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In-Vitro Screenings for Biological and Antioxidant Activities of Aqueous Extract from Sacha Inchi (*Plukenetia volubilis* L.) Husks

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Abstract

Industrial processing of the Sacha inchi (*Plukenetia volubilis* L.) generated a large number of husks, which are one of the major by-products without sufficient utilization. Sacha inchi husk contains rich bioactive compounds unveiling its valorization potential. This study for the first time investigated the biological and antioxidant activities of the Sacha inchi husk extract recovered with distilled water at different temperatures (70, 80, 90 and 100 °C). The results showed that the aqueous extracts from Sacha inchi husks powder obtained at the extraction temperature of 80 °C showed the highest total phenolic content (7.90 mg GAE/g dry extract) and total flavonoid content (5.79 mg CE/g dry extract) and had the highest antioxidant activities (1,055.81, 53.60 and 1.59 µmol TE/g dry extract for DPPH, ABTS and FRAP assays, respectively, and 413.32 µmol EE/g dry extract for metal chelating assay). For antimicrobial activity, all extracts were effective against Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella enteritidis*, and *Vibrio parahaemolyticus*).

In addition, Sacha inchi husks powder extraction at 80 °C exhibited the lowest MIC (≤ 0.625 mg/mL) and MBC (≤ 0.625 mg/mL) against all tested microorganisms. The extracts obtained at the extraction temperature of 80 °C also exhibited maximum inhibition of α-amylase (77.71 %) and α-glucosidase (76.40 %). The highest inhibition of tyrosinase activity was presented by the aqueous extract obtained at 80 °C with an IC_{50} value of 8.40 ± 1.40 mg/mL. Our findings suggested that the aqueous extract of Sacha inchi husks can be served as an economical source of antioxidant, antimicrobial, anti-diabetic and anti-tyrosinase agents.

Keywords: Sacha inchi, Antioxidant, Antimicrobial, Anti-hypoglycemic activity, Anti-tyrosinase

Introduction

The manufacturing of horticultural products generates a significant quantity of waste materials, which is mainly composed of biodegradable organic matter. Their disposal can result in severe health and environmental issues. These wastes are frequently disposed in landfills, but their decomposition can promote the growth of pathogenic microbes or create toxic leachates [1]. To minimize associated environmental effects and material disposal costs, researchers are focusing on finding methods to recover, recycle, and upgrade these leftovers into useful materials.

Sacha inchi (*Plukenetia volubilis* L.) of the family Euphorbiaceae is also known as Sacha peanut, mountain peanut, Inca nut or Inca peanut. It is native to the tropical rainforest of the Amazon region of South America which includes parts of Peru and northwestern Brazil. Sacha inchi is being developed in other parts of the world (e.g., Southeast Asia) because of its great potential as an economic crop. Sacha inchi seeds are composed of seed covering layers (33 - 35 %), including the husk and shell, and the oleaginous seed kernel (65 - 67 %), which is the commercially important part, mainly industrialized into oil [2]. Sacha inchi oil extraction generates by-products, including the husk and shell that are used as animal feed or fertilizer. Chemical compositions, biological activities and antioxidant activities of Sacha inchi shells have been studied [3]. However, limited information on the husk, major solid waste of Sacha inchi production, is available. Several investigations have linked plant-derived by-product extracts with health

benefits, such as anti-inflammatory, antioxidant, antimicrobial, chemopreventive and antimutagenic activities [4]. Hence, it is necessary to prepare Sacha inchi husk powder using optimal extraction conditions, and further characterize its bioactivity for potential utilization by herbal practitioners and for commercial use. The extraction of bioactive compounds depends on several factors, such as the extraction technique, raw materials, and the extraction solvent that are used. The techniques can be classified into conventional or non-conventional. Conventional techniques require the use of organic solvents, temperature, and agitation. Examples of this type of technique include Soxhlet, maceration, and hydrodistillation. Modern techniques, or non-conventional techniques, are green or clean techniques due to reduced use of energy and the implementation of organic solvent, which are beneficial in relation to the environment [5].

For the preparation of plant extracts, water is undoubtedly the safest and the most environmentally friendly and accessible solvent [6]. It is also significantly less expensive than organic solvents, which have been traditionally employed for plant bioactive extractions. Currently, no published information exists relating to the optimized use of water for analyzing the potential bioactive constituents of Sacha inchi husks. The present study aimed to examine the chemical compositions of the husk of Sacha inchi and the aqueous extract of Sacha inchi husk was screened for antimicrobial, antioxidant, anti-hyperglycemic and anti-tyrosinase properties using standard methods. The findings from this work may add to the overall value of the food, cosmetic and medicinal potential of the Sacha inchi husk.

Materials and methods

Chemicals

All reagents and solvents used were of analytical grade and were obtained from Merck (Darmstadt, Germany).

Preparation of Sacha inchi husk powder

Sacha inchi (*Plukenetia volubilis* L.) husks (**Figure 1**) were obtained from Ban Obboon OTOP (One Tumbon One Product), Phatthalung province, Thailand, in January 2021. All experiments were carried out within a year after obtaining the samples. Sacha inchi husk powder was prepared following the method of Chotphruethipong *et al.* [7].

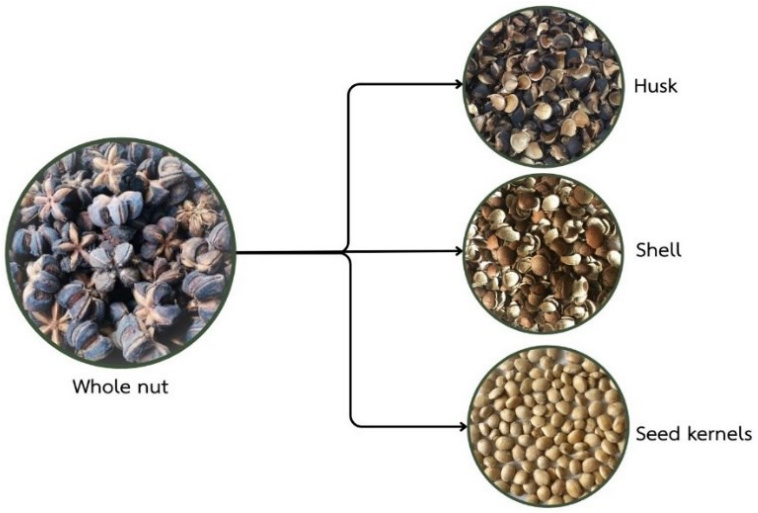


Figure 1 The physical appearances of the husk of Sacha inchi.

Extraction of Sacha inchi husks

The method of Chotphruethipong *et al.* [7], was adopted for the preparation of Sacha inchi husk extract. Distilled water used as an extraction medium was mixed with Sacha inchi husk powder using a solid/solvent ratio of 1:15 (w/v). Extraction was performed at various temperatures (80, 90 and 100 °C) for 60 min using a temperature-controlled shaking water bath, followed by centrifugation in an RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA) at 5,000 g for 30 min at 25 °C. The supernatants were then filtered through Whatman filter paper No.1. The filtrates were evaporated at 40°C by an Eyela rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan) and were freeze-dried. Dried extracts were stored in a desiccator before analysis. The extraction at room temperature (28 - 30 °C) was also compared.

Analyses

Chemical compositions

Protein, fat, carbohydrate, moisture, ash and total dietary fiber content of Sacha inchi husk were determined according to the standard protocols of the Association of Official Analytical Chemists (AOAC) [8].

Total phenolic content and total flavonoid content

The total phenolic content (TPC) of the Sacha inchi husk extracts was determined spectrophotometrically with Folin-Ciocalteu's reagent (FCR) as described by Benjakul *et al.* [9]. The TPC was calculated and expressed as mg gallic acid equivalent (GE)/g dry extract. The total flavonoid (TFC) of the Sacha inchi husk extracts was measured by the aluminum chloride colorimetric method of Yang *et al.* [10]. TFC was expressed as mg catechin equivalent (CE)/g dry extract.

Antioxidant activities

DPPH radical scavenging activity was measured using the method of Benjakul *et al.* [9]. ABTS radical-scavenging activity was determined following the method of Binsan *et al.* [11]. Ferric reducing antioxidant power (FRAP) was measured as per the method of Benzie and Strain [12]. Activities were expressed as μmol Trolox equivalent (TE)/g dry extract. Chelating activity toward ferrous ion (Fe^{2+}) was determined by the method of Boyer and Mccleary [13], and was expressed as mmol EDTA equivalent (EE)/ g dry extract.

Bacterial strains, culture conditions, and cell suspension

The bacteria used included *Escherichia coli* DMST 4212 and *Staphylococcus aureus* DMST 4745, were obtained from the Department of Medical Science, Ministry of Health, Thailand. *Listeria monocytogenes* PSU.SCB.16S.13, *Vibrio parahaemolyticus* PSU.SCB.16S.14 and *Salmonella enteritidis* PSU.SCB.16S.12, were gifted from Food Safety Laboratory, Department of Food Technology, Prince of Songkhla University, Hat Yai, Thailand. Each culture was separately grown overnight (18 h) in Tryptic Soy Broth (TSB; Difco, LePortdeclaix, France) until the OD_{600} of approximately 0.8 was attained. Subsequently, the cultures were supplemented with 15 % glycerol and stored at −80 °C. Before use, each bacterial culture was grown on Tryptic Soy Agar (TSA; Difco Le Port de claix, France) at 37 °C for 24 h. Cells were suspended in TSB and incubated at 37 °C for 4 h, and the concentration was serially diluted to 10^6 CFU/mL.

Minimum inhibitory concentration and minimum bactericidal concentration of aqueous extracts

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extracts from Sacha inchi husks were determined against *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Escherichia coli*, and *Salmonella enteritidis* following the method outlined by Odedina, *et al.* [14]. Four-h cultures were serially diluted to the final concentration of 10^6 CFU/mL. About 100 μl of aqueous Sacha inchi husk extracts of different concentrations (0.31 - 10.0 mg/mL) were placed in a sterile 96-well flat bottom microtiter plate. Subsequently, 100 μl of the bacterial cells were inoculated in triplicates. The microtiter plates were incubated at 37 °C for 24 h. The extracts at various concentrations without the bacterial cells were served as positive controls, while the bacteria cells without extract were used as a negative control. The MIC was defined as the least concentration that resulted in complete inhibition of noticeable growth in the microtiter plate. Aliquots (10 μl) from wells showing no visible growth were spotted on TSA. The plates were incubated for 24 h at 37 °C. The lowest concentration of extracts preventing bacterial growth on TSA plates after incubation at 37 °C for 24 h was regarded as MBC.

In vitro hypoglycemic activity of Sacha inchi husk extracts

In vitro hypoglycemic activity was determined by analyzing the effects of Sacha inchi husk extracts on the inhibition of α-amylase and α-glucosidase enzymes according to the protocols mentioned by Boue *et al.* [15]. For the assay of α-glucosidase, 50 μl of sample buffer or positive control (500 μg/mL acarbose) was combined with 100 μl of α-glucosidase solution (1 U/mL in 0.1 M sodium phosphate buffer, pH 6.9) in a 96-well plate, and was incubated for 10 min. Consequently, a solution of *p*-nitrophenyl-α-D-glucopyranoside (5 mM, 50 μl) was added and the plate was kept at 25 °C. The absorbance at 405 nm was recorded, 5 min before and after the incubation.

The α-amylase assay was performed by adding 500 μl of sample, buffer, or positive control (acarbose) to 0.5 mL of α-amylase solution (13 U/mL in 0.02 M sodium phosphate buffer, pH 6.9), following

incubation for 10 min at 25 °C. Then, soluble starch solution (500 µl) was mixed with the previous mixture and incubated for 10 min at 25 °C. Then, 1000 µl of dinitrosalicylic acid was added and the solution was kept for 5 min in 100 °C water. Distilled water (10 mL) was used for the dilution of the mixture and the absorbance was recorded at 540 nm. The following equation was used to analyze the inhibitory activity of each enzyme:

$$\% \text{ Inhibition} = \left[\frac{(\text{absorbance of control- absorbance of extract})}{\text{absorbance of control}}\right] \times 100$$

Determination of anti-tyrosinase activity

Tyrosinase inhibitory activity of Sacha inchi extracts was evaluated by using L-DOPA and mushroom tyrosinase [16]. In a 96-well plate, the samples dissolved in 10 % DMSO, 20 µl of the samples at 0.001 - 10 mg/mL, 40 µl of 0.1 M phosphate buffer at pH 6.8 and 20 µl of tyrosinase solution in phosphate buffer (46 units/mL) were added. The plate was allowed to react at 25 ± 2 °C for 10 min and then added 20 µl of 2.5 mM L-DOPA in phosphate buffer. The absorbance at 475 nm was measured after incubation at 25 ± 2 °C for 10 min. The positive controls at 0.001 - 10 mg/mL were vitamin C, kojic acid and butylated hydroxytoluene (BHT). All the samples were assayed in triplicate. Tyrosinase inhibition was calculated using the same equation as described above. IC_{50} value of tyrosinase inhibition was obtained from the sample concentration at 50 % inhibition activity.

Statistical analysis

A completely randomized design was used throughout this study. All data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test [17]. Statistical analysis was performed using a SPSS package (SPSS 24.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and discussion

Chemical compositions

Chemical compositions of the Sacha inchi husk, including protein, fat, carbohydrate, total dietary fiber, and ash were investigated as shown in **Table 1**. Sacha inchi husk contained 60.01 % crude protein, 20.08 % carbohydrate, 11.23 % fat, 5.65 % ash and 16.85 % dietary fiber. The moisture content of the sample was 3.03 % which is optimum for the storage of species. High protein and carbohydrates contents were found in cacao pod husk [18], rice husk [19], chickpea husk [20], moth bean husk [21], walnut shell [22], and cashew nut shell [22]. Recently, chemical compositions of the shell of Sacha inchi, including protein (43.12 %), fat (37.87 %), carbohydrate (9.90 %) and ash (4.46 %) were observed [23]. Based on the high protein and dietary fiber content in Sacha inchi husk, it could be an essential source of proteins as well as dietary fiber.

Table 1 Chemical compositions of Sacha inchi husk.

Compositions	Content (%)
Moisture	3.03 ± 0.01
Protein	60.01 ± 0.05
Lipid	11.23 ± 0.04
Carbohydrate	20.08 ± 0.07
Dietary fiber	16.85 ± 0.17
Ash	5.65 ± 0.03

All experiments were carried out in triplicate (n = 3)
All data are presented as mean ± standard deviation (SD)

TPC, TFC and antioxidant activities of Sacha inchi husk aqueous extracts

TPC, TFC and antioxidant activities of Sacha inchi husk aqueous extracts affected by extraction temperature are presented in **Table 2**. TPC and TFC of the aqueous extracts were different, in which the values of 4.58 - 7.90 mg GE/g dry extract and 2.54 - 5.79 mg CE/g dry extract were obtained, respectively. Among the investigated Sacha inchi husk extracts, the highest TPC and TFC were obtained at 80 °C ($p < 0.05$). The TPC and TFC increased when the extraction temperature increased from 70 to 80 °C.

However, the TPC and TFC decreased when the temperature was raised to 100 °C, a result that may be linked to thermally induced decomposition. Water as a polar solvent at room temperature can extract polar compounds. Moreover, due to the decrease of water polarity at higher temperatures, its capability to dissolve polar compounds is reduced [24]. An earlier research paper demonstrated that an increase in water temperature also causes a reduction in surface tension and viscosity, so the diffusion rate and the rate of mass transfer during the extraction were increased [25]. Lim and Murtijaya [26], also mentioned that cool water extracted significantly less polyphenols than boiling water from dried *Phyllanthus amarus* plant material. Previous investigations on the extraction of polyphenolics from sources such as green tea [27], grape pomace [28], peanut skins [29], and olive seeds [30], have identified a temperature-dependent extraction relationship. While higher solvent temperatures typically increase mass transfer rates during extraction, in the case of polyphenols and flavonoids, elevated temperatures trigger competing processes such as decomposition and epimerization [31]. Temperature control during the extraction and isolation process for Sacha inchi husks needs to be strictly controlled to minimize the loss of polyphenols.

For antioxidant activities, as the extraction temperature increased from 70 to 80 °C, higher DPPH and ABTS radical scavenging activities, FRAP and metal chelating activity were detected and all antioxidant activities declined drastically with increasing extraction temperature after 80 °C (**Table 2**). Increasing extraction temperature will enhance the solubility of solute and increase the extraction coefficient, but temperature above 80 °C will affect the stability of the phenolic compounds and the alteration of the plant's membrane integrity may affect the antioxidant capacity. Herrera *et al.* [32], reported that intense thermal treatment is responsible for a significant loss of antioxidants, as most of these compounds are relatively unstable. This is similar to the current work studying water extracts of *Matricaria flos*, where the antioxidant activity decreased at the highest temperature [33]. Horžic *et al.* [34], reported that chamomile infusion at 80 °C reached a higher antioxidant capacity than at 100 °C. Vuong *et al.* [31], also indicated that the antioxidant activity of the papaya leaf extracts increased when the extraction temperature increased to 70 °C and subsequently decreased when the extraction temperature exceeded 90 °C.

Table 2 Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of Sacha inchi husks aqueous extracts obtained at different temperatures.

Temperature of extraction (°C)	TPC (mg GAE/g dry extract)	TFC (mg CE/g dry extract)	DPPH radicals scavenging activity (mmol TE/g dry extract)	ABTS radicals scavenging activity (µmol TE/g dry extract)	FRAP (µmol TE/g dry extract)	Metal chelating activity (µmol EE/g dry extract)
Room temperature	4.58 ± 0.04^{e}	2.54 ± 0.08^{e}	979.47 ± 2.47^{e}	49.01 ± 0.07^{e}	1.31 ± 0.01^{e}	399.98 ± 0.10^{e}
70 °C	6.87 ± 0.13^{b}	3.68 ± 0.21^{b}	$1{,}046.95 \pm 2.95^{b}$	52.98 ± 0.19^{b}	1.49 ± 0.04^{b}	404.90 ± 0.05^{b}
80 °C	7.90 ± 0.60^{a}	5.79 ± 0.54^{a}	$1{,}055.81 \pm 5.07^{a}$	53.60 ± 0.22^{a}	1.59 ± 0.01^{a}	413.32 ± 0.02^{a}
90 °C	6.13 ± 0.02^{c}	3.20 ± 0.09^{c}	$1{,}040.65 \pm 2.82^{c}$	52.67 ± 0.20^{c}	1.43 ± 0.04^{c}	403.41 ± 0.04^{c}
100 °C	5.17 ± 0.08^{d}	2.85 ± 0.02^{d}	$1{,}026.92 \pm 3.35^{d}$	51.51 ± 0.37^{d}	1.36 ± 0.01^{d}	400.28 ± 0.02^{d}

Values represent mean and standard deviation ($n = 3$)
Different superscripts within the same column indicate significant differences ($p < 0.05$)

Antimicrobial activities of Sacha inchi husk aqueous extracts

The MIC of Sacha inchi husk aqueous extracts extracted at various temperatures against Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli*, *Vibrio parahaemolyticus* and *Salmonella enteritidis*) are presented in **Figure 2(a)**. The MIC was 1.25 - 7.5, 2.5 - 7.5, 0.625 - 5.0, 1.25 - 7.5 and 0.625 - 7.5 mg/mL for *Staphylococcus aureus*, *Vibrio parahaemolyticus, Escherichia coli*, *Listeria monocytogenes* and *Salmonella enteritidis*, respectively. Various Sacha inchi husk aqueous extracts extracted using different temperatures had different degrees of inhibition toward the bacteria tested. Generally, Sacha inchi husk aqueous extracts extracted at 80 °C had the lowest MIC against all investigated microorganisms ($p < 0.05$). When the extraction temperature increased from 80 to 100 °C, MIC against all bacteria of the extracts increased. This was plausibly owing to the loss of antimicrobial phenolic compounds from the Sacha inchi husk powder during the heat extraction process. The bacteria cell membrane became permeable and disintegrated in the presence

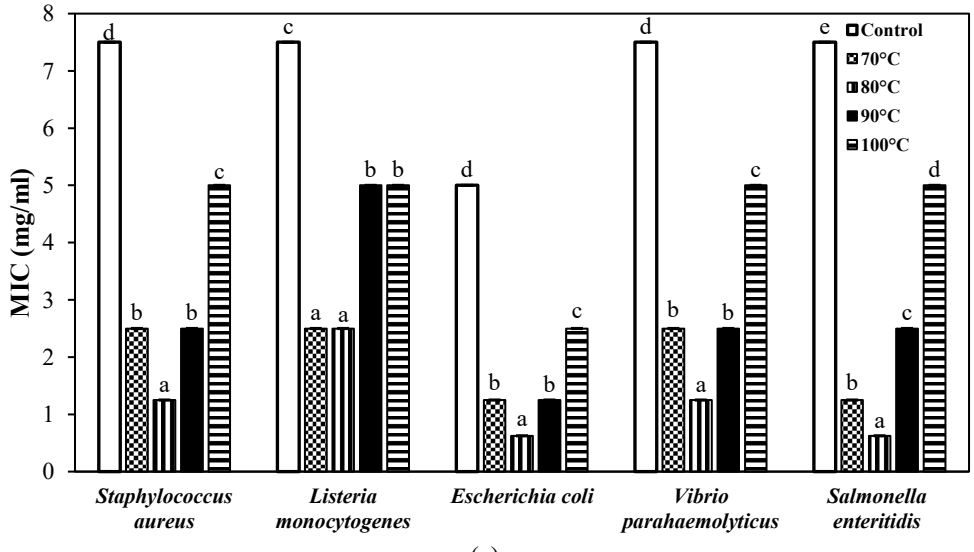
of plant extracts [35]. However, Gram-negative bacteria are more sensitive to plant extracts since their peptidoglycan cell wall was thinner than Gram-positive bacteria [36]. Consequently, higher MIC was recorded for Gram-positive bacteria. Akbary *et al.* [36] classified plant extracts on the basis of their MIC values as strong inhibition: MIC < 0.5 mg/mL, moderate inhibition: 0.6 mg/mL < MIC < 1.5 mg/mL and low inhibition: MIC > 1.6 mg/mL. From the results, the lowest MIC against both Gram-positive and Gram-negative bacteria was observed for Sacha inchi husk aqueous extracts extracted at 80 °C and based on this classification, this extract exerted a moderate inhibition on *Escherichia coli* and *Salmonella enteritidis*, while this extract had the low inhibition on *Staphylococcus aureus*, *Listeria monocytogenes* and *Vibrio parahaemolyticus*.

Figure 2(b) illustrates the MBC of Sacha inchi husk aqueous extracts extracted at different temperatures against some Gram-negative and Gram-positive bacteria. The MBCs were 1.25 - 7.5, 2.5 - 7.5, 0.625 - 5.0, 1.25 - 7.5 and 0.625 - 7.5 mg/mL for *Staphylococcus aureus, Vibrio parahaemolyticus, Escherichia coli, Listeria monocytogenes and Salmonella enteritidis,* respectively. Antibacterial effects of bioactive compounds are better evaluated by comparing the MICs and MB Cs values. A substance is classified as bactericidal when the ratio MBC/MIC is ≤ 2, and is bacteriostatic if the ratio MBC/MIC is > 2 [37]. From the results, the Sacha inchi husk aqueous extracts extracted at 80 °C had a bactericidal effect on both Gram-positive and Gram-negative bacteria. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores [38]. This may therefore explain the demonstration of antimicrobial activity by the husk extracts of Sacha inchi.

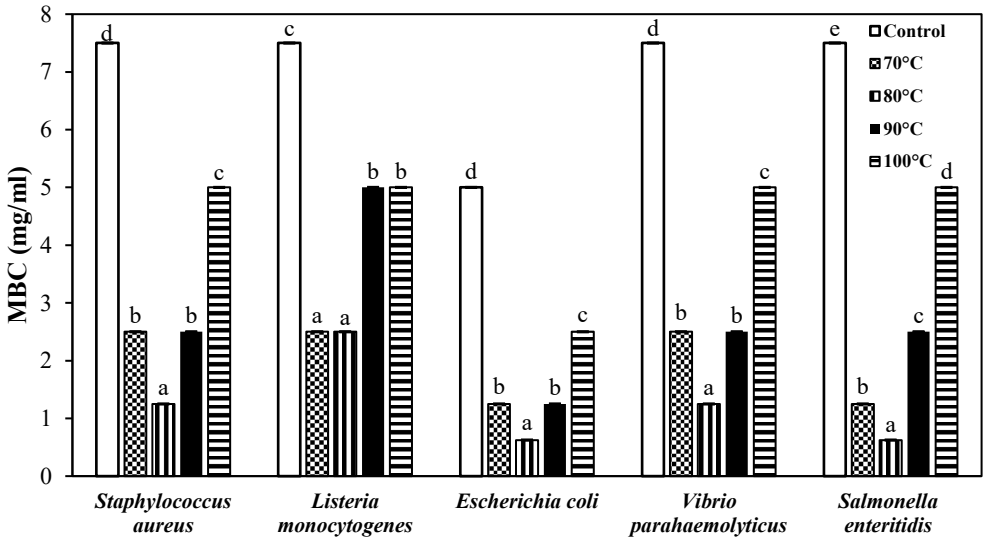
***In vitro* hypoglycemic activity of Sacha inchi husk aqueous extracts**

The α-amylase is a well-known enzyme that is present in the saliva and pancreatic juice and produces absorbable molecules from large insoluble starch molecules, and α-glucosidase breaks down oligosaccharides into monosaccharides. These monosaccharides increase blood glucose levels after being absorbed into the blood circulation. Inhibitors of α-amylase and α-glucosidase postpone the carbohydrate breakdown and ultimately reduce the postprandial blood glucose excursion in the small intestine [39].

In-vitro hyperglycemic activities of Sacha inchi husk aqueous extracts affected by extraction temperature are presented in **Table 3**. All Sacha inchi husk aqueous extracts significantly ($p < 0.05$) inhibited both enzymes (α-amylase and α-glucosidase). The Sacha inchi husk aqueous extract at 80 °C, showed the highest α-amylase and α-glucosidase inhibition. However, in the case of both enzymes (α-amylase and α-glucosidase), acarbose (positive control) showed higher inhibition activity than the Sacha inchi aqueous extracts ($p < 0.05$). Phenolic acids play an important role in the inhibition of α-amylase and α-glucosidase activity during carbohydrate metabolism. Phenolic compounds alter the α-amylase activity by covalently binding it with this enzyme. Such binding results in the formation of lactones or quinones that react with nucleophilic groups on the enzyme and inhibit its activity [40]. Therefore, the inhibitory effect of the Sacha inchi husks aqueous extract is because of the presence of phenolic compounds in the extract.



(a)



(b)

Figure 2 Minimum inhibitory concentration (MIC) (a) and minimum bactericidal concentration (MBC), (b) of Sacha inchi husk aqueous extracts against some Gram-positive and Gram-negative bacteria. Bars represent the standard deviation (n = 3). Different lowercase letters on the bars within the same microorganisms tested indicate significant differences ($p < 0.05$). 70 °C: The aqueous extract extracted at 70, 80 °C: The aqueous extracts extracted at 80, 90 °C: The aqueous extracts extracted at 90, 100 °C: The aqueous extracts extracted at 100 °C, Control: The aqueous extracts extracted at room temperature (28 - 30 °C).

Table 3 *In vitro* hypoglycemic activity of Sacha inchi husk extracts using different extraction temperatures.

Samples	% Inhibition	
	α-amylase	α-glucosidase
Acarbose	98.80 ± 0.01[a]	98.55 ± 0.02[a]
70 °C	73.92 ± 0.01[d]	70.76 ± 0.20[e]
80 °C	77.71 ± 0.05[b]	76.40 ± 0.17[b]
90 °C	77.56 ± 0.01[c]	74.01 ± 0.33[c]
100 °C	72.96 ± 0.08[e]	73.14 ± 0.48[d]

Values represent mean and standard deviation (n = 3)
Different superscripts within the same column indicate significant differences ($p < 0.05$)

Tyrosinase inhibitory activity of Sacha inchi husk aqueous extracts

Tyrosinase (phenol oxidase) is known to be a key enzyme for melanin biosynthesis [41]. Overproduction of melanin may lead to hyperpigmentation, which can cause the skin to appear darker. The use of tyrosinase inhibitors is becoming essential in skin whitening agents and in other skincare and cosmeceutical products. Many antioxidant compounds, such as polyphenols, may also function as tyrosinase inhibitors. Some phenolic compounds and their derivatives such as ferulic acid, caffeic acid and *p*-coumaric acid have been described to possess anti-tyrosinase activity [42]. In the present study, the impact of the Sacha inchi husk extracts prepared at various temperatures on tyrosinase inhibition was examined and shown in **Table 4**. Kojic acid, ascorbic acid and BHT were used as the positive control. Their inhibitory activities were tested against tyrosinase for the oxidation of L-DOPA. The highest tyrosinase inhibitory activity was from kojic acid at an IC_{50} value of 0.12 mg/mL. However, all Sacha inchi husk extracts exhibited inhibitory activity. The anti-tyrosinase activity of Sacha inchi husk extracts increased when the extraction temperature increased from 70 to 80 °C. However, at 90 and 100 °C extraction temperatures, a decrease in tyrosinase inhibition ability was observed. It may be due to the degradation of anti-tyrosinase compounds during high-temperature extraction process [43]. These results indicate that

extraction temperature played an equally significant role in the tyrosinase inhibitory activity of Sacha inchi husk extracts.

Table 4 Tyrosinase inhibitory activity of Sacha inchi husk aqueous extracts prepared at different temperatures in comparison with ascorbic acid, butylated hydroxyl tolulene (BHT), and Kojic acid.

Sample	IC_{50} (mg extract/mL)
Ascorbic acid	0.24 ± 0.01^{b}
BHT	ND
Kojic acid	0.12 ± 0.01^{a}
Room temperature	9.96 ± 0.07^{g}
70 °C	9.11 ± 0.01^{f}
80 °C	8.47 ± 0.07^{c}
90 °C	8.70 ± 0.05^{d}
100 °C	8.92 ± 0.02^{e}

Values represent mean and standard deviation ($n = 3$)
Different superscripts within the same column indicate significant differences ($p < 0.05$)
IC_{50} = the sample concentration providing 50 % of tyrosinase inhibition activity
ND= Not detected

Conclusions

The effect of extraction temperature on the bioactivity of herbal infusions is very important. All the examined assays of bioactivity indicated the influence of the extraction temperature. The highest TPC, TFC and maximum antioxidant, anti-hypoglycemic and anti-tyrosinase capacity of aqueous extracts of Sacha inchi husk at tested temperatures were achieved at 80 °C. Also, the lowest MIC and MBC of Sacha inchi husk extract were observed at 80 °C. The bioactivity of Sacha inchi husk is reported for the first time. The results of this research suggest that Sacha inchi husk can be used as an inexpensive and easily accessible source of effective natural antioxidants, antimicrobial and anti-hypoglycemic agents as well as tyrosinase inhibitors. Moreover, this information could help to open-up future opportunities for the valorization of the husk of Sacha inchi, for producing health-promoting products. Functional foods or supplements from Sacha inchi husk can be developed as potential nutrient-rich sources, with high phenolics for health benefits after assessing their toxicology and bioaccessibility.

Acknowledgments

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References

[1] K Palaniveloo, MA Amran, NA Norhashim, N Mohamad-Fauzi, F Peng-Hui, L Hui-Wen, L Kai-Lin, L Jiale, MG Chian-Yee, L Jing-Yi, B Gunasekaran and SA Razak. Food waste composting and microbial community structure profiling. *Processes* 2020; **8**, 723.

[2] LF Gutiérrez, YQ Segura, Z Sanchez-Reinoso, DL Díaz and JI Abril. Physicochemical properties of oils extracted from γ-irradiated Sacha Inchi (*Plukenetia volubilis* L.) seeds. *Food Chem*. 2017; **237**, 581-7.

[3] N Kodahl and M Sørensen. Sacha inchi (*Plukenetia volubilis* L.) is an underutilized crop with a great potential. *Agronomy* 2021; **11**, 1066.

[4] KB Pandey and SI Rizvi. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Med. Cell. Longevity* 2009; **2**, 270-8.

[5] C Rodríguez-Pérez, R Quirantes-Piné, A Fernández-Gutiérrez and A Segura-Carretero. Optimization of extraction method to obtain a phenolic compounds-rich extract from *moringa oleiferalam* leaves. *Ind. Crop. Prod.* 2015; **66**, 246-54.

[6] R Karjalainen, M Anttonen, N Saviranta, D Stewart, GJ McDougall, H Hilz, P Mattila and R Törrönen. A review on bioactive compounds in black currants (*Ribes nigrum* L.) and their potential health-promoting properties. *Acta Horticulturae* 2009; **839**, 301-7.
[7] L Chotphruethipong, S Benjakul and K Kijroongrojana. Optimization of extraction of antioxidative phenolic compounds from cashew (*Anacardium occidentale* L.) leaves using response surface methodology. *J. Food Biochem.* 2017; **41**, e12379.
[8] GW Latimer and Association of Official Analytical Chemists International. *Official methods of analysis of AOAC International*. 21st ed. AOAC International Gaithersburg, Maryland, 2019.
[9] S Benjakul, P Kittiphattanabawon, P Sumpavapol and S Maqsood. Antioxidant activities of lead (*Leucaena leucocephala*) seed as affected by extraction solvent, prior dechlorophyllisation and drying methods. *J. Food Sci. Tech.* 2014; **51**, 3026-37.
[10] J Yang, TE Martinson and RH Liu. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem.* 2009; **116**, 332-9.
[11] W Binsan, S Benjakul, W Visessanguan, S Roytrakul, M Tanaka and H Kishimura. Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). *Food Chem.* 2008; **106**, 185-93.
[12] IFF Benzie and JJ Strain. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 1996; **239**, 70-6.
[13] RF Boyer and CJ McCleary. Superoxide ion as a primary reductant in ascorbate-mediated ferret in iron release. *Free Radic. Biol. Med.* 1987; **3**, 389-95.
[14] GF Odedina, K Vongkamjan, SP Voravuthikunchai. Potential bio-control agent from *Rhodomyrtus tomentosa* against *Listeria monocytogenes*. *Nutrients* 2015; **7**, 7451-68.
[15] SM Boue, KW Daigle, MH Chen, H Cao and ML Heiman. Antidiabetic potential of purple and red rice (*Oryza sativa* L.) bran extracts. *J. Agr. Food Chem.* 2016; **64**, 5345-53.
[16] T Masuda, D Yamashita, Y Takeda and S Yonemori. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. *Biosci. Biotechnol. Biochem.* 2005; **69**, 197-201.
[17] RGD Steel, JH Torrie and DA Dickey. *Principles and procedures of statistics: A biometrical approach*. McGraw-Hill, New York, 1997.
[18] VT Nguyen and NH Nguyen. Proximate composition, extraction, and purification of theobromine from cacao pod husk (*Theobroma Cacao* L.). *Technologies* 2017; **5**, 14.
[19] R Badar and AQ Shamim. Composted rice husk improves the growth and biochemical parameters of sunflower plants. *J. Bot.* 2014; **2014**, 427648.
[20] D Bose and M Shams-Ud-Din. The effect of chickpea (*Cicer arietinim*) husk on the properties of cracker biscuits. *J. Bangladesh Agr. Univ.* 2010; **8**, 147-52.
[21] MH Kamani, A Martin and MS Meera. Valorization of by-products derived from milled moth bean: Evaluation of chemical composition, nutritional profile and functional characteristics. *Waste Biomass Valorization* 2020; **11**, 4895-906.
[22] TF Akinhanmi, VN Atasie and PO Akintokun. Chemical composition and physicochemical properties of cashew nut (*Anacardium occidentale*) oil and cashew nut shell liquid *J. Agr. Food Environ. Sci.* 2008; **2**, 1-10.
[23] A Cvetanovi'c, J Švarc-Gaji'c, P Maškovi'c, S Savi'c and L Nikoli'c. Antioxidant and biological activity of chamomile extracts obtained by different techniques: Perspective of using superheated water for isolation of biologically active compounds. *Ind. Crop. Prod.* 2015; **65**, 582-91.
[24] S Kittibunchakul, C Hudthagosol, P Sanporkha, P Sapwarobol, P Temviriyanukul and U Suttisansanee. Evaluation of Sacha inchi (*Plukenetia volubilis* L.) by-products as valuable and sustainable sources of health benefits. *Horticulturae* 2022; **8**, 344-56.
[25] A Jiménez-Zamora, C Delgado-Andrade and JA Rufián-Henares. Antioxidant capacity, total phenols and color profile during the storage of selected plants used for infusion. *Food Chem.* 2016; **199**, 339-46.
[26] YY Lim and J Murtijaya. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT Food Sci. Tech.* 2007; **40**, 1664-9.
[27] M Pinelo, M Rubilar, M Jerez, J Sineiro and MJ Nunez. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *J. Agr. Food Chem.* 2005; **53**, 2111-7.
[28] TS Ballard, P Mallikarjunan. KO Zhou and SF Keefe. Optimizing the extraction of phenolic antioxidants from peanut skins using response surface methodology. *J. Agr. Food Chem.* 2009; **57**, 3064-72.

[29] MH Alu'datt, I Alli, K Ereifej, MN Alhamad, A Alsaad and T Rababeh. Optimisation and characterisation of various extraction conditions of phenolic compounds and antioxidant activity in olive seeds. *Nat. Prod. Res.* 2011; **25**, 876-89.
[30] J Shi, G Mazza and ML Maguer. *Solid-liquid extraction technologies for manufacturing nutraceuticals. Functional Foods*. 1st ed. CRC Press, Florida, 2002.
[31] QV Vuong, JB Golding, M Nguyen and PD Roach. Extraction and isolation of catechins from tea. *J. Separ. Sci.* 2010; **33**, 3415-28.
[32] T Herrera, Y Aguilera, M Rebollo-Hernanz, E Bravo, V Benítez, N Martínez-Sáez, SM Arribas, MDD Castillo and MA Martín-Cabrejas. Teas and herbal infusions as sources of melatonin and other bioactive non-nutrient components. *LWT* 2018; **89**, 65-73.
[33] A Cvetanovi'c, J Švarc-Gaji'c, Z Zekovi'c, J Jerkovi'c, G Zengin, U Gaši'c, Z Teši'c, P Maškovi'c, C Soares, MF Barroso. The influence of the extraction temperature on polyphenolic profiles and bioactivity of chamomile (*Matricaria chamomilla* L.) subcritical water extracts. *Food Chem.* 2019; **271**, 328-37.
[34] D Horži'c, D Komes, A Belščak, KK Ganic, D Ivekovic and D Karlovic. The composition of polyphenols and methylxanthines in teas and herbal infusions. *Food Chem.* 2009; **115**, 441-8.
[35] E Abdollahzadeh, M Rezaei and H Hosseini. Antibacterial activity of plant essential oils and extracts: The role of thyme essential oil, nisin, and their combination to control *Listeria monocytogenes* inoculated in minced fish meat. *Food Contr.* 2014; **35**, 177-83.
[36] P Akbary. *In vitro* inhibitory activity of the leaf methanol extract of green tea (*Camellia sinensis*) against *Lactococcus garvieae* and *Aeromonas hydrophila* isolated of rainbow trout (*Oncorhynchus mykiss*). *Adv. Microbiol.* 2014; **4**, 829-34.
[37] MC Marjorie. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 1999; **12**, 564-82.
[38] JH Doughari. Antimicrobial Activity of *Tamarindus indica* Linn. *Trop. J. Pharmaceut. Res*. 2006; **5**, 597-603.
[39] H Rasouli, SMB Hosseini-Ghazvini, H Adibi and R Khodarahmi. Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: A virtual screening perspective for the treatment of obesity and diabetes. *Food Funct.* 2017; **8**, 1942-54.
[40] N Deng, B Zheng, T Li and RH Liu. Assessment of the phenolic profiles, hypoglycemic activity, and molecular mechanism of different highland barley (*Hordeum vulgare* L.) varieties. *Int. J. Mol. Sci.* 2020; **21**, 1175.
[41] S Momtaz, N Lall and A Basson. Inhibitory activities of mushroom tyrosine and DOPA oxidation by plant extracts. *S. Afr. J. Bot.* 2008; **74**, 577-82.
[42] O Taofiq, AM Gonzáles-Paramás, A Martins, MF Barreiro and ICFR Ferreira. Mushrooms extracts and compounds in cosmetics, cosmeceuticals and nutricosmetics-a review. *Ind. Crop. Prod.* 2016; **90**, 38-48.
[43] KN Yoon, N Alam, KR Lee, PG Shin, JC Cheong, YB Yoo and TS Lee. Antioxidant and anti-tyrosinase activities of various extracts from the fruiting bodies of *Lentinus lepideus*. *Molecules* 2011, **16**, 2334-47.