory effects in the examined extracts, being in accordance with previous reports in purslane after harvesting in different growing stages (Petropoulos et al., 2019).

3.5. Correlation of Nr ratios to individual parameters

Linear correlation coefficients were computed and detailed in Table S3, to illustrate the role of Nr levels in the nutrient solution for every single variable. The correlation coefficient (r) and p-values are reported as well. In leaves, Nr had a positive correlation with Proteins, Linoleic acid, Linolenic acid, Arachidic acid, Polyunsaturated fatty acids, β-tocopherol, Oxalic acid, Oleracein C and OxHLIA. However, it had a negative correlation with fiber content, Lauric acid, Tridecanoic acid, Palmitic acid, Palmitoleic acid, Stearic acid, Saturated fatty acids, Hydroxytyrosol hexoside and TBARS.

In stems, Nr had a positive correlation with Proteins, Energy, Linolenic acid, Arachidic acid, Polyunsaturated fatty acids, α-tocopherol, Fructose, Glucose, Hydroxytyrosol hexoside, TPC and TBARS, while features as Ash, Fiber, Lauric acid, Pentadecanoic acid, Heptadecanoic acid, Oleic acid, Monounsaturated fatty acids, β-tocopherol and Oleracein C were negatively correlated (Table S3).

4. Conclusions

The present study outcomes demonstrated new insights on *Portulaca oleracea* response to ammonium to total N ratio in hydroponics. The increased Nr ratios resulted in decreased crop yield but increased nutritive value for the final products. Therefore, the increased Nr levels resulted in increased centesimal composition, increased fatty acids profile, antibacterial and antifungal activity, in both leaves and stems of purslane. However, given that some of the phytochemicals associated with anti-inflammatory and cytotoxic properties were not identified in the present study, this substantiates the absence of these bioactivities at the maximum concentration tested (400 μg/mL) in purslane leaves and stems. The Nr ratio tailoring in the nutrient solution applied to purslane may trigger some health-promoting compounds and decrease the anti-nutrients level, thereby improving the total quality of the final product. The current insights delivered by this study, are particularly relevant for the Mediterranean agricultural sector, which is centered on small farms, as they provide growers with new cropping alternatives for producing superior quality products.

CRediT authorship contribution statement

Tânia C.S.P. Pires: Methodology, Investigation. **Filipa Mandim:** Methodology, Investigation. **Maria Inês Dias:** Writing – review & editing, Methodology. **Beatriz H. Paschoalinotto:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Antonios Chrysargyris:** Methodology, Investigation, Formal analysis, Data curation, Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision. **Mikel Añibarro-Ortega:** Methodology, Investigation. **Nikolaos Tzortzakis:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization, Writing – review & editing. **Lillian Barros:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Financial support has been provided by PRIMA (grant Number Prima2019–11, PRIMA/0009/2019, P2P/PRIMA/1218/0006, 01DH20006, Prima2019–12, STDF Valuefarm, 18–3–2021, TUBITAK-119N494, 301 / October 18th, 2020, PCI2020–112091) a programme supported by the European Union with co-funding by the Funding Agencies RIF – Cyprus. This work was also financially supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); and national funding by FCT, P.I., through the institutional scientific employment program contract for L. Barros (DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002) and M.I. Dias (DOI: 10.54499/CEECINST/00016/2018/CP1505/CT0004); as also for the doctoral scholarship of B.H.P (2023.02731.BD).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.107958.

Data availability

Data will be made available on request.

References

Alonso-Esteban, J.I., Pinela, J., Ćirić, A., Calhelha, R.C., Soković, M., Ferreira, I.C.F.R., Barros, L., Torija-Isasa, E., Sánchez-Mata, M. de C., 2022. Chemical composition and biological activities of whole and dehulled hemp (*Cannabis sativa* L.) seeds. Food Chem. 374, 1–9. https://doi.org/10.1016/j.foodchem.2021.131754.

Al-Quwaie, D.A., Allohibi, A., Aljadani, M., Alghamdi, A.M., Alharbi, A.A., Baty, R.S., Qahl, S.H., Saleh, O., Shakak, A.O., Alqahtani, F.S., Khalil, O.S.F., El-Saadony, M.T., Saad, A.M., 2023. Characterization of *Portulaca oleracea* whole plant: evaluating antioxidant, anticancer, antibacterial, and antiviral activities and application as quality enhancer in yogurt. Molecules 28. https://doi.org/10.3390/molecules28155859.

AOAC, 2016. Official methods of analysis of AOAC International. In: Horwitz, W., Latimer, G. (Eds.), Official Methods of Analysis of AOAC International. MD: AOAC International, Gaithersburg.

Atherton, H.R., Li, P., 2023. Hydroponic cultivation of medicinal plants—plant organs and hydroponic systems: techniques and trends. Horticulturae 9, 194–195. https://doi.org/10.3390/horticulturae9030349.

Bafort, F., Jijakli, M.H., 2024. Vertical farming of medicinal plants. Digit. Agric. 129–177. https://doi.org/10.1007/978-3-031-43548-5_5.

Barroso, T.L.C.T., de Barros Alexandre, J., da Cruz, E.P., Dias, A.R.G., Forster-Carneiro, T., Bastos, C.P., 2024. An updated on applications and future perspectives for the valorization of purslane (*Portulaca oleracea*): a comprehensive review and bibliometric analysis. Eur. Food Res. Technol. 250, 1285–1306. https://doi.org/10.1007/s00217-024-04494-z.

Bian, Z., Wang, Y., Zhang, X., Li, T., Grundy, S., Yang, Q., Cheng, R., 2020. A review of environment effects on nitrate accumulation in leafy vegetables grown in controlled environments. Foods 9, 732. https://doi.org/10.3390/foods9060732.

Bybordi, A., 2012. Effect of different ratios of nitrate and ammonium on photosynthesis, and fatty acid composition of canola under saline conditions. Int. J. Agric. Crop Sci. 4, 622–626.

Camalle, M., Standing, D., Jitan, M., Muhaisen, R., Bader, N., Bsoul, M., Ventura, Y., Soltabayeva, A., Sagi, M., 2020. Effect of salinity and nitrogen sources on the leaf quality, biomass, and metabolic responses of two ecotypes of *Portulaca oleracea*. Agronomy 10. https://doi.org/10.3390/agronomy10050656.

Carbajal, A., 2013. A Manual de Nutrição e Dietética; Departamento de Nutrição. Faculdade de Farmácia, Madrid, Espanha.

Cebani, S., Jimoh, M.O., Sogoni, A., Wilmot, C.M., Laubscher, C.P., 2024. Nutrients and phytochemical density in *Mesembryanthemum crystallinum* L. cultivated in growing media supplemented with dosages of nitrogen fertilizer. Saudi J. Biol. Sci. 31, 103876. https://doi.org/10.1016/j.sjbs.2023.103876.

Ceccanti, C., Landi, M., Incrocci, L., Pardossi, A., Venturi, F., Taglieri, I., Ferroni, G., Guidi, L., 2020. Comparison of three domestications and wild-harvested plants for nutraceutical properties and sensory profiles in five wild edible herbs: is domestication possible? Foods 9, 1065. https://doi.org/10.3390/foods9081065.

Chowdhary, C.V., Meruva, A., Naresh, K., Elumalai, R.K.A., 2013. A review on phytochemical and pharmacological profile of *Portulaca oleracea* Linn. (Purslane). Int. J. Res. Ayurveda Pharm. 4, 34–37. https://doi.org/10.7897/2277-4343.04119.

Chrysargyris, A., Hajisolomou, E., Xylia, P., Tzortzakis, N., 2023. Ammonium to total nitrogen ratio affects the purslane (*Portulaca oleracea* L.) growth, nutritional, and antioxidant status. Heliyon 9, e21644. https://doi.org/10.1016/j.heliyon.2023.e21644.

Chrysargyris, A., Tzortzakis, N., 2021. Tailor-made hydroponic recipes for the production of high quality medicinal and aromatic plants. Acta Hortic. 87–94. https://doi.org/10.17660/ActaHortic.2021.1321.11.

Chrysargyris, A., Tzortzakis, N., 2023. Optimising fertigation of hydroponically grown sowthistle (*Sonchus oleraceus* L.): the impact of the nitrogen source and supply concentration. Agric. Water Manag 289, 108528. https://doi.org/10.1016/j.agwat.2023.108528.

Chrysargyris, A., Xylia, P., Zengin, G., Tzortzakis, N., 2024. Purslane (*Portulaca oleracea* L.) growth, nutritional, and antioxidant status under different nitrogen levels in hydroponics. Horticulturae 10, 1007. https://doi.org/10.3390/horticulturae10091007.

Cosgrove, D.J., Jarvis, M.C., 2012. Comparative structure and biomechanics of plant primary and secondary cell walls. Front. Plant Sci. 3, 1–6. https://doi.org/10.3389/fpls.2012.00204.

D'Imperio, M., Durante, M., Gonnella, M., Renna, M., Montesano, F.F., Parente, A., Mita, G., Serio, F., 2022. Enhancing the nutritional value of *Portulaca oleracea* L. by using soilless agronomic biofortification with zinc. Food Res. Int. 155, 111057. https://doi.org/10.1016/j.foodres.2022.111057.

Eidi, A., Mortazavi, P., Moghadam, J.Z., Mardani, P.M., 2015. Hepatoprotective effects of *Portulaca oleracea* extract against CCl4-induced damage in rats. Pharm. Biol. 53, 1042–1051. https://doi.org/10.3109/13880209.2014.957783.

El-Sayed, M., Awad, S., Ibrahim, A., 2019. Impact of purslane (*Portulaca oleracea* L.) extract as antioxidant and antimicrobial agent on overall quality and shelf life of Greek-style yoghurt, 0–0 Egypt. J. Food Sci.. https://doi.org/10.21608/ejfs.2019.12089.1005.

FAO, 1994. Fats and oils in human nutrition. Report of a joint FAO/WHO expert consultation. FAO Food and nutrition paper 57. Food and Agriculture Organization of the United Nations, Rome.

Farag, M.A., Shakour, Z.T.A., 2019. Metabolomics driven analysis of 11 Portulaca leaf taxa as analysed via UPLC-ESI-MS/MS and chemometrics. Phytochemistry 161, 117–129. https://doi.org/10.1016/j.phytochem.2019.02.009.

Fernández-Poyatos, M.D.P., Llorent-Martínez, E.J., Ruiz-Medina, A., 2021. Phytochemical composition and antioxidant activity of *Portulaca oleracea*: influence of the steaming cooking process. Foods 10, 94. https://doi.org/10.3390/foods10010094.

Fontana, E., Hoeberechts, J., Nicola, S., Cros, V., Palmegiano, G.B., Peiretti, P.G., 2006. Nitrogen concentration and nitrate/ammonium ratio affect yield and change the oxalic acid concentration and fatty acid profile of purslane (*Portulaca oleracea* L.) grown in a soilless culture system. J. Sci. Food Agric. 86, 2417–2424. https://doi.org/10.1002/jsfa.2633.

Fukalova, T.F., Garcia-Martinez, M.D., Raigon, M.D., 2022. Nutritional composition, bioactive compounds, and volatiles profile characterization of two edible undervalued plants: *Portulaca oleracea* L. and *Porophyllum ruderale* (Jacq.) Cass. Plants 11, 1–22.

Ghorani, V., Saadat, S., Khazdair, M.R., Gholamnezhad, Z., El-Seedi, H., Boskabady, M. H., 2023. Phytochemical characteristics and anti-inflammatory, immunoregulatory, and antioxidant effects of *Portulaca oleracea* L.: a comprehensive review. Evid. Based Complement. Altern. Med. 2023. https://doi.org/10.1155/2023/2075444.

Gul, K., Singh, A.K., Jabeen, R., 2016. Nutraceuticals and functional foods: the foods for the future world. Crit. Rev. Food Sci. Nutr. https://doi.org/10.1080/10408398.2014.903384.

Hajisolomou, E., Neofytou, G., Petropoulos, S.A., Tzortzakis, N., 2024. The application of conventional and organic fertilizers during wild edible species cultivation: a case study of purslane and common sowthistle. Horticulturae 10, 1222. https://doi.org/10.3390/horticulturae10111222.

Hameed, M.K., Umar, W., Razzaq, A., Aziz, T., Maqsood, M.A., Wei, S., Niu, Q., Huang, D., Chang, L., 2022. Differential metabolic responses of lettuce grown in soil, substrate and hydroponic cultivation systems under NH_4+/NO_3− application. Metabolites 12, 444. https://doi.org/10.3390/metabo12050444.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I.S., White, P., 2011. Functions of Macronutrients, Marschner's Mineral Nutrition of Higher Plants, Third Edition. Elsevier Ltd. https://doi.org/10.1016/B978-0-12-384905-2.00006-6.

Hosseinzadeh, M.H., Ghalavand, A., Mashhadi-Akbar-Boojar, M., Modarres-Sanavy, S.A. M., Mokhtassi-Bidgoli, A., 2020. Increased medicinal contents of purslane by nitrogen and arbuscular mycorrhiza under drought stress. Commun. Soil Sci. Plant Anal. 51, 118–135. https://doi.org/10.1080/00103624.2019.1695828.

Hu, Q., Li, Y., Liu, C., Huang, L.-P., Zeng, L., Wang, S., Song, H., Peng, H., Huang, J., Chen, C., Yao, L.-H., 2018. Effects of polysaccharide from *Portulaca oleracea* L. on voltage-gated Na+ channel of INS-1 cells. Biomed. Pharm. 101, 572–578. https://doi.org/10.1016/j.biopha.2018.02.136.

Iranshahy, M., Javadi, B., Iranshahi, M., Jahanbakhsh, S.P., Mahyari, S., Hassani, F.V., Karimi, G., 2017. A review of traditional uses, phytochemistry and pharmacology of *Portulaca oleracea* L. J. Ethnopharmacol. 205, 158–172. https://doi.org/10.1016/j.jep.2017.05.004.

Jiao, Z., Wang, H., Wang, P., Sun, H., Yue, S., Xiang, L., 2014. Detection and quantification of cyclo-dopa amides in *Portulaca oleracea* L. by HPLC-DAD and HPLC-ESI-MS/MS. J. Chin. Pharm. Sci. 23, 533–542.

Khammar, A.A., Moghaddam, M., Asgharzade, A., Sourestani, M.M., 2021. Nutritive composition, growth, biochemical traits, essential oil content and compositions of *Salvia officinalis* L. grown in different nitrogen levels in soilless culture. J. Soil Sci. Plant Nutr. 21, 3320–3332. https://doi.org/10.1007/s42729-021-00608-8.

Kolega, S., Miras-Moreno, B., Buffagni, V., Lucini, L., Valentinuzzi, F., Maver, M., Mimmo, T., Trevisan, M., Pii, Y., Cesco, S., 2020. Nutraceutical profiles of two hydroponically grown sweet basil cultivars as affected by the composition of the nutrient solution and the inoculation with *Azospirillum brasilense*. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.596000.

Li, M., Ma, S., 2024. A review of healthy role of dietary fiber in modulating chronic diseases. Food Res. Int. 191, 114682. https://doi.org/10.1016/j.foodres.2024.114682.

Liberal, Â., Almeida, D., Fernandes, Â., Pereira, C., Ferreira, I.C.F.R., Vivar-Quintana, A. M., Barros, L., 2024. Nutritional, chemical, and antioxidant screening of selected varieties of lentils (*Lens culinaris* spp.) from organic and conventional agriculture. J. Sci. Food Agric. 104, 104–115. https://doi.org/10.1002/jsfa.12896.

Machado, R.M.A., Alves-Pereira, I., Lourenço, D., Ferreira, R.M.A., 2020. Effect of organic compost and inorganic nitrogen fertigation on spinach growth, phytochemical accumulation and antioxidant activity. Heliyon 6, e05085. https://doi.org/10.1016/j.heliyon.2020.e05085.

Maggini, R., Tzortzakis, N., Currey, C.J., 2022. Editorial: soilless culture for vegetative biomass production and specialized metabolites: medicinal, aromatic, and edible plants. Front. Plant Sci. 13. https://doi.org/10.3389/fpls.2022.887487.

Mandim, F., Petropoulos, S.A., Dias, M.I., Pinela, J., Kostic, M., Soković, M., Santos-Buelga, C., Ferreira, I.C.F.R., Barros, L., 2021. Seasonal variation in bioactive properties and phenolic composition of cardoon (*Cynara cardunculus* var. altilis) bracts. Food Chem. 336. https://doi.org/10.1016/j.foodchem.2020.127744.

Mihaylova, D., Vrancheva, R., Petkova, N., Ognyanov, M., Desseva, I., Ivanov, I., Popova, M., Popova, A., 2018. Carotenoids, tocopherols, organic acids, charbohydrate and mineral content in different medicinal plant extracts. Z. Naturforsch. C. 73, 439–448. https://doi.org/10.1515/znc-2018-0057.

Monteiro, C.A., Levy, R.B., Claro, R.M., Castro, I.R.R. de, Cannon, G., 2010. A new classification of foods based on the extent and purpose of their processing. Cad. Saude Publica 26, 2039–2049. https://doi.org/10.1590/s0102-311x2010001100005.

Montoya-García, C.O., García-Mateos, R., Becerra-Martínez, E., Toledo-Aguilar, R., Volke-Haller, V.H., Jesús Magdaleno-Villar, J., 2023. Bioactive compounds of purslane (*Portulaca oleracea* L.) according to the production system: a review. Sci. Hortic. (Amst.). 308. https://doi.org/10.1016/j.scienta.2022.111584.

Montoya-García, C.O., Volke-Haller, V.H., Trinidad-Santos, A., Villanueva-Verduzco, C., 2018. Change in the contents of fatty acids and antioxidant capacity of purslane in relation to fertilization. Sci. Hortic. (Amst.). 234, 152–159. https://doi.org/10.1016/j.scienta.2018.02.043.

Murteira, M., Turcios, A.E., Calado, R., Lillebø, A.I., Papenbrock, J., 2022. Relevance of nitrogen availability on the phytochemical properties of *Chenopodium quinoa* cultivated in marine hydroponics as a functional food. Sci. Hortic. 291, 110524. https://doi.org/10.1016/j.scienta.2021.110524.

Nemzer, B., Al-Taher, F., Abshiru, N., 2020. Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (*Portulaca oleracea* L.) genotypes. Food Chem. 320, 126621. https://doi.org/10.1016/j.foodchem.2020.126621.

Nkhumeleni, Z., Phoswa, W.N., Mokgalaboni, K., 2024. Purslane ameliorates inflammation and oxidative stress in diabetes mellitus: a systematic review. Int. J. Mol. Sci. 25, 12276. https://doi.org/10.3390/ijms252212276.

Ojah, E.O., Oladele, E.O., Chukwuemeka, P., 2021. Phytochemical and antibacterial properties of root extracts from *Portulaca oleracea* Linn. (Purslane) utilised in the

management of diseases in Nigeria. J. Med. Plants Econ. Dev. 5, 1–7. https://doi.org/10.4102/jomped.v5i1.103.
Oliveira, I., Valentão, P., Lopes, R., Andrade, P.B., Bento, A., Pereira, J.A., 2009. Phytochemical characterization and radical scavenging activity of *Portulaca oleraceae* L. leaves and stems. Microchem. J. 92, 129–134. https://doi.org/10.1016/j.microc.2009.02.006.
Palaniswamy, U.R., Bible, B.B., McAvoy, R.J., 2004. Oxalic acid concentrations in Purslane (*Portulaca oleraceae* L.) is altered by the stage of harvest and the nitrate to ammonium ratios in hydroponics. Sci. Hortic. 102, 267–275. https://doi.org/10.1016/j.scienta.2004.01.006.
Palaniswamy, U.R., McAvoy, R.J., Bible, B.B., 2000. Omega-3-fatty acid concentration in *Portulaca oleracea* is altered by nitrogen source in hydroponic solution. J. Am. Soc. Hortic. Sci. 125, 190–194. https://doi.org/10.21273/jashs.125.2.190.
Pereira, C., Barros, L., Carvalho, A.M., Ferreira, I.C.F.R., 2013. Use of UFLC-PDA for the analysis of organic acids in thirty-five species of food and medicinal plants. Food Anal. Methods 6, 1337–1344. https://doi.org/10.1007/s12161-012-9548-6.
Pereira, A.G., Cassani, L., Oludemi, T., Chamorro, F., Calhelha, R.C., Prieto, M.A., Barros, L., Simal-Gandara, J., Lucini, L., Garcia-Perez, P., 2023. Untargeted metabolomics and in vitro functional analysis unravel the intraspecific bioactive potential of flowers from underexplored *Camellia japonica* cultivars facing their industrial application. Ind. Crops Prod. 204, 117389. https://doi.org/10.1016/j.indcrop.2023.117389.
Petropoulos, S.A., Fernandes, Â., Dias, M.I., Vasilakoglou, I.B., Petrotos, K., Barros, L., Ferreira, I.C.F.R., 2019. Nutritional value, chemical composition and cytotoxic properties of common purslane (*Portulaca oleracea* L.) in relation to harvesting stage and plant part. Antioxidants 8, 1–15. https://doi.org/10.3390/antiox8080293.
Petropoulos, S.A., Fernandes, Â., Dias, M.I., Pereira, C., Calhelha, R.C., Ivanov, M., Sokovic, M.D., Ferreira, I.C.F.R., Barros, L., 2020. The effect of nitrogen fertigation and harvesting time on plant growth and chemical composition of *Centaurea raphanina* subsp. *mixta* (DC.) runemark. Molecules 25, 3175. https://doi.org/10.3390/molecules25143175.
Petropoulos, S., Karkanis, A., Fernandes, Â., Barros, L., Ferreira, I.C.F.R., Ntatsi, G., Petrotos, K., Lykas, C., Khah, E., 2015. Chemical composition and yield of six genotypes of common Purslane (*Portulaca oleracea* L.): an alternative source of omega-3 fatty acids. Plant Foods Hum. Nutr. 70, 420–426. https://doi.org/10.1007/s11130-015-0511-8.
Petropoulos, S.A., Karkanis, A., Martins, N., Ferreira, I.C.F.R., Thalassinos, G., Petropoulos, S.A., Antoniadis, V., Carrascosa, A., Pascual, J.A., Ros, M., Petropoulos, S.A., Alguacil, M., 2023. The response of Purslane (*Portulaca oleracea*) to soil-added Pb: is it suitable as a potential phytoremediation species? Trends Food Sci. Technol. 74, 69–84. https://doi.org/10.3390/toxics11020153.
Pires, E. de O., Pereira, E., Pereira, C., Dias, M.I., Calhelha, R.C., Ćirić, A., Soković, M., Hassemer, G., Garcia, C.C., Caleja, C., Barros, L., Ferreira, I.C.F.R., 2021. Chemical composition and bioactive characterisation of *Impatiens walleriana*. Molecules 26, 1–13. https://doi.org/10.3390/molecules26051347.
Polyzos, N., Paschoalinotto, B.H., Pires, T.C.S.P., Añibarro-Ortega, M., Calhelha, R., Ferreira, I.C.F.R., Dias, M.I., Barros, L., Petropoulos, S.A., 2024. The impact of deficit irrigation on the agronomic performance and chemical composition of *Scolymus hispanicus* L. Horticulturae 10, 1–18. https://doi.org/10.3390/horticulturae10050479.
Rahimi, V.B., Rakhshandeh, H., Raucci, F., Buono, B., Shirazinia, R., Kermani, A.S., Maione, F., Mascolo, N., Askari, V.R., 2019. Anti-inflammatory and anti-oxidant activity of *Portulaca oleracea* extract on LPS-induced rat lung injury. Molecules 24. https://doi.org/10.3390/molecules24010139.
Regulation (EU) No 1169/2011, 2011. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011. Off. J. Eur. Union 18–63.
Roriz, C.L., Barros, L., Carvalho, A.M., Ferreira, I.C.F.R., 2014. HPLC-profiles of tocopherols, sugars, and organic acids in three medicinal plants consumed as infusions. Int. J. Food Sci. 2014. https://doi.org/10.1155/2014/241481.
Saini, R.K., Keum, Y.-S., 2016. Tocopherols and tocotrienols in plants and their products: A review on methods of extraction, chromatographic separation, and detection. Food Res. Int. 82, 59–70. https://doi.org/10.1016/j.foodres.2016.01.025.
Santiago-Saenz, Y.O., Hernández-Fuentes, A.D., Monroy-Torres, R., Cariño-Cortés, R., Jiménez-Alvarado, R., 2018. Physicochemical, nutritional and antioxidant characterization of three vegetables (*Amaranthus hybridus* L., *Chenopodium berlandieri* L., *Portulaca oleracea* L.) as potential sources of phytochemicals and bioactive compounds. J. Food Meas. Charact. 12, 2855–2864. https://doi.org/10.1007/s11694-018-9900-7.
Sianipar, N.F., Muflikhati, Z., Mangindaan, D., Assidqi, K., 2024. Anticancer potential of tocopherols-containing plants and semi-synthetic tocopherols. Plants 13, 2994. https://doi.org/10.3390/plants13212994.
Silva, R., Carvalho, I.S., 2014. In vitro antioxidant activity, phenolic compounds and protective effect against DNA damage provided by leaves, stems and flowers of *Portulaca oleracea* (Purslane). NPC Nat. Prod. Commun. 9, 45–50.
Simopoulos, A.P., 2004. Omega-3 fatty acids and antioxidants in edible wild plants. Biol. Res. https://doi.org/10.4067/S0716-97602004000200013.
Sonneveld, C., Voogt, W., 2009. Plant nutrition of greenhouse crops, Plant Nutrition of Greenhouse Crops. Springer Netherlands. https://doi.org/10.1007/978-90-481-2532-6.
Subpiramaniyam, S., 2021. *Portulaca oleracea* L. for phytoremediation and biomonitoring in metal-contaminated environments. Chemosphere 280, 130784. https://doi.org/10.1016/j.chemosphere.2021.130784.
Sugier, D., Sugier, P., Jakubowicz-Gil, J., Gawlik-Dziki, U., Zając, A., Król, B., Chmiel, S., Kończak, M., Pięt, M., Paduch, R., 2022. Nitrogen fertilization and solvents as factors modifying the antioxidant and anticancer potential of *Arnica montana* L. flower head extracts. Plants 12, 142. https://doi.org/10.3390/plants12010142.
Szalai, G., Dai, N., Danin, A., Dudai, N., Barazani, O., 2010. Effect of nitrogen source in the fertilizing solution on nutritional quality of three members of the *Portulaca oleracea* aggregate. J. Sci. Food Agric. 90, 2039–2045. https://doi.org/10.1002/jsfa.4049.
Tzortzakis, N., Nicola, S., Savvas, D., Voogt, W., 2020. Editorial: soilless cultivation through an intensive crop production scheme. management strategies, challenges and future directions. Front. Plant Sci. 11, 10–12. https://doi.org/10.3389/fpls.2020.00363.
Tzortzakis, N., Pitsikoulaki, G., Stamatakis, A., Chrysargyris, A., 2022. Ammonium to total nitrogen ratio interactive effects with salinity application on *Solanum lycopersicum* growth, physiology, and fruit storage in a closed hydroponic system. Agronomy 12. https://doi.org/10.3390/agronomy12020386.
Uddin, M.K., Juraimi, A.S., Ali, M.E., Ismail, M.R., 2012. Evaluation of antioxidant properties and mineral composition of purslane (*Portulaca oleracea* L.) at different growth stages. Int. J. Mol. Sci. 13, 10257–10267. https://doi.org/10.3390/ijms130810257.
Uddin, M.K., Juraimi, A.S., Hossain, M.S., Nahar, M.A.U., Ali, M.E., Rahman, M.M., 2014. Purslane weed (*Portulaca oleracea*): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. Sci. World J. 2014. https://doi.org/10.1155/2014/951019.
Voynikov, Y., Nedialkov, P., Gevrenova, R., Zheleva-dimitrova, D., Balabanova, V., Dimitrov, I., 2021. UHPLC-orbitrap-MS tentative identification of 51 oleraceins (Cyclo-dopa amides) in *Portulaca oleracea* L. cluster analysis and MS2 filtering by mass difference. Plants 10, 25–29. https://doi.org/10.3390/plants10091921.
WHO, W.H.O., 2020. Healthy diet: Key facts. (⟨https://www.who.int/news-room/fact-sheets/detail/healthy-diet⟩).
Xiang, L., Xing, D., Wang, W., Wang, R., Ding, Y., Du, L., 2005. Alkaloids from *Portulaca oleracea* L. Phytochemistry 66, 2595–2601. https://doi.org/10.1016/j.phytochem.2005.08.011.
Yang, X., Cui, X., Zhao, L., Guo, D., Feng, L., Wei, S., Zhao, C., Huang, D., 2017. Exogenous glycine nitrogen enhances accumulation of glycosylated flavonoids and antioxidant activity in lettuce (*Lactuca sativa* L.). Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.02098.
Zhang, J., Lv, J., Dawuda, M.M., Xie, J., Yu, J., Li, J., Zhang, X., Tang, C., Wang, C., Gan, Y., 2019. Appropriate ammonium-nitrate ratio improves nutrient accumulation and fruit quality in pepper (*Capsicum annuum* L.). Agronomy 9. https://doi.org/10.3390/agronomy9110683.
Zhou, Y.-X., Xin, H.-L., Rahman, K., Wang, S.-J., Peng, C., Zhang, H., 2015. *Portulaca oleracea* L.: a review of phytochemistry and pharmacological effects. Biomed. Res. Int. 2015, 1–11. https://doi.org/10.1155/2015/925631.