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The Impact of Deficit Irrigation on the Agronomic Performance and Chemical Composition of *Scolymus hispanicus* L.

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Abstract: In the current study, the effects of drought stress on the growth and phytochemical profile of *Scolymus hispanicus* L. (a.k.a. golden thistle) were evaluated. Plants were treated with three irrigation regimes, e.g., plants that received only rainwater (Control; C), deficit irrigation (I1; 50% of field capacity (FC)), and full irrigation (I2; 100% of FC). The fresh weight of the rosette of leaves was not negatively impacted by deficit irrigation, whereas root development was severely restrained compared to control and I2 treatments. Drought stress conditions had a positive effect on the nutritional properties of the golden thistle since the treatments of control and deficit irrigation showed the highest content of macronutrients and energy. Oxalic acid was the richest organic acid, especially under the I1 regime. Similarly, α-tocopherol was the only identified vitamin E isoform, whose content was also doubled in I1 treatment. Raffinose, glucose, and sucrose were the most abundant free sugars in amounts that varied among the irrigation treatments, while the total and distinct free sugar content was the highest for the I1 treatment. The most abundant detected fatty acid compounds were α-linolenic acid, followed by palmitic and linoleic acid, with the highest amount being detected in C, I1, and I2 treatments, respectively. Flavonoids were the only class of polyphenols detected in golden thistle leaves, including mostly kaempferol and quercetin derivatives. The greatest antioxidant potency was shown for the control and I1 treatments (for OxHLIA and TBARS methods, respectively). The evaluated leaf samples recorded a varied antimicrobial effect for the different bacterial strains and fungi, whereas no cytotoxic, hepatotoxic, and anti-inflammatory effects against the tested cell lines were recorded. Finally, the mineral content of leaves was significantly affected by the irrigation regime, with Ca, Mg, Cu, and Zn being the highest for the I1 treatment, while the I2 treatment had the highest content of K, Fe, and Mn and the lowest Na content. In conclusion, deficit irrigation showed promising results since it improved the phytochemical content without compromising the fresh weight of leaves, and thus it could be suggested as a sustainable agronomic practice for producing high-added value products without significant constraints in growth development and yield parameters of golden thistle.

Keywords: drought stress; water management; wild edible plants; golden thistle; bioactive properties; phenolic compounds; mineral composition; organic acids; tocopherols

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1. Introduction

Climate change, along with the ever-increasing world population and the decreasing arable agricultural area due to land degradation, has put high pressure on modern agriculture, which has to secure food availability in a sustainable manner [1,2]. Considering that the Mediterranean climate is impacted by an increasing frequency of prolonged drought periods, water availability has become a major challenge for crop productivity [3–5] since water scarcity is highly associated with detrimental effects on plant productivity [6]. Limited water availability is further aggravated by the intensification of cropping systems, as well as by the lack of a sustainable approach regarding the management of irrigation water in the farming sector [7,8]. Therefore, there is an urgent need to transform the existing farming systems and shift towards eco-friendly practices that will ensure the sustainable and eco-friendly use of natural resources in the short and long term [9–11].

Deficit irrigation has emerged as an innovative agronomic practice to substitute irrigation regimes wherein farmers tend to irrigate beyond the crop requirements to achieve a high crop yield [12]. The main objective of this irrigation practice is to lessen the amounts of water supplied to the crop by subjecting crops to mild water stress without detrimental effects on crop yield, thus resulting in water saving and an increase in water use efficiency [4]. Deficit irrigation is classified into three different strategies depending on when and how water is applied to crops, i.e., regulated deficit irrigation (water reduction is implemented during specific, non-critical stages of the plant); sustained deficit irrigation (water reduction is applied as a percentage of crop evapotranspiration throughout the growing period); and partial root zone drying (only a part of the root is provided with water each time) [13]. Bearing in mind that each crop has varied water requirements throughout its growth cycle, it is crucial to identify those critical stages where plants are sensitive to drought stress and integrate deficit irrigation regimes in a sustainable water management strategy [14,15]. Apart from water-saving benefits, deficit irrigation has been suggested to facilitate the reduction of nutrient losses from the root zone, a lower incidence of diseases due to increased humidity, and a decrease in excessive vegetative vigor [16,17].

Several studies suggested the importance of the Mediterranean region as a hotspot of wild edible plants that could be valorized through their integration into small-scale farming systems [18–21]. Considering the severe impact of climatic change on conventional crop production, these underutilized species could be a promising solution due to their tolerance against biotic and abiotic stressors [22]. So far, various candidates of wild edible plants have been suggested, including *Sonchus oleraceus*, *Portulaca oleracea*, *Cichorium spinosum*, *Crithmum maritimum*, and *S. hispanicus*, among others [19,23–26]. However, despite the recent scientific efforts a significant lack of knowledge about the optimum cultivation practices of wild edible plants has been identified; therefore, further investigation is needed to provide cultivation protocols for the commercialization of these species.

Golden thistle (Asteraceae) is a biennial–perennial wild edible species that mainly can be found in uncultivated fields, sandy soils, and on roadsides [27]. The utilization of golden thistle dates back to the 11th century, while it was associated with the famine period as a vital food source [28,29]. Fresh rosettes and root barks are the mainly edible plant parts that are eaten raw, boiled, and fried in many traditional Mediterranean recipes [30,31]. Additionally, the increasing demand from consumers and the food industry for highly added value products, as well as from the pharmaceutical industries for functional ingredients (e.g., tocopherols, free sugars, n3-fatty acids, and bioactive compounds), has rekindled the interest for the exploitation of golden thistle due to its association with many medicinal properties [32–34]. According to a recent study by Paschoalinotto et al. [19], *S. hispanicus* is a rich source of P and K, dietary fiber, and carbohydrates, α-linolenic, palmitic, and linolenic acids, which constitute a balanced n-6 and n-3 fatty acids ratio, while numerous polyphenols were detected being associated with the antioxidant properties of the plant [19,35]. Moreover, many medicinal and pharmacological properties have been suggested for *S. hispanicus*, namely diuretic, depurative, digestive, choleretic, lithiuretic, antispasmodic, and spasmogenic activities [36–39]. Therefore, considering the current climate change and the

urgent need to introduce alternative/complementary crops to the farming sector, the goal of the present work was to assess the impact of different irrigation regimes on agronomic performance, nutritional value, chemical profile (tocopherols, organic acids, fatty acids, free sugars), and bioactive properties (phenolic compounds, antioxidant activity, cytotoxic effects) of *S. hispanicus* and provide useful feedback regarding the best agronomic practices guides that could facilitate the further commercial valorization of the species.

2. Materials and Methods

2.1. Experimental Conditions

The present study was performed at the experimental farm of the University of Thessaly in central Greece (39°37′18.6″ N, 22°22′55.1″ E) between January and August 2021. Seeds of *Scolymus hispanicus* L. (golden thistle; Geniki Fytotechniki S.A., Athens, Greece) were put in seed trays, and young seedlings (at the stage of 5 true leaves) were transplanted in the field on 22 January [19] (Figure 1). Plants were put at distances of 0.50 × 0.33 cm, while each treatment included 3 plots (n = 3) with 4 rows. The plot dimensions were 4.0 × 1.75 m plot (plant density = 6000 plants/ha), while each treatment included 126 plants. Soil properties are provided in the study of Chaski et al. [40].

Figure 1. Plants of *S. hispanicus* after crop establishment (left photo) and at full growth (right photo; photos are from the personal record of Spyridon A. Petropoulos).

After transplantation, plants received full irrigation (100% of field capacity) until crop establishment (15 February) and then three irrigation treatments were applied, i.e., rain-fed plants (Control), deficit irrigation (I1; 50% of field capacity (FC)), and full irrigation (I2; 100% of FC). During the cultivation period, irrigation was applied once or twice per week, based on the environmental conditions, via a drip irrigation system. The total amount of water that plants received for each treatment was 255 m^3/ha for rain-fed plants through precipitation, while I1 and I2 plants received an additional 984.8 m^3/ha and 1969.6 m^3/ha, respectively. Plants were also fertigated with a solution that contained 200 mg/L of N-P-K twice a month [19]. Weeds were controlled with hoeing, whereas pests and pathogens management was performed based on cultivation practices that are used for similar leafy vegetables of the Asteraceae family (e.g., chicory and endive). Harvest took place on 13 May with 15 plants being harvested from each plot. The aboveground parts of the plants were separated from the roots and then rosettes of leaves and roots were washed with distilled water and left to dry on absorbing paper. Morphological traits, namely the fresh weight of leaves/plant (g), the number of leaves/plant, the weight of roots/plant (g), and the dry matter of roots (%) were estimated from each irrigation treatment (45 plants for each treatment; 135 plants in total). The dry matter of roots was assessed by cutting freshly harvested roots into small pieces and putting them in an oven at 72 °C for approximately 72 h.

2.2. Nutritional Characterization

The nutritional characterization of the lyophilized and ground-to-powder samples of *S. hispanicus* were evaluated using the procedures of the Association of Official Agricultural Chemists (AOAC) [41]. For crude protein content, the macro-Kjeldahl method was applied and calculated as N × 6.25 (AOAC, 991.02). Total fat was estimated using the Soxhlet apparatus (AOAC, 989.05). Total dietary fiber was assessed using both enzymatic and gravimetric methods (AOAC, 993.19 and 991.42). The ash content was assessed by incinerating the samples at 550 ± 10 °C (AOAC, 935.42). Total carbohydrate content was determined by difference. Energy was evaluated based on the following equation: energy (kcal/100 g dw) = 4 × (g protein + g carbohydrates) + 2 × (total dietary fiber) + 9 × (g fat).

2.3. Organic Acids

The organic acid extraction was conducted based on the method by Barros et al. [42]. Briefly, 1 g of dry powder of *S. hispanicus* samples was extracted after stirring with 20 mL of meta-phosphoric acid and then filtrated with Whatman No. 4 paper and 0.2 μm nylon filters before the analysis of the samples. The analysis was conducted using a Shimadzu 20A series UFLC (Shimadzu Corporation) with separation being achieved with a reverse-phase C18 column. Elution was carried out with 3.6 mM sulphuric acid at a flow rate of 0.8 mL/min. Detection was carried out using a photo diode array (PDA), with preferred wavelengths of 215 nm and 245 nm for ascorbic acid. Compounds were quantified by comparing the area of their peaks recorded at 215 nm with respective calibration curves of commercial standards.

2.4. Free Sugars

The extraction of free sugars was carried out on the dry powder samples of *S. hispanicus* and conducted using high-performance liquid chromatography (HPLC; Knauer Smartline 2300 system) [42]. Free sugars were identified and quantified with Clarity 2.4 software (DataApex, Prague, Czech Republic) and after the comparison with respective commercial standards.

2.5. Tocopherols

Tocopherols were analyzed in the dry powder sample using an HPLC system (FP-2020, Jasco, Easton, PA, USA), following previously established methods [19]. The identification of compounds took place by comparing their characteristics with tocopherol standards, while for quantification, the internal standard (IS) method using tocol (Matreya, Pleasant Gap, PA, USA) as the internal standard was applied. Data processing was carried out using Clarity 2.4 software.

2.6. Fatty Acids

Fatty acid methyl esters (FAME) were prepared following previously established methods [19], while analysis took place using gas–liquid chromatography with flame ionization detection on a DANI model GC 1000 instrument. The identification and quantification of fatty acids were carried out using Clarity DataApex 4.0 Software (Prague, Czech Republic) by comparing retention times of compounds with commercial standards.

2.7. Mineral Composition

The mineral composition of the dry powder of *S. hispanicus* was determined following the respective AOAC protocol [41]. In summary, samples were digested using 10 mL of nitric acid for 30 min and then distilled water was added to the solution up to a final volume of 50 mL. Minerals were identified with absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA, USA).

2.8. Phenolic Compounds and Bioactive Properties

For the evaluation of the phenolic profile and bioactive properties, a hydroethanolic extract (80:20, *v*/*v*) was prepared from the lyophilized samples of *S. hispanicus* after maceration. Shortly, 1 g of lyophilized powder from each sample was extracted twice with 30 mL of solvent and stirred for 1 h, while the extract was filtered using Whatman No. 4 filter paper after each extraction. Subsequently, the combined extracts underwent evaporation to remove the ethanol with a rotary evaporator (Buchi, 3000 series, Flawil, Switzerland) and were stored under dry conditions [21]. The obtained extracts were used for the determination of phenolic compounds, antioxidant activities, antimicrobial properties, and hepatotoxic effects using the methods described in the following sections.

2.8.1. Phenolic Profile

The methodology for characterizing the phenolic compounds profile using HPLC-DADESI/MSn was previously outlined in previous works [21]. The lyophilized hydroethanolic extracts of *S. hispanicus* were dissolved in ethanol/water (20:80 *v*/*v*) to obtain a solution with a concentration of 10 mg/mL. The analysis was performed with a diode array detector (DAD; at 280 nm and 370 nm) connected to a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) and the Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). Quantification was achieved with calibration curves obtained from commercial standard compound: quercetin-3-*O*-glucoside (y = 34.843x − 160.173, R^2 = 0.9998, LOD = 0.21 μg/mL, LOQ = 0.71 μg/mL).

2.8.2. Antioxidant Activity

The antioxidant potential of *S. hispanicus* extracts was assessed through two cell-based assays conducted in vitro. The extracts of *S. hispanicus* were dissolved in water to obtain a solution with a concentration of 10 mg/mL. For the oxidative hemolysis inhibition assay (OxHLIA), sheep red blood cell (RBC) solution (2.8%, *v*/*v*) was mixed with 400 μL of extract solution (0.2–4 mg/mL) in phosphate-buffered saline (PBS) [21]. The lipid peroxidation inhibition was assessed via the thiobarbituric acid reactive substances (TBARS) assay, which measures malondialdehyde (MDA) [21]. The assays employed Trolox as a positive control.

2.8.3. Antimicrobial Properties

The antimicrobial efficacy was determined using the broth microdilution method outlined in previous works [21]. The pathogenic bacteria and fungi tested were food-borne bacterial strains (*Enterobacter cloacae* American Type Culture Collection (ATCC) 49741, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterocolitica* ATCC 13076, *Yersinia enterocolitica* ATCC 8610, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 19111, *Staphylococcus aureus* ATCC 11632), clinical bacterial isolates (*E. coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *P. aeruginosa*, *Enterococcus faecalis*, *L. monocytogenes*, and methicillin-resistant *S. aureus* (MRSA)) and two fungal strains (*Aspergillus brasiliensis* ATCC 16404 and *Aspergillus fumigatus* ATCC 204305).

2.8.4. Hepatotoxicity

Hepatotoxicity activity was evaluated with a non-tumor porcine liver primary culture (PLP2) after re-dissolving the hydroethanolic extracts in water. The sulforhodamine B assay was employed for determination [21].

2.9. Statistical Analysis

The experiment was laid out according to the Randomized Complete Blocks (RCB) design with three replications per treatment. The results were expressed as mean values and standard deviations (SD). The Shapiro–Wilk test was used to check the normal distribution of data and then one-way analysis of variance (ANOVA) was performed using Tukey's Honestly Significant Difference (HSD) test (p = 0.05) for the comparison of means. The

statistical analysis was performed with the aid of JMP v. 16.1 (SAS Institute Inc.; Cary, NC, USA).

3. Results and Discussion

3.1. Growth Parameters

The results regarding the growth parameters of *S. hispanicus* are presented in Table 1, where fluctuating trends were recorded for the tested irrigation treatments. Briefly, fully irrigated plants (I2) recorded the largest weight of leaves/plant (118.43 g), being significantly different only from the control treatment (91.13 g). The same trend was also observed for the number of leaves/plant where I2 treatment had the largest number of leaves (17.33), although this time this treatment differed only from deficit irrigation (I1) (13.56). Similarly, the weight of roots for the I2 and the control treatment (224.18 g and 211.1 g) was significantly higher than the I1 treatment (137.9 g), whereas the untreated plants recorded the largest dry matter content in roots (27.0%).

Table 1. The impact of irrigation regimes on growth parameters of *S. hispanicus* plants (mean ± SD).

Treatments	Growth Parameters			
	Weight of Leaves/Plant (g)	Number of Leaves/Plants	Weight of Roots/Plant (g)	Dry Matter of Roots (%)
Control	91.1 ± 22.7 b*	15.2 ± 3.3 ab	211.1 ± 115.5 a	27.0 ± 2.4 a
I1	111.9 ± 26.1 ab	13.6 ± 1.1 b	137.9 ± 44.9 b	16.1 ± 1.8 c
I2	118.4 ± 25.7 a	17.3 ± 3.3 a	224.2 ± 89.2 a	24.2 ± 1.3 b

* Means in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Tukey's test.

Similar values for the recorded growth parameters were measured by Paschoalinotto et al. [19] who studied the impact of different fertilization regimes on pot-grown *S. hispanicus* plants. In contrast, Molina et al. [43] who determined the yield of several wild vegetables recorded a lower weight of the golden thistle plant, a difference that could be due to genetic variability since the genotypes tested in various studies are based on wild harvested plants. Moreover, Papadimitriou et al. [27] recorded a higher number of leaves and leaf fresh weight per plant and lower root fresh weight per plant compared to our work, a finding which could be ascribed to the different cropping systems tested (e.g., hydroponic vs. soil cultivation). Calone et al. [44] also recorded a significant reduction in plant height and the number of shoots in the halophytic species *Salicornia europeae* grown under prolonged water stress, whereas *S. veneta* showed only a height reduction and *S. fruticose* was not impacted by water stress. Overall, our findings indicate that the implementation of deficit irrigation in *S. hispanicus* does not have detrimental effects on fresh yield, whereas rain-fed conditions are not a viable option since fresh yield was significantly reduced. However, the decision for the application of this practice depends on whether the plants will be cultivated as annual or as perennial, since the decrease in root growth recorded after applying deficit irrigation conditions in the first year after crop establishment may have a negative impact on yield in harvestings of successive years. On the other hand, plants grown under rain-fed conditions showed an increased weight of roots, probably as a means to overcome water shortage near the root zone, which could be a beneficial trait in the long-term if plants are cultivated as perennial.

3.2. Nutritional Characterization

The current findings in regards to the effect of different irrigation regimes on *S. hispanicus* nutritional profile are cited in Table 2. Total fat content and energy were the highest for the rain-fed plants (2.71 g/100 g dw and 252.98 kcal/100 g dw, respectively), while crude protein, ash, and total fiber content increased under deficit irrigation conditions (21.1, 20.4, and 43.5 g/100 g dw, respectively). Moreover, the carbohydrate content was

higher in rain-fed and fully irrigated plants (18.1 and 17.2 g/100 dw, respectively) compared to deficit irrigation (12.5 g/100 g dw).

Table 2. The effect of irrigation regimes on nutritional profile and energy content (kcal/100 g dw) of *S. hispanicus* plants (mean ± SD).

Nutritional Profile	Control	I1	I2
Total fat (g/100 g dw)	2.71 ± 0.03 a*	2.41 ± 0.03 b	2.3 ± 0.1 c
Crude protein (g/100 g dw)	17.72 ± 0.10 c	21.1 ± 0.1 a	18.4 ± 0.1 b
Ash (g/100 g dw)	18.8 ± 0.1 c	20.4± 0.1 a	19.6 ± 0.2 b
Total fiber dietary (g/100 g dw)	42.7 ± 0.2 b	43.5 ± 2.6 a	42.6 ± 1.1 b
Carbohydrates (g/100 g dw)	18.1 ± 0.1 a	12.5 ± 1.8 b	17.2 ± 1.0 a
Energy (kcal/100 g dw)	252.98 ± 0.27 a	243.4 ± 3.6 c	247.8 ± 1.8 b

* Means in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Tukey's test.

According to the work of García-Herrera et al. [45], who studied the nutritional profile of wild edible plants of the Asteraceae family of dietary interest, the reported values were in a similar range as in our work. In contrast, Paschoalinotto et al. [19] suggested a decreased content of total fat, crude protein, ash, and total fiber dietary compared to our study, whereas they detected an increased content of carbohydrates content and energy values. This discrepancy could be ascribed to the growing conditions and the agronomic techniques (pot-grown plants grown indoors vs. field conditions). Moreover, Petropoulos et al. [46,47] evaluated the impact of salinity and successive harvestings on the nutritional profile of another wild edible species namely *C. spinosum* and they reported significant differences among the treatments. Consequently, the results of our work and the literature indicate that common cultivation practices can be applied to wild edible plants such as *S. hispanicus* and enhance the nutritional properties of the edible plant parts, thus facilitating their commercial valorization.

3.3. Organic Acids, Free Sugars and Tocopherols Content

The chemical composition of organic acids, tocopherols, and free sugars are presented in Table 3. Regarding the organic acid composition, five compounds were identified namely quinic, oxalic, shikimic, ascorbic, and fumaric acid. Oxalic acid was the most prevalent compound in all the treatments (5.3 to 6.1 g/100 g dw) followed by quinic (4.3 to 5.9 g/100 g dw), shikimic (0.0018 to 0.007 g/100 g dw), and fumaric acid (0.0124 to 0.0588 g/100 g dw); ascorbic acid was detected in trace amounts. Deficit irrigation (I1) increased the oxalic and quinic acid (6.13 and 5.9 g/100 g dw, respectively) and total organic acids content (12.1 g/100 g dw), while rain feeding and I2 treatment had the lowest content of oxalic and quinic acid (5.3 and 4.3 g/100 g dw, respectively), and the rain-fed regime had the lowest amount of total organic acids (9.9 g/100 g dw). On the contrary, rain feeding increased shikimic acid (0.007 g/100 g dw) and fumaric acid (0.0588 g/100 g dw), whereas deficit irrigation resulted in the lowest contents (0.0018 g/100 g dw and 0.0124 g/100 g dw of shikimic and fumaric acid, respectively).

The main organic acids found in our work were also suggested in the study of Sánchez-Mata et al. [34], who reported oxalic acid as the richest organic acid, whereas they also identified citric and malic acid, which were not detected in our study, as well as low amounts of fumaric and ascorbic acid. Similar findings were suggested by Paschoalinotto et al. [19], who detected oxalic, quinic, and malic acid as the major compounds, while shikimic and fumaric acid were detected in lesser amounts. These differences with literature reports could be justified by the different agronomic practices followed in these studies compared to the present work (e.g., wild plants in the work of Sánchez-Mata et al. [34] and pot-grown plants in the case of Paschoalinotto et al. [19]). Moreover, Collado-González et al. [44], who evaluated the effect of water deficit on jujube fruit eating quality, reported a

positive response to moderate/severe water shortage in regard to organic acid composition by detecting an increased content of malic, oxalic, and citric acids.

Table 3. The effect of irrigation regimes regarding the organic acid, tocopherol, and free sugar composition of *S. hispanicus* plants (mean ± SD).

Organic Acids (g/100 g dw)	**Control**	**I1**	**I2**
Oxalic acid	5.55 ± 0.01 b*	6.134 ± 0.002 a	5.3228 ± 0.0001 c
Quinic acid	4.32 ± 0.06 c	5.9 ± 0.2 a	4.62 ± 0.08 b
Shikinic acid	0.007 ± 0.001 a	0.0018 ± 0.0001 c	0.0067 ± 0.0004 b
Ascorbic acid	tr	tr	tr
Fumaric acid	0.0588 ± 0.0001 a	0.0124 ± 0.0001 c	0.05217 ± 0.00002 b
Sum	9.94 ± 0.05 b	12.1 ± 0.2 a	10.01 ± 0.08 b
Tocopherols (g/100 g dw)			
α-Tocopherol	0.023 ± 0.001 b	0.049 ± 0.001 a	0.021 ± 0.001 c
Sum	0.023 ± 0.001 b	0.049 ± 0.001 a	0.021 ± 0.001 c
Free sugars(g/100 g dw)			
Fructose	5.61 ± 0.08 b	5.34 ± 0.05 c	7.65 ± 0.01 a
Glucose	10.5 ± 0.05 b	11.2 ± 0.4 a	9.2 ± 0.2 c
Sucrose	11.8 ± 0.2 b	16.02 ± 0.54 a	8.6 ± 0.4 c
Raffinose	14.8 ± 0.7 b	15.2 ± 0.4 a	14.55 ± 0.06 b
Sum	42.6 ± 0.4 b	47.8 ± 1.3 a	39.995 ± 0.219 c

* Means in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Tukey's test. Calibration curves for organic acids: oxalic acid ($y = 8 \times 10^6x + 331{,}789$, $R^2 = 0.9912$); quinic acid ($y = 692{,}575x + 11{,}551$; $R^2 = 0.9983$); shikinic acid ($y = 5 \times 10^7x + 567{,}119$, $R^2 = 0.9903$); ascorbic acid ($y = 5 \times 10^7x + 449{,}262$, $R^2 = 0.9813$); fumaric acid ($y = 9 \times 10^7x - 100{,}894$, $R^2 = 0.9986$).

Regarding tocopherol composition, α-tocopherol was the only vitamin E isoform identified, while deficit irrigation resulted in the highest α-tocopherol (0.49 g/100 g dw). Similar findings were recorded by Paschoalinotto et al. [19], who tested the same genetic material in a pot experiment and they detected only α-tocopherol in golden thistle leaves. On the other hand, Morales et al. [39] detected α-, β-, γ-, and traces of δ-tocopherol in wild golden thistle leaves, while Petropoulos et al. [33] identified only α- and β-tocopherol and Vardavas et al. [48] α- and γ-tocopherol. In our study, deficit irrigation resulted in an increased content of α-tocopherol, which indicates its important role in plant defense against stressors [49]. According to the work of Oh et al. [50], the implementation of multiple stress or single stress over 6 weeks did not affect α-tocopherol content in lettuce plants. Contrarily, Luoh et al. [51] suggested a varied response of various indigenous African vegetables to water deficit conditions in terms of α- and γ-tocopherol content. Therefore, it seems that the response of α-tocopherol content to irrigation water limitations depends on the species, as well as on agronomic practices (e.g., cropping system) and the severity of water stress implemented on plants.

Regarding the free sugars profile, four compounds were detected in all studied samples namely fructose, glucose, sucrose, and raffinose (Table 3). Sucrose and raffinose were the richest compounds followed by glucose and fructose. Moreover, the implementation of deficit irrigation resulted in the largest amounts for all the detected sugars (16.02, 15.2, and 11.2 g/100 g dw for sucrose, raffinose, and glucose, respectively), except for fructose, where the highest amount was identified in the I2 treatment (7.65 g/100 g dw). Paschoalinotto et al. [19] who evaluated the free sugar profile of pot-grown *S. hispanicus* plants in relation to fertilization regime reported a varied profile with glucose being the most abundant compound, while they also detected fructose, sucrose, and trehalose and no amounts of

raffinose. Moreover, in the same study, the authors suggested lower amounts of individual and total free sugars, thus suggesting that growing conditions may significantly affect their profile and content [19]. This finding is confirmed by other studies with wild edible species where agronomic practices namely fertilization, harvesting time, weed and pest management as well as environmental factors may have a significant impact on free sugars profile [46,47].

3.4. Fatty Acid Composition

The profile of fatty acids is cited in Table 4. The major compounds were α-linolenic acid (C22:0; 56.3 to 58.6%), followed by palmitic (C16:0; 19.3 to 19.9%) and linoleic (C18:2n6c; 12.5 to 14.2%). Moreover, polyunsaturated fatty acids (PUFA) were the most abundant compounds, while saturated (SFA) and monounsaturated fatty acids (MFA) were detected in lower amounts. As for the effect of irrigation regimes, the highest amount of α-linolenic (58.6%), palmitic (19.9%), and linoleic acid (14.2%) was recorded for the control (rain-fed plants), I1, and I2 treatments, respectively. Moreover, the control treatment recorded the highest amounts of PUFA (72.0%), and I1 and I2 treatments had the highest content of SFA and MUFA (24.6 and 4.5%, respectively). All the tested samples recorded health-beneficial fatty acid profiles, as expressed by the ratios of n6/n3 and PUFA/SFA, with values that were lower than 0.4 and higher than 0.45, respectively [52].

Table 4. The effect of irrigation regimes on the composition of fatty acids of *Scolymus hispanicus* plants (mean ± SD).

Fatty Acids	Control	I1	I2
C13:0	0.54 ± 0.02 c*	0.7 ± 0.03 a	0.56 ± 0.01 b
C14:0	0.68 ± 0.02 c	0.91 ± 0.01 b	1 ± 0.02 a
C14:1	0.18 ± 0.01 c	0.283 ± 0.001 a	0.201 ± 0.001 b
C15:0	0.123 ± 0.001 b	0.144 ± 0.001 a	0.112 ± 0.001 c
C15:1	0.165 ± 0.005 b	0.183 ± 0.008 a	0.148 ± 0.004 c
C16:0	19.5 ± 0.5 b	19.9 ± 0.4 a	19.3 ± 0.6 b
C16:1	2.23 ± 0.09 b	2.26 ± 0.1 b	2.39 ± 0.01 a
C17:0	0.31 ± 0.01 c	0.41 ± 0.01 b	0.52 ± 0.01 a
C18:0	1.86 ± 0.07 b	1.79 ± 0.07 c	1.95 ± 0.09 a
C18:1n9c	1.5 ± 0.02 c	1.55 ± 0.08 b	1.76 ± 0.07 a
C18:2n6c	12.5 ± 0.1 c	13.8 ± 0.6 b	14.2 ± 0.5 a
C18:3n6	0.41 ± 0.01 a	0.37 ± 0.01 b	0.404 ± 0.004 a
C18:3n3	58.6 ± 0.4 a	56.4 ± 0.4 b	56.26 ± 0.03 b
C22:0	0.49 ± 0.01 c	0.52 ± 0.01 a	0.501 ± 0.005 b
C23:0	0.32 ± 0.01 a	0.25 ± 0.001 b	0.236 ± 0.01 c
C24:0	0.59 ± 0.01 a	0.51 ± 0.02 b	0.446 ± 0.001 c
SFA	24.4 ± 0.6 b	24.6 ± 0.7 a	24.2 ± 0.7 c
MUFA	4.08 ± 0.13 c	4.3 ± 0.2 b	4.5 ± 0.1 a
PUFA	72.0 ± 0.5 a	70.6 ± 0.9 c	70.8 ± 0.5 b
n6/n3	0.22	0.25	0.26
PUFA/SFA	2.9	2.9	2.9

Fatty acids are expressed as relative percentages of each fatty acid. C13:0—Tridecanoic acid; C14:0—myristic acid; C14:1—tetradecanoic acid; C15:0—pentadecanoic acid; C15:1; C16:0—palmitic acid; C16:1—palmitoleic acid; C17:0—heptadecanoic acid; C18:0—stearic acid; C18:1n9c—oleic acid; C18:2n6c—linoleic acid; C18:3n6—gamma linolenic acid; C18:3n3—α-linolenic acid; C22:0—Behenic acid; C23:0—Tricosanoic acid; C24:0—Lignoceric acid. SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; dw—dry weight; nd—not detected. * Means in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Tukey's test.

Similar findings have been suggested by Morales et al. [38] and Paschoalinotto et al. [19], who also indicated that α-linolenic, linoleic, and palmitic acids were the most abundant fatty acids in *S. hispanicus* leaves, while they also highlighted the high nutritional value of the fatty acid profile. Contrarily, Vardavas et al. [35] suggested a different profile for fatty acids where MUFA (54.8%) was the major class followed by SFA (33.7%) and PUFA (11.4%), while the ratio of n6/n3 was 1.06. These contradictions could be mostly ascribed to differences in growing conditions and the genetic material studied, since both Morales et al. [38] and Vardavas et al. [35] assessed the chemical composition of plants collected in the wild. Moreover, increased amounts of unsaturated fatty acids are correlated with the adaptation strategies of plants, since they are involved with cell membrane fluidity, protecting against biotic and abiotic stressors [53]. Therefore, the severity of stress determines the levels of fatty acids, the double bond index, and the unsaturation level, while significant differences have been recorded between drought-tolerant and drought-sensitive species [54,55]. The slight differences in PUFA content among the studied treatments indicate the induction of different mechanisms for plant defense that trigger the production of bioactive compounds depending on the intensity and duration of water stress [50].

3.5. Mineral Status

The results regarding the mineral composition of *S. hispanicus* plants based on the different irrigation regimes are presented in Table 5. The application of deficit water stress did not have any significant impact on several mineral elements, namely potassium (K), iron (Fe), and manganese (Mn) since the highest content (51.2 Kg/Kg, 1809.4 Fe mg/Kg and 127.9 Mn mg/Kg) was recorded when the plants were fully irrigated. On the other hand, based on the current findings, the mineral contents, namely of calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn), were positively benefited in the case of the deficit irrigation, which recorded most of their content (12.9 Ca g/Kg, 4.9 Mg g/Kg, 11.4 Cu mg/Kg, and 44.02 Zn mg/Kg), whereas the rain-fed plans of golden thistle presented the lowest content (10 Ca g/Kg, 3.98 Mg g/Kg, 8.6 Cu mg/Kg, and 24.8 Zn mg/Kg), respectively. Other than that, the only exception recorded in the current study was for sodium (Na), where the control produced the highest content (7295 mg/Kg), and the fully irrigated plants had the lowest one (2989 mg/Kg).

Table 5. The effect of irrigation regimes on the mineral profile of *S. hispanicus* plants (mean ± SD).

	Control	I1	I2
Minerals			
[K]/(g/Kg)	39.2 ± 39.9 b	35.6 ± 0.3 c	51.2 ± 1.1 a
[Na]/(mg/Kg)	7295.6 ± 7168.7 a	7132.2 ± 271.9 a	2989.98 ± 73.49 b
[Ca]/(g/Kg)	10 ± 10.3 c	12.9 ± 0.3 a	12.6 ± 0.4 b
[Mg]/(g/Kg)	3.90 ± 3.91 c	4.9 ± 0.2 a	4.2 ± 0.2 b
[Fe]/(mg/Kg)	748.66 ± 752.02 c	1333.9 ± 47.4 b	1809.4 ± 46.1 a
[Mn]/(mg/Kg)	80.3 ± 78.4 c	115.95 ± 1.74 b	127.9 ± 3.8 a
[Cu]/(mg/Kg)	8.6 ± 8.3 c	11.4 ± 0.3 a	11.1 ± 0.3 b
[Zn]/(mg/Kg)	24.8 ± 24.2 c	44.02 ± 1.53 a	33.1 ± 0.9 b

Means in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Tukey's test.

The mineral profile of *S. hispanicus* plants was also evaluated by Paschoalinotto et al. [20] and García-Herrera et al. [45], who suggested varied amounts of macro and microminerals. For example, García-Herrera et al. [45] detected higher values of K, Ca, and Mg and lower values of Mn compared to our study in wild *S. hispanicus* plants collected from two sites for two years, while Paschoalinotto et al. [20] reported lower values of Fe

compared to our study in *S. hispanicus* plants that received different fertilization regimes. Papadimitriou et al. [27] also suggested the significant impact of salinity on the mineral status of *S. hispanicus* leaves, while Disciglio et al. [32] and Ghanimi et al. [56] highlighted golden thistle leaves as a rich source of P, Mg and Ca.

3.6. Phenolic Compound Profile

The details about phenolic compound identification and quantification are presented in Table 6. Based on the findings of this work, four compounds were detected in the studied samples, all of which belong to the flavonoids class. Kaempherol-O-hexurunoside (Peak 3) was the richest compound (2.12 to 3.0 mg/g extract) followed by quercetin-O-hexurunoside (Peak 1; 0.83 to 1.25 mg/g extract), kaempherol-O-deoxyhexosil-hexoside (Peak 2; 0.56 to 1.10 mg/g extract), and isorhamnetin-O-hexurunoside (Peak 4; 0.68 to 1.04 mg/g extract). Regarding the effect of the studied irrigation regimes, the I1 treatment resulted in the largest content of individual and total phenolic compounds, apart from isorhamnetin-O-hexurunoside, where the highest content was measured in the I2 treatment.

Table 6. Retention time (Rt), wavelength of the maximum absorption (λmax), deprotonated ion ($[M-H]^-$), main mass fragments (MS^2), and tentative identification and quantification (mg/g extract) of the phenolic compounds detected in *S. hispanicus* samples.

Peak	Rt	λmax	$[M-H]^-$	MS^2	Tentative Identification	Control	I1	I2
1	18.26	354	477	301 (100)	Quercetin-*O*-hexurunoside	1.255 ± 0.001 b*	2.14 ± 0.01 a	0.835 ± 0.002 c
2	21.07	328	593	285 (100)	Kaempherol-*O*-deoxyhexosil-hexoside	1.1063 ± 0.0002 b	1.19 ± 0.01 a	0.56 ± 0.001 c
3	22.13	347	461	285 (100)	Kaempherol-*O*-hexurunoside	3.025 ± 0.007 b	3.67 ± 0.04 a	2.121 ± 0.004 c
4	23.6	346	491	315 (100)	Isorhamnetin-*O*-hexurunoside	0.679 ± 0.006 c	0.683 ± 0.001 b	1.04 ± 0.004 a
					TPC	6.065 ± 0.002 b	7.67 ± 0.03 a	4.56 ± 0.01 c

TPC—Total Phenolic Compounds; calibration curve: Quercetin-3-*O*-glucoside (y = 34,843x − 160,173, R^2 = 0.9998), LOD and LOQ (ug/mL) = 17.01 and 51.54, respectively. * Means in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Tukey's test.

Petropoulos et al. [33] reported similar findings and suggested that flavonoids were the richest class of phenolic compounds (99.6% of total phenolic compounds) in *S. hispanicus* leaves. On the other hand, apart from kaempferol, quercetin, and isorhamnetin derivatives, they also detected luteonin and apigenin derivatives, the former being the most abundant polyphenol. Moreover, Gonçalves et al. [57] a balanced amount of phenolic acids and flavonoids in wild plants of *S. hispanicus* with 5-O-Caffeoylquinic acid and kaempferol-3-O-glucoside being the richest compounds, while the amount of total phenolic compounds was larger than that of our study [57]. Contrarily, Marmouzi et al. [28] suggested that phenolic acids were the richest class of phenolic compounds in all the studied plant parts of *S. hispanicus* (roots, leaves, stems, and flowers). These contradictory results in literature reports could be explained by the different growing and environmental conditions (wild vs. cultivated plants), the extraction method (different solvents and extraction conditions), and genetic variability, which may have an impact on phenolic compound biosynthesis and extraction yield [58,59].

Water deficit resulted in increased content of phenolic compounds, as indicated by the results for rain-fed and I1 treatment of our study. According to the literature, water deficit irrigation could be ascribed to an increase in secondary metabolites via the reallocation of the photosynthesis products as the development of the plant gradually slows down [60]. The increased content of phenolic compounds under drought conditions could be also considered as an adaptive mechanism of plants since phenolic compounds are non-enzymatic antioxidants that are able to chelate metal ions and eliminate reactive oxygen species (ROS), thus resulting in the alleviation of oxidative damage [61,62]. Rodrigues et al. [63], who studied the impact of growing conditions on antioxidant compounds (e.g., flavonoids) in white and red cultivars of Portuguese onions, reported that deficit irrigation treatment had a beneficial impact on the phenolic composition, especially the flavonoids in red onion, a finding which is in accordance with our study. The same trends have been reported by Guarise et al. [64], who found an increased total phenolic compound content by 25% when plants of *Sisymbrium officinale* were irrigated at 50% of the field capacity in comparison to the fully irrigated plants, while Schiattone et al. [65] suggested that limited water supply at 50% of the evapotranspiration increased the content of bioactive compounds in wild rocket leaves, namely total phenols and total carotenoids.

3.7. Bioactive Properties

3.7.1. Antioxidant Activity

The antioxidant activity of *S. hispanicus* plants was evaluated with two different methods (OxHLIA and TBARS) and a varied response was recorded (Table 7). In particular, for the OxHLIA method, the highest activity was obtained by the extracts of rain-fed plants, whereas I1 treatment extracts were the most effective for the TBARS assay. It is worth mentioning that, despite the recorded significant differences, the plant extracts were more efficient in inhibiting hemolysis since the differences between the treatments were smaller than the ones achieved by the TBARS assay. However, for both methods, the observed IC_{50} values were higher than Trolox, which was the positive control.

Table 7. Antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and hepatotoxic activities of the extracts of *S. hispanicus* plants (mean ± SD).

	Control		**I1**		**I2**	
Antioxidant activity (IC_{50} values ug/mL) [A]						
OxHLIA **Δt = 60**	184 ± 9 c*		204 ± 5 b		210 ± 6 a	
TBARS inhibition	501 ± 19 a		201 ± 12 c		432 ± 9 b	
Antimicrobial activity (mg/mL) [B] Food bacteria	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria						
Enterobacter Cloacae	>10	>10	10	>10	10	>10
Escherichia coli	10	>10	10	>10	10	>10
Pseudomonas aeruginosa	10	>10	10	>10	10	>10
Salmonella enterica	10	>10	5	>10	10	>10
Yersinia enterocolitica	>10	>10	10	>10	10	>10
Gram-positive bacteria						
Bacillus cereus	10	>10	>10	>10	>10	>10
Listeria monocytogenes	5	>10	5	>10	10	>10
Staphylococcus aureus	5	>10	5	>10	5	>10

Table 7. *Cont.*

	Control		I1		I2	
Clinical bacteria [B]	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria	10	>10	10	>10	10	>10
Escherichia coli	>10	>10	>10	>10	10	>10
Klebsiella pneumoniae	>10	>10	>10	>10	10	>10
Morganella morganii	>10	>10	>10	>10	>10	>10
Proteus mirabilis	10	>10	10	>10	10	>10
Pseudomonas aeruginosa	10	>10	>10	>10	>10	>10
Gram-positive bacteria						
Enterococcus faecalis	5	>10	5	>10	10	>10
Listeria monocytogenes	5	>10	5	>10	5	>10
MRSA	10	>10	10	>10	10	>10
Antifungal activity (mg/mL) [B]	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	>10	>10	>10	>10	>10	>10
Aspergillus fumigatus	10	>10	10	>10	10	>10
Anti-inflammatory activity (IC_{50} values μg/mL) [C]						
RAW 264.7	>400		>400		>400	
Cytotoxicity Activity (GI_{50} values μg/mL) [D]						
AGS	>400		>400		>400	
CaCo2	>400		>400		>400	
MCF7	>400		>400		>400	
NCI-H460	>400		>400		>400	
Hepatotoxicity (GI50 values μg/mL) [D]						
PLP2	>400		>400		383 ± 3 a	

[A] Trolox IC50 values: 5.8 ± 0.6 μg/mL (TBARS), 21.5 ± 0.2 μg/mL (OxHLIA 60 min); [B] Food bacteria—Streptomicin, 1 mg/mL (*E. Cloacae*, *S. enterica*, *Y. enterocolitica*, *B. cereus*, *L. monocytogenes* and *S. aureus*—MIC/MBC 0.007; *E. coli*—MIC/MBC 0.01; *P. aeruginosa*—MIC/MBC 0.06), Methicilin, 1 mg/mL (*S. aureus*—MIC/MIB 0.007), Ampicillin, 10 mg/mL (*E. Cloacae*, *E. coli*, *S. enterica*, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus*—MIC/MBC 0.15; *P. aeruginosa*—MIC/MBC 0.63); Clinical bacteria—Ampicillin, 10 mg/mL (*E. coli*, *P. mirabilis*, *E. faecalis*, *L. monocytogenes*, MRSA MIC/MBC—<0.15; *K. pneumoniae*, *M. morganii*, *P. aeruginosa* MIC/MBC—>10); Imipenem, 1 mg/mL (*E. coli*, *K. pneumoniae*, *M. morganii*, *P. mirabilis*, *L. monocytogenes* MIC/MBC—<0.0078; *P. aeruginosa* MIC/MBC—0.5/1); Vancomycin, 1 mg/mL (*E. faecalis* MIC/MBC—<0.0078 and MRSA MIC/MBC—0.25/0.5; Antifungal activity—Ketoconazole (*A. brasiliensis* MIC/MFC 0.06/0.125, *A. fumigatus* MIC/MFC 0.5/1); MIC—Minimum Inhibitory Concentration, MBC—Minimum Bactericidal Concentration and MFC—Minimum Fungicidal Concentration; [C] Dexamethasone IC_{50} value: 6.3 ± 0.4 μg/mL; [D] Ellipticine GI_{50} values: 1.23 ± 0.03 μg/mL (AGS), 1.21 ± 0.02 μg/mL (CaCo2), 1.01 ± 0.01 μg/mL (NCI-H460), 1.02 ± 0.02 μg/mL (MCF-7) and 1.4 ± 0.1 μg/mL (PLP2). * Mean values and standard deviations in the same column followed by different Latin letters are significantly different at $p < 0.05$ according to Tukey's test.

The findings of this study are in accordance with the work of Morales et al. [39] and Ozel-Tasci and Gulec [66], who also reported a varied response to different assays for the antioxidant activity of *S. hispanicus* plants that could be due to different compounds involved in the scavenging of radicals for the different methods tested. Similarly, Gonçalves et al. [57] evaluated the phytochemical properties of wild Mediterranean edible plants and they also reported a varied response for the antioxidant activity of golden thistle for different extraction methods. Drought stress conditions could have severe effects on plant physiology that lead to an increase of reactive oxygen species and consequently trigger the defense mechanisms of plants and induce the biosynthesis of enzymatic and non-enzymatic antioxidants [67]. In this context, our results indicated that drought conditions induce the antioxidant mechanisms of *S. hispanicus* plants through the biosynthesis of

bioactive compounds (e.g., α-tocopherol and polyphenols) that contribute to the observed antioxidant properties. According to the literature, phenolic compound content is strongly associated with antioxidant activity, although the ability to scavenge radicals depends on the species and its phytochemical profile [68,69]. Similarly to our study, Sarker and Oba [70] indicated that increasing the intensity of drought stress increased the content of non-enzymatic antioxidants which contributed to the overall antioxidant capacity of *Amaranthus tricolor* cultivars. Moreover, Puente-Garza et al. [71] suggested that *Agave salmiana* plantlets subjected to in vitro drought stress conditions presented increased antioxidant activity due to higher saponins content, whereas the flavonol content decreased and total phenolic compound content remained unaffected.

3.7.2. Antimicrobial Properties

The antibacterial properties of the hydroethanolic extracts of *S. hispanicus* are cited in Table 7. Our findings suggest that tested extracts resulted in varied efficacy in inhibiting the growth of selected bacteria depending on the studied bacterial strain and the irrigation treatment. The highest inhibitory activity for the extracts from rain-fed plants and I1 treatment was recorded only against Gram+ bacteria, namely *Listeria monocytogenes* (food and clinical strains), *Staphylococcus aureus*, and *Enterococcus faecalis*, while I1 extracts were also efficient in inhibiting the Gram- bacterium *Salmonella enterica*. The extracts of I2 treatment were efficient in inhibiting only *Listeria monocytogenes* (clinical strain) and *Staphylococcus aureus*. However, all the studied extracts recorded higher MIC and MBC values than positive controls suggesting lower efficacy against the studied bacteria. For the antifungal properties, none of the tested extracts showed significant inhibitory and fungicidal activity (Table 6).

According to Petropoulos et al. [33], *S. hispanicus* extracts showed significant inhibitory activities against the bacteria *Bacillus cereus*, *Salmonella typhimurium*, *Enterobacte cloacae*, and *Escherichia coli*, as well as against the fungi *Aspergillus ochraceus* with MIC, MBC, and MFC values similar or better than the positive controls. Moreover, Marmouzi et al. [28] reported that the golden thistle leaves recorded significant activity against *Salmonella enterica* and *Pseudomonas aeruginosa*, while Aboukhalaf et al. [72] suggested that the stem and leaves of golden thistle were effective against *E. coli* and *Cryptococcus neoformans*. Polyzos et al. [21] also reported a varied response of hydroethanolic and aqueous extracts of *Cichorium spinosum* against various bacterial strains and fungi, indicating the used solvent may affect the profile of the extracted phytochemicals and consequently the bioactive properties of the obtained extracts. This finding along with the varied growing conditions and the genetic background could justify the differences between the literature reports regarding the antimicrobial effects of *S. hispanicus* leaves.

3.7.3. Hepatotoxicity

The tested extracts did not record any cytotoxic, hepatotoxic, or anti-inflammatory activity with GI_{50} values larger than 400 μg/mL (Table 7). Similarly to our work, Polyzos et al. [21] did not report any cytotoxic effects in tumor and non-tumor cells for the wild edible species *C. spinosum* and *Portulaca oleracea,* respectively. In contrast, Ozel-Tasci et al. [73] suggested significant cytotoxic activity against Caco-2 cell lines for the hydromethanolic extracts from different plant parts of *S. hispanicus*, while Petropoulos et al. [74] and Mikropoulou et al. [75] a differential cytotoxic effect against various cancer cell lines for different wild edible species and different extraction protocols.

4. Conclusions

The results of this work indicate the potential of growing golden thistle under deficit irrigation conditions without compromising yield parameters, while at the same time, key quality features and bioactive properties were significantly improved and water saving by 50% was achieved. In conclusion, our findings highlight the high nutritional value and rich phytochemical properties of golden thistle plants under deficit irrigation conditions

and also offer significant knowledge regarding the agronomic practices that farmers have to follow in the years to come to secure food availability and sustainable management of natural resources. However, more research is required to fine-tune the agronomic practices that will support the commercialization of golden thistle plants in the existing small-scale commercially employed farming systems.

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