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ORIGINAL ARTICLE

Sacha inchi (*Plukenetia volubilis* L.) husks and seed shells are sources of phenolic compounds with potential health benefits

Nicole Valeria Polanía Cuellar[1], Indira Paola Hernández Peñaranda[1], Deicy Villalba Rey[2], Katherin Natalia Acuña Rodríguez[3], Franci Nathalie Gómez Jaimes[1*]

Edited by
Angela Johana Espejo Mojica
editorus@javeriana.edu.co

1. Universidad de Santander, Facultad de Ciencias Exactas, Naturales y Agropecuarias, Bucaramanga, Colombia, 680003
2. Universidad del Tolima, Facultad de Ciencias, Ibagué, Colombia, 730006299
3. Industrias Acuña Ltda., Bucaramanga, Colombia, 680011

*nathalie.gomez@mail.udes.edu.co

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Abstract

Sacha inchi (*Plukenetia volubilis* L.) is an oilseed plant that yields a highly nutritious oil. However, its husks and seed shells are under-utilized byproducts. In this study, ethanolic extracts of sacha inchi husks (SI-husk) and seed shells (SI-shell) were obtained using ultrasound-assisted extraction, evaluating the effects of extraction temperature and solvent-to-solid ratio on the yields. The extracts' total phenolic contents, phenolic profiles, antioxidant activities, and antimicrobial activities against Gram-positive and Gram-negative bacteria were investigated. Under the selected extraction conditions for SI-husk (60 °C, 1:8 s/s, 280 W, 120 min) and SI-shell (60 °C, 1:3 s/s, 280 W, 120 min), the extracts' total phenolic contents were 80.18 ± 0.32 and 50.94 ± 0.48 mg GAE (gallic acid equivalents)/g, respectively. Cyanidin, naringenin, and kaempferol were principally found in the SI-husk extract (79.4 %) and vanillic acid in the SI-shell extract (79.9 %). Both extracts exhibited antioxidant activity, with ORAC values of 360.36 ± 0.21 and 228.11 ± 0.14 µmol TE/g, respectively. The antimicrobial activity of the extracts was evaluated against *S. aureus*, *P. aeruginosa*, and *E. coli* using the agar disk diffusion assay. SI-husk (1 mg) exhibited antibacterial activity against *Staphylococcus aureus*, with an inhibition zone of 10.5 ± 1.8 mm. Our results provide new insights into sacha inchi byproducts as sources of bioactive compounds with potential health benefits.

Keywords: antimicrobial; antioxidants; byproducts; oilseed; plant extracts; ultrasound.

1. Introduction

Sacha inchi (*Plukenetia volubilis* L.) is an oilseed plant within the *Euphorbiaceae* family. This crop is primarily of interest due to its seeds' economic value, which yield a commercially valuable and biologically active oil [1]. During Sacha inchi seed processing, the fruit husks and seed shells are removed and discarded as byproducts. However, these plant materials can be sustainably managed and reused as sources of natural ingredients, added to foods and cosmetics, or utilized as pharmaceutical agents.

Sacha inchi fruit husks and seed shells have been previously reported as a potential source of antioxidant phenolic compounds, with antioxidant activity comparable to other oilseed byproducts. Sacha inchi seed shells contain condensed and hydrolyzable tannins, free and bound phenolic acids, lignans, flavonoids, triterpenoids, and phenolic aldehydes [2, 3]. Moreover, reported seed shell benefits include tyrosinase inhibition and antihypertensive effects [4, 5], and fruit husks primarily contain phenolic acids and flavonoids and exhibit anti-cholesterol esterase, anti-diabetic, and anti-tyrosinase activities [6–8].

To the best of our knowledge, few studies have addressed sacha inchi's antimicrobial activity. An *in vitro* study demonstrated that although sacha inchi oil was not bactericidal against *Staphylococcus aureus*, it hindered bacterial adherence to human skin explants [9]. Furthermore, a combination of sacha inchi oil with a Myoviridae phage had a synergistically inhibited multidrug-resistant *Acinetobacter baumannii* biofilm formation [10]. Recently, aqueous extracts of sacha inchi husks obtained at a variety of temperatures were active against Gram-positive bacteria, *S. aureus* and *Listeria monocytogenes*, and Gram-negative bacteria, *Escherichia coli*, *Salmonella enteritidis*, and *Vibrio parahaemolyticus* [7]. However, the phenolic profiles of sacha inchi ethanol extracts have not yet been studied or characterized.

Plant extracts are obtained using a variety of extraction techniques, among which ultrasound-assisted extraction (UAE) is noteworthy due to its simplicity and low operational cost. UAE is based on cavitation caused by the formation, growth, and vapor or gas asymmetric collapse of ultrasound-induced bubbles within a liquid. Ultrasonic waves (> 20 kHz) facilitate extraction from plant materials, increasing mass transfer while reducing solvent consumption and extraction time [11]. Operating conditions affect UAE efficiency, namely temperature, solvent-to-solid ratio, ultrasonic power, and extraction time, which are generally optimized to obtain high yields.

Based on the considerations above, the aims of this study were threefold. (i) To obtain ethanolic extracts from sacha inchi (*Plukenetia volubilis* L.) husks and seed shells using ultrasound-assisted extraction to evaluate the effect of temperature and solvent-to-solid ratio conditions on the yields; (ii) to quantify the total phenolic content and to identify the leading secondary metabolites present in the extracts using chromatographic analysis; and (iii) to determine the antioxidant activity and the antimicrobial activity of the extracts against Gram-positive and Gram-negative bacteria. The results of this study provide new information on the phenolic composition of sacha inchi byproducts as natural sources of bioactive extracts with potential health benefits.

2. Materials and methods

2.1. Plant material

Commercially available sacha inchi fruits were obtained from Industrias Acuña INAL, in Bucaramanga, Santander, Colombia (**Fig. 1**). The husks and seed shells were separated from the fruits, milled, and sieved using a mesh size of 5 mm. The samples were oven-dried at 50 °C for five days until they reached a moisture content of 10 % and were stored for further extraction.

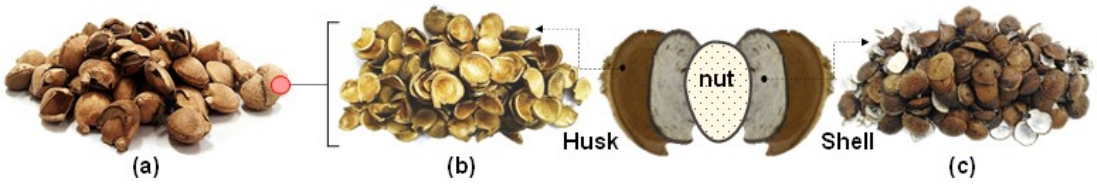

Figure 1. Sacha inchi fruits (**a**) whole nuts, (**b**) husks, and (**c**) seed shells.

2.2. Chemicals

Folin-Ciocalteu reagent, sodium carbonate, phenolic compounds: phenolic acids (gallic 98 %, *p*-hydroxybenzoic 99 %, vanillic > 97 %, ferulic ≤ 100 %, and cinnamic acid ≤ 100 %), flavonoids (ursolic ≤ 100 %, carnosic ≤ 100 %, caffeic ≥ 98 %, *p*-coumaric ≥ 98 %, rosmarinic ≤ 100 %, quercetin ≤ 100 %, naringenin ≤ 100 %, luteolin ≥ 98 %, kaempferol ≤ 100 %, apigenin ≤ 100 %, pinocembrin acids ≥ 95 %), catechins [(-)-epigallocatechin ≥ 98 %, (±)-catechin ≤ 100 %, (-)-epigallocatechin gallate ≤ 100 %, (-)-epicatechin ≥ 95 %, (-)-epicatechin gallate ≥ 99 %], anthocyanins (cyanidin-3-rutinoside ≤ 100 %, pelargonidine-3-glucoside ≥ 98 %, quercetin-3-glucoside ≤ 100 %, kaempferol-3-glucoside ≤ 100 %), phosphate-buffered saline (PBS), Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), dimethyl sulfoxide (DMSO, ≥ 99.9%), and gentamicin sulfate salt (potency ≥ 590 I.U) were purchased from Sigma Aldrich (Saint Louis, MO, USA). Fluorescein, AAPH ([2,2'-azobis(2-amidinopropane) dihydrochloride, 99 %] and Müller-Hinton agar were purchased from Merck (Darmstadt, Germany). Brain heart infusion broth (BHI) was purchased from Oxoid (Cheshire, UK). All reagents were used without further purification, and aqueous solutions were prepared using ultrapure water (12 MΩ·cm at 25 °C).

2.3. Ultrasound-Assisted Extraction

Sacha inchi husk and seed shell extracts were obtained using UAE. Plant samples were extracted separately with absolute ethanol at three different temperatures and solvent (volume, mL)-to-solid (mass, g) ratios. The extractions were carried out using an Elmasonic S30H ultrasonic water bath (Elma Schmidbauer GmbH, Singen, Baden-Württemberg, Germany) operated in continuous mode (37 kHz, 280 W) at intervals of 20 min for 2 h. After the extraction step, the solvent was removed by rotary evaporation, and the extracts (labeled as SI-husk and SI-shell) were collected and refrigerated between 1.5 °C - 2.5 °C for further analysis. The extraction yield was expressed as a percentage (%) based on the weight of the extract and the dry weight (DW) of the plant material.

2.4. Experimental design

The UAE conditions for SI-husk and SI-shell were optimized using two 3^2 factorial experimental designs, with yield as response variable. The yield was defined as the percentage of the ratio between mass differences, namely initial and final mass (m_i-m_f) and initial mass (m_i). The evaluated factor levels were temperature (25 °C, 45 °C, and 60 °C) and solvent-to-solid (s/s) ratios (4:1, 6:1, and 8:1 for SI-husk, and 2:1, 3:1, and 4:1 for SI-shell). All treatments were performed in triplicate and data standard deviations of the data were calculated and reported. A two-way analysis of variance ANOVA was used to evaluate and compare the yields using R 4.0.2 software. The UAE conditions for SI-husk and SI-shell were selected based on the experimental design results.

2.5. Total phenolic compounds

SI-husk and SI-shell total phenolic contents (TPC) were measured using the Folin-Ciocalteu method, as described by Dastmalchi *et al*. [12]. A 100-μL aliquot of each extract was mixed with 6 mL of ultrapure water and 500 μL of Folin–Ciocalteu reagent in a test tube. After 1 min, 1.5 mL of sodium carbonate solution (200 g/L) were added to each tube, and all reactions were brought to a total volume of 10 mL with ultra-pure water. The reaction proceeded for 2 h at 25 °C, and the

absorbance of the mixture was measured at 760 nm using a UV/VIS spectrophotometer 4001/4 GENESYS 20™ (Thermo Scientific, Waltham, MA, USA). Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g), DW.

2.6. Chromatographic analysis

Phenolic compounds in SI-husk and SI-shell were identified by ultra-high-performance liquid chromatography time-of-flight mass spectrometry (UHPLC-TOF-MS). The extracts were analyzed using a Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Sunnyvale, CA, USA) coupled with an Orbitrap mass spectrometer (Exactive Plus, Thermo Scientific). Chromatographic separation was performed using a Hypersil GOLD Aq column (Thermo Scientific, Sunnyvale, CA, USA; 100 x 2.1 mm, 1.9 μm) at 30 °C. The mobile phase was comprised of a combination of A (0.2% ammonium formate in water) and B (0.2% ammonium formate in acetonitrile). The initial condition of the gradient was 100% A, which was changed linearly to 100% B (8 min), and held for 4 min. The composition of the eluent was then restored to 100% A in 1 min, and the total run time was 13 min at 3 min after the run. The mass spectrometer was equipped with an electrospray interface (ESI) operated in the positive-ion mode with a capillary voltage of 4.5 kV. Mass spectra were obtained in the mass range m/z 60- 900. The identification of phenolic compounds was confirmed using the full scan acquisition mode and ion extraction corresponding to the $[M+H]^+$ of the compounds of interest, the measurement of masses with accuracy and precision of $\triangle$ ppm < 0.001, and the use of a standard solution of phenolic compounds. For quantification of the samples, the external standardization technique was used, and the results were expressed as mg of phenolic compound per kilogram of extract (mg/kg), DW. The limit of quantification (LOQ) was calculated as S/N = 10, where S/N is the signal-to-noise ratio.

2.7. Antioxidant capacity: ORAC assay

The antioxidant capacities of the SI-husk and SI-shell extracts were quantified using the ORAC method following Ou *et al*. [13] and Huang *et al*. [14]. Assays were performed using a Fluoroskan fluorimeter (Ascent™; Thermo Scientific, Waltham, MA, USA). DPPH was used as a peroxyl radical generator, fluorescein as a fluorescent probe, and Trolox as the standard. All solutions were prepared in a phosphate buffer solution PBS (75 mM, pH 7.4). Briefly, the ORAC assay was carried out as follows: 25 μL of PBS (blank), diluted extracts, or Trolox standard were mixed with 150 μL of fluorescein solution (8.16 x 10^{-5} mM) and incubated for 20 min at 37 °C. Subsequently, 25 μL of the AAPH solution (0.153 mM) was added to each sample, and the readings were started immediately. Fluorescence was measured every minute for 60 min at 37 °C using filters at an excitation wavelength of 490 nm and an emission wavelength of 510 nm. The ORAC values were calculated based on the net area under the decay curves and expressed as μmol Trolox Equivalents per gram of extract (μmol TE/g), DW.

2.8. Agar disk diffusion assay

Antimicrobial activity was evaluated using the agar disk diffusion assay, as described in the M02-A11 protocol of the Clinical & Laboratory Standards Institute (CLSI) [15]. Before the test, stock solutions of SI-shell and SI-husk were prepared at a concentration of 50 mg/mL in sterile distilled water and 0.2% DMSO, respectively, and continuously vortexed at 1500 rpm for 30 min. Gentamicin, a reference antibiotic, was prepared in sterile distilled water at a concentration of 1 mg/mL. Disks with a diameter of 6 mm were prepared using Wathmann filter paper No. 3. Sterile

disks were loaded with SI-husk and SI-shell solution stocks at a final concentration of 1 mg/disk, and gentamicin at 0.01 mg/disk. The disks were then incubated at 37 °C for 4 h and stored in a freezer at -20 °C until use.

For the agar disk diffusion test, a bacterial inoculum was prepared from a subculture of *S. aureus* ATCC® 25923™, *E. coli* ATCC® 25922™, and *Pseudomonas aeruginosa* ATCC® 27853™ in BHI broth incubated for 22 h at 37 °C. The concentration of each microorganism was adjusted to $1x10^8$ CFU/mL to obtain an absorbance between 0.08 and 0.1 at 620 nm by UV-vis spectrophotometry. Subsequently, the surface of the Mueller-Hinton agar was inoculated by streaking a cotton swab previously impregnated with each microorganism. SI-husk (1 mg), SI-shell (1 mg), and gentamicin (0.01 mg) disks were then applied to the surface of the inoculated agar plate. The cultures were incubated under aerobic conditions for 24 h at 37 °C. The antimicrobial activities of the extracts and antibiotics were determined by measuring the diameter of the zone of inhibition on the agar surface around the disk. Disks containing distilled water or 0.2 % DMSO were employed as negative controls.

3. Results and discussion

3.1. Ultrasound-assisted extraction of sacha inchi husks and seed shells

SI-husks and SI-shells were subjected to UAE at an array of temperatures and s/s ratios (**Table 1**). The highest yield was obtained at 60 °C for both extracts. This temperature had been previously reported to be the most efficient for the extraction of phenolic compounds from sacha inchi byproducts [2].

The highest yield for SI-husk was obtained at 60°C and a 1:8 s/s ratio, with 0.95 ± 0.15% (treatment 9). The yields at 45 °C and 25 °C did not differ when 1:6 and 1:8 s/s ratios were used (**Suppl. Fig. 1a**). These results likely indicate the absence of significant interactions between temperature and s/s ratio. Accordingly, the results of the two-way ANOVA without interaction (**Suppl. Table** 1) showed differences in SI-husk yield based on the temperature and s/s ratio evaluated ($p < 5\%$) (**Suppl. Fig. 1b**). Given these results, a *post-hoc* contrast was performed using Scheffe's test for multiple comparisons, indicating differences in the yield at each temperature level, with the highest yield at 60 °C (**Suppl. Table 2**). In contrast, there were no differences between the yields for 1:8 and 1:6 s/s ratios, and the lowest yield was achieved with a 1:4 s/s ratio.

Table 1. UAE yields for SI-husk and SI-shell at different temperatures and s/s ratios.

Treatment	Temperature (°C)	SI-husk		SI-shell	
		s/s ratio	Yield (% ± sd)	s/s ratio	Yield (% ± sd)
1	25	1:4	0.35 ± 0.05	1:2	0.90 ± 0.05
2	25	1:6	0.44 ± 0.05	1:3	1.26 ± 0.22
3	25	1:8	0.45 ± 0.03	1:4	2.67 ± 0.20
4	45	1:4	0.44 ± 0.02	1:2	1.37 ± 0.11
5	45	1:6	0.59 ± 0.04	1:3	1.50 ± 0.11
6	45	1:8	0.60 ± 0.02	1:4	3.08 ± 0.07
7	60	1:4	0.68 ± 0.06	1:2	1.38 ± 0.09
8	60	1:6	0.77 ± 0.09	1:3	3.12 ± 0.55
9	60	1:8	0.95 ± 0.15	1:4	2.19 ± 0.71

Standard deviation (sd), n = 3; solvent-to-solid ratio (s/s).

For SI-shells, the yields at 45 °C and 25 °C increased at higher s/s ratios, whereas at 60 °C, the behavior concerning the s/s ratio differed. Contrary to SI-husk, these results revealed significant interactions between temperature and s/s ratio (**Suppl. Fig. 1c**). The results of the two-way ANOVA (**Suppl. Table 3**) indicated that the interaction between these two factors was significant ($p < 5$ %). Subsequently, differences were observed in the yield according to the temperature and s/s ratio, but there was a differentiated behavior of the yield according to the temperature for each s/s ratio (**Suppl. Fig. 1d**).

The highest yields for SI-shell extracts were obtained at 60 °C and a 1:3 s/s ratio, with 3.12 ± 0.55 % (treatment 8), and at 45 °C and a 1:4 s/s ratio, with 3.08 ± 0.07 % (treatment 6). Given these results, a *post-hoc* contrast was performed using Tukey's test for multiple comparisons, and no significant differences were found between these treatments ($p = 0.9998$). Similarly, the yield obtained at 25 °C with a 1:4 s/s ratio, with 2.67 ± 0.20 (treatment 3) was not significantly different from these in treatment 6 ($p = 0.29573$) or treatment 8 ($p = 0.39619$).(**Suppl. Table 4**)

Compared to SI-husk extraction (60 °C, 1:8 s/s, 280 W, 120 min), UAE-selected conditions for SI-shell extraction (60 °C, 1:3 s/s, 280 W, 120 min) required lower solvent consumption. This finding may be attributable to the higher diffusion of the compounds, as the concentration gradient is higher when using a lower solid-to-solvent ratio [16]. From this perspective, the lower consumption of solvent in the UAE is also reasonable from an economic and environmental point of view.

3.2. Total phenolic content and phenolic profiles of sacha inchi husk and shell extracts

Plant extracts are widely recognized as sources of bioactive components with prevalent phenolic compounds [17]. Under the UAE-selected conditions, TPC values for SI-husk and SI-shell extracts were 80.18 ± 0.32 mg GAE/g (0.76 mg GAE/100 g husk) and 50.94 ± 0.48 mg GAE/g (1.58 mg GAE/100 g seed shell), respectively (**Table 2**).

Different TPC values have been reported in sacha inchi husks and seed shell extracts highlighting a marked variability. For instance, seed shells subjected to extraction using various solvent mixtures exhibited TPC values ranging from 9.5 ± 0.3 mg to 15.3 ± 0.2 mg GAE/g [2]. Similarly, using aqueous ethanol, seed shell extracts had a TPC value of 129.95 ± 7.58 mg GAE/g, and a value of 41.97 ± 1.05 mg GAE/g after microwave-assisted extraction [3, 4]. Methanolic seed shell extracts showed a TPC value of 323.74 ± 2.25 mg GAE/100 g, and 503.96 ± 5.16 mg GAE/100 mg for the husks. Aqueous extraction of the husks at varying temperatures resulted in TPC values of 74.8 mg GAE/g, and from 4.58 ± 0.04 mg to 7.90 ± 0.60 mg GAE/g dry extract [6, 7].

Thirteen phenolic compounds were identified in SI-husk and SI-shell extracts via UHPLC-ESI$^+$-Orbitrap-MS analysis by comparing the spectra with standard phenolic compounds (**Suppl. Fig. 2**). Flavonoid-type compounds were primarily found in SI-husk; with cyanidin (anthocyanidin), naringenin (flavanone), and kaempferol (flavanol) as the main compounds (79.4 % of the total sample); followed by phenolic acids such as vanillic acid (hydroxybenzoic acid derivative) (**Table 3**). In SI-shell, vanillic acid was the most abundant compound (79.9 %), followed by cyanidin (**Fig. 2**).

Table 2. TPC and ORAC values for SI-husk and SI-shell.

Sample	TPC + sd (mg GAE/g)	ORAC+ sd (μmol Trolox®/ g)
SI-husk	80.18±0.32	360.36 ± 0.21
SI-shell	50.94 ± 0.48	228.11 ± 0.14

Standard deviation (sd), n = 3

Table 3. Phenolic compounds identified in SI-husk and SI-shell extracts by UHPLC-ESI$^+$-Orbitrap-

No	Phenolic compound[a]	Molecular formula	Measured mass $[M+H]^+ m/z$	Theoretical mass $[M+H]^+ m/z$	t_R[b] (min)	SI-husk (mg/kg)	SI-shell (mg/kg)	LOQ[c] (mg/kg)
1	(-)-Epigallocatechin	$C_{15}H_{14}O_7$	307.0812	307.0818	3.80	1.59	0.72	0.50
2	Pelargonidin-3-glucoside	$C_{21}H_{21}O_{10}$	433.1135	433.3885	4.07	1.54	<0.01	0.01
3	Vanillic acid	$C_8H_8O_4$	169.0495	169.0501	4.23	341.27	54.27	0.07
4	Quercetin-3-glucoside	$C_{21}H_{19}O_{12}$	465.1022	465.3865	4.66	1.54	<0.01	0.01
5	*p*-Coumaric acid	$C_9H_8O_3$	165.0546	165.0552	4.70	209.88	<0.02	0.02
6	Ferulic acid	$C_{10}H_{10}O_4$	195.0652	195.0657	4.81	18.33	5.98	0.01
7	Kaempferol-3-glucoside	$C_{21}H_{20}O_{11}$	449.1078	449.1084	4.84	3.30	0.09	0.01
8	Cyanidin	$C_{15}H_{11}O_6$	287.0556	287.2465	4.86	1352.99	6.80	0.01
9	Quercetin	$C_{15}H_{10}O_7$	303.0499	303.0505	5.59	2.43	<0.01	0.01
10	Naringenin	$C_{15}H_{12}O_5$	273.0757	273.0763	5.87	973.21	<0.01	0.01
11	Apigenin	$C_{15}H_{10}O_5$	271.0601	271.0607	6.01	125.49	<0.01	0.01
12	Kaempferol	$C_{15}H_{10}O_6$	287.0550	287.0556	6.08	396.44	<0.01	0.01
13	Pinocembrin	$C_{15}H_{12}O_4$	257.0808	257.0814	6.75	0.59	0.05	0.01

[a] Identification with standard substances. [b] tR: retention time [c] Limit of quantification

Flavonoids were primarily found in the SI-husk extracts and only in minimal amounts in the SI-shell extracts. The flavonoid contents calculated by summing the individual amounts of all constituents in SI-husk and SI-shell were 2854.45 mg/kg (27.15 mg/kg husk) and 6.94 mg/kg (0.35 mg/kg seed shell), respectively. Cyanidin was the most abundant phenolic compound in SI-husk, with 1352.99 mg/kg (12.85 mg/kg husk), and the predominant flavonoid in SI-shell, with 6.80 mg/kg (0.21 mg/kg seed shell). Naringenin and kaempferol were also found as major components of SI-husk extracts, with 973.21 mg/kg (9.25 mg/kg husk) and 396.44 mg/kg (3.77 mg/kg husk), respectively. These compounds were only detected, but not quantified, in SI-shell extracts.

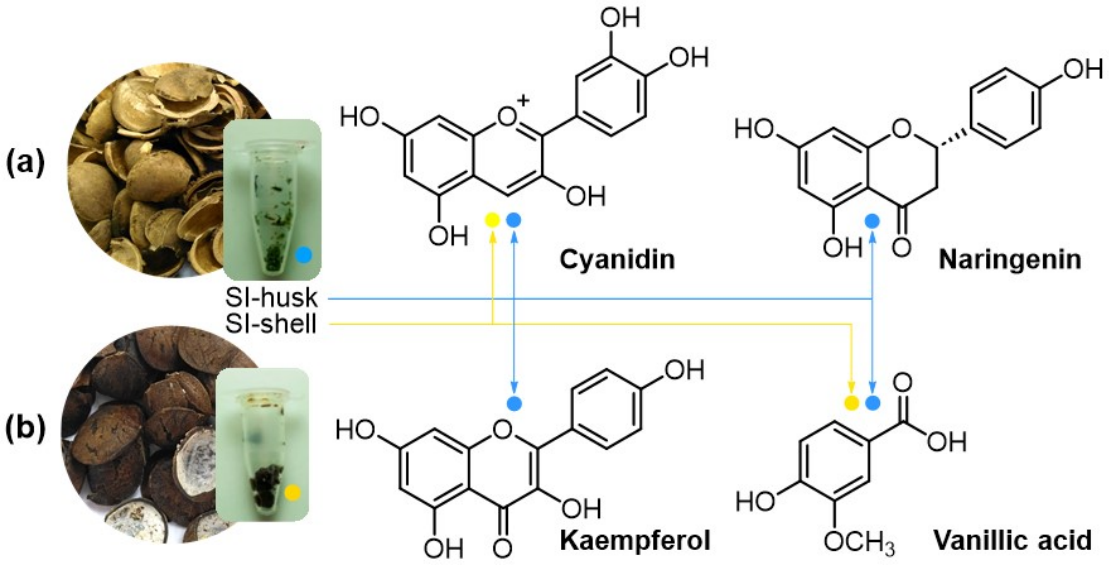

Figure 2. Chemical structures of the main phenolic compounds found in (a) SI-husk and (b) SI-shell extracts.

In contrast to SI-shell extract, the group of phenolic acids (vanillic, *p*-coumaric, and ferulic acid) were mostly found in SI-husk extracts. The sum of free phenolic acids detected in SI-husk and SI-shell extracts was 569.48 mg/kg (1.88 mg/kg husk) and 60.25 mg/kg (5.41 mg/kg seed shell), respectively. Vanillic acid was predominant in SI-shell, with 54.27 mg/kg (1.69 mg/kg seed shell), and the major phenolic acid in SI-husk, with 341.27 mg/kg extract (3.24 mg/kg husk); followed by *p*-coumaric acid, with 209.88 mg/kg extract (1.99 mg/kg husk). Other minor phenolic compounds identified in the extracts were quercetin, apigenin, ferulic acid, quercetin-3-glucoside, pelargonidin-3-glucoside, kaempferol-3-glucoside, epigallocatechin, and pinocembrin.

Interestingly, our study is the first detecting cyanidin in sacha inchi husks and seed shells. Differences in the phenolic composition of these byproducts have been found in related studies, depending on sample processing and analysis techniques. For instance, caffeic acid was predominant in husks, with 4.44 ± 0.04 mg/100 g, while *p*-coumaric acid led in seed shells, with 148.74 ± 2.46 mg/100 g, via HPLC analysis [8]. Regarding flavonoid content, both husks and seed shells contained kaempferol, with 0.27 ± 0.01 mg/100 g and 12.63 ± 0.45 mg/100 g, respectively. Naringenin was only detected in the seed shells, with 29.21 ± 0.17 mg/100 g [8].

Likewise, protocatechuic acid, *p*-coumaric acid, and hydroxycinnamic acid derivatives have been detected in seed shells using HPLC-DAD analysis, and 3,4-dihydroxy-benzaldehyde (DHBA) and hydroxy-4-chromone were identified by UHPLC-DAD-ESI+-MS analysis [2, 3]. In agreement with these reports, phenolic acids were predominant in SI-shell in our study. However, flavonoid-type compounds were the main type of phenolics in SI-husk extracts.

3.3. Antioxidant activity of sacha inchi husk and seed shell extracts

Phenolic compounds are potential pharmaceutical ingredients due to their health-promoting properties, highlighting their antioxidant activity [18]. SI-husk and SI-shell extract antioxidant activities were measured using the ORAC assay. This test is based on the inhibition of oxidation induced by peroxyl radicals, commonly found in food and biological systems [19]. SI-husk and SI-shell extracts showed ORAC values of 360.36 ± 0.21 µmol TE/g (3.42 µmol TE/100 g husk) and 228.11 ± 0.14 µmol TE/g (7.11 µmol TE/100 g seed shell), respectively. In previous reports, sacha inchi seed shells exhibited ORAC values from 92.5 ± 5.4 to 192.6 ± 12.8 µmol TE/g and 9751.06 ± 116.58 µmol TE/100 g DW; as well as 4238.67 ± 89.15 µmol TE/100 g for the husks [2, 8]. We found that the ORAC value from the SI-shell extract is higher than the SI-husk extract, but similar to or lower than the ORAC values reported. Other methods, including Ferric ion Reducing Antioxidant Power (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), have also been used to evaluate the antioxidant activity of sacha inchi husks and seed shells [**?**, 2–4, 6–8]. These byproducts are valuable sources of antioxidant phenolic compounds with potential health benefits and applications in the nutraceutical, cosmetic, and food industries.

The ORAC activity of SI-husk and SI-shell extracts may be attributable to their phenolic composition. Cyanidin, kaempferol, naringenin, and vanillic acid demonstrate antioxidant activity in *in vitro* and *in vivo* studies [20]. They also exhibit pharmacological activities, including antimutagenic, anticancer, anti-inflammatory, antimicrobial, and antiviral effects [21]. Naringenin and kaempferol have been described as inhibitors of SARs-CoV-2 main protease (3CLpro) and could be considered as complementary therapeutic strategies against COVID-19 [22, 23]. Furthermore, methanolic extracts of sacha inchi leaves, presumably containing kaempferol, reduced the viability of HeLa and A549 cells, and induced cell death via apoptosis [24].

3.4. Antimicrobial activity of sacha inchi husk and seed shell extracts

Phenolic compounds from plant extracts have been extensively studied as potential antimicrobial agents [25]. In the present study, the antimicrobial activity of SI-husk and SI-shell extracts against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria was evaluated by the agar disk diffusion method (**Fig. 3**), with the results being based on the inhibition zones.

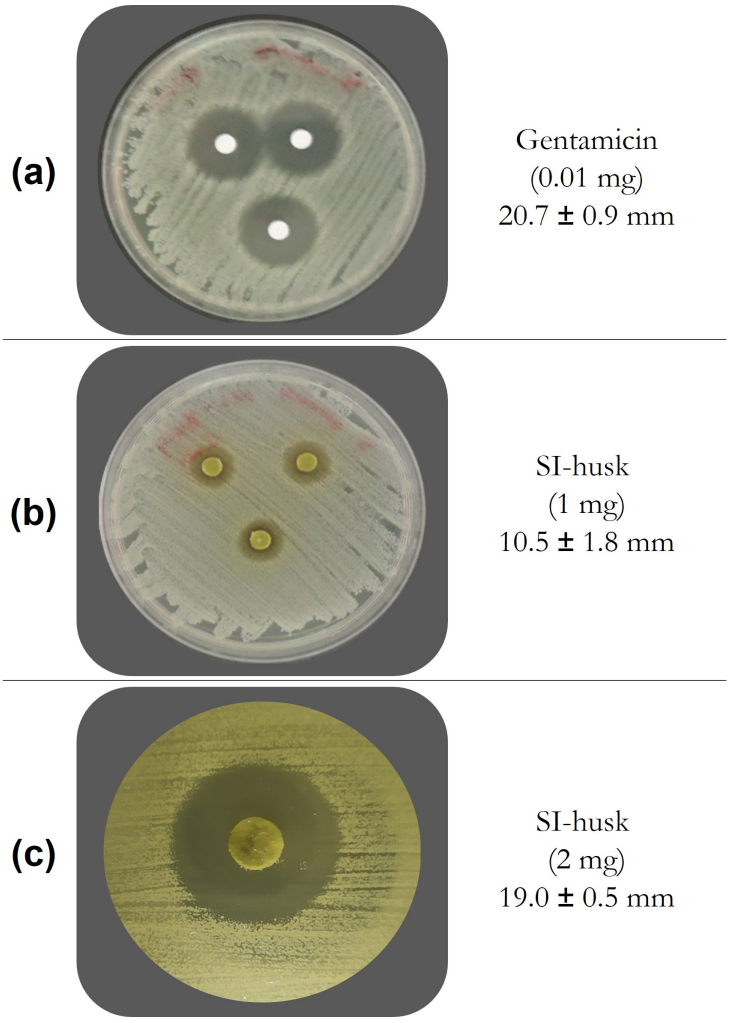

Figure 3. Disk diffusion assay of *S. aureus* exposed to (**a**) gentamicin (0.01 mg), (**b**) SI-husk (1 mg), and (**c**) SI-husk (2 mg).

Only *S. aureus* was susceptible to SI-husk, with an inhibition zone of 10.5 ± 1.8 mm (Fig. 3b), and none of the extracts inhibited the growth of Gram-negative bacteria. In an additional assay, the SI-husk extract increased its zone of inhibition to 19.0 ± 0.5 mm at a concentration of 2 mg (Fig. 3c). Gentamicin demonstrated an inhibition halo of 20.7 ± 0.9 mm in *E. coli*, 18.0 ± 3.3 mm in *P. aeruginosa,* and 20.7 ± 0.9 mm in *S. aureus*. No inhibition of bacterial growth was observed after exposure to disks loaded with 0.2 % DMSO or distilled water.

Some of the primary phenolic compounds found in the SI-husk extarct exhibit antimicrobial activity. Naringenin, kaempferol, and vanillic acid demonstrate inhibitory effects against *S. aureus* and methicillin-resistant *S. aureus* (MRSA); with MIC values of 200 µM, 62.5 µg/mL, and 600 µg/mL, respectively [26–28]. They also display a synergistic effect with antibiotics used for the treatment of these microorganisms [29]. The mechanisms of action of these compounds are based on their ability to interact with the cytoplasmic membrane of *S. aureus*, altering cell morphology and cell membrane permeability [30]. They can also act as inhibitors of the DNA helicase PriA, which is essential for the reactivation of DNA replication, or inhibit the enzyme sortase A (SrtA), responsible for *S. aureus* biofilm formation [31].

In addition to the above-mentioned antimicrobial activities of the primary components of the SI-husk extract, other minor compounds are also active against *S. aureus*. For instance, *p*-coumaric acid inhibits the growth of MRSA (MIC 1 mg/mL) [32]. Also, apigenin demonstrates antimicrobial activity against quinolone-resistant *S. aureus* (MIC 4 mg/L) by inhibiting the DNA gyrase harboring the quinolone resistance mutation gyrA (Ser84Leu) [33].

To build upon these reports, further research into the antimicrobial activity of sacha inchi husks and seed shells is required. To date, sacha inchi oil has been shown to prevent the adhesion of *S. aureus* to human skin explants, and the use of this oil with a Myoviridae phage inhibited the growth of *A. baumannii* [9, 10]. Recently, aqueous extracts of sacha inchi husks obtained at various temperatures (70 °C – 100 °C) were active Against Gram-positive (*S. aureus* and *L. monocytogenes*) and Gram-negative (*E. coli*, *S. enteritidis*, and *V. parahaemolyticus*) bacteria, with a MIC value of ≤ 0.625 mg/mL [7]. Our results provide new insights into the potential of SI-husk as a source of bioactive compounds with antimicrobial activity against *S. aureus*.

4. Conclusions

In this study, we used ultrasonic-assisted extraction as an effective and sustainable method to obtain sacha inchi husk and shell extracts for the revalorization of these byproducts. Under the UAE-selected conditions, we used lower solvent consumption to achieve the highest yields for both byproducts. SI-husk and SI-shells are sources of phenolic compounds, mainly flavonoids and phenolic acids. The main compounds present in SI-husk extracts were cyanidin, naringenin, and kaempferol, whereas SI-shell extracts contained vanillic acid. Given their phenolic composition, SI-husk and SI-shell extracts exhibited antioxidant activity, indicating potential health benefits and applications in the food, cosmetic, and pharmaceutical industries. SI-husk extracts inhibited the growth of *S. aureus*, a human pathogen responsible for multiple moderate-to-severe clinical infections. Our results provide a starting point for future research on the use of this extract as an alternative or complementary natural antimicrobial agent to prevent *S. aureus* and other Gram-positive bacteria-related infections.

5. Acknowledgments

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6. Conflict of interest

The authors declare having no conflicts of interest.

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Cáscaras y envolturas de semillas de sacha inchi (*Plukenetia volubilis* L.) como fuentes de compuestos fenólicos con potenciales beneficios para la salud

Resumen: Sacha inchi (*Plukenetia volubilis* L.) es una planta oleaginosa que produce un aceite altamente nutritivo. Sin embargo, sus cáscaras y envolturas de semillas son subproductos infrautilizados. En este estudio, se obtuvieron extractos etanólicos de las cáscaras (SI-husk) y envolturas de semillas (SI-shell) de sacha inchi mediante extracción asistida por ultrasonido, evaluando la influencia de la temperatura de extracción y la proporción solvente-sólido en el rendimiento. Se investigaron los contenidos totales de compuestos fenólicos, los perfiles fenólicos, las actividades antioxidantes y las actividades antimicrobianas de los extractos contra bacterias Gram-positivas y Gram-negativas. Bajo las condiciones de extracción seleccionadas para SI-husk (60 °C, 1:8 s/s, 280 W, 120 min) y SI-shell (60 °C, 1:3 s/s, 280 W, 120 min), los extractos presentaron contenidos totales de compuestos fenólicos de 80.18 ± 0.32 y 50.94 ± 0.48 mg equivalentes de ácido gálico (GAE)/g, respectivamente. En el extracto de SI-husk se encontraron principalmente cianidina, naringenina y kaempferol (79.4 %) y en el extracto de SI-shell se encontró ácido vanílico (79.9 %). Ambos extractos presentaron actividad antioxidante, con valores ORAC de 360.36 ± 0.21 y 228.11 ± 0.14 µmol TE/g, respectivamente. Se evalúo la actividad antimicrobiana de los extractos contra *S. aureus*, *P. aeruginosa* y *E. coli* mediante el ensayo de difusión en disco de agar. El extracto de SI-husk (1 mg) mostró actividad antibacteriana contra *S. aureus*, con una zona de inhibición de 10.5 ± 1.8 mm. Nuestros resultados brindan nuevos conocimientos sobre los subproductos del sacha inchi como fuentes de compuestos bioactivos con potenciales beneficios para la salud.

Palabras Clave: antimicrobiano; antioxidantes; extractos de plantas; oleaginosas; subproductos; ultrasonido

Cascas e envolturas de sementes de sacha inchi (*Plukenetia volubilis* L.) como fontes de compostos fenólicos com potenciais benefícios para a saúde

Resumo: Sacha inchi (*Plukenetia volubilis* L.) é uma planta oleaginosa que produz um óleo altamente nutritivo. No entanto, suas cascas e envolturas de sementes são subprodutos subutilizados. Neste estudo, extratos etanólicos das cascas (SI-husk) e das envolturas de sementes (SI-shell) de sacha inchi foram obtidos por meio de extração assistida por ultrassom, avaliando a influência da temperatura de extração e da proporção solvente-sólido no rendimento. Foram avaliados os conteúdos totais de compostos fenólicos, os perfis fenólicos, as atividades antioxidantes e as atividades antimicrobianas dos extratos contra bactérias Gram-positivas e Gram-negativas. Sob as condições de extração selecionadas para SI-husk (60 °C, 1:8 s/s, 280 W, 120 min) e SI-shell (60 °C, 1:3 s/s, 280 W, 120 min), os extratos apresentaram teores totais de compostos fenólicos de 80,18 ± 0,32 e 50,94 ± 0,48 mg equivalentes de ácido gálico (GAE)/g, respectivamente. No extrato de SI-husk, foram encontrados principalmente cianidina, naringenina e kaempferol (79,4 %) e no extrato de SI-shell foi encontrado ácido vanílico (79,9 %). Os dois extratos apresentaram atividade antioxidante, com valores ORAC de 360,36 ± 0,21 e 228,11 ± 0,14 µmol TE/g, respectivamente. A atividade antimicrobiana dos extratos foi avaliada contra *S. aureus, P. aeruginosa* e *E. coli* por meio do ensaio de difusão em disco de ágar. O extrato de SI-husk (1 mg) mostrou atividade antibacteriana contra *S. aureus*, com uma zona de inibição de 10,5 ± 1,8 mm. Nossos resultados fornecem novos conhecimentos sobre os subprodutos de sacha inchi como fontes de compostos bioativos com potenciais benefícios para a saúde.

Palavras-chave: antimicrobiano; antioxidantes; extratos de plantas; oleaginosas; subprodutos; ultrassom

Franci Nathalie Gómez Jaimes

Chemist with a master's degree in Materials Engineering. Her research is framed in natural compounds' obtention, structural characterization, and chemical transformation to generate functional new biomaterials. Currently, her work contributes to the research line in nanotechnology and biomaterials of the Group in Basic and Applied Sciences for Sustainability (CIBAS).

ORCID: 0000-0001-8273-4013

Katerin Natalia Acuña Rodríguez

Chemist with a master's degree in Chemistry. Her research interests are focused on using waste biomass and leveraging entrepreneur's ideas first on a pilot scale and then on an industrial scale. She also works as director of corporate innovation in INDUSTRIAS ACUÑA LTDA – INAL, in charge of process management.

ORCID: 0009-0005-1487-2568

Nicole Valeria Polanía Cuellar

Industrial Microbiologist from the University of Santander. Her research has focused on evaluating natural extracts' antioxidant and antimicrobial activities.

ORCID: 0009-0004-0681-6673

Indira Paola Hernández Peñaranda

Microbiologist with a master's degree in basic biomedical sciences. Her research focuses on identifying new alternatives to infectious diseases. She has developed expertise in carrying out scientific research projects. She has extensive knowledge of cell culture techniques, cytotoxicity tests, and in vitro and in vivo tests for compounds with antimicrobial potential. Additionally, she has conducted studies on the mechanisms of action of bacterial membranes, drug release and retention assays in human, animal, and synthetic membranes.

ORCID: 0000-0002-1305-8100

Deicy Villalba Rey

Ph.D. in statistics, is a full-time professor and researcher at the University of Tolima and a peer reviewer recognized by the Ministry of Science, Technology, and Innovation of Colombia. Her main research areas are multivariate analysis, modeling, and experimental design. She has participated in various interdisciplinary research projects in fields such as biological sciences, social studies, economics and finance, education, and agro-industry.

ORCID: 0000-0002-8579-1379