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Bioactive Properties of Huizache (*Caesalpinia cacalaco*) Seed Flour: Phenolic Compounds, Antioxidant Capacity, and Inhibition of Glycolytic Enzymes

Propiedades Bioactivas de Harina de Semilla de Huizache (*Caesalpinia cacalaco*): Compuestos Fenólicos, Capacidad Antioxidante e Inhibición de Enzimas Glucolíticas

Carrillo-Torres, J.A.[1], *Perea-Domínguez, X.P.*[1*], *Ahumada-Cervantes, R.*[1], *Martínez-Álvarez, I.G.*[1], *Valenzuela-García, P.*[2], *Ruiz-Armenta, X.A.*[3], *Gómez-Favela, M.A.*[3*]

[1] *Doctorado en Sustentabilidad, Universidad Autónoma de Occidente, Unidad Regional Guasave, Av. Universidad S/N, Fraccionamiento Villa Universidad, C.P. 81048, Guasave, Sinaloa, México.*
[2] *Departamento de Ingeniería y Tecnología, Universidad Autónoma de Occidente, Unidad Regional Los Mochis, Blvd. Macario Gaxiola y Carretera internacional, México 15, C.P. 811223, Los Mochis, Sinaloa, México.*
[3] *Programa de Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Calz. de las Américas Nte 2771, Cd Universitaria, Col. Burócrata, C.P. 80030 Culiacán, Sinaloa, México.*

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ABSTRACT

Huizache (*Caesalpinia cacalaco*) is a leguminous species native to the Americas, thriving in Mexico's tropical dry forest. This study evaluated the total phenolic content (TPC), antioxidant capacity, and hypoglycemic potential of *C. cacalaco* seed flour extracts. Phytochemical analysis confirmed the presence of phenolics, tannins, and terpenes, while saponins and alkaloids were absent. The TPC content was 249.83 ± 0.68 mg GAE/100 g. Antioxidant activity, assessed via ABTS and DPPH assays, was 3,931.26 ± 202.87 and 2,500.87 ± 14.44 μmol TE/100 g, respectively. Enzyme inhibition tests showed α-amylase inhibition of 31.75 % (free) and 37.42 % (bound), while α-glucosidase inhibition was 43.52 % (free) and 26.15 % (bound). These results suggest that huizache seed flour possesses high antioxidant capacity and hypoglycemic potential, making it a promising source of bioactive compounds for managing chronic diseases.

KEY WORDS: *Caesalpinia cacalaco*; phenolic compounds; antioxidant activity; α-amylase; α-glucosidase.

***Corresponding Author:**

Mario Armando Gómez Favela. Programa de Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Calz. de las Américas Nte 2771, Cd Universitaria, Col. Burócrata, C.P. 80030 Culiacán, Sinaloa, México. Teléfono: (667) 2169112. mario.gomez.fcqb@uas.edu.mx
Xiomara Patricia Perea Domínguez. Departamento de Salud-Licenciatura en Ciencias Biomédicas, Universidad Autónoma de Occidente, Unidad Guasave, Sinaloa. Avenida Universidad S/N, Fraccionamiento Villa Universidad, Guasave, Sinaloa, C.P. 81048. Teléfono: (687) 8780963. xiomara.perea@uadeo.mx

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R E S U M E N

El Huizache (*Caesalpinia cacalaco*) es una leguminosa nativa de América, que prospera en el bosque seco tropical de México. Este estudio evaluó el contenido de compuestos fenólicos totales (TPC), la capacidad antioxidante y el potencial hipoglucemiante de los extractos de harina de semilla de *C. cacalaco*. El análisis fitoquímico confirmó la presencia de fenólicos, taninos y terpenos, mientras que no se observaron saponinas ni alcaloides. El contenido de TPC fue de 249.83 ± 0.68 mg de GAE/100 g. La actividad antioxidante, evaluada mediante ensayos ABTS y DPPH, fue de 3,931.26 ± 202.87 y 2,500.87 ± 14.44 μmol de TE/100 g, respectivamente. Las pruebas de inhibición enzimática mostraron una inhibición de la α-amilasa del 31.75 % (libre) y del 37.42 % (ligado), mientras que la de la α-glucosidasa fue del 43.52 % (libre) y del 26.15 % (ligada). Estos resultados sugieren que la harina de semilla de huizache posee una alta capacidad antioxidante y potencial hipoglucemiante, lo que la convierte en una fuente prometedora de compuestos bioactivos para el manejo de enfermedades crónicas.

P A L A B R A S C L A V E : *Caesalpinia cacalaco*; huizache; compuestos fenólicos; actividad antioxidante; α-amilasa; α-glucosidasa.

Introduction

Legume-rich diets are inversely associated with the risk of developing cardiovascular diseases and related factors such as obesity, hypertension, and type 2 diabetes. This association is primarily attributed to their fiber, mineral, tocopherol, phytosterol, and antioxidant content (Becerra-Tomás *et al*., 2018; Tosh & Yada, 2010). Promoting a plant-based diet offers a favorable nutritional profile, as it prioritizes complex carbohydrates, fiber, vitamins, and minerals over total and saturated fats (Soto-Aguilar *et al*., 2022). The bioactive metabolites present in different plant species and at various maturity stages are diverse and can be tailored to user needs, providing a potential solution to food scarcity (Benincasa *et al*., 2019).

Among these metabolites, phenolic compounds have been extensively studied in various legumes, including common bean (*Phaseolus vulgaris*) (Salas *et al.*, 2015; Sharma & Giri, 2022) chickpea (*Cicer arietinum* L.) (Niño- Medina *et al*., 2017; Ponce- Fernández *et al*., 2019), and sesame (*Sesamum indicum* L.) (Ruiz-Armenta *et al*., 2022), due to their numerous health benefits, such as cancer prevention, diabetes management, chronic inflammation reduction, and cardiovascular disease mitigation (Acosta-Estrada *et al*., 2014). The primary function of phenolic compounds is to neutralize and/or reduce the effects of reactive oxygen species (ROS) in cells, thereby mitigating oxidative stress (OS) and reducing the risk of cellular damage and chronic disease complications. Antioxidants are among the main protective mechanisms against free radical damage in the body, and their intake has been associated with improved health and reduced disease incidence (Cereceres-Aragón *et al*., 2019; Lu *et al*., 2018).

Mexico harbors a vast number of underutilized wild legumes that, despite their nutritional properties and bioactive compound content, remain largely untapped. Their high potential for sustainable utilization warrants further exploration (Pío-León *et al*., 2013). Huizache or Tehuixtle (*Caesalpinia cacalaco*), a leguminous species of the Fabaceae family native to the Americas, thrives in Mexico's tropical dry forests. Its fruits are oblong-ovoid pods, constricted between seeds enclosed in two thin, indehiscent valves that turn dark yellowish-brown upon drying. The seeds are transversely ovoid and nearly devoid of endosperm at maturity, turning reddish-brown upon drying (Miranda & Hernández, 1963). These seeds hold potential for the development of functional foods.

Veloz-García *et al*. (2004) reported that *C. cacalaco* pods are a rich source of tannins, evaluated their antimutagenic activity against aflatoxin B1, and their antioxidant capacity. They found that the antimutagenic activity was dose-dependent, showing a 64.42% inhibition, and a high antioxidant activity.

In recent years, there has been growing interest in nutraceutical foods, which are expected to promote health beyond basic nutrition by conferring physiological benefits (Quelal *et al*., 2020). Currently, no studies have characterized the nutraceutical potential of huizache seed flour. The objective of this study was to evaluate the total phenolic compound content, antioxidant activity, and hypoglycemic potential of *C. cacalaco* seed flour extracts, thereby expanding its potential applications as an ingredient, additive, or dietary supplement.

Material and methods

Material

Huizache (*C. cacalaco*) seeds were collected in the municipality of Elota, Sinaloa, Mexico (Latitude: 24.03080625, Length: -106.861236459879; 23°55'13''Norte, 106° 48' 0'' Oeste). The seeds were manually extracted from the pods and carefully cleaned with a brush to remove any surface impurities. They were then ground using a hammer mill to produce a whole-grain flour. The ground material was sieved through a mesh screen with 1.00 mm openings (No. 60 mesh) to separate larger particles and obtain a more uniform particle size distribution. The resulting flour was packaged in polyethylene bags and stored at 4 °C until used.

Extract Preparation

For the phytochemical screening, a hydroalcoholic extraction was performed. A total of 500 mg of the sample was weighed, and 20 mL of 80 % ethanol was added. The mixture was agitated at 100 rpm for 1 hour, followed by centrifugation at 2,500 rpm at 10 °C, and the supernatant was collected (Flieger *et al*., 2021). This procedure was performed in triplicate.

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Phytochemical Screening

Once the extracts were obtained, a phytochemical screening of huizache flour extracts was conducted following the methodology described by Harborne (1998) and Solomon *et al*. (2013), to qualitatively identify the presence or absence of various compounds, including phenolic compounds, tannins, saponins, triterpenes, and alkaloids. All assays were performed in triplicate.

Phenolic Compounds (Magnesium Test)

To determine the presence of phenolic compounds, 500 µL of the hydroalcoholic extract was placed in test tubes, followed by the addition of a magnesium chip (FagaLab CAS Number 2248-100) and 200 µL of 1N HCl. The reaction was observed, and discoloration was considered a positive indicator of phenolic compounds.

Tannins (Ferric Chloride, Sodium Chloride, and Gelatin Test)

For the determination of tannins, solutions of 0.02 % (w/v) $FeCl_3$ (FagaLab CAS Number 2143-100), 0.1 % (w/v) gelatin (MerckMillipre CAS Number 9000-70-8), and 0.01 % (w/v) NaCl (FagaLab CAS Number 2153-2500) were prepared. In three separate test tubes, 1 mL of the hydroalcoholic extract was added. In the first tube, 50 µL of $FeCl_3$ solution, 50 µL of gelatin solution, and 50 µL of NaCl solution were added. In the second tube, 50 µL of $FeCl_3$ solution and 50 µL of gelatin solution were added, while in the third tube, 50 µL of $FeCl_3$ solution and 50 µL of NaCl solution were added. The reaction was observed, and the formation of a precipitate in the gelatin-NaCl tube was considered a positive indication of tannin presence.

Saponins (Foam Test)

A total of 500 µL of the hydroalcoholic extract was placed in test tubes, followed by the addition of 1 mL of boiling water. Each tube was vortexed and left to rest for 15 minutes. The formation and persistence of foam indicated the presence of saponins.

Triterpenes (Salkowski Test)

For the triterpene test, 1 mL of the hydroalcoholic extract was placed in test tubes, followed by the addition of 1 mL of $CHCl_3$ concentrated and 1 mL of H_2SO_4 concentrated along the inner wall of the tubes. The tubes were gently agitated, and a reddish-purple coloration was considered a positive indication of triterpenes.

Alkaloids (Mayer and Wagner's Reagent Test)

For alkaloid detection, Wagner's reagent was used. A total of 500 µL of the hydroalcoholic extract was placed in a test tube, followed by the addition of 250 µL of 0.1N HCl and 750 µL of Wagner's reagent. The presence of turbidity or precipitate formation was considered a positive indicator of alkaloid presence.

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Extraction of Free and Bound Phenolics

The seeds were removed from the pods, cleaned, and milled using a hammer mill to obtain whole flour, which was subjected to phytochemical extraction with 80 % EtOH. The free fraction was obtained after centrifugation. The extraction of bound phenolic compounds followed the methodology described by Ruiz-Armenta *et al*. (2022). The residual pellet underwent hydrolysis with 10 mL of 4 M NaOH for 4 hours, with brief agitation every 30 minutes. Subsequently, 2 mL of concentrated HCl was added to neutralize the solution, followed by agitation for 10 minutes at moderate speed. Next, 10 mL of hexane was added, agitated for 10 minutes at moderate speed, and centrifuged at 4,000 × g at 10°C for 10 minutes to remove lipids. Finally, 10 mL of ethyl acetate was added, agitated for 10 minutes at moderate speed, and centrifuged at 4,000 × g at 10°C for 10 minutes to extract the released compounds. This last step was repeated four times. Both extract fractions were evaporated to dryness using a rotary evaporator and reconstituted in methanol for the determination of total phenolic compounds, antioxidant activity (ABTS and DPPH), and antidiabetic potential (inhibition of α-amylase and α-glucosidase).

Quantification of total phenolic compounds

The quantification of total phenolic compounds was performed according to the methodology reported by Ruiz-Armenta *et al*. (2022). Aliquots of 20 µL of free or bound extracts were oxidized with 1,800 µL of Folin-Ciocalteu reagent (Sigma-Aldrich CAS Number F9252), forming a blue coloration after 90 minutes of incubation. The absorbance of this reaction was measured using a spectrophotometer (UV-GENESYS model 10, Serial AQ7-2H7G229001, Thermo Electron Scientific Instruments LLC, Madison, Wisconsin, USA) at 760 nm. Standard curves were prepared using gallic acid (Sigma-Aldrich Cas Number 5995-86-8) in methanol at concentrations of 25, 50, 100, 200, 400, 600, 800, and 1,000 ppm. Results were expressed as mg of gallic acid equivalents (GAE) per gram of dry sample.

Determination of Antioxidant Capacity

ABTS Assay (2,2’-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))

The ABTS radical cation decolorization assay was performed following the method proposed by Re *et al.* (1999), which is based on the reduction of the $ABTS^{+}$ radical by antioxidants present in the extracts. A solution of ABTS (Sigma-Aldrich CAS Number A1888) was prepared by mixing it with potassium persulfate ($K_2S_2O_8$) (Sigma-Aldrich CAS Number: 7727-21-1) at a concentration of 2.45 mM in methanol. The resulting solution was stored in the dark for 16 hours to allow radical formation. The radical solution was then diluted with methanol to obtain an absorbance of 0.7 ± 0.02 at 734 nm. A volume of 20 µL of either blank (methanol) or extract was mixed with 1,980 µL of the $ABTS^{+}$ solution, homogenized gently, and its absorbance was read using a spectrophotometer (UV-vis Genesys 10 UV, Thermo Electron Corporation, Madison, WI, USA) at 734 nm. The reading was taken 5 minutes after adding the radical to the first sample. Trolox (Sigma-Aldrich CAS Number 135806-59-6) was used as the reference standard (0–800 µM), and

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the antioxidant activity of the samples was expressed as micromoles of Trolox equivalents (TE) per 100 grams of dry sample.

DPPH Assay (2,2-Diphenyl-1-picrylhydrazyl)

The DPPH radical scavenging activity of free and bound phytochemical extracts was determined using the method reported by Brand-Williams *et al*. (1995). The DPPH radical (Sigma-Aldrich CAS Number D9132-1G) was prepared at a concentration of 150 μM and protected from light. A 96-well microplate was used, where 20 μL of sample, blanks, and standards were placed. Then, 200 μL of DPPH solution was added, and absorbance was read at 517 nm using a spectrophotometer (Thermo Scientific Multiskan GO). Antioxidant activity was calculated using a Trolox standard curve (0–800 μM). Data were expressed as micromoles of Trolox equivalents per 100 grams of dry sample (μmol TE/100 g of sample).

Hypoglycemic Potential

The hypoglycemic potential of *C. cacalaco* seed flour extracts was evaluated through the inhibition of α-amylase and α-glucosidase, following the methodology described by Quintero-Soto *et al.* (2021).

α-Amylase Inhibition

For α-amylase inhibition, 10 μL of sample (30 mg/mL) and 10 μL of α-amylase solution (2 U/mL) were mixed and incubated for 10 minutes at 25°C. Then, 10 μL of a 1% starch solution was added, and the mixture was incubated for an additional 10 minutes at 25°C. Subsequently, 20 μL of a 3,5-dinitrosalicylic acid solution was added, followed by incubation for 5 minutes at 25°C. Finally, 200 μL of distilled water was added to the reaction mixture, and absorbance was measured at 540 nm using a spectrophotometer (UV-vis Genesys 10 UV, Thermo Electron Corporation, Madison, WI, USA). The assay was performed in triplicate. The results were expressed as the percentage of α-amylase enzyme inhibition, calculated as follows:

$$\frac{\%\ \text{inhibition} = ([A_{540}\,\text{blank} - A_{540}\,\text{extract}])\ {*}100}{(A_{540}\ \text{blank})}$$

Where: A_{540} blank = absorbance of the blank at 540 nm; A_{540} extract = absorbance of the extract at 540 nm.

α-Glucosidase Inhibition

For α-glucosidase inhibition, 50 μL of the sample (15 mg/mL) was mixed with 100 μL of α-glucosidase solution (1 U/mL), and the mixture was incubated for 10 minutes at 37°C. Subsequently, 50 μL of p-nitrophenyl-α-D-glucopyranoside (5 mM) was added, and the mixture was incubated again for 10 minutes at 37°C. Finally, absorbance was measured at 405 nm

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using a spectrophotometer (UV-vis Genesys 10 UV, Thermo Electron Corporation, Madison, WI, USA). The assay was performed in triplicate. The results were expressed as the percentage of α-glucosidase enzyme inhibition, calculated as follows:

$$\% \text{ inhibition} = \frac{([A_{405} \text{ blank-} A_{405} \text{ extract}]) * 100}{(A_{405} \text{ blank})}$$

Where: A_{405} blank = absorbance of the blank at 405 nm; A_{405} extract = absorbance of the extract at 405 nm.

Statistical analysis

The results of this research are presented as the mean of three replicates ± their corresponding standard deviation.

Results and discussion

Phytochemical Screening

Phytochemical screening is an assay that evaluates the potential of plants and enables the discovery and identification of molecules of pharmacological interest. Phytochemicals are synthesized from secondary metabolism and are not directly related to the primary processes of plant development and growth (Aronés-Jara *et al.,* 2022). The results of the phytochemical screening revealed the presence of phenolic compounds, tannins, and triterpenes, while saponins and alkaloids were absent in huizache flour (Table 1).

Table 1. Phytochemical screening of hydroalcoholic extract from huizache (*C. cacalaco*) seed flour

Phenolic compounds	Tannins	Saponins	Triterpenes	Alkaloids
+	+	-	+	-

Presence (+); Absence (-). Source: Own elaboration

Nawaz *et al.* (2020), detected the presence of tannins, flavonoids, cardiac glycosides, anthocyanins, terpenoids, and carotenoids in polar extracts using organic solvents (hexane, petroleum ether, chloroform, ethanol, acetone, and water) from bean seed flour (*Phaseolus vulgaris*), without observing saponins, phlobatannins, or anthraquinones in any of the extracts. Likewise, Haleshappa *et al*. (2022) demonstrated the qualitative presence of triterpenes, alkaloids, tannins, flavonoids, and glycosides in ethyl acetate, petroleum ether, and ethanol extracts of *Simarouba glauca* seeds. Gazwi *et al.* (2023), performed the phytochemical screening of pea seed coat (*Pisum sativum*), using water as a solvent with an extraction time of three hours under constant agitation at 25°C, reporting the presence of alkaloids, flavonoids, glycosides, tannins, and phenols, as well as the absence of terpenes and saponins. The phytoconstituents identified may be responsible for the health benefits of peas, as they have been reported to possess analgesic, antibacterial, and antioxidant properties.

Plants synthesize a wide variety of secondary metabolites containing a phenol group; these substances are known as phenolic compounds. Their structure consists of an aromatic ring with a hydroxyl group, and chemically, they form a highly diverse group ranging from simple phenolic acids to complex polymers such as condensed tannins and lignin (Martin-Gordo, 2017). Secondary metabolites, including tannins, alkaloids, saponins, flavonoids, glycosides, and resins, significantly contribute to plants' medicinal and physiological activities. Their chemical composition is influenced by the plant's defense mechanisms, soil conditions, and climate. For instance, tannin content varies according to the season, humidity, and light exposure (Camacho-Escobar *et al*., 2020). In recent decades, different health benefits of these metabolites have been reported. Terpenes, for example, are primarily used in the treatment of various diseases and can serve as anticancer (Bai & Tang, 2020), antimicrobial (Liu *et al*., 2020), anti-inflammatory (Kim *et al*., 2020), antioxidant (Querio *et al*., 2018), and antiallergic agents (Yang *et al*., 2021) through their derivatives' activity (Zhao *et al*., 2016). Products or organisms such as plants, citrus fruits, resinous trees, as well as certain actinobacteria and filamentous fungi, including the genera Streptomyces and Aspergillus, which contain these compounds, are an excellent source of vitamins A, E, and K, as well as coenzyme Q10 (Rodríguez-Concepción *et al*., 2018). Additionally, tannins in legumes have been studied for their antioxidant properties, cholesterol-lowering effects, blood pressure-lowering effects, and immune-stimulating effects. These effects occur because tannins can form complexes with carbohydrates, proteins, and specific mineral ions in food. The formation of these complexes depends on suitable conditions such as pH, temperature, and concentration (Grosse-Brinkhaus *et al*., 2016). Polyphenols, including tannins, are among the primary antioxidants in the human diet (Montes-Ávila *et al.*, 2017).

Conversely, the absence of saponins in this study is considered beneficial, as these compounds have been reported to impact human and animal diets negatively. Samtiya *et al*. (2020) stated that saponin intake can lead to weight loss, diarrhea, anorexia, and, in severe cases, death. High saponin intake in humans can reduce the bioavailability of minerals for absorption, making them antinutritional compounds.

This study investigated the nutraceutical properties of *C. cacalaco* flour to assess its potential use in food development. The results indicate the presence of important bioactive compounds such as phenolic compounds, tannins, and triterpenes, while saponins and alkaloids were absent (Table 1). These characteristics align with those of another significant native legume, mesquite (*Prosopis spp*.), as described by Valencia *et al*., (2020), who reported the presence of terpenes, saponins, and anthraquinones. The secondary metabolites found in plants enable them to produce and accumulate chemically diverse compounds, which, although not essential for

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plant life, play a crucial role in adaptation to environmental stress and defense against potential predators and pathogens (Lustre-Sanchez, 2022). Plants under stress or extreme conditions affecting their growth, development, or productivity produce specific secondary metabolites that directly influence their survival capacity (Gull *et al*., 2019).

Quantification of Total Phenolic Compounds

The total phenolic compound contents of the present work were 209.99 ± 4.78, 39.84 ± 2.01, and 249.83 ± 6.79 mg GAE/100 g, for the free, bound, and total fractions, respectively (Figure 1). These values are higher than those reported by Niño-Medina *et al.* (2017) for bean varieties (*Phaseolus vulgaris*), who obtained 1.50 mg GAE/g, 0.11 mg GAE/g and 1.61 mg GAE/g of sample in the free, bound and total fractions, respectively; this trend was repeated in the free and total fractions reported by Rochín-Medina *et al.* (2021) for the same bean variety, who obtained values with a variation between 7.32 to 45.87 mg GAE/100 g in the free fraction and values whose range was between 67.90 to 204.37 mg GAE/100 g for the total fraction; opposite case for the bound fraction whose values ranged between 43.55 to 158.50 mg GAE/100 g. De Camargo *et al.* (2022) reported free fraction values of 221.4 mg GAE/100 g, insoluble fraction values of 1,243.31 mg GAE/100 g, and a total value of 1,560.8 mg GAE/100 g. Comparing the total phenolic compound content of *C. cacalaco* seed flour with that of other arboreal legumes such as carob (*Ceratonia siliqua*) and mesquite (*Prosopis spp.*) suggests the potential future use of this flour. Carbas *et al*. (2019) reported values of 17.7±0.9 mg GAE/g and 0.5±0.01 mg GAE/g for carob and mesquite flour, respectively, both of which are already used as commercial ingredients in supplements such as Caromax® (Nutrinova, GmbH, Germany). The high polyphenol content provides valuable health benefits, such as cholesterol-lowering effects, antioxidant properties, and reduced risk of gastrointestinal cancer (*Stavrou et al.*, 2018).

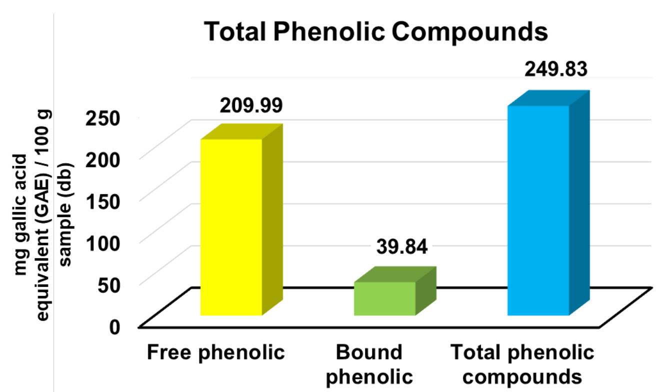

Figure 1. Free, bound and total phenolic compounds from huizache (*Caesalpinia cacalaco*) seed flour (mg gallic acid equivalent (mg GAE)/100 g sample (db)); db= dry base.

Source: Own elaboration

Furthermore, most of the phenolic compounds obtained from *C. cacalaco* were in their free form (84.05%). Free phenolics do not physically or chemically interact with other molecules and are generally soluble in aqueous/organic solvents, while conjugated phenolics are covalently bound to low-molecular-weight compounds such as sugars or fatty acids and are also soluble in aqueous/organic solvents (Rocchetti *et al*., 2022). The variation in phenolic compound content depends on the environmental conditions where the plant grows (temperature, precipitation, soil type, relative humidity, UV exposure, etc.) (Soto-Vasquez, 2015). Additionally, differences can be attributed to the various solvents used in the extraction process, temperature, and exposure time. The polarity of the extracted compounds depends on the solvent used, with highly polar solvents such as ethanol disrupting cell membranes, thereby enabling the extraction of the highest amount of phenolic compounds due to their polar nature (Garrido *et al.*, 2013).

Determination of Antioxidant Capacity

The antioxidant activity values for the free, bound, and total fractions in the ABTS assay were 2,745.22 ± 231.70, 1,186.04 ± 28.82, and 3,931.26 ± 202.87 μmol TE/100 g of sample, respectively. For the DPPH assay, the values were 1,245.45 ± 22.50, 1,255.42 ± 8.06, and 2,500.87 ± 14.44 μmol TE/100 g of sample for the same fractions (Table 2). The determination of antioxidant capacity is a mechanism-dependent reaction. In the present study, antioxidant analysis was performed using chemical reaction-based assays (DPPH and ABTS). Antioxidant activity is strongly correlated with the content of phenolic compounds, as they are the primary contributors to this activity (Szychowski *et al*., 2018). Therefore, if an extract has a higher phenolic content, it is expected to have a greater antioxidant capacity. Accordingly, the high phenolic content of *C. cacalaco* flour suggests its potential for the development of functional antioxidant products, as the DPPH value found was 2,500.87 ± 14.44 μmol TE/100 g of sample.

Table 2. Antioxidant activity, evaluated by ABTS and DPPH assays, of free, bound, and total phenolic extracts from huizache (*C. cacalaco*) seed flour.

Antioxidant activity	Free	Bound	Total
ABTS	2,745.22 ± 231.70	1,186.04 ± 28.82	3,931.26 ± 202.87
DPPH	1,245.45 ± 22.50	1,255.42 ± 8.06	2,500.87 ± 14.44

The values are the product of the average of three measurements ± the standard deviation. ABTS (μmol Trolox equivalents (TE)/100 g sample); DPPH (μmol TE/100 g sample).

Source: Own elaboration

Carbas et al. (2019) reported total antioxidant values of 5,890 ± 0.2 and 290 ± 1.0 μmol TE/100 g for *Ceratonia siliqua* and *Prosopis spp*., respectively, which are commercially utilized

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flours. Rochín-Medina *et al*. (2021) reported lower values than those obtained in this study for different varieties of common bean (*Phaseolus vulgaris*), with DPPH values ranging from 53.05 to 376.10 µmol TE/100 g, 190.54 to 1,064.63 µmol TE/100 g, and 1,282.70 µmol TE/100 g for the free, bound, and total fractions, respectively. Similarly, Niño-Medina *et al*. (2017) and Tungmunnithum *et al*. (2021) reported total antioxidant activity values by ABTS of 5.16 µmol TE/g for chickpea grain and 89.5 µmol TE/g for bean seed, respectively, which are lower than those obtained in this study.

The antioxidant activity of plant extracts cannot be evaluated using a single approach due to the complex nature of phytochemicals, as the determination of antioxidant activity largely depends on the reaction mechanism involved (Nazir *et al*., 2019). The activity of antioxidants can be tested using various analytical techniques relating to the different properties of the test compounds on the one hand, and to the different stages of the oxidative process and a different understanding of the role of antioxidants on the other (Munteanu & Apetrei, 2021). Research using stable free radicals ABTS and DPPH, which can be determined spectrophotometrically and are discolored upon deactivation, is particularly popular. However, their popularity, ease of use and susceptibility to modification have resulted in the appearance of many publications in which, because of the different versions of the assays used, the results are not comparable (Hano *et al*., 2017).

Hypoglycemic Potential

Inhibition of α-Amylase and α-Glucosidase

The α-amylase and α-glucosidase inhibition values are presented in Table 3. In this study, a concentration of 30 mg/mL of extract (free or bound phenolic fraction) was used to evaluate α-amylase enzyme inhibition, while a concentration of 15 mg/mL of extract (free or bound phenolic fraction) was used for α-glucosidase enzyme inhibition. The inhibition values for α-amylase were 31.75% and 37.42% for the free and bound phenolic fractions, respectively. For α-glucosidase inhibition, the values were 43.52% for the free phenolic fraction and 26.15% for the bound phenolic fraction.

Table 3. Hypoglycemic activity of free and bound phenolic extracts from huizache (*C. cacalaco*) seed flour.

Hypoglycemic activity	Free	Bound
α- amylase inhibition (%)	31.75 ± 1.27	37.42 ± 1.49
α- glucosidase inhibition (%)	43.52 ± 3.04	26.15 ± 1.94

The values are the product of the average of three measurements ± the standard deviation.

Source: Own elaboration

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One of the most effective strategies for reducing postprandial hyperglycemia (PPHG) in diabetes mellitus, particularly in insulin-independent diabetes mellitus, is to decrease glucose absorption by inhibiting carbohydrate-hydrolyzing enzymes in the digestive system, such as α-glucosidase and α-amylase (Kashtoh & Beak, 2022). Various plant species, such as Gymnema (*Gymnema sylvestre*), cinnamon (*Cinnamomum verum*), bitter melon (*Momordica charantia*), turmeric (*Curcuma longa*), blueberry (*Vaccinium myrtillus*), and prickly pear cactus (*Opuntia ficus-indica*), have been used to counteract the effects of diabetes or, at least, to offer an alternative to medication or to reduce the dosage (Day *et al.,* 1990; Zare *et al.*, 2019; Devangan *et al.*, 2021).

Regarding α-amylase and α-glucosidase inhibition, studies have demonstrated that specific legume components possess this effect. Tungmunnithum *et al*. (2021) reported lower inhibition values for 10 varieties of cooked Fabaceae extracts, with α-amylase and α-glucosidase inhibition ranging between 15.6–25.2% and 16.26%, respectively, using 200 and 100 µL of sample with 200 and 100 µL of enzyme (13 U/mL and 10 U/mL, respectively). Quintero-Soto *et al.* (2021) determined the antidiabetic potential of hydrolyzed chickpea (*Cicer arietinum* L.) proteins, obtaining higher inhibition values than those found in this study. They reported average inhibition rates of 54% for α-amylase using 5 µL of sample with 5 µL (13 U/mL) of enzyme, and an average inhibition rate of 56% for α-glucosidase using 25 µL of sample with 50 µL (1 U/mL) of enzyme.

Similarly, Doué *et al.* (2021) evaluated the in vitro antidiabetic potential of germinated soybean (*Glycine max*) seed extracts and *Vigna subterranea* protein isolates through α-amylase and α-glucosidase inhibition assays using 30 µL of extract and 50 µL of enzyme (1.5 U/mL) for both assays. They reported average α-amylase inhibition values of 19.73% in soybean and 21.76% in *Vigna subterranea*, while α-glucosidase inhibition values were 16.55% and 28.47%, respectively.

Some phytochemicals accumulated in Fabaceae seeds have been shown to inhibit α-glucosidase and α-amylase and/or stimulate glucose uptake (Maliehe *et al*., 2019). Inhibition of these enzymes reduces serum glucose levels, which is beneficial for controlling postprandial blood glucose (Yan *et al*., 2019). The inhibitory activity increases with higher enzyme concentrations, enhancing the inhibitory potential of the sample or tested inhibitor. This trend has been observed in numerous studies on antidiabetic peptides in legumes (Ngoh & Gan, 2016; Wang *et al*., 2019).

Variations in results may be attributed to enzymatic inhibition activity being influenced by climatic conditions and altitude variations, species type, and environmental factors such as precipitation, soil conditions, sunlight exposure, and experimental methodologies (Sharma & Giri, 2022).

Conclusions

Huizache seed flour exhibited a high content of total phenolic compounds and demonstrated significant antioxidant and hypoglycemic potential. Therefore, this flour could serve as a valuable source of these bioactive compounds for combating various chronic degenerative diseases. Additionally, this study provides new insights into a species with limited research on phenolic quantification and antioxidant potential. This work is the first to evaluate the nutraceutical potential of Huizache seed flour. It is recommended that future studies explore its potential in other biological activities related to its ethnomedicinal uses.

Authors' contributions

Conceptualization of the work: C.T.J.A., G.F.M.A..; Writing and preparation of the manuscript: C.T.J.A., G.F.M.A.; Writing, reviewing and editing: C.T.J.A., A.C.R., M.A.I.G., V.G.P., R.A.X.A. P.D.X.P., G.F.M.A.; Creation and editing of the figure: C.T.J.A., G.F.M.A.

"All authors of this manuscript have read and agreed to the published version".

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Conflicts of interest

The authors declare no competing interests. The manuscript has not been submitted for publication in other media with substantial circulation. All previously published works cited in the manuscript have been duly acknowledged. All authors have made substantial contributions to the manuscript and approved the final submission. No interest conflicts exist between the authors and the reviewers who assessed this manuscript. The preparation of this manuscript strictly adhered to the journal formatting provided in the authors' guide.

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