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The Phytochemical Profile and Antioxidant and Gastroprotective Effects of Three Varieties of *Chenopodium quinoa* Willd. Sprouts Cultivated in Peru

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Abstract: *Chenopodium quinoa* sprouts possess a superior nutritional profile relative to conventional quinoa seeds, which is mainly attributable to their germination process. Sprouting quinoa is able to preserve its substantial nutritional value while enhancing its bioavailability and digestibility. The aim of this study was to evaluate the gastroprotective effects of hydroalcoholic extracts of three varieties of quinoa sprouts (pasankalla, yellow maranganí, and black coito). The chemical compounds were determined using LC-MS (Liquid Chromatography–Mass Spectrometry). Antioxidant activity was determined using two analytical methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). To evaluate the gastroprotective effects of these hydroalcoholic extracts in Holtzman male rats, a gastric lesion was induced with 96% ethanol after the administration of the hydroalcoholic extract of the three varieties of *C. quinoa* sprouts. Our phytochemical analysis results reveal the presence of amino acids (valine, leucine, isoleucine, phenylalanine, tryptophane, proline, tyrosine, and arginine, among others) and their derivatives, organic acids, monosaccharides, lipids, nucleobases/nucleosides, steroids, triterpene saponins, and coumarins. The pasankalla, yellow maranganí, and black coito varieties exhibited antioxidant capacities of 36.70, 32.32, and 34.63 µmol Trolox equivalent (TE)/mg of extract for the DPPH radical and 56.61, 41.56, and 52.09 µmol TE/mg of extract for the ABTS radical, respectively. The percentage of antisecretory efficiency at a dose of 500 mg/kg for the pasankalla, yellow maranganí, and black coito varieties was 34.13%, 30.67%, and 26.67%, respectively, and the anti-ulcer effect, expressed as a percentage of inhibition of ulcer formation, was 74.7%, 67.4%, and 69.5%, respectively. In contrast, the groups treated with ranitidine and sucralfate exhibited percentages of 59.0% and 67.4%, respectively. The pasankalla quinoa exhibits more significant antioxidant activity and a stronger gastroprotective effect compared to the other varieties examined in this study. In conclusion, the hydroalcoholic extracts of the three varieties of *C. quinoa* sprouts exhibited a gastroprotective effect, and the pasankalla variety at a dose of 500 mg/kg exhibited a stronger protective effect on the gastric mucosa of the rats.

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1. Introduction

Gastrointestinal disorders remain a significant challenge in global healthcare, exhibiting no substantial reduction in their frequency or occurrence rates from 1990 to 2019 [1]. A common gastric disease affecting the general population is gastric ulcers, the pathogenesis of which involves an imbalance between mucosal defense factors (e.g., mucus secretion, bicarbonate production, and prostaglandin synthesis) and damage factors (e.g., increased acid secretion, *Helicobacter pylori* infection, and use of nonsteroidal anti-inflammatory drugs) [2]. Bioactive compounds found in plants have shown promise in managing gastric ulcers due to their diverse pharmacological properties. These natural compounds can exert gastroprotective effects through various mechanisms, including antioxidant activity, anti-inflammatory effects [3], the enhancement of mucosal defense [4], and the inhibition of *H. pylori* growth [5]. For instance, flavonoids and polyphenols from various plant sources have demonstrated the ability to increase mucus production, stimulate prostaglandin synthesis, and scavenge free radicals [6], thereby reducing oxidative stress in the gastric mucosa. The multifaceted approach of phytochemicals in targeting different aspects of gastric ulcer pathogenesis makes them promising candidates for both prevention and treatment strategies.

According to Regulation 208/2013, EC, the term "Sprouts" refers to "the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed" [7]. Moreover, sprouts can develop in varied conditions, including both light and dark environments [8], and are abundant in nutrients, including amino acids, peptides, carbohydrates, lipids, enzymes, phenolic compounds, flavonoids, minerals, and vitamins [9]; however, they exhibit low levels of anti-nutritional factors such as tannins, lectins, trypsin inhibitors, and galactosides [10]. Sprouts contain a wide variety of phytochemicals with bioactive properties. In cruciferous sprouts, such as broccoli, cabbage, and radish, glucosinolates (glucoraphanin and sinigrin) stand out, and they are metabolized into isothiocyanates such as sulforaphane and allyl-isothiocyanate, compounds recognized for their antioxidant and chemopreventive effects [11]. In legume sprouts, such as alfalfa, chickpeas, and lentils, high levels of flavonoids (kaempferol and quercetin), saponins (soyasaponins), and phenolic acids (ferulic acid and caffeic acid) are reported, which have anti-inflammatory and protective effects on the cardiovascular system [12]. Likewise, in cereal sprouts, such as wheat and oats, phenolic compounds (vanillic acid, syringic acid) and tocopherols (vitamin E) predominate [13,14]. Around the world, the use of sprouts has been gaining significant interest as of late, and countries such as those on the Asian continent represent the largest consumers since ancient times. However, their popularity has also grown in Western countries [15]. Furthermore, seed germination triggers various biochemical changes that modify the composition of primary and secondary metabolites. This process opens up avenues for future investigations exploring how specific seeds, such as quinoa sprouts, might offer unique health advantages [16].

In contrast, quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal widely used in the Andean region (Bolivia and Peru), classified under the *Chenopodiaceae* subfamily, and is characterized by its herbaceous nature [17]. The seeds produced by the quinoa plant are small, circular, and compressed, measuring between 1.5 and 4.0 mm in diameter and 0.5 mm in thickness. These seeds exhibit a diverse range of colors, including black, white, gray–purple, yellow, red, and violet. The process of distinguishing between quinoa

varieties relies on examining the morphological features of the plant and the coloration of both the plant itself and its seeds [18]. Quinoa is considered a "superfood" due to its high nutrient content, and it offers health-promoting qualities, such as anti-diabetic, antioxidant, anti-obesity, and cardioprotective effects [19]. In addition, the examination of quinoa sprouts has revealed the presence of phytocompounds such as phenolic acids, flavonoids, terpenoids, carbohydrates, saponins, and lipids [20]. In their study, Barakat et al. demonstrated an increase in the levels of lysine and other essential amino acids such as leucine, phenylalanine, threonine, and valine in these sprouts [21]. To date, there is insufficient research into the potential beneficial effects of quinoa sprouts. More recently, Al-Qabba et al. determined the potential antioxidant effect of red and yellow quinoa sprouts in a model of hepatoxicity induced with CCl_4 in rats [22].

Despite these benefits, the potential use of sprouted grains, particularly quinoa, in treating gastric ulcers remains underexplored. Investigating quinoa sprouts offers numerous potential benefits for researchers and consumers alike. Quinoa sprouts are rich in nutrients, containing higher levels of vitamins, minerals, and antioxidants compared to unsprouted quinoa seeds [23]. These sprouts may provide an enhanced bioavailability of nutrients, making them more easily absorbed by the body. Furthermore, studying quinoa sprouts may lead to the development of novel functional foods or nutraceuticals, contributing to advancements in nutrition science and public health initiatives. Hence, the aims of this study were as follows: (i) to determine the phytochemical profile of phytoconstituents using LC-MS analysis of three varieties of quinoa sprouts (pasankalla, yellow maranganí, and black coito), (ii) to determine the antioxidant activity using two methods (DPPH and ABTS), and (iii) to evaluate the gastroprotective effect of the hydroalcoholic extract using a preclinical model of gastric ulcer induced with ethanol in male Holtzman rats.

2. Materials and Methods

2.1. Collection of the C. quinoa Seeds

Quinoa seeds of the pasankalla, yellow maranganí, and black coito varieties were collected from the city of Chontaca, Acocro district, Huamanga province, in the department of Ayacucho, Peru (located at an altitude of 3125 m). The three varieties of quinoa sprouts are shown in Figure 1 and were certified by the Agrarian Research Institute (INIA, Ayacucho, Peru) with ID. 09/03/2020-INIA.

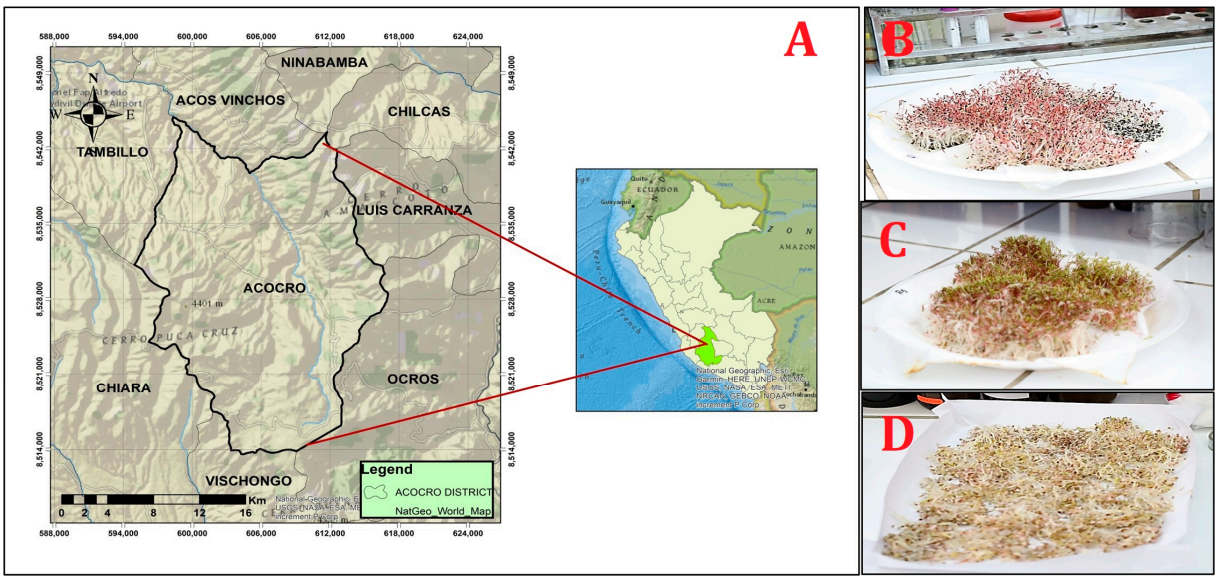

Figure 1. (**A**) Geographical location of the Acocro district. (**B**) Black coito, (**C**) yellow maranganí, and (**D**) pasankalla sprouts from *C. quinoa*.

2.2. Obtention of the C. quinoa Sprouts

The quinoa seeds (250 g) were washed and left in 0.02% sodium hypochlorite aqueous solution for two hours and then incubated at room temperature under shade in Styrofoam containers on filter papers (Whatman N°1) moistened with distilled water for 5 days. After incubation, the heights of sprouts of a representative sample of each variety were measured. Once the sprouts were obtained, they were dried in an oven with air ventilation at 40 °C until a constant weight was achieved. Thereafter, they were pulverized and stored in amber bottles under refrigeration conditions [20].

2.3. Preparation of the Hydroalcoholic Extract of C. quinoa Sprouts

The germinated seeds of each variety were crushed and macerated with 70% ethanol for 10 days with constant stirring at a ratio of 1:5 and then filtered and concentrated by rotary evaporation (Rotavapor® R-300 system, Buchi, Flawil, Switzerland). The concentrated extract was frozen at −60 °C for 24 h and sublimed at −80 °C for 48 h in a lyophilizer (Daihan Scientific, Model: FD-8 8LT/HR—90 °C, Gangwon, Korea). Lastly, each hydroalcoholic extract was stored in an amber bottle at 4 °C.

2.4. Phytochemical Analysis Through Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis of the Hydroalcoholic Extract of Three Varieties of C. quinoa Sprouts

2.4.1. Preparation of the Sample

A methanolic solution (2 mg/mL) of the hydroalcoholic extracts was prepared and centrifuged for 10 min at 10,000 rpm. Next, the supernatant was diluted to obtain a 1 mg/mL solution using a methanol–water (1:1) mixture. From this diluted solution, 800 µL of the supernatant was collected in vials for LC-MS analysis on a Dionex UltiMate 3000 liquid chromatograph (Thermo Fisher Scientific, Bremen, Germany) coupled to a Thermo QExactiveTM mass spectrometer. A Plus Orbitrap (Thermo Fisher Scientific, Bremen, Germany) with an electrospray ionization source was also used.

2.4.2. Chromatographic Parameters

In our analysis, we employed an XBridge Amide BEH water column (150 mm × 4.6 mm × 3.5 µm). The mobile phases consisted of Solvent A (0.1% formic acid in water) and Solvent B (0.1% formic acid in ACN). The elution gradient was programmed as follows: 0–2 min, 95% B; 2–17.0 min, 50% B; 17–20.0 min, 50% B; 20.0–21.0 min, 95% B; 21.0–27.0 min, 95% B. The flow rate was set at 500 µL/min, with an 8 µL injection volume and a column temperature of 40 °C [20].

2.4.3. Mass Spectrometry Settings

A combined full scan and fragmentation (MS/MS) experiment was conducted using both positive and negative electrospray ionization modes (ESI+ and ESI−). The ESI source was configured with the following parameters: spray voltage—3.9 kV (+) and 3.6 kV (−); sheath gas flow rate—50 (arbitrary units); auxiliary gas flow—10 (arbitrary units); tube lens voltage—50 V; probe heater temperature—400 °C; capillary temperature—300 °C.

1. (. ESI+) mode: Full MS settings, resolution of 35,000; automatic gain control (AGC) target, 5×10^5; maximum injection time (IT), 100 ms; scan range, 100–1200 m/z.

Data-dependent acquisition (DDA) MS^2 settings: resolution of 17,500; AGC target, 1×10^5; maximum IT, 50 ms; loop count, 3; isolation window, 1–2 m/z; topN, 3; stepped normalized collision energy (NCE), 15, 30, and 40.

2. (. ESI−) mode: Full MS settings, resolution of 35,000; AGC target, 5×10^5; maximum IT, 100 ms; range, 100–1200 m/z.

Data-dependent acquisition (DDA) MS2 settings: resolution of 17,500; AGC target, 1×10^5; maximum IT, 50 ms; loop count, 3; isolation window, 1–2 m/z; topN, 3; NCE, 15, 20, and 40.

Data acquisition and processing were performed using Thermo XcaliburTM software version 3.0 (Thermo Fisher Scientific Inc., Waltham, MA, USA) with the Qual Browser. Metabolite annotations were carried out using MS-Dial software version 4.70 (Riken, Osaka University, Suita City, Japan) in conjunction with the MS-Dial metabolomics MPS spectral kit library (accessible at: https://systemsomicslab.github.io/compms/index.html; last updated on 8 August 2024) [20].

2.5. Determination of Antioxidant Capacity Using the Free Radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

First, 150 µL methanolic solutions of the hydroalcoholic extract at concentrations of 5.0, 10.0, and 20.0 mg/mL were mixed with 2850 µL of the methanolic DPPH solution with an absorbance of 0.6 ± 0.02. The samples were then incubated in the dark for 30 min. Absorbance was measured at 517 nm using a GENESYS 10 UV-VIS spectrophotometer (Thermo Scientific, Waltham, MA, USA) [24]. A standard curve was prepared with Trolox at concentrations ranging from 0 to 800 µM. Trolox equivalent antioxidant capacity (TEAC) was calculated using the following formula and expressed as µmol Trolox equivalent (TE)/mg extract:

$$\mathrm{TEAC_{DPPH}} \frac{\mu\mathrm{mol\ TE}}{\mathrm{mg}} = \mathrm{EC_{50}\ TROLOX\ (\mu M)/EC_{50}extract\ (mg/mL)}$$

To calculate the half-maximal inhibitory concentration (EC_{50}), a curve of the percentage of inhibition versus the concentration of the methanolic extract was plotted. The inhibition percentage was calculated using the following formula:

$$\%\ \mathrm{inhibition} = (\mathrm{AbC} - \mathrm{AbS})/\mathrm{AbC} \times 100$$

where AbC is the absorbance of the control and AbS is the absorbance of the sample.

2.6. Determination of Antioxidant Capacity Using the Sequestration Method with the Radical Cation of the 2.2′-Azinobis-(3-ethylbenzothiazoline)-6-sulfonic Acid (ABTS)

A stock ethanolic solution of 7 mM ABTS was prepared. Thereafter, potassium persulfate was added to produce a final stock solution of 2.45 mM and allowed to react for 12–16 h at room temperature in the dark. To evaluate the antioxidant capacity of the ABTS radical, 150 µL of the extract (1.0, 5.0, and 10.0 mg/mL) was mixed with 2850 µL of ABTS radical with an absorbance of 0.7 ± 0.02 nm and incubated in the dark for 7 min, and absorbance was read at 734 nm [25]. A standard curve was prepared with Trolox at concentrations ranging from 0 to 800 µM. The Trolox equivalent antioxidant capacity (TEAC) was expressed as µmol TE/mg extract.

$$\mathrm{TEAC_{ABTS}\ (\mu mol\ TE/(mg) = EC_{50}\ TROLOX\ (\mu mol/mL)/EC_{50}extract\ (mg/mL)}$$

To calculate the half-maximal effective concentration (EC_{50}), a curve of the percentage of inhibition versus the concentration of the methanolic extract was plotted. The inhibition percentage was calculated using the following formula:

$$\%\ \mathrm{inhibition} = (\mathrm{AbC} - \mathrm{AbS})/\mathrm{AbC} \times 100$$

where AbC is the absorbance of the control and AbS is absorbance of the sample.

2.7. Animals

Male Holtzman rats (160~180 g, 12 weeks old) were acquired from the bioterio of the National Institute of Health (Chorrillos, Lima, Peru). The rats were maintained in polypropylene cages in controlled conditions (temperature 22~26 °C, humidity 60~70%, alternating 12 h light and 12 h dark), and they were fed a normal rodent diet and had ad libitum access to water. The animal experiment was performed in accordance with guidelines established by the ethical committee of the Universidad San Cristóbal de Huamanga and approved with ID 006-VRI-UNSCH.

2.8. Ethanol-Induced Gastric Ulcer

The protocol was performed according to the method described by Arroyo et al. [26]. The animals were acclimatized for 10 days in a bioterio under standard conditions and randomized into 13 treatment groups of 8 animals per group:

- Group I, control—physiological saline (PS), 4 mL/kg;
- Group II, 96% ethanol (1 mL/animal);
- Group III, Ranitidine 100 mg/kg;
- Group IV, Sucralfate 50 mg/kg;
- Group V, rats administered a dose of 125 mg/kg of the hydroalcoholic extract of pasankalla quinoa;
- Group VI, rats administered a dose of 250 mg/kg of the hydroalcoholic extract of pasankalla quinoa;
- Group VII, rats administered a dose of 500 mg/kg of the hydroalcoholic extract of pasankalla quinoa;
- Group VIII, rats administered a dose of 125 mg/kg of the hydroalcoholic extract of yellow maranganí quinoa;
- Group IX, rats administered a dose of 250 mg/kg of the hydroalcoholic extract of yellow maranganí quinoa;
- Group X, rats administered a dose of 500 mg/kg of the hydroalcoholic extract of yellow maranganí quinoa;
- Group XI, rats administered a dose of 125 mg/kg of the hydroalcoholic extract of black coito quinoa;
- Group XII, rats administered a dose of 250 mg/kg of the hydroalcoholic extract of black coito quinoa;
- Group XIII, rats administered a dose of 500 mg/kg of the hydroalcoholic extract of black coito quinoa.

One hour before the administration of 96% ethanol, the extracts were dissolved in physiological saline and orally administered to rats in each group at a single dose. After 60 min of ethanol administration, the animals were intraperitoneally euthanized with the administration of 100 mg/kg sodium pentothal (Halatal®, Montana Laboratory, Lima, Peru) as indicated by the guidelines of the Federation for Laboratory Animal Science Associations (FELASA). The stomach was removed from each rat, the gastric juice volume was measured, and the pH was determined with a Hanna Instrument brand pH meter, washed with physiological saline, and finally spread on a technopor to evaluate lesions on the gastric mucosa. Ulcer index (UI) scores were calculated using an arbitrary scale based on the method of Zhou et al. [27], which is based on the number of lesions that have occurred in the gastric mucosa, as follows: no lesions (normal stomach) = 0; hyperemia = 0.5–1; hemorrhagic spots = 1–2; 1–5 small ulcers = 2–3; several small ulcers = 3–4; 1–5 small and 1–3 large ulcers = 4–5; several small and large ulcers = 5–6; stomach full of ulcers or perforations = 6.

The percentage of ulcer inhibition (% UI) was calculated using the following formula:

$$\%UI = \frac{UIc - UI_P}{UIc} \times 100$$

where UI_C is the mean ulcer index of the control group and UI_P is the mean ulcer index of the experimental group with different treatments.

2.9. Histopathological Analysis

For histopathological analysis, the stomach samples were preserved in 10% formalin. Using a microtome, sections of 3–4 µm thickness were prepared and placed in a cassette holder, following a phase of ascending 85% ethanol until absolute ethanol was used. The samples were subsequently rinsed with xylol and the tissues were fixed and stained with hematoxylin and eosin. Lastly, the samples were analyzed microscopically and photographed [28].

2.10. Statistical Analysis

All assays for antioxidant compounds were performed in triplicate. The results are expressed as mean ± standard deviation. Differences between the means (using Student's *t*-test) of the following variables were assessed: the ability to scavenge DPPH and ABTS free radicals. Differences were considered significant when the *p*-value was <0.05. The gastroprotective effects in the animals are presented in tables and figures, and statistical evaluation of these results was carried out in a database using GraphPad Prism v4.0. Analysis of variance (ANOVA) and Tukey's multiple comparison test were used to detect possible differences between treatments, with a significance level of 5% ($\alpha = 0.05$).

3. Results

3.1. Chromatographic Analysis Through LC-MS Analysis of the Hydroalcoholic Extract of Three Varieties of C. quinoa

Phytochemical analysis was performed via LC-MS analysis for the three varieties of quinoa sprouts, as shown in Supplementary Material Tables S1–S3 and their chromatograms in both ESI (−) and ESI (+) ionization mode in Figures 2 and 3.

Our results indicate that the black coito extract contained 36 phytoconstituents, as visualized in Table S1, of which 25 were observed in ESI (−) and 11 were observed in ESI (+). They were classified as (i) primary metabolites, such as amino acids and derivatives (*n* = 14), sugars and sugar alcohols (*n* = 5), lipids (*n* = 2), and nucleobases/nucleosides (*n* = 4), and (ii) secondary metabolites, such as triterpene saponins (*n* = 5), coumarins (*n* = 1), steroids (*n* = 1), and others (*n* = 4).

Here, 30 phytoconstituents were identified in the pasankalla extract, of which 23 were observed in ESI (−) and 7 were observed in ESI (+), as presented in Table S2. They were classified as (i) primary metabolites, such as amino acids and derivatives (*n* = 10), organic acids (*n* = 3), sugars and sugar alcohols (*n* = 5), and nucleobases/nucleosides (*n* = 2), and (ii) secondary metabolites, such as triterpene saponins (*n* = 4), steroids (*n* = 1), coumarins (*n* = 1), and other compounds (*n* = 3).

Here, 24 compounds were identified in the maranganí extract, of which 17 were observed in ESI (+) and 7 were observed in ESI (−). They were classified as (i) primary metabolites, such as amino acids and derivatives (*n* = 13), organic acids (*n* = 1), and nucleobases/nucleosides (*n* = 1), and (ii) secondary metabolites, such as triterpenoid saponins (*n* = 6) and others (*n* = 5).

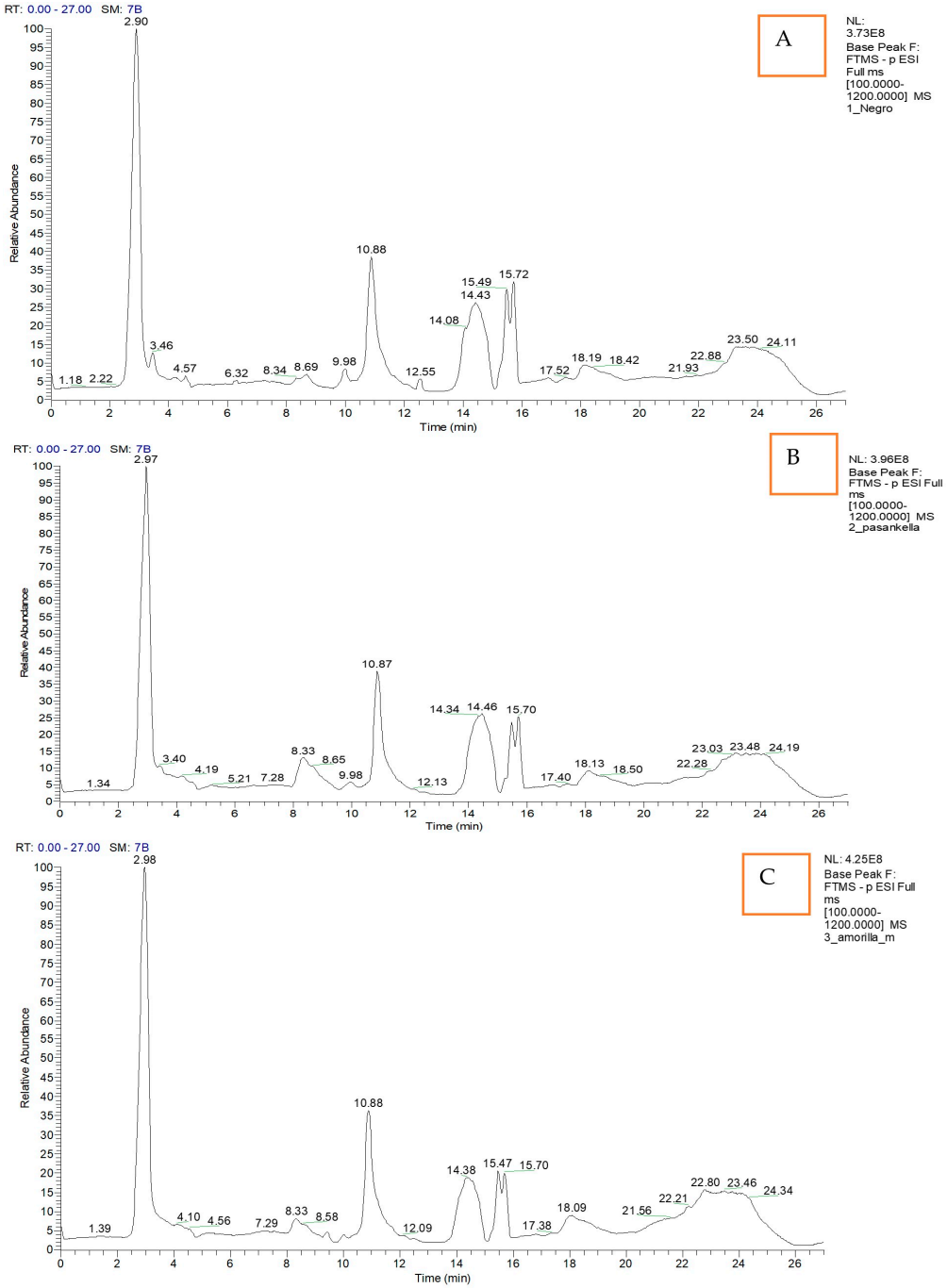

Figure 2. Chromatographic profile (LC-MS) of the hydroalcoholic extract *of C. quinoa* sprouts. (**A**) Black coito, Rt (min)—10.88, N-(2-Furoyl) glycine; 14.08, 3-Glu-(1-2) Ara-28-Glu Hederagenin; 14.43, sorbose; 15.49, 7-Hydroxy-4-methylcoumarin-3-acetic acid; 15.72: Xylose. (**B**) Pasankalla, Rt (min)—10.97, $C_{36}H_{56}O_{10}$; 14.34, Purine; 15.70, glucose. (**C**) Yellow maranganí, Rt (min)—10.88, 2S,3S,4S,5R,6R)-6-[[(3S,6aR,6bS,8aS,14bR)-8a-carboxy-4-(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl]oxy]-3,4,5-trihydroxyoxane-2-carboxylic acid in ESI (−) ionization mode.

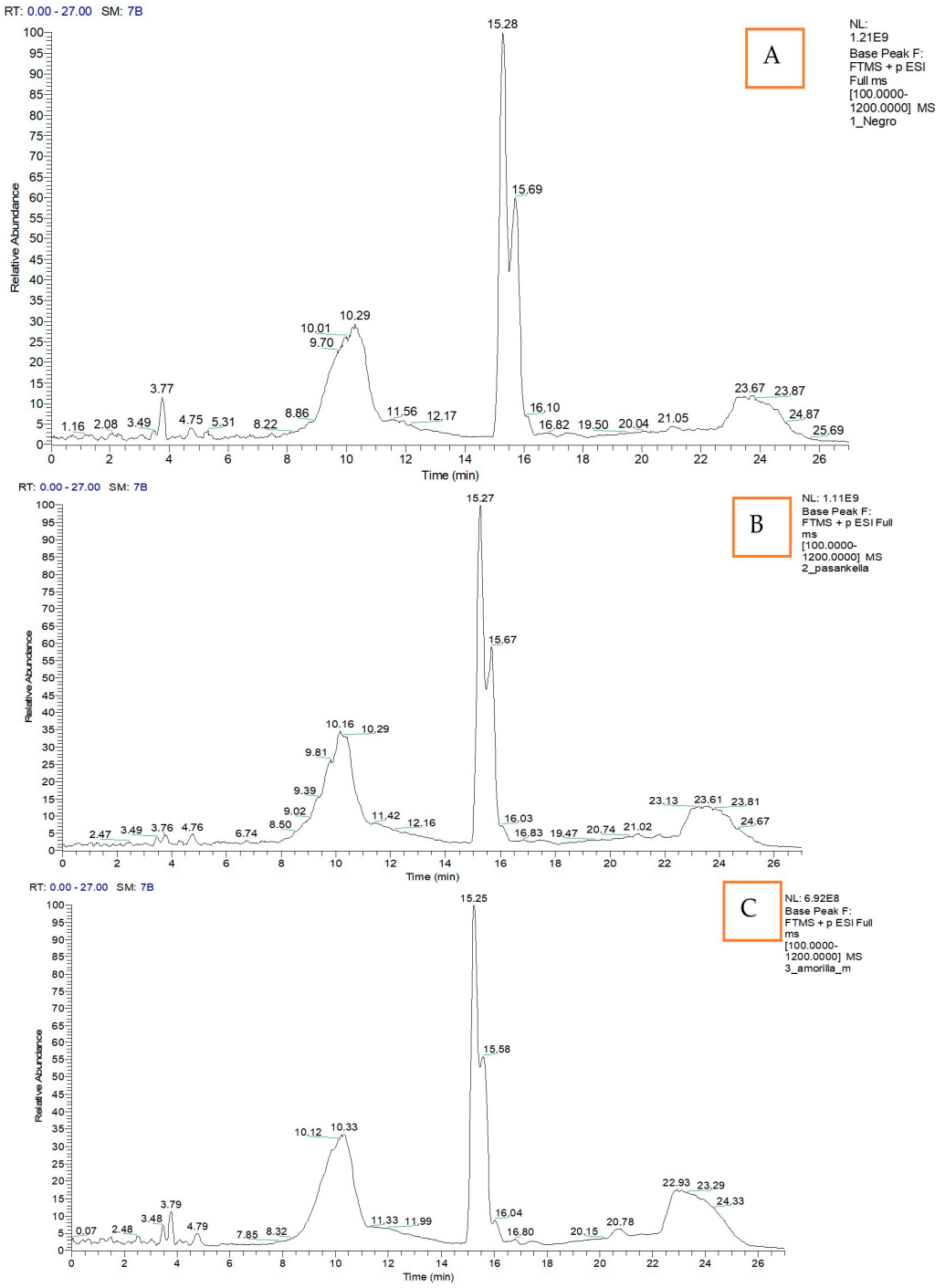

Figure 3. Chromatographic profile (LC-MS) of the hydroalcoholic *extract of C. quinoa* sprouts. (**A**) Black coito, Rt (min)—15.28, betaine; 15.69, choline. (**B**) Pasankalla, Rt (min)—15.27, valine. (**C**) Yellow maranganí, Rt (min)—15.25, betaine; 15.58, choline in ESI (+) ionization mode.

Based on the results presented in Figure 4, the dendrogram shows how the quinoa varieties (yellow maranganí, black coito, and pasankalla) are grouped according to similarity in their metabolite profiles. The results suggest that varieties that are closer to one another in the dendrogram share similar chemical compositions. In this instance, the varieties pasankalla and black coito share similar metabolites such as triterpenoids and amino acids. More dissimilar varieties are connected by longer branches, such as the yellow maranganí with the other analyzed varieties.

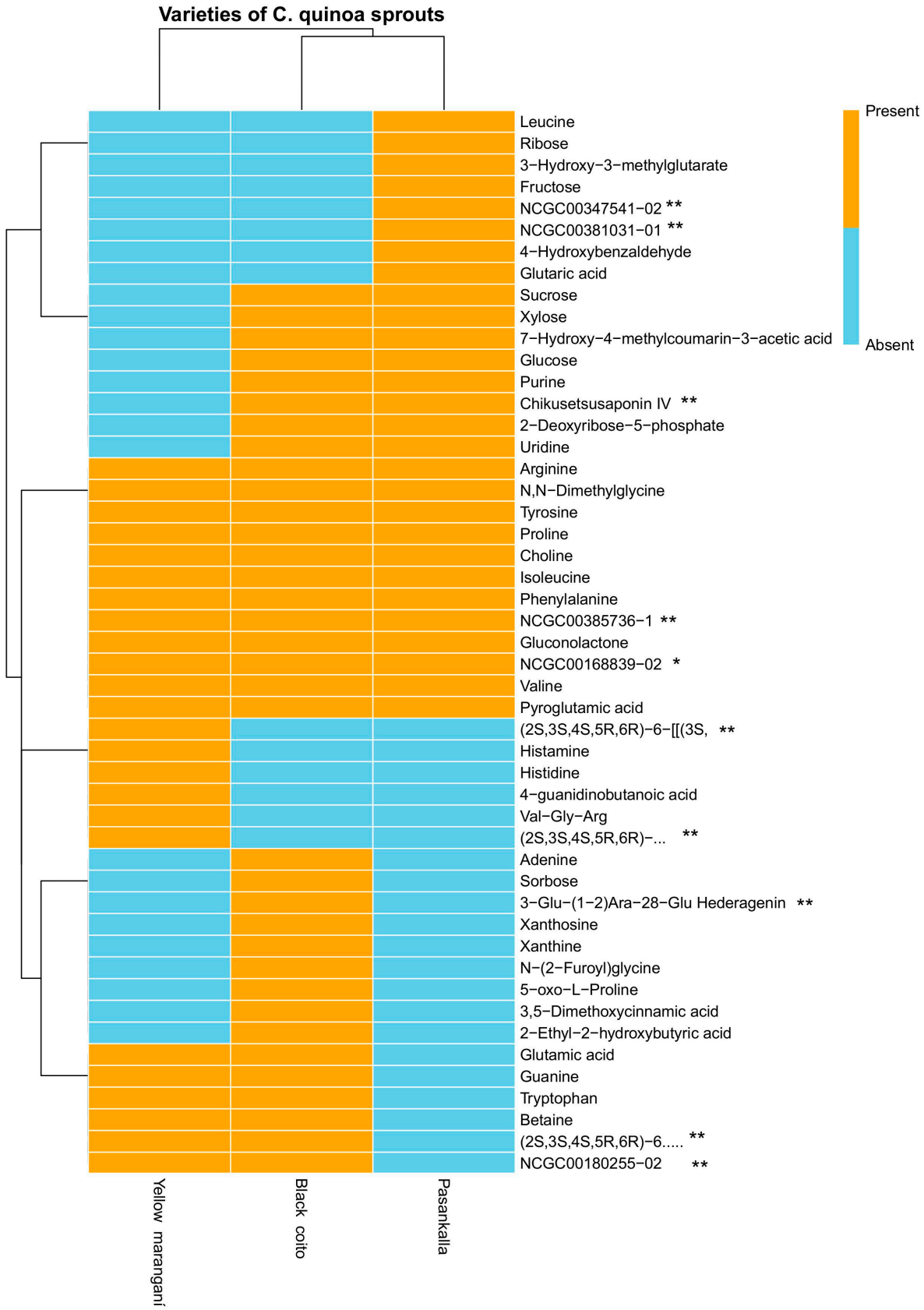

Figure 4. Heatmap visualization showing both the chemical compounds shared and not shared among the three varieties of *C. quinoa* sprouts: yellow maranganí, black coito, and pasankalla. * Steroid saponins; ** triterpenoid saponins.

3.2. Antioxidant Activity of the Hydroalcoholic Extract of the Three Varieties of C. quinoa

Table 1 shows the TEAC of the hydroalcoholic extracts of the germinated seeds of the pasankalla, yellow maranganí, and black coito varieties with the DPPH radical, which are 36.70 ± 0.73, 32.32 ± 0.41, and 34.63 ± 0.16 µmol TE/mg, and for the ABTS radical they are 56.61 ± 1.3, 41.56 ± 0.66, and 52.09 ± 0.79 µmol TE/mg, respectively. The pasankalla variety had the highest antioxidant capacity using both methods, followed by the black and yellow varieties (ANOVA test; $p < 0.0001$).

Table 1. Trolox equivalent antioxidant capacity (TEAC) of DPPH and ABTS radicals from the hydroalcoholic extract of quinoa sprouts.

Variety	TEAC (µmol TE/mg) *	
	DPPH Mean ± SD	**ABTS Mean ± SD**
Pasankalla	36.70 ± 0.73	56.61 ± 1.3
Yellow maranganí	32.32 ± 0.41	41.56 ± 0.66
Black coito	34.63 ± 0.16	52.09 ± 0.79
p-value (ANOVA test)	$p < 0.0001$	$p < 0.0001$

* The results are shown as the mean ± standard deviation (SD) of three determinations.

3.3. Evaluation of the Anti-Ulcer Effect of the Hydroalcoholic Extract of Three Varieties of C. quinoa Sprouts

The results presented in Figure 5 show the volume and pH of the gastric contents, in addition to the ulcer index of the experimental groups. Regarding the volume values, the group treated with sucralfate showed the lowest values compared to the animals that received 96% ethanol ($p = 0.0212$); in comparison, the results for the other groups were not significant. Conversely, the percentage of pH efficiency at a dose of 500 mg/kg for the pasankalla, yellow maranganí, and black varieties was 34.13%, 30.67%, and 26.67%, respectively, compared to the control group. Furthermore, these values were lower than those for ranitidine and sucralfate (43.68% and 39.44%, respectively). However, rats in the pasankalla group administered a dose of 250 mg/kg and ranitidine exhibited increased pH values of 4.41 ± 0.23 ($p = 0.0021$) and 4.82 ± 0.51 ($p = 0.0208$) compared to the group who developed gastric ulcers (96% ethanol). In the other evaluations, the pasankalla group administered a dose of 125 mg/kg exhibited a significant increase in pH values compared to the ranitidine group ($p = 0.0135$); however, the results for the other groups treated with the other varieties of quinoa at different doses were not significant.

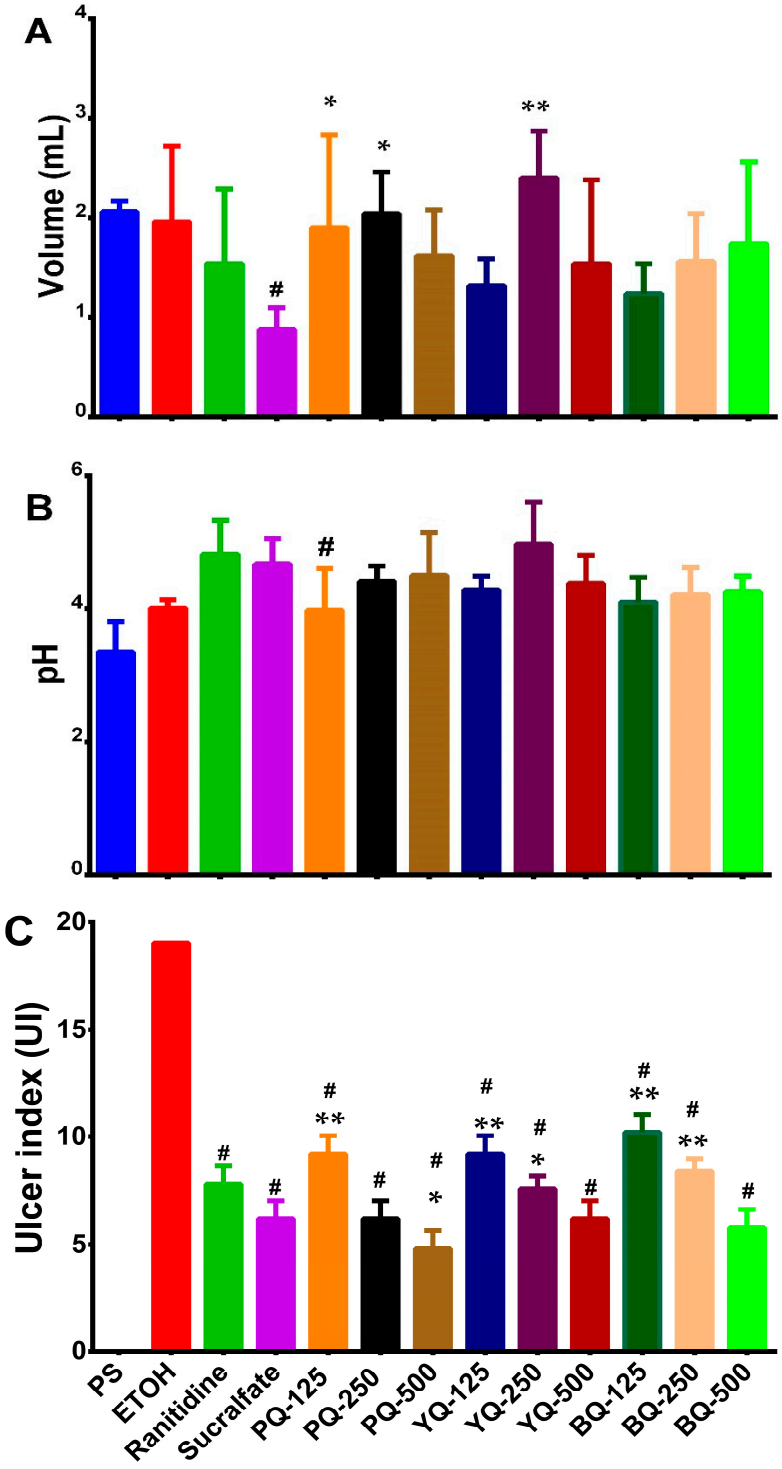

Figure 5. Effects of ranitidine, sucralfate, and quinoa sprouts in rats exposed to ethanol (n = 8 per group). (**A**) Gastric juice volume, (**B**) pH of gastric contents, and (**C**) ulcer index. Data are expressed as the mean ± standard deviation. * Significant difference at $p < 0.01$ and ** at $p < 0.0001$ compared with the sucralfate group. # Significant difference at $p < 0.0001$ compared with the ethanol group. Note: PS, physiological saline, 4 mL/kg; ETOH, 96% ethanol (1 mL/animal); PQ-125, pasankalla quinoa 125 mg/kg; PQ-250, pasankalla quinoa 250 mg/kg; PQ-500, pasankalla quinoa 500 mg/kg; YQ-125, yellow maranganí quinoa 125 mg/kg; YQ-250, yellow maranganí quinoa 250 mg/kg; YQ-500, yellow maranganí quinoa 500 mg/kg; BQ-125, black coito quinoa 125 mg/kg; BQ-250, black coito quinoa 250 mg/kg; BQ-500, black coito quinoa 500 mg/kg.

Additionally, the ulcer index scores varied, with values ranging from 0 to 19, indicating the degree of protection or damage to the stomachs isolated from the animals. In the evaluation, the group treated with pasankalla, black coito, and yellow maranganí at a dose of 500 mg/kg exhibited values ranging between 4.8 ± 0.54 ($p < 0.0001$), 5.8 ± 0.84 ($p < 0.0001$), and 6.2 ± 0.84 ($p < 0.0001$), respectively, with these values being lower than those of the positive control group treated with 96% ethanol. Furthermore, rats treated with the pasankalla variety presented greater ulcerogenic inhibition (74.7%), followed by black coito (69.5%) and yellow maranganí (67.4%), at a dose of 500 mg/kg, whose rate of inhibition was higher than those of ranitidine and sucralfate (59.0% and 67.4%, respectively).

Figure 6 shows the results of the histopathological study of the stomachs, in which rats in the control group (Figure 6A) presented no lesions; rats in the group treated with 96% ethanol (Figure 6B) presented with acute hemorrhagic necrosis (red arrow); rats in the group treated with ranitidine (Figure 6C) presented gastric mucosal hyperplasia (red arrow); rats in the group treated with sucralfate (Figure 6D) presented mild chronic (red arrow) and acute erosive gastritis (green arrow); rats in the groups treated with the hydroalcoholic extracts of the pasankalla variety at a dose of 125 mg/kg (Figure 6E) presented moderate erosive gastritis (red arrow) and mild chronic submucosal inflammation (blue arrow), at 250 mg/kg (Figure 6F) they presented moderate acute erosion (red arrow) and necrosis of the apical mucosa (yellow arrow), and at a dose of 500 mg/kg (Figure 6G) they presented mild erosion (red arrow). Rats treated with the yellow maranganí variety at a dose of 125 mg/kg (Figure 6H) presented mucosal hyperplasia (red arrow); at a dose of 250 mg/kg (Figure 6I), they presented severe hemorrhagic necrosis (red arrow), mild chronic submucosal inflammation (blue arrow), and severe hemorrhagic gastritis (green arrow); and at a dose of 500 mg/kg (Figure 6J), they presented moderate chronic submucosal inflammation (red arrow). The rats treated with the black variety at the dose of 125 mg/kg (Figure 6K) presented moderate hemorrhagic necrosis (red arrow), while at the dose of 250 mg/kg (Figure 6L), they presented moderate hemorrhagic necrosis (red arrow), mild dispersed chronic submucosal inflammation (green arrow), and moderate acute hemorrhagic gastritis (blue arrow), and at 500 mg/kg (Figure 6M), they presented mild acute necrosis (red arrow), mild submucosal inflammation (yellow arrow), and moderate hemorrhagic mucosal necrosis (blue arrow).

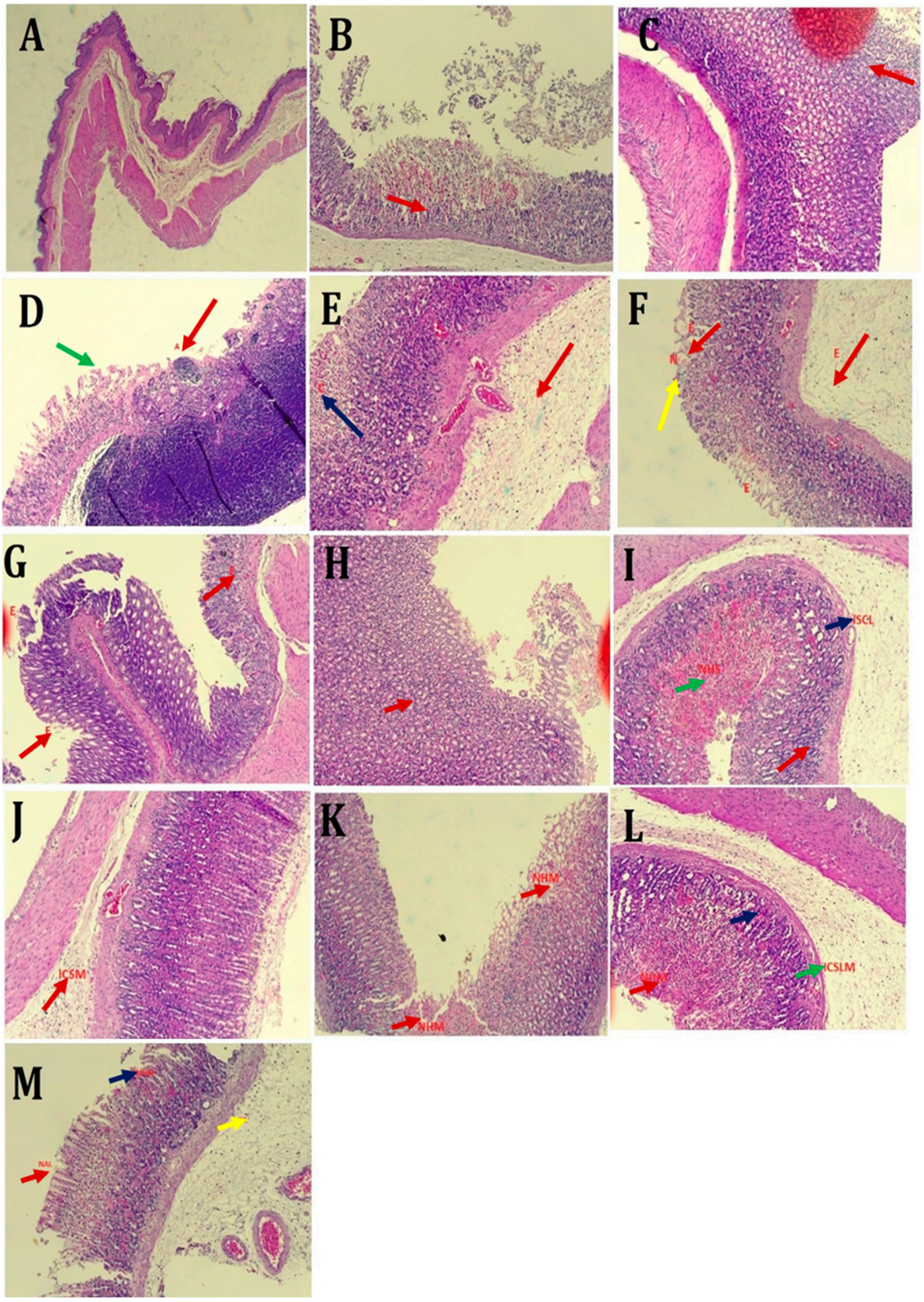

Figure 6. Effects of ranitidine, sucralfate, and quinoa sprouts on histopathological lesions in the gastric mucosa of rats exposed to ethanol (H&E staining, 10×) (n = 8 per group). (**A**) PS. 4 mL/kg, (**B**) 96% ethanol (E), (**C**) (E) + ranitidine 100 mg/kg, (**D**) (E) + sucralfate 50 mg/kg, (**E**) (E) + pasankalla quinoa extract 125 mg/kg, (**F**) (E) + pasankalla quinoa extract 250 mg/kg, (**G**) (E) + pasankalla quinoa extract 500 mg/kg, (**H**) (E) + yellow maranganí quinoa extract 125 mg/kg, (**I**) (E) + yellow maranganí quinoa extract 250 mg/kg, (**J**) (E) + yellow maranganí quinoa extract 500 mg/kg, (**K**) (E) + black coito quinoa extract 125 mg/kg, (**L**) (E) + black coito quinoa extract 250 mg/kg, and (**M**) (E) + black coito quinoa extract 500 mg/kg.

4. Discussion

Based on the results of the phytochemical analysis of the three varieties of quinoa sprouts, the phytoconstituents found in the LC-MS analysis are similar to those found in our previous study on the methanolic extract of the pasankalla variety, in which saponins, sugars, amino acids (valine, pyroglutamic acid, 5-oxo-L-proline, phenylalanine, tryptophan, isoleucine, leucine, proline, tyrosine, glutamic acid, arginine, and histidine), and higher antioxidant activity were noted [20]. In addition, based on the results visualized in the heat map, the yellow maranganí and pasankalla varieties show a greater degree of similarity in their phytochemical compounds. Conversely, the heat map shows a comparison of the phytochemical compounds in the different varieties of quinoa sprouts. Orange colors indicate the presence of a compound, whereas sky-blue colors indicate their absence. Some metabolites, such as the triterpenoid saponins and amino acids (leucine, valine, etc.), show clear patterns of abundance that may be characteristic of certain varieties. The variety black coito has a higher abundance of triterpenoids than yellow maranganí; this finding could be related to its antioxidant or bittering properties. In addition, the map reinforces the clusters in the dendrogram, showing how chemical profiles match the proximity of varieties. These findings may indicate specific metabolic associations, such as higher saponin content in certain varieties compared to others rich in amino acids. However, some phenolic compounds and flavonoids were not detected in the hydroalcoholic extracts of any of the varieties, in contrast with the methanolic extract used in our previous study mentioned above. This finding might be explained by the germination time and other conditions, such as the seed varieties used in this study, which lacked certain phenolic compounds. In their study, Lan et al. revealed that the germination time of 72 h enhanced the phenolic content of some varieties of quinoa compared to 48 h of germination; in this study, however, the total germination time was five days, which could have resulted in a decreased presence of some phytoconstituents and, overall, flavonoids [29]. The results of the study by Guajardo-Flores et al. confirm that in black bean sprouts, a germination time of 24 h is optimal for determining the major quantity of flavonoids and saponins [30], in contrast with five days for the germination process. Moreover, quinoa contains a diversity of nutrients and chemical compounds, which vary between quinoa seeds of different colors, such as pasankalla and black varieties, which have been demonstrated to possess the highest contents of phenolic compounds and flavonoids [31]. In addition, sprouted seeds improve their nutritional and medicinal properties by increasing their phenolic compound and flavonoid content [32].

In this study, the in vitro antioxidant activity against DPPP and ABTS radicals exhibited a stronger effect in the pasankalla variety followed by the black coito and yellow maranganí, with these results being similar to those reported by Enciso et al., with values ranging between 37.65 $\pm$ 0.88 for pasankalla, 27.51 $\pm$ 0.29 for yellow maranganí, and 28.04 $\pm$ 0.10 µmol TE/mg ethanol extract for black coito in the DPPH assay; however, they differed in the ABTS assay, with values ranging between 78.79 $\pm$ 0.86 for pasankalla, 78.11 $\pm$ 1.69 for yellow maranganí, and 69.41 $\pm$ 0.87 µmol TE/mg for black coito [20], with these values being lower than in our findings based on the results presented in Table 1. Although the freeze-drying method is effective for preserving the phenolic and antioxidant activities of fruits [33], other factors such as the conditions during the germination process might have decreased the TEAC, such as the absence of flavonoids, which were not identified in the phytochemical analysis of the hydroalcoholic extracts.

In contrast, ethanol has been demonstrated to cause damage to the gastric mucosa, in both preclinical and clinical studies, primarily through the generation of severe gastric hemorrhagic lesions [34]. Therefore, in this study, the model of ethanol-induced gastric lesions was used in rats and was directly associated with increased reactive oxygen species

(ROS) levels, which cause lipid peroxidation in the membranes via the oxidation of unsaturated fatty acids [35]. The results of studies involving the use of alternative models have shown that absolute ethanol increases the concentration of inflammatory indicators such as TNF-α, NF-κB, COX-2, and iNOS in rat stomach tissues, with accompanying decreases in the gastric levels of superoxide dismutase, catalase, and glutathione peroxidase [36]. In contrast, antioxidants can eliminate free radicals, preventing gastric ulcers [37], and generally trigger antioxidant activity through several mechanisms [38], such as the chelation of free radicals and ROS, blocking lipid peroxidation [39], or promoting the secretion of mucus and bicarbonate [40] and its anti-inflammatory effect [41].

With regard to gastroprotective effects, the results of this study show differences in terms of mucosal gastric protection in the groups treated with quinoa sprouts compared to the group exposed to ethanol, such as the increased pH, a decrease in volume and content, a reduction in the ulcer index, and histological findings. Furthermore, in their study, Wang et al. [42] demonstrated the gastroprotective effect of quinoa seeds through their anti-inflammatory and antioxidant effects. The anti-ulcer and antisecretory activities of the three varieties of quinoa sprouts are supported by the results of a histopathological study that showed the protection of the mucosal layer against ulcers and inflammation. Although we did not quantify the levels of each amino acid in this study, several amino acids might act as anti-ulcer agents. In studies examining amino acids, they have been found to exhibit anti-ulcer effects, such as L-lysine [43], due to their antioxidant activity. L-arginine exhibited an anti-ulcer effect on mucosal injury induced by indomethacin, and its main mechanism involves the enhancement of mucosal nitric oxide/PGE2 content, inhibiting gastric inflammation and oxidative stress [44]. In another study, histidine, arginine, and lysine increased the anti-inflammatory activity of plant-derived bioactive peptides modulating COX-2, iNOS, IL-6, and TNF-α in RAW264.7 macrophages [45]. Leucine and isoleucine modulate the PI3K/Akt signaling pathway and ERK kinases in the downstream MAPK pathways, resulting in anti-inflammatory effects [46]. Other amino acids such as phenylalanine and tryptophan have been shown to stimulate gastric acid secretion [47], which would exert contrasting gastroprotective activity.

Saponins, a diverse group of naturally occurring compounds found in various plant species, have garnered significant attention owing to their potential gastroprotective effects. These bioactive molecules, which are characterized by their amphipathic nature and ability to form stable foams in aqueous solutions, have demonstrated promising results in protecting the gastric mucosa against various ulcerogenic factors [48]. The gastroprotective properties of saponins are attributed to multiple mechanisms, including the enhancement of mucus production, the inhibition of gastric acid secretion, and the modulation of inflammatory responses such as chikusetsusaponin IV [49] and oleane-12-en-3β-ol-28-oic acid 3β-D-glucopyranoside [50]. Additionally, saponins have been shown to possess antioxidant properties that contribute to their ability to neutralize free radicals and reduce oxidative stress in the gastric environment. The authors of several studies have reported the efficacy of saponins derived from plants, such as ginseng [51], licorice [52], and quinoa [53], in alleviating gastric ulcers and promoting mucosal healing. A growing body of evidence supporting the gastroprotective effects of saponins highlights their potential as natural therapeutic agents for the prevention and treatment of gastrointestinal disorders.

Based on the results observed in our study, the pasankalla variety exhibits more significant antioxidant activity and gastroprotective effects than the other varieties, black coito or yellow maranganí, and these results are correlated with its antioxidant activity. The profile of phytochemical compounds identified in pasankalla differed from those of black coito and yellow maranganí in the presence of two saponins named olean-12-en-28-oic acid, 3-(beta-D-glucopyranuronosyloxy)-23-hydroxy-, (3beta,5xi,9xi,18xi)- (1)

and (2S,3R,5R,10R,13R,14S,17S)-2,3,14-trihydroxy-10,13-dimethyl-17-[(2R,3R)-2,3,6-trihydroxy-6-methylheptan-2-yl]-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one, as well as the amino acid leucine, which is an essential amino acid not produced by animals. Leucine undergoes substantial metabolic processes in the small intestine's absorptive epithelial cells. Studies have revealed its influence on the proliferation of intestinal mucosal cells, the migration of cells within the intestine, and the overall intestinal structure. Moreover, the addition of leucine to diets has been found to promote intestinal development and enhance the expression of amino acid transporters [54], which are important factors for the healthy maintenance of the digestive system. However, it is possible that other compounds may have synergized this gastroprotective effect.

As some of the limitations of this investigation, we highlight the specific germination conditions. We used a five-day germination period, which may have decreased the presence of certain chemical compounds, such as flavonoids, and this may have significantly contributed to increased antioxidant and gastroprotective activity in the animals. Regarding variability in quinoa sprouts, these differences in phytochemical profiles could be influenced by factors such as growing region, climatic conditions, and post-harvest handling. Although the results of this study demonstrate positive effects in rats, these results cannot be directly extrapolated to humans without further clinical studies. In addition, the better effect observed at 500 mg/kg is high, which requires further confirmation. However, the effective dose in preclinical models (such as rats) cannot be directly extrapolated to humans due to differences in the metabolism, distribution and absorption of bioactive compounds. According to the dose conversion formula based on body surface area [55], the dose in humans would be significantly lower than that used in rats. Furthermore, we only developed an acute model of gastric ulcer in animals, and administered a single dose of the different quinoa sprout extracts.

5. Conclusions

In the present study, we determined the presence of amino acids and saponins in three quinoa sprout varieties. The yellow maranganí and pasankalla varieties were highly significantly similar in their phytochemical compound profiles, sharing several metabolites, mainly amino acids, in addition to similarities between the black coito and yellow maranganí varieties. Under experimental conditions, extracts of yellow maranganí, pasankalla, and black coito quinoa sprouts showed significant gastroprotective effects in a preclinical model of ethanol-induced gastric ulcers in male Holtzman rats. However, at a dose of 500 mg/kg, the pasankalla variety was the most effective in reducing the ulcer index, similar to the results for ranitidine and sucralfate, which was also confirmed by our histopathological findings. Regarding antioxidant activity, pasankalla quinoa exhibited more significant antioxidant activity against DPPH and ABTS radicals. The gastroprotective effect appears to be mediated by antioxidant and antisecretory activities attributable to the presence of amino acids and triterpene saponins, which might protect the gastric mucosa against oxidative stress and inflammation produced by ethanol. Although these findings are promising, further in-depth studies are required to validate these effects in humans and explore their molecular mechanisms. In addition, it is also necessary to determine the impacts of the extracts in models of gastric ulcers induced by other agents, such as stress, NSAIDs, or *Helicobacter pylori* infections, and standardize the composition of the extracts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/scipharm93010010/s1. Table S1: Phytoconstituent profile of the hydroalcoholic extract of black coito quinoa sprouts. Table S2: Phytoconstituent profile of the hydroalcoholic extract of pasankalla quinoa sprouts. Table S3: Phytoconstituent profile of the hydroalcoholic extract of yellow maranganí quinoa sprouts.

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Informed Consent Statement: Not applicable.

Data Availability Statement: All relevant data supporting the findings of this study are contained within this article.

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Conflicts of Interest: The authors declare no conflicts of interest.

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