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Soil Quality Effects on *Chenopodium album* Flavonoid Content and Antioxidant Potential

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Antioxidant capacity, total phenolic content and flavonoid glycosides profile were compared in *C. album* samples grown in intensively cultivated (IC) and nondisturbed (ND) soils to evaluate differences in their nutraceutical potential. Petroleum ether, methanol, and aqueous extracts were sequentially obtained from *C. album* dried samples. Methanol crude extract exhibited the highest antioxidant potential and phenolic content, which were significantly enhanced by soil deterioration. This feature was enhanced in its ethyl acetate/*n*-buthanol subextract that also yielded higher amounts of the fraction containing flavonoid glycosides in samples grown in IC soils. Compounds were isolated by activity-guided fractionation, and chemical structure−antioxidant activity relationships were established. Chemical structures were elucidated by chemical and spectroscopic methods. Six known flavonoid glycosides were isolated, and their antioxidant activity was determined by DPPH assay. **1**, quercetin-3-*O*-(2′′,6′′-di-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside; **2**, kaempferol-3-*O*-(2′′,6′′-di-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside; **3**, quercetin-3-*O*-*β*-D-glucopyranosyl-(1′′′→6′′)-*β*-D-glucopyranoside; **4**, rutin; **5**, quercetin-3-*O*-*β*-D-glucopyranoside; and **6**, kaempferol-3-*O*-*β*-D-glucopyranoside. Triosides **1** and **2** were identified for the first time in *C. album*. Our results suggest that this edible weed, ubiquitously present in cultivated fields, should be considered as a nutraceutical food and an alternative source for nutrients and free radical scavenging compounds, particularly when collected from cultivated fields that seem to increase some of its advantages.

KEYWORDS: Edible weed; lambsquarters; *Chenopodium album*; antioxidant; DPPH; flavonoid glycosides; soil quality

1. INTRODUCTION

Free radicals such as superoxide anions ($O^{\bullet}{}_2{}^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$) can trigger reactions damaging essential biomolecules in living organisms such as lipids, proteins, and nucleic acids. Diabetes and degenerative diseases, cancer, heart and vascular diseases, neurological, and ocular disorders, besides the aging process itself, are some of the consequences of their action (*1, 2*). They also represent a problem in processed food affecting their quality, stability, and safety (*3*). Antioxidants, either as nutraceuticals, pharmaceutical supplements, or as food additives, can terminate radical reactions. Current concerns about potential mutagenic activity and possible carcinogenic effects of synthetic antioxidants, such as buthylated hydroxytoluene (BHT) and buthylated hydroxyanisole (BHA), have encouraged the search for naturally occurring antioxidants (*4, 5*).

Plants produce a diversity of chemical structures by biosynthetic processes that have been modulated through genetic effects over time in order to ensure their success in ecosystems. Among them, flavonoids, anthocyanins, carotenoids, and vitamins are compounds with free radical scavenging properties. Polyphenols and particularly flavonoid derivatives behave as reducing agents, mostly donating hydrogen and quenching singlet oxygen. They seem to have additive effects on endogenous scavenging compounds (*6*). Flavonoids are versatile bioactive secondary metabolites present in almost all plant species. Most representative family members include flavones, flavanes, flavonols, catechins, and anthocyanidins. Their antioxidant potential toward ROS depends on structural characteristics such as the number and substitution pattern of hydroxyl groups and the extent at which these groups are glycosilated (*7*).

Many edible fruits and vegetables contain free radical inhibitors with preventive potential regarding a large number of human diseases that have been defined as nutraceuticals (*8*). However, many other natural sources such as edible weeds have not been used yet with this purpose, even when they have been known by ancient civilizations and used as a part of their daily

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diet. Stephen De Felice coined the term nutraceutical in 1989 (*9*) to define a food (or a food component) providing medical or health benefits, including prevention and/or treatment of diseases (*8*). Even when weeds are considered to be a problem in agriculture, from the productivity stand point, it is possible to take advantage of them as alternative vegetables in foods or as a renewable resource to obtain nutraceuticals or other bioactive principles.

White quinoa, *Chenopodium album* (Chenopodiaceae), is a broadleaf weed species called fat hen in Europe and lambsquarters in United States of America. It has developed cross- and multiple resistance to synthetic herbicides, and still causes significant economic losses in agriculture all over the world (*10*). Having nutritional values, its leaves have been consumed raw in salads or cooked by different civilizations in America and are still part of human diet in Mexico and other developing countries. *C. album* leaves are rich in proteins (4.2%) with a high proportion of essential aminoacids such as lysine, leucine, and isoleucine, and significant amounts of calcium and vitamins A (11000 IU/100 g) and C (80 mg/100 g) (*11*).

Cinnamic acids amides (*12*), flavonoids (*13, 14*), and apocarotenoids (*15*) have also been isolated from this species.

Environmental conditions, including biotic and abiotic factors, modulate the rate at which primary and secondary metabolisms occur (*16*). Stresses such as drought, extreme temperatures, low soil quality, and/or the presence of herbicides and pathogens are now common features in intensively cultivated agroecosystems, with direct consequences in the proportion at which particular secondary metabolites are produced. It has been shown that different kinds of stresses enhance secondary metabolism pathways related to chemical defense strategies (*16*). Changes in environmental conditions particularly in soil quality can trigger variations on secondary metabolites production. In a previous work (*17*), it was reported that *C. album* samples grown in Argentina in intensively cultivated soils produced larger amounts of nonpolar compounds such as long chain linear and branched hydrocarbons, fatty acid esters, and long chain alcohols and aldehydes. The aim of this work was to study the free radical scavenging ability and flavonoid contents in plant samples grown under the same conditions. Extracts and subextracts from *C. album* aerial parts were studied in relation to the quality of the soil where the samples were grown in order to separate and identify the corresponding bioactive compounds. A literature survey revealed no previous references on the antioxidant potential of this weed. Nevertheless, flavonol glycosides with antioxidant activity were isolated from seeds of another species within this genus, *Chenopodium quinoa*, considered a pseudocereal in Latin America (*18*).

2. MATERIALS AND METHODS

2.1. General Methods. Analytical and spectroscopy degree solvents were purchased from Sintorgan (Chemical Center SRL, Argentine). Kieselgel 60 F_{254} TLC aluminum sheets for thin layer chromatography were purchased from Merck (Research AG, Buenos Aires, Argentina). Sephadex LH-20 for column chromatography was purchased from Pharmacia Fine Chemical Co., Ltd., Germany.

Chemical standards and reagents, such as DPPH (1,1-diphenyl-2-picryl-hydrazyl), Folin−Ciocalteu reagent, 2,6-dichloroquinone-4-chlorimide, quercetin, ascorbic acid, BHT (butylated hydroxytoluene), and chlorogenic acid hemihydrate were purchased from Sigma Aldrich S.A. (Buenos Aires, Argentina).

Column chromatography was performed on Sephadex LH-20, using a glass column (60 cm × 4.5 cm id).

HPLC analysis was performed in a SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, Rheodyne manual injector, and UV micrometrics Mod 787 detector using a 5 μm-Bondapak C-18 column (5 μm, 30 cm × 7.8 mm id) from Waters Corp., MA, USA.

A Hewlett-Packard 5890A Gas Chromatograph (Hewlett-Packard, Avondale, PA, USA), equipped with a flame-ionization detector was used to analyze peracetylated alditols, with a GC column: SP-2330 (25 m × 0.2 mm id).

IR spectra were recorded on a FTIR spectrometer (Nicolet Magna-550 Series II, Midac, Co., USA).

^{1}H- and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, on a Bruker AM 500 Spectrometer (Bruker, Germany). Chemical shifts (δ) were determined in CD_3OD and expressed in ppm. ^{13}C NMR multiplicity was established by DEPT.

2.2. Plant Material. *Chenopodium album* samples were collected at their vegetative stage, during summer time, from fields in the maize production area of Argentina (32−35° S and 58−62° W), characterized by silty clay loam soil. Plant samples were collected from four fields with a long cropping history (more than 15 years since last pasture) exhibiting low stability indexes (less than 20%) (*19*). Samples with similar height were chosen at random within each field from two kinds of sites: (i) intensively cultivated (IC) and (ii) nondisturbed (ND). In most cropping fields, there is an area surrounding cultivated soil that remains without being disturbed by human activity. ND samples were collected from noncultivated areas at the edge of each field, where soil is not disturbed. These intensively cultivated and nondisturbed soils were previously classified as deteriorated and nondeteriorated, on the basis of their chemical and physical properties (*20*).

IC and ND samples were collected the same day from each field, under identical environmental conditions, with the exception of soil exploitation level.

A voucher specimen of each sample was deposited (no. 24038) in the Botany Herbarium of the Agronomy School, University of Buenos Aires.

2.3. Extraction and Isolation. *C. album* aerial parts were separated from roots and immediately submitted to drying conditions at 40 °C under ventilation. Dry material was milled and continuously extracted in a Soxhlet device with light petroleum ether to separate lipidic components and then with methanol. The remaining plant residue was finally extracted with distilled water overnight at room temperature. Organic extracts were evaporated to dryness under vacuum conditions, while the aqueous extract was lyophilized. All of them were tested on their antioxidant potential by DPPH assay. Methanol crude extract, which showed the highest free radical scavenging activity, was further processed in order to separate active compounds by polarity.

C. album methanol extract was dissolved in distilled water, partitioned with chloroform (3×) and further extracted with ethyl acetate/*n*-butanol (1:1) (EtOAc/*n*-BuOH) (3×). Organic layers were evaporated to dryness under vacuum. EtOAc/*n*-BuOH fraction, the subextract with higher antioxidant activity, was further purified by column chromatography through Sephadex LH-20 using MeOH/H_2O (2:1) as eluent. Fractions (9 mL) were collected and analyzed by chromatography on Kieselgel 60 F_{254} TLC aluminum sheets with *n*-BuOH/AcOH/H_2O (12:3:5) as mobile phase in order to characterize those enriched in flavonoid derivatives. Chromatograms were visualized by UV-light and/or by reaction with Gibbs reagent (2,6-dichloroquinone-4-chlorimide in chloroform (2%)). After spraying with Gibbs reagent, TLC sheets were placed in a chamber saturated with ammonia fumes and then heated at 120 °C for 5 min. Fractions showing positive reactions were joint and evaporated together to give a flavonoid enriched extract (F), which was further chromatographed by reverse-phase HPLC.

2.4. Comparative HPLC. *C. album* flavonoid enriched extract F was dissolved in methanol (100 μg mL^{-1}) and 1 μL of each solution (corresponding to F_{IC} and F_{ND} repetitions) was analyzed by RP-HPLC, eluting with acetonitrile/water/acetic acid (23:77:1, v/v/v) at a flow rate 1.0 mL min^{-1}, with UV detection at λ 350 nm.

2.5. Identification of Flavonoid Glycosides. RP-HPLC was further performed to isolate flavonoid glycosides, under the conditions established in the previous paragraph. Structures were elucidated by chemical and spectroscopic methods and confirmed by comparison with literature data. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD.

2.6. Acid Hydrolysis of Glycosides: Monosaccharide Composition. Acid hydrolysis of purified flavonoid glycosides was performed in order to establish monosaccharide composition, as previously reported (*21*). Each pure glycoside (1−2 mg) was heated in a screw cap vial with 2 N trifluoroacetic acid (0.5 mL) at 120 °C for 1 h. The aglycone was extracted with $CHCl_3$, and the remaining aqueous solution was evaporated under reduced pressure. The resulting sugar mixture was treated with 0.5 M NH_3 (0.5 mL) and $NaBH_4$ (2 mg) at room temperature for 18 h. The reaction mixture was first acidified with 1 M AcOH (pH 5−6), and then MeOH (0.5 mL) was added. The solution was evaporated in vacuo to obtain the alditol mixture, which was peracetylated with Ac_2O (0.5 mL) in pyridine (0.5 mL) at 100 °C for 45 min, cooled, and poured into $CHCl_3/H_2O$ (1:1). Aqueous phase was extracted with $CHCl_3$ (3 × 0.5 mL). Chloroform extract was sequentially washed with distilled water (0.5 mL), $NaHCO_3$ saturated solution (0.5 mL), again with distilled water (0.5 mL), and evaporated to dryness under nitrogen. Peracetylated alditols were analyzed by GC (SP-2330 column; nitrogen as gas carrier; temperature range, 200−230 °C; rate, 2 °C min^{-1}). Standard peracetylated alditols were used as reference compounds.

2.7. Total Phenolic Content. Total phenolic content of *C. album* extracts and fractions was evaluated by means of the Folin−Ciocalteau reagent, using chlorogenic acid as standard (*22*). The sample solution (10 μL) containing 100 μg extract was poured into a volumetric flask, which was thoroughly shaken for 3 min, after adding distilled water (4.6 mL) and Folin−Ciocalteau reagent (0.1 mL). 2% Na_2CO_3 solution (0.3 mL) was then added, and the mixture was submitted to intermittent shaking for 2 h. Absorbance was measured at 760 nm. These determinations were carried out per triplicate, and mean values were calculated. The same procedure was repeated with standard chlorogenic acid solutions (0−100 μg mL^{-1}) in order to obtain a standard curve.

2.8. Antioxidant Activity. DPPH Spectrophotometric Assay. The free radical scavenging potential of extracts, subextracts, and pure compounds was confirmed by spectrophotometric assay, quantifying their capacity to bleach a purple solution of DPPH in methanol (*23*). Different concentrations of their methanol solutions (50 μL) were added to 0.004% DPPH methanol solution (5 mL). After a 30 min incubation period at room temperature, each absorbance (A) was determined at 517 nm. Inhibition percentage of DPPH (I %) was calculated as follows:

$$\mathrm{I}\ \% = (A_{\mathrm{control}} - A_{\mathrm{sample}}/A_{\mathrm{control}}) \times 100$$

where $A_{control}$ is the absorbance of a control solution containing only the reagents, and A_{sample} is the absorbance of the corresponding sample reaction. The effective dose responsible of 50% inhibition (ED_{50}) was obtained from a plot of inhibition percentage versus extract concentration. These determinations were carried out in triplicate, and mean values were calculated. BHT (butylated hydroxytoluene) and ascorbic acid were used as positive controls.

2.9. Statistical Analysis. Data is reported as mean values ± standard deviation of four independent extractions, and activity measurements were done in triplicate. Analysis of variance was performed for all data using the General Linear Model. Mean values were compared by least significant difference (LSD) at 0.05 probability level.

3. RESULTS

Stress conditions often enhance secondary metabolite production, and among them, that of free radical scavenging compounds. We found that soil deterioration increases flavonoid content in *C. album*, particularly those derivatives related to quercetin, also diminishing its glycosilation degree.

C. album samples collected from intensively cultivated (IC) and nondisturbed (ND) soils were sequentially extracted with petroleum ether (E), methanol (M), and distilled water (A). The corresponding TLC chromatograms stained with Gibbs reagent showed significant amounts of phenolic compounds in methanol and aqueous extracts. *C. album* methanol crude extract (M) exhibited the highest antioxidant potential. Its TLC chromatogram showed various radical scavenging compounds as light yellow spots on a violet background, even at the lowest concentration (0.10 mg mL^{-1}). Crude extracts (1000 μg mL^{-1}) were also compared in their antioxidant potential by DPPH spectrophotometric assay, a fast method to detect free radical scavenging compounds. **Figure 1** shows higher DPPH inhibition percentage values for both A and M extracts, also identifying the latter as the only one with significant activity enhancement when obtained from samples grown in intensively cultivated soils.

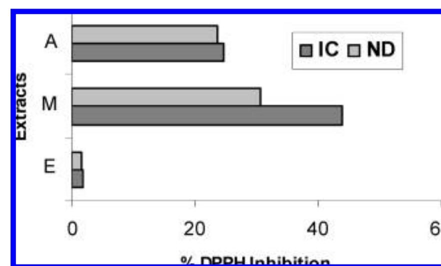

Figure 1. DPPH inhibition percentage of crude extracts from *C. album* samples grown in IC and ND soils. *E* = petroleum ether extract; M = methanol extract; A = aqueous extract (*P* < 0.05).

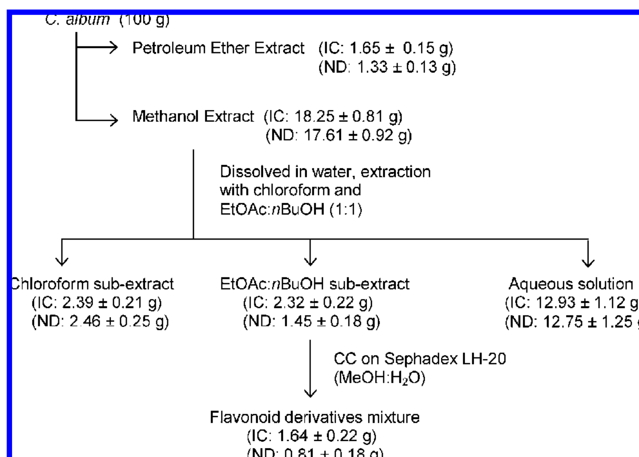

Figure 2. *C. album* flavonoid glycoside extraction and isolation scheme.

3.1. Free Radical Scavenging Activity and Total Phenolic Content. Methanol crude extract from samples grown in IC soils (M_{IC}) showed free radical scavenging activity 37% higher than M_{ND}, with DPPH ED_{50} values of 122.3 and 167.0 μg mL^{-1}, respectively. Since M activity was the only one exhibiting a significant increment in samples from intensively cultivated soils, this extract was further submitted to fractionation as indicated in **Figure 2**. Its aqueous solution was extracted first with chloroform and then with EtOAc/*n*-BuOH (1:1). When staining the TLC subextracts chromatogram, it was found that free radical scavenging substances included in the latter reacted much faster with DPPH than those in the chloroform subextract. EtOAc/*n*-BuOH subextract exhibited the highest antioxidant activity, which was also significantly enhanced by soil deterioration. These results were further confirmed by spectrophotometric analysis.

Total phenolic content was evaluated in extracts and subextracts by the Folin−Cicolteau modified method (*22*), which expresses data in terms of chlorogenic acid μmol (CA) mg^{-1} dry extract. Only EtOAc/*n*-BuOH subextract, with the highest phenolic content, showed significant increments in samples from intensively cultivated soils (IC, 0.75 ± 0.17; and ND, 0.52 ± 0.10) (**Table 1**). Free radical scavenging capacity was directly correlated with total phenolic content, with correlation coefficients of −0.9872 and −0.8097 for IC and ND samples,

Table 1. Total Phenolic Content and Free Radical Scavenging Activity of *C. album* Extracts, Sub-Extracts, and Isolated Compounds

sample	total phenolic content[a]	DPPH ED_{50}[b]
MeOH extract (crude) ND	0.20 ± 0.14	167.00 ± 3.95
MeOH extract (crude) IC	0.22 ± 0.08	122.30 ± 2.91
$CHCl_3$ ND	0.36 ± 0.06	196.25 ± 5.19
$CHCl_3$ IC	0.38 ± 0.06	108.11 ± 2.61
EtOAc-*n*BuOH ND	0.52 ± 0.10	87.80 ± 1.99
EtOAc-*n*BuOH IC	0.75 ± 0.17	22.47 ± 0.94
compound **1**		6.50 ± 0.89
compound **2**		6.92 ± 0.72
compound **3**		6.17 ± 0.71
compound **4**		4.90 ± 0.68
compound **5**		4.71 ± 0.49
compound **6**		6.41 ± 0.66
quercetin		2.63 ± 0.19
ascorbic acid		5.94 ± 0.28
BHT		10.81 ± 0.57

[a] Expressed as μmol AC mg $^{-1}$ dry extract. [b] Expressed in μg mL^{-1}.

Table 2. Relative Mean Abundance (MA) of Flavonoid Glycosides in F_{IC} and F_{ND}

	glycoside	MA_{IC}[a]	MA_{ND}[a]
1	quercetin 3-*O*-(2″,6″-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside	2.35 ± 0.53(a)	10.71 ± 0.56(b)
2	kaemferol 3-*O*-(2″,6″-di-*O*-α-L-rhamnopyranosyl)- β-D-glucopyranoside	21.10 ± 1.99(a)	23.10 ± 0.78(a)
3	quercetin-3-*O*-β-D-glucopyranosyl (1‴→6″)-β-D- glucopyranoside	7.47 ± 0.53(b)	2.18 ± 0.24(a)
4	quercetin-3-*O*-α-L-rhamnopyranosyl-(1‴→6″)-β-D-glucopyranoside	24.64 ± 2.46(b)	15.44 ± 1.51(a)
5	quercetin-3-*O*-β-D-glucopyranoside	3.42 ± 0.27(b)	1.62 ± 0.13(a)
6	kaemferol-3-*O*-β-D-glucopyranoside	2.53 ± 0.22(a)	2.92 ± 0.12(b)

[a] Different letters correspond to significant differences ($P < 0.05$).

respectively. Free radical scavenging activity of extracts, subextracts, and compounds by DPPH assay, and total phenolic content are shown in **Table 1**.

The EtOAc/*n*-BuOH subextract obtained from M_{IC} was almost three times more active toward DPPH (ED_{50}: 22.47 μg mL^{-1}) than the corresponding M_{ND} subextract (ED_{50}: 87.80 μg mL^{-1}), showing almost half-of the BHT free radical scavenging potential.

EtOAc/*n*-BuOH subextracts were further fractionated by column chromatography with Sephadex LH-20 to give flavonoid glycosides enriched fractions (F) (**Figure 2**). Starting from the same amount of dry plant material, the F_{IC} mean value (1.63 g) resulted twice as much as F_{ND} mean value (0.81 g). These fractions were submitted to RP-HPLC to obtain glycoside relative abundances (**Table 2**).

3.2. Comparative HPLC. *C. album* flavonoid glycoside fraction F was dissolved in methanol (100 μg mL^{-1}) and submitted to RP-HPLC with acetonitrile/water/acetic acid (23:77:1, v/v/v) as eluent and UV detection at λ = 350 nm. HPLC chromatograms showed signals at retention time (*Rt*) values within 0.72−5.36 min.

Six flavonoid derivatives were further identified as glycosides **1**−**6** (**Figure 4**). The signal at 4.03 min exhibited the highest abundance difference between samples from IC and ND soils. Triosides **1** (0.77 min) and **2** (1.69 min) were eluted first, while both monosides **5** (5.05 min) and **6** (5.36 min) were the latter. With similar oligosaccharide chains, quercetin trioside (**1**) eluted prior to kaempferol trioside (**2**). Relative abundances of glycosides in F_{IC} and F_{ND} HPLC chromatograms are presented in **Table 2**.

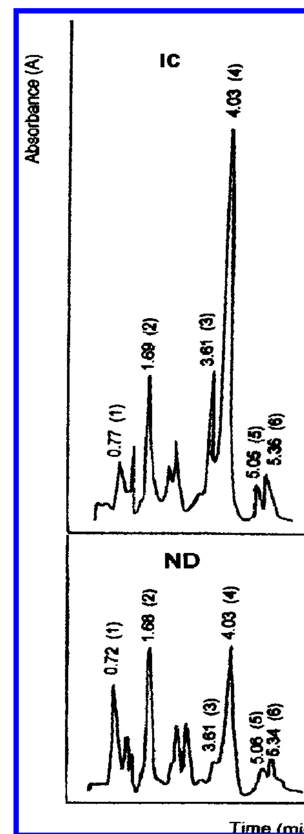

Figure 3. F_{IC} and F_{ND} HPLC profiles.

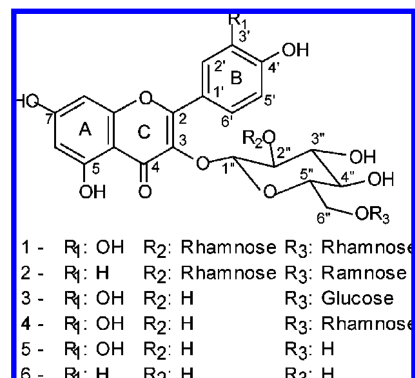

Figure 4. Flavonoid glycosides isolated from *Chenopodium album*.

Higher abundances of diosides (**3** + **4**), rutin being one of them, were also found in F_{IC} (32.11%) compared to those in F_{ND} (17.62%). Total triosides abundance percentage in F was lower under stress conditions related to soil deterioration. Both triosides **1** and **2**, which represented 33.80% of F_{ND}, were less abundant in F_{IC} (23.44%). Quercetin derivative **1** was responsible for it since there were no significant differences in the amount of kaempferol trioside **2**.

Monosides **5** and **6** were minor components in samples from IC and ND soils, nevertheless the relative amount of quercetin derivative (**5**) in samples grown in IC soils (3.42%) was more than twice as much its value in samples from ND soils (1.62%). Even when the relative abundance of kaempferol monoside decreased 11% as a result of low soil quality, the total amount of monosides was enhanced 33% because quercetin monoside (**5**) abundance was increased more than 100%.

3.3. Isolation and Identification of Flavonoid Glycosides. Six pure known compounds **1**−**6** were isolated by RP-HPLC

(**Figure 3**). All compounds showed free radical scavenging activity, and two of them (**1** and **2**) are reported for the first time in *C. album*.

Compounds were isolated as light- or dark-yellow amorphous powders. Structures were determined by chemical and spectroscopic methods, and identified by direct comparison of their physical properties with those previously reported. Compound (**1**−**6**) IR spectra showed bands at 1650 and 3380 (cm^{-1}), which were consistent with carbonyl and hydroxyl groups. ^{1}H and ^{13}C NMR, and DEPT spectra of isolated glycosides exhibited chemical shifts and coupling constants corresponding to quercetin (**1**, **3**, **4**, and **5**) and kaempferol (**2** and **6**).

Monosaccharide composition of sugar moieties was determined by hydrolysis with aqueous 2 N trifluoroacetic acid followed by GC analysis of the corresponding peracetylated alditols.

β-anomeric configuration for glucose and α-anomeric configuration for rhamnose were suggested by its large $J_{H1,H2}$ coupling constants at 7.4−7.7 Hz and 1.5−1.7 Hz, respectively (*24*). All isolated compounds were glycosilated at C-3 in agreement with literature data (*14, 25–27*).

1*: Quercetin-3-O-(2″,6″-Di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside.* Light-yellow amorphous powder. IR (KBr): 1650, 3380 (cm^{-1}). Gas chromatography analysis of peracetylated alditols showed the presence of rhamnose and glucose in 2:1 ratio. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.60 (dd, 1, J = 8.7 Hz,1.9 Hz, H-6′), 7.58 (d, 1, J = 1.9 Hz, H-2′), 6.88 (d, 1, J = 8.7 Hz, H-5′), 6.41 (d, 1, J = 1.8 Hz, H-8), 6.22 (d, 1, J = 1.8 Hz, H-6), 5.62 (d, 1, J = 7.7 Hz, H-1″), 5.19 (d, 1, J = 1.7 Hz, H-1‴), 4.52 (d, 1, J = 1.5 Hz, H-1⁗), 3.12−4.24 (m, sugar protons), 1.08 (d, 3, J = 6.3 Hz, CH$_3$-6⁗), 0.98 (d, 3, J = 6.1 Hz, CH$_3$-6‴). ^{13}C NMR (125 MHz, CD$_3$OD): δ 179.0 (C-4, s), 165.3 (C-7,d), 162.8 (C-5, s), 159.3 (C-9, d), 158.2 (C-2, s), 149.8 (C-4′, s), 145.4 (C-3′, d), 135.7 (C-3, s), 123.6 (C-6′, d), 123.1 (C-1′, s), 117.9 (C-2′, d), 115.8 (C-5′, s), 105.8 (C-10, s), 102.6 (C-1‴, d), 102.4 (C-1⁗, d), 100.7 (C-1″, d), 100.0 (C-6, s), 94.7 (C-8, s), 80.0 (C-2″,d), 78.8 (C-3″, d), 77.0 (C-5″, d), 74.0 (C-4‴, d), 73.9 (C-4⁗, d), 72.5 (C-3⁗, d), 72.4 (C-2‴, d), 72.3 (C-3‴, d), 72.2 (C-2⁗, d), 71.9 (C-4″, d), 69.8 (C-5‴, d), 69.5 (C-5⁗, d), 68.7 (C-6″, t), 18.0 (C-6⁗, c), 17.5 (C-6‴, c).

2*: Kaempferol-3-O-(2″,6″-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside.* Light-yellow amorphous powder. IR (KBr): 1650, 3380 (cm^{-1}). Gas chromatography analysis of peracetylated alditols showed the presence of rhamnose and glucose in 2:1 ratio. ^{1}H NMR (500 MHz, CD$_3$OD): δ 8.01 (br d, 2, J = 8.9 Hz, H-6′, H-2′), 6.89 (br d, 2, J = 8.8 Hz, H-3′, H-5′), 6.37 (d, 1, J = 2.1 Hz, H-8), 6.18 (d, 1, J = 2.1 Hz, H-6), 5.59 (d, 1, J = 7.6 Hz, H-1″), 5.15 (d, 1, J = 1.5 Hz, H-1‴), 4.45 (d, 1, J = 1.5 Hz, H-1⁗), 3.15 − 4.20 (m, sugar protons), 1.06 (d, 3, J = 6.2 Hz, CH$_3$-6⁗), 0.94 (d, 3, J = 6.2 Hz, CH$_3$-6‴). ^{13}C NMR (125 MHz, CD$_3$OD): δ 179.3 (C-4, s), 166.0 (C-7, d), 162.6 (C-5, s), 161.5 (C-4′, s), 159.1 (C-9, d), 158.6 (C-2, s), 135.4 (C-3, s), 132.6 (C-2′, d), 132.5 (C-6′, d), 122.8 (C-1′, s), 116.4 (C-3′ and 5′, s), 105.8 (C-10, s), 102.9 (C-1‴, d), 102.7 (C-1⁗, d), 101.0 (C-1″, d), 99.7 (C-6, s) 94.8 (C-8, s), 80.2 (C-2″,d), 79.0 (C-3″,d), 77.2 (C-5″, d), 74.2 (C-4‴,d), 74.0 (C-4⁗, d), 72.7 (C-3⁗, d), 72.4 (C-2‴and 3‴, d), 72.1 (C-2⁗, d), 72.0 (C-4″, d), 70.0 (C-5‴, d), 69.2 (C-5⁗, d), 68.3 (C-6″, t), 18.3 (C-6⁗, c), 17.3 (C-6‴, c).

3*: Quercetin-3-O-β-D-glucopyranosyl-(1‴ 6″)-O-β-D-glucopyranoside.* Dark-yellow amorphous powder. IR (KBr): 1650, 3380 (cm^{-1}). Gas chromatography analysis of peracetylated alditols showed the presence of glucose. ^{1}H NMR (500 MHz, CD$_3$OD: δ 7.61 (dd, 1, J = 8.8 Hz,1.9 Hz, H-6′), 7.57 (d, 1, J = 1.9 Hz, H-2′), 6.88 (d, 1, J = 8.8 Hz, H-5′), 6.39 (d, 1, J = 1.8 Hz, H-8), 6.18 (d, 1, J = 1.8 Hz, H-6), 5.05 (d, 1, J = 7.6 Hz, H-1″), 5.31 (d, 1, J = 7.4 Hz, H-1‴), 3.15−4.20 (m, sugar protons). ^{13}C NMR (125 MHz, CD$_3$OD): δ 179.4 (C-4, s), 165.8 (C-7,d), 162.9 (C-5, s), 159.0 (C-9, d), 158.5 (C-2, s), 149.5 (C-4′, s), 145.1 (C-3′, d), 135.6 (C-3, s), 123.2 (C-6′, d), 122.9 (C-1′, s), 117.6 (C-2′, d), 115.9 (C-5′, s), 105.7 (C-10, s), 105.0 (C-1″, d), 104.1 (C-1‴, d), 99.9 (C-6, s) 94.2 (C-8, s), 78.4 (C-3″,d), 78.1 (C-5‴,d), 77.9 (C-3‴, d), 77.2 (C-5″, d), 76.0 (C-2″,d), 74.2 (C-2‴, d), 71.1 (C-4″, d), 70.9 (C-4‴, d), 69.0 (C-6″, t), 62.6 (C-6″, t).

4*: Quercetin-3-O-α-L-rhamnopyranosyl-(1‴ 6″)-O-β-D-glucopyranoside.* Light-yellow amorphous powder. IR (KBr): 1650, 3380 (cm^{-1}). Gas chromatography analysis of peracetylated alditols showed the presence of rhamnose and glucose in 1:1 ratio. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.64 (dd, 1, J = 8.7 Hz,1.8 Hz, H-6′), 7.60 (d, 1, J = 1.8 Hz, H-2′), 6.89 (d, 1, J = 8.7 Hz, H-5′), 6.39 (d, 1, J = 2.0 Hz, H-8), 6.20 (d, 1, J = 2.0 Hz, H-6), 5.12 (d, 1, J = 7.5 Hz, H-1″), 5.42 (d, 1, J = 1.5 Hz, H-1‴), 3.12−4.24 (m, sugar protons), 1.11 (d, 3, J = 6.2 Hz, CH$_3$-6‴). ^{13}C NMR (125 MHz, CD$_3$OD): δ 179.2 (C-4, s), 165.9 (C-7,d), 162.7 (C-5, s), 159.4 (C-9, d), 158.3 (C-2, s), 149.7 (C-4′, s), 145.6 (C-3′, d), 135.7 (C-3, s), 123.3 (C-6′, d), 122.9 (C-1′, s), 117.8 (C-2′, d), 115.6 (C-5′, s), 105.7 (C-10, s), 104.8 (C-1″, d), 102.1 (C-1‴, d), 99.8 (C-6, s) 94.3 (C-8, s), 78.2 (C-3″,d), 77.2 (C-5″,d), 75.7 (C-2″, d), 74.1 (C-4‴, d), 72.5 (C-3‴, d), 72.0 (C-2‴, d), 71.1 (C-4″, d), 70.3 (C-5‴, d), 68.9 (C-6″, t), 17.9 (C-6‴, c).

5*: Quercetin-3-O-β-D-glucopyranoside.* Light-yellow amorphous powder. IR (KBr): 1650, 3380 (cm^{-1}). Gas chromatography analysis of peracetylated alditols showed the presence of glucose. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.64 (dd, 1, J = 8.7 Hz,1.7, H-6′), 7.58 (d, 1, J = 1.7 Hz, H-2′), 6.83 (d, 1, J = 8.7 Hz, H-5′), 6.36 (d, 1, J = 1.9 Hz, H-8), 6.20 (d, 1, J = 1.9 Hz, H-6), 5.20 (d, 1, J = 7.5 Hz, H-1″), 3.17−4.20 (m, sugar protons). ^{13}C NMR (125 MHz, CD$_3$OD): δ 179.4 (C-4, s), 165.8 (C-7,d), 162.9 (C-5, s), 159.3 (C-9, d), 158.5 (C-2, s), 149.8 (C-4′, s), 145.2 (C-3′, d), 135.6 (C-3, s), 123.4 (C-6′, d), 123.2 (C-1′, s), 118.0 (C-2′, d), 115.9 (C-5′, s), 105.9 (C-10, s), 104.3 (C-1″, d), 99.9 (C-6, s) 94.3 (C-8, s), 78.3 (C-5″,d), 78.1 (C-3″, d), 75.4 (C-2″, d), 71.3 (C-4″, d), 62.3 (C-6″, t).

6*: Kaempferol-3-O-β-D-glucopyranoside.* Light-yellow amorphous powder. IR (KBr): 1650, 3380 (cm^{-1}). Gas chromatography analysis of peracetylated alditols showed the presence of glucose. ^{1}H NMR (500 MHz, CD$_3$OD): δ 7.98 (br d, 2, J = 8.7 Hz, H-6′, H-2′), 6.85 (br d, 2, J = 8.6 Hz, H-3′, H-5′), 6.36 (d, 1, J = 2.1 Hz, H-8), 6.19 (d, 1, J = 2.1 Hz, H-6), 5.24 (d, 1, J = 7.4 Hz, H-1″), 3.17−4.23 (m, sugar protons). ^{13}C NMR (125 MHz, CD$_3$OD): δ 179.4 (C-4, s), 166.1 (C-7, d), 162.5 (C-5, s), 161.6 (C-4′, s), 159.5 (C-9, d), 158.2 (C-2, s), 135.4 (C-3, s), 132.8 (C-2′, d), 132.2 (C-6′, d), 122.6 (C-1′, s), 116.3 (C-3′, s), 116.2 (C-5′, s), 105.7 (C-10, s), 104.7 (C-1″, d), 99.6 (C-6, s), 94.9 (C-8, s), 78.3 (C-3″,d), 78.1 (C-5″, d), 75.6 (C-2″, d), 71.2 (C-4″, d), 62.2 (C-6″, t).

DISCUSSION

EtOAc/BuOH subextract as well as flavonoid derivatives isolated from *C. album* showed a concentration-dependent antiradical activity. Rutin (**4**), the most abundant glycoside in *C. album*, exhibited a very high DPPH scavenging activity, with an ED$_{50}$ value of 4.90 μg mL^{-1}. Triosides **1** and **2**, which are less active, showed ED$_{50}$ values of 6.50 and 6.90 μg mL^{-1},

respectively, both with free radical scavenging potential higher than that of BHT (ED $_{50}$: 10.8 μg mL^{-1}) (**Table 1**). All isolated compounds showed lower antioxidant activity than quercetin (ED$_{50}$: 2.63 μg mL^{-1}) and similar to that exhibited by ascorbic acid (ED$_{50}$: 5.94 μg mL^{-1}). Lower antioxidant activities were observed for kaempferol glycosides **2** and **6**. Quercetin glycosides were more active than the corresponding kaempferol derivatives, and all of them exhibited higher activity than the synthetic antioxidant BHT.

Flavonoid antioxidant activity has been studied by numerous authors, and many attempts have been made to establish a relationship between their structure and radical-scavenging potential (*28, 29, 7*). Radical scavenging activity depends on flavonoid molecular structure and particularly on hydroxyl group substitution pattern. A structural feature considered essential for it is the presence of an *o*-dihydroxy group (catechol structure) in the B ring, with electron donating properties. The presence of a hydroxyl group at C-3 in the C ring increases the antioxidant potential of flavonoids, and the α-β unsaturated carbonyl group also enhances the radical scavenging capacity of these compounds. Its simultaneous occurrence with hydroxyl groups at C-3 and C-5 contributes to the increase their potential as antioxidants (*7, 30*).

Sugar chain attachment occurring at C-3 (compounds **1**−**6**) seems to attenuate the free radical scavenging potential of aglycone. Since the flavonoid moiety is responsible for the free radical scavenging potential of these glycosides, its glycosilation, which increases their molecular weight, also contributes to lower the corresponding antioxidant activity.

C. album flavonoids increased their level in samples growing in continuously cultivated soils, particularly in their methanol extract. Even when similar amounts of this extract were obtained from samples collected in IC and ND soils, they exhibited significant differences in their antioxidant activity. Higher concentrations of flavonoid derivatives, in addition to *C. album* nutritional features, enhance the nutraceutical value of this edible weed when grown in IC soils.

Synthetic antioxidants demonstrated to be dangerous to human health, encouraging the search for naturally occurring antioxidants and flavonoids, are a group of natural products playing this role in plants. Species such as common weeds with nutritional quality are potential sources to find them. Current concerns about aging and diseases triggered by free radical action have also encouraged the addition of vegetables with significant amounts of flavonoids to the human diet. Numerous epidemiological studies demonstrated inverse associations between fruit and vegetable intake, and chronic diseases such as cancer and cardiovascular problems. Flavonoid derivatives, among other natural antioxidants, had been recognized as responsible for this protective effect. Our results suggest that *C. album*, an ethnic food containing important amounts of them, could be considered a functional food, particularly when growing in intensively cultivated soils. Flavonoid content was duplicated by soil deterioration, a common feature in most cropping lands. We also found that total phenolic content as much as the free radical scavenging potential is related to the relative amount of flavonoid glycosides in *C. album* samples, facts that characterize this edible weed as a potential renewable resource for obtaining natural antioxidants.

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