horticulturae

Article

The Optimization of Ultrasound-Assisted Extraction for Bioactive Compounds from *Flourensia cernua* and *Jatropha dioica* and the Evaluation of Their Functional Properties

Nestor Everardo Aranda-Ledesma [1], Pedro Aguilar-Zárate [2], Israel Bautista-Hernández [1,3], Romeo Rojas [1], Claudia Lizeth Robledo-Jiménez [1] and Guillermo Cristian Guadalupe Martínez-Ávila [1,*]

[1] Laboratory of Chemistry and Biochemistry, School of Agronomy, Autonomous University of Nuevo Leon, General Escobedo 66054, Nuevo León, Mexico; nestor.arandald@uanl.edu.mx (N.E.A.-L.); s-ibhernandez@ucp.pt (I.B.-H.); romeo.rojasmln@uanl.edu.mx (R.R.); claudia.robledojmn@uanl.edu.mx (C.L.R.-J.)

[2] Laboratorio Nacional CONAHCYT de Apoyo a la Evaluación de Productos Bióticos (LaNAEPBi), Unidad de Servicio, Tecnológico Nacional de Mexico/I.T. de Ciudad Valles, Ciudad Valles 79010, San Luis Potosí, Mexico; pedro.aguilar@tecvalles.mx

[3] Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, 4169-005 Porto, Portugal

* Correspondence: guillermo.martinezavl@uanl.edu.mx

Abstract: *Flourensia cernua* and *Jatropha dioica* are non-timber forest species that grow in the desert regions of northern Mexico. Currently, they are recognized as a viable alternative to traditional remedies used to address certain ailments. However, the low yields of bioactive compounds obtained propitiate the use of more effective extraction techniques such as ultrasound-assisted extraction (UAE) in combination with statistical models such as Taguchi to maximize the yields of bioactive compounds. The objective of the research was to determine the optimal conditions for maximizing the yields of purified polyphenolic compounds from *F. cernua* and *J. dioica* using the Taguchi statistical model. In addition, we evaluated the total flavonoid content, antioxidant activity (ABTS•+, FRAP, and OH• hydroxyl radical inhibition), and the in vitro evaluation of α-amylase inhibition and ex vivo hemolysis inhibition. Furthermore, a functional group analysis was conducted using FTIR. The optimization process revealed that the maximum yields of bioactive compounds for *F. cernua* and *J. dioica* were achieved with a particle size of 0.25 mm and 0.30 mm, temperatures of 60 °C for both, a water–ethanol concentration of 50% for both, and extraction times of 30 min and 5 min, respectively. The matrix bioactive compounds obtained contain flavonoid-type phenolic compounds, which exhibit properties such as inhibiting free radicals and acting as reducing agents; inhibiting α-amylase activity, which is involved in carbohydrate metabolism; and protecting red blood cells from damage by reactive oxygen species. Finally, the FTIR analysis allowed for the identification of functional groups associated with the polyphenolic compounds (O–H, C–H, C–C, C=O). These results suggest that both non-timber forest species are a rich source of bioactive compounds with potential for application in the agri-food and pharmaceutical industries However, it is necessary to carry out compound identification as well as toxicity analyses to guarantee the safety of these bioactive compounds.

Keywords: *Flourensia cernua*; *Jatropha dioca*; flavonoids; Taguchi L9

Citation: Aranda-Ledesma, N.E.; Aguilar-Zárate, P.; Bautista-Hernández, I.; Rojas, R.; Robledo-Jiménez, C.L.; Martínez-Ávila, G.C.G. The Optimization of Ultrasound-Assisted Extraction for Bioactive Compounds from *Flourensia cernua* and *Jatropha dioica* and the Evaluation of Their Functional Properties. *Horticulturae* **2024**, *10*, 709. https://doi.org/10.3390/horticulturae10070709

Academic Editors: Dasha Mihaylova, Aneta Popova and Jiri Gruz

Received: 3 May 2024
Revised: 27 June 2024
Accepted: 28 June 2024
Published: 4 July 2024

1. Introduction

During the last 20 years, research into the benefits of polyphenolic compounds for human health has gained significant importance. Both preclinical and clinical evidence have shown that the consumption of these secondary metabolites (i.e., quercetin, resveratrol, cyanidin) protects against the development of chronic diseases such as neurodegenerative and cardiovascular diseases, cancer, and diabetes, which have a global impact on society [1]. It has also been demonstrated that these compounds serve as a valuable tool against emerging diseases like the SARS-CoV-2 pandemic. In this lane, polyphenolic compounds such as epigallocatechin gallate and theaflavins have shown the ability to inhibit key enzymes necessary for virus replication. Hence, they could potentially be used for the prevention or treatment of virus symptoms [2]. In light of this compelling evidence, the exploration of new and natural sources of bioactive compounds becomes crucial in addressing the health conditions and negative effects that impact the global population.

In this sense, in Mexico, there is a great diversity of medicinal plants that have gained wide cultural acceptance in recent years. This is due to their therapeutic properties, as well as the lack of new drugs and their adverse effects. The use of these plants and their extracts plays an important role in the treatment of several diseases [3]. In the northern semi-desert region of the country, there are several endemic species with antidiabetic, anticancer, and antimicrobial properties. Among the most common species are *Lippia graveolens*, *Euphorbia antisyphilitica* Zucc., *Turnera diffusa*, and *Larrea tridentata*, which have been widely studied and applied in the food industry [4]. However, it is also possible to find other less studied species with potential for application in the biotechnology industry, such as *Flourensia cernua* and *Jatropha dioica*.

F. cernua is a species of the genus *Flourensia* that, along with *F. microphylla* and *F. retinophylla*, grow wildly in extreme climatic conditions. They possess an adapted metabolism and produce several phytochemicals, including phenols, terpenes, waxes, and fatty acid esters, which enable them to survive such conditions [5]. The traditional medicine applications of this species include the treatment of gastrointestinal ailments, as well as respiratory disorders [6]. Extracts of *F. cernua* have been reported to exhibit activity against phytopathogens and medically significant microorganisms. Recent studies have evaluated its cytotoxic and antiproliferative capacity on human adenocarcinoma cells (A549), as well as its anti-inflammatory and hepatoprotective effects. These studies demonstrated that *F. cernua* extracts have the potential to be applied in the treatment of lung cancer and liver-related diseases [5,7,8]. Likewise, *J. dioica* is an endemic species from arid and desert regions of Mexico. In traditional medicine, its stems and roots have been used as infusions against issues such as hair loss, dental problems, and even ailments associated with cancer [9]. The presence of flavonoids, terpenes, alkaloids, and reducing sugars responsible for its antioxidant potential has been detected. Additionally, its extracts have demonstrated antimicrobial and antiviral activity, as well as a hepatoprotective effect at the tubular level against damage induced by diabetes [10,11].

In recent years, alternative extraction techniques have been developed, characterized by saving inputs and environmentally friendly, which allow for the obtention of high-quality bioactive compounds. Among these techniques, ultrasound-assisted extraction (UAE) stands out for its simplicity and low investment costs. It is characterized by its reduced extraction time because the acoustic cavitations of UAE cause the rupture of plant cell walls, increasing mass transfer and facilitating solvent penetration into cellular content. Currently, reports on the use of UAE for the extraction of bioactive compounds from plant species from the Mexican semi-desert are limited. However, Wong-Paz et al. [12] conducted an optimization of the extraction process of *J. dioica* and *F. cernua*, along with other forest species (*Turnera diffusa* and *Eucalyptus camaldulensis*), demonstrating the capacity of this unconventional technique to obtain bioactive compounds of a phenolic nature from these species. It is necessary to consider that the efficiency of UAE is influenced by several factors, such as the solvent-to-solvent ratio, the concentration and type of solvent, particle size, temperature, and extraction time [13]. In this sense, to maximize the yields of bioactive compounds, several statistical techniques can be used, one of which is the Taguchi model. This orthogonal design allows for the simultaneous evaluation of multiple factors with a minimal number of experimental trials, thereby saving cost and time. Furthermore, the Taguchi design improves the quality of the evaluated processes due to the versatility of its analysis models, making it more efficient than full factorial and fractional factorial designs. This is because a large amount of information can be obtained from a few experiments [14,15].

In light of the above, the aim of this study was to optimize the ultrasound-assisted extraction process of bioactive compounds using the Taguchi L9 statistical model, evaluate the functional properties through in vitro and ex vivo assays, and characterize the bioactive compounds of two non-timber forest species using FTIR.

2. Materials and Methods

2.1. Plant Material

The plant material (*J. dioica* and *F. cernua*) was obtained from the wild at Ejido San Jeronimo, Melchor Ocampo, Zacatecas, Mexico. Upon collection, the plant material was transported to the laboratory, where it underwent washing with distilled water, and dried in a Jeio Tech model ON-12G.9 (Geumcheon-gu, Seoul, Republic of Korea) oven at 50 °C for 24 h.

2.2. Reagents

Ethanol, catechin, gallic acid, linoleic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Amberlite XAD-16, ferrous chloride ($FeCl_2$), ferric chloride ($FeCl_3$), monobasic potassium phosphate (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4), potassium ferricyanide ($C_6N_6FeK_3$), sodium chloride (NaCl), aluminum chloride ($AlCl_3$), anhydrous sodium carbonate (Na_2CO_3), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), ferrous sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), salicylic acid ($C_7H_6O_3$), 2,2-Azobis (2-amidinopropane dihydrochloride (AAPH), potassium chloride (KCl), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), porcine pancreatic α-amylase, starch, dinitrosalicylic acid ($C_7H_4N_2O_7$), phenol (C_6H_6O), sodium sulfite (Na_2SO_3), and sodium nitrite ($NaNO_2$) were purchased from Sigma-Aldrich (Burlington, MA, USA).

2.3. Taguchi L9 Design

The Taguchi methodology was used to optimize the extraction process of bioactive compounds. Four factors with three levels each (Table 1) were evaluated. These factors were determined after rigorous research and analysis of several references on the optimization of the extraction process of bioactive compounds. There are currently no reports on optimization using these factors for *F. cernua* and *J. dioica* species. The result is an L9 array (3^4) with a total of nine runs combining the four factors and their three levels (Table 2). The experimental data were analyzed using Statistica 7 software (Statsoft, Tulsa, OK, USA).

Table 1. Factors and levels used in Taguchi L9 design.

Levels	Factors			
	Particle Size (mm)	**Temperature (°C)**	**Concentration Ethanol-Water (%)**	**Time (min)**
L1	0.42	40	30	5
L2	0.30	50	50	15
L3	0.25	60	70	30

In the present study, the quality characteristic "bigger is better" was used, which is described by the loss function $L(y) = k \times (1/y^2)$ and represented by the following equation:

$$E[L(y)] = -10 * log_{10}[({}^{1}/{}_{n}) * \Sigma({}^{1}/{}_{y_i^2})]$$

where the factor -10 measures the ratio of the inverse of "poor quality" and n represents the number of samples. Once the analysis of variance (ANOVA) was performed (S2 for

J. dioica and S4 for *F. cernua*), their percentage contribution was determined from the following equation:

$$P = \frac{SS_i}{SS_T} * 100\% = \frac{SS_i - MS_i * df_i}{SS_T} \times 100\%$$

where P is the contribution percentage, SS_i is the individual sum of squares, SS_T is the total sum of squares, MS_i is the initial mean square, and df_i represents the degrees of individual freedom.

Table 2. Orthogonal array for the Taguchi L9 (3^4) experimental design and extraction yields for *J. dioica* and *F. cernua*.

Run	Particle	Temperature	Concentration	Time	***J. dioica* Yield ($mg \cdot g^{-1}$)**	***F. cernua* Yield ($mg \cdot g^{-1}$)**
8	3	2	1	3	10.3 ± 2.0	117.6 ± 10.6
5	2	2	3	1	11.3 ± 3.1	41.9 ± 9.4
6	2	3	1	2	11.9 ± 0.2	84.1 ± 3.7
1	1	1	1	1	10.5 ± 1.6	72.5 ± 18.4
2	1	2	2	2	11.8 ± 2.8	115.1 ± 6.6
9	3	3	2	1	13.8 ± 0.6	101.3 ± 4.3
7	3	1	3	2	11.6 ± 1.5	107.2 ± 20.7
4	2	1	2	3	12.8 ± 1.5	91.0 ± 11.2
3	1	3	3	3	11.0 ± 3.5	108.2 ± 10.3

2.4. Extraction Process of Polyphenolic Compounds

A conventional extraction (CE) process based on the methodology reported by Ascacio-Valdés et al. [16] was conducted for comparison. Briefly, 10 g of the sample (previously adjusted to a particle size of 0.42 mm) was weighed in amber bottles with 100 mL of distilled water. The bottles were then placed in a water bath at 60 °C for 30 min. Finally, the obtained extract was filtered through a Whatman #41 filter paper.

For the optimization of the ultrasound-assisted extraction process, 5 g of the sample—previously dried, adjusted to the appropriate particle size, and crushed using an electronic High-Speed Multifunction GRINDER model HC-500 (Sisli, Istanbul, Turkey)—was used. Then, the plant material was mixed with a volume of 120 mL of solvent (at the respective concentration) in amber-colored vials. These vials were then placed in a Digital Ultrasonic Cleaner CO-Z (Shanghai, China) ultrasound bath at a 40 kHz ultrasonic frequency, following the times and temperatures outlined by the Taguchi model. The obtained extract was filtered through a Whatman #41 filter paper. Subsequently, ethanol was removed using a rotary evaporator Yamato RE200 model (Santa Clara, CA, USA) coupled with a BM200 water bath set at a temperature of 60 °C for varying time intervals between 10 and 25 min (depending on the solvent concentration indicated in Table 1) to facilitate the purification process.

The purification was carried out using Amberlite XAD-16 as a stationary phase due to its high adsorption–desorption ratio, which allows the concentration of phenolic compounds to increase by three- or five-fold compared to the crude extract after this process [17]. Ethanol is the mobile phase. Twenty milliliters of the aqueous extract was added to the stationary phase, and distilled water was used as the eluent to discard unwanted compounds. Ethanol was then used as an eluent to isolate the purified polyphenolic compounds. The solvent was evaporated at 50 °C for 48 h and the polyphenolic compounds were recovered as a fine powder. This approach was taken because it has been reported that in a solution, the stability of the phenolic compounds can be affected by the presence of carbohydrates, proteins, metal ions, and even autoxidation processes [18].

2.5. Preparation of Samples

Polyphenolic compounds extracted from *F. cerna* and *J. dioica* were dissolved in a 50% water–ethanol mixture. The IC_{50} values for ABTS•+ radical inhibition were determined through serial dilutions of the same sample. Subsequently, the concentrations of flavonoids, ferric reducing power (FRAP), hydroxyl radical ($OH^\bullet$) inhibition, and α-amylase inhibition were determined based on the IC_{50} concentration. Finally, the anti-hemolytic activity was determined at a concentration of 500 $\mu g \cdot L^{-1}$.

2.6. Total Flavonoids Determination

The flavonoid content was determined according to the method previously reported by De la Rosa et al. [19], with slight modifications. A sample volume of 31 µL was mixed with sodium nitrite (9.3 µL at 5%) and distilled water (9.3 µL). The solution was mixed and then incubated for 3 min at 40 °C. Subsequently, aluminum chloride (9.3 µL at 10%) was added and incubated for another 3 min. Finally, sodium hydroxide (12.50 µL at 0.5 $mol \cdot L^{-1}$) was added, and the mixture was incubated for 30 min in the dark. The absorbance was measured at 510 nm using a Synergy HTX MULTI-MODE READER microplate (Avenue Santa Clara, CA, USA). Results were expressed as Quercetin Equivalents (QE $\mu g \cdot mL^{-1}$) based on a calibration curve prepared with the same standard.

2.7. Antioxidant Activity

2.7.1. ABTS•+ Radical Scavenging Assay and IC_{50} Determination

The evaluation of ABTS•+ radical inhibition was conducted according to the methodology proposed by Berg et al. [20], with slight modifications. The ABTS•+ radical cation was generated by an aqueous solution of ABTS•+ (7 $mmol \cdot L^{-1}$) mixed with potassium persulfate (2.45 $mmol \cdot L^{-1}$) in the dark at room temperature for 12 h prior to use. The working solution of ABTS•+ was adjusted in ethanol with an absorbance of 0.700 ± 0.002 nm. A volume of 5 µL of the sample was mixed with 95 µL of the ABTS•+ solution to initiate the reaction. After 1 min of reaction time, the absorbance was measured at 734 nm. Results were expressed as Trolox Equivalents (TE $\mu g \cdot mL^{-1}$) based on a calibration curve prepared with the same standard.

2.7.2. Ferric Reducing Power (FRAP)

The ferric reducing antioxidant power was determined according to the methodology proposed by Celik et al. [21], with slight modifications. Samples (5 µL) were mixed with phosphate buffer (12 µL, 1 $mol \cdot L^{-1}$, pH 7). Then, potassium ferricyanide (22 µL at 1%) was added to the reaction mixture, which was homogenized and incubated at 50 °C for 20 min. Following this incubation, trichloroacetic acid (12 µL at 10%), distilled water (45 µL), and ferric chloride (10 µL at 0.1%) were added. The absorbance was measured at 700 nm. Results were expressed as Gallic Acid Equivalents (GAE $\mu g \cdot mL^{-1}$) based on a calibration curve prepared with the same standard.

2.7.3. Hydroxyl Radical ($OH^\bullet$) Scavenging Activity

Hydroxyl radical inhibition was determined based on the methodology reported by Ge et al. [22]. Hydroxyl radicals were generated using the Fenton reaction, which involved the addition of iron sulfate ($FeSO_4$ 200 µL, 6 $mmol \cdot L^{-1}$) and hydrogen peroxide (H_2O_2 200 µL, 24 $mmol \cdot L^{-1}$). Subsequently, the sample (400 µL) was added, and the reaction was brought to a final volume of 2.5 mL with salicylic acid (8 $mmol \cdot L^{-1}$). The mixture was incubated at 37 °C for 30 min and the absorbance was measured at a wavelength of 510 nm. The results were expressed as the percentage of inhibition (%), using the following equation:

$$Inhibition\ (\%) = \left[\frac{(A_1 - A_0)}{A_2}\right] \times 100$$

where A_1 is the absorbance of the sample, A_0 is the absorbance of the control, and A_2 is the absorbance of the reagent bank in the absence of salicylic acid.

2.8. Enzyme Inhibition Assays

Inhibition of α-Amylase Assay

The inhibition of α-amylase was determined based on the methodology reported by Aranda-Ledesma et al. [7]. Initially, the sample (50 µL) was mixed with the enzyme (50 µL, 1 mg·mL^{-1}) previously dissolved in saline phosphate buffer. The mixture was incubated at 37 °C for 10 min. Subsequently, starch (50 µL at 1%) was added, and the mixture was incubated again at 37 °C for 10 min. Following this, DNS solution (containing 1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, and 1% sodium hydroxide) was added. The mixture was heated in a water bath at 100 °C for 5 min. Finally, the sample was dissolved by adding distilled water (1.5 mL), and the absorbance was measured at 540 nm. The results were expressed as the percentage of inhibition (%), using the following equation:

$$\text{Inhibition } (\%) = \left[\frac{(A_c - A_s)}{A_c}\right] \times 100$$

where A_c represents the absorbance value of the control and A_s is the reaction value according to each sample.

2.9. Ex Vivo Assay

Anti-Hemolytic Activity

The determination of anti-hemolytic activity was conducted based on the methodology of Monroy-García et al. [23], with slight modifications. Initially, 5 mL of blood from a healthy male donor, obtained via venipuncture in purple tubes with anticoagulant (EDTA), was utilized. The blood was centrifuged at 1500 rpm at 25 °C for 12 min to separate erythrocytes from plasma, followed by three washes with 10 mL of PBS (containing sodium chloride (137 mmol·L^{-1}), potassium chloride (2.7 mmol·L^{-1}), disodium phosphate (10 mmol·L^{-1}), and monobasic potassium phosphate (1.8 mmol·L $^{-1}$); pH 7.4). The erythrocyte concentration was then adjusted to a density of 8 × 10^9 cells·mL, using PBS as diluent.

To induce RBC oxidation, AAPH (2-2-Azobis (2-amidinopropane dihydrochloride) was used to form peroxyl radicals. The erythrocyte suspension (100 µL) was mixed with the samples (100 µL) previously dissolved in PBS and AAPH (100 µL, 300 mmol·L^{-1}). The mixture was incubated at 37 °C for 1.5 h with constant shaking. Finally, the mixture was diluted with PBS (800 µL) and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was then recorded at 540 nm. The results were expressed as the percentage inhibition (%) using the following equation:

$$Inhibition\ (\%) = \left[\frac{(APPH - A_s)}{(APPH)}\right] \times 100$$

where $APPH$ is the absorbance of $APPH$ and A_s is the absorbance of each sample.

2.10. Fourier-Transform Infrared Spectroscopy (FT-IR)-ATR Assay

An Agilent FTIR model Cary 630 coupled with a zinc selenide (ZnSe) crystal coupled ATR reader was used for the analysis of functional groups. Samples were analyzed using Agilent MicroLab PC (v5.7) licensed software over a spectral range of 4000 to 650 cm^{-1}, a resolution of 2 cm^{-1}, and a 32-scan cycle. Graph construction was performed using OriginPro 8 v8.0724 (B724) software, OriginLab.

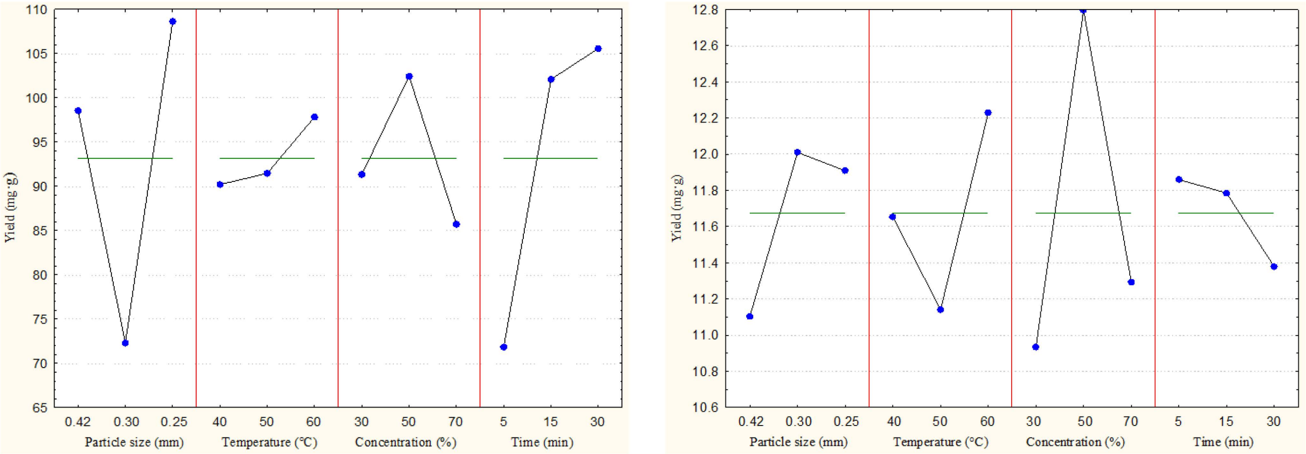
3. Results and Discussion

3.1. Extraction Process Optimization

The Taguchi design can be applied across different biological, food, and biotechnological areas and allows the identification of experimental factors that exert the most favorable effect on desired characteristics, such as yields of bioactive compounds. This approach not only reduces costs but also increases the quality of the final product obtained [24].

3.1.1. Effect of Individual Impact on Extraction Factors

In the optimization results of the *F. cernua* (Figure 1a) case of particle size, a significant difference is observed between 0.42 mm and 0.30 mm; in the case of temperature and time, an upward increase is observed between levels, so it would be recommended to increase these levels to obtain superior results. On the other hand, in the case of concentration, a decrease was observed in levels 1 and 3 (concentration of ethanol–water 30 and 70%). Meanwhile, for *J. dioica* (Figure 1b), the particle size did not show a significant difference between levels 1 and 2 (0.30 and 0.25 mm), while in the case of the temperature, a significant difference was observed at level 3 (60 °C); so, a temperature adjustment could improve the expected yields. Solvent concentration at level 2 (water–ethanol 50%) showed a significant difference, and finally, with respect to the time, a decrease was observed as the levels increased.



(**a**) (**b**)

Figure 1. Individual factors performance at different levels; (**a**) *F. cernua*, (**b**) *J. dioica.* The green lines correspond to the means, and the blue dots represent the behavior of the levels within each factor.

Table 3 shows the relative contribution of factors in the polyphenolic extraction process from *F. cernua*. The impact on the extraction process was as follows: particle size (37.98%) > extraction time (37.15%) > solvent concentration and temperature, with a residual/error sum of squares (SS) value of 5.66%.

Table 3. Analysis of variance (ANOVA) for *F. cernua* extraction process.

Factors	SS	df	MS	F	*p*	Contribution (%)
Particle size	6331.96	2	3165.98	22.38	0.000013	37.97
Temperature	301.59	2	150.79	1.06	0.365248	1.80
Solvent concentration	1301.55	2	650.77	4.60	0.024354	7.80
Time	6193.86	2	3096.93	21.89	0.000015	37.14
Particle size by time	1603.13	4	400.78	5.95	0.005188	9.61
Error	943.83	14	67.42			5.66
Total	16,675.91	26				100

In the same way, Table 4 shows that the solvent concentration had the most significant influence on the polyphenolic extraction process of *J. dioica* (16.40%), followed by temperature (4.97%), particle size, and time. In addition, it is observed that although the individual influence of particle size and time is low, the interaction of these two factors has an influence of 21.37%, so it is not recommended to eliminate or modify these factors. In this case, the influence of the sum of squares (SS) residual/error was 13.91%.

Table 4. Analysis of variance (ANOVA) for *J. dioica* extraction process.

Factors	SS	df	MS	F	*p*	Contribution (%)
Particle size	4.44	2	2.22	0.55	0.584827	4.14
Temperature	5.33	2	2.66	0.66	0.527751	4.97
Solvent concentration	17.58	2	8.79	2.20	0.146677	16.40
Time	1.21	2	0.60	0.15	0.860186	1.13
Particle size by temperature	18.80	4	4.69	1.09	0.395095	17.53
Particle size by time	22.91	4	5.72	1.43	0.272815	21.37
Temperature by time	22.02	4	5.50	1.36	0.296846	20.54
Error	14.91	6	2.48			13.91
Total	107.21	26				100

3.1.2. Effect of Particle Size

In the case of *F. cernua*, higher yields were achieved using plant material with a particle size of 0.25 mm, whereas for *J. dioica*, a higher yield was obtained with a particle size of 0.30 mm. For both plants, the interaction of particle size with other factors was important according to the percentage of contribution shown in Tables 3 and 4. It has been reported that the use of a smaller particle size (<0.40 mm) in the extraction process of bioactive compounds enhances yields. This is because the sieving process leads to the breakdown of plant material, thereby increasing the surface area and contact area of the sample with the solvent, which allows for the entrainment of a greater number of these metabolites [25]. However, it is important to note that very small particle sizes (<0.15 mm) can lead to decreased yields because fine particles tend to agglomerate, which have to form a precipitate even under agitation and, therefore, the diffusion of the solvent through the plant tissue is hindered [26]. Therefore, the use of a particle size within the range of 0.15 mm to 0.40 mm can provide positive results in increasing yields, consistent with the findings of this research.

3.1.3. Effect of Temperature

Regarding temperature, higher yields were obtained at 60 °C for both species. Concerning the UAE system, an initial rise in temperature leads to increased yields. However, a prolonged elevation in temperature results in a decrease due to the weakening of the cavitation of the ultrasound waves. Furthermore, temperature increase has a dual effect on both the sample and the extraction solvent: it enhances the solubility and desorption of the sample in the solvent while decreasing the solvent's viscosity, facilitating greater solvent diffusion through the plant tissue [27]. Hence, there must be a balance between the temperature used to achieve optimal yields while avoiding degradation, as it has been reported that a significant portion of phenolic compounds causes degradation at high temperatures, particularly above 80 °C [28].

3.1.4. Effect of Solvent Concentration

On the other hand, concerning solvent concentration, a water-to-ethanol ratio of 50% yielded the highest results in both cases. Binary solvents, specifically hydroalcoholic mixtures, have been reported as highly effective solvents for bioactive compound extraction. In a UAE extraction system, water serves as a medium absorber of microwave waves, while ethanol exhibits stronger absorption compared to other solvents such as methanol. Thus, ethanol possesses the capacity to absorb ultrasound waves and convert them into heat. In addition, the polarity of a binary water–ethanol system aligns closely with the molecular polarity characteristic of phenolic compounds, thereby facilitating their extraction [29]. Moreover, it has been reported that high ethanol concentrations, near 100%, adversely affect the extraction process by inducing the dehydration of plant tissues, which leads to the denaturation of plant components, resulting in lower yields [25].

3.1.5. Effect of Time

Finally, regarding the influence of extraction time, a higher yield was achieved for *F. cernua* at 30 min, whereas for *J. dioica*, the highest yield was obtained at 5 min. It has been reported that the optimal yields of bioactive compounds are typically obtained within the first 25 and 30 min, attributed to an enhanced energy supply. This increase in energy leads to a higher release of bioactive compounds, but also a higher probability of a degradation of these [30]. However, it has been reported that the extraction of polyphenolic compounds in other species has been extended up to periods of 4 h, obtaining positive results [25]; considering this, it is necessary to carry out more studies in the analyzed species used in the present investigation using longer extraction times.

Once the Taguchi analysis was performed, using the experimental matrix L9 (3^4), the parameters to maximize the yields of phenolic compounds were determined. The theoretical and actual yields (Table 5) showed a significant resemblance with standard deviation (SD) values of 1.91 and 0.32 for *F. cernua* and *J. dioica*, indicating the effectiveness of the optimization process in maximizing the yields of bioactive compounds for both species and ensuring the reproducibility of the experiment. Additionally, these optimal yields were significantly higher compared to the control (conventional extraction) and previous studies [7,31].

Table 5. Conditions for maximizing yields of bioactive compounds.

Sample	Particle Size (mm)	Temperature (°C)	Concentration (%)	Time (min)	Theoretical Yields (mg·g^{-1})	Actual Yield (mg·g^{-1})	CE Yield (mg·g^{-1})
F. cernua	0.25	60	50	30	134.99	132.75 ± 1.91	41.36 ± 3.61
J. dioica	0.30	60	50	5	13.87	13.14 ± 0.32	8.30 ± 1.23

3.2. Total Flavonoids

Flavonoid-type compounds protect plants from stress conditions such as UV damage and oxidative stress, and they act as growth regulators [32]. The flavonoid content, as presented in Table 6, showed a significant difference ($p < 0.05$) between samples. Polyphenolic compounds from *F. cernua* had the highest flavonoid content (61.33 QE µg·mL^{-1}) compared to those of *J. dioica*.

Table 6. Total flavonoids and antioxidant activity of bioactive compounds.

Sample	Flavonoids (QE µg·mL^{-1})	ABTS$\bullet^{+}$ IC$_{50}$ (TE µg·mL^{-1})	FRAP (GAE µg·mL^{-1})	Hydroxyl Radicals (Inhibition %)
F. cernua	61.33 ± 2.25 ^{a}	547.04 ± 4.58 ^{a}	22.90 ± 0.25 ^{a}	38.32 ± 0.91 ^{a}
J. dioica	36.16 ± 1.5 ^{b}	355.61 ± 11.81 ^{b}	15.28 ± 2.25 ^{b}	39.75 ± 3.39 ^{a}

Different letters show a significant difference in Student's t test results ($p < 0.05$).

In the case of *F. cernua*, the presence of flavonoid-type compounds has been previously reported by Aranda-Ledesma et al. [7], who reported values from 133.77 to 258.22 QE µg·mL^{-1} at a concentration of 1 mg mL^{-1}, which are higher values than those registered in this study. However, the phenolic concentration used by these authors represents a significant variable to consider when comparing both studies.

Conversely, Terrazas-Hernández et al. [33], reported slightly lower flavonoid concentrations (23.59–45.30 QE µg·mL^{-1}) in both sterilized and non-sterilized methanol extracts of *J. dioica* root. The variation in flavonoid content could be attributed to the type of plant tissue analyzed. In the present research, stems were used, whereas Terrazas-Hernandez et al. [34] utilized roots, a difference that may result in varied secondary metabolite production, including flavonoids.

The biological potential of flavonoids is associated with their structure, specifically the total number of hydroxyl groups ($OH^{\bullet}$), their positional arrangement, and the effects of conjugation and resonance. Flavonoids have been found to exhibit a stronger antioxidant activity than vitamins C and E, which are commonly used as supplements. Moreover, the inclusion of flavonoids in the diet, particularly for the elderly, has been shown to have benefits for bone tissue regeneration and cardiovascular health [34]. Therefore, both plants analyzed in this study could serve as sources of bioactive flavonoid-type compounds, which could be used for innovative research within food science and pharmaceuticals.

3.3. Antioxidant Activity

Given the diversity and complexity of oxidation processes in plant and animal organisms, as well as in food systems, it is imperative to analyze the biological antioxidant capacity of bioactive compounds through different methodologies to ensure a reliable antioxidant profile [35]. In this sense, three assays were conducted to evaluate the antioxidant potential of polyphenolic compounds from both *F. cernua* and *J. dioica*.

3.3.1. ABTS•+ Radical Inhibition and IC_{50} Determination

The ABTS•+ radical inhibition assay is a method widely accepted by researchers for evaluating the antioxidant potential of extracts of food extracts, plant-derived substances, and pure compounds [36].

The determination of IC_{50}, based on the inhibition of ABTS•+ radicals, revealed a significant difference ($p < 0.05$) between the samples, with *J. dioica* presenting a lower IC_{50} value (355.61 TE $\mu g \cdot mL^{-1}$). This indicates that *J. dioica* requires a lower concentration of polyphenolic compounds to inhibit 50% of the free radicals compared to *F. cernua*, which showed an IC_{50} value of 547.04 TE $\mu g \cdot mL^{-1}$. While the potential of *J. dioica*'s bioactive compounds to inhibit free radicals has been previously documented, specific reports on IC_{50} value determination are absent. Gutiérrez-Tlahque et al. [9] reported that ethyl acetate and hexane extracts from *J. dioica* stems inhibited 29.1% to 36.1 of radicals, respectively at a concentration of 5 $mg \cdot mL^{-1}$. This concentration was higher than those used in this study and did not achieve a 50% inhibition of the reaction, suggesting that polar-green solvents (i.e., water–ethanol) are more effective for extracting polyphenolic compounds from this plant material, avoiding the environment and health risks associated with other toxic solvents.

In the case of the samples from *F. cernua*, Aranda-Ledesma et al. [7] reported inhibition values ranging from 105.34 to 124.39 TE $\mu g \cdot mL^{-1}$ at concentrations of 1000 $\mu g \cdot mL^{-1}$ of purified polyphenolic compounds. Similarly, Alvarez-Perez et al. [37] reported a radical inhibition near 50% at a concentration of 500 $\mu g \cdot mL^{-1}$, aligning with the findings from this study. These observations suggest that anti-radical activity is related to the concentration and specific types of phenolic compounds present in the samples. In *F. cernua*, flavonoids such as luteolin 7-O-rutinoside and Apigenin-6-C-glucosyl-8-C-arabinoside, found in higher proportions [7,37], are likely responsible for this activity.

The variations observed between samples and the literature are attributed to the presence of OH groups attached to aromatic rings or an unsaturated carbon atom of the phenolic compounds. The number of electrons exchanged will depend on this characteristic. Therefore, the oxidation of the OH group of an aromatic/enol ring involves the transfer of an electron to the radical ABTS•+ [38]. The presence of free radicals is a natural part of organismal metabolism. However, an imbalance and increase in free radicals, due to environmental factors, bodily activities, or even emotional changes, can lead to organismal alterations. Polyphenolic compounds have the capacity to neutralize free radicals, thereby preventing tissue damage caused by radicals and peroxides [39].

3.3.2. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay is widely used to measure reduction potential in biological studies of various substances such as foods, essential oils, biological fluids, spices, and fruits and vegetables due to its simplicity and stability [40].

The analysis of Ferric Reducing Power Activity revealed a significant difference ($p < 0.05$) between samples, with *F. cernua* exhibiting the highest reducing potential (22.90 GAE $\mu g \cdot mL^{-1}$). Purified polyphenolic compounds from *F. cernua* have been reported to possess reducing activity, with values ranging from 84.02 to 90.50 GAE $\mu g\ mL^{-1}$ [7]. On the other hand, higher values (416.63 GAE $\mu mol \cdot mL^{-1}$) have been reported in samples from hydroalcoholic extracts of *J. cardiophylla* [41]. The presence of Fe^{+3} ions is associated with several oxidative and detrimental processes in organisms, leading to degenerative effects. Bioactive compounds capable of reducing Fe^{+3} to a ferrous state (Fe^{+2}) can potentially mitigate these harmful processes [40]. The FRAP assay allows for the measurement of the total antioxidant activity of reducing phenolic compounds (reducing agents) in a single test. This reaction is based on the transfer of electrons to the polyphenolic compounds (which are oxidized), while simultaneously reducing the oxidizing ferric salt. This results in a color change from pale yellow (ferric salt) to deep blue (in its ferrous form) [42]. Furthermore, oxidative processes not only impact human health but also affect food products, particularly in the meat industry. The oxidation of lipids and proteins represents the second most common cause of deterioration in these foods, compromising their organoleptic properties [43]. In this context, phenolic compounds from semidesert plants could serve as potential reducing agents for the food industry.

3.3.3. Hydroxyl Radical ($OH^{\bullet}$) Scavenging Activity

In the hydroxyl radical inhibition assay, no significant difference ($p < 0.05$) was observed between samples. This suggests that both species have very similar inhibitory activity, as indicated by their comparable inhibition percentages, ranging from 38.32% for *F. cernua* to 39.75% for *J. dioica* polyphenolic compounds. As shown in Table 6, where there is a significant difference in the presence of flavonoids between the two samples, their potential to inhibit the hydroxyl radical does not display a significant disparity. This implies that the activity of the hydroxyl radical is not solely determined by the number of phenolic compounds but rather by their structural quality. Previous studies have highlighted the necessity for phenolic compounds to possess available ortho positions to effectively neutralize the hydroxyl radical through aromatic hydroxylation [44].

The current literature on the ability of polyphenolic compounds from forest plant origins to inhibit the hydroxyl radical is limited, with most existing reports outdated. However, there are several reports demonstrating the efficacy of bioactive compounds derived from barley in inhibiting the radical, with percentages exceeding 90% for different barley grains according to Ge et al. [18] and up to 80% for bagasse, comparable to results with a standard antioxidant like vitamin C [45]. In the inhibition of OH radicals, flavonoids are the main phenolic compounds involved in this activity. However, specific structural characteristics must be met, such as the hydroxylation of the B ring and a C2-C3 double bond connected to a group, a C3 hydroxyl, and a C4 carbonyl group. Moreover, the hydroxylation of ring A and the presence of gallate and galacturonate residues, which are characteristic of the flavonoid structure, enhance the inhibition activity of the radical [46].

Given that high concentrations of the hydroxyl radical induce severe damage to tissue stability and synthesis by affecting all known macromolecules (nucleic acids, carbohydrates, amino acids, and lipids) [47], the use of bioactive compounds of natural origin to regulate hydroxyl radical levels is crucial. Despite the limited utilization of bioactive compounds from forest species, the potential demonstrated in this study suggests they are viable in mitigating such negative effects. Furthermore, it has been reported that the ability of a matrix of bioactive compounds to inhibit hydroxyl radicals is directly associated with the inhibition of lipid oxidation and the inhibition of hemolysis [48]. Therefore, the positive effects of inhibiting the radical may translate into beneficial effects by attenuating other oxidative processes induced by the presence of reactive oxygen species (ROS).

3.4. In Vitro and Ex Vivo Assays

3.4.1. Inhibition of α-Amylase Assay

The evaluation of bioactive compounds' potential on α-amylase enzyme activity is regarded as a focal point in contemporary medicine, serving as a therapeutic agent to regulate blood levels in diabetic patients [32].

The inhibitory potential against α-amylase (Table 7) showed a significant difference ($p < 0.05$), with *F. cernua* presenting a higher inhibition percentage of 72.07% compared to the 49.73% obtained from the bioactive compounds of *J. dioica*. In the case of the samples obtained from *F. cernua*, only one report on the inhibitory potential against this enzyme is available, where values ranging from 10.14 to 44.93% were reported at a concentration of 1000 $\mu g \cdot mL^{-1}$ according to Aranda-Ledesma et al. [7]. These reported activities are lower than those reported in the present study using a concentration of almost half, which could be influenced by the optimization process, as mentioned earlier. In this sense, the use of the Taguchi L9 design might have impacted the activity shown by the samples, as it not only reduces the number of experiments but also enhances and ensures the quality of the products obtained [23,28].

Table 7. In vitro and ex vivo assays.

Sample	In Vitro	Ex Vivo
	α-Amylase Inhibition (%)	Hemolysis Inhibition (%)
F. cernua	72.07 (4.26) ^{a}	33.21 (1.56) ^{b}
J. dioica	49.73 (5.90) ^{b}	56.04 (2.90) ^{a}

Different letters show a significant difference in Student's *t* test results ($p < 0.05$).

On the other hand, it has been demonstrated that hydroalcoholic and ethyl acetate extracts of *J. dioica* possess the potential to inhibit the activity of digestive enzymes (α-amylase and α-glucosidase), and the ability to block glucose absorption has also been evaluated by obtaining positive results from hydroalcoholic extracts [49].

Moreover, seed extracts (aqueous, methanol, and hexane) of *J. curcas* have been reported to exhibit ≈38–78% inhibition at concentrations of 4 $mg \cdot mL^{-1}$ [50]. This suggests that species belonging to the genus *Jatropha* may possess activity not only to inhibit enzymes involved in sugar degradation but also to regulate glucose absorption at the intestinal level. It has been reported that the inhibition of the enzyme α-amylase by phenolic compounds is highly related to their binding affinity with the enzyme. Models suggest that these bonds are mainly due to covalent bonds including hydrogen bonds and hydrophobic interactions, causing conformational modifications to the molecule [51]. The α-amylase is one of the most important enzymes in carbohydrate metabolism and glucose conversion, making it a key contributor to increased blood glucose levels postprandially. Hence, its control is vital, especially in diabetic patients [50]. Therefore, the incorporation of phenolic compounds obtained from forest species into the diet of people with diabetes could potentially replace the intake of commonly used glucose-controlling drugs such as acarbose and metformin. Although these drugs have proven to be effective, they often carry various side effects that can lead to additional health complications [52].

3.4.2. Anti-Hemolytic Activity Assay

Since approximately 90% of assays evaluating the antioxidant biological potential of polyphenolic compounds are performed in vitro, this may sometimes lead to perspectives that are not ideal for the application of these compounds in human health [53]. In this sense, the hemolysis of human red blood cells serves as a widely utilized model to assess free radical damage in biological membranes [54]. Therefore, there is a growing need to evaluate the biological potential in ex vivo systems.

In the anti-hemolytic activity assay, a significant difference was observed ($p = 0.05$), with *J. dioica* presenting a higher percentage of inhibition at 56.04% compared to *F. cernua* at 33.21%. Notably, a greater inhibition by the bioactive compounds of *J. dioica* compared to *F. cernua* was evident in terms of anti-hemolytic activity. In this sense, flavonoids are known to be associated with the protection of red blood cells during hemolytic processes. Nevertheless, within this group, tannins have been reported as potent anti-hemolytic agents [55]. *F. cernua* has been reported to contain tannins in concentrations ranging between 0.3 and 0.4% [56], while *J. dioca* has concentrations between 1.95 and 2.80% [57]. Hence, the anti-hemolytic effect could be related to the type of metabolites rather than their quantity.

At present, the analysis of the capacity of pure polyphenolic compounds of natural origin remains a limited area of exploration. However, the potential of extracts from plant species such as avocadoes (*Persea americana*), oranges (*Citrus sinensis*), and garlic (*Allium sativum*), which have been locally used to treat metabolic disorders, has been evaluated. These extracts demonstrated, in ex vivo assays, protection against erythrocyte hemolysis of over 80% [58]. In addition, it has been reported that this inhibition is due to the presence of polyphenolic compounds in plants, as these compounds effectively interact with the erythrocyte membrane using hydrogen bonds. For this, the presence of at least one free hydroxyl group attached to an aromatic ring is necessary, thereby preventing the oxidation process and providing protection to proteins and membrane l [59,60].

Although the antioxidant potential and the biological effect on the α-amylase enzyme and the inhibition of hemolysis are attributed to flavonoids and their different classes, a significant effect between these and other compounds present in the matrix is not ruled out. For example, there is the interaction of flavonoids with phenolic acids that has been shown to influence the antioxidant potential and, therefore, enhance biological activity [61].

3.5. FTIR-ATR Assay

The FT-IR technique is one of the most crucial methods used for the identification of functional groups in different chemical and biological matrices. It is characterized by its non-destructive nature, reproducibility, and ease of use, making it a valuable tool for qualitatively evaluating the composition of bioactive compounds [62]. The IR spectra (Figure 2) of both samples show a broad signal at 3312 cm^{-1}, indicative of the stretching of the O–H group of polyphenolic compounds. Additionally, the signal at 3922 cm^{-1} corresponds to the stretching of C-H bonds, possibly originating from CH_2 or CH_3 groups. The band located at 1723 cm^{-1} indicates the presence of a C=O interaction of aromatic rings. On the other hand, the signal at 1607 cm^{-1} denotes the presence of C-C bonds characteristic of the aromatic rings of phenolic compounds. The signal located at 1166 cm^{-1} corresponds to the stretching of C–O bonds. Finally, the signals at 1015 and 829 cm^{-1} indicate the presence of C–OH bonds of secondary alcohols.

In recent years, regarding the analysis of functional groups via FITR, Rajhard et al. [63] analyzed several compounds of phenolic compounds and found that all compounds share at least six characteristic peaks. These include the presence of OH groups in the region around 3340 cm^{-1}, C–H bonds in the region of 2978–2896 cm^{-1}, and characteristic signals of phenolic compounds (C–C, C=O, and C–OH) in the region between 1600 and 800 cm^{-1}. These findings align with the results reported in the present investigation. Likewise, Wongsa et al. [62] noted that C–H and C=C signals indicate the presence of one or more aromatic ring characteristics typical of polyphenolic compounds, while the signal at approximately 1200 cm^{-1} (C–O) suggests the presence of a pyran ring characteristic of flavonoids, concluding that these signals are highly related to aromatic compounds. Thus, it can be inferred that the bioactive compounds obtained from the extraction and purification processes, specifically those identified as phenolic in nature, have been successfully isolated. However, further specific analyses are needed to determine the exact identity of these molecules.

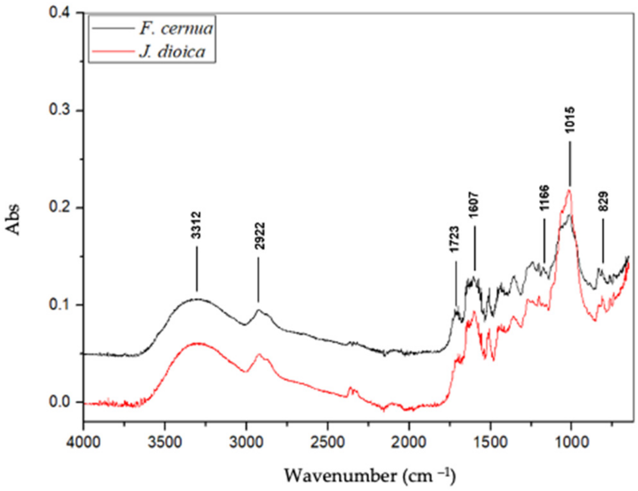


Figure 2. IR spectra of polyphenolic compounds.

4. Conclusions

The implementation of the Taguchi L9 statistical model facilitated the determination of conditions to enhance the yields of bioactive compounds derived from the non-timber forest species *F. cernua* and *J. dioica*. These matrices contain flavonoid-type compounds that demonstrated antioxidant activity through different assays. In addition, they showed the capacity to inhibit the activity of the α-amylase enzyme involved in the assimilation of starch and its derivatives, while exerting a protective effect on erythrocytes against peroxyl radicals generated from AAPH. The results indicate that the non-timber forest species *F. cernua* and *J. dioica* contain secondary metabolites with biological potential. However, it is necessary to establish a complete profile of the compounds present in these matrices, expand the analyses using ex vivo models, and conduct rigorous toxicity analyses to guarantee their safety. In addition, the mechanisms underlying the enzyme inhibition and anti-hemolytic activity of the bioactive compounds should be made clear. This will enable their future integration into the food and pharmaceutical industries.

Author Contributions: N.E.A.-L.: Writing—Original Draft, Writing—Review and Editing, Formal analysis, Methodology, and Investigation; P.A.-Z.: Formal Analysis, Methodology, and Writing—Review and Editing; I.B.-H.: Data Curation, Methodology, and Investigation; R.R.: Funding Acquisition, Resources, and Methodology; C.L.R.-J.: Formal Analysis, Visualization, and Writing—Review and Editing; G.C.G.M.-Á.: Data Curation, Writing—Review and Editing, Supervision, Funding Acquisition, Investigation, and Resources. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Program for the Support of Science, Technology, and Innovation UANL-ProACTI 2023 (funding number: 13-BQ-2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to the student thesis being in its finishing process.

Acknowledgments: Aranda-Ledesma thanks the National Council of Humanities, Science and Technology (CONAHCyT) of Mexico for his postgraduate scholarship.

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

References

1. Rudrapal, M.; Khairnar, S.J.; Khan, J.; Bin Dukhyil, A.; Ansari, M.A.; Alomary, M.N.; Alshabrmi, F.M.; Palai, S.; Deb, P.K.; Devi, R. Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Front. Pharmacol.* **2022**, *13*, 283. [CrossRef] [PubMed]
2. Belojević, G. Effects of polyphenols on human health. *Galen. Med. J.* **2023**, *2*, 46–52. [CrossRef]
3. Agustina, R.-M.; Dealmy, D.-G.; Verónica, B.-R.; Jolanta, E.M.; Hady, K.; Ansoumane, K.; Sergio, A.R.G.; Jesús, R.R.A.; José, C.T.-C. *Jatropha dioica*, an Aztec plant with promising pharmacological properties: A systematic review. *Afr. J. Pharm. Pharmacol.* **2020**, *14*, 169–178. [CrossRef]
4. Palomo-Ligas, L.; Flores, S.D.N.; García-Ortiz, J.D.; Pérez-Juárez, C.M.; Flores-Gallegos, A.C.; Rodríguez-Herrera, R.; Cruz-Requena, M. Molecular Effects of Bioactive Compounds from Semi-Desert Plants and Their Uses as Potential Ingredient in Food Products. *Mol. Mech. Funct. Food* **2022**, *1*, 142–192. [CrossRef]
5. De Rodríguez, D.J.; Torres-Moreno, H.; López-Romero, J.C.; Vidal-Gutiérrez, M.; Villarreal-Quintanilla, J.; Carrillo-Lomelí, D.A.; Robles-Zepeda, R.E.; Vilegas, W. Antioxidant, anti-inflammatory, and antiproliferative activities of *Flourensia* spp. *Biocatal. Agric. Biotechnol.* **2023**, *47*, 102552. [CrossRef]
6. Ganchev, D. Antisporulation Action of Tarbush Plant (*Flourensia Cernua*) Towards Conidiospores of Plant Pathogens. *Malays. J. Sustain. Agric.* **2022**, *6*, 81–84. [CrossRef]
7. Aranda-Ledesma, N.E.; González-Hernández, M.D.; Rojas, R.; Paz-González, A.D.; Rivera, G.; Luna-Sosa, B.; Martínez-Ávila, G.C.G. Essential Oil and Polyphenolic Compounds of *Flourensia cernua* Leaves: Chemical Profiling and Functional Properties. *Agronomy* **2022**, *12*, 2274. [CrossRef]
8. García-Carmona, E.L.; Tijerina-Márquez, R.; Torres-González, L.; Moreno-Peña, D.; Rodríguez-Rodríguez, D.R.; Espíndola-Vela, P.; Muñoz-Espinosa, L.E.; Pérez-Rodríguez, E.; Zapata-Chavira, H.; Cordero-Pérez, P. Evaluation of the hepatoprotective effect of *Flourensia cernua* against the damage induced ischemia-reperfusion in Wistar rats. *Ann. Hepatol.* **2022**, *29*, 101417. [CrossRef]
9. Gutiérrez-Tlahque, J.; Aguirre-Mancilla, C.L.; López-Palestina, C.; Sánchez-Fernández, R.E.; Hernández-Fuentes, A.D.; Torres-Valencia, J.M. Constituents, antioxidant and antifungal properties of *Jatropha dioica* var. *dioica*. *Nat. Prod. Commun.* **2019**, *14*, 1–5. [CrossRef]
10. Ramírez-Moreno, A.; García Garza, R.; Pedroza Escobar, D.; Soto Dominguez, A.; Flores-Loyola, E.; Castillo Maldonado, I.; Keita, H.; Sharara Núñez, I.; Keita, H.; Delgadillo Guzmán, D. Antioxidant Effect of *Jatropha dioica* Extract on Immunoreactivity of Claudin 2 in the Kidney of Rats with Induced Diabetes. *Nat. Prod. Commun.* **2023**, *18*, 1–8. [CrossRef]
11. Tijerina-Márquez, R.; Mendoza-Hernández, O.H.; Espinosa-Cantú, C.B.; Rivas-Galindo, V.M.; Moreno-Peña, D.; Torres-González, L.; Muñoz-Espinosa, L.E.; Pérez-Rodríguez, E.; Cura-Esquivel, I.A.; Cordero-Pérez, P. Evaluation of the hepatoprotective effect of an hydroalcoholic extract of *Jatropha dioica* against the damage induced by valproic acid in Wistar rats. *Ann. Hepatol.* **2024**, *29*, 101416. [CrossRef]
12. Paz, J.E.W.; Márquez, D.B.M.; Ávila, G.C.M.; Cerda, R.E.B.; Aguilar, C.N. Ultrasound-assisted extraction of polyphenols from native plants in the Mexican desert. *Ultrason. Sonochem.* **2015**, *22*, 474–481. [CrossRef]
13. Clodoveo, M.L.; Crupi, P.; Muraglia, M.; Corbo, F. Ultrasound assisted extraction of polyphenols from ripe carob pods (*Ceratonia siliqua* L.): Combined designs for screening and optimizing the processing parameters. *Foods* **2022**, *11*, 284. [CrossRef]
14. Gutiérrez-Sánchez, M.d.C.; Aguilar-Zárate, P.; Michel-Michel, M.R.; Ascacio-Valdés, J.A.; Reyes-Munguía, A. The Ultrasound-Assisted Extraction of Polyphenols from Mexican Firecracker (*Hamelia patens* Jacq.): Evaluation of Bioactivities and Identification of Phytochemicals by HPLC-ESI-MS. *Molecules* **2022**, *27*, 8845. [CrossRef] [PubMed]
15. Michel, M.R.; Aguilar-Zárate, M.; Rojas, R.; Martínez-Ávila, G.C.G.; Aguilar-Zárate, P. The Insecticidal Activity of Azadirachta indica Leaf Extract: Optimization of the Microencapsulation Process by Complex Coacervation. *Plants* **2023**, *12*, 1318. [CrossRef] [PubMed]
16. Ascacio-Valdés, J.A.; Aguilera-Carbó, A.; Martínez-Hernández, J.L.; Rodríguez-Herrera, R.; Aguilar, C.N. Euphorbia antisyphilitica residues as a new source of ellagic acid. *Chem. Pap.* **2010**, *64*, 528–532. [CrossRef]
17. Zadeh, N.S.; Zeppa, G. Recovery and concentration of polyphenols from roasted hazelnut skin extract using macroporous resins. *Foods* **2022**, *11*, 1969. [CrossRef] [PubMed]
18. Pasquet, P.; Julien-David, D.; Zhao, M.; Villain-Gambier, M.; Trébouet, D. Stability and preservation of phenolic compounds and related antioxidant capacity from agro-food matrix: Effect of pH and atmosphere. *Food Biosci.* **2024**, *57*, 103586. [CrossRef]
19. De la Rosa, L.A.; Vazquez-Flores, A.A.; Alvarez-Parrilla, E.; Rodrigo-García, J.; Medina-Campos, O.N.; Ávila-Nava, A.; González-Reyes, S.; Pedraza-Chaverri, J. Content of major classes of polyphenolic compounds, antioxidant, antiproliferative, and cell protective activity of pecan crude extracts and their fractions. *J. Funct. Foods.* **2014**, *7*, 219–228. [CrossRef]
20. Van den Berg, R.; Haenen, G.R.M.M.; Van den Berg, H.; Bast, A.A.L.T. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511–517. [CrossRef]
21. Çelik, S.E.; Özyürek, M.; Güçlü, K.; Apak, R. Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/persulphate and FRAP methods. *Talanta* **2010**, *81*, 1300–1309. [CrossRef] [PubMed]
22. Ge, X.; Jing, L.; Zhao, K.; Su, C.; Zhang, B.; Zhang, Q.; Han, L.; Yu, X.; Li, W. The phenolic compounds profile, quantitative analysis, and antioxidant activity of four naked barley grains with different color. *Food Chem.* **2021**, *335*, 127655. [CrossRef] [PubMed]

23. Monroy-García, I.N.; Carranza-Torres, I.E.; Carranza-Rosales, P.; Oyón-Ardoiz, M.; García-Estévez, I.; Ayala-Zavala, J.F.; Morán-Martínez, J.; Viveros-Valdez, E. Phenolic profiles and biological activities of extracts from edible wild fruits *Ehretia tinifolia* and *Sideroxylon lanuginosum*. *Foods* **2021**, *10*, 2710. [CrossRef] [PubMed]
24. Navidad-Murrieta, M.S.; Pérez-Larios, A.; Sanchéz-Burgos, J.A.; Ragazzo-Sánchez, J.A.; Luna-Bárcenas, G.; Sáyago-Ayerdi, S.G. Use of a taguchi design in *Hibiscus sabdariffa* extracts encapsulated by spray-drying. *Foods* **2020**, *9*, 128. [CrossRef] [PubMed]
25. Yazdi, A.P.G.; Barzegar, M.; Sahari, M.A.; Gavlighi, H.A. Optimization of the enzyme-assisted aqueous extraction of phenolic compounds from pistachio green hull. *Food Sci. Nutr.* **2019**, *7*, 356–366. [CrossRef] [PubMed]
26. Bindes, M.M.M.; Cardoso, V.L.; Reis, M.H.M.; Boffito, D.C. Maximisation of the polyphenol's extraction yield from green tea leaves and sequential clarification. *J. Food Eng.* **2019**, *241*, 97–104. [CrossRef]
27. Kumar, K.; Srivastav, S.; Sharanagat, V.S. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrason. Sonochem.* **2021**, *70*, 105325. [CrossRef] [PubMed]
28. Salacheep, S.; Kasemsiri, P.; Pongsa, U.; Okhawilai, M.; Chindaprasirt, P.; Hiziroglu, S. Optimization of ultrasound-assisted extraction of anthocyanins and bioactive compounds from butterfly pea petals using Taguchi method and Grey relational analysis. *J. Food Sci. Technol.* **2020**, *57*, 3720–3730. [CrossRef] [PubMed]
29. Kaderides, K.; Papaoikonomou, L.; Serafim, M.; Goula, A.M. Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. *Chem. Eng. Process.* **2019**, *137*, 1–11. [CrossRef]
30. Nishad, J.; Saha, S.; Kaur, C. Enzyme-and ultrasound-assisted extractions of polyphenols from *Citrus sinensis* (cv. Malta) peel: A comparative study. *J. Food Process. Preserv.* **2019**, *43*, e14046. [CrossRef]
31. Aranda Ledesma, N.E. Caracterización Avanzada de Candelilla y Otras Plantas del Semi-Desierto Mexicano. Master's Thesis, Universidad Autónoma de Nuevo León, Monterrey, Mexico, 2022.
32. Khound, P.; Sarma, H.; Sarma, P.P.; Jana, U.K.; Devi, R. Ultrasound-Assisted Extraction of Verbascoside from *Clerodendrum glandulosum* Leaves for Analysis of Antioxidant and Antidiabetic Activities. *ACS Omega* **2023**, *8*, 20360–20369. [CrossRef] [PubMed]
33. Terrazas-Hernández, J.; Santos-López, E.M.; Cariño-Cortés, R.; Jiménez-Alvarado, R.; López-Palestina, C.U.; Hernández-Fuentes, A.D. Effects of Sterilization on Bioactives of *Jatropha dioica* and *Opuntia oligacantha* Extracts, and on Antimicrobial Capacity against *Streptococcus mutans*. *Appl. Sci.* **2018**, *8*, 2516. [CrossRef]
34. Ullah, A.; Munir, S.; Badshah, S.L.; Khan, N.; Ghani, L.; Poulson, B.G.; Emwas, A.-H.; Jaremko, M. Important flavonoids and their role as a therapeutic agent. *Molecules* **2020**, *25*, 5243. [CrossRef] [PubMed]
35. Hernández-Rosas, N.A.; García-Zebadúa, J.C.; Hernández-Delgado, N.; Torres-Castillo, S.; Figueroa-Arredondo, P.; Mora-Escobedo, R. Perfil de polifenoles, capacidad antioxidante y efecto citotóxico in vitro en líneas celulares humanas de un extracto hidroalcohólico de pétalos de *Calendula officinalis* L. *TIP Rev. Espec. En Cienc. Quím.-Biol.* **2019**, *21* (Suppl. S1), S54–S64. [CrossRef]
36. Hmidani, A.; Bouhlali, E.D.T.; Ajebli, M.; Khouya, T.; Benlyas, M.; Alem, C. In vitro investigation of antioxidant and antihemolytic activities of three Lamiaceae species from Morocco. *Beni-Suef Univ. J. Basic Appl. Sci.* **2019**, *10*, 1–8. [CrossRef]
37. Alvarez-Pérez, O.B.; Ventura-Sobrevilla, J.M.; Ascacio-Valdés, J.A.; Rojas, R.; Verma, D.K.; Aguilar, C.N. Valorization of *Flourensia cernua* DC as source of antioxidants and antifungal bioactives. *Ind. Crops Prod.* **2020**, *152*, 112422. [CrossRef]
38. Abramovič, H.; Grobin, B.; Ulrih, N.P.; Cigić, B. Relevance and standardization of in vitro antioxidant assays: ABTS, DPPH, and Folin–Ciocalteu. *J. Chem.* **2018**, *2018*, 1–9. [CrossRef]
39. Ji, M.; Gong, X.; Li, X.; Wang, C.; Li, M. Advanced research on the antioxidant activity and mechanism of polyphenols from Hippophae species—A review. *Molecules* **2020**, *25*, 917. [CrossRef] [PubMed]
40. Wojtunik-Kulesza, K.A. Approach to optimization of FRAP methodology for studies based on selected monoterpenes. *Molecules* **2020**, *25*, 5267. [CrossRef]
41. Leon-Bejarano, M.; Ovando-Martínez, M.; Molina-Domínguez, C.C.; Trasviña-Mendoza, R.A.; Medina-Juárez, L. Identificación y Actividad Antioxidante de los Compuestos Fenólicos de *Jatropha cardiophylla* (Torr.) Müll. Arg. *TIP Rev. Espec. Cienc. Quím.-Biol.* **2021**, *24*, 1–9. [CrossRef]
42. Benzie, I.F.; Devaki, M. The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: Concepts, procedures, limitations and applications. In *Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications*; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2018; pp. 77–106. [CrossRef]
43. Gutiérrez-Del-Río, I.; López-Ibáñez, S.; Magadán-Corpas, P.; Fernández-Calleja, L.; Pérez-Valero, Á.; Tuñón-Granda, M.; Miguélez, E.M.; Villar, C.J.; Lombó, F. Terpenoids and polyphenols as natural antioxidant agents in food preservation. *Antioxidants* **2021**, *10*, 1264. [CrossRef] [PubMed]
44. Lipinski, B. Hydroxyl radical and its scavengers in health and disease. *Oxid. Med. Cell Longev.* **2011**, *2011*, 1–9. [CrossRef] [PubMed]
45. Ikram, S.; Zhang, H.; Ming, H.; Wang, J. Recovery of major phenolic acids and antioxidant activity of highland barley brewer's spent grains extracts. *J. Food Process. Preserv.* **2020**, *44*, e14308. [CrossRef]
46. Treml, J.; Šmejkal, K. Flavonoids as potent scavengers of hydroxyl radicals. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 720–738. [CrossRef] [PubMed]
47. Zhu, M.; Huang, Y.; Wang, Y.; Shi, T.; Zhang, L.; Chen, Y.; Xie, M. Comparison of (poly) phenolic compounds and antioxidant properties of pomace extracts from kiwi and grape juice. *Food Chem.* **2019**, *271*, 425–432. [CrossRef] [PubMed]

48. Shabbir, M.; Khan, M.R.; Saeed, N. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenus royleanus* leaves. *BMC Complement. Altern. Med.* **2013**, *13*, 1–13. [CrossRef] [PubMed]
49. Adame-Miranda, S.J.; Granados-Guzmán, G.; Silva-Mares, D.A.; Acevedo-Fernández, J.J.; Waksman-Minsky, N.; Salazar-Aranda, R. Evaluation of antihyperglycemic activity of plants in northeast Mexico. *Cell. Mol. Biol.* **2021**, *67*, 212–218. [CrossRef] [PubMed]
50. Haq, A.; Mushtaq, S.; Khan, A.; Islam, A.; Khan, H.; Malik, Z.A.; Younas, F.; Khan, S.; Shah, A.A.; Badshah, M. Evaluation of phytochemical, bioactive, and antifungal potential of *Jatropha curcas* seed oil and de-oiled seed cake extracts against phytopathogenic fungi. *Plant Pathol. J.* **2021**, *103*, 863–873. [CrossRef]
51. Sun, L.; Wang, Y.; Miao, M. Inhibition of α-amylase by polyphenolic compounds: Substrate digestion, binding interactions and nutritional intervention. *Trends Food Sci.* **2020**, *104*, 190–207. [CrossRef]
52. Ćorković, I.; Gašo-Sokač, D.; Pichler, A.; Šimunović, J.; Kopjar, M. Dietary polyphenols as natural inhibitors of α-amylase and α-glucosidase. *Life* **2022**, *12*, 1692. [CrossRef] [PubMed]
53. Bertelli, A.; Biagi, M.; Corsini, M.; Baini, G.; Cappellucci, G.; Miraldi, E. Polyphenols: From theory to practice. *Foods* **2021**, *10*, 2595. [CrossRef] [PubMed]
54. Saffidine, K.; Zerargui, F.; Guemmaz, T.; Baghiani, A. Evaluation of Antioxidant, Antihemolytic Activity, Analgesic and Anti-Inflammatory Potential of *Aristolochia clematitis* Extracts. *Trop. J. Nat. Prod. Res.* **2023**, *7*, 2996–3001. [CrossRef]
55. Dima, J.; Raghda, L.; Jalil, G.A. Evaluation of hemolytic and anti-hemolytic activity of the aerial parts of *Sonchus oleraceus* extracts. *Int. J. Pharm. Sci. Nanotechnol.* **2017**, *10*, 3745–3751. [CrossRef]
56. Estell, R.E.; Fredrickson, E.L.; Havstad, K.M. Chemical composition of *Flourensia cernua* at four growth stages. *Grass Forage Sci.* **1996**, *51*, 434–441. [CrossRef]
57. Ventura-Sobrevilla, J.; Gutiérrez-Sánchez, G.; Bergmann, C.; Azadi, P.; Boone-Villa, D.; Rodriguez-Herrera, R.; Aguilar, C.N. Glycosylation of polyphenols in tannin-rich extracts from *Euphorbia antisyphilitica*, *Jatropha dioica*, and *Larrea tridentata*. In *Green Chemistry and Biodiversity*; Apple Academic Press: Williston, VT, USA, 2019; pp. 81–96. [CrossRef]
58. Azantsa, B.G.K.; Mbong, M.-A.A.; Takuissu, G.R.; Matsinkou, R.S.; Djuikoo, I.L.N.; Youovop, J.F.; Ngondi, J.L.; Oben, J.E. Anti-hemolytic, anti-lipid peroxidation and antioxidant properties of three plants locally used to treat metabolic disorders: *Allium sativum*, *Persea americana* and *Citrus sinensis*. *J. Food Res.* **2019**, *8*, 89–100. [CrossRef]
59. De La Cruz-Jiménez, L.; Hernández-Torres, M.A.; Monroy-García, I.N.; Rivas-Morales, C.; Verde-Star, M.J.; Gonzalez-Villasana, V.; Viveros-Valdez, E. Biological activities of seven medicinal plants used in Chiapas, Mexico. *Plants* **2022**, *11*, 1790. [CrossRef]
60. Fernandes, S.; Ribeiro, C.; Paiva-Martins, F.; Catarino, C.; Santos-Silva, A. Protective effect of olive oil polyphenol phase II sulfate conjugates on erythrocyte oxidative-induced hemolysis. *Food Funct.* **2020**, *11*, 8670–8679. [CrossRef] [PubMed]
61. Hajimehdipoor, H.; Shahrestani, R.; Shekarchi, M. Investigating the synergistic antioxidant effects of some flavonoid and phenolic compounds. *Res. J. Pharm.* **2014**, *1*, 35–40.
62. Wongsa, P.; Phatikulrungsun, P.; Prathumthong, S. FT-IR characteristics, phenolic profiles, and inhibitory potential against digestive enzymes of 25 herbal infusions. *Sci. Rep.* **2022**, *12*, 6631. [CrossRef]
63. Rajhard, S.; Hladnik, L.; Vicente, F.A.; Srčič, S.; Grilc, M.; Likozar, B. Solubility of luteolin and other polyphenolic compounds in water, nonpolar, polar aprotic and protic solvents by applying FTIR/HPLC. *Processes* **2021**, *9*, 1952. [CrossRef]