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Fertilization of Pot-Grown *Cichorium spinosum* L.: How It Can Affect Plant Growth, Chemical Profile, and Bioactivities of Edible Parts?

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Abstract: *Cichorium spinosum* L. is a perennial wild edible plant that is usually found near the coasts of the Mediterranean basin. In the present study, we evaluated the effect of the fertilization regime on the growth and chemical profile of pot-grown *C. spinosum* plants, as well as the effect of extraction protocol (aqueous and hydroethanolic extracts on bioactive properties). For this purpose, plants were fertilized via a nutrient solution that differed in the amounts (mg/L) of N:P:K, e.g., 100:100:100 (C111), 200:100:100 (C211), 200:200:200 (C222), 300:100:100 (C311), 300:200:200 (C322), and 300:300:300 (C333) mg/L of N:P:K, as well as a control treatment with no fertilizer added (C0). The fertilization regime had a beneficial effect on the growth parameters of spiny chicory, while it improved its nutritional value, as indicated by the polyunsaturated (PUFA)/saturated (SFA) ratio being higher than 0.45 and the omega-6 (n-6)/omega-3 (n-3) ratio being lower than 4.0. Seven phenolic compounds were detected, including two phenolic acids and five flavonoids, while a varied composition was recorded depending on the fertilization regime and the extraction protocol. In regards to the studied bioactive parameters, antioxidant activity was significantly affected by the applied fertilizers and the extraction protocol, while there was no significant effect on the cytotoxicity, hepatotoxicity, and anti-inflammatory activity. The antimicrobial properties of *C. spinosum* showed varying trends depending on the bacterial strain, the fertilization regime, and the extraction protocol, whereas we recorded the extracts' weak antifungal activity against the studied fungi. In conclusion, even though the fertilization of *C. spinosum* plants had beneficial effects on growth and nutritional value, a significant effect of the extraction protocol on the chemical profile and bioactivities of the edible leaves was also recorded, indicating the application of tailor-made fertilization regimes combined with the most suitable extraction method for the achievement of high-quality final products.

Keywords: spiny chicory; wild edible plants; chemical fertilizers; healthy food; bioactive compounds; phenolic compounds; antimicrobial properties

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1. Introduction

The Mediterranean Basin is an abundant hotspot of native edible plants that have been used since ancient times for different culinary and medicinal purposes [1]. They have been mainly associated with the "famine" period when the inhabitants of rural areas used to collect them from the wild due to scarcity of food; hence they are termed "famine

food" [2]. Wild edible plants are usually used as leafy greens and they are included in many traditional dishes and recipes of the highly appreciated "Mediterranean diet" [3,4]; however, the ever-growing marketing trends for healthy and functional foods have renewed the interest in these species due to their rich phytochemical profile and interesting bioactive properties [5,6]. *Cichorium spinosum* is a wild edible green of the Mediterranean basin, also known as "stamnagkathi", which abounds in the coastal areas of this region [7]. "Stamnagkathi" has a perennial growth with a woody and spiny elongated inflorescence, while its leaves form a ground rosette [8,9]. Spiny chicory plants are mainly handpicked in the wild for consumption in fresh or cooked dishes at growth stages depending on the environmental conditions and consumer requirements, whereas the harvest of cultivated plants can take place several times during each cultivation period on the condition that they reach the marketable size [10].

Soil fertilization has been considered a common cultivation practice implemented by farmers to increase the yield production of crops; hence increasing farmers' income but also sustaining food production due to the increasing human population [11]. Nitrogen (N) is an essential primary macronutrient whose deficiency in cropping systems could have serious impacts on plant growth and development and subsequently on crop performance [12]; however, the irrational application of N could have severe effects on the quality of the product, the economy and the environment as well [13]. In particular, the application of N fertilizers in amounts that exceed crop demands could provoke nitrate accumulation in the soil and decrease N use efficiency by the crop, while at the same time leaching of N in excessive amounts could contaminate the aquifer [14,15]. Considering these side effects of irrational fertilization use in the context of the ongoing climate crisis and the increasing land degradation due to anthropogenic activities, it is of major importance to shift to innovative and sustainable farming practices with an eco-friendly environmental footprint.

Therefore, wild edible plants could be a promising cropping alternative, especially in low-quality soils where conventional species cannot grow or their yield could be severely affected, with low input requirements and a sustainable footprint [2,16]. According to Papadimitriou et al. [17], who studied the effect of the N:P:K ratio and the salinity level of nutrient solution on the growth and yield of golden thistle (*Scolymus hispanicus*) in a hydroponic system, it was suggested that wild edible golden thistle could be cultivated in such systems at various seasons of the year using reduced nutrient inputs. The chemical profile and nutritional value of *C. spinosum* have already been reported in the literature [18,19], while several studies have highlighted the rich nutritional profile of spiny chicory due to the high content of tocopherols, total phenols, omega-3 fatty acids, ascorbic acid, and beta carotene [10,20,21]. Common cultivation practices such as the sowing date or the stage of harvest may also have a significant effect on the chemical profile and mineral composition of wild edible plants and subsequently the quality of the edible product [22,23]. Apart from growing conditions and cultivation practices, the nutrient solution applied to wild edible plants could also have significant effects on morphological traits and the overall growth of the plant as well as on its chemical profile and bioactivities [24–26]. Likewise, Kaymak et al. [27], who evaluated the effect of the form of applied nitrogen on the growth, crop performance, and accumulation of nitrates of cultivated purslane (*Portulaca oleracea* L.), reported that different nitrogen forms significantly affected the growth and the morphological traits of the plant; especially the ammonium nitrate treatment which resulted in the highest yield and root length, whereas ammonium sulfate and calcium ammonium nitrate treatments gave the highest plant height. Moreover, the nitrogen form provided to the plants via fertigation had a remarkable effect on the chemical profile and bioactivities of *C. spinosum*, with NO_3-N (100:0) resulting in the highest amounts of tocopherols and organic acids, while NO_3-N:NH_4-N in a ratio of 3:1 resulted in the highest phenolic compounds content [18]. Considering the lack of reports in the literature regarding the optimum agronomic practices for the cultivation of wild edible plants, the main goal of the current study was to determine the effect of the nutrient solution composition on the growth and morphological parameters and the chemical composition and bioactivities

of *C. spinosum* plants. The results of the study could provide useful input regarding the exploitation of the species as complementary/alternative and help towards its integration in small-scale and sustainable farming systems.

2. Materials and Methods

2.1. Plant Material, Experimental Treatments, and Growing Conditions

The experiment was performed at the unheated glasshouse of the University of Thessaly in Velestino, Greece. *Cichorium spinosum* seeds (Geniki Fytotechniki, S.A., Greece) were sown in seed trays containing peat, and seedlings (3–4 true leaves) were transferred in 2 L plastic pots filled with a mixture of peat (Klassman-Deilmann KTS2, Geeste, Germany) and perlite (1:1, *v*/*v*) on March 2021 [28]. The experimental treatments included seven fertilization regimes which varied in the N:P:K ratio in the nutrient solution. Stock solutions were prepared with Atlas 20-20-20 (4.8% ammonium N, 5.0% nitric N, 10.2% ureic N; 20% P_2O_5; 20% K_2O)+ TE (trace elements) fertilizer (Gavriel; S.A., Volos, Greece) for the preparation of 100:100:100 ppm (C111), 200:200:200 ppm (C222), and 300:300:300 ppm (C333), while for the rest of the solutions (200:100:100 ppm (C211), 300:100:100 ppm (C311), 300:200:200 ppm (C322)) the extra amount of nitrogen was achieved with the addition of ammonium nitrate fertilizer (34.5% of N; Gavriel; S.A., Volos, Greece). The control treatment included tap water with no fertilizers added (C0). The application of treatments was performed manually once or twice per week via the above-mentioned solutions, while each treatment included fifteen plants (n = 15) with one plant per pot and 105 plants in total. The total amount of nutrient solution for all the treatments was 1.05 L, and the experiment layout was arranged according to a completely randomized design (CRD) [28]. Chlorophyll content (expressed as SPAD values) of leaves was recorded before harvesting (26th of April), while after harvest the following parameters were measured: leaves/plant (g), dry matter content of leaves (%), leaf area (cm^2), and specific leaf area (m^2/kg) [28]. A portion of fresh leaves was used for the determination of dry weight, while the rest were stored in a freezer until lyophilization (Sublimator model EKS, Christian Zirbus Co., Germany) and further storage in a deep freezer (−80 °C) in the form of fine powder for all the chemical analyses described in the following sections [28].

2.2. Nutritional Profile

The nutritional profiles (crude protein, total fat, total dietary fiber, carbohydrates, and ash) were evaluated in the dry powder of C. spinosum through the AOAC procedures [29]. Briefly, the macro-Kjeldahl method was employed to determine the crude protein content (N × 6.25); the Soxhlet apparatus was used to estimate crude fat by extracting a known weight of the samples with petroleum ether; the total dietary fiber assay was determined through a combination of enzymatic and gravimetric methods; while the content of ash was determined by incineration of the samples at 550 ± 10 °C. Total carbohydrates and total energy were assessed, respectively, by difference and based on the equation: energy (kcal/100 g dw (dw)) = 4 × (g protein + g carbohydrates) + 2 × (total dietary fiber) + 9 × (g fat).

2.3. Organic Acids

Organic acids were assessed in the dry powder of C. spinosum, based on a previously described procedure and optimized by Pereira et al. [30]. The analysis was performed using a Shimadzu 20A series UFLC and detection was performed in a PDA, using 215 nm as preferred wavelengths. The quantification of the detected organic acids found was accomplished by comparison of the peaks' area with calibration curves created from commercial standards of each compound. The results are presented in g per 100 g of dw.

2.4. Tocopherols

The extraction of tocopherols, performed in the dry powder of the plant (*C. spinosum*), and the chromatographic characterization were performed based on a previously described procedure [31], using an HPLC methodology coupled to a fluorescence detector (Knauer,

Smartline system 1000, Berlin, Germany), and the identification of the compounds was performed by comparison with authentic standards. The response to the fluorescence signal of each standard allowed the identification of the three isoforms of this vitamin, and the exact quantities were determined using the internal standard method (tocol), as well as calibration curves obtained using the commercial standards of each compound (α-tocopherol ($y = 1.295x$, $R^2 = 0.991$; LOD = 18.06 ng/mL; LOQ = 60.20 ng/mL); β-tocopherol ($y = 0.396x$, $R^2 = 0.992$; LOD = 25.82 ng/mL, LOQ = 86.07 ng/mL); γ-tocopherol ($y = 0.567x$; $R^2 = 0.991$; LOD = 14.79 ng/mL, LOQ = 49.32 ng/mL)). The results are presented in mg per 100 g of dry weight.

2.5. Free Sugars

The composition of free sugars was performed by high-performance liquid chromatography (HPLC, Knauer Smartline 2300, Knauer, Berlin, Germany), coupled with a refractive index detector (RI detector, Knauer Smartline 2300, Knauer, Berlin, Germany) [32]. The extraction was performed in the dry powder samples of *C. spinosum*. The identification and quantification of free sugars were carried out with the use of Clarity 2.4 software (DataApex, Prague, Czech Republic), as well as by comparison with commercial standards, namely D (−)-fructose, D (+)-glucose, D (+)-raffinose pentahydrate, D (+)-sucrose, and D (+)-trehalose (Sigma-Aldrich, St. Louis, MO, USA).

2.6. Fatty Acids

Fatty acid methyl esters (FAME) were determined in the dry powder samples of C. spinosum after trans-esterification of the lipid fraction obtained through Soxhlet extraction as previously described in the study by Spréa et al. [32] and analyzed by gas-liquid chromatography with flame ionization detection, using a YOUNG IN Crhomass 6500 GC System instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Zebron-Fame column. The identification and quantification of fatty acids were performed by comparing the relative retention times of peaks detected in samples with commercial standards (standard mixture 47885-U, Sigma, St. Louis, MO, USA). The results were analyzed using the Clarity DataApex 4.0 Software (Prague, Czech Republic) and fatty acid content is presented in relative percentages.

2.7. Hydroethanolic and Aqueous Extracts Preparation

For the preparation of the hydroethanolic extracts (80:20, *v*/*v*), by maceration, 1 g of the lyophilized powder of each sample was used and stirred with 30 mL of solvent for 1 h. The obtained extracts were then filtered using Whatman nº 4 filter paper and re-extracted with 30 mL of solvent for 1 h. Finally, the combined extracts were submitted to an evaporation process, in order to eliminate the ethanol fraction, using a rotary evaporator (Buchi, 3000 series, Flawil, Switzerland) under a vacuum at 50 °C. The aqueous extracts were prepared by adding 2 g of each dried sample to 50 mL of boiling distilled water (100 °C), allowing them to stand for 5 min at room temperature, and then filtering them through Whatman no. 4 filter paper. The hydroethanolic and aqueous extracts were frozen and lyophilized to obtain a dry powder, then stored in a dry place at room temperature and protected from light before further analysis (phenolic compound profile analysis and bioactive activities).

2.8. Phenolic Compounds

The protocol used for the phenolic compounds profile characterization by HPLC-DAD-ESI/MSn was previously described in the study of Bessada et al. [33]. The lyophilized hydroethanolic and aqueous extracts of *C. spinosum* were redissolved in ethanol:water (20:80 *v*/*v*) to obtain a stock solution of 10 mg/mL. The diode array detector (DAD) used 280 nm and 370 nm as preferred wavelengths, while it was connected in line with a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) further equipped with an ESI source working in negative mode. For data acquisition and analysis,

the Xcalibur® data system was used (Thermo Finnigan, San Jose, CA, USA). Quantification was performed using 7-level calibration curves obtained from commercial standard compounds (*p*-coumaric acid ($y = 301950x + 6966{,}7$, $R^2 = 1$, LOD (limit of detection) = 0.68 µg/mL and LOQ = 1.61 µg/mL) and quercetin-3-*O*-glucoside ($y = 34.843x - 160.173$, $R^2 = 0.9998$, LOD = 0.21 µg/mL; LOQ (limit of quantification) = 0.71 µg/mL). The results are presented as mg per g of extract.

2.9. Antioxidant Activity

The antioxidant activity of *C. spinosum* extracts was evaluated in vitro by applying two cell-based assays. The lyophilized hydroethanolic and aqueous extracts of *C. spinosum* were re-dissolved in water to obtain a stock solution of 10 mg/mL, which were further diluted to obtain a range of eleven concentrations below the stock solution for the TBARS assay. For OxHLIA, it was re-dissolved in PBS, while water was used for complete hemolysis. Freshly prepared solutions of sheep red blood cells (2.8%, *v*/*v*) and porcine brain homogenate (1:2 *w*/*w*) were used to evaluate their ability to inhibit oxidative hemolysis (OxHLIA) induced by the temperature-dependent free-radical initiator 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and lipid oxidation via formation of thiobarbituric acid reactive substances (TBARS) by monitoring a colored adduct of malondialdehyde with 2-thiobarbituric acid, respectively. Both assays used Trolox as a positive control and are fully described by Mandim et al. [34,35]. Results were expressed as IC_{50} values (µg/mL); for OxHLIA, a 60 min Δt was considered for results calculation.

2.10. Antimicrobial Activity

The lyophilized hydroethanolic and aqueous extracts of *C. spinosum* were re-dissolved in water to obtain a stock solution of 10 mg/mL, while the samples were serially diluted to obtain the concentration ranges (10 at 0.15 mg/mL). The capacity to inhibit/kill pathogenic bacteria and fungi was evaluated against food-borne bacterial strains (*Enterobacter cloacae* ATCC 49741, *Eschericia 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 isolated bacterial strains (*E. coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *P. aeruginosa*, *Enterococcus faecalis*, *L. monocytogenes*, methicillin-resistant *S. aureus* (MRSA)); finally against two fungal strains (Aspergillus brasiliensis ATCC 16404, Aspergillus fumigatus ATCC 204305). All the ATCC microorganisms were obtained from Frilabo (Porto, Portugal) and the clinical isolates were provided by the northeastern local health unit (Bragança, Portugal) and Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal). The antimicrobial capacity was performed using the broth microdilution method previously described in the study by Pires et al. [36].

2.11. Anti-Inflammatory Activity

The anti-inflammatory activity was evaluated using the Griess Reagent System Kit (Promega, Madison, WI, USA) as described in detail in the study by Silva et al. [37], according to the inhibitory capacity against the nitric oxide (NO) production of lipopolysaccharide (LPS)-induced murine macrophage cells (RAW 264.7). The lyophilized hydroethanolic and aqueous extracts of *C. spinosum* were re-dissolved in water to obtain stock solutions of 8 mg/mL, which were further diluted to obtain a range of six concentrations below the stock solution.

2.12. Hepatotoxicity and Cytotoxicity Assays

For cytotoxicity assay, the cell lines tested included four tumor cell lines, e.g., cervical carcinoma—HeLa, breast carcinoma—MCF-7, hepatocellular carcinoma—HepG2, and non-small cell lung cancer (NCI-H460). The hepatotoxicity activity was determined using a non-tumor porcine liver primary culture (PLP2) [34], while the cytotoxicity and hepatotoxicity of the lyophilized hydroethanolic and aqueous extracts of *C. spinosum* re-dissolved in water

to obtain the concentration ranges (8 at 0.125 mg/mL) and were determined using the sulforhodamine B assay [38]. The results were expressed in GI_{50} values (g/mL), which correspond to the sample concentration providing 50% of inhibition of cell growth.

2.13. Statistical Analysis

Growth-related parameters were measured in 15 plants for each treatment (n = 15), whereas for chemical analyses the plant tissues (e.g., leaves) collected from each treatment were used to prepare three separate batch samples (n = 3) obtained from five plants per batch sample. All the analyses were carried out three times (n = 3). The statistical analysis of the data was performed with JMP v. 16.1 (SAS Institute Inc., Cary, NC, USA), while, prior to analysis, the Shapiro–Wilk test was used to determine the normal distribution of the raw data. After that, the one-way analysis of variance (ANOVA) was used for the analysis of data, while when significant effects were recorded means were compared with the Tukey HSD test at p = 0.05. All the results are presented as mean values and standard deviations (mean ± SD).

3. Results and Discussion

3.1. Growth Parameters

The results of the effect of the fertilization regime on the growth parameters of *C. spinosum* plants are presented in Table 1, indicating a varied effect on the tested parameters. In particular, the highest and lowest number of leaves per plant (30.36) were recorded for the C322 and C211 treatments (30.4 and 24.2 leaves/plant, respectively); however, C322 treatment did not differ significantly from C111, C333, and the control treatments, while C211 differed significantly from the rest of the treatments. Similarly, the highest and lowest fresh weight of leaves per plant were recorded for the C211 and C222 treatments (12.9 and 9.9 g/plant, respectively), which both differed significantly from the rest of the treatments. On the other hand, the application of fertilizers resulted in a significant decrease in dry matter of leaves (%) compared to the control treatment (C0), which recorded the highest overall value (8.3%) and was significantly different from the rest of the treatments. Regarding the SPAD index values, the C0 (94.8) and C222 (98.1) treatments presented the highest amounts of chlorophyll without being significantly different from the C322 treatment (87.9), whereas the C311 (62.1) treatment recorded significantly lower content than the rest of the treatments. Similarly, the C211 treatment was noted for the highest leaf area (324.7 cm^2), whereas C311 achieved the lowest index (250.4 cm^2) without differing from the C333 and C222 treatments (255.8 and 260.2, respectively). Moreover, C311 (39.2 m^2/kg) and C222 (37.6 m^2/kg) recorded significantly higher specific leaf area values, whereas the lowest values were reported for C211 and C0 treatments (28.2 m^2/kg and 27.1 m^2/kg, respectively). The results of the study are in agreement with other reports where similar values were reported regarding the number and dry matter of leaves of *C. spinosum* [22], although a slightly higher fresh weight was observed in comparison to our study, probably due to the different growing and harvesting period and the genotype tested. Moreover, the findings of our study indicate the C211 treatment affected positively the fresh weight of plants by achieving higher yields than fertilization regimes with higher amounts of N:P:K, which further confirms the low nutrient requirements of *C. spinosum*, especially regarding the amounts of P and K. Similar trends have also been suggested by Petropoulos et al. [23] who suggested that higher amounts of ammonium nitrogen in the feeding solution had no beneficial effect on plant fresh weight. Similarly, Chatzigianni et al. [9] reported that increasing the content of ammonium nitrogen in nutrient solution did not affect *C. spinosum* growth, thus suggesting the tolerance of the species to high amounts of ammonium nitrogen. To the best of our knowledge, this is the first report regarding the effect of P and K fertilization on the species and our results indicate its low requirements in terms of fresh biomass production since the highest and the second-highest values of fresh weight of leaves were recorded for the C211 and C311 treatments. This finding also indicates that the species is more responsive to nitrogen fertilization compared to P and K.

Table 1. The effect of fertilization regimes on the number and weight (g) of leaves per plant, the dry matter content of leaves (%), the chlorophyll content of leaves (SPAD index), the leaf area (m^2), and specific leaf area (m^2/kg) of *C. spinosum* plants.

	Traits					
Treatments	**Number of Leaves/Plant**	**Weight of Leaves/Plant (g)**	**Dry Matter of Leaves (%)**	**SPAD Index**	**Leaf Area (cm^2)**	**Specific Leaf Area (m^2/kg)**
C0	29.5 ± 1.3 ^{a}	11.5 ± 1.0 ^{c}	8.3 ± 2.2 ^{a}	94.8 ± 12.2 ^{a}	297.1 ± 8.5 ^{b}	27.1 ± 1.7 ^{e}
C111	29.1 ± 1.1 ^{a}	11.6 ± 1.2 ^{c}	6.6 ± 1.0 ^{e}	82.8 ± 7.8 ^{bc}	282.8 ± 7.9 ^{c}	31.2 ± 1.7 ^{bc}
C211	24.2 ± 1.4 ^{c}	12.9 ± 1.3 ^{a}	6.7 ± 0.1 ^{d}	74.2 ± 6.6 ^{c}	324.7 ± 8.6 ^{a}	28.2 ± 1.7 ^{e}
C222	27.3 ± 0.7 ^{b}	9.9 ± 1.1 ^{e}	6.1 ± 1.2 ^{e}	98.1 ± 13.1 ^{a}	260.2 ± 11.4 ^{d}	37.6 ± 2.0 ^{a}
C311	27.3 ± 1.3 ^{b}	12.0 ± 1.7 ^{b}	5.6 ± 2.6 ^{f}	62.1 ± 7.0 ^{d}	250.4 ± 6.8 ^{d}	39.2 ± 1.4 ^{a}
C322	30.4 ± 1.7 ^{a}	11.5 ± 1.3 ^{c}	7.9 ± 1.0 ^{b}	87.9 ± 7.2 ^{ab}	278.4 ± 8.3 ^{c}	30.8 ± 1.6 ^{cd}
C333	29.7 ± 1.3 ^{a}	10.8 ± 0.8 ^{d}	6.9 ± 2.6 ^{c}	76.1 ± 7.8 ^{c}	255.8 ± 8.0 ^{d}	29.8 ± 1.5 ^{d}

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

3.2. Nutritional Value

The effects of different fertilization programs on the nutritional and energetic value of *C. spinosum* leaves are presented in Table 2. Total fat content was significantly higher for the C222 treatment (6.8 g/100 g dw), while the lowest total fat content was observed for the C311 treatment (4.8 g/100 g dw). Significant statistical differences were also recorded in crude protein content with the C0 (control treatment) and C311 having the highest content (22.24 g/100 g dw and 22.09 g/100 g dw, respectively), while the C222 treatment (19.29 g/100 g dw) recorded the lowest one, respectively. Equally, the C0 treatment (13.99 g/100 g dw) gave the highest content of ash, whereas the lowest content was detected in the C322 treatment (12.59 g/100 g dw). Carbohydrate content was the highest for the C222 treatment (20.20 g/100 g dw) and the lowest for the C0 treatment (12.64 g/100 g dw). Finally, the energetic value of the leaves was affected by the different fertilization regimes and values ranged between 277.57 kcal/100 g dw (C311) and 300.78 kcal/100 g dw (C222), although the C311 treatment did not differ significantly from the C0 treatment (278.72 kcal/100 g dw). Our results indicate that the fertilization regime is very important for the quality of the final product, while most of the parameters (except for protein and ash content) were beneficially affected by low to moderate amounts of P and K and moderate to high amounts of N, e.g., C222 and C311 treatments. Similar results have also been reported in the study by Petropoulos et al. [39], who evaluated the nutritional profile and chemical composition of *C. spinosum* ecotypes and recorded slightly higher values of macronutrient contents compared to our study, a difference which could be mainly attributed to the different fertilization regimes and secondarily to the growing and conditions and the genotypes tested. Moreover, Petropoulos et al. [18,23] also suggested that the composition of the nutrient solution may affect the chemical composition of the species and improve the nutritional value of the edible leaves depending on the fertilization regime.

Table 2. Nutritional profile and energetic value of *C. spinosum* (mean ± SD).

	C0	**C111**	**C211**	**C222**	**C311**	**C322**	**C333**
Total fat (g/100 g dw)	5.3 ± 0.2 ^{d}	5.6 ± 0.1 ^{c}	6.1 ± 0.1 ^{b}	6.8 ± 0.1 ^{a}	4.8 ± 0.1 ^{e}	5.6 ± 0.2 ^{c}	5.6 ± 0.2 ^{c}
Crude protein (g/100 g dw)	22.24 ± 0.38 ^{a}	20.49 ± 0.10 ^{c}	20.23 ± 0.05 ^{d}	19.29 ± 0.03 ^{f}	22.09 ± 0.35 ^{a}	19.75 ± 0.13 ^{e}	21.58 ± 0.42 ^{b}
Ash (g/100 g dw)	13.99 ± 0.27 ^{a}	13.37 ± 0.30 ^{e}	13.51 ± 0.12 ^{d}	13.04 ± 0.01 ^{f}	13.48 ± 0.08 ^{d}	12.59 ± 0.01 ^{c}	13.69 ± 0.12 ^{b}
Total fiber dietary (g/100 g dw)	45.85 ± 0.28 ^{b}	45.15 ± 0.04 ^{c}	44.64 ± 0.30 ^{d}	40.63 ± 0.17 ^{f}	46.35 ± 0.87 ^{a}	45.14 ± 0.12 ^{c}	42.77 ± 0.52 ^{e}
Carbohydrates (g/100 g dw)	12.64 ± 0.44 ^{f}	15.36 ± 0.08 ^{d}	15.56 ± 0.26 ^{d}	20.20 ± 0.07 ^{a}	13.25 ± 0.84 ^{e}	16.91 ± 0.33 ^{b}	16.41 ± 0.15 ^{c}
Energy (Kcal/100 g dw)	278.72 ± 0.47^{c}	284.36 ± 0.97 ^{b}	286.96 ± 1.12 ^{b}	300.78 ± 0.66 ^{a}	277.57 ± 1.81 ^{c}	287.47 ± 0.50 ^{b}	287.45 ± 0.41 ^{b}

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

3.3. Organic Acids, Tocopherols, and Free Sugars Content

Organic acid composition is presented in Table 3. According to our results, significant statistical differences were recorded in the effect of fertilization regimes on the organic acid composition of the plant, while the main detected organic acids were quinic and citric acid, followed by malic and oxalic acid. In particular, the oxalic acid content was the highest for the C0 treatment (2.75 mg/100 g dw) and the lowest for the C222 treatment (0.53 mg/100 g dw), while all the treatments differed significantly from each other. The quinic acid showed the highest content for the control treatment (C0) (3.90 mg/100 g dw) and the least content for the treatments of C222 (2.98 mg/100 g dw) and C322 (2.96 mg/100 g dw), which did not differ with each other. Likewise, the treatment C311 (2.56 mg/100 g dw) recorded the highest content of malic acid and the treatment C222 (1.82 mg/100 g dw) had the lowest content, respectively. Regarding the citric acid content, the leaves of the plants treated with the C322 treatment had the highest content (2.82 mg/100 g dw), whereas the lowest amounts were detected for the C111 treatment (2.12 mg/100 g dw). Finally, the total organic acids content was the highest for the C0 (11.39 mg/100 g dw) and C311 (11.34 mg/100 g dw) treatments with no significant statistical differences between them, whereas the application of the C222 treatment (7.99 mg/100 g dw) resulted in the lowest total organic acid content. Therefore, our results indicate that fertilization regimes that contained high amounts of nitrogen (e.g., 311 and 322 treatments) increased only malic and citric acid, whereas the total amounts of organic acids were not significantly greater than the control treatment. However, it is worth mentioning that oxalic acid content was significantly lower for the C222 and would improve the quality of the final product. The determination of the organic acid composition is highly suggested for the *C. spinosum* leaves as it could have a significant impact on the taste and quality of the product, while at the same time high concentrations of oxalic acid could provoke unpleasant health effects as it is considered an anti-nutritional factor. Based on the current study, the organic acid composition was clearly affected by the tested fertilizer regimes, whereas it was observed that the oxalic acid content was decreased when plants were treated with the tested nutrient solutions, especially in the case of C222 and C322 treatments where the lowest amounts were detected. These findings have also been confirmed by other authors who reported that higher amounts of ammonium nitrogen resulted in a decreased content of oxalic acids since the nitrogen form is important for the accumulation of organic acids for osmoregulation purposes [24,40,41].

Table 3. Organic acid, tocopherol, and free sugar content of *C. spinosum* (mean ± SD).

	C0	C111	C211	C222	C311	C322	C333
Organic acids				(mg/100 g dw)			
Oxalic acid	2.75 ± 0.01 ^{a}	2.09 ± 0.02 ^{c}	1.51 ± 0.01 ^{e}	0.53 ± 0.01 ^{g}	2.70 ± 0.02 ^{b}	0.56 ± 0.01 ^{f}	1.78 ± 0.01 ^{d}
Quinic acid	3.90 ± 0.14 ^{a}	3.13 ± 0.02 ^{d}	3.07 ± 0.1 ^{c}	2.98 ± 0.12 ^{e}	3.42 ± 0.02 ^{b}	2.96 ± 0.01 ^{e}	3.46 ± 0.04 ^{b}
Malic acid	2.22 ± 0.07 ^{c}	2.13 ± 0.06 ^{e}	2.12 ± 0.03 ^{e}	1.82 ± 0.01 ^{f}	2.56 ± 0.01 ^{a}	2.52 ± 0.01 ^{b}	2.17 ± 0.01 ^{d}
Citric acid	2.51 ± 0.05 ^{c}	2.12 ± 0.02 ^{e}	2.17 ± 0.01 ^{d}	2.66 ± 0.02 ^{b}	2.65 ± 0.03 ^{b}	2.82 ± 0.02 ^{a}	2.63 ± 0.03 ^{b}
Sum	11.39 ± 0.12 ^{a}	9.48 ± 0.01 ^{c}	8.87 ± 0.14 ^{d}	7.99 ± 0.14 ^{e}	11.34 ± 0.07 ^{a}	8.87 ± 0.01 ^{d}	10.04 ± 0.02 ^{b}
Tocopherols				(mg/100 g dw)			
α-Tocopherol	1.19 ± 0.03 ^{f}	3.37 ± 0.11 ^{b}	1.83 ± 0.02 ^{d}	3.32 ± 0.12 ^{b}	1.39 ± 0.02 ^{e}	3.56 ± 0.1 ^{a}	2.10 ± 0.06 ^{c}
β-Tocopherol	nd	4.68 ± 0.07 ^{a}	3.47 ± 0.04 ^{b}	4.61 ± 0.28 ^{a}	nd	nd	nd
γ-Tocopherol	3.29 ± 0.11 ^{c}	nd	nd	nd	2.45 ± 0.03 ^{d}	5.55 ± 0.23 ^{a}	4.39 ± 0.14 ^{b}
Sum	4.49 ± 0.14^{f}	8.05 ± 0.04 ^{b}	5.30 ± 0.02 ^{e}	7.94 ± 0.16 ^{c}	3.84 ± 0.05 ^{g}	9.11 ± 0.33 ^{a}	6.49 ± 0.2 ^{d}
Free sugars				(mg/100 g dw)			
Fructose	2.74 ± 0.07 ^{e}	3.50 ± 0.08 ^{b}	2.14 ± 0.02 ^{f}	2.86 ± 0.02 ^{d}	2.88 ± 0.02 ^{d}	3.72 ± 0.03 ^{a}	3.01 ± 0.06 ^{c}
Glucose	3.27 ± 0.05 ^{d}	3.96 ± 0.17 ^{a}	2.71 ± 0.09 ^{e}	2.60 ± 0.08 ^{f}	2.62 ± 0.09 ^{f}	3.71 ± 0.09 ^{b}	3.67 ± 0.04 ^{c}
Sucrose	3.11 ± 0.04 ^{b}	2.71 ± 0.11 ^{c}	1.74 ± 0.08 ^{e}	3.07 ± 0.11 ^{b}	3.09 ± 0.11 ^{b}	4.52 ± 0.06 ^{a}	2.46 ± 0.08 ^{d}
Trehalose	0.77 ± 0.03 ^{a}	0.42 ± 0.01 ^{d}	0.41 ± 0.01 ^{d}	0.69 ± 0.01 ^{b}	0.69 ± 0.01 ^{b}	0.56 ± 0.02 ^{c}	0.68 ± 0.03 ^{b}
Sum	9.88 ± 0.04 ^{c}	10.59 ± 0.04 ^{b}	6.98 ± 0.01 ^{e}	9.21 ± 0.05 ^{d}	9.26 ± 0.03 ^{d}	12.50 ± 0.16 ^{a}	9.82 ± 0.15 ^{c}

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

Based on the findings of this study, the contents of individual and total tocopherols were significantly affected by the different fertilizer regimes applied to the *C. spinosum* plants (Table 3). The main detected isoform of vitamin E was α-tocopherol, while the treatments of C322 and C0 had the highest content and the lowest content (3.56 mg/100 g dw and 1.19 mg/100 g dw, respectively), both being significantly different from the rest of the treatments. Equally, the second most abundant tocopherol was γ-tocopherol, which was detected in the treatments C322 (5.55 mg/100 g dw), C333 (4.39 mg/100 g dw), C0 (3.29 mg/100 g dw), and C311 (2.45 mg/100 g dw), with contents being significantly different from each other. Similarly, β-tocopherol was only detected in the treatments C111 (4.68 mg/100 g dw), C222 (4.61 mg/100 g dw), and C211 (3.47 mg/100 g dw) which differed significantly from these treatments (e.g., C111 and C222). Finally, total tocopherol content was significantly higher for the C322 treatment (9.11 mg/100 g dw) compared to the rest of the treatments, whereas the treatment C311 had the significantly lowest numbers of total tocopherols (3.84 mg/100 g dw). Our results indicate that the tested fertilizers increased the individual and total tocopherol content over the control treatment, except for the case of the C311 treatment where a significant decrease was detected, suggesting a negative effect of the excessive amount of nitrogen in the nutrient solution. Moreover, the fertilization regime of C322 resulted in the highest content of individual (except for β-tocopherol) and total tocopherols, indicating that high amounts of N and balanced content of P and K have a beneficial effect on tocopherols biosynthesis. According to Petropoulos et al. [18], tocopherol content was significantly affected by the composition of the nutrient solution and particularly by nitrogen form. Similarly, the increased nitrogen rates resulted in higher amounts of individual and total tocopherols in the leaves of the wild edible species *Centaurea raphanina* subsp. *mixta* [42], while Hussain et al. [43] also mentioned that nitrogen rates could have a significant impact on the tocopherol content of oilseed rape seeds.

Significant statistical differences were also recorded in free sugar composition in relation to the fertilization regimes, while the main detected sugars were sucrose, glucose, and fructose, followed by trehalose (Table 3). In particular, sucrose content was significantly higher for the C322 treatment (4.52 g/100 g dw) and significantly lower for the C211 treatment (1.74 g/100 g dw), while glucose content varied from 3.96 g/100 g dw (C111 treatment) to 2.60 g/100 g dw (C222 treatment). Fructose content was the highest and lowest for the C322 treatment (3.72 g/100 g dw) and C211 treatment (2.14 g/100 g dw), respectively, while both treatments differed significantly from the rest of the tested fertilizer regimes. Trelahose content ranged between 0.77 g/100 g dw (C0) and 0.41 g/100 g dw (C211). Finally, the total free sugars content was significantly higher in the C322 treatment (12.50 g/100 g dw) due to the highest amounts of individual sugar, whereas the C211 treatment recorded significantly lower content (6.98 g/100 g dw) than the rest of the treatments. In general and similarly to tocopherols composition, C322 was the treatment where the highest amounts of total and individual sugars (except for trehalose and glucose) were detected, indicating the beneficial effects of N, P, and K on three-sugar biosynthesis. The findings of the study are in agreement with previous reports [10,23,39], which also identified glucose, fructose, sucrose, and trehalose as the main detected sugars in similar ranges of concentration. Moreover, a recent study by Petropoulos et al. [18,23] reported fluctuating trends in sugar content depending on the nitrogen form of the nutrient solution, while Cocetta et al. [44] and Rosales et al. [45] highlighted the effect of growing conditions on sugar composition and content of vegetable crops. On the other hand, the findings of our study indicate that the application of the C322 treatment increased the content of fructose, sucrose, and total sugars, and could be associated with an improved taste of the edible leaves. This observation was also suggested by Fallovo et al. [46], who recorded a significant effect of nitrogen supply forms on the phytochemical content of two leafy vegetables and proposed the regulation of nitrogen supply form as a simple practice to improve vegetable quality.

3.4. Fatty Acid Composition

Wild edible plants are regarded as a valuable source of polyunsaturated fatty acids (ω-3 and ω-6 fatty acids) and are highly appreciated as an integral element of the Mediterranean

diet. Based on the results of our study, the main detected fatty acids were alpha-linolenic (C18:3n3; ranging from 62.16% to 54.18%), followed by linolenic (C18:2n6c; ranging from 18.55% to 16.06%) and palmitic acid (C16:0; ranging between 15.11% and 13.44%) (Table 4). These results are in accordance with previous findings which suggested the same compounds as the main detected fatty acids in *C. spinosum* plants [10,22,47,48], although differences in the range of content were recorded which could be justified by the different studied genotypes and environmental conditions but also to the cultivation practices such as fertilizer treatments [23]. The effect of the tested fertilization regimes on the fatty acid composition showed a varied response, depending on the amounts of N:P:K applied. In particular, the C111 treatment resulted in significantly higher content for linolenic (18.55%) and palmitic acids (15.11%) compared to the rest of the treatments, whereas the C211 (16.06%) and C322 (13.48%) treatments had the lowest content for the above-mentioned fatty acids, respectively. Equally, α-linolenic significantly increased for treatment C322 (62.11%), whereas the significantly lowest content was recorded for C111 (54.67%) and C311 (54.18%). Polyunsaturated fatty acids (PUFA) were the richest class of fatty acids in all the tested fertilizers due to the high content of α-linolenic acid. This finding is also in accordance with previous reports on the fatty acids composition of *C. spinosum* leaves [22]. Moreover, the recorded ratios of n6/n3 fatty acids and PUFA/SFA were lower than 4.0 and higher than 0.45, respectively, for all the tested treatments, thus indicating a high nutritional value for the edible leaves of *Cichorium* species [49]. Regarding the particular effect of the tested treatments on fatty acid composition, C322 had the highest content of PUFA (79.2%), which could be associated with the highest tocopherol content observed for this treatment and its protective role against lipid oxidation and the regulation of unsaturated fatty acid metabolism [50], whereas the lowest SFA and MUFA were recorded for the same treatment.

3.5. Phenolic Compound Composition

Phenolic compound characteristics (retention time, wavelength of maximum absorption in the visible region, and mass spectral data) and tentative identities are presented in Table 5, whereas the quantification data of the detected compounds in the hydroethanolic and aqueous extracts of *C. spinosum* leaves are presented in Table 6. According to our results, the polyphenol profile was characterized by the presence of nine phenolic compounds for both extracts, including two phenolic acids and seven flavonoids, except for the case of aqueous extracts, where isorhamnetin-*O*-acetylhexoside was not detected in any of the samples and quercetin-*O*-acetylhexoside which was detected only in the C311 treatment. The most abundant compounds were 4-*O*-*p*-coumaroylquinic acid and isorhamnetin-*O*-hexuronoside (peaks 2 and 7) in both hydroethanolic and aqueous extracts. The application of fertilizers and the extraction protocol had a significant effect on phenolic compound composition, since the increased amounts of nutrients (C333) resulted in significantly increased phenolic acid contents in both extracts, while total flavonoids were significantly higher in the case of C311 and C222 for the hydroethanolic and aqueous extracts, respectively. In contrast, low amounts of nutrients resulted in decreased total phenolic compound (TPC) content, as indicated by the significantly lower TPC values recorded for C211 (4.682 mg/g extract) and C111 (2.520 mg/g extract) in hydroethanolic and aqueous extracts, respectively. These results are in agreement with the findings of Petropoulos et al. [22], who also identified *p*-Coumaroylquinic acid as the richest phenolic acid, whereas the derivatives of quercetin, kaempherol, and isorhamnetin have also been identified, and confirmed. Moreover, the effect of fertilizer regime on TPC content has also been confirmed by Sinkovič et al. [49] who, in contrast to our study, reported that the application of mineral fertilizers decreased TPC in various genotypes of *C. intybus.* However, Chatzigianni et al. [9] suggested that nitrogen form may also have an effect on phenolic compound biosynthesis in *C. spinosum* plants, with ammonium nitrogen being beneficial for secondary metabolite accumulation, whereas Otálora et al. [51] recorded a decrease in TPC in *C. endivia* var. *latifolium* plants when treated with foliar urea. Therefore, these differences could be associated with the environmental conditions, the genotype, and the cultivation practices, as already reported in the literature [52,53].

Table 4. Fatty acid composition of *C. spinosum* (mean ± SD).

	C0	C111	C211	C222	C311	C322	C333
Fatty acids	(Relative Percentage—%)						
C11:0	nd	nd	0.096 ± 0.001	nd	nd	nd	nd
C12:0	0.112 ± 0.004 ^{e}	0.150 ± 0.003 ^{c}	0.123 ± 0.002 ^{d}	0.287 ± 0.008 ^{a}	0.256 ± 0.006 ^{b}	nd	nd
C13:0	0.769 ± 0.021 ^{e}	1.092 ± 0.002 ^{b}	0.966 ± 0.011 ^{c}	1.434 ± 0.048 ^{a}	0.788 ± 0.016 ^{d}	0.624 ± 0.002 ^{g}	0.737 ± 0.015 ^{f}
C14:0	0.185 ± 0.009 ^{g}	0.395 ± 0.001 ^{c}	0.450 ± 0.013 ^{b}	1.094 ± 0.010 ^{a}	0.282 ± 0.004 ^{d}	0.200 ± 0.001 ^{f}	0.246 ± 0.007 ^{e}
C14:1	0.292 ± 0.003 ^{d}	0.452 ± 0.001 ^{a}	0.421 ± 0.002 ^{b}	0.333 ± 0.009 ^{c}	0.262 ± 0.008 ^{e}	0.213 ± 0.005 ^{f}	0.337 ± 0.004 ^{c}
C15:0	nd	0.159 ± 0.001 ^{c}	0.288 ± 0.003 ^{b}	0.478 ± 0.003 ^{a}	0.088 ± 0.004 ^{d}	0.075 ± 0.003 ^{e}	nd
C15:1	0.221 ± 0.003 ^{c}	0.244 ± 0.011 ^{a}	0.210 ± 0.006 ^{d}	0.227 ± 0.008 ^{b}	0.233 ± 0.009 ^{b}	0.150 ± 0.001 ^{f}	0.194 ± 0.004 ^{e}
C16:0	13.495 ± 0.537 ^{e}	15.116 ± 0.002 ^{a}	14.277 ± 0.249 ^{c}	14.280 ± 0.067 ^{c}	14.949 ± 0.062 ^{b}	13.448 ± 0.204 ^{f}	13.781 ± 0.334 ^{d}
C16:1	1.138 ± 0.029 ^{f}	1.627 ± 0.004 ^{a}	1.431 ± 0.041 ^{d}	1.589 ± 0.053 ^{b}	1.040 ± 0.021 ^{g}	1.501 ± 0.043 ^{c}	1.317 ± 0.036 ^{e}
C17:0	0.224 ± 0.222 ^{d}	0.236 ± 0.001 ^{c}	0.215 ± 0.007 ^{e}	0.182 ± 0.006 ^{f}	0.244 ± 0.019 ^{b}	0.174 ± 0.005 ^{g}	0.265 ± 0.001 ^{a}
C17:1	nd	0.088 ± 0.004 ^{b}	0.114 ± 0.006 ^{a}	0.113 ± 0.001 ^{a}	nd	nd	nd
C18:0	2.662 ± 0.021 ^{c}	2.573 ± 0.037 ^{c}	2.557 ± 0.068 ^{c}	2.037 ± 0.036 ^{d}	3.323 ± 0.023 ^{a}	1.561 ± 0.068 ^{e}	2.803 ± 0.094 ^{b}
C18:1n9c	2.384 ± 0.067 ^{c}	2.546 ± 0.005 ^{b}	1.162 ± 0.052 ^{f}	1.496 ± 0.020 ^{e}	3.436 ± 0.025 ^{a}	1.091 ± 0.030 ^{g}	2.277 ± 0.086 ^{d}
C18:2n6c	18.077 ± 0.127 ^{c}	18.551 ± 0.044 ^{a}	16.066 ± 0.234 ^{g}	16.759 ± 0.231 ^{f}	18.423 ± 0.028 ^{b}	16.983 ± 0.180 ^{e}	17.826 ± 0.623 ^{d}
C18:3n3	58.616 ± 0.568 ^{c}	54.677 ± 0.121 ^{f}	59.586 ± 0.628 ^{b}	57.601 ± 0.117 ^{e}	54.185 ± 0.002 ^{g}	62.116 ± 0.193 ^{a}	58.236 ± 0.362 ^{d}
C20:0	0.451 ± 0.004 ^{c}	0.424 ± 0.001 ^{d}	0.377 ± 0.013 ^{f}	0.411 ± 0.006 ^{e}	0.492 ± 0.006 ^{a}	0.265 ± 0.010 ^{g}	0.470 ± 0.011 ^{b}
C20:1	nd	nd	nd	nd	nd	nd	nd
C20:2	nd	nd	nd	nd	nd	nd	nd
C21:0	nd	0.313 ± 0.001 ^{b}	nd	nd	0.417 ± 0.018 ^{a}	nd	nd
C20:4n6	nd	nd	nd	nd	nd	nd	nd
C22:0	0.363 ± 0.006 ^{e}	0.374 ± 0.001 ^{d}	0.449 ± 0.021 ^{a}	0.412 ± 0.006 ^{c}	0.428 ± 0.012 ^{b}	0.359 ± 0.014 ^{e}	0.443 ± 0.013 ^{a}
C20:3n3	nd	nd	0.070 ± 0.001	nd	nd	nd	nd
C20:3n6	nd	0.083 ± 0.004	nd	nd	nd	nd	nd
C22:2	0.083 ± 0.002 ^{f}	0.127 ± 0.001 ^{c}	0.189 ± 0.001 ^{a}	0.194 ± 0.006 ^{a}	0.122 ± 0.001 ^{d}	0.114 ± 0.003 ^{e}	0.149 ± 0.007 ^{b}
C23:0	0.172 ± 0.006 ^{d}	0.172 ± 0.035 ^{d}	0.237 ± 0.003 ^{b}	0.293 ± 0.007 ^{a}	0.214 ± 0.006 ^{c}	0.212 ± 0.008 ^{c}	0.209 ± 0.005 ^{c}
C24:0	0.760 ± 0.021 ^{d}	0.609 ± 0.001 ^{f}	0.719 ± 0.013 ^{e}	0.785 ± 0.026 ^{c}	0.824 ± 0.013 ^{b}	0.917 ± 0.041 ^{a}	0.715 ± 0.020 ^{e}
SFA	19.19 ± 0.60 ^{e}	21.61 ± 0.07 ^{b}	20.75 ± 0.39^{c}	21.69 ± 0.18 ^{b}	22.30 ± 0.03 ^{a}	17.83 ± 0.05 ^{f}	19.67 ± 0.20 ^{d}
MUFA	4.03 ± 0.10 ^{c}	4.87 ± 0.01 ^{b}	3.22 ± 0.01^{e}	3.64 ± 0.07 ^{d}	4.97 ± 0.06 ^{a}	2.95 ± 0.07 ^{f}	4.12 ± 0.05 ^{c}
PUFA	76.8 ± 0.7 ^{b}	73.4 ± 0.1 ^{e}	75.9 ± 0.4^{c}	74.6 ± 0.1 ^{d}	72.7 ± 0.01 ^{f}	79.2 ± 0.02 ^{a}	76.2 ± 0.3 ^{b}

Means in the same row followed by different Latin letters are significantly different at $p < 0.05$, according to Tukey's HSD test. Undecylic acid (C11:0); lauric acid (C12:0); tridecylic acid (C13:0); myristic acid (C14:0); myristoleic acid (C14:1); pentadecylic acid (C15:0); ginkgolic acid (C15:1); palmitic acid (C16:0); palmitoleic acid (C16:1); margaric acid (C17:0); heptadecenoic acid (C17:1); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6c); α-linolenic acid (C18:3n3); arachidic acid (C20:0); gondoic acid (C20:1); eicosadienoic acid (C20:2); heneicosylic acid (C21:0); arachidonic acid (C20:4n6); behenic acid (C22:0); eicosatrienoic acid (C20:3n3); dihomo-gamma-linolenic acid (C20:3n6); docosadienoic acid (C22:2); tricosylic acid (C23:0); lignoceric acid (C24:0); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 5. Retention time (Rt), wavelength of the maximum absorption (λ_{max}), deprotonated ion ([M-H]$^-$), main mass fragments (MS^2), and tentative identification of the phenolic compounds found in the hydroethanolic and aqueous extracts of *C. spinosum* samples.

Peak	Rt	λ_{max}	[M-H]$^-$	MS^2	Tentative Identification
1	8.51	292	337	191(100),173(12),163(71),155(3),119(34)	3-*O*-*p*-Coumaroylquinic acid
2	10.64	292	337	191(5),173(100),163(39),155(10),119(23)	4-*O*-*p*-Coumaroylquinic acid
3	17.24	352	477	301(100)	Quercetin-*O*-hexuronoside
4	17.82	345	461	285(100)	Luteolin-*O*-hexuronoside
5	18.97	342	505	463(24),301(100)	Quercetin-*O*-acetylhexoside
6	20.71	342	461	285(100)	Kaempherol-*O*-hexuronoside
7	22.07	340	491	315(100)	Isorhamnetin-*O*-hexuronoside
8	23.19	343	489	285(100)	Kaempherol-*O*-acetylhexoside
9	24.48	344	519	315(100)	Isorhamnetin-*O*-acetylhexoside

Table 6. Quantification (mg/g extract) of the phenolic compounds found in the hydroethanolic and aqueous extracts of *C. spinosum* samples (mean ± SD).

Hydroethanolic Extracts (mg/g Extract)							
Peak	**C0**	**C111**	**C211**	**C222**	**C311**	**C322**	**C333**
1	0.541 ± 0.003 ^{a}	0.471 ± 0.002 ^{c}	0.459 ± 0.003 ^{d}	0.453 ± 0.001 ^{d}	0.518 ± 0.003 ^{b}	0.315 ± 0.001 ^{e}	0.468 ± 0.002 ^{c}
2	0.864 ± 0.004 ^{d}	0.938 ± 0.008 ^{b}	0.813 ± 0.001 ^{f}	0.893 ± 0.016 ^{c}	0.845 ± 0.004 ^{e}	0.696 ± 0.001 ^{g}	1.016 ± 0.005 ^{a}
3	0.496 ± 0.001 ^{b}	0.461 ± 0.003 ^{e}	0.459 ± 0.001 ^{e}	0.465 ± 0.002 ^{d}	0.533 ± 0.001 ^{a}	0.497 ± 0.004 ^{b}	0.484 ± 0.002 ^{c}
4	0.541 ± 0.007 ^{c}	0.530 ± 0.006 ^{d}	0.510 ± 0.003 ^{e}	0.497 ± 0.001 ^{f}	0.610 ± 0.001 ^{b}	0.532 ± 0.001 ^{d}	0.631 ± 0.002 ^{a}
5	0.481 ± 0.002 ^{c}	0.447 ± 0.003 ^{e}	0.409 ± 0.003 ^{f}	0.46 ± 0.001 ^{d}	0.508 ± 0.001 ^{a}	0.497 ± 0.004 ^{b}	0.441 ± 0.001 ^{e}
6	0.580 ± 0.004 ^{c}	0.558 ± 0.001 ^{d}	0.555 ± 0.003 ^{d}	0.518 ± 0.005 ^{e}	0.63 ± 0.001 ^{a}	0.581 ± 0.004 ^{c}	0.588 ± 0.001 ^{b}
7	0.673 ± 0.001 ^{c}	0.637 ± 0.004 ^{d}	0.588 ± 0.004 ^{e}	0.588 ± 0.001 ^{e}	0.695 ± 0.003 ^{b}	0.672 ± 0.0004 ^{c}	0.737 ± 0.007 ^{a}
8	0.443 ± 0.003 ^{e}	0.472 ± 0.001 ^{c}	0.434 ± 0.001 ^{f}	0.470 ± 0.005 ^{c}	0.508 ± 0.001 ^{a}	0.503 ± 0.002 ^{b}	0.455 ± 0.001 ^{d}
9	0.465 ± 0.001 ^{c}	0.430 ± 0.001 ^{f}	0.454 ± 0.005 ^{d}	0.449 ± 0.007 ^{e}	0.491 ± 0.001 ^{a}	0.472 ± 0.004 ^{b}	0.416 ± 0.001 ^{g}
TPA	1.404 ± 0.002 ^{b}	1.409 ± 0.01 ^{b}	1.272 ± 0.002 ^{e}	1.346 ± 0.015 ^{d}	1.362 ± 0.006 ^{c}	1.010 ± 0.002 ^{f}	1.484 ± 0.002 ^{a}
TF	3.678 ± 0.002 ^{c}	3.536 ± 0.003 ^{d}	3.410 ± 0.001 ^{f}	3.447 ± 0.001 ^{e}	3.975 ± 0.005 ^{a}	3.754 ± 0.001 ^{b}	3.753 ± 0.001 ^{b}
TPC	5.082 ± 0.002 ^{c}	4.945 ± 0.013 ^{d}	4.682 ± 0.003 ^{g}	4.794 ± 0.015 ^{e}	5.338 ± 0.007 ^{a}	4.764 ± 0.001 ^{f}	5.237 ± 0.003 ^{b}

Table 6. *Cont.*

Aqueous Extracts (mg/g Extract)							
Peak	C0	C111	C211	C222	C311	C322	C333
1	0.4625 ± 0.0001 ^{b}	0.385 ± 0.001 ^{d}	0.371 ± 0.004 ^{e}	0.3403 ± 0.005 ^{f}	0.447 ± 0.003 ^{c}	0.384 ± 0.011 ^{d}	0.521 ± 0.01 ^{a}
2	0.573 ± 0.006 ^{f}	0.584 ± 0.001 ^{e}	0.591 ± 0.001 ^{d}	0.662 ± 0.002 ^{c}	0.659 ± 0.014 ^{c}	0.684 ± 0.007 ^{b}	0.821 ± 0.001 ^{a}
3	0.534 ± 0.002 ^{c}	nd	0.497 ± 0.001 ^{d}	0.608 ± 0.001 ^{a}	0.537 ± 0.001 ^{c}	0.496 ± 0.001 ^{d}	0.563 ± 0.001 ^{b}
4	0.595 ± 0.001 ^{c}	0.521 ± 0.001 ^{f}	0.54 ± 0.000 ^{e}	0.636 ± 0.001 ^{b}	nd	0.567 ± 0.001 ^{d}	0.668 ± 0.01 ^{a}
5	nd	nd	nd	nd	0.565 ± 0.001	nd	nd
6	0.639 ± 0.001 ^{c}	0.489 ± 0.003 ^{f}	0.605 ± 0.001 ^{e}	0.666 ± 0.001 ^{b}	0.665 ± 0.008 ^{b}	0.624 ± 0.009 ^{d}	0.678 ± 0.002 ^{a}
7	0.716 ± 0.005 ^{e}	0.541 ± 0.002 ^{g}	0.674 ± 0.006 ^{f}	0.795 ± 0.003 ^{b}	0.777 ± 0.001 ^{d}	0.758 ± 0.007 ^{c}	0.829 ± 0.005 ^{a}
8	0.554 ± 0.001 ^{b}	nd	0.499 ± 0.001 ^{e}	0.569 ± 0.001 ^{a}	0.504 ± 0.001 ^{d}	0.504 ± 0.001 ^{d}	0.539 ± 0.001 ^{c}
9	nd	nd	nd	nd	nd	nd	nd
TPA	1.036 ± 0.006 ^{d}	0.969 ± 0.001 ^{e}	0.962 ± 0.003 ^{e}	1.003 ± 0.007 ^{d}	1.106 ± 0.018 ^{b}	1.068 ± 0.005 ^{c}	1.342 ± 0.009 ^{a}
TF	3.038 ± 0.008 ^{c}	1.551 ± 0.005 ^{f}	2.816 ± 0.003 ^{e}	3.273 ± 0.001 ^{a}	3.049 ± 0.01 ^{b}	2.949 ± 0.001 ^{d}	3.277 ± 0.008 ^{a}
TPC	4.074 ± 0.002 ^{d}	2.520 ± 0.005 ^{g}	3.778 ± 0.002 ^{f}	4.275 ± 0.008 ^{b}	4.155 ± 0.027 ^{c}	4.017 ± 0.005 ^{e}	4.619 ± 0.001 ^{a}

Means in the same row followed by different Latin letters are significantly different at $p < 0.05$, according to Tukey's HSD test. nd—not detected; TPA—total phenolic acids; TF—total flavonoids; TPC—total phenolic compounds. Standard calibration curves used for quantification: *p*-coumaric acid ($y = 301{,}950x + 6966{,}7$, $R^2 = 1$, LOD = 0.68 μg/mL and LOQ = 1.61 μg/mL, peaks 1 and 2) and quercetin-3-*O*-glucoside ($y = 34.843x - 160.173$, $R^2 = 0.9998$, LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL, peaks 3 to 9).

3.6. Bioactive Properties

3.6.1. Antioxidant Activity

The results of the bioactive properties of the hydroethanolic and aqueous extracts are presented in Tables 7 and 8. The results of the OxHLIA assay for the hydroethanolic extracts showed that the highest antioxidant activity was observed when low amounts of nutrients were applied (e.g., C111 treatment; 53 μg/mL), followed by C222 (61 μg/mL) and C311 (65 μg/mL), while the latter treatment (C311) was also the most effective for the TBARS assay (151 μg/mL) (Table 7). Similarly, and for the aqueous extracts, the highest antioxidant activity was recorded for the C222 treatment for both assays (20 μg/mL and 116 μg/mL for OxHLIA and TBARS, respectively). These results agree with those previous studies [7,10,22] which reported varied IC_{50} values for TBARS and OxHLIA assays. The high antioxidant activity recorded in the current research could be attributed to the high flavonoid, organic acid, and tocopherol content recorded in C311 and C222 treatments for the hydroethanolic and aqueous extracts, respectively, since bioactive compounds content is highly associated with antioxidant activity of natural matrices [54]. Other factors such as the fertilization regime may also affect antioxidant activity through the regulation of bioactive compound biosynthesis [9,12,18].

3.6.2. Antimicrobial Properties

The antibacterial effects of the hydroethanolic and aqueous extracts are presented in Tables 7 and 8. The present findings depicted mild antimicrobial effects depending on the extraction method, tested bacteria, and fertilizer regime since, despite the differences between the tested extracts, none of them showed higher activity than the positive controls (Ketoconazole and Ampicillin). In any case, the hydroethanolic extracts obtained from plants treated with C111 and C211 were more efficient against the food gram-negative bacteria namely *S. enterocolitica* and *S. aureus* compared to the rest of the treatments, while C322 and C333 treatments were effective against the most tested bacteria (*E. coli*, *S. enterocolitica*, *Y. enterocolitica*, *B. cereus*, *L. monocytogenes*, and *S. aureus*). Moreover, regarding the tested clinical bacteria, the treatments of C111, C211, and C311 presented efficiency against *E. faecalis*. On the other hand, the aqueous extracts of the treatments C111, C211, C222, C322, and C333 recorded higher effectiveness (MIC values) against the food gram-negative bacteria namely *E. cloacae* and *Y. enterocolitica* compared to the rest of the tested fertilizer regimes. Likewise, the aqueous extracts of C0, C111, C211, C222, and C311 treatments presented higher effectiveness (MIC values) against the food gram-positive bacteria, namely *S. aureus* and *L. monocytogenes*, whereas all the extracts were similarly effective regardless of the fertilizer regime based on the minimal bactericidal concentration (MBC) values. Similarly, regarding the tested clinical bacteria, the extracts from the C0, C111, C211, C222, C311, and C322 treatments were the most effective against the gram-positive bacteria MRSA and *E. faecalis*, while the extracts from all the tested treatments especially in the case of C0, C211, C222, C322, and C333 recorded the highest effectiveness against *E. coli*.

The antifungal activities of the tested extracts are presented in Tables 7 and 8, where a varied response was recorded depending on the extraction method used, the fertilizer regime, and the tested fungi. Regarding the hydroethanolic extracts, the treatments of C0, C311, and C322 presented higher efficiency (MIC values) than the rest of the treatments against *A. fumigatus*, while all the fertilizer regimes recorded similar minimal fungicidal concentrations (MFC) values against the two tested fungi. Similarly, the aqueous extracts obtained from plants treated with C0, C111, C211, C322, and C333 were more efficient (MIC values) than the rest of the fertilizer regimes against *A. fumigatus*, while the treatments C222 and C322 showed higher efficiency (MIC values) against *A. brasiliensis*. All the treatments showed similar MFC values against the two tested fungi.

Table 7. Antioxidant, antimicrobial, anti-inflammatory, hepatotoxic, and cytotoxic activities of the hydroethanolic extract of *C. spinosum* (mean ± SD).

	C0		**C111**		**C211**		**C222**		**C311**		**C322**		**C333**	
Antioxidant activity														
IC_{50} values (μg/mL) [A]														
OxHLIA Δ*t* = 60	322 ± 20 [b]		53 ± 3 [g]		339 ± 18 [a]		61 ± 2 [f]		65 ± 2 [e]		103 ± 4 [d]		123 ± 7 [c]	
TBARS inhibition	479 ± 9 [b]		411 ± 15 [d]		408 ± 6 [d]		363 ± 16 [e]		151 ± 6 [f]		465 ± 15 [c]		547 ± 27 [a]	
Antimicrobial activity (mg/mL) [B–G]														
Food borne bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria														
Enterobacter cloacae	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
Escherichia coli	10	>10	>10	>10	10	>10	10	>10	10	>10	5	>10	10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella enterocolitica	10	>10	5	>10	5	>10	>10	>10	10	>10	5	>10	5	>10
Yersinia enterocolitica	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	5	>10
Gram-positive bacteria														
Bacillus cereus	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	5	>10
Listeria monocytogenes	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	5	>10
Staphylococcus aureus	10	>10	5	>10	5	>10	10	>10	10	>10	5	>10	5	>10
Clinical bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria														
Escherichia coli	0.3	>10	5	>10	0.6	>10	1.25	>10	0.6	>10	2.5	>10	5	>10
Klebsiella pneumoniae	10	>10	10	>10	10	>10	5	>10	10	>10	10	>10	>10	>10
Morganella morganii	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
Proteus mirabilis	10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Gram-positive bacteria														
Enterococcus faecalis	10	>10	5	>10	5	>10	10	>10	5	>10	10	>10	10	>10
Listeria monocytogenes	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
MRSA	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	10	>10
Fungal strains (mg/mL)	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus brasiliensis	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10
Aspergillus fumigatus	5	>10	10	>10	10	>10	10	>10	5	>10	5	>10	10	>10
Anti-inflammatory activity [H]														
RAW 264,7	>400		>400		>400		>400		>400		>400		>400	
Hepatotoxicity (GI50 values ug/mL) [I]														
PLP2	>400		>400		>400		>400		>400		>400		>400	

Table 7. *Cont.*

	C0	C111	C211	C222	C311	C322	C333
Cytotoxicity activity (GI_{50} values ug/mL) [I]							
AGS	>400	>400	>400	>400	>400	>400	>400
CaCo2	>400	>400	>400	>400	>400	>400	>400
VERO	>400	>400	>400	>400	>400	>400	>400
MCF7	>400	>400	>400	>400	>400	>400	>400

Means in the same row followed by different Latin letters are significantly different at $p < 0.05$, according to Tukey's HSD test. [A.] Trolox IC_{50} values: 5.8 ± 0.6 µg/mL (TBARS), 21.8 ± 0.3 µg/mL (OxHLIA 60 min); [B] Ketoconazole MIC/MFC (mg/mL): (*A. brasiliensis* 0.06/ 0.125, *A. fumigatus* 0.5/1), [C] Ampicillin for clinical bacteria tested at a maximum of 10 mg/mL: ((*E. coli*, *P. mirabilis*, *E. faecalis*, *L. monocytogenes*, MRSA <0.15/<0.15), (*K. pneumoniae* 10/>10), (*M. morganii*, *P. aeruginosa* >10/>10); [C.] Ampicilin for food-borne bacteria, tested at a maximum of 10 mg/mL: ((*E. cloacae*, *E. coli*, *S. enterocolitica*, *Y. enterocolitica*, *L. monocytogenes*, *S. aureus* 0.15/0.15), (*P. aeruginosa* 0.63/0.63)); [D] Imipenem for clinical bacteria tested at a maximum of 1 mg/mL: ((*E. coli*, *K. pneumoniae*, *M. morganii*, *P. mirabilis*, *L. monocytogenes* <0.0078/<0.0078), (*P. aeruginosa* (0.5/1); [E] Streptomycin for food-borne bacteria tested at a maximum of 1 mg/mL: ((*E. cloacae*, *S. enterocolitica*, *Y. enterocolitica*, *B. cereus*, *L. monocytogenes*, *S. aureus* 0.007/0.007), (*E. coli* 0.01/0.01), (*P. aeruginosa* (0.06/0.06)); [F] Methicilin for food-borne bacteria tested at a maximum of 1 mg/mL: (*S. aureus* 0.007/0.007); [G] Vancomycin for clinical bacteria tested at a maximum of 1 mg/mL: ((*E. faecalis* <0.0078/<0.0078), (MRSA 0.25/0.5)); [H] Dexametaxone IC_{50} value: 6.3 ± 0.4 µg/mL; [I] Ellipticine GI_{50} values: 1.4 ± 0.1 µg/mL (PLP2), 1.23 ± 0.03 µg/mL (AGS), 1.21 ± 0.02 µg/mL (CaCo2), 1.41 ± 0.06 µg/mL (VERO), and 1.02 ± 0.02 µg/mL (MCF-7).

Table 8. Antioxidant, antimicrobial, anti-inflammatory, hepatotoxic, and cytotoxic activities of the aqueous extract of *C. spinosum* (mean ± SD).

	C0		C111		C211		C222		C311		C322		C333	
Antioxidant activity IC_{50} values (µg/mL) [A]														
OxHLIA Δt = 60	131 ± 5 [c]		97 ± 5 [d]		25 ± 2 [e]		20 ± 1 [f]		207 ± 12 [b]		278 ± 9 [a]		207 ± 13 [b]	
TBARS inhibition	357 ± 11 [a]		143 ± 2 [e]		167 ± 6 [c]		116 ± 5 [f]		225 ± 8 [b]		163 ± 8 [c,d]		159 ± 7 [d]	
Antimicrobial activity (mg/mL) [B–G]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Food borne bacteria														
Gram-negative bacteria														
Enterobacter cloacae	>10	>10	10	>10	10	>10	5	>10	>10	>10	10	>10	10	>10
Escherichia coli	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella enterocolitica	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
Yersinia enterocolitica	10	>10	5	>10	5	>10	10	>10	10	>10	5	>10	5	>10
Gram-positive bacteria														
Bacillus cereus	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
Listeria monocytogenes	10	>10	10	>10	10	>10	5	>10	10	>10	10	>10	10	>10
Staphylococcus aureus	5	>10	5	>10	5	>10	2.5	>10	5	>10	10	>10	10	>10

Table 8. *Cont.*

	C0		C111		C211		C222		C311		C322		C333	
Clinical bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria														
Escherichia coli	2.5	>10	5	>10	2.5	>10	2.5	>10	5	>10	2.5	>10	2.5	>10
Klebsiella pneumoniae	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10
Morganella morganii	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10
Proteus mirabilis	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Gram-positive bacteria														
Enterococcus faecalis	5	10	10	>10	5	>10	5	>10	5	>10	10	>10	10	>10
Listeria monocytogenes	10	10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
MRSA	5	5	5	>10	5	>10	10	>10	5	>10	5	>10	>10	>10
Fungal strains (mg/mL)	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus brasiliensis	10	>10	10	>10	10	>10	5	>10	10	>10	5	>10	10	>10
Aspergillus fumigatus	5	>10	5	>10	5	>10	10	>10	10	>10	5	>10	5	10
Anti-inflammatory activity (IC_{50} values μg/mL) [H]														
RAW 264.7	>400		>400		>400		>400		>400		>400		>400	
Hepatotoxicity (GI_{50} values μg/mL) [I]														
PLP2	>400		>400		>400		>400		>400		>400		>400	
Cytotoxicity activity (GI_{50} values μg/mL) [I]														
AGS	>400		>400		>400		>400		>400		>400		>400	
CaCo2	>400		>400		>400		>400		>400		>400		>400	
VERO	>400		>400		>400		>400		>400		>400		>400	
MCF7	>400		>400		>400		>400		>400		>400		>400	

Means in the same row followed by different Latin letters are significantly different at $p < 0.05$, according to Tukey's HSD test. [A] Trolox IC_{50} values: 5.8 ± 0.6 μg/mL (TBARS), 21.8 ± 0.3 μg/mL (OxHLIA 60 min); [B] Ketoconazole MIC/MFC (mg/mL): (*A. brasiliensis* 0.06/ 0.125, *A. fumigatus* 0.5/1), [C] Ampicillin for clinical bacteria tested at a maximum of 10 mg/mL: ((*E. coli*, *P. mirabilis*, *E. faecalis*, *L. monocytogenes*, MRSA <0.15/<0.15), (*K. pneumoniae* 10/>10), (*M. morganii*, *P. aeruginosa* >10/>10); [C.] Ampicilin for food-borne bacteria tested at a maximum of 10 mg/mL: ((*E. cloacae*, *E. coli*, *S. enterocolitica*, *Y. enterocolitica*, *L. monocytogenes*, *S. aureus* 0.15/0.15), (*P. aeruginosa* 0.63/0.63)); [D] Imipenem for clinical bacteria tested at a maximum of 1 mg/mL: ((*E. coli*, *K. pneumoniae*, *M. morganii*, *P. mirabilis*, *L. monocytogenes* < 0.0078/<0.0078), (*P. aeruginosa* (0.5/1); [E] Streptomicin for food-borne bacteria tested at a maximum of 1 mg/mL: ((*E. cloacae*, *S. enterocolitica*, *Y. enterocolitica*, *B. cereus*, *L. monocytogenes*, *S. aureus* 0.007/0.007), (*E. coli* 0.01/0.01), (*P. aeruginosa* (0.06/0.06)); [F] Methicilin for food-borne bacteria tested at a maximum of 1 mg/mL: (*S. aureus* 0.007/0.007); [G] Vancomycin for clinical bacteria tested at a maximum of 1 mg/mL: ((*E. faecalis* <0.0078/<0.0078), (MRSA 0.25/0.5)); [H] Dexametaxone IC_{50} value: 6.3 ± 0.4 μg/mL; [I] Ellipticine GI_{50} values: 1.4 ± 0.1 μg/mL (PLP2), 1.23 ± 0.03 μg/mL (AGS), 1.21 ± 0.02 μg/mL (CaCo2), 1.41 ± 0.06 μg/mL (VERO), and 1.02 ± 0.02 μg/mL (MCF-7).

Overall, our findings do not suggest consistent trends in the antimicrobial efficacy of the extracts, since a variable response was recorded for the various fertilizer treatments. However, many authors have also suggested that *Cichorium* extracts may show variable antimicrobial properties due to differences in plant parts used for the extraction, as well as the concentration and the solvent of the extracts [55–57]. In particular, Quave et al. [58] reported that they did not observe any inhibition against *S. aureus* when they tested the ethanolic extracts from basal leaves and roots of *Cichorium intybus* plants, whereas in our study there was a mild efficiency of the extracts of the C0, C111, C211, C222, and C311 treatments against this bacterium. Moreover, Rani et al. [59] suggested an efficient activity of the methanolic extracts from *C. intybus* leaves against *Salmonella typhi*, whereas other authors observed contradictory effects regarding the inhibitory effects of the aqueous and hydroethanolic extracts of *C. intybus* seeds against *Salmonella enteritidis*, *S. infantil* and *S. typhimurium* [55,60]. A promising inhibitory effect of the methanolic extracts of *C. intybus* seeds against *E. coli* was reported [61–63], a finding which is in agreement with our results and the effectiveness of hydroethanolic and aqueous extracts from C0, C211, C222, C322, and C333 treatments. In contrast, Petropoulos et al. [64] suggested that *E. Cloacae* was the most sensitive bacterium to *C. spinosum* leaves' extracts, a finding which was also recorded in the current study, whereas the same authors did not observe any antimicrobial effects against *E. coli*. Similarly, in our study, there was not any recorded inhibition effect of *C. spinosum* extracts against *Pseudomonas aeruginosa*, a finding which has also been observed by other authors who tested the methanolic and ethanolic extracts of *C. intybus* [55,65]. Finally, the weak antifungal activity recorded in the present study was also suggested by Petropoulos et al. [64], whereas other studies suggested good antifungal activity of the aqueous and ethanolic extracts of *C. intybus* against *Aspergillus niger*, *A. fumigatus*, *A. flavus*, and *Fusarium solani* [66,67].

3.6.3. Cytotoxic Effects

None of the tested samples showed cytotoxic, hepatotoxic, or anti-inflammatory activity since all the extracts (hydroethanolic or aqueous) were not effective at the maximum tested concentration (400 mg/mL) (Tables 7 and 8). The lack of efficient activity of *C. spinosum* hydroethanolic extracts against tumor cell lines (MCF-7, HeLa, HepG2, and PLP2) has been previously reported by Petropoulos et al. [18], while nitrogen fertilization slightly increased the cytotoxic effects in hydroethanolic extracts of cultivated plants of *C. raphanina* subsp. *mixta* [42,68,69]. These findings indicate that although specific bioactive compounds (e.g., tocopherols, organic acids, and phenolic compounds) may be positively affected by the tested fertilization regimes, it seems that these compounds do not contribute to the cytotoxic efficacy of the leaf extracts. Another explanation for the lack of activity could be attributed to the implemented extraction protocols since according to the literature this could affect the content of phytochemicals and the bioactivities of the obtained extracts [64,68,70,71]. Therefore, although in the present study we tested both hydroethanolic and aqueous extracts it seems that further research and more extraction protocols are needed.

4. Conclusions

Our results highlight the low nutrient requirements of *Cichorium spinosum* since the C211 treatment significantly increased plant fresh weight and subsequently the yield of the crop. Moreover, the studied nutrient solutions had a beneficial effect on the nutritional value of the edible leaves, especially in the case of the C222 treatment, indicating that tailor-made nutritional recipes may improve the final product quality. Regarding the tested bioactive properties, a variable effect was suggested depending on the fertilization regime and the extraction protocol. For example, high inputs of nutrients (e.g., C333 treatment) increased total phenolic acid content in both aqueous and hydroethanolic extracts, while the same treatment was the most beneficial for flavonoid and total phenolic compound content in the case of aqueous extracts. For tocopherols and sugars, lower amounts of P and K compared to N (e.g., C322 treatment) resulted in a significant increase, while the same

treatment increased the content of α-linolenic acid. In regards to antioxidant activity, the effect of the fertilization regime and extraction protocol varied depending on the assay (e.g., TBARS and OxHLIA), although the C222 treatment was consistently the most effective in the case of aqueous extracts for both assays. Finally, the antimicrobial effects varied depending on the tested factors and the studied bacteria and fungi, whereas none of the extracts showed significant cytotoxic effects. Overall, the present results highlight the high nutritional value of spiny chicory, which could be further exploited by cultivating the species as a complementary/alternative crop for the production of produce with high added value. Finally, wild edible species can be promising cropping alternatives within the climate change scenario; however, more studies are needed to evaluate and standardize the cultivation practices for the commercial cultivation of such species and also to determine the bioactivities of the plant extracts, which could be further exploited for medicinal purposes.

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